Eco-morphological diversity of larvae of soldier flies and their closer relatives in deep time (#44242)

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Eco-morphological diversity of larvae of soldier flies and their closer relatives in deep time

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Stratiomyomorpha (Soldier flies and allies) is an ingroup of Diptera, with a fossil record stretching back to the Early Cretaceous. Stratiomyomorpha includes at least 3000 species in the modern fauna, with many species being crucial for ecosystem functions, especially as saprophages. Larvae of many stratiomyomorphans are especially important as scavengers and saproxyls in modern ecosystems. Taking into account the long history of the group, it is natural to assume that this also was the case for the stratiomyomorphan larvae in deep time. Yet, fossil larvae of the group are extremely scarce. Here we present 23 new records of fossil stratiomyomorphan larvae, belonging to 6 discrete morphotypes. Specimens are originating from Cretaceous amber from Myanmar, Eocene Baltic amber, Miocene Dominican amber, and compression fossils from the Eocene of Messel (Germany) and the Miocene of Slovenia. We discuss the implications of these new records for an understanding of the stratiomyomorphan ecomorphology in deep time as well as their palaeoecology.

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Abstract

Stratiomyomorpha (Soldier flies and allies) is an ingroup of Diptera, with a fossil record stretching back to the Early Cretaceous. Stratiomyomorpha includes at least 3000 species in the modern fauna, with many species being crucial for ecosystem functions, especially as saprophages. Larvae of many stratiomyomorphans are especially important as scavengers and saproxyls in modern ecosystems. Taking into account the long history of the group, it is natural to assume that this also was the case for the stratiomyomorphan larvae in deep time. Yet, fossil larvae of the group are extremely scarce. Here we present 23 new records of fossil stratiomyomorphan larvae, belonging to 6 discrete morphotypes. Specimens are originating from Cretaceous amber from Myanmar, Eocene Baltic amber, Miocene Dominican amber, and compression fossils from the Eocene of Messel (Germany) and the Miocene of Slovenia. We discuss the implications of these new records for an understanding of the stratiomyomorphan ecomorphology in deep time as well as their palaeoecology.

Introduction

Stratiomyomorpha is a group of flies (Diptera), which includes more than 3000 species of soldier flies and allies in the modern day fauna (Pape, Blagoderov, and Mostovski 2011). The major ingroups of Stratiomyomorpha include: 1) Stratiomyidae, the group of true soldier flies, 2) Xylomyidae, the group of wood soldier flies and 3) Pantophthalmidae, the group of giant timber flies (Marshall 2012). The group has a fossil record reaching back about 125 million years into the past to the Barremian (Lower Cretaceous) (Mostovski 1998; Whalley and Jarzembowski 1985) .

Representatives of Stratiomyomorpha are widespread in modern ecosystems and diverse in their biology (Marshall 2012). Most notably many subgroups have very different larval biologies: fully aquatic larvae are known in Stratiomyinae (ingroup of Stratiomyidae); other larvae develop inside dead wood (saproxylic life style) as in the groups Pachygastrinae (ingroup of Stratiomyidae), Xylomyidae and Pantophthalmidae); finally some larvae do not live inside wood, but are generally considered terrestrial, they are often saprophagous, feeding on decaying organic matter, as in the groups Clitellariinae, Sargiinae or Hermetiinae (all ingroups of Stratiomyidae) (Marshall 2012; Hauser, Woodley, and Fachin 2017).

Representatives of Stratiomyomorpha are playing important roles in their respective habitats: 1) the larvae often act as important saprophages, involved in the cycling of organic matter and 2) adults are important pollinators (Hauser, Woodley, and Fachin 2017).

One species of soldier flies, namely *Hermetia illucens* (Linnaeus,1758), with its fast growing scavenger-type larvae, is considered as an important source of protein for feeding cattle in industrial agriculture or for production of human food (Hauser, Woodley, and Fachin 2017). Many merolimnic species are important algal mat grazers, involved in carbon cycling (i.e. those with aquatic larvae) (Mángano, Buatois, and Claps 1996) .

Due to the direct impact on their environment it may not be surprising that ichnofossils of the larval activity, rather than body fossils, provide most of the geological record of Stratiomyidae. The Jurassic ichnogenus *Helminthopsis* was interpreted as originally caused by larvae of soldier flies of the group *Stratiomys* or at least a closely related group (Mángano, Buatois, and Claps 1996). This possibly expands the potential range of the geological records of the group from the Barremian (Cretaceous) to the mid Jurassic (Mángano, Buatois, and Claps 1996; Pickerill, Han, and Jiang 1998).

Body fossils of larval forms of Stratiomyomorpha have so far been considered to be extremely rare. So far there are only five records:

1) Whalley & Jarzembowski (1985) reported four stratiomyomorphan larvae, differentiated into two morphotypes, from the Early Cretaceous Montsech (Lerida, Spain, 125.45 to 122.46 Ma) lithographic limestones of Spain. 2) Two morphotypes of larvae from the Burmese amber, reported by Liu et al. (2020).

3) Kühbander & Schleich (1994) reported a stratiomyomorphan larva, interpreted as a larva of the group *Odontomyia,* from the Miocene Randecker Maar in Germany (17 million years old). Numerous additional specimens were recorded later from the same deposit (Rasser et al., 2013).

4) Karl & Bellstedt (1989) reported a single body fossil of a larva of the group Stratiomyidae from the Holocene of Eastern Germany.

5) Sixteen fossil larvae of Stratiomyidae from the late Eocene of the Isle of Wight are present in the collection of the Natural History Museum London (UK). They can be attributed to the aquatic Stratiomyini, and are tentatively assigned to the species *Odontomyia brodiei* (Cockerell, 1915) which is known from fossils of adults from the same deposit (Krzeminski et al. 2019).

6) Numerous Odontomyia sp. Larvae

Larval forms are crucial for the success and diversification of any group of Holometabola, with the super diverse lineages of beetles (Coleoptera), wasps (Hymeoptera), butterflies (Lepidoptera) and flies (Diptera). The severe lack of fossil larvae of Stratiomyidae is hampering progress in our understanding of the evolution of the group. Here we report new records of larvae of Stratiomyomorpha based on new fossil specimens. We furthermore discuss the ecological roles of the extinct larval forms based on morphometric comparison of modern and fossil forms.

Materials & Methods

Material

Twenty-three specimens of fossil larvae are in the focus of this study, 20 of them are preserved in amber, and three are preserved as compression fossils. The majority of the specimens in amber originated from Myanmar (“Burmese amber”), and most belong to a single morphotype (“morphotype 1”). All these specimens were purchased on ebay.com from different sellers and are now deposited at the research collection of the Palaeo-Evo-Devo Research Group, Ludwig-Maximilians-Universität, Munich, Germany (PED) (PED -0152, PED-0349, PED-0362, PED-0031, PED-0041, PED-0243, PED-0113, PED-0025).

A second morphotype (morphotype 2) also preserved in amber from Myanmar is represented by 5 larval specimens, preserved in a single piece of amber (accession number NHMLA-LACM ENT 366281). This specimen is deposited in the collection of the Los Angeles County Museum of Natural History, Los Angeles, California, USA (LACM).

Specimen PED-0462, representing the morphotype 3 was commercially acquired by YW, and originated from Dominican Republic, and now is deposited in the PED research collections.

Specimen PED-0463, representing morphotype 4 was collected by RG at the locality Činžat is situated in the Ribnica-Selnica graben, northern Slovenia, and now is deposited at the research collection of the Palaeo-Evo-Devo Research Group, Ludwig-Maximilians-Universität, Munich, Germany (PED).

Specimen PED-0464, representing morphotype 5, was obtained commercially from Mr. Jonas Damzen (http://www.amberinclusions.eu) and stems from Yantarnyj, Kaliningrad district (formerly Palmnicken, Königsberg), it is now deposited in the PED research collection.

Finally, two compression fossils originated from the Messel pit fossil site in Germany, representing morphotype 6, are deposited under coll-no. SF-MeI 4666 in the collection of the Forschungsinstitut und Naturmuseum Senckenberg (SF), Frankfurt-am-Main, Germany.

For comparative purposes, we used extant larval representatives of Stratiomyidae from the collection of the Zoological State Collection, Munich (Zoologische Staatssammlung München, ZSM), in particular larvae of: *Pachygaster atra* (Panzer, 1798), *Oxycera nigricornis* Olivier, 1811, as well as *Odontomyia* sp. The latter is deposited in the collection of the Palaeo-Evo-Devo Research Group, Ludwig-Maximilians-Universität, Munich, Germany (PED-0465). For the full list of material please see Table 1.

The morphological terminology largely follows Borkent and Sinclair (2017). Yet, to enhance the understanding for non-experts, we amended some of the special morphological terms with more general terms. As Insecta is an accepted ingroup of Crustacea s.l. “crustacean”-terms are given in brackets were necessary to provide wider frame correspondence. Data on the fossil record of Stratiomyomorpha were downloaded from the Paleobiology Database on 09 November, 2019, using the group name “Stratiomyomorpha” without any other filtering parameters.

Imaging methods: Amber specimens were imaged using a Keyence VHX-6000 Digital microscope, with ring-light type illumination and/or cross-polarised, coaxial illumination. All images were recorded as composite images. Images were assembled using stitching and panorama functions to overcome the limitation of the field of view under higher magnifications. Each image details were recorded by a stack of images of shifting focus to overcome the limitation of the depth of field (Haug, Haug, and Ehrlich 2008; Haug et al. 2011; Haug, Müller, and Sombke 2013). Fusion into sharp images and panorama stitching was performed with the built-in software as e.g. in Baranov, Schädel, and Haug (2019). We also employed the built-in HDR function of the digital microscope; therefore every single frame is a composite from several images taken under different exposure times (cf. Haug et al. 2013). Additionally, some specimens were imaged using a Keyence BZ-9000 fluorescence microscope with either 2×, 4×, 10×or 20× objectives. Observations were conducted at an emitted wavelength of 532 nm since it was the most compatible with the fluorescence capacities of the fossil specimens (Haug et al. 2011). To counteract the limitation in the depth of the focus we recorded stacks of images which then were digitally fused to single in-focus images using CombineZP (GNU). Compression fossils were photographed with a Leica MZ12.5 stereomicroscope with an attached Nikon D300 camera.

The cuticle fossil of Pachygastrinae (“morphotype 4”) was additionally imaged using a scanning electron microscopy (SEM). Scanning was performed using a Carl Zeiss Leo 1430VP scanning electron microscope in the Zoologische Staatssammlung München (Germany). Scanning was performed with beam’s electric current 80 µA; filament electric current 2500 A; and electric potential 10-20 kV. Scanning was performed in low vacuum (<2e-005 mbar).

Morphometry and outline analysis

Maximum dorsal head capsule length and width of some larvae were measured from the tip of the labrum to the outer edge of the head capsule. Actual measurements were done from the photos, using ImageJ, public domain (Schindelin et al. 2012).

As a proxy for shape diversity we conducted an analysis of the shapes, more precisely outlines of all specimens and comparative extant specimens with Fourier Elliptical Transformation using R package Momocs (Bonhomme et al. 2014), and compared morphospace occupancy.

For the outline analysis we used black and-white .jpg files, containing the outlines of all available fossil stratiomyomorphan larvae as well as all extant stratiomyomorphan larvae for which we were able to obtain a full-body image in the dorso-ventral aspect in the literature. Only specimens with a relatively straight body were included, as any specimens imaged in curled or bent position will heavily bias the morphospace. Full body images of the larvae were obtained from the following published sources (Bucánková, Kovac, and Rozkosny 2009; Bull 1976; Xerez and Garcia 2008; Dubrovsky [illegible]4; James 1965; Marques and Xerez 2009; Marshall 2012; McFadden 1967; Pujol-Luz and Xerez 1999; Pujol-Luz, Lopes, and Viana 2016; Rudolf Rozkosny 1997; R Rozkosny and Kovac 1998; James [illegible]1) , see supplementary table 1 for the full information. In total representatives of 23 extant stratiomyomorphan species were analysed (see supplementary table 1, supplementary images).

Black-and white outlines where produced using a polygonal tool and mask functionality of the FIJI (Schindelin et al. 2012) . Jpg outlines were analysed in R using the *momocs* package (Bonhomme et al. 2014), with the shapes being characterized by 19 harmonics. Source code, list of the material used for the outline production and all the underlying data are available as supplementary material (supplement 3). In order to determine the habitat affinity of the fossil larvae, we plotted them into the morphospace of the extant larvae, with demarcated saproxylic, aquatic and terrestrial habitats. Based on the position of the fossil larvae in this divided morphospace, we have attempted to assess their habitat affinity and morphology. All data analyses were conducted in R version 3.4.1 (2017-06-30) - "Single Candle" (R Core Team 2014).

Data availability

All the specimens used in the paper are deposited in public collections (see Table 1). All the outline jpg images are provided in the supplementary materials to this paper, together with the R code used to conduct the analysis.

Geological context

Ambers and Messel:

The geological context of the Burmese (Cruickshank and Ko 2003; Yu et al. 2019), Dominican (Iturralde-Vinent 2001) and Baltic (Wichard, Gröhn, and Seredszus 2009) ambers as well as Messel shale (e.g. Büchel and Schaal 2018) is explained in detail in various previous works.

Činžat:

Locality of the Činžat is much less well known to the wider audience than the three above mentioned, so we are discussing it in the further details. The studied locality Činžat is situated in the Ribnica-Selnica graben (Jelen and Rifelj 2002) filled with sediments once deposited in the Central Paratethys sea (Rögl, 1998), within the westernmost parts of the Styrian Basin, approximately 15 km west of Maribor. Here, strata of the Ivnik Beds (Mioč 1972) are exposed in a belt from Maribor, on the northern slopes of the igneous Pohorje pluton, towards the town of Radlje.

Fossil bearing micaceous laminated siltstones cover older pre-Cenozoic rocks and sequences of loosely bound conglomerates, alternating with sandstones and siltstones of the Ivnik Beds. A late Burdigalian age (Miocene) coinciding with the 'Karpatian' stage of the regional scale was identified based on a benthic foraminiferan association and nannoplankton sampling (Gašparič and Hyžný 2015).

The fossil fauna includes decapod crustaceans, bivalves, gastropods and echinoids which are randomly distributed within the siltstone layers of the Činžat section, although certain layers and variations in lithology are more likely to contain macrofaunal fossil remains. Interbedded layers of sandstones and conglomerates contain no macrofossils. The faunal association suggests low energy deep-water depositional environment with epibathyal water depth exceeding 125 m (Gašparič and Hyžný 2015).

Results

In total we can distinguish six different morphotypes among the studied fossil larvae.

Morphotype 1 (Stratiomyomorpha)

Material examined*:* **13 specimens (**PED-0025, PED-0031_1, PED-0031_2, PED-0031_3, PED-0031_4, PED-0041, PED-0113, PED-0152_1, PED-0152_2, PED-0152_3, PED-0243, PED-0349, PED-0362) in 8 amber pieces (see Table 1, Supplementary fig. 1). Most of the measurement were performed on the two best preserved specimens PED-0031_1 and PED-0041 **(Figs. 1A-C, 2 A-C, 3 A-C, Supplementary figures 1,2, 3, 4, 5, 6, 7).** *Syninclusions:* see Table 1.

