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Synchrotron X-ray imaging of inclusions in amber

Imagerie par rayonnement X synchrotron d'inclusions dans l'ambre

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ABSTRACT

Over the past six years, organic inclusions preserved in amber samples from outcrops worldwide have been discovered and imaged in 3D using propagation phase contrast based X-ray synchrotron imaging techniques at the European Synchrotron Radiation Facility (ESRF). A brief description of the techniques and protocols used for detecting and 3D non-destructive imaging of amber inclusions is provided. The latest results from the major amber projects in the ESRF are given, illustrating the increasing utility of the imaging capabilities of X-ray synchrotron phase contrast microtomography.

RÉSUMÉ

Au cours des six dernières années, de nombreuses inclusions d'organismes préservés dans des ambres d'origines géographiques diverses ont été imagées en trois dimensions, voire découvertes, grâce à la microtomographie à haute résolution en contraste de phase à rayonnement X synchrotron, à l'Installation Européenne de Rayonnement Synchrotron (ESRF, Grenoble, France). Une brève description des techniques et protocoles utilisés pour la détection et l'imagerie 3D non destructive des inclusions de l'ambre est fournie. Les

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derniers résultats des principaux projets engagés sur l'ambre à l'ESRF présentés ici montrent l'intérêt croissant des possibilités d'imagerie par microtomographie en rayonnements X synchrotron.

1. Introduction

Amber results from the fossilization of various tree resins and is found in sediments dating from the Paleozoic to Holocene. Although the oldest ambers with biological remains (microorganisms) date from the Triassic (Schmidt et al., 2006), macroinclusions have not been found in sediments older than the Lower Cretaceous (c.a. Grimaldi and Engel, 2005; Martínez-Delclòs et al., 2004). Due to the exceptional preservation of organisms in amber, these Lagerstätten often preserve a significant part of the ecosystems in which the amber-producing trees lived. Further, ethological behaviour may be captured (such as evidence of phoresis or parasitism). Various studies have demonstrated that it is possible to obtain information about the soft anatomy of organisms preserved in amber (Grimaldi et al., 1994; Penney et al., 2007; Poinar and Hess, 1982), although most of these techniques led to the destruction of the sample (e.g. Grimaldi et al., 1994).

Amber opacity varies greatly among deposits and between samples from the same outcrop. In some cases, the transparency of the resin is high enough to allow the study of the inclusions by classical optic methods (binocular, electronic and confocal microscopes). However, in other cases, the inclusions may be obscured by amber impurities, white foams (due to presence of microbubbles) or oxidation or hydration processes (Martínez-Delclòs et al., 2004), greatly decreasing access to anatomical data of the animal or plant preserved in the resin. In extreme cases, the amber pieces are completely opaque, making it virtually impossible to recognize the presence of inclusions inside (Martínez-Delclòs et al., 2004; Perrichot, 2004). Recently, a new approach for studying amber inclusions within opaque pieces was published, namely X-ray synchrotron radiation using phase propagation contrast (Tafforeau et al., 2006). This technique was later optimized and applied to a large quantity of opaque French Lower Cretaceous amber material from Charentes (Lak et al., 2008a, 2008b), establishing the basis for the study of amber with synchrotron radiation and its later application to different ambers worldwide.

2. Methods

Using third generation synchrotrons, it is possible to obtain a partially coherent X-ray beam due to the small source size and the long distance between the source and the sample (140 meters in the case of the ESRF ID19 beamline). As a result of the coherence, interfaces in the sample create interferential pattern propagation in space. It is then possible to detect enhanced contrast of the sample interfaces just by increasing the distance between the sample and the detector (propagation phase contrast effect). Since the phase contrast effect is far more sensitive than the absorption one, the technique allows for detection of inclusions that would have remained invisible using only absorption contrast (Lak et al., 2008a, 2008b; Tafforeau et al. 2006). This property can be also achieved in some conventional microtomographic devices, but only in small samples at high resolution and low energy (Penney et al., 2007). Nowadays, propagation phase contrast is the most frequently used technique for high-resolution tomographies of inclusions in amber (chiefly performed at the ESRF), with voxel size ranging from 0.35 to 15 μm in the case of larger samples.

