



## First fossil Micropholcommatidae (Araneae), imaged in Eocene Paris amber using X-Ray Computed Tomography

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### Abstract

We apply Very-High-Resolution X-Ray Computed Tomography (VHR-CT) to a minute fossil spider (~1 mm long) from Eocene amber of the Paris Basin, France. We demonstrate that the newly described genus and species of Micropholcommatidae, *Cenotextricella simoni*, retains excellent details of the somatic and male pedipalpal morphology that allows unqualified comparison with extant species. Thus, in addition to calibrating the tree of life, such fossils can now be incorporated into cladistic matrices and their resultant phylogenies. This is the first fossil record of the family Micropholcommatidae, extending the known geological range of the family by 53 million years (lowermost Eocene) and that of the symphytognathoid clade by approximately 5–9 million years.

**Key words:** Cenozoic, new species, palaeontology, spider, Symphytognathoidea, Tetracellinae

### Introduction

Some neontologists criticize palaeontology in their belief that preservation of fossils is inadequate in terms of the important taxonomic characters they reveal (see Grimaldi *et al.* 2000). For example, Wanless (1984) considered a small number of Baltic amber spiders in his review of the salticid spider subfamily Spartaeinae, but emphasized the taxonomic inferiority of many amber specimens as originally preserved. The novel application to fossils of existing imaging technologies, designed for non-palaeontological purposes, is yielding remarkable results (Grimaldi *et al.* 2000; Polcyn *et al.* 2002; Alonso *et al.* 2004; Siveter *et al.* 2004; Ascaso *et al.* 2004; Tafforeau *et al.* 2006), although in some cases, these methods destroy the specimen (Siveter *et al.* 2004). Very-High-Resolution X-Ray Computed Tomography (VHR-CT) (Grimaldi *et al.* 2000; Polcyn *et al.* 2002; Alonso *et al.* 2004; Henderickx *et al.* 2006; Dierick *et al.* 2007) provides a non-destructive, minimum preparation method for imaging minute morphological details, including internal morphology, and generates 3-dimensional reconstructions that can be sectioned and viewed from multiple angles (essentially permitting digital ‘dissection’ of the specimen within the amber). Here we apply VHR-CT to a spider bioinclusion in ~53 million-year-old Eocene Paris amber (Nel *et al.* 1999; Nel 2004) and show that the specimen is preserved with life-like fidelity, revealing sufficient detail of palpal morphology for phylogenetic comparison with extant taxa, thus demonstrating that such fossils are not as taxonomically sub-standard as previously thought.

Studies on the spider fauna of the Paris Basin amber deposit are still in their initial stages with only three families described to date: Oonopidae (Penney 2007a), Pholcidae and Selenopidae (Penney 2007b). The fossil described here is a new species of minute spider (Micropholcommatidae), representing the first identified fossil of this family and the oldest representative of the symphytognathoid clade, the systematic limits of which are poorly delimited (Schütt 2003; Griswold *et al.* 2005). It is also the first northern hemisphere record of the family. Clearly, the inclusion of fossils in phylogenetic analyses has much to offer modern systematic studies, particularly when considering the relationships of extant species with highly disjunct distributions, as in the extant Micropholcommatidae (Australasia and South America; Platnick 2007). The application of such imaging technologies to fossils will also narrow the interdisciplinary void, which often exists between neontologists and palaeontologists. In addition to merely calibrating the tree of life, such fossils can now be incorporated into cladistic matrices and their resultant phylogenies.

## Material and methods

**Material:** The specimen (MNHN PA 327) in lowermost Eocene amber from the Paris Basin was loaned from the Muséum National d'Histoire Naturelle, Paris, France.

**Horizon, locality and geological setting:** Lowermost Eocene amber from the Paris Basin, Le Quesnoy, Chevière, region of Creil, Oise department, France. The amber-bearing strata occur under the River Oise Quaternary deposits. They prograde towards the north-east and lie at the bottom of two channels, which cut into the underlying Thanetian marine greensands. The Sparnacian (lowermost Eocene) beds consist of a succession of lenticular bodies with two main facies. The first consists of clayey sands rich in frequently pyritised lignite, together with amber, and the second consists of grey clayey sands with less lignite and with a continental fossil vertebrate fauna (Nel 2004). Palaeoenvironmental reconstructions based upon the fossils identified to date suggest that some 53 million years ago the region consisted of a wet river forest surrounded by semi-deciduous or deciduous woodland, in a warm climate with wet and dry seasons (Nel *et al.* 1999; Nel 2004).

