High-resolution phase-contrast X-ray computed tomography (CT) reveals the phoretic deutonymph of a fossil astigmatid mite (Acariformes: Astigmata) attached to a spider’s carapace (Araneae: Dysderidae) in Eocene (44–49 Myr ago) Baltic amber. Details of appendages and a sucker plate were resolved, and the resulting three-dimensional model demonstrates the potential of tomography to recover morphological characters of systematic significance from even the tiniest amber inclusions without the need for a synchrotron. Astigmatids have an extremely sparse palaeontological record. We confirm one of the few convincing fossils, potentially the oldest record of Histiostomatidae. At 176 μm long, we believe this to be the smallest arthropod in amber to be CT-scanned as a complete body fossil, extending the boundaries for what can be recovered using this technique. We also demonstrate a minimum age for the evolution of phoretic behaviour among their deutonymphs, an ecological trait used by extant species to disperse into favourable environments. The occurrence of the fossil on a spider is noteworthy, as modern histiostomatids tend to favour other arthropods as carriers.

Keywords: Acariformes; Astigmata; Histiostomatidae; Acaridae; amber; Eocene

1. INTRODUCTION

Astigmatids (Acariformes: Sarcoptiformes: Astigmata) are a diverse assemblage of often tiny mites, with about 5000 species in over 70 families [1]. They include a number of economically significant taxa, such as pests of stored food, as well as the largely parasitic Psoroptida which includes feather, fur and scabies mites. Astigmatids occupy a range of ecological niches, but can be broadly categorized as mites that use ephemeral habitats. Most taxa breed rapidly under ideal circumstances, and in order to facilitate dispersal many of the more basal (i.e. non-parasitic) lineages employ phoresy: attaching themselves to larger arthropod carriers that transport them to (hopefully) better conditions. Specifically, it is the third free-living juvenile instar, or deutonymph, that disperses in this way [2,3]. These typically adhere to the carrier via a distinct attachment organ or ‘sucker plate’ composed of various suckers and mechanoreceptors (conoids) on the underside of the body.

The small size, poorly sclerotized cuticle and sometimes cryptic habits of astigmatids render their fossil record (summarised in [4]) almost non-existent. ‘Tyroglyphidae’ (i.e. Acaridae) has been reported as subfossils from Japanese Mizunami copal. The Neogene of Sicily has an extinct genus of dubious familial affinity. Mexican Chiaapas amber (Miocene, 16 Ma) has yielded the deutonymph of Winterschmidtiidae. The contemporary Dominican amber has produced sarcoptoid mites associated with mammalian hairs. Provisionally assigned to Listrophoridae, they are more reminiscent of the related fur mite family Atopmelidae [1]. Most recently, a fossil mite from Baltic amber (Eocene, 44–49 Myr ago) was re-identified as the oldest unequivocal example of Astigmatida [5] and assigned to a new (fossil) family, Glascaridae, with an unusual, female-mediated mode of reproduction.

A further mite from Baltic amber—this time attached to a spider—was provisionally identified as the ‘phoretic larva of an Acari: Astigmata...’ (M. Judson in [6]). However, the original drawing lacks detail and it was not formally assigned to a family. In the hope of confirming what would be one of the oldest astigmatid mites, we therefore acquired the specimen. However, traditional optical microscopy yielded no further data. Dorsally, the deutonymph presents an uninformative, shield-like surface (figure 1a,b). Taxonomically relevant features are largely hidden underneath. Therefore, we employed phase-contrast X-ray computed tomography (CT) as an alternative method for visualization. In recent years, laboratory and synchrotron-based tomography have been successfully applied to amber inclusions ([7,8], and references therein). Nevertheless, this phoretic, juvenile mite represented a significant technical challenge given (i) its small size and (ii) its attachment to another inclusion. Previous work has successfully imaged both large oribatid mites [7] and small spiders [8] in the 1–2 mm body size range. Yet, at 176 μm, our fossil is almost an order of magnitude smaller and, to our knowledge, is the smallest inclusion in amber to be investigated tomographically to date. Despite this, we were still able to recover sufficient features (figures 1 and 2a) to make systematic inferences and to show that tomography is useful and applicable to even the tiniest amber arthropods.

