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Original Article

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The hidden world of fossil larvae: description and morphological insights of an immature scorpionfly (Mecoptera: Panorpidae) from the Baltic amber

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ABSTRACT

So far, there has been no information of the pre-imaginal stages in the well-preserved fossil record of the Mecoptera. The first and well-preserved mecopteran larva was discovered in Eocene Baltic amber. The application of synchrotron X-ray microtomography enabled the reconstruction of the body structure with high accuracy, providing a comprehensive set of morphological data that classical stereoscopic microscopy could not capture. The larva is eruciform, with distinct segmentation of the body. All the most important morphological structures are documented, including the chaetotaxy system. Shape of antenna and annulated processes on the abdominal segments point to the third/four stage of development. There is no doubt that the larva belongs to Panorpidae, the most abundant family of extant Mecoptera. The morphological characters point to the closest affinity to larvae of *Cerapanorpa* and *Panorpa* but the fossil larva cannot be assigned to any extant genus of Panorpidae. Based on the results of synchrotron X-ray microtomography, it is possible to discuss the assignement of this larva to a lower systematic level than the family, and draw conclusions about the nature of the habitat preferences of Eocene panorpid.

Keywords: fossil resin; Eocene; scorpionflies; immature stage; morphology; synchrotron microtomography

INTRODUCTION

Scorpionflies (Mecoptera) are one of the oldest holometabolous insects, including nine families with more than 800 species. However, more than half of the extant species belong to one family, Panorpidae, exclusively spread in the northern hemisphere and oriental region (Bicha 2018). Fossil evidence indicates that the diversity of scorpionflies was incomparably greater in the past (Ren *et al.* 2009, Lin *et al.* 2019). The earliest fossil records of Mecoptera have been known since the Upper Permian, giving rise to the evolutionary lineage Anthliophora, from which Diptera and Siphonaptera evolved. In the Mesozoic, scorpionflies were much more abundant than today, being one of the most important and diverse elements of the entomofauna (Grimaldi and Engel 2005). The Cretaceous Terrestrial Revolution caused a remodelling of the biota (Benton *et al.* 2022), which resulted in a decline in diversity of Mecoptera. The ecological niches formerly occupied by Mecoptera were successfully filled by groups that then underwent radiation, among others, flies (Diptera; Wiegmann *et al.* 2011, Peña-Kairath *et al.* 2023). However, even in the Eocene, scorpionflies were much more diverse than they are today (Archibald *et al.* 2013, Lin *et al.* 2019). In fossil inclusions, larger insects, to which scorpionflies belong, are quite rare. So far, only three families based on adults have been recorded from Baltic amber. Within the family Panorpidae, two species of the modern genus *Panorpa* Linnaeus, 1758 (*P. mortua* Carpenter, 1954 and *P. obsoleta* Carpenter, 1954) and two species belonging

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to the extinct genus *Baltipanorpa* Krzemiński and Soszyńska-Maj, 2012 (*B. damzeni* Krzemiński and Soszyńska-Maj, 2012 and *B. oppressiva* Soszyńska-Maj and Krzemiński, 2022) have been described from this amber (Carpenter 1954, Krzemiński and Soszyńska-Maj 2012, Soszyńska-Maj *et al.* 2022). Eocene species of the genus *Panorpa* do not differ particularly from modern representatives of the genus. In contrast, both representatives of the genus *Baltipanorpa* are characterized by the unique development of a notal organ in males, which in modern scorpionflies plays a fundamental role during copulation. This extensively developed organ of males was probably the most oppressive mating strategy known in Mecoptera, which has not survived to the present day (Soszyńska-Maj *et al.* 2022). In addition to the Panorpidae, representatives of two other families have also been described in Baltic amber: Panorpodidae (one genus, three species; Carpenter 1955, Soszyńska-Maj and Krzemiński 2013, 2015) and Bittacidae (two genera, five species; Pictet 1854, Pictet-Baraban and Hagen 1856, Carpenter 1954, Krzemiński 2007).

Although we have a great deal of fossil evidence regarding imagines of Mecoptera, so far nothing is known about the preimaginal stages, both among compression fossils and amber. The first well-preserved inclusion in Eocene Baltic amber has provided a chance for the description of the morphology of the fossil mecopteran larva. Over the past 30 years lots of information about the larvae of extant Mecoptera has been accumulated, resulting in the availability of morphological data representing most families and genera (e.g. Byers and Yeates 1999, Hua and Cai 2009, Chen and Hua 2011, Ma *et al.* 2014, Beutel *et al.* 2019a, b, Jiang *et al.* 2019). The morphology of larval stages is quite complicated; many details are required to describe and determine them. Studying immature stages in the fossil resin is even more challenging. The preservation state of inclusions, combined with advanced imaging techniques, such as synchrotron X-ray microtomography, made it possible to describe the morphology of the fossil immature stage of Mecoptera with great accuracy and to compare it with extant genera and species.

MATERIALS AND METHODS

Geological context

Although the Baltic amber forests covered nearly all of Europe, the age of Baltic amber is is still debated arousing the interest of many scientists (paleontologists, geneticists, etc.). The views on this subject are changing with new research results. Not long ago, the widely accepted and generally agreed view was that Baltic amber is Middle or/and Late Eocene (Lutetian–Priabonian) in age (47.8–33.9 Mya) (Standke 2008). Some authors accept a Lutetian age (based on glauconite dating), whereas others suggest a Priabonian age (based on microfossils). The vast majority of Baltic amber pieces come from the so-called Blue Earth layer, which is mainly mined in the Kaliningrad area (Russia) on the Samland Peninsula (Standke 2008), but also amber pieces are frequently found along the Baltic Sea (Weitschat and Wichard 2010). According to the latest research based on pollen and dinoflagellate data, as well as on lithostratigraphic studies, the Blue Earth layer is suggested to be Late Eocene (only Priabonian) in age (37.8–33.9 Mya) (Kasiński *et al.* 2020). However, much of the Baltic amber has been secondarily redeposited in Pleistocene glaciation across the North European Plain. For a broader discussion on this topic, see Sadowski *et al.* (2022) and Sadowski and Hofmann (2023).