**Botanical origin of the amber:** The wood structure of the resin-producing tree is similar to that of the Detarieae tribe of the Caesalpiniaceae family (angiosperms, eudicotyledons), especially to the modern genus *Daniellia* Benn (De Franceschi & De Plog 2003). The infra-red spectrum of the fossil resin bears little resemblance to that of Baltic amber (Nel *et al.* 2004).

**Methods:** The specimen was scanned at Ghent University's High Resolution Micro-Tomography Facility (UGCT: <http://www.ugct.ugent.be>) using a Feinfocus nano-focus transmission type X-ray tube set at a voltage of 60 kVp and a spot size of 1 µm. The X-ray detector was a Photonic Science X-Ray Imager-VHR 1:1 CCD camera system with straight fibre-optic coupling to a gadolinium oxysulphide scintillator (5 mg/cm<sup>2</sup>), resulting in a sensitivity for X-rays between 5 and 35 keV. The sample manipulator featured 7 axes, including a high precision air bearing rotation stage (MICOS, UPR160F-AIR) and a XY piezo stage with 50 nm resolution and 20 mm travel range for accurate centring on the axis of rotation. The complete tomography setup was controlled with LabView based software. A series of 800 projections of 1002 × 667 pixels (each 36 µm<sup>2</sup>) was recorded covering a field of view of 2.7 mm over 360 degrees. Each projection angle was exposed three times over 1.5 seconds. The source-object distance was 21 mm and the source-detector distance was 280 mm, resulting in a geometrical magnification of 13.33× and thus a voxel size of 2.7 µm. The projections were saved as uncompressed 16-bit TIFF files. Reconstruction of the tomographic projection data was performed using the in-house developed Octopus-package (Vlassenbroeck *et al.* 2007), which comes with a special adaptation of the Feldkamp (FDK) cone-beam algorithm for fast reconstruction. This resulted in a reconstructed 3-dimensional volume of 1002 × 1002 × 555 voxels with a voxel size of 2.7 µm<sup>3</sup>. Because the specimen consisted of a small inclusion inside a relatively large amber matrix the contour of the amber was not always

inside the field-of-view as is theoretically required for a correct tomographic acquisition. Therefore, a specific adaptation was made to the reconstruction algorithm to reduce the resulting artefacts. Volume rendering and segmentation was performed using VGStudio Max (Volume Graphics). Full details of the entire process are given in Dierick *et al.* (2007).

## Taxonomy

### Family Micropholcommatidae Hickman, 1944

#### Subfamily Textricellinae Hickman, 1945

#### *Cenotextricella* Penney gen. nov.

**Type species.** *Cenotextricella simoni* sp. nov.

**Etymology.** ‘*Ceno*’ from Cenozoic, the geological era from which the type species originates, plus ‘*textri-cella*’ from *Textricella*, a closely allied extant genus.

**Diagnosis.** Male *Cenotextricella* can be distinguished from other (extant) Micropholcommatidae by the presence of a proximal, retrolateral knob-like pedipalpal patellar apophysis (Fig. 9), by the short, curved embolus wrapped around a conductor in retrolateral view (Figs 7, 9), and by the shape of the highly modified tibia (Figs 7–10). Some extant Australian and New Zealand *Textricella* possess a similar patellar apophysis, and sometimes also a modified tibia (e.g. *T. complexa* Forster), but these palpal segments are otherwise nothing like those observed in the type specimen. Females of *Cenotextricella* are unknown.

