Report from the Experiment EC-639

Preliminary title of the manuscript: *Microfossils from the amber: record revealed by nanotomography*

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Introduction

The study of organisms trapped in fossilized amber has allowed detailed characterization of the morphology of extinct organisms, and has provided many insights on the evolution of macroscopic organisms such as insects. However, smaller organisms such as protists are much more challenging to identify because of their relative lack of conspicuous features. In particular, the existence of certain structures resembling organisms, named here inclusions, can easily bias our interpretation of past communities. Here, our study revealed structure of microinclusions trapped to the resin ca 45 million years ago. We made an experiment with the nanotomography that was applied to the Baltic amber, and our study revealed that some structures identified with light microscopy as testate amoebae (Amoebozoa, Arcellinida) remained as not identified inclusions. We have tried to explain the rarity of the protists' fossils in the Baltic amber that has been studied for over 150 years. Furthermore, we want to revise identification issues connected with potential pitfalls of testate amoebae identification found in the amber.

Methods

We studied several fragments of the Baltic amber that was obtained from Sambia coast (Eastern Baltic, Russia). There were three protist-like specimens found that resembled testate amoebae (similar to Arcella or Centropyxis). The specimens were examined and documented under the light microscope. Then microinclusions were scanned in ESRF by X-ray synchrotron – using nanotomography method developed by Peter Cloetens. Furthermore, we scanned other fossils that were found in the amber: one mite and microscopic insect.

The X-ray microscope implemented on the nano-imaging station ID22NI of the ESRF was used to perform nano-tomography with a voxel size between 60 and 390 nm (Mokso et al., 2007;

Requena et al., 2009). The technique uses as illumination source the focused spot of crossed multilayer-coated bent mirrors (Kirkpatrick–Baez mirror optics). The beam size at the focal point was of about 80 nm in both directions. The geometric magnification *M* of the setup is given by $M = (z_1 + z_2)/z_1$, where $z_1$is the distance between the focal point and the sample, and $z_2$between the sample and the detector. The multilayer system provides a medium monochromaticity of $\Delta E/E \approx 1.0 \cdot 10^{-2}$. Tomography scans consisting of 1500 or 2000 two-dimensional radiographs were performed by rotating the sample around a vertical axis by one complete turn. The transmitted intensity was acquired with a CCD based detector system developed at ESRF, which had 1500 x 1500 pixels and an effective pixel size of 1 µm. Radiographs were taken at four sample-source distances $z_1$while keeping the focus-to-detector distance $z_1 + z_2$fixed at 525 mm. All samples were scanned at a X-ray energy of 17 keV. From radiographs measured at the same angular position but different distances the phase of the X-rays exiting the sample was retrieved (Cloetens et al., 1999) and used as input in the Filtered Back-Projection Algorithm method to reconstruct the 3D distribution of the refractive index decrement.

Results and Prospects

The results of the scanning are 3D models of the microfossils found in the Eocene amber. Finally it is difficult to assign the remains to any taxonomic group; however we can state that we recorded organic microfossils are very similar to testate amoebae in structure. The fossils are flattened-spherical with a visible opening – like an aperture in case of testate amoebae. Colour of shells is brown, shape circular and hemispherical in the lateral view.

Light microscope showed a specific structure of shells at first look that reminded *Arcella* sp. shell construction with organic building units and numerous pores. Nevertheless, a closer look revealed the undulation rather than pores that was visible in lower magnification. Then samples were analysed in ESRF in aim to study shell structure in detail. All three testate amoebae-like inclusions were scanned and they will be the matter of the planned publication. The nanotomographic study revealed that these structures are not testate amoebae. Our example here reveals how far these structures can bias our interpretations of the fossil record. We are planning therefore to critically review previous findings of testate amoebae found in amber samples, and draw conclusions on possible misinterpretations and its consequences in our vision of their fossil records.

In the extended group of scientists [Prof. Sina Adl (Canada), Prof. Vincent Girard (Germany)] it is planned to continue the work on the Baltic as well as French amber (Adl et al., 2011; Girard and Adl, 2011) where testate amoebae and other microorganisms were certainly identified. We expect to deepen the understanding of phylogeny and taxonomy of testate amoebae and relate results to the existing modern data calibrate the molecular clock (Berney and Pawlowski, 2006).

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