SRμCT-based study of external and internal structures of adults and larvae of Endopterygota (Hexapoda)

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Background
The investigations at DESY were carried out in the framework of an extensive phylogenetic project (DFG: BE 1789/4-1) aiming at the clarification of the relationships within the most species rich group of organisms, the holometabolous insects or Endopterygota (ca. 800.000 spp.) [1]. More than 30 ingroup taxa representing all conventional endopterygote orders and four outgroup taxa will be included in the phylogenetic analyses. With a combination of different traditional (dissection, histology) and modern techniques (SEM, CLSM, μCT), an extensive and well documented morphological data set will be compiled and finally combined with molecular data. Following a total evidence approach the phylogeny of Endopterygota will be reconstructed with cladistic software programmes and evolutionary scenarios will be developed on the basis of the results.

Material and specimen preparation
Adults of Neuroptera (2 genera), Megaloptera, Hymenoptera, Trichoptera, Lepidoptera, Mecoptera, Siphonaptera, and Diptera were examined, and the larval head of Nevrorthus (Neuroptera). All specimens were either fixed in ethanol (70%, 100%) or FAE (Formaldehyde-ethanol-acetic acid) and critical point dried. Adults were cut into two halves at the anterior abdominal segments. Protruding structures such as wings antennae and legs were cut off near their base in order to minimise the field of view for a maximal scan resolution. The specimens were mounted on cylindrical metal carriers with superglue.

Results
The synchrotron radiation based micro computed tomography (SRμCT) applied to adult and immature specimens yielded results of superior quality, allowing a highly efficient computer based three dimensional reconstruction of external and internal features (Figs 1, 2). The maximum resolution obtained was 4.4μm. In contrast to experiments carried out at other institutions (e.g., Center for Synchroton Radiation Research and Instrumentation [CSRRI], Illinois Institute of Technology) [2] it is possible to use a stable SR-beam at low photon energy (8 KeV) at the beamline BW2, which allows to discern different tissue types such as cuticle, musculature and elements of the nervous system very easily by its specific density. The distinctly different greyscales allow not only an efficient reconstruction of separate internal body parts (e.g. nervous system), but are essential for the morphological interpretation of complex structures.

Outlook
SRμCT is likely a key innovation in insect morphology and has the potential to trigger a major renaissance in this discipline. Most systematists agree that most reliable results are obtained with a combination of molecular data and an extensive and well documented set of morphological characters. The use of SRμCT will contribute to a major progress in insect phylogeny in the near future.

Acknowledgements
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References

[1] Beutel, R. G., and H. Pohl, H. Systematic Entomology, 31, 202-219, (2006)
[2] Beutel, R. G., S.-Q. Ge, and T. Hörnschemeyer, T. Cladistics, (online early)