Specimen repository

The inclusion of the scorpionfly larva (MAIG 6003) in Baltic amber is housed in the Museum of Amber Inclusions, Department of Invertebrate Zoology and Parasitology, Faculty of Biology, University of Gdańsk, Gdańsk, Poland (Curator E. Sontag).

Methods

To confirm the validity of the newly described inclusion, Fourier transform infrared spectroscopy, attenuated total reflectance (FT-IR, ATR) spectrum was obtained with the use of a Nicolet iS5 FTIR. The amber IR spectra are archived in the base of Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Kraków (ISEA PAS), according to procedure recommended for museum materials (Zakrzewska *et al.* (2020) . The obtained spectra show characteristic features, i.e. the 'Baltic shoulder' situated between 1190 and 1280 cm^{-1} flanked by a strong absorbance peak at 1170 cm^{-1} reflecting the succinate content (Wolfe *et al.* 2016).

The images were taken with a M205C Leica stereomicroscope (Leica Microsystems, Wetzlar, Germany) with an integrated high-resolution Leica DFC495 digital camera and the associated software for focus stacking (Leica Application Suite 4.4.0).

Fast synchrotron X-ray microtomography was performed at the Imaging Cluster of the KIT Light Source using a parallel polychromatic X-ray beam produced by a 1.5 T bending magnet that was filtered by 0.5 mm aluminum with a resulting spectrum peak at about 15 keV. We employed a fast indirect detector system consisting of a 13 µm LSO:Tb scintillator (Cecilia *et al.* 2011), a diffraction limited optical microscope (Optique Peter) (Douissard *et al.* 2012), and a 12-bit pco.dimax S4 high speed camera with 2016 × 2016 pixels resolution. The magnification was set to 10×, resulting in an effective pixel size of 1.22 µm. We took three individual scans to cover the complete specimen. For each scan, we recorded 200 dark field images, 200 flat field images, and 3000 equiangularly spaced radiographic projections in a range of 180° with 10 ms exposure time each, resulting in scan durations of 34 s each. We used the control system concert (Vogelgesang *et al.* 2016) for automated data acquisition and online reconstruction of tomographic slices for data quality assurance. The final tomographic 3D reconstructions were performed by tofu (Faragó *et al.* 2022) and additionally included phase retrieval (Paganin *et al.* 2002), ring removal, 8-bit conversion, and blending of phase and absorption 3D reconstructions in order to increase contrast between the background and homogeneous regions, while at the same time highlighting the edges. In AMIRA 5.6, the three tomographic volumes were registered and merged into a single image volume. Afterwards, the specimen was digitally isolated from the background using the VolumeEdit module and setae were pre-segmented with the software's segmentation editor. The pre-segmented label provided the basis for semi-automatic segmentation with BIOMEDISA (Lösel *et al.* 2020). The result served as mask to invert the greyscale of the setae in FIJI (Schindelin *et al.* 2012). Volume renderings of the processed tomogram were created with DRISHTI 2.5.1 (Limaye 2012).

The description and terminology follow Cai and Hua (2009), Chen and Hua (2011), Jiang and Hua (2013), Ma *et al.* (2014), and Jiang *et al.* (2014), Jiang and Hua (2015a, b). Information about the morphology of larvae of extant Panorpidae is provided according to Cai and Hua (2009), Chen and Hua (2011), Jiang and Hua (2013), Ma *et al.* (2014), Jiang and Hua (2015a), Jiang *et al.* (2019), and Liu *et al.* (2021).

RESULTS

Systematics

Order Mecoptera Packard 1886

Family: Panorpidae Latreille 1805

Diagnosis: Larva with eruciform body shape, compound eye, mandibulate mouthparts, antenna with swollen pedicels, thoracic legs four-segmented with triangular tibial lobe, abdominal segments with dorsal annulated processes and eight pairs of abdominal prolegs, dorsal annulated processes on the abdominal segments A1–A7 short, dorsal annulated processes on the abdominal segments A8–A10 strong and long, ventral prolegs small.

General morphology of the larva: The larva is eruciform, with three pairs of thoracic legs and eight pairs of abdominal prolegs (Figs 1A, 2A–C, 3A–C); intersegmental membranes of the segments are not clearly visible; the antennae are three-segmented (Figs 2B, 4B, C, 5A, B); mandibulate mouthparts (Figs 4C, 5B); compound eyes situated dorsolaterally between the vertex and gena (Figs 2B, 3A–C, 4A), the right eye protruding, left eye slightly damaged, flattened (Fig. 4B, C); ommatidia of the right eye are distinctly visible (Figs 2B, 4A); the thoracic segments each bear pairs of four-segmented legs (Figs 2B, 3A, 4A, D), the first thoracic segment features a prominent, prothoracic shield covering the entire dorsal surface of the segment (Figs 2B, 3A, 4D); the abdominal segments are equipped with paired erect subdorsal annulated processes on A1–A9 and a single mid-dorsal annulated process on A10 (Figs 2A, C, 3A–C, 4E); the respiratory system not visible, but one prothoracic, and one spiracle of the abdominal segment A1 present (Fig. 4A, D).

Size: 4.9 mm length.

Head capsule: Head well-sclerotized, width 0.9 mm (Figs 2A, B, 4A–C); the coronal and frontal sutures join together, forming an inverted Y-shaped ecdysial line, with the stem medially extending from the occipital foramen and the lateral arms diverging downward to the frontoclypeal suture (Figs 4B, 5A); the anterior and posterior tentorial pits not visible; nine pairs of setae were identified that are distributed on the cranium symmetrically (Figs 4A, B, 5A, B); the slightly trapezoid clypeus is separated from the frons by the distinct frontoclypeal suture, clypeus is subdivided into the basal sclerotized postclypeus and the apical anteclypeus, between postclypeus and antyclypeus is a slightly sunken line with a transverse row of four setae (Figs 4B, 5A).