The success of the first study (Tafforeau et al., 2006) prompted further development of X-ray synchrotron imaging for other amber inclusions and deposits (e.g., Lak et al., 2008a). Eighty percent of French Cretaceous amber pieces are opaque (Perrichot, 2004), and as such, it is often difficult to know whether there are inclusions within a given piece. To combat this, Lak et al. (2008b) used optimized phase contrast-based microradiographic protocol to survey large quantities of opaque pieces for possible inclusions. Amber pieces were immersed in water to optimize the phase contrast effect by reducing the relative residual absorption contrast. The microradiographs of the opaque blocks were normalized and then surveyed at real resolution size to look for inclusions. Later, select specimens were imaged by propagation phase contrast X-ray synchrotron microtomography (PPC-SRμCT) using local tomography protocol (the amber block being, in most of the cases, far larger than the inclusion itself). Energy was adapted depending upon the size and density pattern of the amber blocks (i.e. presence of pyrite requiring higher energy). Resolution was selected to optimize the level of detail necessary for each inclusion. In some cases, we used a multiscale approach, starting with a complete block tomography (when containing several organisms), followed by detailed scans of selected inclusions, and finishing with higher resolution scans of diagnostic parts of these inclusions.

Using this method of microradiographic survey and later microtomography, more than 350 specimens were recognized inside the blocks of opaque amber from France, thus opening a new window for palaeoentomology.

After acquisition of the scans, the volumes are reconstructed using a filtered back-projection algorithm adapted for local tomography applications (PyHST software, ESRF). The later 3D processing is performed on powerful workstations using the software VGStudioMax (Volume Graphics, Heidelberg, Germany). The segmentation protocol, which virtually extracts the organisms, is based on controlled 3D region growing, fol-

