Entomologie heute 26 (2014): 151-160

Scanning the Past – Synchrotron X-Ray Microtomography of Fossil Wasps in Amber

Die Vergangenheit scannen – Synchrotron-Röntgenmikrotomographie fossiler Wespen in Bernstein

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Summary: Synchrotron-based X-ray microtomography has become an important technique to examine fossil insects in amber. The method allows a non-destructive visualization of amber inclusions without distracting particles or reflections and even facilitates the examination of internal anatomical characters. Recent experiments on fossil wasps from various types of amber revealed a remarkable degree of variation regarding the conditions of the fossils. While many inclusions are just insect-shaped voids, others contain undefined particles, degenerated organs or even well preserved internal anatomical structures.

Keywords: Synchrotron, X-rays, microtomography, amber inclusions, Hymenoptera

Zusammenfassung: Synchrotron-basierte Röntgenmikrotomographie eignet sich hervorragend, um fossile Insekten in Bernstein zu untersuchen. Die Methode erlaubt die zerstörungsfreie Visualisierung von Bernsteininklusen, ohne dass störende Partikel oder Reflektionen die Sicht auf das Objekt behindern, und ermöglicht sogar die Untersuchung innerer anatomischer Merkmale. Aktuelle Experimente an fossilen Wespen aus verschiedenen Bernsteinarten zeigten eine bemerkenswerte Variation hinsichtlich des Erhaltungszustands der Fossilien. Während viele Einschlüsse lediglich als Blasen in Insektenform erhalten waren, enthielten andere undefinierte Partikel, degenerierte Organe oder sogar gut erhaltene innere anatomische Strukturen.

Schlüsselwörter: Synchrotron, Röntgenstrahlung, Mikrotomographie, Bernstein-Inklusen, Hymenoptera

1. Introduction

In contrast to fossil vertebrates, which are almost exclusively preserved as bony remains, insect fossils occur in wider variety, i.e. as compressions and impressions, mineral replications, charcoalified remains and inclusions in amber (Grimaldi & Engel 2005 and references therein). In addition, trace fossils like damaged plants (e.g. Stephenson & Scott 1992; McLoughlin 2011; Labandeira et al. 2014), fossil galls (McLoughlin 2011) and coprolites (e.g. Vršanský et al. 2013) provide evidence for the behavior of extinct insects and their physical interactions with plants.

Amber inclusions are characterized by a three-dimensional preservation state and a high level of completeness. The sources of amber are ancient tree resins, which are complex mixtures, composed of multiple individual components (Langenheim 2003). As the chemical composition of resin differs considerably between tree species, amber from different deposits shows a great deal of variation, including the preservation quality of insects, which were trapped inside (Martínez-Delclòs et al. 2004). In Eocene

Baltic amber, the body cavity of an insect is often preserved merely as a void (Grimaldi & Engel 2005), while well-preserved soft tissue, single cells and even organelles were recognized in insect fossils from Miocene Dominican amber (Grimaldi et al. 1994).

Until recently it was difficult to visualize the internal anatomy of insect inclusions in amber. Azar (1997) dissolved Lebanese amber using chloroform; other authors were able to cut pieces of amber along a plane (e.g. Henwood 1992; Grimaldi et al. 1994). The precious samples, however, got utterly destroyed in course of the studies.

Synchrotron-based X-ray imaging provides powerful techniques to visualize internal characters of insects (Westneat et al. 2008; van de Kamp et al. 2013) and is frequently employed to study the anatomy (Socha & De Carlo 2008; Kim et al. 2012), physiological processes (Betz et al. 2008; Westneat et al. 2003; Socha et al. 2007) and functional morphology (van de Kamp et al. 2011, 2014; dos Santos Rolo et al. 2014) of insects. Synchrotron X-ray computed microtomography (SR-µCT) in particular has become increasingly popular among entomologists in recent years, as it allows examining the three-dimensional morphology of insects with a high spatial resolution below 1 µm (Betz et al. 2007; Weide & Betz 2008). The non-destructive technique was already employed in a couple of studies to examine insect inclusions in amber (e.g. Lak et al. 2009; Soriano et al. 2010; Riedel et al. 2012; Henderickx et al. 2013; Peris et al. 2014), partly revealing well-preserved anatomic characters (e.g. Pohl et al. 2010; Perreau & Tafforeau 2011).

