

Confocal digital photomacro/micrography applied to Collembola taxonomy



Courtesy of Brian Valentine

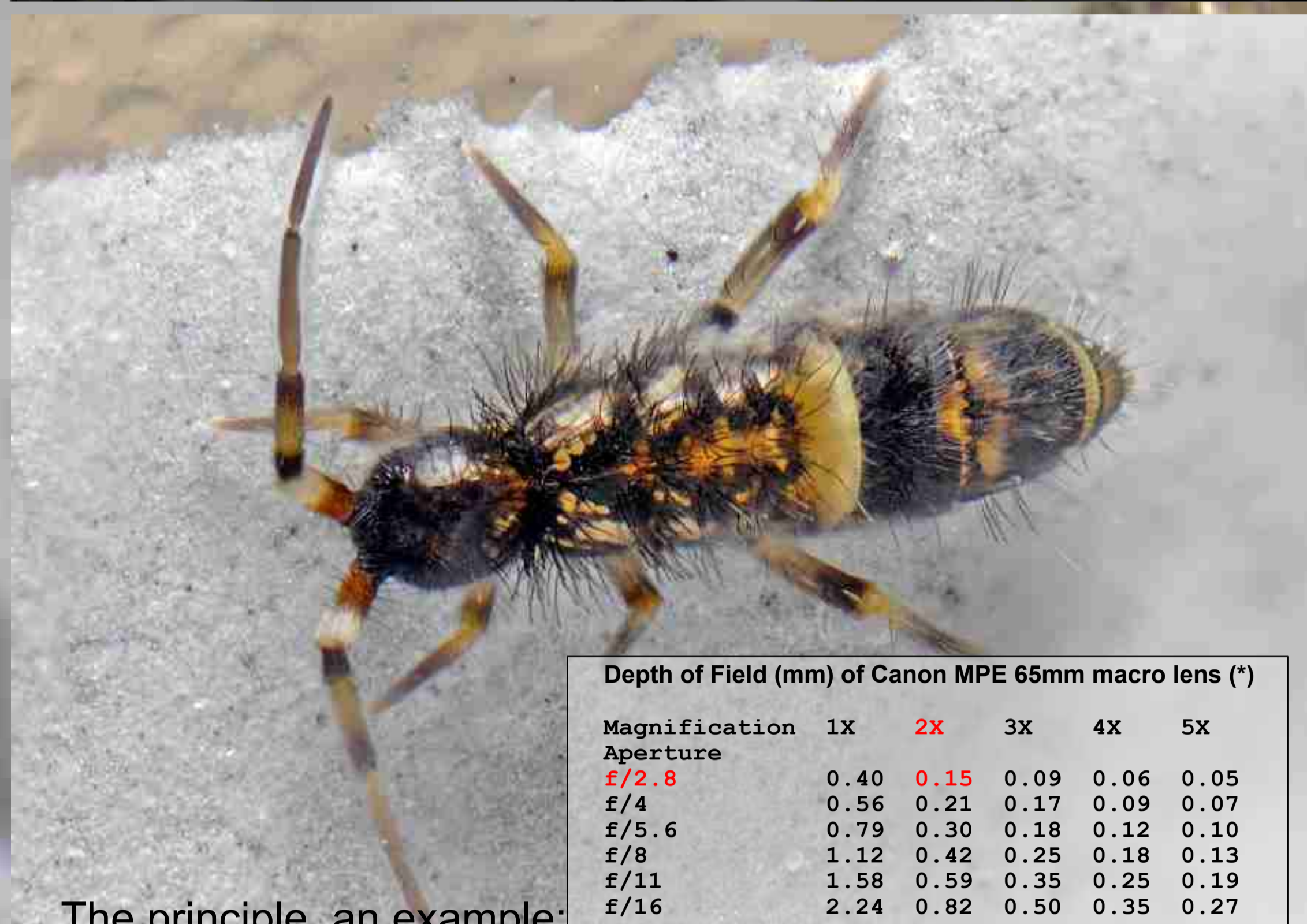


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Current digital imaging technology can help to overcome in a time- and cost-effective manner the lacking colour and spatial information into conventional taxonomic illustrations. High resolution digital SLR camera sensors capture the colour sufficiently accurate. While computer imaging techniques, such as Extended Depth of Focus (EDoF) imaging techniques, can produce images that may support the interpretation of spatial information by means of 2D/3D reconstruction.