Description:

Habitus. Medium sized larva with slightly dorso-ventrally flattened, spindle shaped body, fully covered with oval pallets of calcium carbonate (Figs 1 A-C, 3A).

Body length 2.3-1.1 mm (n=9). Body differentiated into presumably 20 segments, ocular segment plus 19 post-ocular segments (Figs 1 A-C, 3A). Anterior segments forming distinct head capsule.

Head capsule sclerotized anteriorly, posterior part reduced to several longitudinal structures (unpaired metacephalic rod, paired tentorial arms) retracted into the anterior trunk (prothorax and mesothorax). Dimensions of head capsule (including metacephalic rod and the tentorial arms protruding far back into the prothorax; Fig. 2A-C).

Ocular segment recognisable by its appendage derivative, clypeo-labrum complex. Clypeus (clypear sclerite) longer than wide. Labrum roughly triangular, much longer than wide, strongly sclerotized (Figs. 2A-C, 3B, C). Segment with small apparent stemmata, postero-laterally.

Post-ocular segment 1 recognisable by its pair of appendages, antenna [antennula]. Antenna prominent, robust 25 µm long (n=1) (Figs. 3B, C)

Post-ocular segment 2 (intercalary segment) without externally recognizable structures.

Post-ocular segment 3 recognisable by its pair of appendages, mandibles (Figs. 2A-C). Mandible present as a pair of vertically oriented "mouth-hooks", integrated into mandibular-maxillary complex. Mandibles square-shaped in anterior view and sturdy.

Post-ocular segment 4 recognisable by its pair of appendages, maxilla [maxillula] (Figs. 2A-C, 3B, C).

Proximal part of maxilla conjoined with mandible, fully sclerotized with strong, multi-branched setae on the dorso-distal surface, as well as laterally. Distal part, palp, conical, with two elements (palpomeres)

Post-ocular segment 5 recognizable by its appendages, forming the labium [conjoined left and right maxillae]. Labium bearing a large ventral "grinder", which is occupying almost entire ventral side of the head capsule and heavily sclerotized (Figs. 2A-C, 3B, C).

Trunk (thorax+abdomen) with 11 visible units, interpreted as 3 thorax segments, 7 abdomen segments and a trunk end (abdomen unit 8). Trunk is yellowish-brown, except for the very first unit which is light-yellow. Cuticle is covered with round deposits of calcium carbonate pallets. Trunk without any parapods, creeping welts or protuberances on the trunk units. Units of the posterior trunk do however bear complex armament on dorsal and ventral sclerites (tergites and sternites; Figs. 1A-C).

Trunk bears 2 pairs of spiracles (openings of the tracheal system). Tracheal system therefore of the amphipneustic type (Hennig, 1968, but also see discussion) (Figs 1A-C, Supplementary figures 4A-D).

Anterior trunk, thorax with three segments, pro-, meso- and methathorax.

Prothorax 450-770 µm long (n=2), without protrusions. Armament represented by the calcium carbonate pallets and large rhombic sclerite on the sternite, with two rows of small, flat spikes arranged anteriorly sternite. Distinct spiracle (anterior spiracle) on shallow depression at the postero-lateral part of the prothorax (Figs 1A-C).

Mesothorax yellowish-brown, 360-540 µm long (n=2). With two rows of triangular, flat spines on the anterior edge of sternite. Numerous pallets of calcium carbonate. Without any spiracle openings.

Metathorax yellowish-brown, 400-660 µm long (n=2), bears two rows of the triangular, flat spines on the anterior edge of sternite, as well as numerous pallets of calcium carbonate. Methathorax without spiracle openings.

Posterior trunk, abdomen with 8 distinct units. Anterior seven representing true segments.

Abdomen unit 1 rectangular in the dorso-ventral plain, 440-760 µm long (n=2). Bearing numerous calcium carbonate pallets, as well as two rows of the small triangular spikes on the anterior edge of the sternite. Posterior edge of dorsal sclerite, tergite, with a row of 12 very strong, dorso-ventrally triangular spines.

Abdomen units 2-7 rectangular (370-920 µm long). Bearing numerous calcium carbonate pallets. Posterior edge of dorsal sclerites, tergites, each with a row of very strong triangular spines, 10-12 such spines on abdomen units 2-6, 7 on abdomen unit 7.

Trunk end (abdomen unit 8, undifferentiated abdomen segments 8-11?) roughly trapezoid in the dorso-ventral view, 620-750 µm long (n=2). With three pairs of the small lateral setae, two pairs of the strong black setae on two mounds at the middle of tergite; and two pairs of strong needle-like setae on two smaller mounds at the distal edge of on dorsal tergite (syn-tergite?). Tergite also bears posterior spiracles in the transversal cleft, ventrally. Posterior spiracles sitting in the large, transversal cleft, surrounded by the elevated oval sclerotized area, of markedly darker color than the rest of the cuticle.

Morphotype 2 (Stratiomyomorpha: Stratiomyidae)

Material examined*:* LACM ENT 366281(5 specimens in a single piece). Most measurements based on a single specimen, well preserved and visible in dorsal aspect. (Figs. 4A-B, 5 A-D, Supplementary figure 8 A-B).

Syninclusions: NA

Description:

Habitus. Medium sized larva with somewhat dorso-ventrally flattened, spindle shaped body, covered with oval pallets of the calcium carbonate (Figs. 4 A-B).

Body covered by white film, precluding observation of the many fine details. Length 3.3-3.7 mm (n=3). Body differentiated into presumably 20 segments, ocular segment plus 19 post-ocular segments (Figs. 4 A-B, Supplementary figure 8 A, B). Anterior segments forming distinct head capsule.

Head capsule sclerotized anteriorly, posterior part reduced to several longitudal structures (unpaired metacephalic rod, paired tentorial arms), retracted into the prothorax. Dimensions of head capsule: 480 µm long, 340 µm wide (n=1).
Surface of head capsule covered with pallets of calcium carbonate (Figs. 5 A-D)

Ocular segment recognisable by its appendage derivative, clypeo-labrum complex. Clypeus (clypear sclerite) fused with the frontal sclerite. Labrum roughly beak-like (100 µm long, 70 µm wide), much longer than wide, strongly sclerotized (Figs. 5A-D). Segment with small apparent stemmata, anterolaterally.

Post-ocular segment 1: not externally recognisable, possible structures (antennae) not apparrent**.**

Post-ocular segment 2 (intercalary segment) without externally recognizable structures.

Post-ocular segment 3 recognisable by its pair of appendages-, mandibles (Figs. 5A-D). Mandible present as a pair of vertically oriented "mouth-hooks", integrated into mandibular-maxillary complex. Mandibles square-shaped in anterior view and sturdy.

Post-ocular segment 4 recognisable by its pair of appendages, maxilla [maxillula]. Maxilla conjoined with mandible, fully sclerotized with strong, multi-branched setae on the dorso-distal surface, as well as laterally. Distal lobe brown in colour, distal ends chisel-like. Palp not visible on any of the specimens available. (Figs. 5A-D).

Post-ocular segment 5 not recognizable, its appendages, presumably forming the labium [conjoined left and right maxillae] not visible in any of the specimen available (Figs. 5A-D).

Trunk (thorax+abdomen) with 11 visible units, interpreted as 3 thorax segments, 7 abdomen segments and a trunk end (abdomen unit 8). Trunk is yellowish-brown, except for the very first unit which is light-yellow. Cuticle is covered with round deposits of calcium carbonate pallets. Trunk dorsoventrally flattened, spindle-shaped, total length 1.9-2.7 (n=3) mm long; densely covered with calcium carbonate pallets. Trunk without any parapods, creeping welts or protuberances, yet, numerous strong setae present ventrally. Trunk bears 2 pairs of spiracles (openings of the tracheal system). Tracheal system therefore of the amphipneustic type (Hennig, 1968, but also see discussion) (Figs. 4 A-B, Supplementary figure 8 A, B).
Anterior trunk, thorax with three segments, pro-,meso- and methathorax.

Prothorax, ring-like, 240µm long, 630 µm (n=1), wide, with entral excision at place of headcapsule insertion. Small spiracles on the postero-lateral surface. Prothorax bears no protrusions. Armament represented by the calcium carbonate pallets. Anterior spiracle sits on the conical protrusion, ca. 35 µm long, spiracle itself with a single longitudinal opening (Figs. 4 A-B, Supplementary figure 8 A, B).

Mesothorax110 µm long, 780 µm wide (n=1), ring shaped, with no visible protrusion, bearing numerous oval pallets of calcium carbonate. Without spiracle openings.

Metathorax 180 µm long, 820µm wide (n=1), ring shaped, with one pair of the long, wavy setae. Without spiracle openings.

Posterior trunk, abdomen with 8 distinct units. Anterior seven representing true segments.

Abdomen units 1-7 wider than long (200-260 µm long; 900-1000 µm wide). All units bearing several wavy lateral setae; unit 7 additionally bears two lateral wavy setae. Trunk end - (abdomen unit 8, undifferentiated abdomen segments 7-11?) roughly square shaped in dorsal or ventral view, (502 µm long, 525 µm wide); with two pairs of the large, wavy setae. Pair of large spiracles (posterior spiracles) sitting on large elevated mounds posteriorly on the tergite of the unit.(Figs. 4 A-B, Supplementary figure 8 A, B).

Morphotype 3 (Stratiomyomorpha: Stratiomyidae: Pachygastrinae)

Material examined: Piece of Dominican amber with a single fossil larva from the PED collection (collection number PED-0001; Figs. 6 A-B, 7. A-D, Supplementary figures 9 A-B, 10 A-B). The larva is well preserved, anterior trunk obscured ventrally by a large air bubble. Also head capsule details inaccessible.
Description:

Habitus. Medium sized larva with dorso-ventrally flattened body, and triangular posterior end in the dorso-ventral plain). Body armoured with cuticular scales, with calcium carbonate. Total length 9.5 mm. Body differentiated into presumably 20 segments, ocular segment plus 19 post-ocular segments (Figs. 6A-B). Anterior segments forming distinct head capsule.

-Head capsule partially sclerotized, longer than wide, posterior part of the head capsule is retracted into the trunk. Dimensions of head capsule: 720 µm long, 550 µm. Surface of head capsule covered with small cuticular scales with calcium carbonate (Fig. 7A-B).

Ocular segment recognisable by its appendage derivative, clypeo-labrum complex. With 2 pairs of setae, 2 labral setae and 2 fronto-clypeal setae. Clypeus continuous with labrum, clypeus narrow, labrum expanding distally (Figs. 5A, C). Segment with pair of apparent stemmata (larval eyes).

Post-ocular segment 1 recognisable by its pair of appendages, antennae [antennula], inserting ventro-laterally at the anterior end of the head capsule (Figs. 7B). Antenna short, consists of two elements.

Post-ocular segment 2 (intercalary segment) without externally recognizable structures.

Post-ocular segment 3 recognisable by its pair of appendages, mandibles. Mandible heavily sclerotized, with basal plates. Main part of the mandible hook-shaped, continuous with appendages of the following post-ocular segment, integrated into mandibular-maxillary complex. Inner surface forms longitudinal striated "molar" area (Figs. 7A, B).

Post-ocular segment 4 recognisable by its pair of appendages, maxilla [maxillula]. Fleshy, with numerous maxillary setae, conjoined with mandible (mandibulo-maxilar complex) (Figs. 7A, B).

Post-ocular segment 5 recognizable by its appendages, forming the labium (conjoined left and right maxillae). Labium bearing 3 pairs setae (2 ventral setae and 4 ventro-lateral). Proximal part of labium forms a funnel connected to the oral cavity. Labium distally with 2 projections, probably palps. Labium highly modified, connected to the cibarial (pharyngeal) skeleton of the head capsule (Fig. 7A, B).

Trunk (thorax+abdomen) with 11 visible units, interpreted as 3 thorax segments, 7 Abdominal units and a trunk end (abdominal segment 8) (Figs. 6 A-B, 7. A-D, Supplementary figures 9 A-B, 10 A-B). Trunk spindle shaped in a dorso-vetral plain, parallel sided in the middle region, triangular at the hind-end. All bearing calcium carbonate cuticular scales, also with many long setae.

Anterior trunk, thorax with three segments, pro-, meso- and methathorax. Posterior trunk - without creeping welts or parapodia**.** Thoracic "leg" setae groups seemingly with 2 setae in each group (Figs. 6 A-B, Supplementary figures 9 A-B, 10 A-B).

Prothorax 760 µm long. Prothorax with numerous setae: 4 antero-dorsal, 6 dorsal, 2 dorsolateral, 4 lateral, 2 ventro-lateral and 6 ventral (Figs. 6 A-B, Supplementary figures 9 A-B, 10 A-B). Prothorax bears a pair of spiracles.

Mesothorax 800 µm long, with numerous setae: 6 dorsal, 2 dorsolateral, 4 lateral, 2 ventro-lateral and 6 ventral.

Metathorax 500 µm long, with numerous setae: 6 dorsal, 2 dorso-lateral, 4 lateral, 2 ventro-lateral and 6 ventral setae (Figs. 6 A-B, Supplementary figures 9 A-B, 10 A-B).

Posterior trunk (abdomen) with 8 apparent units flattened dorso-ventrally, mostly oval in the dorsal plain, with triangular posterior hind-end, (Figs. 6 A-B **Abdomen units 1-7 with numerous setae:** 6 dorsal setae, 2 dorso-lateral setae, 4 lateral setae, 2 ventro-lateral setae 4 ventral, on each segment (Figs. 6 A-B, Supplementary figures 9 A-B, 10 A-B)., Supplementary figures 9 A-B, 10 A-B). **Trunk end** (abdomen unit 8, undifferentiated abdomen segments 8-11?) triangular in general shape, dorso-ventrally, bears well visible anus on the ventro-terminal part. Probably bears a pair of small spiracles, but not apparent. Carries numerous setae: ventral setae pairs v1 through v4, two pairs of anal setae and 8 dorso-lateral setae. Terminal end elongated into the two rod-shaped protrusions, each carrying an anal setae. No cuticular "teeth" along anal opening (Fig. 6 A, B).

Morphotype 4 (Stratiomyomorpha: Stratiomyidae: Pachygastrinae)

Material examined: small slab of the Činžat shale with a cuticular fossil of a larva. Specimen split in half along the medio-lateral surface of the sternites, so that tergites of the trunk units 5-8 of the posterior trunk are folded upon the tergites 1--4. Coloration of specimen very well preserved (Figs. 8 A-B, 9 A-D, Supplementary figure 12 A-B, 13 A-D).