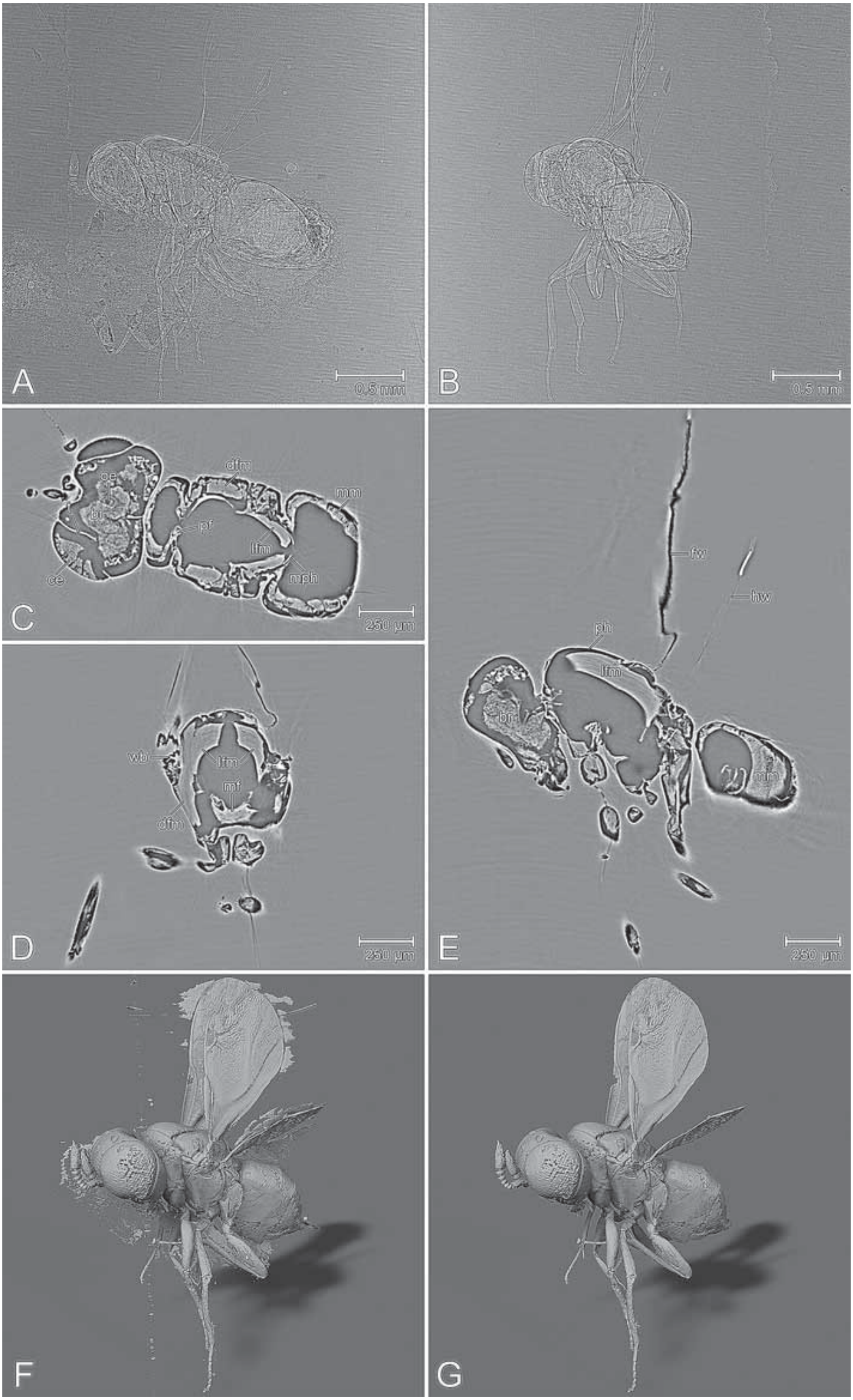
Here, we present some examples from recent experiments carried out at the ANKA Synchrotron Radiation Facility of Karlsruhe Institute of Technology and discuss tomographic scans of fossil wasps from the Eocene (Baltic and French Oise amber) and from the Miocene (Dominican amber).