**Description.** See the description (below) for the type species.

**Distribution:** Early Tertiary (lowermost Eocene) Paris Basin amber forest.

#### *Cenotextricella simoni* Penney sp. nov.

Figs 1–12

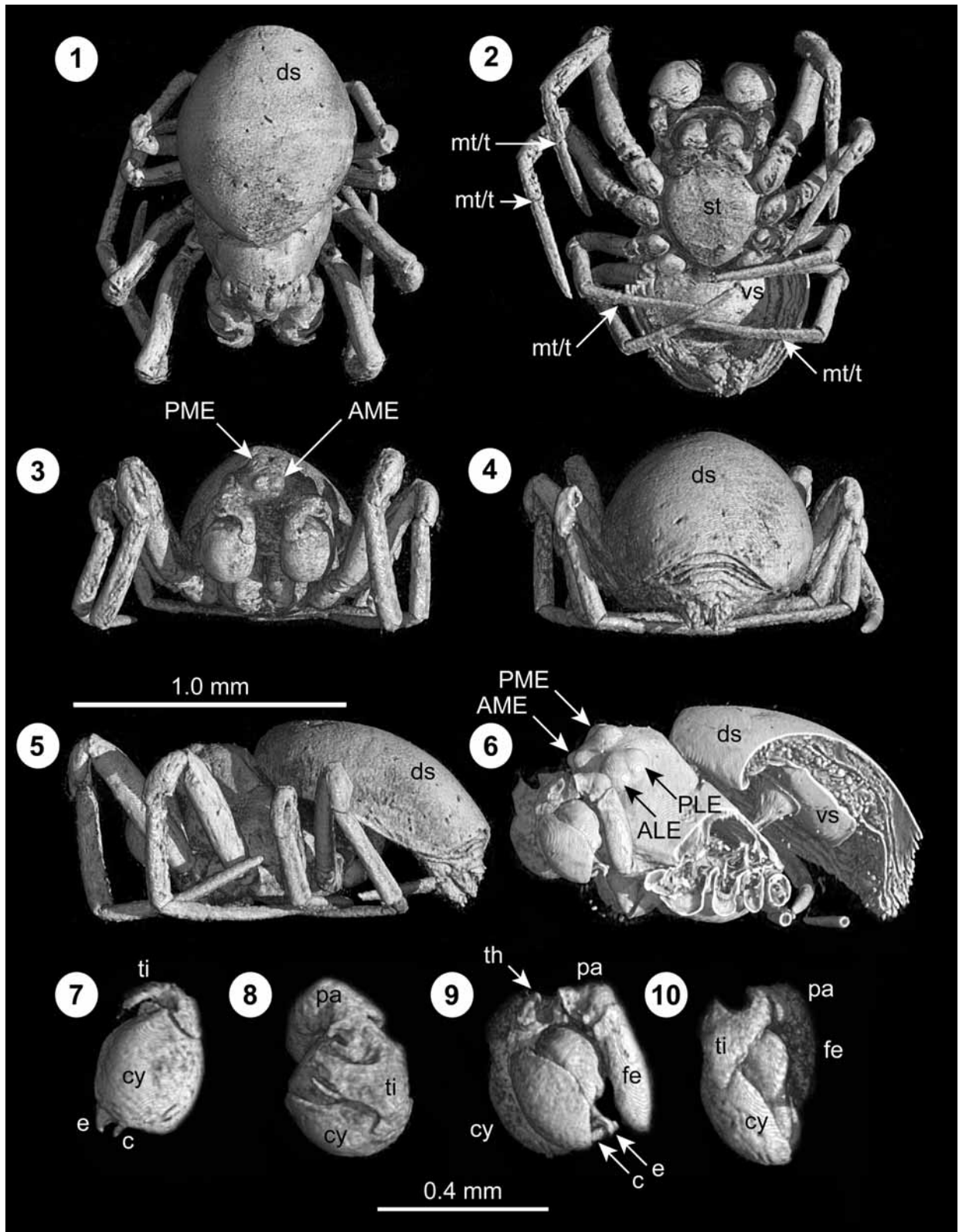
**Type material.** Holotype male: in lowermost Eocene amber from the Paris Basin (MNHN PA 327).