Description:

Habitus. Medium sized larva with dorso-ventraly flattened body, and rounded posterior end. Body armoured with calcium carbonate cuticular scales. Total length 6.4 mm. Body differentiated into presumably 20 segments, ocular segment plus 19 post-ocular segments (Figs. **8 A-B**). Anterior segments forming distinct head capsule.

Anterior body visible in ventral prospective only, of the posterior trunk also the dorsal region can be seen. Sclerites ventrally on anterior trunk, as well as dorsally on posterior trunk bearing distinctly "leopard" pattern of coloration, consisting from the dark-grey and brownish - yellow spots of the irregular shape (**8 A-B, Supplementary figure 12 A-B**).

Head capsule sclerotized, much longer than wide, posterior part of the head capsule is retracted into the trunk. Dimensions of head capsule: 1000 µm long, 250 µm. Figs. 9A-D, **Supplementary figure 13 B**).

Ocular segment Ocular segment recognisable by its appendage derivative, clypeo-labrum complex. Clypeus continuous with labrum, clypeus narrow, blade-shaped (Figs. xxx).

With pair of apparent hemispherical stemmata (larval eyes), at about midlength of the headcapsule, dorso-laterally. Segment surface bears multiple small setae.

Post-ocular segment 1 recognisable by its pair of appendages, antennae [antennula]. Antenna inserted dorso-laterally at the distal end of the head capsule (Figs. 9A-D, **Supplementary figure 13 B**). Antenna short, with two elements.

Post-ocular segment 2 (intercalary segment) without externally recognizable structures.

Post-ocular segment 3 recognisable by its pair of appendages, mandibles, fused in the mandibulo-maxillary complex. Mandible heavily sclerotized, with basal plates, the main part of the mandible hook-shaped, continuous with appendages of the following post-ocular segment. Inner surface forms longitudinal striated "molar" area (Figs. 9A-D, **Supplementary figure 13 B**).

Post-ocular segment 4 recognisable by its pair appendage, maxilla [maxillula]. Maxilla fleshy, with numerous maxillary setae, connected to mandible (mandibulo-maxillary complex) (Fig. 5A).

Post-ocular segment 5 recognizable by its pair of appendages, forming the labium [conjoined left and right maxillae]. Labium bears 3 pairs setae (2 ventral setae and 4 ventro-lateral), on the ventral and lateral surface respectively. Proximal part of labium forms a three-pronged structure, adjacent to the oral cavity(Figs. 9A-D, **Supplementary figure 13 B**).

Trunk (thorax+abdomen) with 11 visible units, interpreted as 3 thorax segments, 7 abdomen segments and a trunk end (abdomen unit 8). Trunk bears 2 pairs of spiracles (openings of the tracheal system). Tracheal system therefore of the amphipneustic type (Hennig, 1968, but also see discussion) (**8 A-B, Supplementary figure 12 A-B** , 13 A, B).

Anterior trunk, thorax consists of three segments, pro-,meso- and metathorax. Tergites and sternites sclerotized, bearing calcium carbonate pallets

Prothorax 300 µm long. Bears a pair of large spiracles (100 µm in diameter at the oppening) . Prothorax with several small setae on the dorsal surface(**8 A-B, Supplementary figure 12 A-B** , 13 A, B)).

Mesothorax 300 µm long, ring-shaped, bearing no spiracles, with the lighter area in the center of the sternite (probably due to the sediment filling the depressions of the fossil) No spiracles present, unit bare.

Metathorax 250 µm,
ring-shaped, with the lighter area in the centercentre of the sternite (probably due to the sediment filling the depressions of the fossil).

Posterior trunk, abdomen with 8 distinct units. Anterior seven representing true segments. Posterior trunk mostly oval with rounded posterior hind-end **(8 A-B, Supplementary figure 12 A-B** , 13 A, B).

Abdomen units 1-7 (320-610 µm long). Cuticle is split along the lateral side, medio-laterally; therefore units 5-7 (and trunk end) folded over the ventral parts of the units 1-4. This damage reveals the inner dorsal surface of abdomen units 5-7 (and trunk end) for the direct observation.

Trunk end (abdomen unit 8, undifferentiated abdomen segments 8-11?) semicircular in general shape, when viewed in dorso-ventral plain, bears anus on the ventro-terminal part. Probably bears a pair of small spiracles, but not apparent. No cuticular “teeth” along the anal opening (Figs. 8A-B, Supplementary figure 13C).

Morphotype 5 (Stratiomyomorpha: Stratiomyidae: Stratiomyinae)

Material examined: a single fossil larva in a piece of Baltic amber from the PED collection (collection number PED- 7568-100). The larva is poorly preserved, covered with air bubbles and cracks in amber; only rear end of the trunk visible well enough to provide any distinguishable features (Figs. 10 A - B). Piece of amber contains several syninclusions: non-biting midge male (Diptera, Chironomidae); window-gnat (Diptera, Anisopodidae), two dark-winged fungus gnats (Diptera, Sciaridae), large spider (Araneae).

Description:
Habitus. Medium sized larva with spindle shaped body, when viewed in the dorso-ventral plain, rear end of trunk with prominent coronet of large setae. Body mostly obscured by cracks and bubbles in the amber; only rear end clearly visible. Total length 4.3 mm. Body differentiated into presumably 20 segments, ocular segment plus 19 post-ocular segments (Figs. 10 A - B). Body armoured with a calcium carbonate cuticular scales.

Trunk (thorax+abdomen) spindle shaped, parallel sided, rounded at the hind-end, when viewed dorsoventrally. Anterior part of the trunk entirely obscured by cracks. Subdivison units are unclear. Posterior trunk is densely covered by strong setae. Trunk end (undifferentiated abdominal segments 8-11?) rounded in general shape, carries strong coronet formed by 19 unbranched setae (Figs. 10 A - B). Additionally - bears a pair of large spiracles, surrounded by the setose coronet and upper and lower sclerotized "lips". Respiratory system probably metapneustic (we cannot be sure as we cannot see prothorax and head in details), with spiracles sitting at the distal part of the last trunk segment (Fig. 10 B).

Morphotype 6 (Stratiomyomorpha: Stratiomyidae)

Material examined: two fossil larvae on one slab from the Grube Messel, stored in the SF collection (collection number SF-MeI 4666; Figs. 11 A-C). The fossils originate from the Messel Formation, they were collected in the year 1994 in grid square E8, 0.9m to 1.1m below local stratigraphic marker horizon alpha. The larvae are poorly preserved, only traces of the head capsules and the rest of the bodies can be seen; no traces of any setae are preserved; nevertheless both specimens show a well preserved coloration pattern of the tergites.

Description:

Habitus. Medium sized larva with spindle shaped body. Accessible only in dorsal aspect.

Body length 3.0 to 3.5 mm (n=2). Body differentiated into presumably 20 segments, ocular segment plus 19 post-ocular segments (Figs. 11A-C). Anterior segments forming distinct head capsule.

Head capsule partially sclerotized, longer than wide, posterior part of the head capsule retracted into the anterior trunk (prothorax). Head capsule visible only in vague outlines, with several longitudinal structures (unpaired metacephalic rod, paired tentorial arms), these are heavily sclerotized. Posterior part of the head capsule more heavily sclerotized (Figs. 11A-C). Width of head capsule ca. 270µm. Other units of the body difficult to measure due to the poorly visible borders between the segments.

Anterior segments not well apparent, without prominent structures.

Post-ocular segment 5 recognisable by an internally located pharyngeal grinding mill (visible in both fossil specimens; Fig.11C).

Trunk (thorax+abdomen) spindle shaped, parallel sided, rounded at the hind-end. Made up of three thorax segments and 7 abdomen segments plus trunk end. Units of the trunk are much wider than long. No setae preserved. No traces of the spiracles or the distal setae coronet present (Fig. 11C). **Anterior trunk, thorax, consisting of** pro,-meso and methathorax.

Prothorax with general outlines visible; heavily sclerotized posterior part of the head capsule can be seen retracted into prothorax (Fig. 11A-C).

Mesothorax bears two distinct pigment dots at the hind edge (Fig. 11A, C).

Metathorax bearing no spiracles (Fig. 11A-C).

Posterior trunk (abdomen) with 8 units (Fig. 11A-C). Trunk bears no apparent parapodia or creeping welts.

Abdominal units 1-6 bearing distinct lines of pigmentation, 2 medially on all tergites, and 2 laterally on most tergites (Fig. 11A-C).

Abdominal unit 7 only general outlines can be seen (Fig. 11A-C).

Trunk end (Abdominal unit 8) only general outline can be seen; square in general shape, with rounded posterior edge, when viewed in the dorso-ventral view (Fig. 11A-B). No spiracles or anus can be discerned.

Discussion

Systematic interpretation

All specimens can easily be identified as larval forms of Diptera. This interpretation can be based on the general body shape of the specimen, the absence of ambulatory legs on the thorax, as well as the spiracle arrangement. The six morphotypes differ in the following character combinations, and their systematic relationships are discussed.

Morphotype 1: This morphotype is interpreted to be a representative of the group soldier and timber flies (Stratiomyomorpha) based on the following combination of characters (see Figs. 1A-C, 2 A-C, 3 A-C, Supplementary figures 1,2, 3, 4, 5, 6, 7): larva elongated and flattened, comprising head, thorax and 8 abdominal units; body covered with calcium carbonate scales (synapomorphy of the Stratiomyidae+Xylomyidae) (**Figs. 1A-C, 2 A-C, 3 A-C, Supplementary figures 1,2, 3, 4, 5, 6, 7).** The thorax of these specimens is covered with shagreen-type nodules, rather than by hardened sclerites as in Xylomyidae (Fig. 1B). Despite the overall similarity with larvae of Stratiomyidae, the fossil larvae of this morphotype 1 exhibit a number of traits unknown among modern forms of Soldiers flies (or Stratiomyomorpha in general): 1) The head capsule of the fossil larvae is extremely elongated with tentorial arms and metacephalic rod reaching back up to the posterior edge of the prothorax (Fig. 2B). 2) The larvae possess long, triangular spines on the tergites of the trunk, as well as smaller rounded spines on the sternites of the trunk. This condition is unique among known larvae of Stratiomyomorpha, and probably represents an autapomorphy of the morphotype (Fig. 1C).

This new morphotype clearly differs from two other types of Stratiomyomorphan larvae recently reported from the Burmese amber (Liu et al. 2019), by the presence of the extremely long and strong spines on the trunk in the new form, as well as by the absence of the long setae on the posterior trunk (abdomen) and Anterior trunk (thorax (Figs. 1A-C).

While the combination the features is unknown from the any other representatives of the stratiomyomorphans, extremely elongated head capsules and large tergal spines are known in larvae of Asiloidea, especially in Mydidae and Bombyliidae flies (Marshall, 2012). This makes the interpretation of these larvae partly challenging, due to the “chimaera-like” combination of the traits, as a probable result of the “push of the past” effect (Baranov, Schädel, and Haug 2019; Haug and Haug 2019). This effect seems quite common among fossil representatives of Holometabola, representing phenomena occurring when initial diversification events in extant hyperdiverse groups (Budd and Mann 2018).In total we have found 13 larvae of this type, with seven of them being preserved in just two amber pieces (four in PED-0031 and three in PED-0152). Almost all larvae (except PED-0031_1 and PED-0031_2) show signs of severe, most probably pre-mortem damage, such as squashing, full-body piercing, and splitting the body medially (along the pleural region). In some cases we even see complete mutilation with entire parts of the body (i.e. thorax) being absent from some specimens. The high abundance of this morphotype, as well as their high incidence of damage indicates that these larvae were both frequent, and probably a preferred prey to the other inhabitants of the amber forest in Myanmar. We discuss further aspects of the ecology further below.

Morphotype 2: This morphotype is similar to morphotype 1 including general features and also shagreen type nodules, indicating an ingroup position within Stratiomyidae (Figs. 4A-B, 5 A-D, Supplementary figure 8 A-B). A further interpretation within Stratiomyidae is more challenging, due to the relatively poor preservation. Yet, the absence of a coronet of so-called 'hydrofuge' setae on the terminal end and a relatively short body both point towards a terrestrial mode of life (McDFadden, 1967). Also this new morphotype clearly differs from two other types of Stratiomyomorphans larvae recently described from amber from Myanmar (Liu, Hakim, and Huang 2020), by the much longer headcapsule (in relation to the body), absence of the any spines on the tergae, as well as by the absence of the long setae on the posterior trunk and thorax.

Morphotype 3: This morphotype is similar to morphotype 1 including general features and also shagreen type nodules, indicating an ingroup position within Stratiomyidae. Within Stratiomyidae, the specimen is interpreted as an ingroup of Pachygastrinae based on the following combination of characters: coronet of so-called 'hydrofuge' setae not present on the segmenttrunk end; larva uniformly coloured; thoracic and abdominal tergites with transversal rows of 6 setae each; labium not sclerotized and weakly developed; dorsal part of the mandibular-maxillary complex sclerotized; small larva<10 mm. Within Pachygastrinae the specimen appears most similar to larvae of the group *Gowdeyana* Curran, 1928 in lacking cuticular “teeth” along the anal opening; thoracic “leg group” setae paired; all setae in the dorsal transversal row are subequal (Figs. 6 A, B).

In general, the larva is relatively unusuall for Pachygastrinae, as it is larger than most last-stage Pachygastrinae larvae (9.5 mm vs 3-8 mm) and has a peculiar trunk end, elongated, ending with two large spines around the anus. It is possible that this larva belongs to an extinct lineage of Pachygastrinae, and large spines on the trunk and trunk end could represent an autapomorphy of this lineage. Yet, one should bear in mind that larvae for less than 10% of extant species Pachygastrinae are known (Bucánková, Kovac, and Rozkosny 2009). Hence the possibility remains that the larva may also belong to an extant ingroup of which the larvae are not yet known.

Currently there are two species of Pachygastrinae known as adults from Dominican and Mexican amber: *Pachygaster hymenaea* Grund & Hauser, 2005 (**Suplementary figures 11 A, B**) and *P. antiqua* James, 1971. The new fossil larva does not fit into the group *Pachygaster,* as in contrast to larvae of *Pachygaster*, the new larva does not have three setae in the ""thoracic leg group" of setae. Also the new larva is notably larger than any known larva of *Pachygaster* (Grund and Hauser 2005).

Morphotype 4: This morphotype is similar to morphotype 1 including general features and also shagreen type nodules, indicating an ingroup position within Stratiomyidae. Within Stratiomyidae, the specimen is interpreted as an ingroup of Pachygastrinae based on numerous characters: coronet of so-called 'hydrofuge' setae not present on the segmenttrunk end; larva uniformly coloured; thoracic and abdominal tergites with transversal rows of 6 setae each; labium not sclerotized and weakly developed; dorsal part of the mandibular-maxillary complex sclerotized; small larva<10 mm. Further identification is impossible, due to the insufficient preservation of the thoracic "leg group" setae.