Confocal Photomacrography: 2D and 3D Reconstruction of Habitus Illustrations by means of Focus Stacking



Depth of Field (mm) of Canon MPE 65mm macro lens (*)					
Magnification	1X	2X	3X	4X	5X
Aperture					
f/2.8	0.40	0.15	0.09	0.06	0.05
f/4	0.56	0.21	0.17	0.09	0.07
f/5.6	0.79	0.30	0.18	0.12	0.10
f/8	1.12	0.42	0.25	0.18	0.13
f/11	1.58	0.59	0.35	0.25	0.19
f/16	2.24	0.82	0.50	0.35	0.27

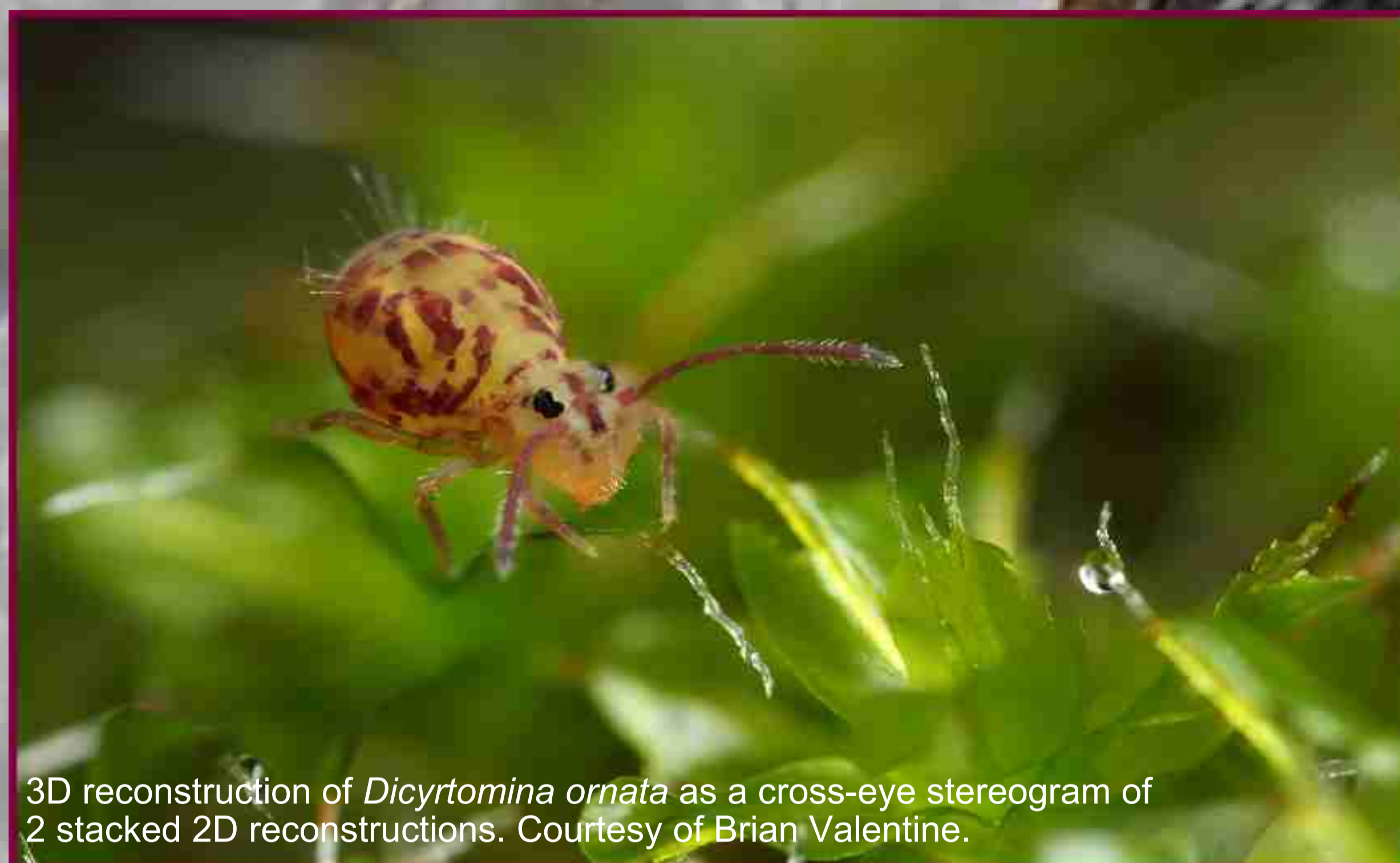
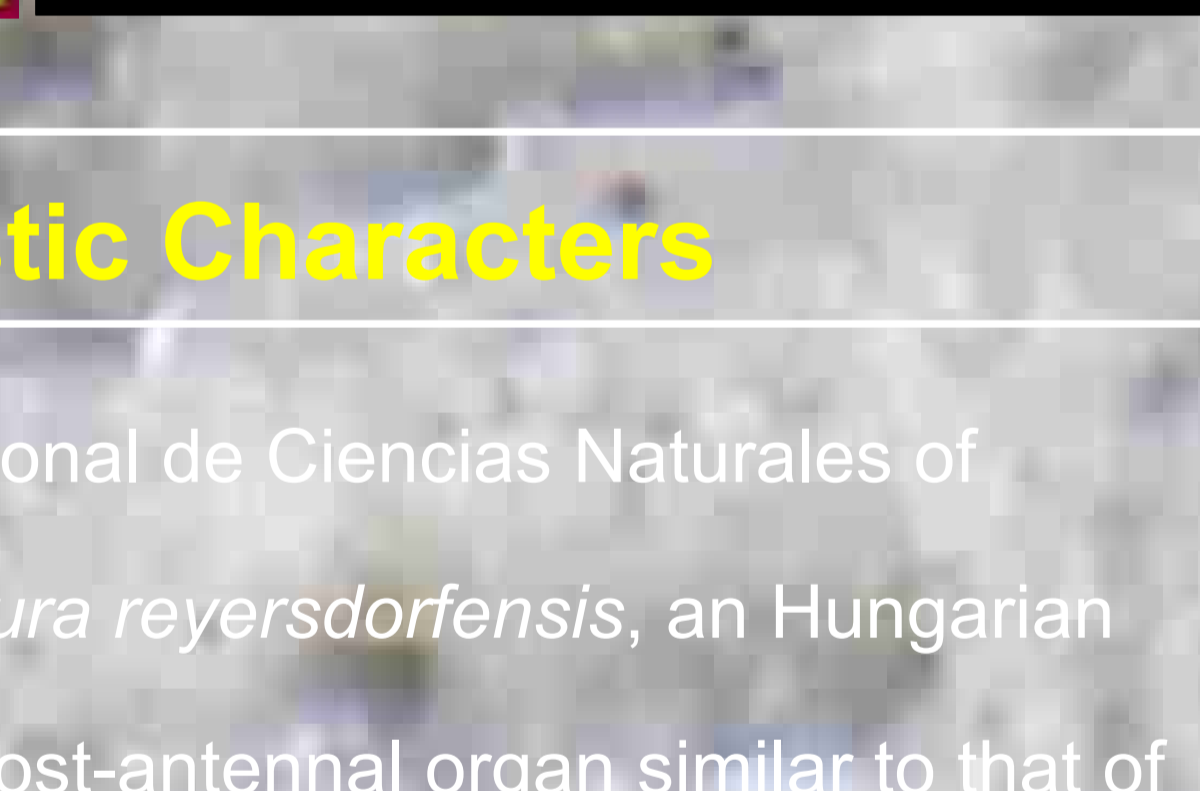
The principle, an example:
Orchesella cincta stacked 2D reconstruction
 Stack of 7 frames taken at different focus (n=7).