**Etymology.** The specific epithet is a patronym in honour of the famous French arachnologist Eugene Simon.

**Diagnosis.** As for the genus.

**Description.** Male holotype. Body length 1.08 mm. Carapace elevated in the cephalic region, bearing eight eyes; anterior medians smallest and contiguous, remainder subequal; lateral eyes contiguous and not on a raised mound, posterior medians slightly separated (Figs 1, 3, 6). Sternum as wide as long, evenly rounded posteriorly, coxae 4 clearly separated (Fig. 2). Labium three times wider than long, unnotched; endites directed across labium but do not meet in the midline. Chelicerae vertical, not fused along their length and with promarginal dentition (Figs 2, 3, 6). Abdomen ovoid, with a large dorsal scutum (with sparse but distinct setae, not detected by the VHR-CT) and a ventral scutum in the epigastric region surrounding the pedicel (Figs 1, 2, 4–6). Relative order of leg length 1,2,4,3, with distinctly shortened metatarsi (0.5× tarsi length) on all legs (Figs 1–5). Legs with erect, bristle-like setae (not picked up by the VHR-CT) as follows: one distal on each patella, one proximal on each tibia, one distal on tibiae 1, 2 and 4 (Figs 11, 12). Spinnerets situated posteriorly in a compact group, with a distinct colulus (Figs 2, 4, 5). Pedipalp (Figs 6–10) with embolus wrapped around a conductor (Figs 6, 9); tibia complex, flattened and abutting on the cymbium, with broad prolateral extension (Fig. 8) and a distinct tibial hook visible in retrolateral view (Fig. 9); patella with a proximal, retrolateral knob-like projection (Figs. 6, 9).

**Distribution:** Early Tertiary (lowermost Eocene) Paris Basin amber forest.



**FIGURES 1–10.** VHR-CT scans of *Cenotetricella simoni* sp. nov. (male holotype, MNHN PA 327). (1) dorsal view; (2) ventral view; (3) anterior view; (4) posterior view; (5) lateral view; (6) lateral sectioned view; (7) right pedipalp dorsal view; (8) right pedipalp posterior view; (9) left pedipalp retrolateral view; (10) right pedipalp prolateral view. Abbreviations: ALE, anterior lateral eye; AME, anterior median eye; c, conductor; cy, cymbium; ds, dorsal abdominal scutum; e, embolus; fe, femur; mt/t, metatarsus/tarsus joint; pa, patella; PLE, posterior lateral eye; PME, posterior median eye; st, sternum; th, tibial hook; ti, tibia; vs, ventral abdominal scutum.



**FIGURES 11–12.** Photographs of *Cenotextricella simoni* sp. nov. (male holotype, MNHN PA 327) using traditional light microscopy. (1) dorsal view; (2) ventral view. See Fig. 5 for scale.

## Discussion

The Micropholcommatidae are currently poorly delimited, but can be considered to consist of two subfamilies: the Micropholcommatinae and Textricellinae (Platnick & Forster 1986). Based on somatic morphology and the morphology of the male pedipalp, *Cenotextricella* appears to belong in the Textricellinae. This is the first fossil record of the Micropholcommatidae, extending the known geological range of the family by 53 million years (to the lowermost Eocene). The known geological age of the superfamily Symphytognathoida can also be extended by approximately 5–9 million years, from the previously oldest recorded specimens of Anapidae in Baltic amber (Wunderlich 2004). All extant micropholcommatid taxa (33 species in eight genera; Platnick 2007) are tiny (<1.5 mm), so the discovery of the new fossil species is a remarkable find and represents the only northern hemisphere record of the family.

The taxonomic status of the Textricellinae has received a considerable degree of attention in recent decades. Initially erected by Hickman (1945), the family Textricellidae (along with the Micropholcommatidae) was synonymized with the Symphytognathidae by Forster (1959). However, monophyly of the Symphytognathidae was questioned by several workers, who considered that most characters used to justify it were simplifications, reductions or losses that could have been the consequence of miniaturization (Schütt 2003). Micropholcommatidae and Textricellidae were thus removed from synonymy with Symphytognathidae (Forster & Platnick 1977) and placed in the superfamily Palpimanoidea (Forster & Platnick 1984). Shortly thereafter Micropholcommatidae and Textricellidae were considered synonymous with each other (Platnick & Forster 1986) (the former name taking precedence) on the basis that the genus *Teutoniella* Brignoli could not be assigned to either taxon. Several workers did not accept the assignment to Palpimanoidea (e.g. Schütt 2003), and a more recent cladistic analysis suggested that some palpimanoid taxa belong in the superfamily Araneoidea (Griswold *et al.* 2005). The Micropholcommatidae were recently synonymized with Anapidae (Schütt 2003), but this conclusion was not accepted by Platnick (2007) due to the limited number of genera included in the analysis and is not followed here for the same reason.