Compound eyes, antennae, and mouthparts: Eyes with about 30 ommatidia (Figs 2B, 4A); antennae located between compound eye and clypeus (Figs 2B, 3A–C, 4B, C), with three segments: basal scape, pedicel, and distal flagellum (Figs 4C, 5A); the scape is inserted into the antennal socket supported by a raised antennal sclerite (Fig. 5A, B), flagellum is the longest and more slender than the pedicel and the basal scape; the mouthparts are typical mandibulate type, the labrum trapezoid and slightly notched midapically, and articulated proximally with the anterior region of the clypeus (Figs 4B, C, 5B), with only one pair of long labral setae (outer) (Fig. 4C); the membranous epipharynx barely visible; the paired mandibles strongly sclerotized and curved inward with pointed tips, uniting to the subgena at the clypeal base (Figs 4C, 5B); the mandibles taper toward the apex, with two long, subequal setae visible on their lateral surfaces (Figs 4C, 5B); the paired maxillae each consist of the basal cardo-stipes, galea, lacinia, and a three-segmented maxillary palp (Figs 4C, 5B); the cardo-stipes is kidney-shaped and distally connected to the galea and lacinia medially and bears the palp laterally, setae on the maxillae not visible; the galea broadly connected with the lacinia at the base (Figs 4C, 5B), comparatively small; the maxillary palp inserted on a palpifer, the basal two segments roughly equal in length and shorter than the distal one (Figs 4C, 5B); the labium is greatly reduced in size, ligula absent, most parts of the labium retracted into the capsule, a pair of two-segmented labial palps visible between the cardo-stipes bases (Figs 4C, 5B); the salivary duct spens between the divided prementum; the distal segment of the labial palp much longer than the basal one (approximately 1.5 times) (Fig. 4C).

Thorax: Prothorax with prothoracic shield and paired spiracles and specific chaetotaxy (Figs 2B, 3A, B, 4A, D); the prothoracic shield saddle-like and broad, with several setae along the margin; the prothoracic spiracle situated at the posterolateral corner of the prothoracic shield (Fig. 4A, D); meso- and metathorax similar in morphology and chaetotaxy, and lack of spiracles; the thoracic legs with four segments: coxa, femur, tibia and tarsus (Figs 2B, 4A, D); the triangular tibial lobe present (Figs 2B, 4A).

Abdomen: Abdomen with 11 segments, each segment with short setae on the pinacula and erect subdorsal annulated processes inserted on stout basal protuberances (Figs 2A, 3A, B); the annulated processes paired and moderately long on segments A1–A7, strong and elongated on segments A8–A9 (Figs 2A, C, 3A–C, 4E), the last abdominal segment bears single annulated process on the mid-dorsal line of A10 (Figs 3A–C, 4E); A1–A7 roughly similar to each other in morphology and chaetotaxy (Fig. 1A), A8–A10 have much longer processes, thinner, and differ considerably from the others in chaetotaxy (Fig. 3A, 4E, 5C); A1–A8 with paired lateral spiracles and ventral prolegs (Figs 2A, C, 3A, C, 4E); the spiracles barely visible (Figs 2A, C, 4A, E); the stout prolegs unsegmented, and not arranged in a longitudinal line with the thoracic legs (Figs 2A, C, 3A, C, 4E); A11 reduced.

The chaetotaxy: The position of numerous small setae was not possible to detect. The chaetotaxy of the prothorax different from the meso- and metathorax, which are similar to each other (Figs 4D, 5C); the first seven abdominal segments (A1–A7) bear similar chaetotaxy, while terminal segments (A8–A10) individually distinct (Figs 4E, 5C).

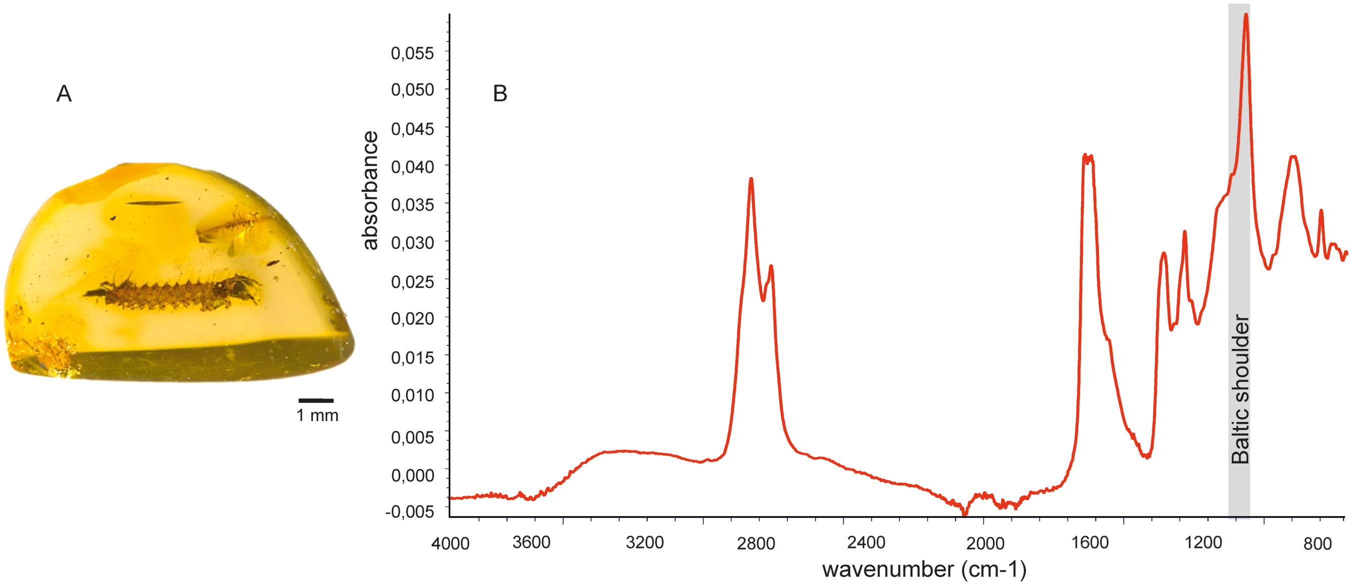

Figure 1. The Baltic amber with Panorpidae larva, MAIG 6003 (A) and its spectrum (B).