Taking into account the exceptional preservation of this cuticle fossil, it is important to remember the possibility of contamination of the geological record by holometabolan larvae, in particular flies (Rasnitsyn 2008).. Fly larvae are known to crawl into the narrow fissures within shales and other types of rocks, effectively creating a hard to spot contamination in the fossil record. The specimen in question has its cuticle interlaced with numerous grains of the sedimentary matrix. In this aspect it is similar to the contamination of late Cretaceous sandstone by an extant fly *Protophormia terranovae,* as reported by Rasnitsyn (2008: p. 249, figs. 96-97). Yet, the fossil in general seems not to be entirely dissimilar from other euarthropodan fossils known from the same formation, in terms of its preservation (Gašparič and Hyžný 2015). Additionally, the specimen was collected form fresh split rock sample and imprint was observed on negative (unfortunately not collected). Still, we cannot entirely rule out that this particular larva is an extant contamination of the shales.
(Gašparič and Hyžný 2015).

Morphotype 5: This morphotype is similar to the other morphotypes including general features and also shagreen type nodules, indicating an ingroup position within Stratiomyidae. Within Stratiomyidae, the specimen is interpreted as an ingroup of Stratiomyinae or Nemotelinae, based on the presence of a coronet of hydrofuge setae. The apical position of this coronet on the trunk end is not compatible with an interpretation as an ingroup of Nemotelinae (Hauser, Woodley, and Fachin 2017).

Not much more information could be gained from the larva, except that the hydrofuge setae coronet indicates an aquatic, rather than a terrestrial habitat of the animal (see discussion). It is impossible to associate this larvae with either of the two known species of Stratiomyidae known as adults from Baltic amber, *Hermetiella bifurcate* Meunier, 1908 and *Cacosis sexannulata* Meunier, 1910, as the larva is too poorly preserved.

Morphotype 6: This morphotype is too poorly preserved for any detailed systematic interpretation. We have tentatively placed it in Stratiomyidae based on the general body shape, apparent remains of the retracted head capsule and leopardine colour pattern. Unfortunately all these characters do not allow an unambiguous placement. Not much more information could be gained from the larvae, since the poorly preserved body falls into the "unknown" habitat category at the morphospace (Fig. 12).

The fossil record of Stratiomyidae

Given the important role of larval dipterans, their numerous ecosystem functions and their often very specific association with certain microhabitats (McFadden 1967), their fossil records can provide a wealth of palaeo-ecological information. The larvae of Stratiomyidae reported here expand the record of this group to still less than 20 occurrences. Hence these new larval stratiomyomorphan specimens widen our understanding of the respective palaeo-ecosystems from which they originated. Even on "the adult side" representatives of Stratiomyidae are pretty rare in the fossil record, with only 73 occurrences (specimens) have been ever recorded (according to PBDB, for the search parameters see Methods). All known larval records are listed in table 1, together with the material used in this paper. Burmese amber seems to be particularly rich in stratiomyomorphan larvae, as the number of morphotypes known from this deposit now has reached four. Liu et al. (Liu, Hakim, and Huang 2020) have described two morphotypes of stratiomyomorphan larvae from amber from Myanmar. Both of these morphotypes are characterized by features intermediate between the two stratiomyomorphan ingroups-Stratiomyidae and Xylomyidae (Liu, Hakim, and Huang 2020). Such intermediate characteristics are also apparent in the one of the new morphotype.s It is easiest interpreted as a result of the "Push of the past" phenomenon (Budd and Mann 2018). In contrast the new morphotype 2 has a much less conspicuous morphology, and seemingly belongs to the Stratiomyidae s.str. as characterised by (Hauser, Woodley, and Fachin (2017).

The record of Pachygastrinae from Dominican amber, new morphotype 3, is only the third record of the group Stratiomyomorpha from this otherwise very productive, deposit (Grund and Hauser 2005). Only a single specimen of the species *Pachygaster hymenea* Grund et Hauser, 2005 and a single specimen of *Nothomyia* sp. (Poinar and Poinar 1999) has so far been reported from Dominican amber. This could indicate that representatives of Stratiomyidae were either very rare in the Miocene of Hispaniola, or alternatively their autecology was prohibiting them from being preserved in amber (Solórzano Kraemer et al. 2018).

The modern fauna of the isle of Hispaniola includes 13 species of Stratiomyidae (Perez-Gelabert 2008). This relationship of fossil specimens to extant species is quite different from the situation with chironomid Diptera. For Chironomidae (non-biting midges), there are more fossil species known from Dominican amber, than there are extant species on the entire island of Hispaniola (Grund 2006).

The situation with Chironomidae in Dominican amber, can be explained by the fact that more attention was given to fossils of Chironomidae of Hispaniola, than to the extant ones. The same explanation cannot be applied to the discrepancy in species richness of fossil and extant species of Stratiomyidae. Soldier flies are mid-size or even large flies, hence they have a much lower chance of being overlooked in the amber records than Chironomidae. Modern representatives of Stratiomyidae in the Neotropics and other tropical regions are associated with open areas in the forests or forest canopy (Woodley 2009; Hauser, Woodley, and Fachin 2017). We can therefore hypothesize that Dominican amber was capturing primarily animals associated with tree trunks, rather than canopy fauna or fauna of the open meadows within the forest. A similar capture pattern was shown for the Madagascar copal (Solórzano Kraemer et al. 2018).insects

Modern larvae of Pachygastrinae are commonly living under the wet bark on the forest floor (Hauser, Woodley, and Fachin 2017). It is therefore quite possible for one of such larvae to be preserved in amber, by resin dripping on the forest floor next to a tree trunk. Similar scenarios were hypothesized by Solorzano Kraemer et al. (Solórzano Kraemer et al. 2018).

The cuticle fossil from the Činžat also is a representative of Pachygastrinae. Taking into account that Činžat represents a deep -water, low energy sedimentary environment (Gašparič and Hyžný 2015), it is difficult to explain how a larva of Pachygastrinae ended up there, as they are mostly saproxylic, hence associated with wood. One possible explanation could be that the specimen drowned with driftwood after a storm event.

The other three new fossil larval morphotypes are likely representatives of the group Stratiomyinae. Modern larvae of this group are fully aquatic, breathing with the terminal coronet of setae. These structures are also apparent, or indicated in the fossils indicating an aquatic lifestyle of these fossil larvae.

The larva preserved in Baltic amber is poorly preserved, and only identifiable as a larva of a soldier fly by the presence of the coronet setae on the rear end. Overall the fossil resembles extant aquatic benthic predatory larvae of the group *Odontomyia* (Supplementary figure 14 A, B). There is quite a number of larval forms of Insecta that have an aquatic lifestyle and have been recorded from Baltic amber. This includes immatures of Odonata (damselflies), Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) (Wichard, Gröhn, and Seredszus 2009). (Martínez-Delclòs, Briggs, and Peñalver (2004) suggested that aquatic individuals of Insecta can well be entrapped by still sticky resin pouring into water. This was probably the case for the larva from Baltic amber. This further supports that at least part of the Baltic amber deposits was formed directly next to water, probably in a swampy environment (Wichard, Gröhn, and Seredszus 2009).

The possible record of larvae of Stratiomyinae from the Eocene of the former Maar Lake Messel might represents a rare find of aquatic insect larvae from this deposit. Unfortunately larvae are too poorly preserved for the detailed interpretation. Aquatic insects are generally rare in the oilshale of Messel and also in other maar lake deposits , because the fossil-bearing sediments (the so-called oilshales) formed only in the deeper parts of the former maar lake, not in its shore-region (Wedmann 2018). Aquatic insects like stratiomyid larvae were living in the shallow water in the shore region, and they could be only preserved as fossils when they drifted into the deeper, anoxic parts of the meromictic lake where later the oilshale was formed.

Eco-morphological consideration

The ecomorphotype, or a shape of an organism adapted to a certain ecological condition, is used here as a proxy for the diversity of forms within a group of organisms. Outlines of the entire body, or parts of it have been shown as superior proxies for the shape of an organism in many cases, when landmarks are hard to define, or when such landmarks do not reflect the shape of the organisms well enough (Tatsuta et al. 2018). One of the most often used methods for the outline capture in geometric morphometry is a Fourier Elliptical Transformation (Tatsuta, Takahashi, and Sakamaki 2018; Polášek et al. 2018). This method allows to access the diversity of ecomorphotypes of a group of organisms via examining body outlines. Here we used all available fossil and extant stratiomymorphan larvae to trace the changes in the larval morphospace occupancy of the group and, consequentially indirectly ecological diversity throughout its history.

Ecomorphology of extant stratiomyomorphan larvae

New material examined in this study has shed a light on the far greater larval diversity of the group Stratiomyomorpha in deep time than was expected from the previously known geological records. We analyse the diversity of the ecological morphotypes of stratiomyomorphan larvae through time comparing it to modern ecomorphotype diversity. Here we use ecomorphotypes as a stable shape of an organism that evolved in response to certain ecological conditions (Rotheray 2019).

Stratiomyomorphan larvae occupy three main types of habitats: 1) aquatic, 2) terrestrial, mostly upper soil, leaf litter, and lower vegetation, and 3) living or decaying or living wood, hence a saproxylic life style. Many of the extant Stratiomyomorpha, in particular larvae of Pachygastrinae, are saproxylic and live under the bark of the dead wood (McFadden 1967; Marshall 2012). Larvae of Xylomyidae and Panthopthamlmidae are also saproxylic (Marshall 2012). Many other larvae of Stratiomyidae, besides those of Pachygastrinae, are occupying aquatic habitats. These larvae are leading a predatory lifestyle (notably Stratiomyinae; Rozkosny, 1997). In the fossil record, we have some indisputably aquatic larvae, i.e. larvae *Odontomyia* sp. from Randecker Maar (Kühbander and Schleich, 1994) or larvae of *Stratiomyia* from the Holocene of Germany (Karl and Bellstedt 1989).The original habitats of other fossil larvae is less clear (Whalley and Jarzembowski 1985; Liu, Hakim, and Huang 2020).

We have attempted to compare ecomorphotypes of the extant aquatic benthic predators, terrestrial and saproxylic stratiomyomorphan larvae with the morphotypes of the fossil larvae. In doing so we hoped to elucidate the changes in the stratiomyomorphan larval morphospace through deep time, as a response to the changing environmental conditions.

Our analysis has shown that stratiomyomorphan larvae are showing essentially four main morphotypes: 1) elongated aquatic benthic predatory larvae, roughly circular to oval in the cross-section, as larvae of *Stratiomyia*, *Oxycera*, *Odontomyi*a, 2) terrestrial larvae with spindle-shaped, massive bodies, flattened dorsoventrally, as larvae of *Hermetia*, *Inops,* and two types of saproxylic larvae, 3) spindle-shaped and dorsoventrally flattened as larvae of most Pachygastrinae, Xylomyidae 4) massive, cylindrical larvae with heavily sclerotized head and trunk end (Figs. 12A, B).

Ecomorphology of fossil stratiomyomorphan larvae. The fossil larvae are widely distributed in the stratiomyomorphan morphospace (Figs. 12A, B). Most Cenozoic larvae (from Slovenian shales, Messel and Baltic amber) fall within the area occupied by modern forms. Also some of the Cretaceous forms fall within the area occupied by modern forms, namely morphotype 2 preserved amber from Myanmar, as well as the two larval types reported by Liu et al. (2020) from the same locality.

In contrast to these, the new morphotype 1, also preserved in amber from Myanmar plots outside of the area of the morphospace occupied by extant forms (Fig. 12) . This comes not as a large surprise, taking into account that stratiomyomorphan new morphotype 1 is a rather unique type of dipteran larva, with a character combination unknown among the extant or other fossil larvae of Stratiomyomorpha (xxx). In particular morphotypes 2, 4 as well as the larvae from Liu et al. (2020) firmly fell into the part of the morphospace, occupied by the saproxylic ecomorphotypes (Figs. 12A). The larva from Baltic amber has predictably fallen into the “aquatic benthic predators” part of the morphospace (Fig 12A).

As morphotype 1 falls outside the area occupied by extant forms it is very difficult to speculate about the original habitat of these larvae (Fig. 12A, B). The extremely high abundance of morphotype 1 larvae (at least for dipteran larvae in an amber deposit) can be explained by a possible close association with tree trunks, possibly living under the bark of the trees, as seen in many extant larvae of Stratiomyidae (McFadden, 1967, Marshall, 2012). Additionally, morphotype 1 larvae possess large structures resembling grinders on the mandible-maxillary complex, similar to saproxylic larvae of Xylomyidae, some Pachygastrinae and Pantopthalmidae (Marshall 2012), Figs. 2A-C, 3 A-C), which points on the trophic association between the larvae and dead wood (McFadden 1967).

In general, it is notable that the area occupied by terrestrial larva in the ecomorphospace is deeply nested within the area occupied by saproxylic larvae. The area occupied by aquatic benthic predators larvae is pretty clearly separated from the area of terrestrial, and hence also saproxylic larvae. While we currently lack a stable phylogenetic framework for reconstructing the character evolution of larvae within Stratiomyomorpha it seem reasonable to suggest that the transition between the two main larval habitats, aquatic benthic predators and saproxylic, occurred via waterlogged wood. While no larvae of Stratiomyidae or Stratiomyomorpha are known from waterlogged wood (Marshall 2012; Ulyshen 2018), many other dipterans are (Marshall 2012; Krivosheina 2012). The transition of stratiomyomorphans through the waterlogged wood might happen via the intermittent ecomorphotype, occupying habitats similar to forms of Axymyidae or Chironomidae from groups like *Brillia, Xylotypus* or *Orthocladius (Symposiocladius) lignicola* (Cranston and Oliver 1988).

Our analysis has shown that the morphospace of stratiomyomorphan larvae has not become significantly smaller over time. Only a small part of the occupied area of the morphospace was lost, when we are concerned with general body shape (the area part related to the new morphotype 1). A possible reason for the apparently stable morphology and, linked to that, ecology of the larvae most likely lies in the habitat. The association with dead wood and aquatic habitats, probably made them more resilient to ecosystem changes during the Cretaceous Angiosperm revolution, than other groups of Diptera (Peñalver et al. 2015).

Conclusions

The fossil record of dipteran larvae and pupae is generally skewed towards abundant forms from low-energy sedimentary basins, such as lake environments (Rasnitsyn and Quicke 2002). Therefore, groups with primarily aquatic immatures and high abundance, such as Chaoboridae and Chironomidae are over-represented in the fossil record (Rasnitsyn and Quicke 2002; Zherikhin, Ponomarenko, and Rasnitsyn 2008). Aquatic larvae of other dipteran ingroups, while rare have occasionally provided unprecedented insights into the evolution and palaeocology of the group (Whalley and Jarzembowski 1985; Chen et al. 2014).

Terrestrial larvae of Diptera have been until recently considered extremely rare (Grimaldi and Engel 2005). Recent works, however, have shown that certain groups of terrestrial dipteran larvae can be quite abundant, at least in amber (Baranov, Schädel, and Haug 2019). Therefore it is not entirely surprising to find new immature representatives of Stratiomyidae in Cretaceous, Neogenic and Palaeogenic ambers and also in deposits with compression fossil. Further in-depth studies of amber and compression fossils collections will certainly lead to more new discoveries pertaining larval biology of Stratiomyomorpha and other groups of Diptera.

