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ORIGINAL PAPER

Reconstructing the anatomy of the 42-million-year-old fossil †*Mengea tertiaria* (Insecta, Strepsiptera)

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Abstract Fossilization in amber is unique in preserving specimens with microscopic fidelity; however, arthropod inclusions are rarely examined beyond the exoskeleton as this requires destructive sampling when traditional techniques are used. We report the first complete, digital 3D, non-destructive reconstruction of the anatomy of an insect fossil, a specimen of †*Mengea tertiaria* embedded in a 42-Ma Baltic amber. This was made possible using Synchrotron μ-CT. The species belongs to the stem group of the phylogenetically enigmatic and extremely specialized Strepsiptera. Most internal structures of the fossil are preserved, but small parts of the lumen had decayed due to incomplete infiltration of the resin. Data on internal organs provided additional information for resolving phylogenetic relationships. A sister group relationship between †*Mengea* and all extant lineages of the group was confirmed with characters previously not accessible. The newly gained information also yielded some insights in the biology of †*Mengea* and the early evolutionary history of Strepsiptera. The technique has a tremendous potential for a more accurate interpretation of diverse fossil arthropods preserved in ambers from 130 Ma to the present.

Keywords Amber · Internal anatomy · SRμ-CT · 3-dimensional reconstruction · Arthropoda · Strepsiptera

Introduction

Amber is resin that has polymerized over millions of years. Often, small organisms become mired and engulfed in it as long as it is fluid (Grimaldi and Engel 2005; Schlee and Glöckner 1978). Amber occurs throughout the world in geological strata ranging the Late Carboniferous (ca. 320 Ma) (Poinar 1992) to the Pleistocene, although the oldest ambers with arthropod and other inclusions are from the Cretaceous of Austria, England, Japan, and Lebanon (which is particularly abundant) (Grimaldi 2003; Schlee 1970). The most extensive and best studied deposits are from northeastern Europe, especially the Middle Eocene (Lutetian) Baltic amber Lagerstätten (Larsson 1978), which may be estimated to have a minimum age of approximately 42–49 and a probable maximum age of 54 Ma (Kosmowska-Ceranowicz 1996; Odin and Luterbacher 1992; Ritzkowski 1997). The life-like external preservation in amber has fascinated numerous paleontologists and zoologists, among them the famous systematist Willi Hennig. He devoted 15 studies to flies preserved in amber in the framework of his effort to reconstruct the phylogeny of Diptera (Hennig 1965). Internal preservation is even more impressive than the specimens as such, with soft tissues usually retaining intracellular fidelity (Grimaldi et al. 1994; Henwood 1992; Kohring 1998), presumably caused by low molecular weight

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mono- and sesquiterpenes infiltrating the body cavities of trapped insects. All studies thus far have required destructive procedures that cleave through the body of an arthropod inclusion, either mechanically or by freeze fracturing, in order to expose internal tissues. Certain solvents, like chloroform and toluene, will cause the disintegration of some amber (e.g., Lebanese amber; Azar 1997), allowing the extraction of cuticular fragments of arthropods. The recent use of μ-CT has allowed non-destructive visualization of external details of arthropods in amber that are obscured by debris, bubbles, or a turbid suspension of particles (Lak et al. 2008; Tafforeau et al. 2006). Some internal tissues of amber arthropods have been visualized with μ-CT such as the rectum and egg of a mite (Heethoff et al. 2009); however, what we present here is the first reconstruction of the entire internal anatomy of a fossil insect. This approach does not only allow a refined interpretation of the phylogeny and evolution of the enigmatic Strepsiptera. It opens the way to assessing previously inaccessible characters in myriads of arthropods preserved in amber, and thus may lead to breakthroughs in the phylogenetic placement of many phylogenetically obscure or ambiguous taxa.