2. Method

Tomographic scans were performed at the TOPO-TOMO beamline of the ANKA Synchrotron Radiation Facility using an indirect detector system composed of a scintillating screen, a white beam macroscope (Elya Solutions, s. r. o.) and a 12 bit pco.dimax high speed camera. For each tomogram, 2.500 radiographs covering an angular range of 180° were acquired using a filtered white beam with the spectral peak at about 15 keV. For amber specimen SMNS

Fig. 1: Well-preserved chalcid wasp (MNHN 16311; Chalcidoidea: Pteromalidae: Pireninae) from French Oise amber (54-56 mya). **A**, **B** Two of 2,500 radiographic projections, which were acquired in the tomographic scan. **C**-**E** Virtual slices of the tomographic volume from different viewing angles. **F**, **G** Surface reconstruction based on the tomographic volume (F: result of automated greyscale segmentation; G: reconstruction after manual removal of artifacts). br = brain; ce = compound eye; dfm = dorsoventral flight muscle; fw = forewing; hf = hindwing; lfm = longitudinal flight muscle; mm = metasomal muscles; mf = mesofurca; mph = mesophragma; oe = oesophagus; pf = profurca; ph = phase shift; t = tentorium; wb = wing base.

Abb. 1: Gut erhaltene Erzwespe (MNHN 16311; Chalcidoidea: Pteromalidae: Pireninae) aus französischem Oise-Bernstein (ca. 55 Mio Jahre alt). **A**, **B** Zwei von insgesamt 2.500 2D-Projektionen, die während des tomographischen Scans aufgenommen wurden. **C**-**E** Virtuelle Schnitte durch den tomographischen Volumendatensatz aus verschiedenen Perspektiven. **F**, **G** Oberflächenrekonstruktion basierend auf den Tomographiedaten (F: Resultat der automatischen Graustufen-Segmentierung; G: Rekonstruktion, nachdem die Artefakte manuell entfernt wurden). br = Gehirn; ce = Komplexauge; dfm = dorsoventraler Flugmuskel; fw = Vorderflügel; hf = Hinterflügel; lfm = Longitudinaler Flugmuskel; mm = metasomale Muskeln; mf = mesofurca; mph = mesophragma; oe = Oesophagus; pf = profurca; ph = Phasengrenze; t = Tentorium; wb = Flügelbasis.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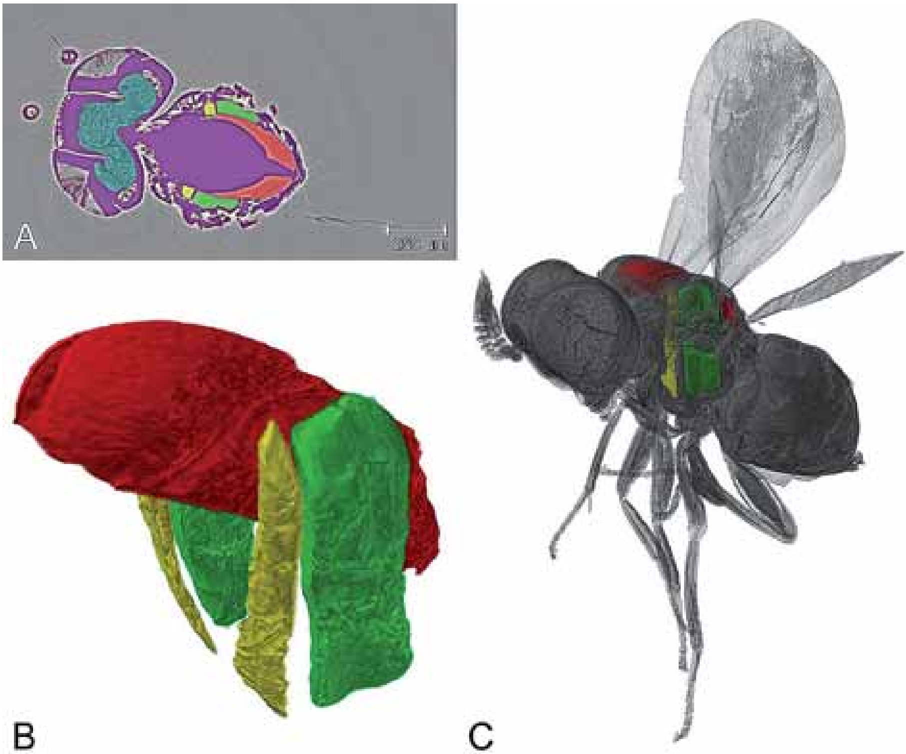
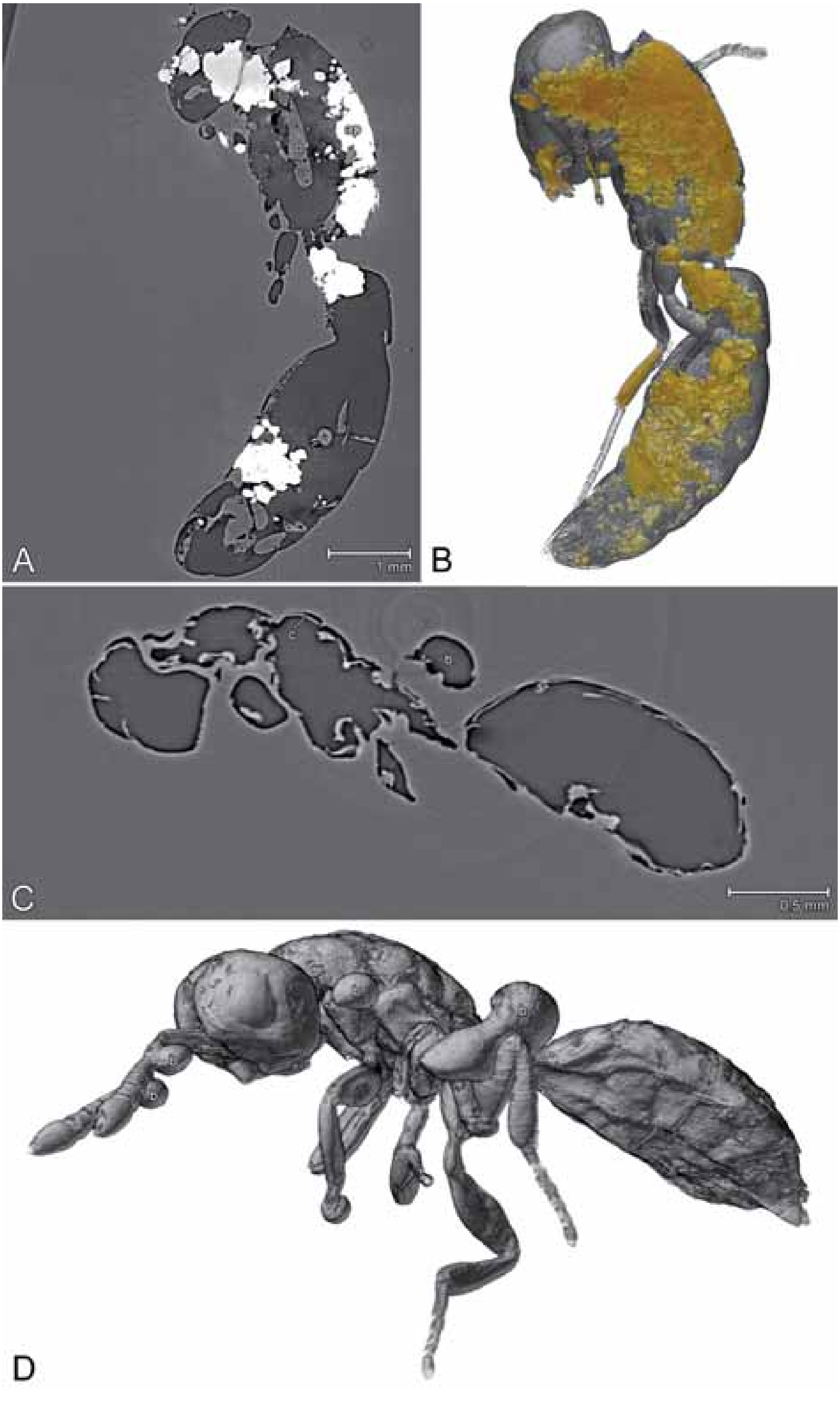


Fig. 2: Segmentation of the tomographic volume from Fig. 1. **A** Slice showing labels of body cavity (violet), brain (turquoise), longitudinal (red) and dorsoventral flight musculature (yellow and green). **B** Volume rendering of the segmented flight muscles. **C** Flight musculature inside a semi-transparent rendering of the wasp.