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List of figures

Figure 1. Larva of morphotype 1, specimen PED-0031-2. A) Ventro-lateral view; B) Ventro-lateral view, marked; C) Dorso-lateral view. Abbreviations: *hc*-head capsule, *as*-anterior spiracle; *pt*-prothorax, *mt*-metathorax, *a2-a6* - posterior trunk units 2-6, *te*- trunk's end, *ps* - posterior spiracle.

Figure 2. Larva head of morphotype 1, specimen PED-0031-2. A) Lateral view; B) Lateral view, marked; C) Lateral view, linedrawing. Abbreviations: *hs*-head soft tissues, *hc* - head capsule, *lb*-labrum; *la* - labium; *mk* - mandibular-maxillar complex, *ta* - tentorial arm, *mr* -metacephalic rode, *ct* - cut off through the part of the head capsule, *pt*-prothorax, *cc* - calcium carbonate pallet.

Figure 3. Larval head of morphotype 1, specimen PED-0152-2. A) Dorsal view, habitus; B) Ventral view, head; C) Ventral view, head - marked. Abbreviations: *mp* - maxillary palp, *bm* - base of mandibular-maxillar complex ("grinder"), *lb* - labrum.

Figure 4. Larva of morphotype 2, specimen LACM ENT 366281-1. A) Dorsal view, habitus; B) Dorsal view, habitus - marked Ventral view, head; C) Ventral view, head - marked. Abbreviations: *hc*-head capsule, *as*-anterior spiracle; *ms*- mesothorax, *mt*-metathorax, *a1-a7* - posterior trunk units 1-7, *te*- trunk's end, *ps* - posterior spiracle.

Figure 5. Larval head of morphotype 2, specimens LACM ENT 366281-1(A-B) and LACM ENT 366281-2 (C-D). A) Dorsal view, head, LACM ENT 366281-1; B) Dorsal view, head - marked, LACM ENT 366281-1; C) Lateral view, head, LACM ENT 366281-2; D) Lateral view, head -marked, LACM ENT 366281-2. Abbreviations: Abbreviations: *hs*-head soft tissues, *hc* - head capsule, *lb*-labrum; *mk* - mandibular-maxillar complex, *ey* - eyes; *as* - anterior spiracle.

Figure 6. Morphotype 3, habitus, ventral, larva PED-0462. A) Habitus, ventral view; B Habitus, ventral view, markes. Abbreviations: hc- head capsule, pt - prothorax, ms - mesothorax, mt - metathorax, a1-a7 - abdominal units 1-7, *te*- trunk's end.

Figure 7. A-B) Fossil Pachygastrinae, larva of morphotype 3, PED-0462 and C-D), extant *Pachygaster atra, head.* A & B) Ventral view of the headcapsule unmarked and marked; C & D) *Pachygaster atra*, Ventral view of the headcapsule unmarked and marked; Abbreviations: *an*-antenne; *as*- anal setae; *pt* -prothorax, *ey*-eyes; *lb*-labrum; *mp*-maxilar palp; *mb*- base of mandibular-maxillar complex ("grinder"); *v1*-3 - ventral setae 1-3, *la*- labium.

Figure 8. Pachygastrinae, larva, morphotype 4, PED-0463. A) habitus, ventral view; B) habitus, ventral view, marked. Abbreviations: *hc*-head capsule; *ey* - eyes; *as*-anterior spiracle; *pt* - prothorax, *ms*- mesothorax, *mt*-metathorax, *a1-a7* - posterior trunk units 1-7, *te*- trunk's end, *ps* - posterior spiracle, *fc* - folded cuticle.

Figure 9. Pachygastrinae, larva, morphotype 4, PED-0463, head ventrally. A) head and prothorax, ventral view; B) head and prothorax, ventral view, marked; C) head, ventral view; D) head, ventral view, marked. Abbreviations: *an* - antenna, *hc* - head capsule, *ey* - eyes; *as* - anterior spiracle, *lb*-labrum; *la* - labium; *mk* - mandibular-maxillar complex.

Figure 10. Fossil Stratiomyinae larva, morphotype 5 (PED-0464). A) Habitus; B) Close-up photo of coronet of the hydrofuge setae.

Figure 11. Fossil stratiomyinae larvae, morphotype 6 (SF-MeI 4666). A) compression fossil, habitus; B) compression fossil, marked; C) Messel shale with Stratiomyiinae larvae, SF-MeI 4666, overview. Abbreviations: *hc* -head capsule; *phc* - pharyngeal grinding mill; *pt*-prothorax; mt -methathorax; *a1-a6*-abdominal units; *te*- trunk's end.

Figure 12. Ecomorphospace occupied by the extant and fossil Stratiomyomorpha larvae. Both plots presenting the same morphospace, split by the different grouping variables. Total captured variation = 75%; 62.1% at PC1 and 12.9 % at PC2. A) Morphospace split by the larval habitat: violet - saproxylic, blue-terrestrial, green -"unknown" (fossils), red- aquatic; B) Morphospace split by the geological age/deposit of the larvae: blue - extant, red- burmese amber, the rest of the deposits are represented by the single labelled dots.

SUPPLEMENTARY FIGURES

Supplementary figure S1. All reprensetatives of the morphotype 1 Stratiomyomorpha, covered in this artivle. All specimens are for scale.

Supplementary figure S2. A) Syninclusions in the amber piece PED-0031; B) Morphotype 1 larvae (PED-0031-3) in the PED-0031 amber piece, habitus; C) Morphotype 1 larvae (PED-0041), habitus, dorso-lateral; D) Morphotype 1 larvae (PED-0041), habitus, ventro-lateral. Legend: 1-beetle larva, 2-possible cuticle of the morphotype 1 larva, 3 - morphotype larva, 4-morphotype 1 larva, 5- mite, 6 -possible scale insect, 7 -partial Insecta inclusion, 8 - Bibionomorpha fly, 9 - beetle larva, 10- Aranea, 11- Myriapoda.

Supplementary figure S3. Syninclusions in the amber piece PED-0031. A-B) Coleoptera larvae; C) Myriapoda; D) Possible Collembola; E) Bibonomorpha fly; F)Mite; G) Coleoptera, adult; H) Possible scale insect.

Supplementary figure S4. Trunk end of the morphotype 1. A)Specimen PED-0041; B) Specimen PED-0031-2; C) Specimen PED-0362 ; D) Specimen PED-0243.

Supplementary figure S5. Larvae of the morphotype 1. A) Specimen PED-0349; B) Specimen PED-0362; C) First fragment of the specimen PED-0025; D) Second fragment of the specimen PED-0025.

Supplementary figure S6. Larvae of the morphotype 1 in the amber piece PED-0152. A) Overview of the amber piece; B-D) Photos of the individual larvae in the piece. Photo D was taken using Keyence BZ-9000 fluorescence microscope. Legend: 1-4 - larvae of the morphotype 1.

Supplementary figure S7. Larva of the morphotype 1, specimen PED-0025. A) Habitus, overview; B) Trunk's end, enlarged, with tergal spines clearly visible.

Supplementary figure S8. Larva of morphotype 2. A) Overview of the amber piece LACM ENT 366281, with 5 larvae; B) Habitus, ventral view of the morphotype 2 larva from the LACM ENT 366281.

Supplementary figure S9. Fossil Pachygastrinae larva, morphotype 3 (PED-462). A) dorsal view; B) dorsal view, marked. Abbreviations: *hc*- head capsule, *pt* - prothorax, *ms* - mesothorax, *mt* - metathorax, *a1-a7* - abdominal units 1-7, *te* - trunk's end.

Supplementary figure S10.A) Fossil Pachygastrinae larva, morphotype 3, (PED-462), dorsal view; B) *Pachygaster atra*, dorsal view. Abbreviations: *hc*- head capsule; *as* - anterior spiracle; *ds*-dorsal setae; *ad* -antero-dorsal setae; *lt*-lateral setae.

Supplementary figure S11. *Pachygaster hymenea*, Patrick Müller collection. A) Habitus; B) Close-up photo of wing .

Supplementary figure S12. Pachygastrinae, larva, morphotype 4, PED-463. A) habitus, ventro-lateral view, right-hand side; B) habitus, ventro-lateral view, left-hand side.

Supplementary figure S13. Pachygastrinae, larva, morphotype 4, PED-463, Scanning Electron Microscopy. A). Habitus, ventro-lateral view; B) Head, ventrally; C) Posterior trunk, ventrally; D) Cuticle with Calcium carbonate pallets upclose.

Supplementary figure S14. Extant Larva of *Odontomyia* sp. from the PED research collection. A) Dorsal view; B) ventral view. Abbreviations: hc-head capsule, pt-prothorax; mt- metathorax; a1-a6 - abdominal units 1-6; te- trunk end.

Figure 1

Figure 1. Larva of morphotype 1, specimen PED-0031-2

A) Ventro-lateral view; B) Ventro-lateral view, marked; C) Dorso-lateral view. Abbreviations: *hc*-head capsule, *as*-anterior spiracle; *pt*-prothorax, *mt*-metathorax, *a2-a6* - posterior trunk units 2-6, *te*- trunk's end, *ps* - posterior spiracle.

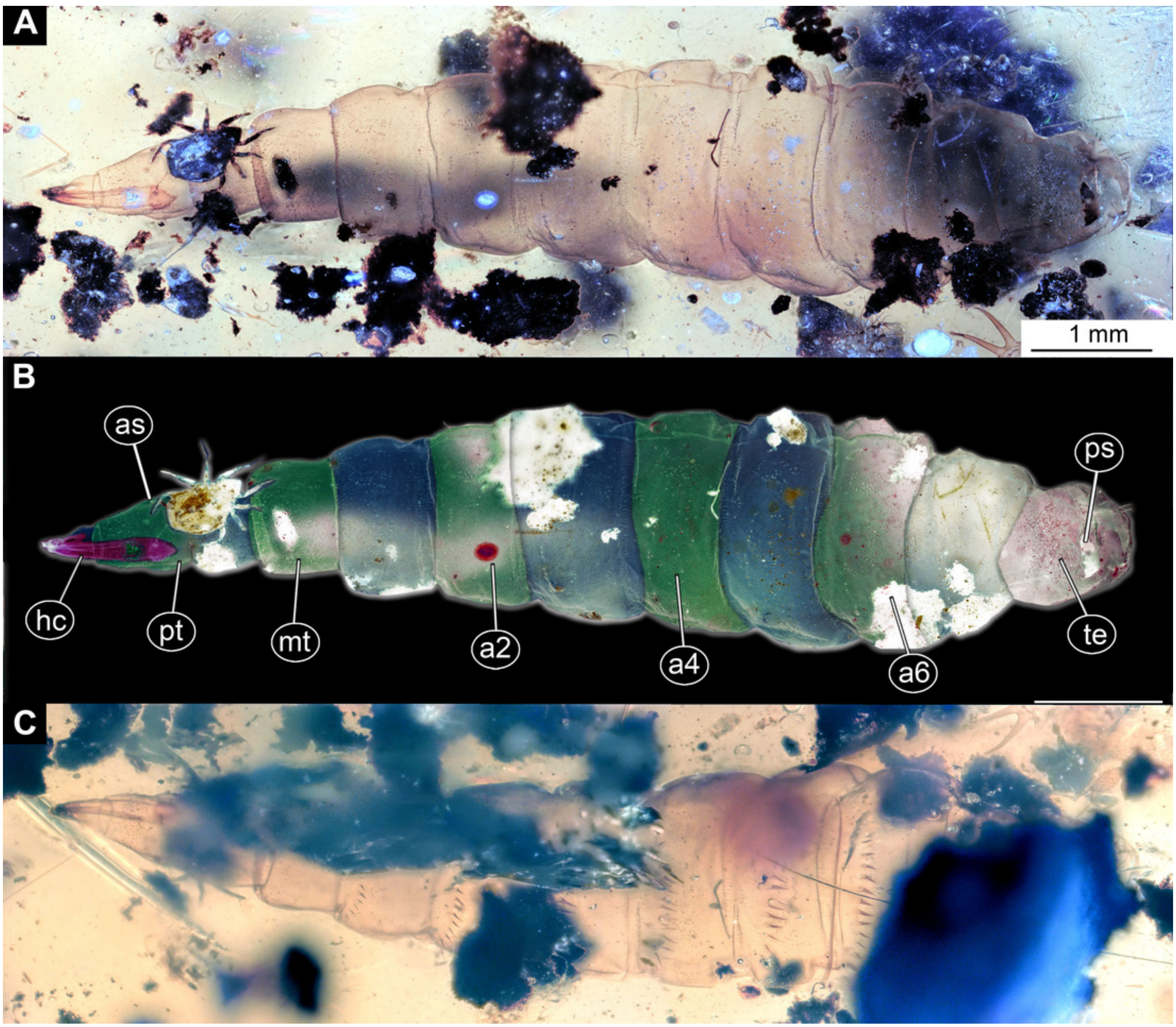

Figure 2

Figure 2. Larva head of morphotype 1, specimen PED-0031-2.

A) Lateral view; B) Lateral view, marked; C) Lateral view, linedrawing. Abbreviations: *hs*-head soft tissues, *hc* - head capsule, *lb*-labrum; *la* - labium; *mk* - mandibular-maxillar complex, *ta* - tentorial arm, *mr* -metacephalic rode, *ct* - cut off through the part of the head capsule, *pt*-prothorax, *cc* - calcium carbonate pallet.