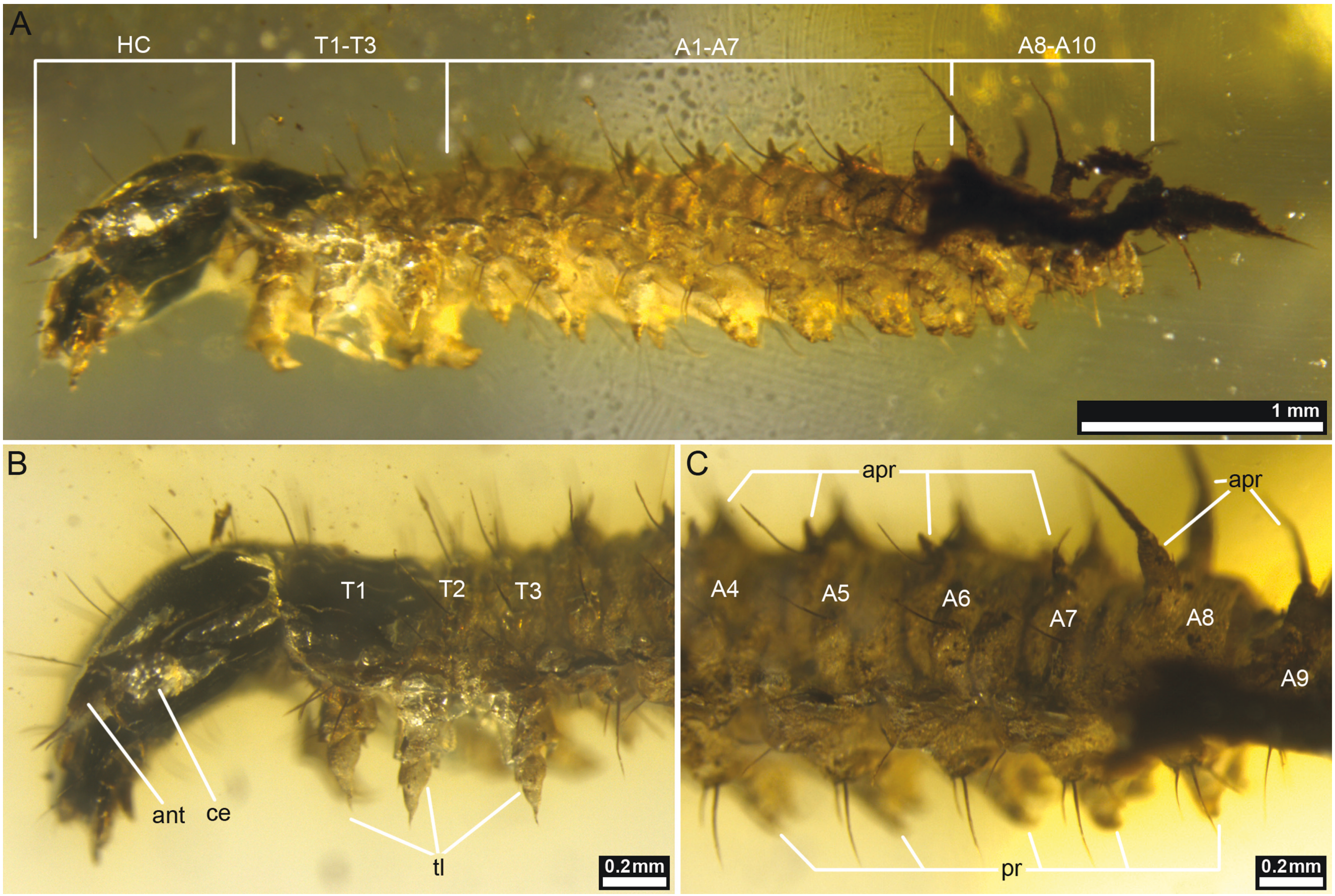

Figure 2. Stereoscope images of Panorpidae larva, MAIG 6003. A, habitus. B, head capsule and thoracic segments, in lateral view. C, abdominal segments A4–A9, in dorso-lateral view. Abbreviations: A, abdominal segments; ant, antenna; apr, annulated processes; ce, compound eye; HC, head capsule; pr, prolegs; T, thoracic segments 1-3; tl, thoracic legs.

Prothorax (T1) (Figs 4D, 5C)*:* Three setae (xd1, xd2, and sd2) along the anterior edge of the prothoracic shield are, one seta (sd1) situated at the posterior edge of shield, seta d1 absent; the distance between xd2 and sd2 slightly longer than that of xd2 and xd1; L1 on the lateral pinaculum, anteroventral to the spiracle; two long setae (sv1 and sv2) on a crescent pinaculum