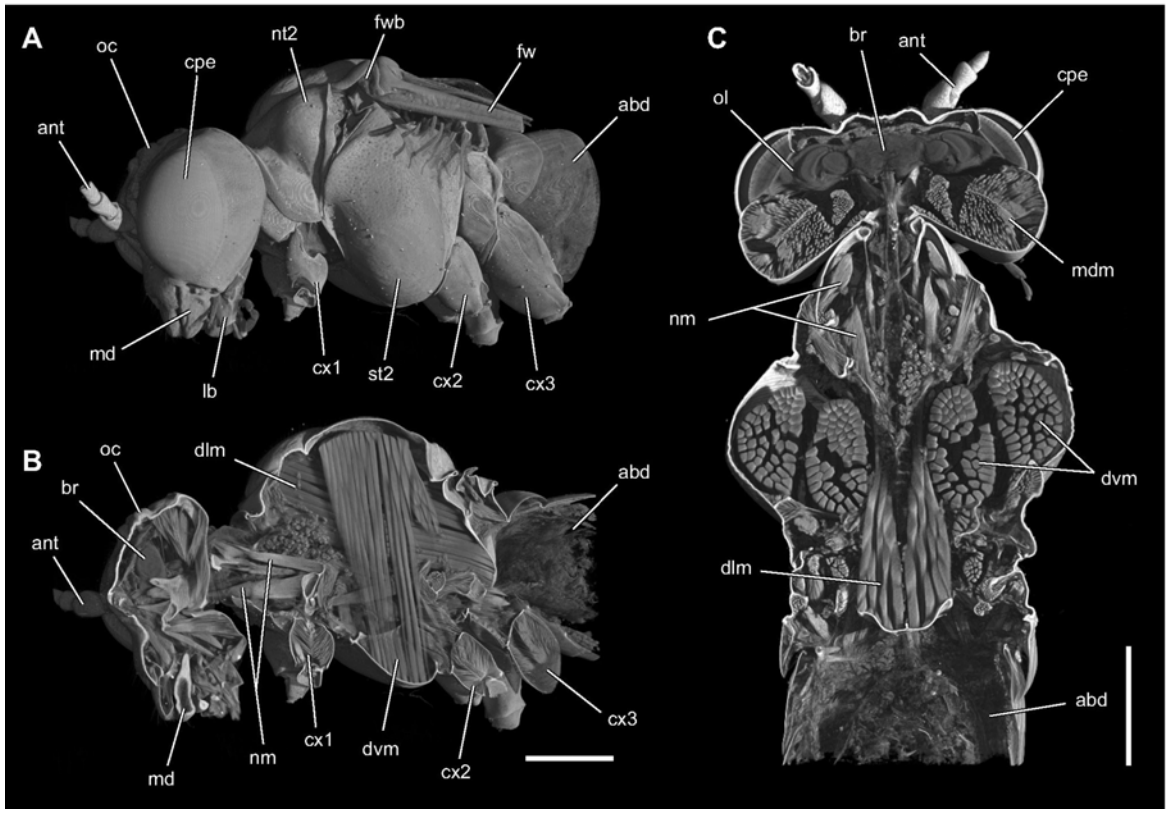

Figure 1: External and internal anatomy of the head and thorax of the sawfly *Tenthredo vespa*. Reconstruction based on SRμCT data (2x binned) using myVGI. **A.** Lateral view. **B.** Sagittal section. **C.** Horizontal section. abd – abdomen, ant – antenna, br – brain, cpe – compound eye, cx1/2/3 – pro-/meso-/metacoxae, dvm/dlm – dorso-ventral and dorsal longitudinal parts of indirect flight muscles, fw – fore wing, fwb – fore wing base, lb – labium, md – mandible, mdm – mandibular muscle, nm – neck muscles, nt2 – mesonotum, ol – optical lobes of the brain, oc – ocellus, st2 – mesosternum. Scale bar: 1mm.

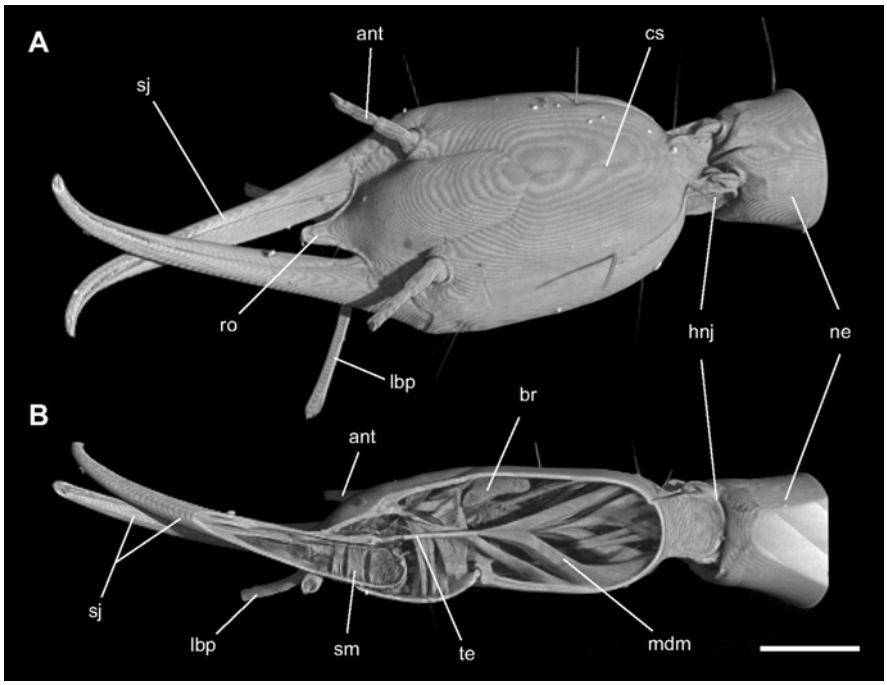

Figure 2: Head of the minute aquatic larva of *Nevrorthus* (Neuroptera). Reconstruction based on SRμCT data (2x binned) using myVGI. **A.** Dorsolateral view. **B.** Sagittal section. ant – antenna, br – brain, cs – coronal suture, hnj – head-neck joint, lbp – labial palp, mdm – mandibular muscle, ne – neck, ro – rostrum, sj – sucking jaw, sm – sucking muscles, te – tendon. Scale bar: 500µm.