Synchrotron Imaging Procedure.

The holotype specimen of *Gymnopollisthrips minor* gen. et sp. nov. was imaged at the BM05 beamline of the European Synchrotron Radiation Facility at Grenoble, France, using propagation phase-contrast X-ray synchrotron microtomography. This technique reveals structural detail on samples lacking sufficient contrast for X-ray absorption techniques (1–3). To facilitate the 3D processing of the data, an algorithm for single-distance phase retrieval developed by Paganin et al. (4) was implemented. The algorithm developed by Paganin et al. already has been successfully applied for imaging on fossils (5) but, in the present case, was modified because the sample to image profile did not fit conditions for the application of such an algorithm.

The sample consisted of an amber piece embedded in epoxy resin and glued on a cover glass. The geometry of the sample clearly was not adapted for a single-phase retrieval process because of very strong absorption anisotropy during sample rotation. Because the insect structures were principally small in size, all structures larger than 50 pixels were removed, subtracting to each projection a copy of itself after a median filtering of 50 pixels. This operation of subtraction led to complete normalization of the background and optimization of the contrast of the insect itself, using the Paganin phase retrieval. To compensate for the blurring induced by the phase retrieval, a less-than-sharp mask filter was applied on the radiographs after the phase retrieval. Residual ring artifacts were corrected on the reconstructed slices and the volume was converted into a 16-bit tagged image file format (TIFF) stack file. The 3D images were manually rendered into a movie using a region-growing technique with VGStudiomax (version 2.1) software (Volume Graphics).

The scan was performed using a propagation distance of 24mm and beam energy of 20 keV, implemented by a double-crystal multilayer monochromator. The movie consisted of 1,500 projections, each of which was acquired every 0.8 s over 180 degrees. To obtain a sufficiently high resolution of the fine structures in the specimen, a detector giving an isotropic voxel size of 0.75 μm was used. The scanned amber piece was translucent and potentially of high scientific value; consequently, it would have been risky to perform an elevated high-resolution scan. The equipment setup and protocol mentioned above typically are used for high-resolution scanning of opaque amber pieces (2, 3, 5). In the case of non opaque amber, it is common to observe strong darkening of the amber during synchrotron imaging. Although the darkening can later be removed using UV light exposure, it always is preferable to avoid the potentially damaging effects of amber darkening and to reduce the dosage to an acceptable minimum level whenever possible, especially when dealing with potential holotype material. Accordingly, a high efficiency and high-resolution detector was developed that would reduce the X-ray dose by nearly a factor of 50. This lower dose, compared with the much more elevated standard, produces a comparable dynamic level in the data but requires scanning times of approximately 1 h rather than a few minutes. As a consequence of a dramatic reduction in total X-ray flux, and the scanning time limited by the speed of the detector instead of the X-ray flux, it appeared more reasonable to use the beam of a bending magnet (BM05), instead of the far higher flux of an undulator, such as the ID19 beamline (5, 6).

The detector apparatus consisted of a single optical element from a 20× microscope objective with a 0.4-mm opening that was coupled with a 9 μm lutetium oxyorthosilicate (LSO) scintillator (6). The light was projected on a CCD FreLoN camera (7) containing a new-generation CCD chip (E2V) with a 15-μm pixel size, yielding five times greater efficiency than the Atmel chip generally used for these type of experiments. The combination of the LSO scintillator and a E2V FreLoN camera also allowed higher efficiency, attributable to scintillator emission as green light (550 nm emission ray), complimenting the maximum efficiency of the E2V CCD chip, compared with the red light (715 nm) of the usually used Gd3Ga5O12 (GGG) scintillators. The principal drawbacks of this type of detector are: (i) a notably slow readout time of 0.6 s per frame; (ii) distortion attributable to the optical configuration that is corrected by retrodistortion of the pictures; and (iii) a loss of about 200 pixels on the total field of view compared with an optical system using both a 10× objective and a 2× eyepiece. Considering that the dose reduction and the longer scans did not result in darkening of the amber piece and the resulting data quality was similar to the alternative rapid and high-dose

setup, this modified system was preferable for scanning by avoiding specimen damage. Because of adjustments to scan duration and dose intensity, some pollen grains were not detected by our procedure and, thus, not imaged in the resulting movie.

References

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