Abb. 2: Segmentierung des Tomographiedatensatzes aus Abb. 1. **A** Schnitt mit Markierungen für Körperhöhle (violett), Gehirn (türkis), longitudinale (rot) und dorsoventrale Flugmuskulatur (gelb und grün). **B** Volumenrendering der segmentierten Flugmuskeln. **C** Flugmuskulatur im halbtransparenten Rendering der Wespe.

Fig. 3: Poorly-preserved fossil wasps. **A**, **B** Potter wasp (MNHN 3145; Vespoidea: Vespidae: Eumeninae) from French Oise amber (54-56 mya), partly filled with highly-absorbing material, maybe due to mineralization (A: slice of tomographic volume; B: volume rendering showing 3D distribution of highly-absorbing particles (yellow)). **C**, **D** Chalcid wasp (SMNS Do-4171-B; Chalcidoidea: Pteromalidae: Cerocephalinae) from Dominican amber (15-20 mya), which is almost completely hollow (C: slice of tomographic volume; D: volume rendering). ap = highly-absorbing particles; b = air bubbles; c = cuticle remains; o = organ remains.

Abb. 3: Schlecht erhaltene fossile Wespen. **A**, **B** Solitäre Faltenwespe (MNHN 3145; Vespoidea: Vespidae: Eumeninae) aus französischem Oise-Bernstein (54-56 Mio Jahre alt), teilweise gefüllt mit stark absorbierendem Material (A: Schnitt durch Tomographiedatensatz; B: Volumenrendering, das die 3D-Verteilung der stark absorbierenden Partikel (gelb) verdeutlicht). **C**, **D** Erzwespe (SMNS Do-4171-B; Chalcidoidea: Pteromalidae: Cerocephalinae) aus dominikanischem Bernstein (15-20 Mio Jahre alt), die fast vollständig hohl ist (C: Schnitt durch Tomographie-Datensatz; D: Volumenrendering). ap = stark absorbierende Partikel; b = Luftblasen; c = Überreste der Cuticula; o = Überreste von Organen.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D

BB-2621, the magnification was set to 5X, resulting in an effective pixel size of 2.44 µm. All other samples were tomographed with a magnification of 3X and a pixel size of 3.67 µm. A frame rate of 25 images per second resulted in a scan duration of 100 seconds for each tomogram. Before reconstruction, the projections were processed with the phase retrieval ImageJ plugin ANKAphase (Weitkamp et al. 2011). Volume reconstruction was done by the PyHST software developed at the European Synchrotron Radiation Facility in Grenoble, France. Volume rendering, image segmentation and surface reconstruction were done with the software Amira (FEI). Surface meshes (Figs 1F, G & 4 B) were visualized with the 3D animation software CINEMA 4D 14 (Maxon Computer GmbH). Amber specimens are deposited in the collections of the Muséum national d'histoire naturelle, Paris (MNHN) and the State Museum of Natural History Stuttgart (SMNS).

3. Specimens examined

3.1. MNHN 16311:

Chalcid wasp (Chalcidoidea: Pteromalidae: Pireninae) from French Oise amber; 54-56 mya; body length 1.2 mm (Figs 1, 2).
While the imprint of the wasp reveals remarkable details of the body surface, the original cuticle seems to have degenerated over time. The body cavity appears as a void inside the amber, but inner organs, including brain, compound eyes and most of the musculature are astonishingly well-preserved. The entire digestive system, however, is lacking.
A surface model of the wasp was created automatically by grey-scale segmentation of the tomogram (Fig. 1F) and artifacts were removed (Fig. 1G). Parts of the internal mesosomal anatomy, such as the flight musculature were manually segmented (Fig. 2).

3.2. MNHN 3145:

Potter wasp (Vespoidea: Vespidae: Eumeninae) from French Oise amber; 54-56 mya ; body length 7.2 mm (Fig. 3A, B).
Rather poorly-preserved fossil. The imprint of the original wasp inside the amber is distinct, but the level of surface details is not as high as in the above specimen. Remains of wings cannot be recognized.
The cuticle is damaged; some fragments inside the body cavity may represent remains of organs, but their original positions and shapes are largely gone. Very prominent are highly-absorbing parts inside the void, which may be a result of soft tissue mineralization and are distributed throughout the whole imprint.