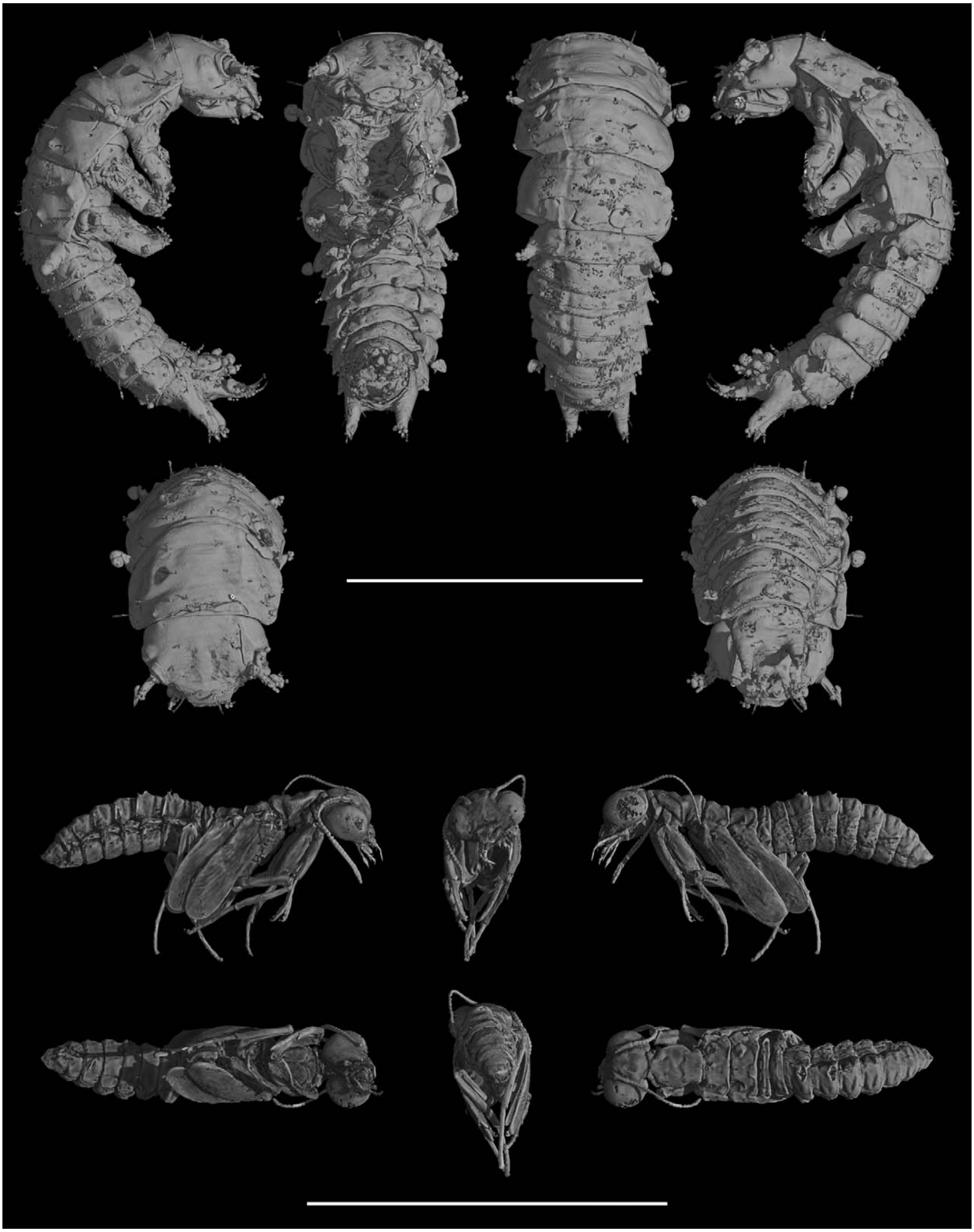

Fig. 1. 3D reconstructions of Lower Cretaceous French amber insects using PPC-SRμCT at ID19 beamline, ESRF, Grenoble. 1: Coleopteran larva, specimen MNHN A33496 (ARC-331.5), voxel size 0.55 μm, propagation distance 70 mm, 35 keV, scale bar 500 μm. 2: Raphidiopteran pupa, specimen MNHN A33497 (ARC-332.1), voxel size 7.46 μm, propagation distance 800 mm, 25 keV, scale bar 5 mm.

Fig. 1. Reconstructions 3D d'insectes dans l'ambre Crétacé inférieur de France, effectuées par PPC-SRμCT sur la ligne de lumière ID19, ESRF, Grenoble. 1 : Larve de Coléoptère, spécimen MNHN A33496 (ARC-331.5), taille de pixel 0,55 μm, distance de propagation 70 mm, 35 keV, barre d'échelle 500 μm. 2 : Pupe de Raphidioptère, spécimen MNHN A33497 (ARC-332.1), taille de pixel 7,46 μm, distance de propagation 800 mm, 25 keV, barre d'échelle 5 mm.