(*) Only as example. The actual lens used = AF-S VR Micro-Nikkor 105 mm f/2.8 G IF-ED with Soligor automatic extensionrings



Also small displacements of body parts are handled automatically by the digital image processing software.

Methods and Material: To cover the Extended Depth of Field (EDoF) of the photographed specimen, the number of required frames is a function of the aperture and magnification of the optics used (see chart). The focus stacking software performs an automated alignment of the frames. Therefore a DoF frame overlap of minimum 25% is required. The number of required frames = (EDoF/DoF)+1. For an aperture of f/8, the minimum number of frames to cover an EDoF of 1mm is: at 1x 1, at 2x 3, at 3x 5, at 4x 7, and at 5x 9. All single frames of the specimens are taken by hand without making use of a tripod. Digital image processing software used: Helicon Focus Mac Version 3.79 (of Helicon Soft Co) CombineZM Windows XP Version April 26, 2008 (of Alan Hadley)



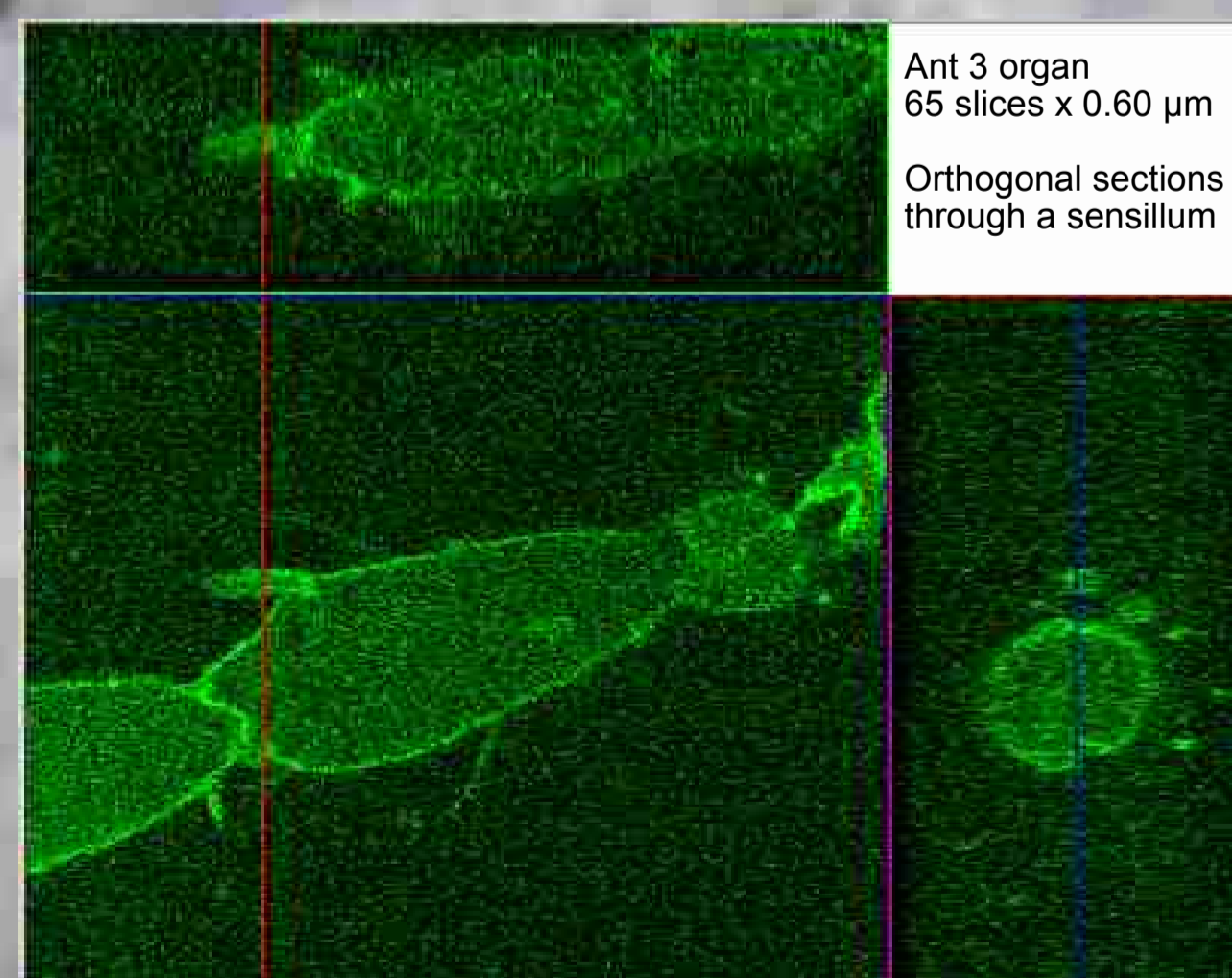
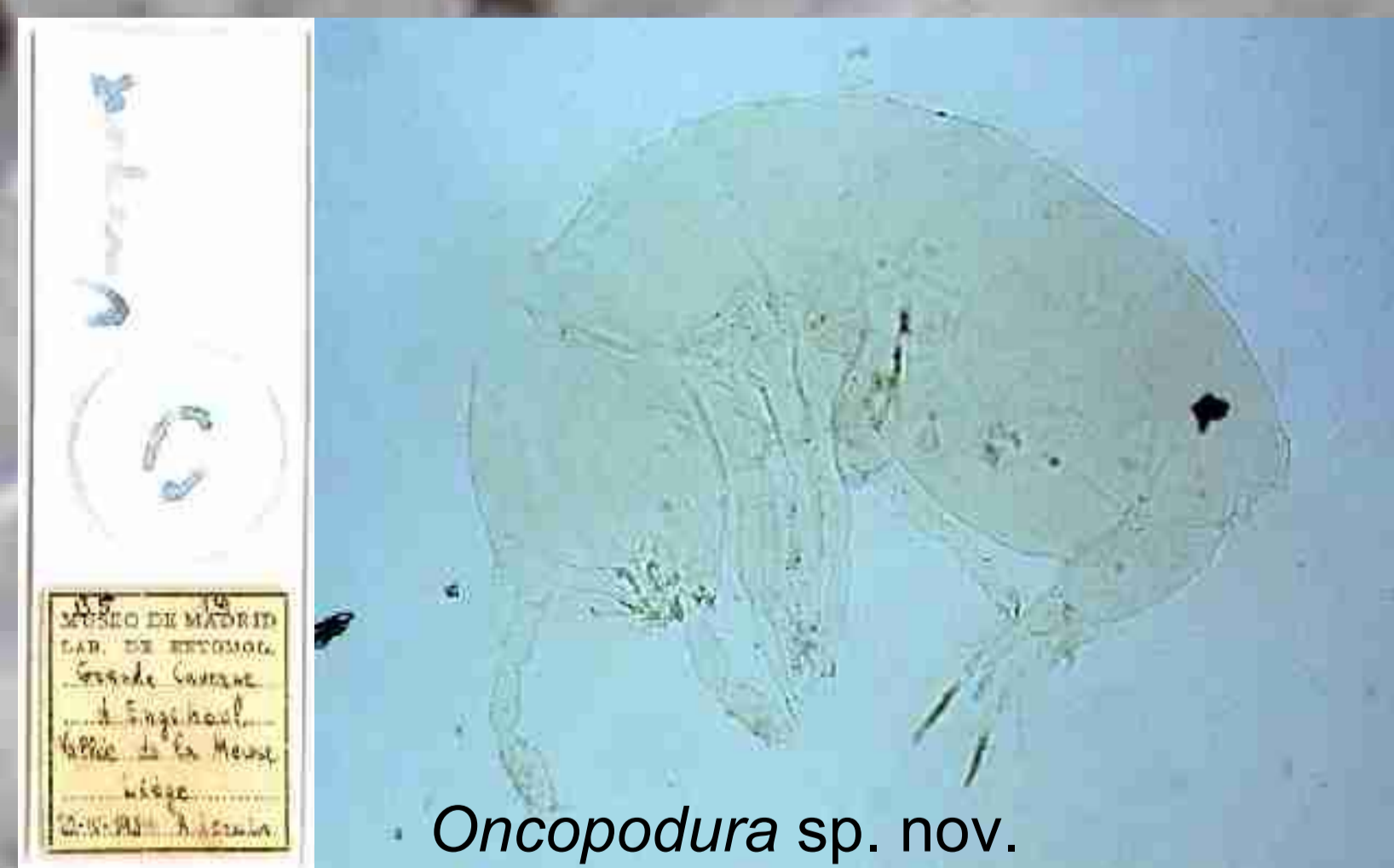
3D reconstruction of *Dicyrtomina ornata* as a cross-eye stereogram of 2 stacked 2D reconstructions. Courtesy of Brian Valentine.