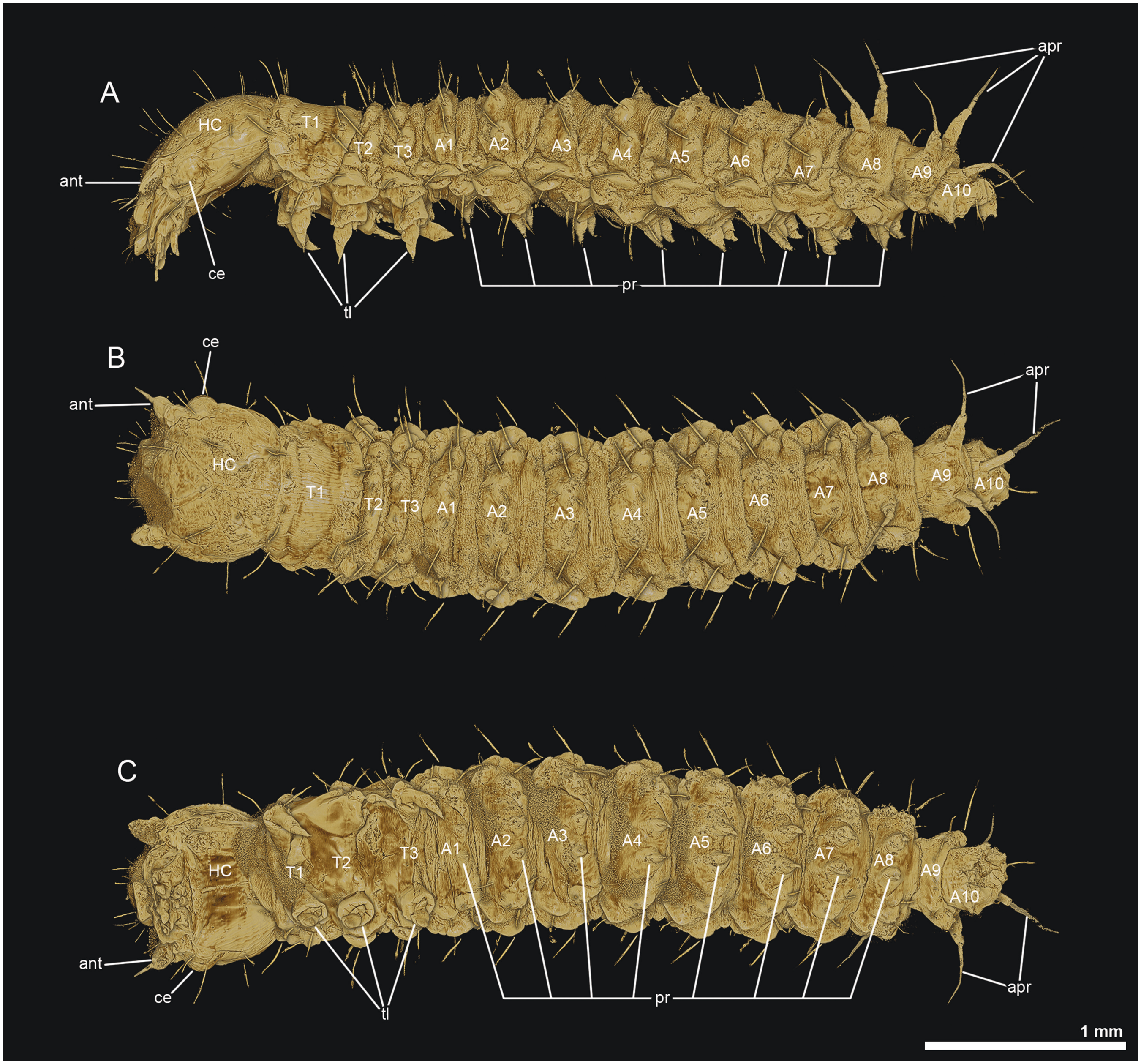

Figure 3. Habitus of the Panorpidae larva, MAIG 6003, volume renderings based on synchrotron X-ray microtomography data. A, in lateral view, right side. B, in dorsal view. C, in ventral view. Abbreviations: A, abdominal segments; ant, antenna; apr, annulated processes; ce, compound eye; HC, head capsule; pr, prolegs; T, thoracic segments; tl, thoracic legs.

dorsal to the coxal cavity; microsetae on this pinaculum not visible; spiculate ventral seta (v1) not visible.

Meso- and metathorax (T2 and T3) (Figs 4D, 5C)*:* Two clavate setae (d1? or d2?, and sd1) and one microseta (msd1) on dorsal pinaculum; microseta md1 not visible; ventral to the dorsal pinaculum three pinacula, each accompanied respectively by one long seta (sd2, l1, and l2); microseta ml2 present; subventral setae (sv1 and sv2) located on two detached subventral pinacula.

Abdominal segments A1–A7 (Figs 4E, 5C)*:* The annulated processes short on A1–A7, three setae (d1, d2, and sd1) and one detectable microseta (msd1) on the dorsal pinaculum; ventral to the dorsal pinaculum, another pinaculum accompanied by long seta (sd2); posterior to the spiracle a long lateral seta (l1); L2 located together with ml2 on a lower lateral pinaculum; three setae (sv1–sv3) on two subventral pinacula; ventral setae (v1 and v2) not visible; seta d2 two times shorter than d1 in length; setae sd1 and sd2 roughly in the same length as l1; setae sv2 and sv3 half as long as sv1.

Abdominal segment A8 (Figs 4E, 5C)*:* The setae on the basal protuberance of the annulated process are not visible; setae l1 and l2 long; subventral pinacula with long setae (sv1–sv3); seta l1 about 1.5 times as long as l2 and markedly shorter than sv1; anteriorly to the ventral prolegs, setae v1 and v2 present.

Abdominal segment A9 (Figs 4E, 5C)*:* The setae on the basal protuberance of the annulated process are not visible; setae l1 and sv1 almost equal in length.