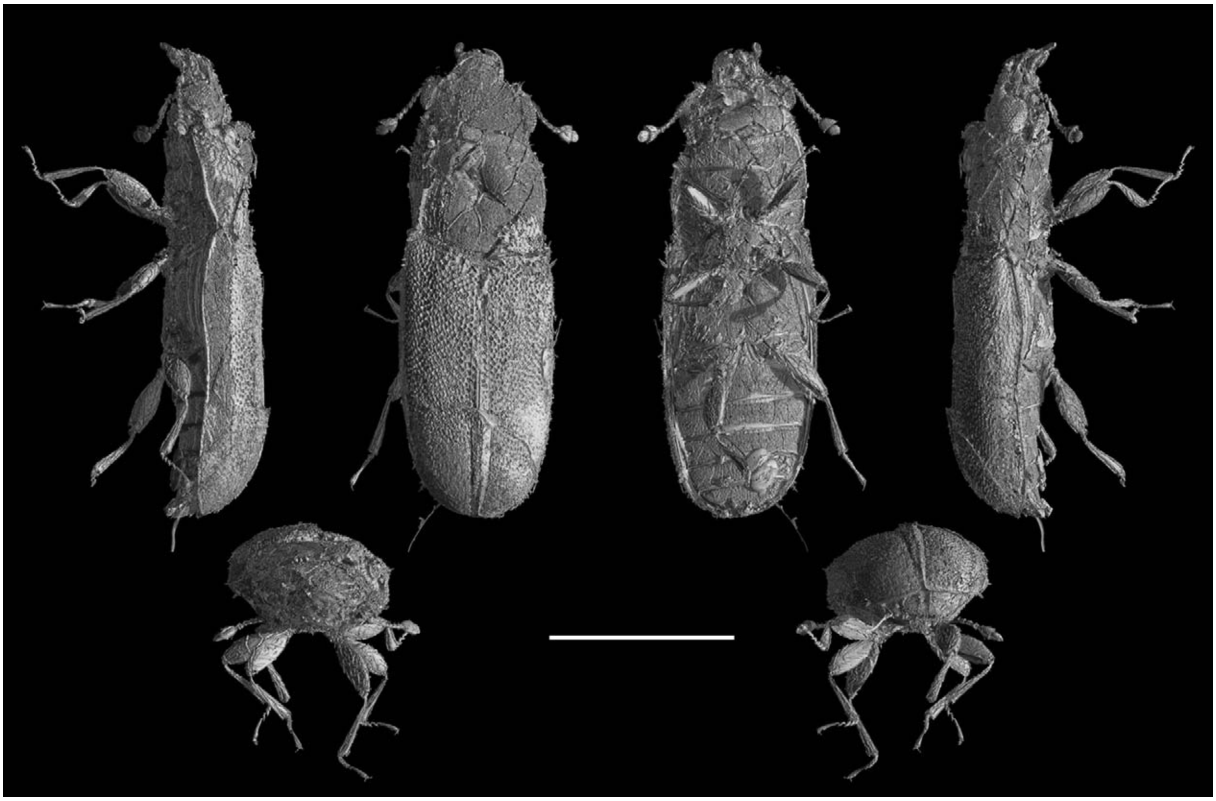

Fig. 2. 3D reconstruction of a monotomid beetle (*Rhizophtoma elateroides* Kirejtshuk et al., 2009), specimen 1512, Coll. D. Azar, from Lower Cretaceous Lebanese amber using PPC-SRμCT at BM05 beamline, ESRF, Grenoble. Voxel size 1.03 μm, propagation distance 50 mm, 20.5 keV. Scale bar 500 μm.
Fig. 2. Reconstruction 3D d'un Coléoptère Monotomidé (*Rhizophtoma elateroides* Kirejtshuk et al., 2009), spécimen 1512, Coll. D. Azar, dans l'ambre Crétacé inférieur du Liban, effectuée par PPC-SRμCT sur la ligne de lumière BM05, ESRF, Grenoble. Taille de pixel 1,03 μm, distance de propagation 50 mm, 20,5 keV. Barre d'échelle 500 μm.

lowed by manual refinement of the rough first result (Fig. 1).

After publication of an exemplar, the microtomographic data is available online in the free access paleontological database at http://paleo.esrf.eu, including original reconstructions of the scan, all the scan parameters, the segmented slices, the VGStudioMax files, .stl surface files for 3D printing, plates and animations with anaglyphic versions. When possible, 3D prints in ABS plus plastic are constructed at the ESRF, which are used as accessible physical representations of the virtual holotypes.

The specimens included in these publications are deposited in the following institutions: French and Lebanese amber at the Division of Palaeontology of the Natural History Museum of Paris (MNHN, France), with French amber being provisionally housed at the Geological Department of the University of Rennes 1 (France); Spanish amber in the Fundación Conjunto Paleontológico Dinopolis (CPT) and in the Museo de Ciencias Nacional de Alava (MCNA), Spain; and Australian amber at the Queensland Museum (Australia).

3. Results

In the last 6 years, amber pieces from virtually the world over have been scanned or surveyed in the ESRF, but long-term projects have been launched only with amber collections from the Cretaceous of France, Spain, and Lebanon, and from the Tertiary of Australia.