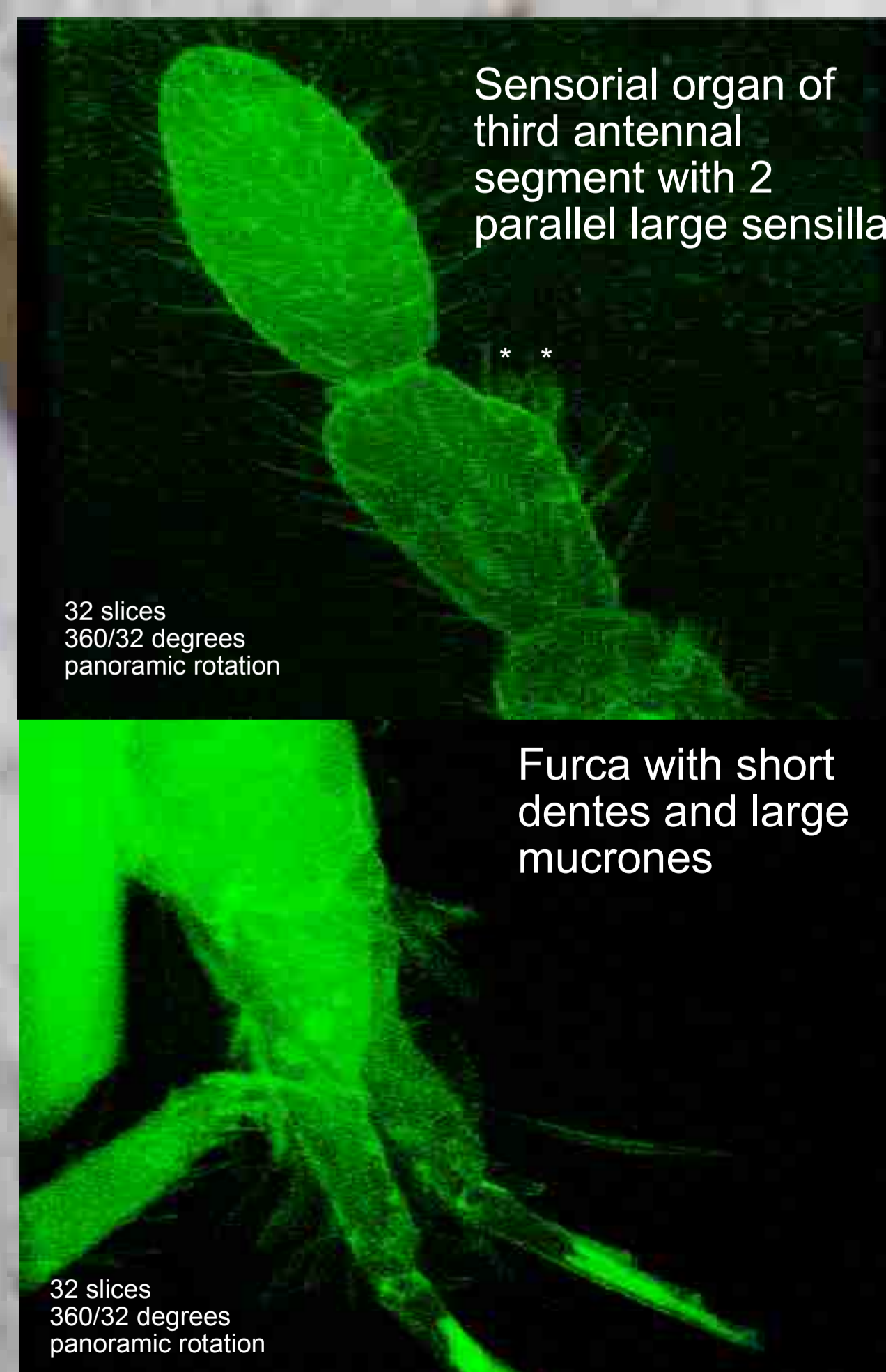
The authors thank Brian Valentine for the kind permission to reproduce his pictures, and Andrew Robertson for his contribution of the DoF chart of the Canon MPE 65 mm lens.

