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

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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 (the Barremian, about 125 MYA). 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. Yet, fossil larvae of the group are extremely scarce. Here we present 23 new records of fossil stratiomyomorphan larvae, representing ~~6~~ six discrete morphotypes. Specimens originate 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 our understanding of 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 Stratiomyomorpha has a fossil record reaching back about 125 million years into the past, to the Barremian (Lower Cretaceous; Whalley and Jarzembowski 1985; Mostovski 1998). A ~~now~~ now-extinct group of flies with long proboscides (Zhangosolvidae) from the Cretaceous has also been interpreted as an ingroup of Stratiomyomorpha (Peñalver et al., 2015).

Representatives of Stratiomyomorpha are widespread in modern ecosystems and diverse in their biology (Woodley, 2001~~, ~~; Marshall 2012). Larvae of different ingroups of Stratiomyomorpha vary in habitat preferences. Fully aquatic larvae are known in Stratiomyinae~~-~~, Rhaphiocerinae, etc. (ingroups of Stratiomyidae); other larvae develop in the terrestrial habitats as in the groups Pachygastrinae~~-~~, Clitellariinae, Sargiinae or Hermetiinae (ingroups of Stratiomyidae), and Xylomyidae~~-~~, while larvae of timber flies (Pantophthalmidae) are saproxylic, burrowing in living wood (James 1981, Rozkošný, 1981, 1982, Pujol-Luz. 2014). Xylomyidae is a small group of lies with predacious or saprophagous larvae living under tree bark (James 1981). Pantophthalmidae, the group of timber flies, including one of the largest extant representatives of Diptera, with larvae burrowing in living wood (Rapp, 2007, 2011).

Representatives of Stratiomyomorpha are carrying vital ecosystem functions 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~~ essential source of protein for feeding cattle in industrial agriculture or for the production of human food (Hauser, Woodley, and Fachin 2017~~-~~; Lessard et al. 2019). Many merolimnic species of Stratiomyomorpha, i.e., those with aquatic larvae, are important algal mat grazers, involved in carbon cycling (Mángano, Buatois, and Claps 1996).

Ichnofossils attributed to larvae of Stratiomyomorpha are quite common in the fossil record (Mángano, Buatois, and Claps 1996; Pickerill, Han, and Jiang 1998), while body fossils of this group have been~~ extremely rar~~scarce (Evenhuis 1994). So far, ~~there are only five deposits~~ only five deposits are yielding any of them:

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 limestone of Spain.

2) Two morphotypes of larvae from Myanmar amber (~100 MYA) were reported by Liu et al. (2019).

3) Kühbander & Schleich (1994) reported a stratiomyomorphan larva, interpreted as a larva of the group *Odontomyia*, from the Miocene Randecker Maar in Germany (~17 MYA). 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 (>1 MYA).

5) Sixteen fossil larvae of Stratiomyidae from the late Eocene of the Isle of Wight (129.4 ± 1.5 MYA) are present in the collection of the Natural History Museum London (UK). They can be interpreted as aquatic forms of the group Stratiomyini and have been tentatively suggested to be representatives of the species *Odontomyia brodiei* (Cockerell 1915), which is known from fossils of adults from the same deposit (Krzeminski et al. 2019).

Larval forms are crucial for the success and diversification of any ingroup of Holometabola, due to the ecological niche separation of the life stages (Grimaldi and Engel 2005). This applies to the ~~super~~ super-diverse lineages of beetles (Coleoptera), wasps (Hymenoptera), butterflies (Lepidoptera), and flies (Diptera), but also ~~to~~ the less species-rich groups. ~~The severe lack~~ Lack of fossil larvae of Stratiomyomorpha 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 a morphometric comparison of modern and fossil forms.

Materials & Methods

Material

Twenty-three specimens of fossil larvae are in the focus of this study. Twenty of them are preserved in amber, and three are preserved as compression fossils. These larvae are representing six morphotypes: two from Myanmar amber, one from Baltic amber, one from Dominican amber, one from the Činžat shale of Slovenia, and the last one from the Messel lake deposits. Most of the specimens in amber originated from Myanmar ("Burmese amber"), and most represent a single morphotype ("morphotype 1"). Working with Burmese amber requires special ethical consideration~~,~~; for details, see the ongoing discussion (Haug et al., 2020). All these specimens were purchased on ebay.com from different sellers and are now deposited at the 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 five 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 morphotype 3, was commercially acquired by Y.W. and originated from the Dominican Republic. It is now deposited in the collection of the Palaeo-Evo-Devo Research Group, Ludwig-Maximilians-Universität, Munich, Germany PED research collections (PED).

Specimen PED-0463, representing morphotype 4, was collected by R.G. at the locality Činžat, situated in the Ribnica-Selnica Graben, northern Slovenia. The specimen is now deposited at the 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 stemmed from Yantarnyj, Kaliningrad district (formerly Palmnicken, Königsberg). It is now deposited in the collection of the Palaeo-Evo-Devo Research Group, Ludwig-Maximilians-Universität, Munich, Germany (PED).

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 a full list of materials, please see Table 1.