2. MATERIAL AND METHODS

The mite was scanned using the Xradia MicroXCT system at the University of Manchester’s ‘Henry Moseley X-ray Imaging Facility’. Two scans, each comprising 1200 projections, were acquired at 10× and 20× optical magnifications and energies of 40 kV and 75 kV, resulting in pixel sizes of 1.7 and 0.87 μm. In each case, phase-contrast was exploited to increase edge contrast, enabling the extraction of fine anatomical features. Further methodological details can be found in Penney et al. [8]. Baltic amber is conventionally dated to Eocene (Lutetian); an overview of its geological setting and palaeoenvironment can be found in Weitschat & Wichard [9].

3. SYSTEMATIC PALAEOONTOLOGY

Cohort: Astigmata Canestrini, 1891.

Material: Syninclusion of the holotype of *Dasumiana emicans* Wunderlich, 2004 (Araneae: Dysderidae) from Eocene Baltic amber; Senckenberg Museum für Naturkunde Görlitz, Germany, no. SMNG 07/36290 (ex Wunderlich coll. F540/BB/AR/DYS/CJW).

Description: Deutonymph; total length of dorsal idiosoma, measured medially from anterior propodosoma (i.e. proterosoma) to posterior hysterosoma: 176 µm. Body outline oval in ventral view; lateral walls bulged ventrally (a desiccation effect?). Dorsal cuticle damaged in both propodosoma and hysterosoma. Propodosoma divided into four pieces by three fissures—a larger one in dorsal view on the left, a smaller one on the right and a twice-interrupted one medially. Hysterosoma with artificial indent in dorsal view on the left; a smaller one in a more anterior and medial position. Longitudinal fissure on right side runs the entire length of hysterosoma, representing an elongation of the right propodosomatal fissure. Hysterosoma about five times longer than anterior—posterior length of the propodosoma. Anterior margin of propodosoma anterior-medially pointed. Outline of hysterosoma anteriorly diverging until half-length of hysterosoma; then rounded distally. Dorsal setation, cupules and opisthonotal gland openings equivocal. Ventrally, gnathosomatal remnants (gr = ‘palposoma’ = fusion of mouth opening, chelicerae and pedipalps without ability to assimilate food) slender and of equal length to the anteriorly directed parts of legs I.

Legs I (le1) bulge sideward; demarcations between individual articles of leg I barely visible. Articles of leg II on the right partly distinguishable. Laterally directed leg parts may represent complete tarsus. Leg II (le2) clearly visible and directed anteriorly. As in leg I, setation equivocal. Second left leg (viewed laterally) with thorn-shaped process (tp) on the inner surface article potentially representing tibial setae gT or hT. Distal parts of leg II appear distorted and tarsus obviously broken off. Diagenetic processes during fossilization may have shrunk these legs and left partly covers left leg II, such that this leg defines a rim (r) on the inside (on which the thorn originates). Left leg II not fully visible in its natural shape. Legs III (le3) and IV (le4) directed forwards; leg appearing stilt beyond trochanter—femur articulation. Trochanter (t) and all four hind legs clearly visible, but femur, genu, tibia and tarsus cannot be differentiated. All hind legs appear unusually short, possibly having broken during fossilization. Legs IV

Figure 1. X-ray computed tomography of the tiny, phoretic deutonymph of a fossil mite (Acariformes: Astigmata: ?Histiostomatidae) from Eocene Baltic amber. (a) Whole specimen (arrowed) attached to the carapace of a spider; the same in (b) dorsal, (c) oblique lateral, (d) ventral, (e) anterior and (f) ventro-lateral view; for abbreviations see description.
nearly touch the sucker plate (sp) with the posterior parts of trochanter.

Sucker plate clearly visible and nearly hemispherical; evidently having moved forward relative to posterior end of hysterosoma. Sucker plate in life consists ventrally of individual sucking structures (one pair of movable and one pair of larger main suckers, two pairs of conoids and at least five sucking panels around the conoids) which appear to be completely lost. Possibly, the movable suckers (ms) have remained. Instead, the cuticular skeleton (cs) underpinning the whole sucking apparatus seems to be preserved. Four symmetrically arranged holes (one to four) recognizable, the first pair rather lateral, the posterior one slightly more medial; possibly openings of decayed conoids. Genital opening (gp) and anus (a) apparently visible medially at the anterior sucker plate margin. Two rounded heads visible behind genital opening, possibly representing conoids or remnants of extended movable suckers (ms). Remnants of apodemes discernible in ventral area between hysterosoma and propodosoma; apparently part of medially running sternum (st) usually almost reaching the palposoma. Parts of apodemes p1 (running until the posterior area of legs I) also apparently present.