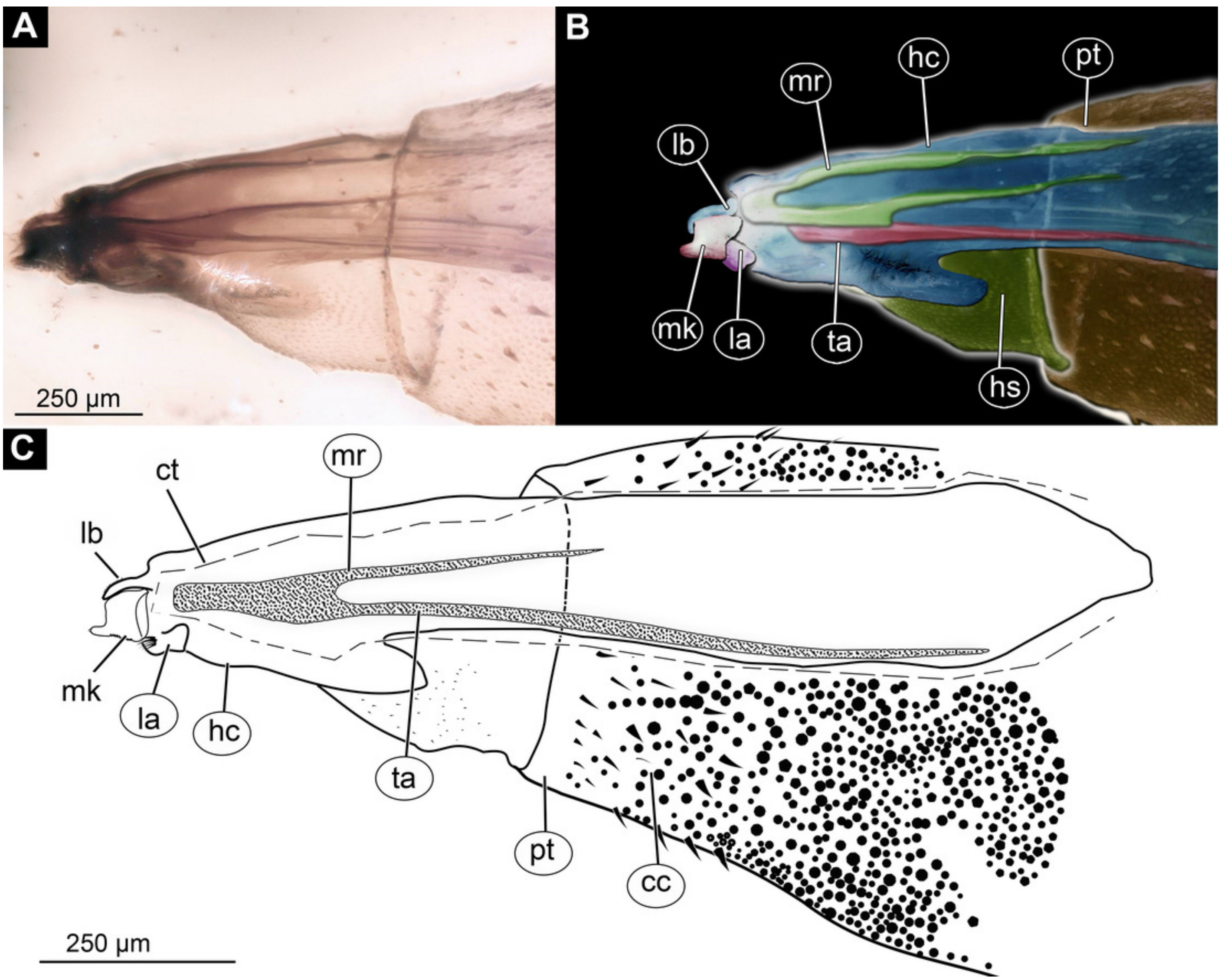

Figure 3

Figure 3. Larval head of morphotype 1, specimen PED-0152-2.

A) Dorsal view, habitus; B) Ventral view, head; C) Ventral view, head - marked. Abbreviations: *mp* - maxillary palp, *bm* - base of mandibular-maxillar complex (“grinder”), *lb* - labrum.

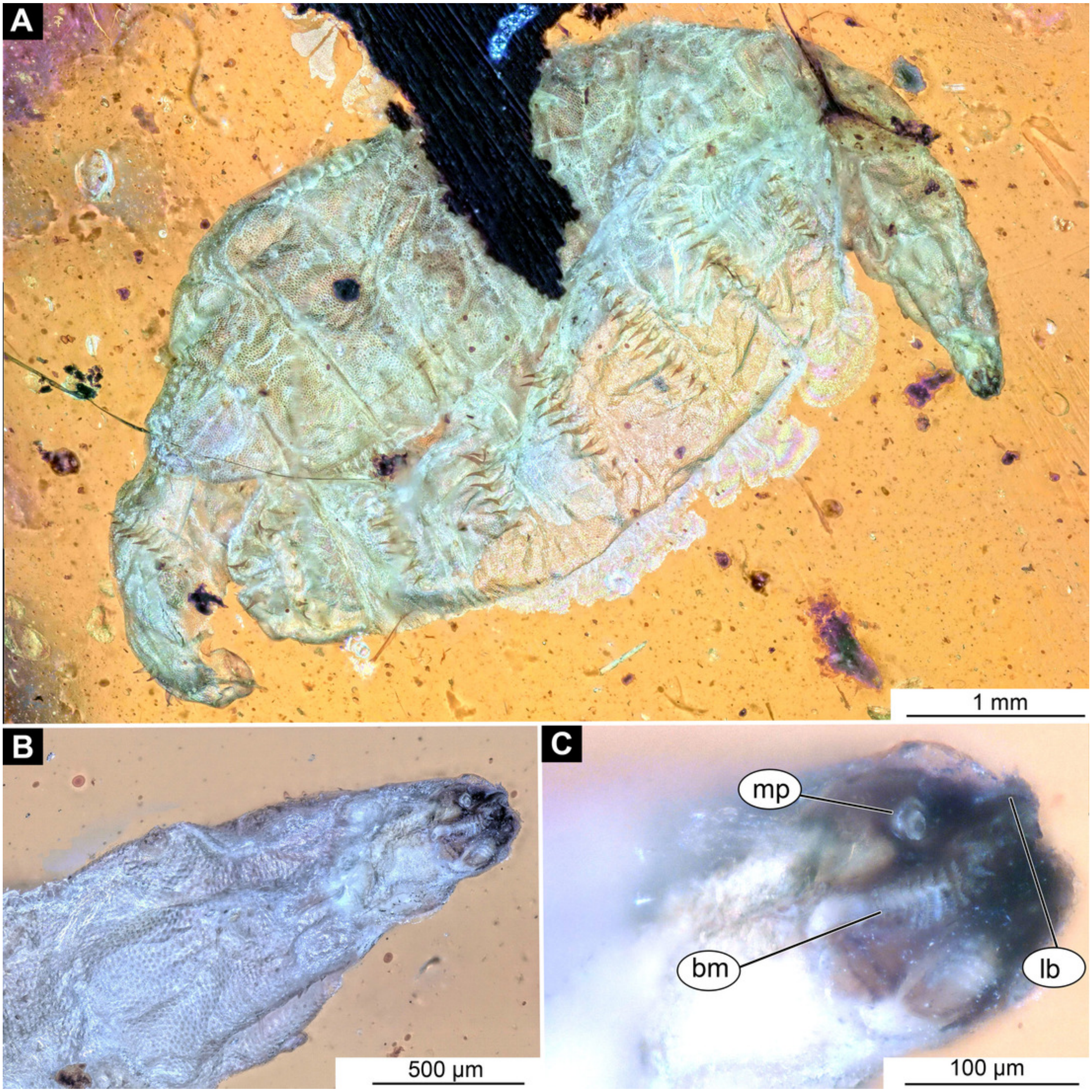
A
1 mm
B
500 µm
C
mp
bm
lb
100 µm

Figure 4

Figure 4. Larva of morphotype 2, specimen LACMALACM ENT 366281-1.

Figure 4. Larva of morphotype 2, specimen LACMALACM ENT 366281-1. A) Dorsal view, habitus; B) Dorsal view, habitus - marked Ventral view, head; C) Ventral view, head - marked. Abbreviations: *hc*-head capsule, *as*-anterior spiracle; *ms*- mesothorax, *mt*-metathorax, *a1*-*a7* - posterior trunk units 1-7, *te*- trunk's end, *ps* - posterior spiracle.

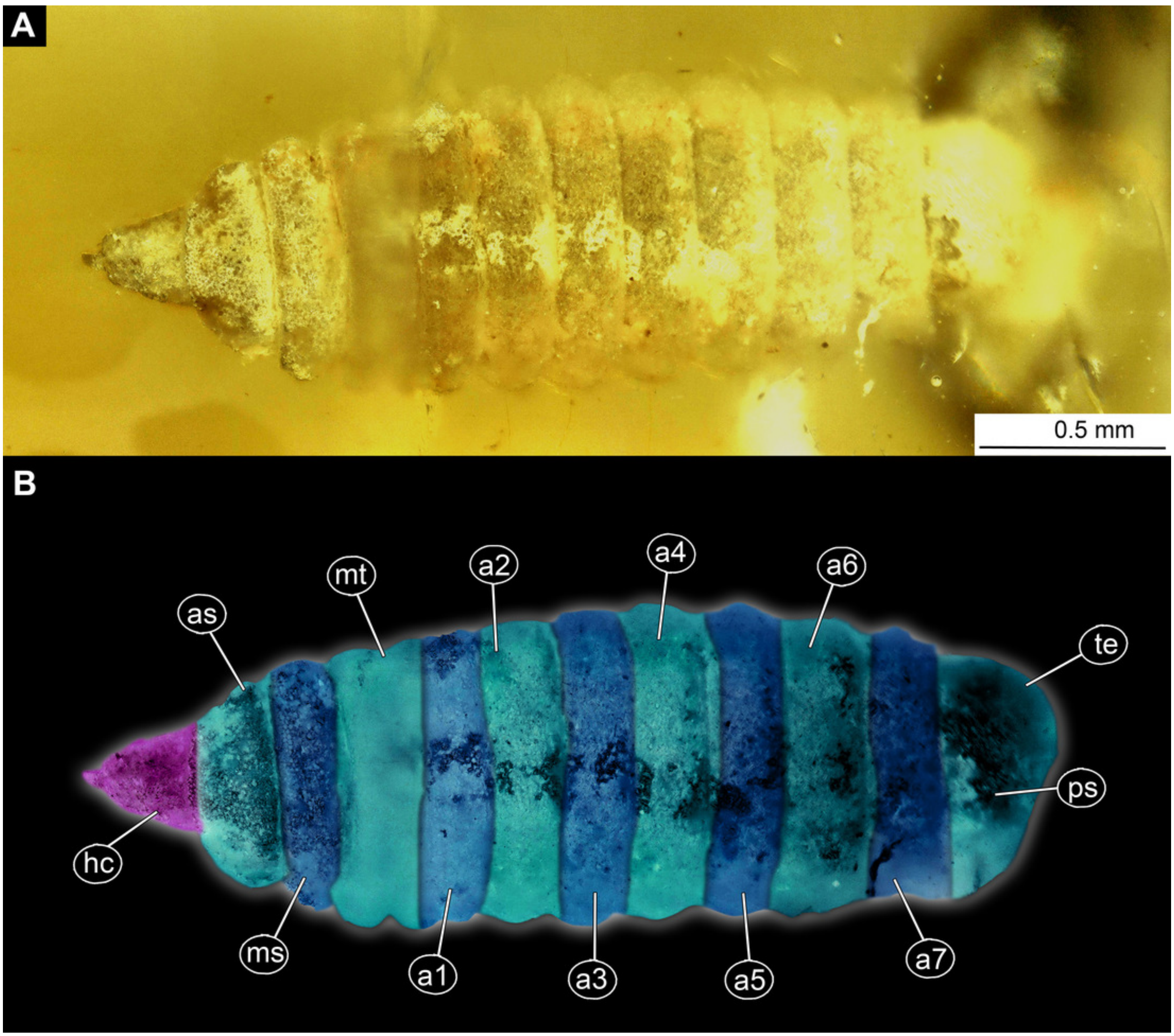

Figure 5

Figure 5. Larval head of morphotype 2, specimens LACM ENT 366281-1(A-B) and LACM ENT 366281-2 (C-D).

A) Dorsal view, head, LACM ENT 366281-1; B) Dorsal view, head - marked, LACM ENT 366281-1; C) Lateral view, head, LACM ENT 366281-2; D) Lateral view, head -marked, LACM ENT 366281-2. Abbreviations: Abbreviations: *hs*-head soft tissues, *hc* - head capsule, *lb*-labrum; *mk* - mandibular-maxillar complex, *ey* - eyes; *as* - anterior spiracle.

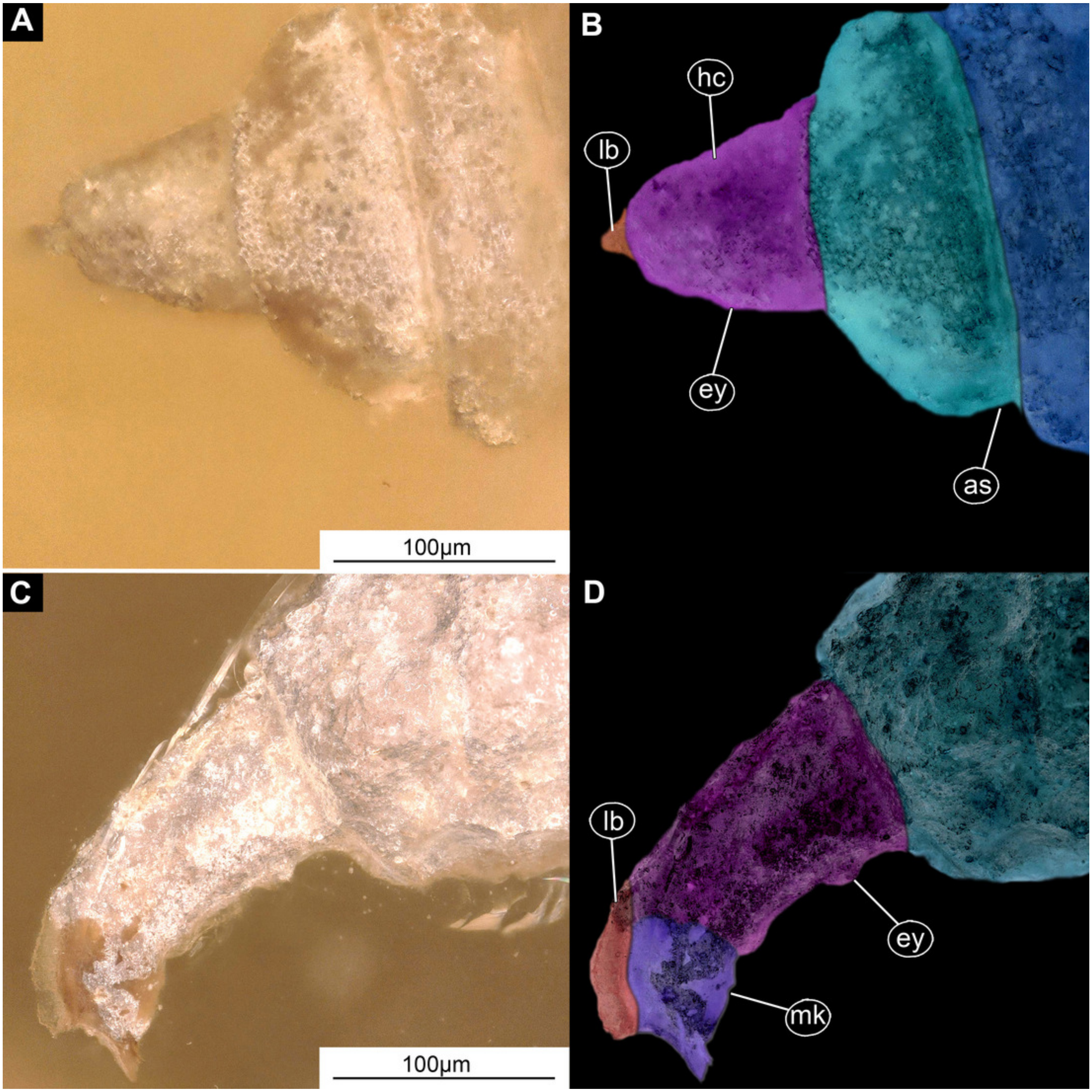
A
B
hc
lb
ey
as
100µm
C
D
lb
ey
mk
100µm

Figure 6

Figure 6. Morphotype 3, habitus, ventral, larva PED-0462.