The French Cretaceous amber material was the first to be surveyed and microtomographied by synchrotron X-ray imaging (Lak et al., 2008b; Tafforeau et al., 2006), and since the beginning of this work, several hundred plant and animal specimens have been recognized. The French Cretaceous amber outcrops are located mainly in the Southwest of France, and their ages range from Albian to Cenomanian (Néraudeau et al., 2002, 2003, 2005, 2008, 2009; Perrichot and Néraudeau, 2009; Perrichot et al., 2007, 2010).

As noted, the large quantity of opaque amber pieces (approximately 80%) from the Lower Cretaceous French material spurred study using X-ray synchrotron microtomography (Lak et al., 2008a, 2008b). Because synchrotron imaging was first applied to the French deposit, the material has, at this stage, been reconstructed in 3D and published most extensively (Lak et al., 2008b, 2009; Lak

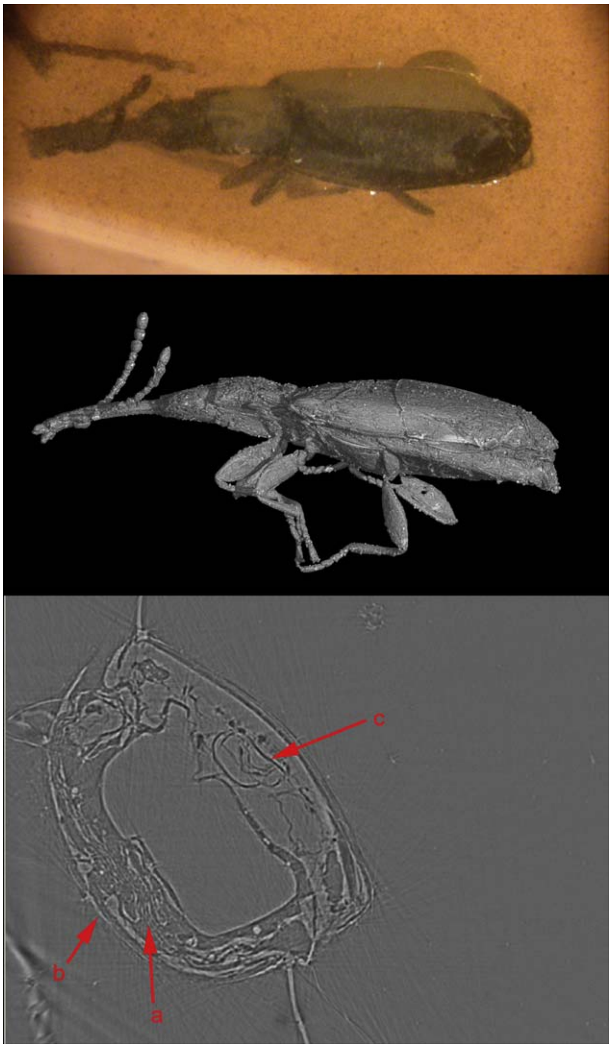

Fig. 3. Coleoptera (Nemonychidae), specimen CPT-4106, from the Lower Cretaceous Spanish amber. 1: Photography of the specimen with binocular. 2: 3D reconstruction of the complete body. 3: Transverse plane projection, showing the hind wings folded (a) and preserved under the elytra (b) and digestive tract (c).

Fig. 3. Coléoptère (Nemonychidae), spécimen CPT-4106, dans l'ambre Crétacé inférieur d'Espagne. 1 : Photo du spécimen à la loupe binoculaire. 2 : Reconstruction 3D du corps complet. 3 : Projection transverse plane montrant les ailes postérieures pliées (a) et préservées sous les élytres (b) et le tube digestif (c).

and Nel, 2009; Perrichot et al., 2008; Tafforeau et al., 2006; Vršanský, 2009).