3.3. SMNS Do-4171-B:

Chalcid wasp (Chalcidoidea: Pteromalidae: Cerocephalinae) from Dominican amber; 15-20 mya; body length 2.1 mm (Fig. 3C, D).
Surface preservation of the wasp is similar to the specimen described before. Inside the imprint, minor cuticle fragments can be recognized, but anatomical parts have entirely degenerated. Small air bubbles are connected to the imprint of the wasp.

3.4. SMNS BB-2621:

Chalcid wasp (Chalcidoidea: cf. Pteromalidae) from Baltic amber; 38-54 mya; body length 2.3 mm (Fig. 4A, B).

Abb. 4: Erzwespe (SMNS BB-2621; Chalcidoidea: cf. Pteromalidae) aus Baltischem Bernstein (38-54 Mio Jahre alt). **A** Schnitt durch den Tomographiedatensatz; man beachte den gut erhaltenen ursprünglichen Abdruck des Insekts und die zusammengefallene Cuticula. **B** Oberflächenrendering, basierend auf einer automatischen Grauwertsegmentierung; man beachte die feinen Risse, die die Wespe umgeben. b = Luftblase; cr = Risse; oi = ursprünglicher Abdruck; sc = eingefallene Cuticula.

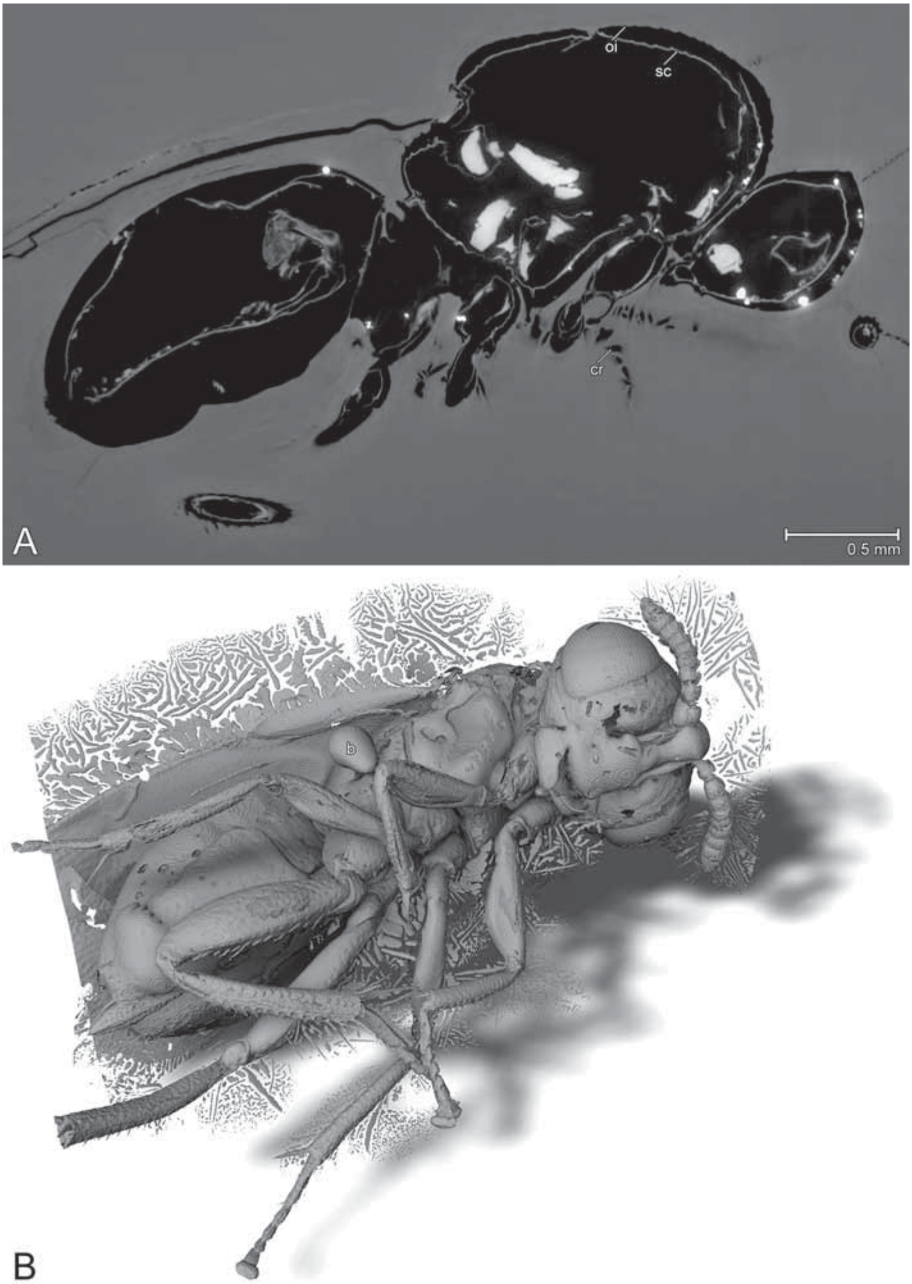

Fig. 4: Chalcid wasp (SMNS BB-2621; Chalcidoidea: cf. Pteromalidae) from Baltic amber (38-54 mya). **A** Slice of the tomographic volume; note the well-preserved original imprint of the insect and the shrunken original cuticle. **B** Surface rendering based on automated greyscale segmentation; note the multiple cracks surrounding the wasp. b = air bubble; cr = cracks; oi = original imprint; sc = shrunken cuticle.

While the surface of the specimen is generally well-preserved, multiple tiny cracks, which are connected to the imprint, make it difficult to isolate the wasp digitally from the tomographic volume. Internally, the cuticle is detached from the original imprint and shrunken. Some remains of organs are visible but lost their original structure.

4. Discussion

X-ray microtomography revolutionized the examination of insect fossils in amber. By providing three-dimensional views of complete samples without distracting particles and reflections and the possibility to picture the anatomy of extinct species, it is of great value for entomologists reconstructing evolutionary history (e.g. Pohl et al. 2010; Riedel et al. 2011).

At ANKA, we scanned multiple different fossil insects from various amber deposits. The examples highlighted in this article can only roughly reflect the spectrum of conditions found in different samples.