Confocal Laser Scan Photomicrography: 3D Reconstruction of Morphological Diagnostic Characters

An undescribed species of *Oncopodura* was found by Leruth (1939) while making his inventory of the fauna of Belgian caves. The 'lost' collection of Collembola specimens of 1932-1934 of Leruth was backtraced by Michel Dethier and recently recovered from the Museo Nacional de Ciencias Naturales of Madrid in Spain. In the Delhez collection of Belgian cave fauna, inventorised by Michel Dethier, some *Oncopodura* specimens were tentatively identified as *Oncopodura reyersdorfensis*, an Hungarian cave species (Delhez, F., Dethier, M. & Hubart, J.-M., 1999; Hubart, J.-M et Dethier, M, 1999). During a more recent exploration of Belgian caves (1999-2001), Michel Dethier recovered new specimens of an undescribed *Oncopodura*, with a post-antennal organ similar to that of *Oncopodura reyersdorfensis*, which was tentatively published as a new undescribed species (Janssens, F. & Dethier, M. 2005). The new species of *Oncopodura* is currently being described as a new species to science endemic to Belgian caves (Janssens, F. & Deharveng, L. 2009).



Ant 3 organ
 65 slices x 0.60 µm
 Orthogonal sections through a sensillum

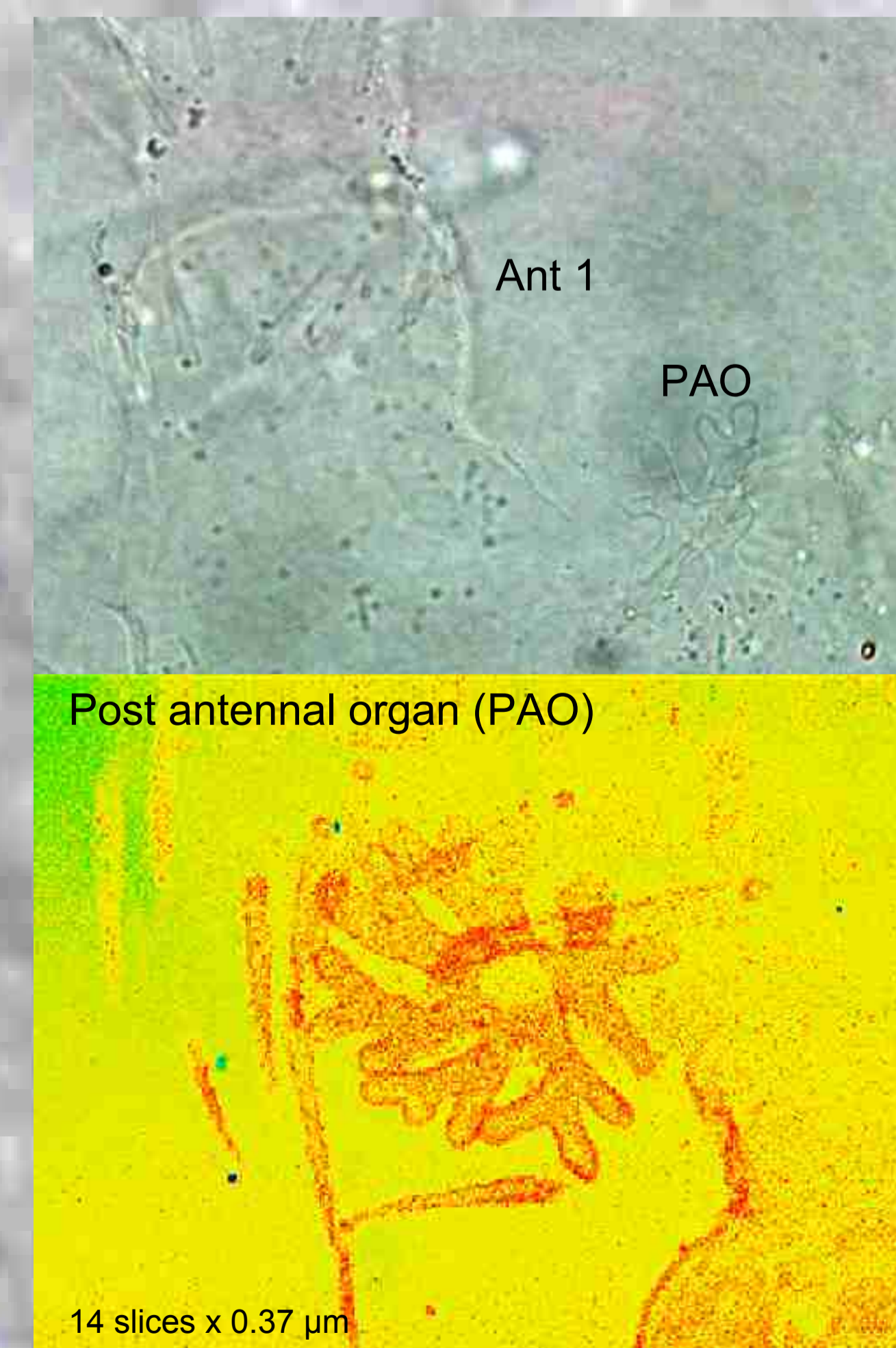


Sensorial organ of third antennal segment with 2 parallel large sensilla

32 slices
 360/32 degrees
 panoramic rotation

Furca with short dentes and large mucrones

32 slices