A) Habitus, ventral view; B Habitus, ventral view, markes. Abbreviations: hc- head capsule, pt - prothorax, ms - mesothorax, mt -metathorax, a1-a7 - abdominal units 1-7, *te*- trunk’s end.

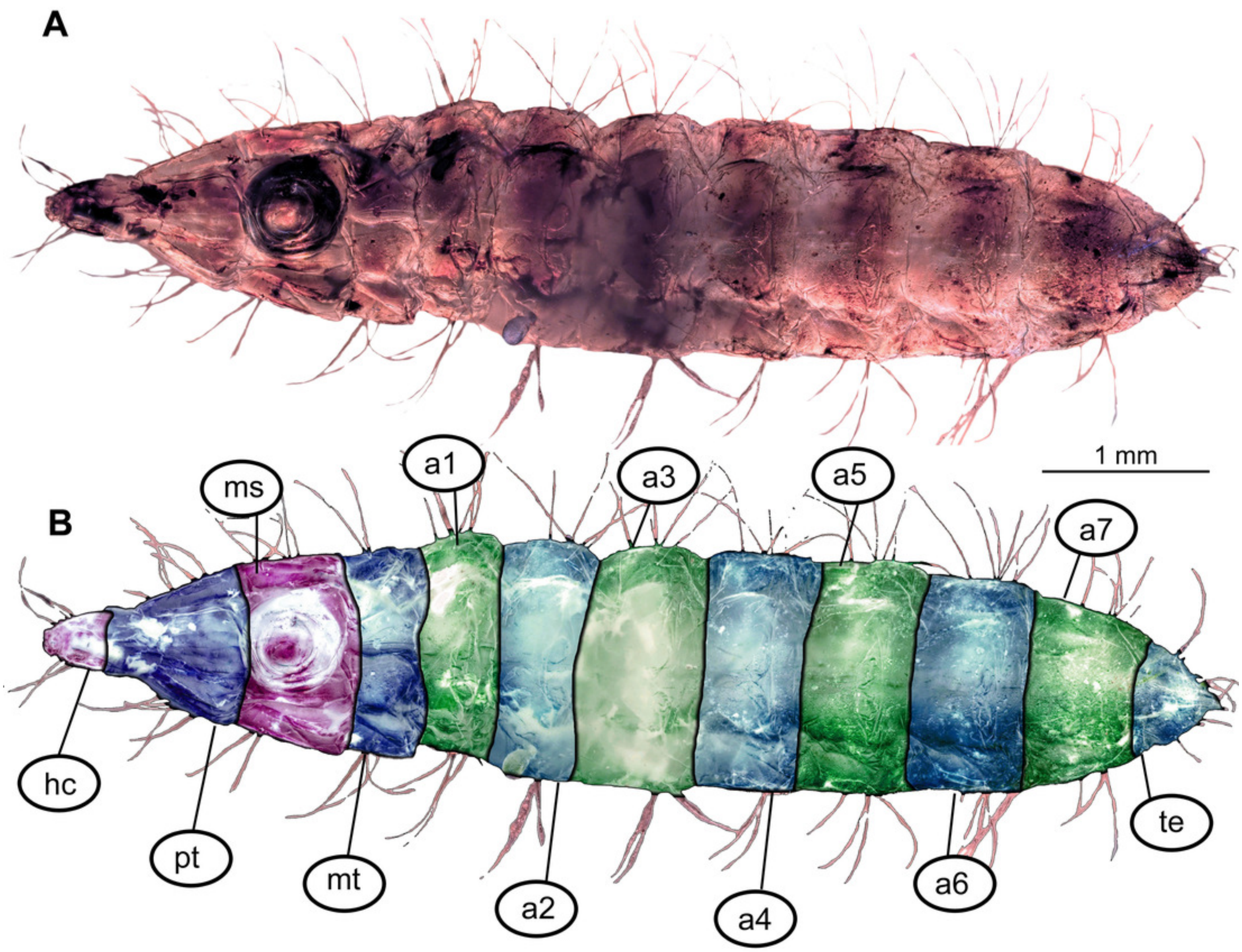

Figure 7

Figure 7. A-B) Fossil Pachygastrinae, larva of morphotype 3, PED-0462 and C-D), extant *Pachygaster atra, head.*

A & B) Ventral view of the headcapsule unmarked and marked; C & D) *Pachygaster atra*, Ventral view of the headcapsule unmarked and marked; Abbreviations: *an*-antenne; *as*- anal setae; *pt* -prothorax, *ey*-eyes; *lb*-labrum; *mp*-maxilar palp; *mb*- base of mandibular-maxillar complex ("grinder"); *v1*-3 - ventral setae 1-3, *la*- labium.

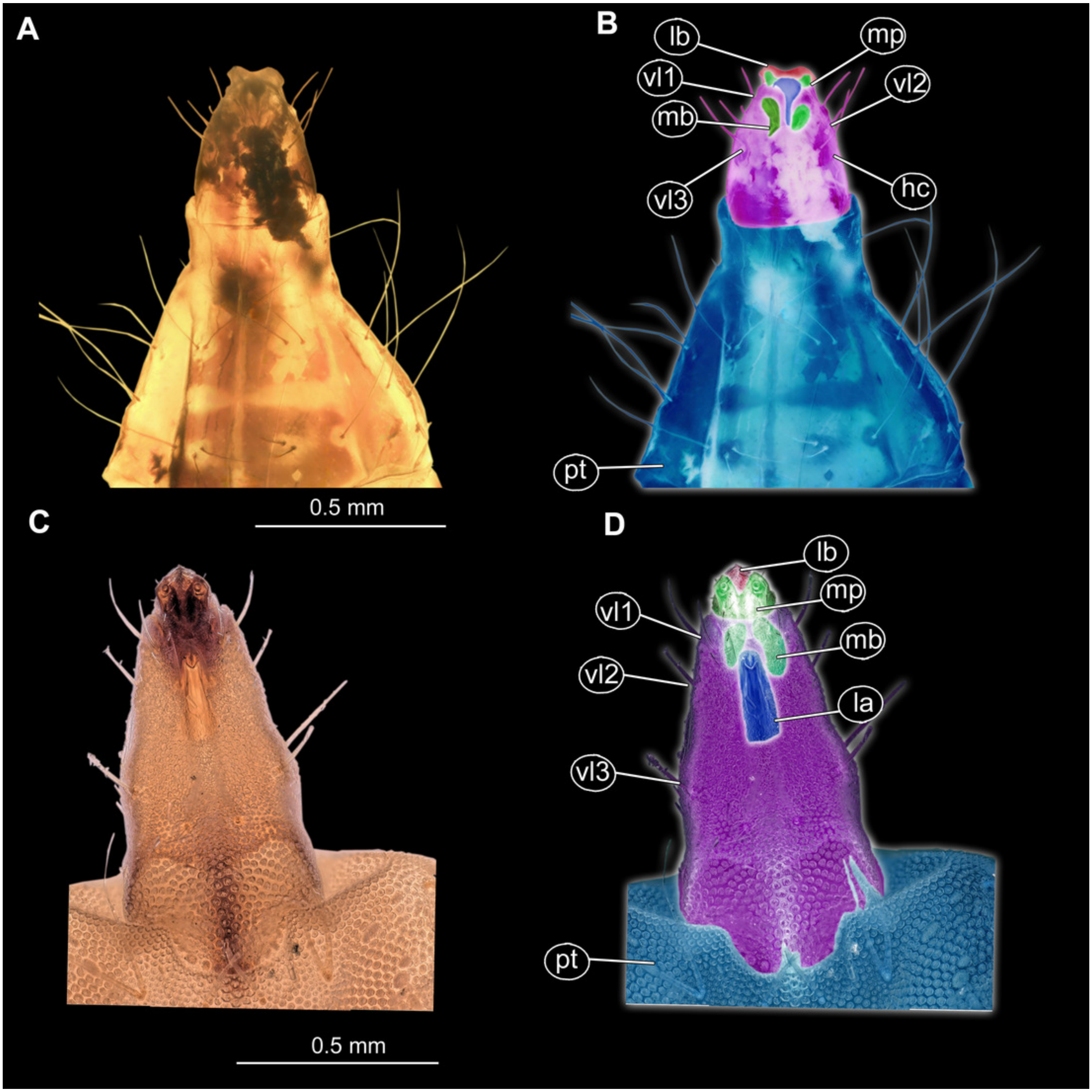
A
B
lb
mp
vl1
vl2
mb
vl3
hc
pt
0.5 mm
C
D
lb
mp
vl1
mb
vl2
la
vl3
pt
0.5 mm

Figure 8

Figure 8. Pachygastrinae, larva, morphotype 4, PED-0463.

A) habitus, ventral view; B) habitus, ventral view, marked. Abbreviations: *hc*-head capsule; *ey* - eyes; *as*-anterior spiracle; *pt* - prothorax, *ms*- mesothorax, *mt*-metathorax, *a1-a7* - posterior trunk units 1-7, *te*- trunk's end, *ps* - posterior spiracle, *fc* - folded cuticle.

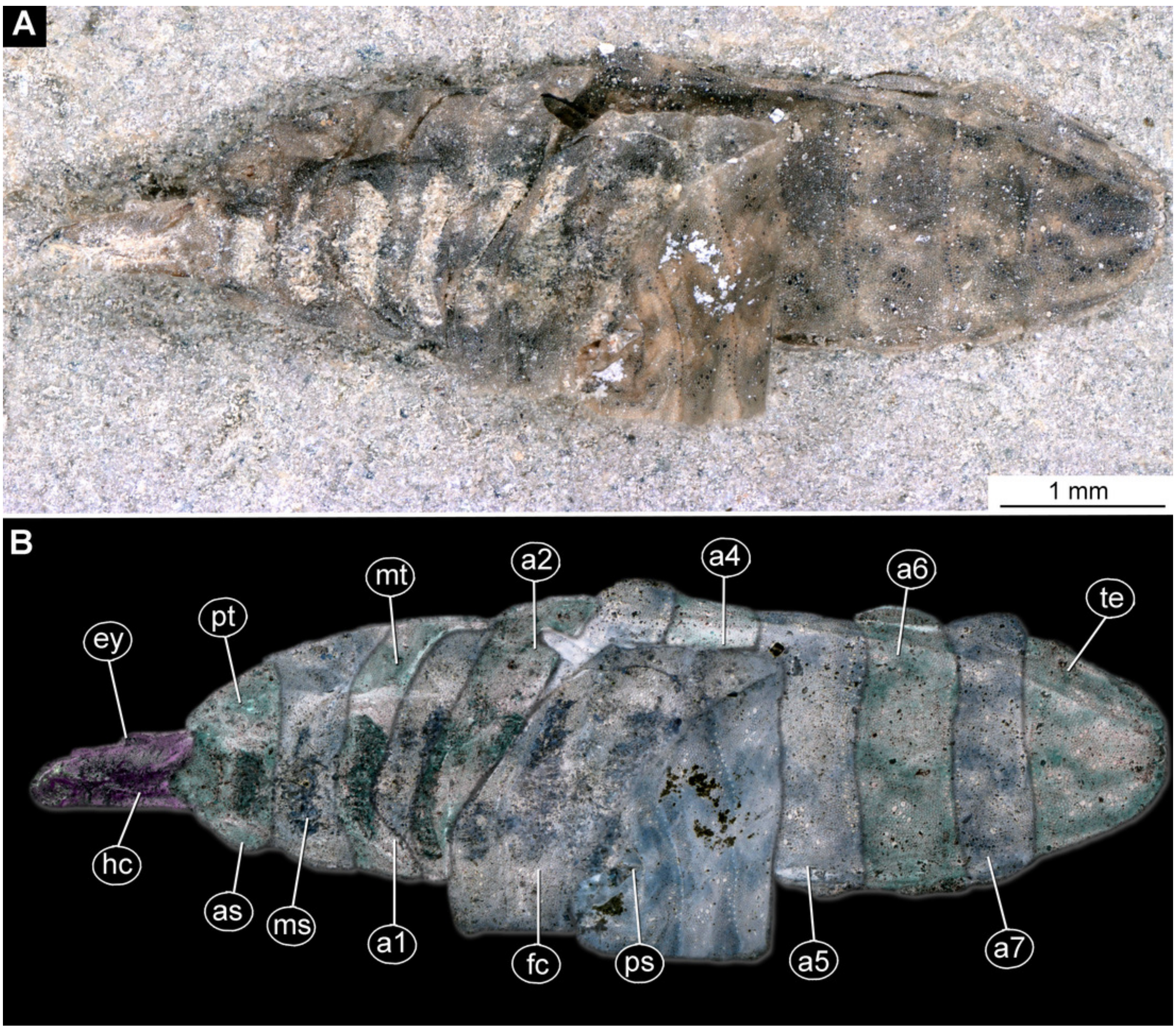

Figure 9

Figure 9. Pachygastrinae, larva, morphotype 4, PED-0463, head ventrally.

A) head and prothorax, ventral view; B) head and prothorax, ventral view, marked; C) head, ventral view; D) head, ventral view, marked. Abbreviations: *an* - antenna, *hc* - head capsule, *ey* - eyes; *as* -anterior spiracle, *lb*-labrum; *la* - labium; *mk* - mandibular-maxillar complex.