From our experience, it is quite impossible to predict the degree of preservation of an insect by the outer shape of the inclusion. Many amber inclusions turned out to be merely insect-shaped voids (SMNS Do-4171-B). The cuticle may appear degenerated (MNHN 16311), shrunken (SMNS BB-2621) or in its original shape. Other inclusions contained undefined particles (MNHN 145) and some showed an astonishingly well-preserved anatomy suitable for taxonomic studies based on internal characters. It is noteworthy that preservation varies between anatomical structures. While the musculature of MNNH 16311 is well-preserved and reflects its original condition, other organs and the exoskeleton are completely degenerated. In rare cases, specimens, who look fine under a stereo microscope, were not visible at all using X-rays.

X-ray examination of insects in amber is still in its infancy, but our preliminary results already revealed a great variety regarding the conditions of amber fossils – even in the same type of amber. Large comprehensive studies on the different kinds of amber are still missing and it is too early to link the degree of internal preservation with the different amber types. Studies of Pohl et al. (2010) and Perreau & Tafforeau (2011) already revealed well-preserved organs in Baltic amber, which was previously considered to contain mostly voids (Grimaldi & Engel 2005).

Advances in X-ray imaging like high throughput synchrotron tomography facilitate the scanning of large amount of samples in comparatively short time and will surely fasten the acquisition of data. Careful examination of the tomographic volumes, however, will remain time-consuming.

Even if SR-μCT is often described as being non-destructive, it should be mentioned that intense synchrotron-based X-rays – and in particular the polychromatic radiation employed for high-throughput experiments – usually results in browning of the amber. From our experience, the browning is not persistent and fades after a couple of months.

Acknowledgements

The research was partially funded by the German Federal Ministry of Education and Research by grants 05K10CKB and 05K12CK2 (UFO/UFO2 – Ultra fast X-ray imaging of scientific processes with on-line assessment and data-driven process control). Additional support was received from the SYNTHESYS Project (http://www.synthesys.info) which is financed by European Community Research Infrastructure Action under the FP7 "Capacities" Program (FR-TAF-341). Günter Bechly (SMNS) and André Nel (MNHN) are thanked for loan of material and access to their collections. The ANKA Synchrotron Radiation Facility is acknowledged for providing beamtime.

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Zeitschrift/Journal: Entomologie heute

Jahr/Year: 2014

Band/Volume: 26

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Artikel/Article: Scanning the Past – Synchrotron X-Ray Microtomography of Fossil Wasps in Amber. Die Vergangenheit scannen – Synchrotron-Röntgenmikrotomographie fossiler Wespen in Bernstein 151-160