The subject of our study was a male specimen of †*Mengea tertiaria* (Mengeidae) in Baltic amber. This species is belonging to the enigmatic order Strepsiptera, which is arguably the most problematic group of insects in terms of its systematic placement (e.g., Kristensen 1991, "the Strepsiptera problem"). Strepsipterans are highly dimorphic. The females are wingless, usually blind, and morphologically strongly simplified. They develop as endoparasites of other insects. Only females of the basal extant Mengenillidae leave their host and are free-living, whereas the adult females of Stylopidia are permanently endoparasitic and only penetrate the abdominal intersegmental membranes of the host with their anterior body part. The primary larvae belong to the smallest known Metazoans. The free-living, winged males come closest to what may be addressed as a *normal* insect morphology but are extremely short lived and characterized by numerous autapomorphies. The sense organs and flight apparatus are well developed and highly specialized, allowing them to find the females within their very short lifespan, whereas the mouthparts, digestive tract, and excretory organs are highly reduced. The specific importance of the extinct genus *Mengea* is apparent considering its suggested placement in the stem group of Strepsiptera and as sister group of all extant lineages of the order (Strepsiptera s.str.) (Grimaldi et al. 2005; Pohl and Beutel 2005). It plays a key role in understanding the early evolution of the group, and in the reconstruction of the ground plan, which is essential for the systematic placement within the holometabolous insects. This makes a better knowledge of hitherto obscured structural features of †*Mengea* (and other strepsipteran amber fossils) highly desirable. A new phylogenetic evaluation of characters is presented here to corroborate (or falsify) the previously suggested phylogenetic placement of the extinct genus (Pohl and Beutel 2005; Bravo et al. 2009) and some interpretations concerning its biology suggested by the new anatomical data.

Material and methods

Fossil material and preparation of the amber

†*M. tertiaria* male, Baltic amber, Yantarny, Kaliningrad region, Samland peninsula, Russia, ex coll. H. Pohl, Jena. The fossil will be finally deposited at the Phyletisches Museum Jena (Germany). The specimen was originally embedded in a 16×19×4 mm piece of amber. To perform μ-CT the amber was trimmed to a small cone of 5×4×2 mm. The specimen was chosen from among four since its leg and thoracic musculature was vaguely visible through the cuticle using light microscopy (LM) (Fig. 1a). The amber is more transparent at the periphery and is somewhat opaque centrally, thus obscuring important structures of the insect using LM.

Micron-level computer tomography

The amber was scanned using the microtomography instrument operated by the GKSS Research Center Geesthacht, Germany, using 11.5 KeV at Beamline BW2 of the storage ring DORIS-3 at Deutsches Elektronen-Synchrotron DESY, Hamburg, Germany. An X-ray photon energy of 11.5 KeV was selected for performing absorption-contrast SRμ-CT. To increase the density resolution, the tomographic scan was performed over 360° setting the rotation axis to the side of the detector. Prior reconstructions of the projection were combined to obtain 720 projections at equal intervals between 0 and 180° (Beckmann 2008; Beckmann et al. 2006, 2008; Thurner et al. 2004).

Three-dimensional reconstruction

The 3D reconstruction was done with Amira 4.1.2 (Mercury Systems) and Maya 7.0 (Alias Systems Corp.). Illustrations were finally edited with Photoshop CS 2 (Adobe Systems Inc.). Decayed areas were assigned to associated structures whenever this was possible with reasonable certainty.

Results

Structures in approximately 80% of the interior volume of the insect are preserved. The resin apparently did not