The latest discoveries from these amber deposits include different larval stages of various groups of insects, including coleopterans and raphidiopterans (Fig. 1). Formal systematic study of these inclusions is complicated because the larval stages of these families do not resemble the adult stages, but they may contribute to the general ecosystem reconstruction. Data from the specimens are available online on the ESRF paleontological database described above. Furthermore, a great number of new larval and adult forms of dipterans, coleopterans, heteropterans, hymenopterans and other groups are currently under study.

Following the success of the French pilot study in late 2008, work began on the Lower Cretaceous amber collection from Lebanon using PPC-SRμCT at the ESRF. Until now, the Lebanese amber deposits, with more than 300 outcrops, are considered to be the oldest with arthropod inclusions (Azar, 2007), and so far numerous representatives of different groups of arthropods, vertebrates and plants have been recognized. Although the amber is fairly transparent, some pieces were surveyed by microtomography to study the internal features of the inclusions and/or to resolve anatomical details invisible with conventional techniques (as was the case, for example, for the oldest representative of the beetle family Monotomidae) (Fig. 2) (Kirejtshuk et al., 2009).

In 2009, a new project studying the fossil content of the Spanish amber outcrops began, and approximately one hundred specimens have already been scanned on the ID19 and BM05 beamlines of the ESRF using PPC-SRμCT. The Spanish amber dates from the Barremian-Cenomanian. The first outcrop with paleobiological content was described in 2000 from Albian deposits (Alonso et al., 2000), and since then several outcrops have been discovered that yield a rich collection of animals and plants (Delclòs et al., 2007; Peñalver et al., 2007; Najarro et al., 2009).

Spanish amber samples are generally translucent, so X-ray synchrotron microradiographic techniques to detect inclusions are not needed. Even so, partial opacity of samples and/or debris within a piece can make the study of morphological details extremely difficult (Fig. 3). In other cases, data on the internal anatomy is required to perform a thorough systematic study. The preservation of internal structures in some Spanish samples has allowed for unprecedented, exhaustive study of the inclusions, for example making it possible to see the hind wings underneath the elytra of beetles (Fig. 3). Other Spanish specimens that have been reconstructed include a female spider of the genus *Orchestina* (family Oonopidae; Fig. 4) and a mymarommatoid wasp of the genus *Galloromma* (family Gallorommatidae; Fig. 4), both of which are currently under study (e.g., Ortega-Blanco et al., in press).

The Australian Tertiary amber is the newest project currently underway at the ESRF. This material is found on eastern Cape York Peninsula, and although its precise age is the subject of current investigation, its entomological content and geological context suggest a Tertiary age (Bickel, 2009). All the material scanned at the ESRF involved fully opaque pieces; hence the same technique applied to the French Cretaceous opaque amber samples was implemented. From this survey, several arthropod and plant inclusions were recognized, including hymenopterans, hemipterans, dipterans and a diverse group of beetles, some within the family Scolytidae (Fig. 5).

Because of the utility of this method and its relevance for amber research, new projects on other significant amber deposits will begin in the near future at the ESRF.