Terminology: The morphological terminology ~~largely~~ mostly follows Rozkošný (1981, 1982) and Sinclair (1992) for the head capsule morphology. Yet~~, to enhance the understanding for~~ , to help 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~~ names are given in brackets where necessary to provide ~~wider~~ more comprehensive frame correspondence. It is important to note that many structures cannot be discerned externally in the Diptera larvae, i.e., it is impossible to see any tergite boundaries in the head capsule of the post-embryonic larvae. Nevertheless, it is well possible to reconstruct the sequence of the segments in the head capsule, using the arrangement of the appendages (Baranov et al., 2019).

Database use

Data on the fossil record of the group 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-polarized, coaxial illumination. All images were recorded as composite images to counteract the limitations of depth of the focus. Models were assembled using stitching and panorama functions to overcome the weakness of the field of view under higher magnifications. Each image detail was 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). Also, here we recorded stacks of images which then were digitally fused to single in-focus images using CombineZP (GNU). Compression fossils from Messel were photographed with a Leica MZ12.5 stereomicroscope with an attached Nikon D300 camera.

The cuticle fossil, specimen PED-0463 (“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 the beam current 80 µA; filament electric current 2500 A; and electric potential 10-20 kV. Scanning was performed in a low vacuum (<2e-005 mbar).

Morphometry and outline analysis

~~Maximum dorsal head capsule length and width of some larvae~~, The 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, a public domain (Schindelin et al. 2012).

As a proxy for the overall shape diversity, we compared the outlines of the larvae in the dorsoventral aspect. To do so, we have analyzed the shapes, more precisely sketches of all specimens, and extant comparative 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 dorsoventral aspect from the literature. Only specimens with a relatively straight body were included, as any examples imaged in curled or bent position will heavily bias the morphospace. Full~~-~~-body images of the larvae were obtained from numerous published sources (Schremmer 1951; Hennig 1952; James 1965; McFadden 1967; Bull 1976; Tuskey 1976; James 1981; Schremmer 1986; Beuk 1990; Rozkošný 1997; Rozkošný and Kovac 1998; Pujol-Luz and Xerez 1999; Stubbs and Darke 2001; Stuke

2003; Pujol-Luz, Xerez and Viana 2004; de Xerez and Garcia 2008; Bucánková, Kovac, and Rozkošný 2009; Marques and de Xerez 2009; Marshall 2012; Pujol-Luz and Pujol-Luz 2014a & b; Pujol-Luz, Lopes, and Viana 2016; Godoi and Pujol-Luz 2018), see ~~supplementary~~ Supplementary Table 1 for the full information. In total, 69 stratiomyomorphan specimens were analyzed (see ~~supplementary~~ supplemental Table 1, supplementary images).

Black-and white outlines w~~h~~ere produced using a polygonal tool and mask functionality of the program FIJI (Schindelin et al. 2012). Jpg outlines were analyzed in R using the *momocs* package (Bonhomme et al. 2014), with the shapes being characterized by 36 harmonics. Source code, list of the material used for the outline production, and all the underlying data are available as supplementary material (supplement 3). To~~-~~ estimate the habitat affinity of the fossil larvae, we plotted them into a single morphospace with the extant larvae. For the latter, we demarcated saproxylic, aquatic, and terrestrial habitats. Based on the position of the fossil larvae in this morphospace, we have attempted to assess their habitat affinity. 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

The geological context of Myanmar (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)~~, as well as Messel shale (e.g., Büchel and Schaal 2018), has been explained in detail in various previous works.

The Locality of Činžat is much less well known to the broader audience than the three above mentioned, so we are discussing it in ~~the~~ further detail~~s~~. 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 foraminifera 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 individual layers and variations in lithology are more likely to contain macrofaunal fossil remains. Interbedded layers of sandstones and conglomerates ~~contain~~ have 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. Description, general notes: to provide the necessary background, we first give a generalized description of the characteristics of larvae of Stratiomyidae (and to the same extent Stratiomyomorpha) segment by segment.

General shared appearance

Habitus. Small to ~~medium~~ medium-sized larva with slightly dorsoventrally flattened, ~~spindle~~ spindle-shaped body. ~~Body~~ The body is fully covered with oval pellets, supposedly of calcium carbonate composition (although it is impossible to ~~conclusively ascertain this aspect for the fossil forms~~ confirm this aspect for the fossil forms) (Figs 1 A-B, 2 A-C, 3A-C, 4A).

Body length from 2 mm to slightly less than 20 mm. ~~Body~~ The body differentiated into presumably 20 segments, ocular segment plus 19 post-ocular segments (Figs 1A-B, 2 A-C, 4 A-C). Anterior segments are forming a distinct head capsule.

The ~~Head~~ head capsule sclerotized anteriorly, posterior part (one third to one half) reduced to several longitudinal structures, retracted into the anterior trunk (prothorax). The Head capsule is formed by an ocular segment plus five post-ocular segments.

Ocular segment recognizable by its appendage derivative, clypeo-labral complex. The Clypeus (clypeal sclerite) is longer than it is ~~wide~~ complete. Labrum was roughly triangular, much longer than wide, strongly sclerotized (Figs. 2A-C, 3B, C). ~~Segment~~ The segment with small stemmata (“eye prominences”), anteromedially.