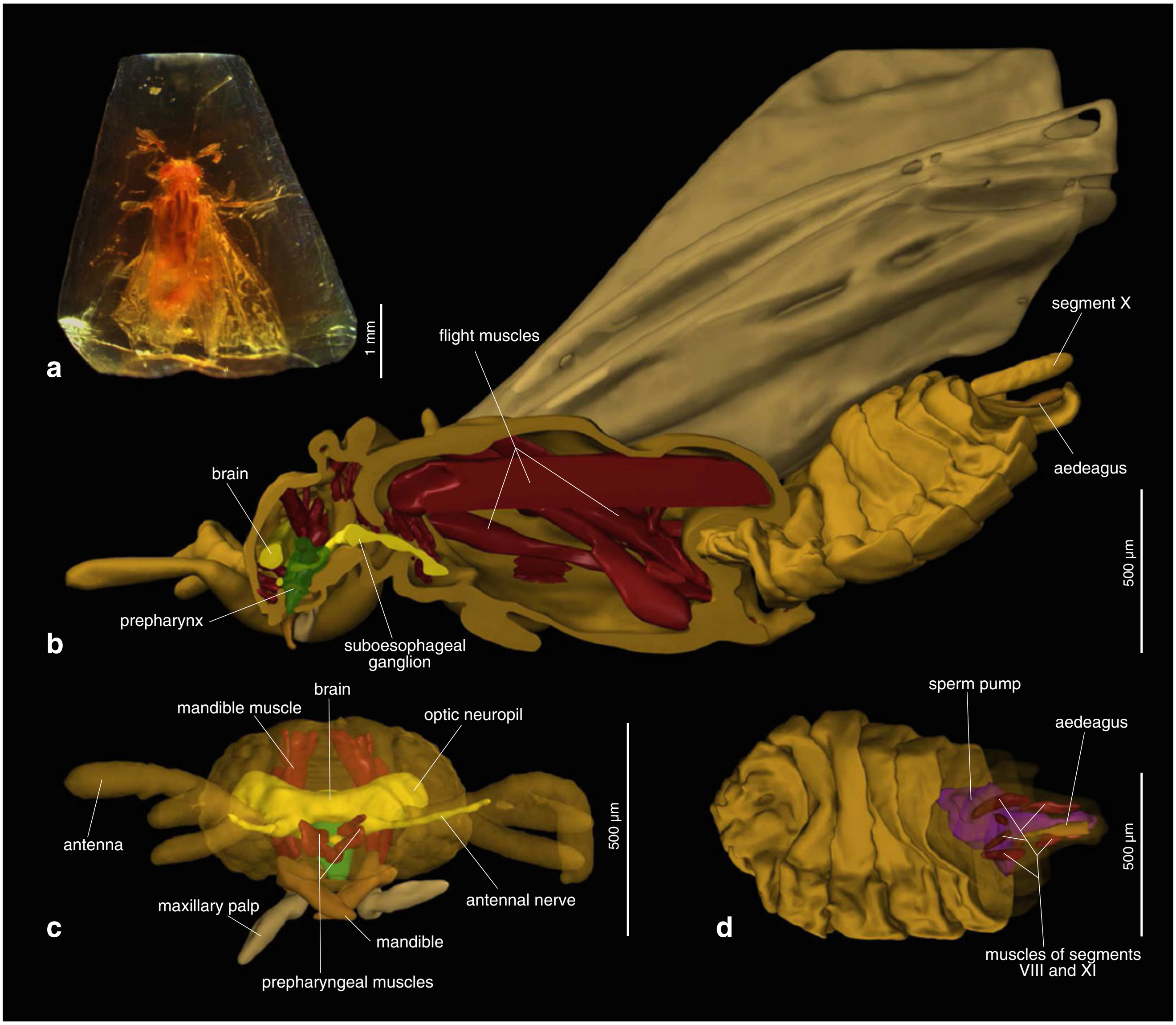

Fig. 1 †*Mengea tertiaria* (Strepsiptera) in Eocene Baltic amber. **a** Dorsal view of the specimen within amber matrix. **b–d** µ-CT 3-dimensional reconstructions. **b** Midsagittal section of head and thorax. **c** Head, frontal view (cuticle shown as transparent). **d** Abdomen, dorsal view, cuticle of terminal segments are transparent

infiltrate the remaining portions, which led to the decay of some muscles and nervous tissue. Several important features of the head hitherto unknown for †*M. tertiaria* were revealed by the reconstruction. A well-developed, free labrum is present, in contrast to all extant strepsipterans except for *Bahiaxenos relictus* (Bahiaxenidae), which was recently discovered in Brazil (Bravo et al. 2009). The large and flabellate antennae are not only equipped with numerous dome-shaped chemoreceptors but also with Hofeneder's organ, a sensorial groove of antennomere 4. Like in other strepsipterans, the well-developed mandible lacks a molar part. The maxilla is simplified as it is generally the case in the order, and a prementum, glossae, paraglossae and the labial palps are absent as in all other extinct and extant members of the group (Pohl and Beutel 2005). A mouthfield sclerite, which belongs to the ground plan of Strepsiptera s.str. (excl. stem group) is present, but a balloon gut, a typical feature of extant strepsipterans (excl. *Eoxenos*) is absent. Like in other strepsipterans, the prepharyngeal dilator muscles are strongly developed (Fig. 1c), whereas dilators of the pharynx are distinctly reduced compared to the condition found in most other insect lineages. The conservation of the digestive tract as such is poor. In contrast, the brain and other elements of the central nervous system are fully preserved. It is dumbbell shaped, with the optic neuropils well developed (Fig. 1b, c). In contrast to extant species of *Elenchus*, which are about equally large (total length ca. 2 mm), the brain of †*Mengea* does not show effects of miniaturization (Beutel et al. 2005). The antennal nerves (Fig. 1c) are well preserved and thick. The subesophageal ganglion is fused with the thoracic ganglia (Fig. 1b). The thorax does not distinctly differ from extant strepsipterans in internal or external features. The indirect flight muscles are very large (Fig. 1b). Most internal organs of the abdomen are preserved. The internal and external genitalia of †*Mengea* are nearly identical to those of the extant genus *Mengenilla* (Hünefeld and Beutel 2005), with a strongly developed musculature of the proximal ductus ejaculatorius (Fig. 1b, d) and unusual muscles of the abdominal segments VIII and IX (Fig. 1d). The aedeagus is straight (Fig. 1b), a ground plan feature only preserved in the most basal lineages of extant Strepsiptera.