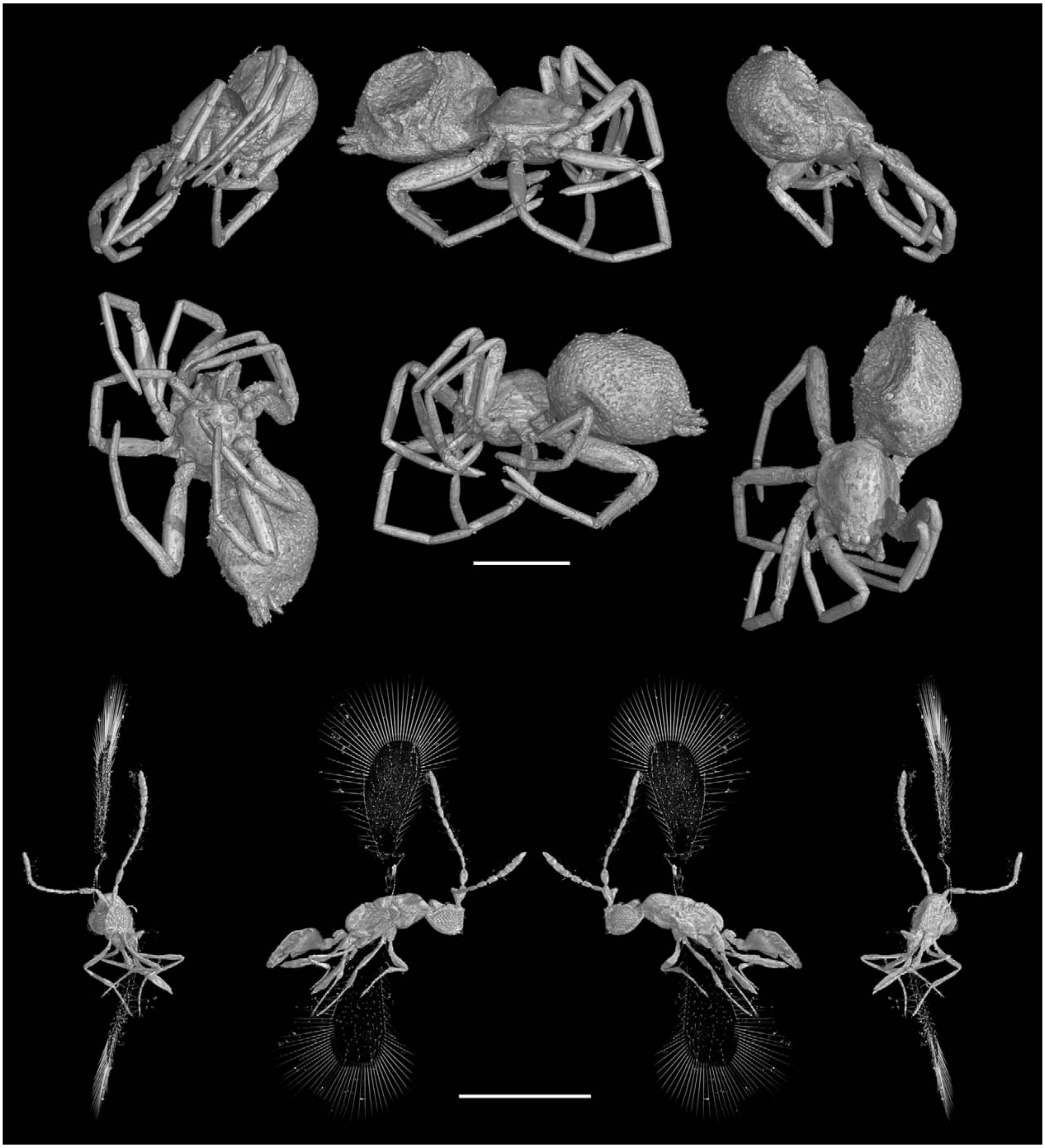

Fig. 4. 3D reconstructions of Lower Cretaceous Spanish amber arthropods using PPC-SRμCT at BM05 beamline, ESRF, Grenoble. 1: Female spider from San Just outcrop (*Orchestina*, Oonopidae), specimen CPT-4100, general habitus, voxel size 0.7 μm, propagation distance 100 mm, 20 keV. Scale bar 500 μm. 2: *Galloromma* sp., specimen MCNA12630, a wasp of the mymarommatoid family Gallorommatidae from Peñacerrada I outcrop. Voxel size 0.56 μm, propagation distance 25 mm, 25 keV. Scale bar 500 μm.

Fig. 4. Reconstructions 3D d'arthropodes dans l'ambre Crétacé inférieur d'Espagne, effectuées par PPC-SRμCT sur la ligne de lumière BM05, ESRF, Grenoble. 1 : Araignée Oonopidae femelle (*Orchestina* sp.) du gisement de San Just, spécimen CPT-4100, aspect général, taille de pixel 0,7 μm, distance de propagation 100 mm, 20 keV. Barre d'échelle 500 μm. 2 : *Galloromma* sp., spécimen MCNA12630, guêpe mymarommatoïde de la famille Gallorommatidae du gisement de Peñacerrada I. Taille de pixel 0,56 μm, distance de propagation 25 mm, 25 keV. Barre d'échelle 500 μm.