 360/32 degrees
 panoramic rotation



Ant 1
 PAO

Post antennal organ (PAO)

14 slices x 0.37 µm

Microscopy studies of slide mounted specimens of a new species of *Oncopodura* recorded from Belgian caves were carried out on a confocal laser scanning microscope with appropriate excitation (wavelength 488 nm 1%) and emission filter sets (Laser Scanning Systems LSM 510 Meta; Carl Zeiss), making use of the natural fluorescence of the slide preparations. The 3D reconstructions are visualised by the LSM Image Browser Version 4.2.0.121 (Carl Zeiss MicroImaging GmbH 1997-2006). The pictures here are screenshots of the LSM Image Browser.

The authors thank Michel Dethier of the Faculté universitaire des Sciences agronomiques, Gembloux, Belgique and Mercedes París of the Museo Nacional de Ciencias Naturales, Madrid, Spain for making available the Collembola collection of Leruth (1932-1934). The authors thank Dr. Jean-Pierre Timmermans and Jan Van Daele of the Laboratory of Cell Biology & Histology of the University of Antwerp, Belgium for the use and assistance of the confocal microscopy facilities.

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Background = *Tomoceris vulgaris* from the Netherlands (n=9)
 Photomicrographs by A. H. Baas unless otherwise mentioned. Photomicrographs by J. Van Daele and F. Janssens. Poster design by F. Janssens and W. Hooftman.
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