All the aforementioned systematic works were conducted despite barely any taxonomic work (i.e. revisions) on the extant species since their original descriptions. The whole group is clearly in need of extensive systematic revision, to the extent where the 'families' as currently delimited are still considered working hypotheses (Schütt 2003). Fortunately, both molecular and morphological studies are currently underway (M. Rix pers. comm. 2007).

Classically, neontologists have ignored fossil taxa when revising extant faunas and many continue to do so. Indeed, much of the time the existence of fossil species is not even mentioned, despite the wealth of material available (e.g. Wunderlich 2004). The results presented here demonstrate that even minute fossils can provide a degree of morphological detail that should not be ignored. The novel applications of high quality imaging technologies, such as VHR-CT to palaeontological specimens means that neontologists' criticisms of the quality and phylogenetic utility of fossil species become largely semantic. However, technological advances have not yet advanced to the point where traditional techniques can be abandoned. For example, the VHR-CT was unable to detect the leg bristles, which are clearly visible using light microscopy (Figs 11, 12).

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## References

- Alonso, P.D., Milner, A.C., Ketcham, R.A., Cookson, M.J. & Rowe, T.B. (2004) The avian nature of the brain and inner ear of *Archaeopteryx*. *Nature*, 430, 666–669.
- Ascaso, C., Wierzbos, J., Corral, J.C., López, R. & Alonso, J. (2004) New applications of light and electron microscopic techniques for the study of microbiological inclusions in amber. *Journal of Paleontology*, 77, 1182–1192.
- De Franceschi, D. & De Ploëg, G. (2003) Origine de l'ambre des faciès sparnaciens (Éocène inférieur) du Bassin de Paris: le bois de l'arbre producteur. *Geodiversitas*, 25, 633–647.
- Dierick, M., Cnudde, V., Masschaele, B., Vlassenbroeck, J., Van Hoorebeke, L. & Jacobs, P. (2007) Micro-CT of fossils preserved in amber. *Nuclear Instruments and Methods in Physics Research A*, doi,10.1016/j.nima.2007.05.030
- Forster, R.R. (1959) The spiders of the family Symphytognathidae. *Transactions of the Royal Society of New Zealand*, 86, 269–329.
- Forster, R.R. & Platnick, N.I. (1977) A review of the spider family Symphytognathidae (Arachnidae, Araneae). *American Museum Novitates*, 2619, 1–29.
- Forster, R.R. & Platnick, N.I. (1984) A review of the archaeid spiders and their relatives, with notes on the limits of the superfamily Palpimanoidea. *Bulletin of the American Museum of Natural History*, 178, 1–106.
- Grimaldi, D., Nguyen, T. & Ketcham, R. (2000) Ultra-High-Resolution X-Ray Computed Tomography (UHR CT) and the study of fossils in amber. In: Grimaldi, D. (Ed) *Studies on Fossils in Amber, with Particular Reference to the Cretaceous of New Jersey*. Backhuys Publishers, The Netherlands, pp. 77–91.
- Griswold, C.E., Ramírez, M.J., Coddington, J.A. & Platnick, N.I. (2005) Atlas of phylogenetic data for entelegyne spiders (Araneae: Araneomorphae: Entelegynae) with comments on their phylogeny. *Proceedings of the California Academy of Science*, 56 Suppl. 2, 1–324.
- Henderickx, H., Cnudde, V., Masschaele, B., Dierick, M., Vlassenbroeck, J. & van Hoorebeke, L. (2006) Description of a new fossil *Pseudogarypus* (Pseudoscorpiones: Pseudogarypidae) with the use of X-ray micro-CT to penetrate opaque amber. *Zootaxa*, 1305, 41–50.
- Hickman, V.V. (1944) On some new Australian Apneumonomorphae with notes on their respiratory system. *Papers and Proceedings of the Royal Society of Tasmania*, 1943, 179–195.
- Hickman, V.V. (1945) A new group of apneumone spiders. *Transactions of the Connecticut Academy of Arts and Sciences*, 36, 135–148.