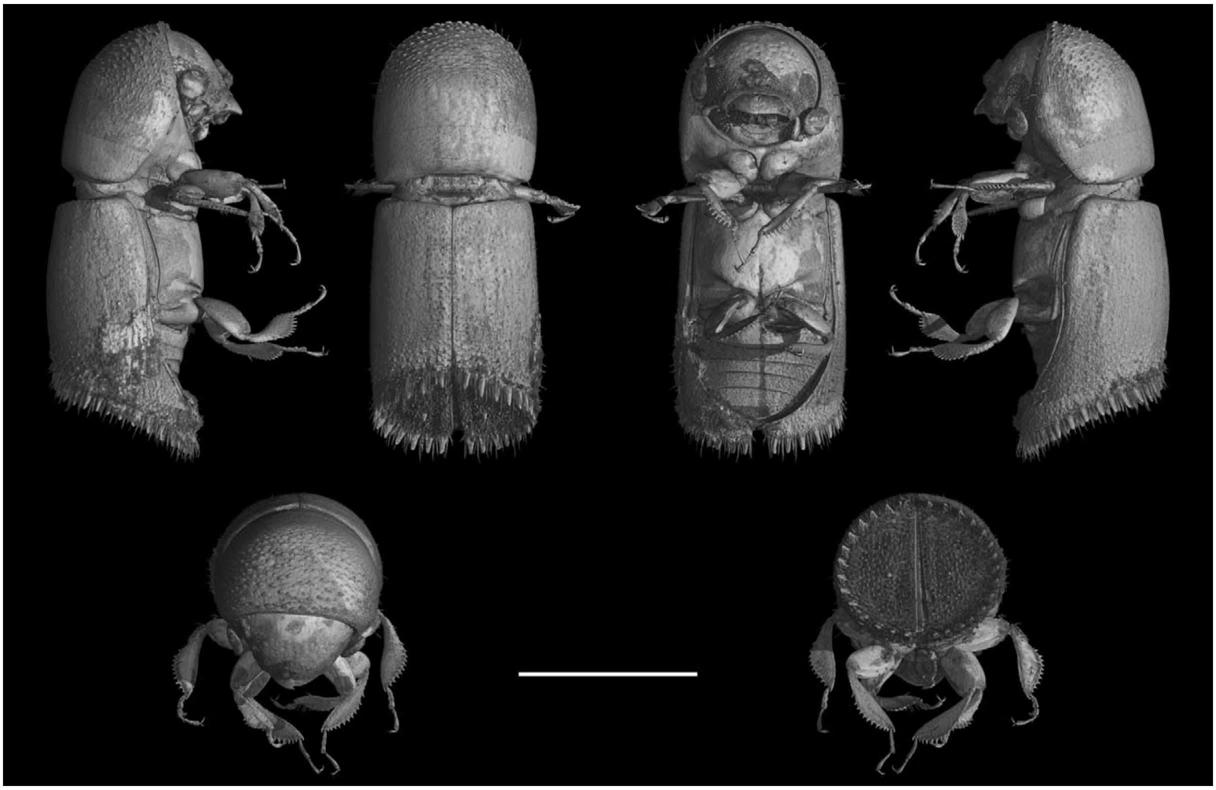

Fig. 5. 3D reconstruction of a scolytid beetle (Scolytidae) from Tertiary Australian amber using PPC-SRμCT at ID19 beamline, ESRF, Grenoble. Voxel size 1.37 μm, propagation distance 150 mm, 30 keV. Scale bar 1 mm.
Fig. 5. Reconstruction 3D d'un Coléoptère Scolytidé dans l'ambre tertiaire d'Australie, effectuée par PPC-SRμCT sur la ligne de lumière ID19, ESRF, Grenoble. Taille de pixel 1,37 μm, distance de propagation 150 mm, 30 keV. Barre d'échelle 1 mm.

All projects are closely related through what is now one of the largest worldwide collaborations on the study of amber.

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