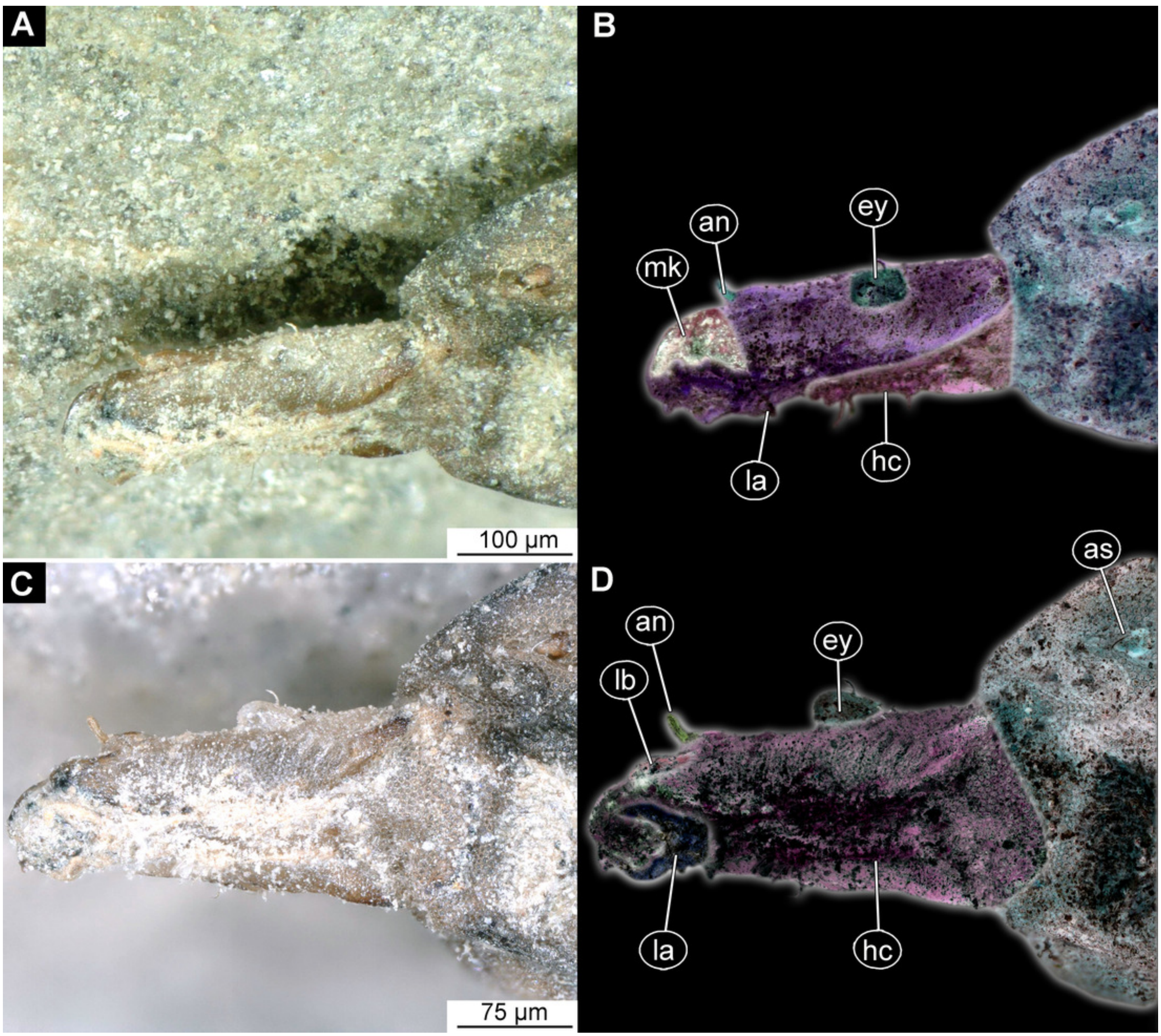

Figure 10

Figure 10. Fossil Stratiomyinae larva, morphotype 5 (PED-0464).

A) Habitus; B) Close-up photo of coronet of the hydrofuge setae.

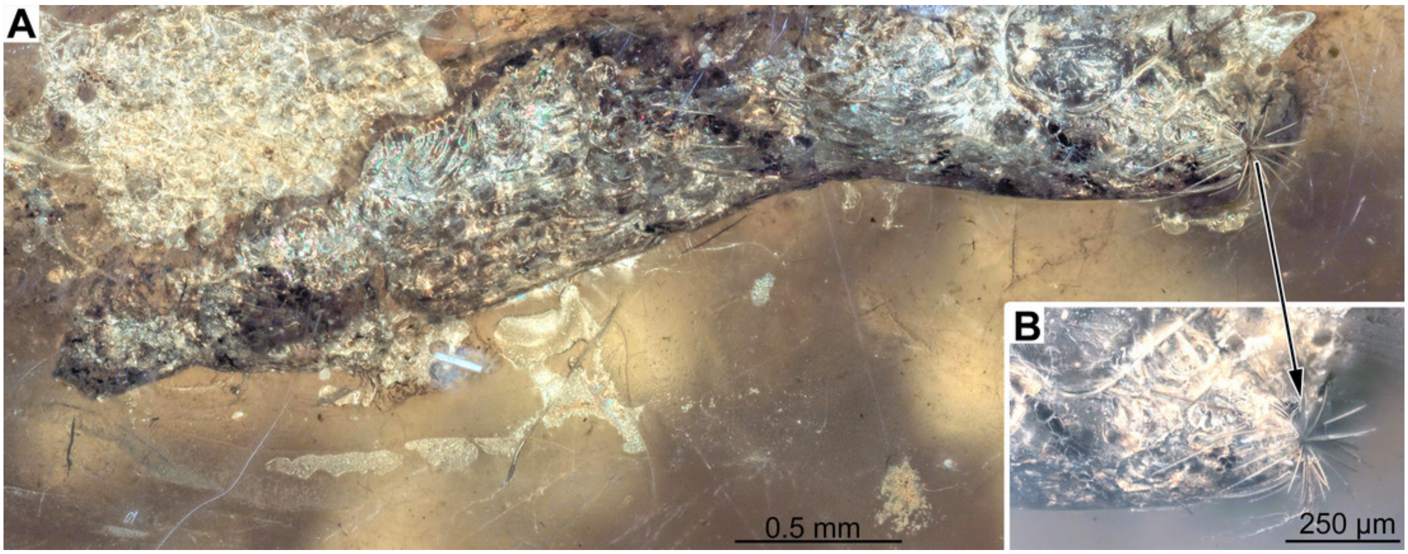

Figure 11

Figure 11. Fossil stratiomyinae larvae, morphotype 6 (SF-MeI 4666).

A) compression fossil, habitus; B) compression fossil, marked; C) Messel shale with Stratiomyiinae larvae, SF-MeI 4666, overview. Abbreviations: *hc* -head capsule; *phc* -pharyngeal grinding mill; *pt*-prothorax; mt -methathorax; *a1*-*a6*-abdominal units; *te*- trunk's end.

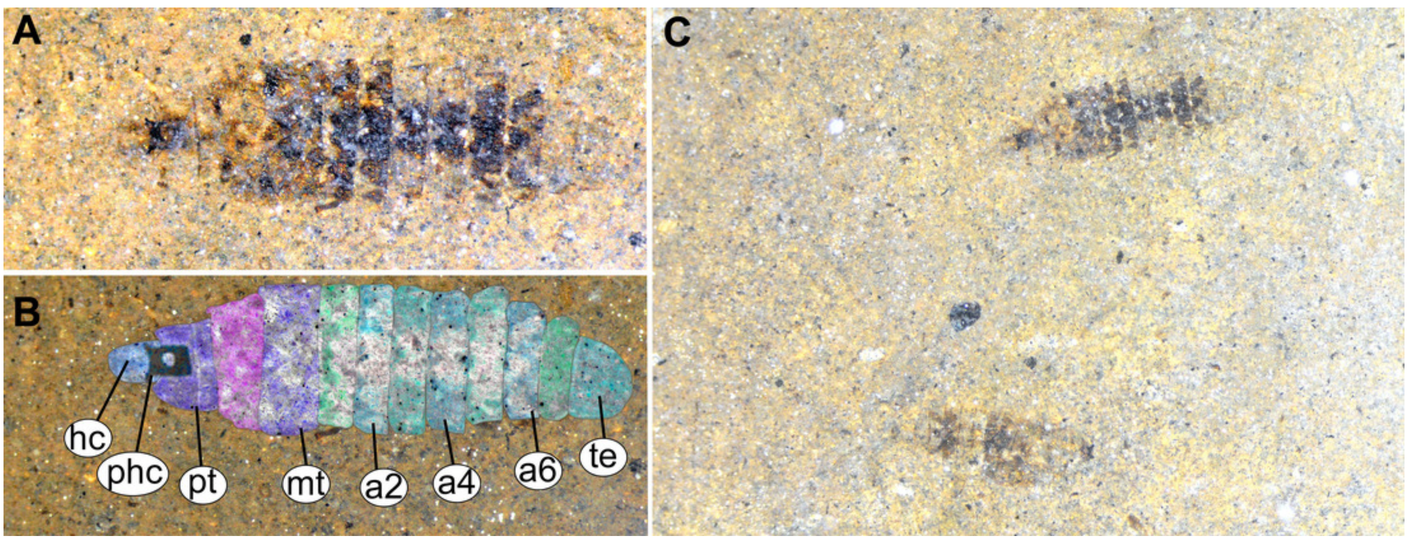

Figure 12

Figure 12. Ecomorphospace occupied by the extant and fossil Stratiomyomorpha larvae. Both plots presenting the same morphospace, split by the different grouping variables. Total captured variation = 75%; 62.1% at PC1 and 12.9 % at PC2.

A) Morphospace split by the larval habitat: violet - saproxylic, blue-terrestrial, green - “unknown” (fossils), red- aquatic; B) Morphospace split by the geological age/deposit of the larvae: blue - extant, red- burmese amber, the rest of the deposits are represented by the single labelled dots.

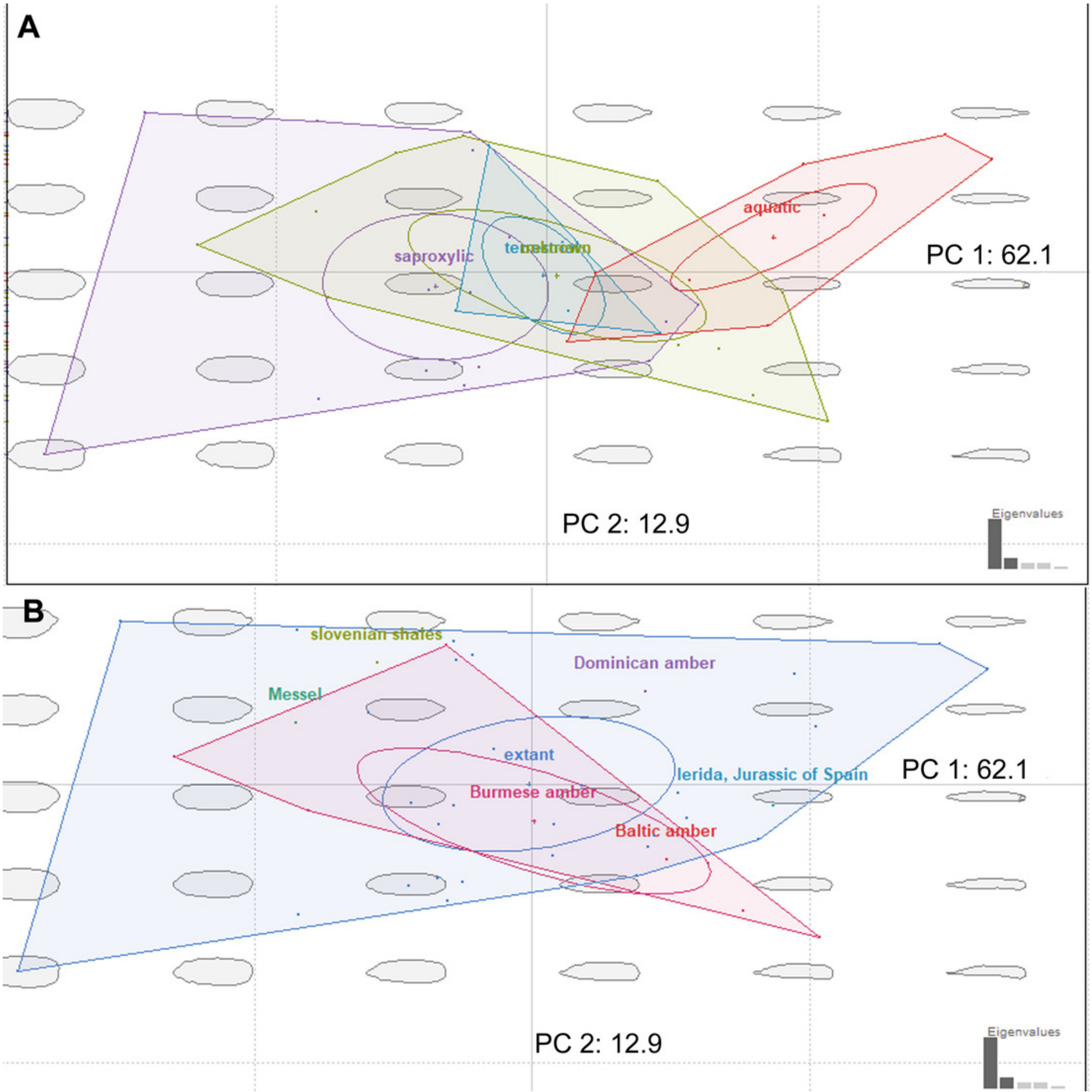
A
aquatic
saproxylic
terrestrial
unknown
PC 1: 62.1
PC 2: 12.9
Eigenvalues
B
slovenian shales
Dominican amber
Messel
extant
lerida, Jurassic of Spain
Burmese amber
Baltic amber
PC 1: 62.1
PC 2: 12.9
Eigenvalues

Table 1(on next page)

Table 1. Material used in the paper.

Please note that ZSM does not provide numbers for most of their extant insect material, including specimens used in this article.

Taxon	ID number	Larvae	Syninclusions	Deposited at	Age
Morphotype 1	PED - 0152	3	NA	PED	Cretaceous, Cenomanian 100.5 ± 0.4 MYA
Morphotype 1	PED-0349	1	NA	PED	Cretaceous, Cenomanian 100.5 ± 0.4 MYA
Morphotype 1	PED-0362	1	Hymenoptera	PED	Cretaceous, Cenomanian 100.5 ± 0.4 MYA
Morphotype 1	PED-0031	4	Diplopoda, beetle, 2 beetle larvae, collembola, aranea, probable scale insect, 2 mites	PED	Cretaceous, Cenomanian 100.5 ± 0.4 MYA
Morphotype 1	PED-0041	1	NA	PED	Cretaceous, Cenomanian 100.5 ± 0.4 MYA
Morphotype 1	PED-0243	1	NA	PED	Cretaceous, Cenomanian 100.5 ± 0.4 MYA
Morphotype 1	PED-0113	1	NA	PED	Cretaceous, Cenomanian 100.5 ± 0.4 MYA
Morphotype 1	PED-0025	1	NA	PED	Cretaceous, Cenomanian 100.5 ± 0.4 MYA
Morphotype 2	LACM ENT 366281	5	NA	LACM	Cretaceous, Cenomanian 100.5 ± 0.4 MYA
Morphotype 3	PED-0462	1	NA	PED	Miocene, 20.44-13.92 MYA
Morphotype 4	PED-0463	1	NA	PED	Miocene, 20.44-15.97 MYA
Morphotype 5	PED-0464	1	non-biting midge male (Diptera, Chironomidae); window-gnat (Diptera, Anisopodidae), two dark-winged fungus gnats (Diptera, Sciaridae), large spider (Araneae).	PED	Eocene, 37.8-33.9 MYA
Morphotype 6	SF-Mel 4666	2	NA	SF	Eocene, ca. 48.2 MYA

Pachygaster atra (Panzer, 1798)	not provided	>100	NA	ZSM	extant
Oxycera nigricornis Olivier, 1811	not provided	>100	NA	ZSM	extant
Odontomyia sp	PED-465	1	NA	PED	extant

Table 1. Material used in the paper. Please note that ZSM does not provide numbers for most of their extant insect material, including specimens used in this article.