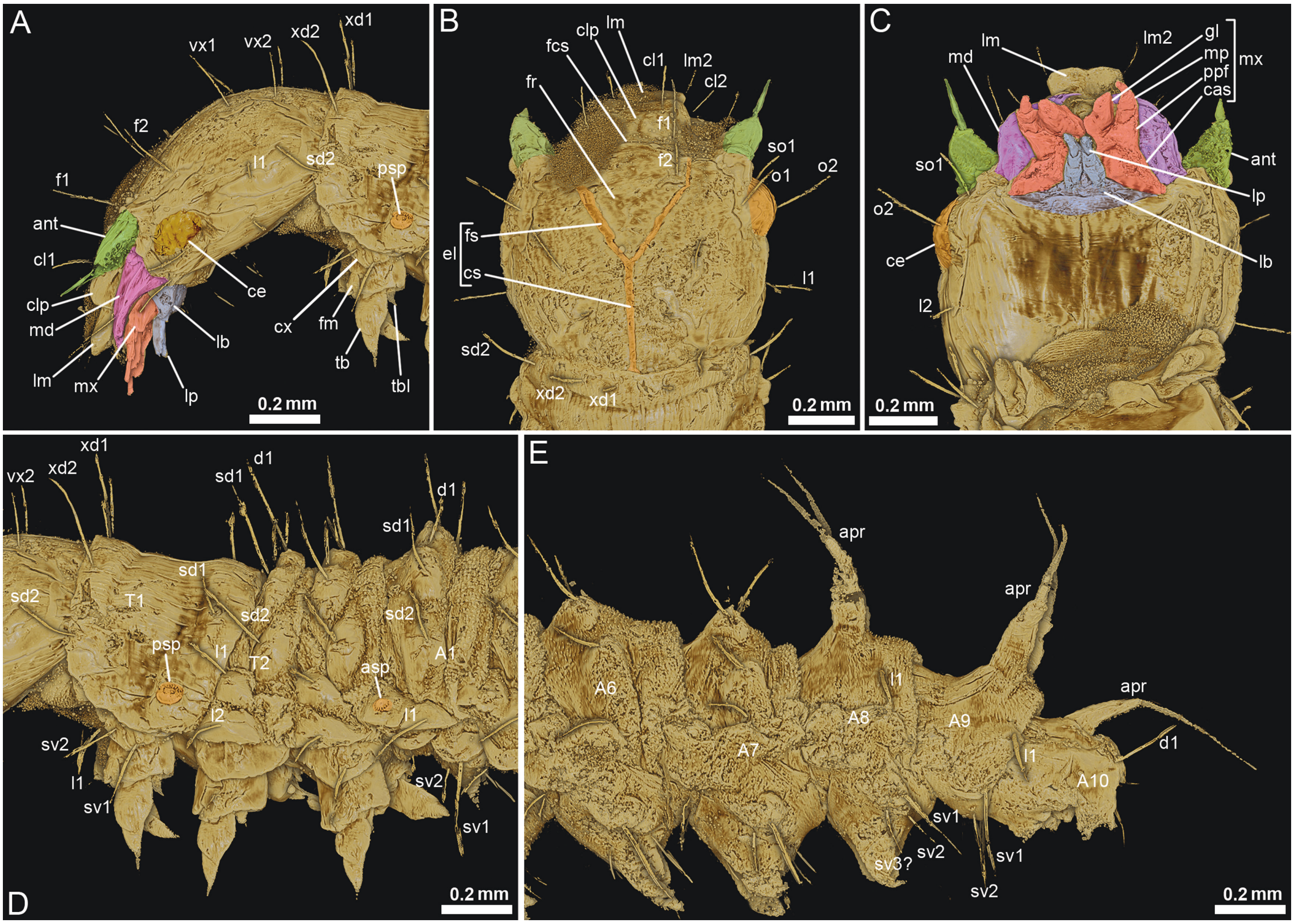

Figure 4. Panorpidae larva, MAIG 6003, volume renderings based on synchrotron X-ray microtomography data. A, head capsule, in lateral view. B, head capsule, in dorsal view. C, head capsule, in ventral view. D, thorax, in lateral view. E, last abdominal segments, lateral view. Abbreviations: A, abdominal segments; ant, antenna; apr, annulated processes; asp, abdominal spiracle; cas, cardo-stipes; ce, compound eye; clp, clypeus; cl, clypeal setae; cs, coronal suture; cx, coxa; d, dorsal setae; el, ecdysial line; f, frontal setae; fcs, frontoclypeal suture; fm, femur; fr, frons; fs, frontal suture; gl, glossa; l, lateral setae; lb, labium; lm, labrum; lm2, labral seta; lp, labial palp; mx, maxilla; md, mandible; mp, maxillary palp; o, ocular setae; ppf, palpifer; psp, prothoracic spiracle; sd, subdorsal setae; so, subocular seta; sv, subventral setae; T, thoracic segments; tb, tibia; tbl, tibial lobe; vx, vertical setae; xd, prothoracic setae.

Abdominal segment A10 (Figs 4E, 5C)*:* The setae on the basal protuberance of the single mid-dorsal annulated process are not visible; seta d1 and microsetae msd1 and msd2 well visible; other elements of chaetotaxy are not visible.

DISCUSSION

Larvae of holometabolous insects are an important developmental stage, which is a precious source of environmental data that we do not get by accessing only adults. Moreover, learning about the morphology of larval stages can yield important information that changes phylogenetic views within a group (Meier and Lim 2009). However, the information of pre-imaginal stages is quite rare. It is estimated that only about 2% of holometabolous larvae are well known (Newton 1990, Jiang *et al.* 2019). Extant scorpionflies with nine families have three general types of larvae: eruciform (caterpillar-shaped) larvae, characteristic of two small relict families from Australia (Apteropanorpidae and Choristidae), and two of the largest and most species-rich contemporary families (Panorpidae and Bittacidae); scarabaeiform larvae (Boreidae and Panorpodidae); larvae of Nannochoristidae are the only ones that are aquatic, and represent a completely different type of morphology. The immature stages of the two remaining families of Mecoptera are unknown (Byers and Thornhill 1983, Byers and Yeats 1999). Therefore, the detailed description of the first fossil larva provides significant new insights into the evolution of Mecoptera. The morphological structures of the larva described herein not only clarify the taxonomic position of this group, but also determine the preferences and adaptations to a particular habitat.

Generally, several larval structures point without doubt to the order Mecoptera, such as the eruciform general body shape, compound eye present, and characteristic antenna with swollen pedicels (Beutel *et al.* 2014). Due to clear distinctions observed in the general body shape, mouthpart structure, and leg morphology, the fossil larva's association with Boreidae and Nannochorstidae can be confidently excluded (Potter 1938, Suzuki 1990, Byers and Yeates 1999, Kluge 2003, Beutel *et al.*