Discussion

The use of μ-CT allows an efficient assessment of previously obscured features. One important character now clearly revealed is the presence of a so-called mouthfield sclerite, which is also present in all known extant strepsipterans. Beutel and Pohl (2006) argued that the presence of the mouthfield sclerite is the anteriormost part of an air uptake apparatus and is combined with a balloon gut. This interpretation is rendered invalid by the character combination in the fossil, i.e., by the apparent absence of the balloon-like system, which fills out large parts of the body and reduces weight during the mating flight in extant strepsipterans. Another feature newly confirmed for †*Mengea* is the complete reduction of the tentorium. This shows that this derived condition has already evolved in the stem group and is possibly a ground plan feature of the entire Strepsiptera s.l. (i.e., including stem group taxa). The muscular system of the head and thorax of †*Mengea* is strikingly similar to what is found in extant representatives of the order. It differs only by the presence of one additional muscle in the metathorax. Whether this belongs to the ground plan of the Strepsiptera or is an autapomorphy of †*Mengea* cannot be decided at present as detailed data on the muscle system are only available for two extant taxa (*Eoxenos*, *Mengenilla*).

The inclusion of new anatomical data in previously used data matrices (Pohl and Beutel 2005; Bravo et al. 2009) confirmed the systematic position of †*Mengea* as sister group of Strepsiptera s.str. (extant groups), and the new findings allow some statements on the biology of adults of †*Mengea*. Extant male strepsipterans do not consume food as the digestive tract is modified as a balloon gut and filled with air (see above). Since this derived condition is absent in †*Mengea* it cannot be excluded that food uptake took place; however, the simplified maxilla and the completely reduced labium and also the complete absence of cuticular remains in the digestive tract suggest that the extinct species did not consume solid food, if anything at all. Clearly, what remains of mouthparts would be unsuitabe for consumption of fungal or plant material. The very large flight muscles of the metathorax indicate strong, maneuvered flight. Features related to the antennae, legs, and external genitalia even allow some conclusions on the female of †*Mengea*; it is very likely that they possessed pheromone glands in order to attract the male chemotactically. This is suggested by the presence of numerous chemoreceptors and Hofeneder's organ on the antennae and the strongly developed antennal nerves. Extant males of Strepsiptera use olfactory signals to search the females, which emit odor from pheromone glands (Nassonows' glands) (Grabert 1953; Lauterbach 1954). The female of †*Mengea* was certainly free-living. All strepsipteran males with endoparasitic females (Stylopidia) have at least the fourth tarsomere equipped with hairy adhesive soles to guarantee efficient attachment to the pterygote host during copulation (Pohl and Beutel 2004). The males of †*Mengea*, like those of the extant Mengenillidae with free-living females, possess rod-shaped tarsi with strong claws without any specialized adhesive hairs (Pohl and Beutel 2004). A straight, tapered aedeagus is present in †*Mengea* and also typical for Mengenillidae (Pohl and Beutel 2008). In the extant species, it is inserted in the soft-skinned abdomen of the female and injects liquid semen by the sperm pump into the female's hemocoel. In contrast, higher strepsipterans have a hook or anchor-shaped aedeagus and fertilize the permanently endoparasitic female in the birth opening or the mouth of her exposed cephalothorax. The hook-like shape of the aedeagus provides an additional, enhanced anchorage during copulation. There is no direct evidence for an endoparasitic development of †*Mengea* even though this appears very likely. If the larvae where indeed endoparasitic, it appears plausible that non-pterygote insects such as lepismatids (Zygentoma) were the hosts, like in all basal extant strepsipterans (*Eoxenos*, *Mengenilla*) (Silvestri 1941, 1943; H. Pohl, pers. obs.).

Like most arthropods in amber, particularly in Cretaceous deposits, specimens of the strepsipteran stem group species †*Cretostylops engeli* (100 Ma amber from Myanmar) (Grimaldi et al. 2005) and †*Protoxenos janzeni* (Baltic amber) (Pohl et al. 2005), are represented by unique or very few specimens. For obvious reasons they cannot be studied using destructive techniques, but detailed information can be easily gained using the technique described here. This will likely give further insights in the early evolution of the Strepsiptera in the near future (Pohl et al. 2005).

Non-destructive visualization using μ-CT has profound implications for the study of arthropod evolutionary history in general, as more data for a phylogenetic placement of critical taxa in Cretaceous and Tertiary amber become available. Improved morphological data are certainly essential in many cases, as DNA sequence data as source of phylogenetic information are unavailable when fossil insect taxa are involved. A better anatomical knowledge does also allow to develop more complex evolutionary scenarios, and to trace features of the biology of extinct species which is not accessible to direct observation.

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