4. DISCUSSION

CT yielded a high-quality, three-dimensional model and movie (see electronic supplementary material: http://db.tt/NSWA3JD). Significantly, we could also demonstrate here that—using phase-contrast enhancement—micron-scale localized tomography is possible with laboratory-based equipment, and does not need the aid of a synchrotron. The morphology recovered allows us to provisionally identify this inclusion as either the first (and so far only, to our knowledge) fossil mite assignable to Histiostomatidae; or alternatively to Acaridae. It does not allow a meaningful comparison with the taxonomic characters (e.g. the positions of individual setae) used for living species, which would permit formal assignment to a genus. Significantly, the troCHANTERS and femora of legs III–IV offer the only clearly visible articulation, and in the preserved quiescent position, the entire resting legs (beyond the trochanters) appear stiff—no further articles—and explicitly fold forwards beyond the femur–trochanter (figures 1c,d and 2a); exactly as in modern histiostomatids (figure 2b). This key feature would have been impossible to resolve using traditional microscopy as the posterior limbs are concealed ventrally. We should note that the short legs III and IV also resemble the habitus of certain Acaridae, particularly the genus *Schwiebea*. However, in acarids, all articles in legs III and IV are expressed along their length. Also, the entire legs do not appear so stiff and are not folded forwards as a single, straight unit as in our fossil. We cannot completely exclude our specimen from Acaridae, but given the preserved shape of legs III and IV, we favour an assignment to Histiostomatidae. In this interpretation, the hind legs presumably broke (the distal ends look artificial) resulting in their unusual shortness. Histiostomatidae can thus potentially be dated to at least 44–49 Myr ago, which is unsurprising since fossil-calibrated molecular data [10] infer an origin for crown-group Astigmata perhaps as far back as the mid-Permian (approx. 270 Ma).

Modern histiostomatids—and species of *Schwiebea* (Acaridae)—are usually phoretically associated with insects or myriapods [2,3], particularly beetles (Coleoptera). With respect to the fossil, we are not aware of published records of associations with spiders. S.W. received specimens of the cobweb spider *Achaearanea tepidariorum* (Theridiidae) from laboratory cultures at the University of Freiburg (Germany) immersed in...
deutonymphs of *Histiostoma* sp., but it remains unclear whether this is a natural association. Histostomatids usually feed on bacteria—their mouthparts were modified for filter-feeding—and typically develop in moist humid substrates, often on the surface of a slimy bacterial film growing over rotting fruit or wood, insect cadavers, etc. *Schwibeeae* prefers similar carriers and habitats, feeding on decaying plant material. Life cycles are rapid in both groups, going from a three-legged (hatching) larva to adult in a few weeks. Under suitable conditions, the dispersing deutonymph can, in some Histostomatidae, be suppressed; the protonymph moulting directly into the tritonymph. Mate-guarding of female tritonymphs has been observed in Histostomatidae [2], whereby males can develop faster than females. Our fossil deutonymph, sitting on its carrier and with remnants of a sucking apparatus, provides a minimum age for the evolution of phoretic behaviour in Astigmata. Given the antiquity of arthropods in general, such behaviour in the Eocene is not hugely surprising. Yet, it does suggest that intimate associations between these mites and other arthropods, leading to specialized adaptations in both their life cycle and juvenile morphology, have a long geological history. It further implies that the Baltic amber forest imposed ecological conditions which made phoresy by astigmatids both necessary and desirable.

Finally, formal descriptions of fossil astigmatids remain rare in the literature [4]. Given the predisposition of at least the basal lineages to use other arthropods for transport, we suspect that this is largely an artefact. Their fossilized remains in amber probably get overlooked, or cannot be identified beyond ‘Acari’, because of their small size and difficulties in resolving details using traditional microscopy. Provisional study by S.W. of other amber mites attached to arthropods indicates the presence of further fossil Astigmata. In some cases, tomography may be the only way to achieve a definitive identification and in spite of the technical challenges, we hope to have shown here that the rewards justify the effort.

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