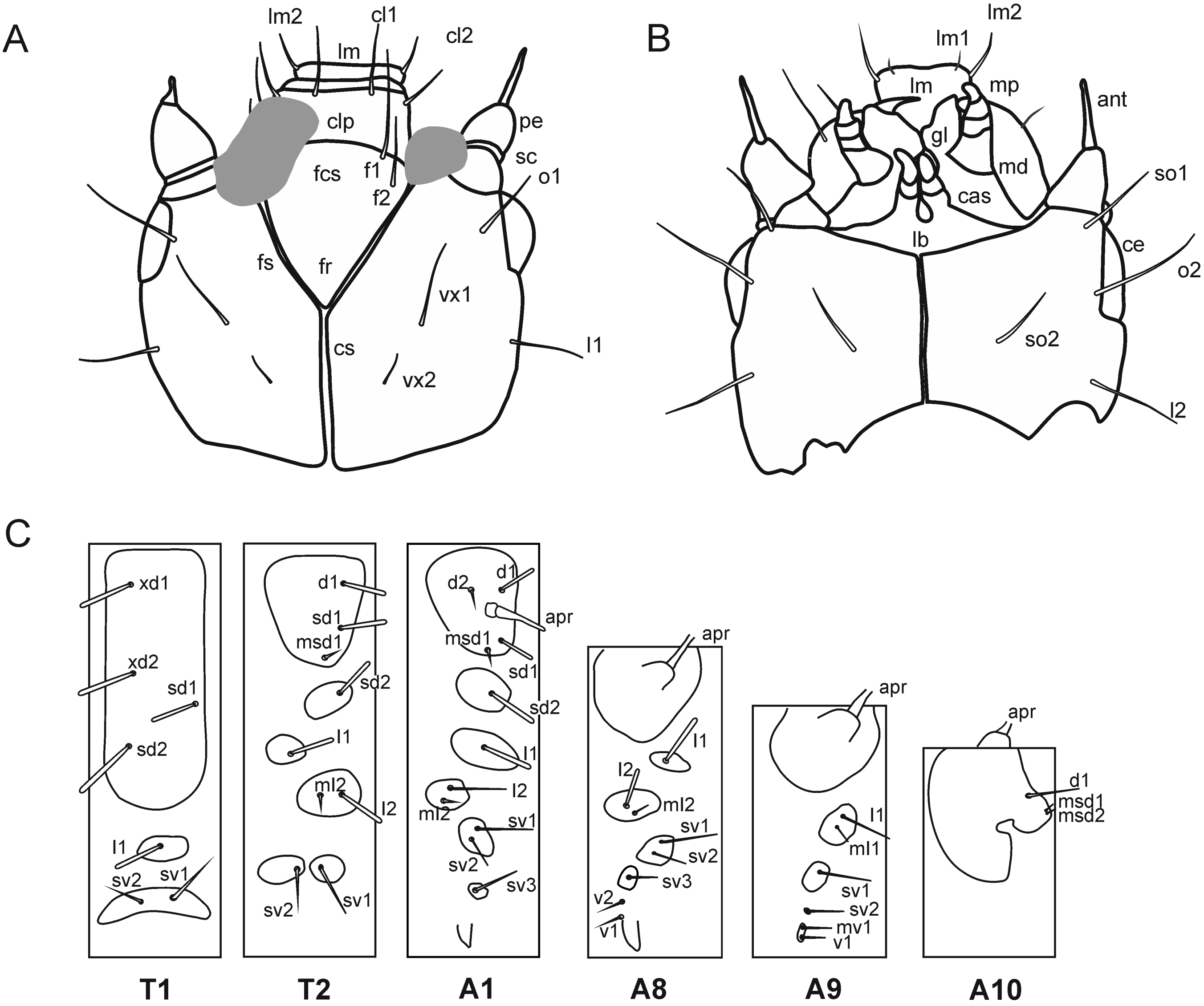

Figure 5. Head capsule morphology and chaetotaxy of Panorpidae larva, MAIG 6003. A, head capsule, in dorsal view. B, head capsule, in ventral view. C, chaetotaxy of T1, T2, A1 and A8–A10 segments. Abbreviations: A, abdominal segments; ant, antenna; cas, cardo-stipes; clp, clypeus; cl, clypeal setae; cs, coronal suture; d, dorsal setae; el, ecdysial line; f, frontal setae; fcs, frontoclypeal suture; fl, flagellum; fr, frons; fs, frontal suture; gl, glossa; l, lateral setae; lb, labium; lm, labrum; lm, labral setae; lp, labial palp; mx, maxilla; md, mandible; ml, minute lateral setae; mp, maxillary palp; msd, minute subdorsal setae; o, ocular setae; pe, pedicel; ppf, palpifer; psp, prothoracic spiracle; sc, scape; sd, subdorsal setae; so, subocular seta; sv, subventral setae; T, thoracic segments; v, ventral setae; vx, vertical setae; xd, prothoracic setae.

2019a, b). The Eocene larva also can be easily distinguished from that of Bittacidae (Jiang and Hua 2015a) and Panorpodidae (Jiang *et al.* 2014, Jiang and Hua 2015a). Larvae of Bittacidae feature different proportions of the particular antennal segments, with a proportionally shorter flagellum in relation to the length of pedicel (Jiang and Hua 2015a). However, the most obvious differences are related to the size of the setae, the annulated processes, and prolegs, which are much longer and more prominent in the larvae of Bittacidae than in any other known larvae of Panorpidae (Beutel *et al.* 2019a, b, Jiang *et al.* 2019). The larvae of Panorpodidae are eyeless and without any prominent body appendages on the abdominal segments (Jiang *et al.* 2014). The larvae of Australian Choristidae and Apteropanorpidae closely resemble the larvae of Panorpidae in general appearance. However, choristid larvae have no annulated setae and apteropanorpids have no dorsal setae, respectively (Riek 1970, Byers and Yeats 1999).

The Eocene larva is without a doubt specific for Panorpidae, based on the form of the labial palp, as well as the shape of the dorsal annulated processes and the ventral prolegs of the abdominal segments (Cai and Hua 2009, Chen and Hua 2011, Jiang and Hua 2013, 2015a, Ma *et al.* 2014, Jiang *et al.* 2019, Liu *et al.* 2021). This highly numerous and diverse family includes 10 extant genera and one exclusively fossil. The morphology of larval stages has been described in seven genera, although the degree of accuracy of these descriptions varies considerably (Table 1). The larvae of the most isolated genus *Leptopanorpa* MacLachlan, 1875, endemic to Java, Sumatra, and Bali, are still unknown (Wang and Hua 2020), as well as the larvae of *Lulilan* Wilmann, 2022 and *Phile* Willmann, 2022 described

Table 1. Selected morphological characters of Panorpidae larvae with known pre-imaginal instars. Character: (1) furrows on the lateral side of head capsule, (2) antenna, (3) tibial lobe on thoracic legs, (4) prolegs size, (5) dorsal annulated processes size (AI–AVII), (6) seta d1 on prothorax. Legend: ?—insufficient description provided by author.

	Characters					
Taxon	**1**	**2**	**3**	**4**	**5**	**6**
Cerapanorpa Gao, Ma and Hua, 2016	absent	elongated	present	regular	mid-size	present?
Dicerapanorpa Zhong and Hua, 2013	absent	elongated	present	regular	long	present
Furcatopanorpa Ma and Hua, 2011	absent	?	absent?	regular	short	?
Megapanorpa Wang and Hua, 2018	absent	elongated	?	regular	short	?
Neopanorpa van der Weele, 1909	present	robust	absent	small	short	present?
Panorpa Linnaeus, 1758	absent	elongated	present	regular	short	present
Sinopanorpa Cai and Hua, 2008	absent	robust	absent	regular	mid-size	present
Eocene larva	absent	elongated	present	regular	short	absent