- Nel, A. (Ed) (2004) *The Ambers of France: Geology and State of the Art of their Palaeontological Content. Geologica Acta*, 2, 1–94.
- Nel, A., De Plog, G., Dejax, J., Dutheil, D., De Franceschi, D., Gheerbrant, E., Godinot, M., Hervet, S., Menier, J.-J., Aug, M., Bignot, G., Cavagnetto, C., Duffaud, S., Gaudant, J., Hua, S., Jossang, A., de Lapparent de Broin, F., Pozzi, J.-P., Paicheler, J.-C., Bouchet, F. & Rage, J.-C. (1999) Un gisement sparnacien exceptionnel plantes, arthropods et vertèbres (ocne basal, MP7): Le Quesnoy (Oise, France). *Comptes-Rendus de l'Académie des Sciences, (IIa)*, 329, 65–72.
- Nel, A., De Ploëg, G., Millet, J., Menier, J.-J. & Waller, A. (2004) The French ambers: a general conspectus and the lowermost Eocene amber deposit of Le Quesnoy in the Paris Basin. *Geologica Acta*, 2, 3–8.
- Penney, D. (2007a) A new fossil oonopid spider, in lowermost Eocene amber from the Paris Basin, with comments on the fossil spider assemblage. *African Invertebrates*, 48, 71–75
- Penney, D. (2007b for 2006) The oldest fossil pholcid and selenopid spiders, in lowermost Eocene amber from the Paris Basin, France. *Journal of Arachnology*, 34, 592–598.
- Platnick, N.I. (2007) *The World Spider Catalog, Version 7.0*. American Museum of Natural History, New York. Available from: <http://research.amnh.org/entomology/spiders/catalog81-87/index.html>.
- Platnick, N.I. & Forster, R.R. (1986) On *Teutoniella*, an American genus of the spider family Micropholcommatidae (Araneae, Palpimanoidea). *American Museum Novitates*, 2845, 1–9.
- Polcyn, M.J., Rogers, J.V., Kobayashi, Y. & Jacobs, L.L. (2002) Computed tomography of an *Anolis* lizard in Dominican amber: systematic, taphonomic, biogeographic, and evolutionary implications. Available from: [http://palaeo-electronica.org/paleo/2002\\_1/amber/issue1\\_02.htm](http://palaeo-electronica.org/paleo/2002_1/amber/issue1_02.htm).
- Schütt, K. (2003) Phylogeny of Symphytognathidae. *Zoologica Scripta*, 32, 129–151.
- Siveter, Derek J., Sutton, M.D., Briggs, D.E.G. & Siveter, David J. (2004) A Silurian sea spider. *Nature*, 431, 978–979.
- Tafforeau, P., Boistel, R., Boller, E., Bravin, A., Brunet, M., Chaimanee, Y., Cloetens, P., Feist, M., Hoszowska, J., Jaeger, J.-J., Kay, R.F., Lazzari, V., Marivaux, L., Nel, A., Nemoz, C., Thibault, X., Vignaud, P. & Zabler, S. (2006) Applications of X-ray synchrotron microtomography for non-destructive 3D studies of paleontological specimens. *Applied Physics A*, 83, 195–202.
- Vlassenbroeck, J., Dierick, M., Masschaele, B., Cnudde, V., Van Hoorebeke, L. & Jacobs, P. (2007) Software tools for quantification of X-ray microtomography at the UGCT. *Nuclear Instruments and Methods in Physics Research A*, 580, 442–445.
- Wanless, F.R. (1984) A review of the spider subfamily Spartaeinae nom. n. (Araneae, Salticidae) with descriptions of six new genera. *Bulletin of the British Museum of Natural History (Zoology Series)*, 46, 135–205.
- Wunderlich, J. (2004) The fossil spiders of the family Anapidae s.l. (Araneae) in Baltic, Dominican and Mexican amber and their extant relatives, with the description of the new subfamily Comarominae. *Beiträge Araneologie*, 3B, 1020–1111.