from Nepal recently (Willmann 2022). The descriptions and graphical documentation of larvae in *Furcatopanorpa* Ma and Hua, 2011 and *Megapanorpa* Wang and Hua, 2018 are rare and superficial (Ma and Hua 2011, Wang and Hua 2018). Recent researches have demonstrated significant morphological variations among larvae of various panorpids, particularly in terms of head capsules, thoracic legs, abdominal prolegs, spiracles, and chaetotaxy (Jiang *et al.* 2019). The larvae of *Neopanorpa* van der Weele, 1909 (van der Weele 1909) are the most distinct among extant Panorpidae, which are distinguished by the presence of the furrows on the head capsule, the shape of the antenna, and the absence of a tibial lobe (Chen and Hua 2011, Jiang and Hua 2015b). The two last characters of *Neopanorpa* are shared with *Sinopanorpa* Cai and Hua, 2008 (Cai *et al.* 2008). *Sinopanorpa* is another genus where the larvae considerably differ from the Eocene larva. The larvae of *Sinopanorpa* also possess slightly elongated dorsal annulated processes on A1–A7 (Chen and Hua 2011). However, these differences of characters are subtle and of rather low taxonomic importance. The length of the dorsal annulated processes is well applicable in the case of the larva of *Dicerapanorpa* Zhong and Hua, 2013 (Zhong and Hua 2013), where processes are distinctly longer than any other extant member of Panorpidae (Ma *et al.* 2014, Jiang *et al.* 2019). The extremely good preservation state of the Eocene larva, combined with advanced imaging technology, such as the synchrotron X-ray microtomography, made it possible to precisely determine the larva to the lowest taxonomic level. The fossil larva shows the highest similarity to that of *Cerapanorpa* Gao, Ma and Hua, 2016 (Gao *et al.* 2016) and *Panorpa* (Table 1). These larvae share the following characters: (i) the absence of furrows on the lateral surface of head capsule, (ii) antennae elongated and filiform, (iii) legs with tibial lobes, and (iv) forelegs regular sized. The dorsal annulated processes of the *Cerapanorpa* are slightly longer than in *Panorpa* and the fossil larva; however, the difference is not distinctive and the size of these structures may also differ in particular larval stages of the same species (Cai and Hua 2009, Jiang and Hua 2013).

Unfortunately, searching for distinctive character states of the Eocene larva has not been very effective. The main differences between the fossil larva and extant species are: (i) the absence of the seta d1 on the prothorax, (ii) flattened head capsule, and (iii) small size in relation to the stage of development. The absence of the seta d1 on the prothorax of the Eocene larva is beyond doubt, as the dorsal surface of the prothorax is well-preserved and easily observable. The distribution of these characters for the larvae of extant species is disputable, because detailed information about thorax chaetotaxy is not visible in *Cerapanorpa*, *Furcatopanorpa*, *Megapanorpa*, and *Neopanorpa* (Hua and Cai 2009, Jiang and Hua 2015a, Wang and Hua 2018, Jiang *et al.* 2019). The head capsule of the Eocene larva is slightly flatter than extant species, but this may be the result of post-mortem flattening of the capsule, which is suggested by the presence of irregular cracks on the lateral surfaces of the head and the collapsing of the left eye. The Eocene larva reached the third/four instar, which is confirmed by the shape of the dorsal annulated processes on A8–A9 (e.g. Cai and Hua 2009). However, the total length of the larva (4.9 mm) is similar to the length of the first/second instar larvae of extant species (3.2–5.5 mm) (Cai and Hua 2009, Chen and Hua; 2011, Jiang and Hua; 2013, 2015a, Ma *et al.* 2014, Liu *et al.* 2021). The relatively small size of the larva may be due to either the small size of the unknown adult form or the body of the larva shrank during desiccation inside the resin. Alive and freshly prepared larvae of extant panorpids clearly exhibit well-defined membranous surfaces on the thoracic and abdominal segments (Cai and Hua 2009, Chen and Hua 2011, Jiang and Hua 2013, Ma *et al.*; 2014, Wang and Hua 2018, Jiang *et al.* 2019, Wang and Hua 2018, Liu *et al.* 2021). The absence of visible membranous surfaces in these regions of the fossil specimen provides additional evidence of its shrinkage and desiccation. The last genus from the family Panorpidae, *Baltipanorpa*, is known exclusively from

Baltic amber and its immature stage is unknown (Krzemiński and Soszyńska-Maj 2012, Soszyńska-Maj *et al.* 2022). Since no contemporary representatives are available, assigning a larva to an imago is impossible for this fossil.

Extant Panorpidae larvae are saprophagous and edaphic. The morphological differences summarized in Table 1 are an expression of adaptation to living on the ground surface (epedaphic) or to tunnelling in the soil (euedaphic) (Jiang *et al.* 2019). Based on the environmental preferences and adaptations of the larvae of different genera, we can infer the likely habitat preferences of the Eocene larva. Many features indicate larval preference for an epedaphic microhabitat, probably excluding a lifestyle associated with burrowing in soil, such as lack of furrows on the lateral side of the head capsule, elongated antenna, the presence of the tibial lobe on the thoracic legs, regular development of the prolegs, and short dorsal annulated processes.

Synchrotron X-ray microtomography enriched the morphological information with a package of additional data, which, despite the very good degree of preservation of the specimen, were not visible during examination by classical stereoscopic microscopy. Obtained details of the mouth apparatus, head, thorax, and abdomen, as well as chaetotaxy, made it possible to accurately describe the larva and place it in the systematics of Panorpidae. Chaetotaxy provides particularly significant characters, such as length, shape, and number of setae, which are diverse among the genera of Panorpidae (Jiang and Hua 2013, Jiang *et al.* 2019). Assigning the larva to a specific genus was not possible simply because the larvae of three modern and one fossil genus are not known. It is also impossible to rule out the possibility that the described larva belongs to a genus not previously described. Therefore, the systematic position of the studied larva was limited to the family level.

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AUTHOR CONTRIBUTIONS

The authors confirm contribution to the paper as follows: study conception and design: AS, KS, WK; analysis and interpretation of results: KS, AS, TK, KK; draft manuscript preparation: KS, AS, TK; funding: ES, WK. All authors reviewed the results and approved the final version of the manuscript.

CONFLICT OF INTEREST

None declared.

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DATA AVAILABILITY

All data needed to evaluate the conclusions are present in the paper. Additional information related to this study may be requested from the authors.

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