Post-ocular segment 1, with a pair of antennae [antennulae in ~~generalised~~ generalized terminology]. The antenna of Stratiomyomorpha larvae stout, comprising two elements, sitting in dorsoanterior position, or more towards the center of the dorsal surface of the head capsule. In many fossil specimens not preserved or not visible (Figs. 4B, C)

Post-ocular segment 2 (intercalary segment) without externally recognizable structures, not identifiable in the post~~-~~embryonic development of most Diptera larvae. It might be argued that discussion of such seemingly absent structure in the description is unnecessary, or mixing conjectures with observed structures. We will say, on the contrary: there is the knowledge and hence expectation of the presence of this segment based on prior ~~knowledge~~experience. Yet, we do not see it. In broader comparison, this is, in fact, informative and needs to be included in the taxa description.

Post-ocular segments 3 and 4 were recognizable by their appendages mandibles and maxillae [maxillula in generalized terminology]. Mandibles and maxillae form a single compound, the mandibular-maxillary complex (autapomorphy of Stratyomyomorpha~~);~~), comprising elements largely indistinguishable, apart from the distal parts of the maxillae (maxillary palp). Maxillary palps quite stout, but prominent (Schremmer 1951, Rozkošný 1981). The proximal ~~part~~ portion of the mandibular-maxillary complex~~,~~ fully sclerotized~~;~~ , with strong, multi-branched setae on its dorsodistal surface, as well as laterally. Distal part or palp, conical, with two elements (palpomeres). ~~Apical~~ The apical part of the mandibular-maxillary complex bears an arrangement of the setae (“brush”) of varying complexity (autapomorphy of Stratyomyomorpha). Basal ~~part~~ interest bearing a ventral "grinder~~",~~ ,"," which is heavily sclerotized (Figs. 3A-C, 4B, C). On the ventral side, the mandibular-maxillary complex forms ventral plates, occupying the ventral side of the head capsule (Sinclair 1992).

Trunk (thorax + abdomen) with eleven visible units, interpreted as ~~3~~ three thorax segments, ~~7~~ seven abdominal segments, and a trunk end (abdomen unit 8). Cuticle covered with round deposits of calcium carbonate pellets, forming a honeycomb-like pattern (autapomorphy of Stratyomyomorpha). Remark: It is difficult to ascertain that the cuticle of the fossils is indeed covered in calcium carbonate pellets. It cannot be excluded that such cuticle scales are simple chitin as in larvae of the Ephydridae or Oestridae (Marshall 2012).

Trunk units are without any parapods, creeping welts, or protuberances. Different arrangements of spiracles possible: 1) Trunk bears nine pairs of spiracles (openings of the tracheal system): one pair of spiracles on the prothorax, and eight pairs on the posterior trunk (abdomen). This type of ~~the~~ tracheal system is called peripneustic (Hennig, 1952). 2) most spiracles reduced, amphipneustic (spiracles present on prothorax and trunk end), or 3) methapneustic (spiracles on trunk end only) (McFadden 1967) (Figs 2A-C, Supplementary figures 4A-D).

~~Anterior~~ The anterior trunk or thorax has ~~with~~ three segments, pro-, meso- and met~~h~~athorax. Armament is represented by the calcium carbonate pellets and large rhombic sclerites on the sternites, occasionally with some modified, spike-like setae. Prothorax with 2–3 pairs of anterodorsal setae (Rozkošný 1981).

Mesothorax and metathorax have ~~with~~ numerous dorsal and ventral setae, as well as ~~numerous~~ multiple pellets of calcium carbonate (Figs. 1A, B). All three units (= segments in this case) of the anterior trunk (thorax) are having very uniform setation. Each of the thoracic segments bears three pairs of dorsal setae (D1–D3), one pair of dorsolateral setae, and one pair of ventrolateral setae. Additionally, each thoracic segment bears two pairs of ventral setae (Figs 1 A, B). ~~Inner~~ The inner pair of ventral setae simple~~,~~ ; the outer pair contains several branched setae. The latter also is known as a "thoracic leg group" setae (Rozkošný 1981).

Posterior trunk (abdomen) units 1–7 with setae arranged in a uniform pattern. This pattern includes three pairs of dorsal setae, in addition to a single pair of dorsolateral and a pair of ventrolateral setae on each of the abdominal units. Each of these units also bears one or two pairs of lateral setae. These lateral setae can be quite prominent. Additionally, three (sometimes four) pairs of ventral setae arranged in a transverse row on the sternites of abdominal units 1–7 (Rozkošný 1981). The trunk end (abdomen unit 8) bears two pairs of lateral setae, which are often quite long~~,~~ .

Additionally, the trunk end bears one pair of subapical setae~~,~~ and one pair of apical setae. ~~Setae~~ The setae of both groups are usually ~~quite~~ relatively short. Dorsal setae are present but rarely prominent on the trunk end. ~~Large~~ A large anal cleft (anus) present on the ventro-terminal part of the trunk end. Around this cleft, the ventral seta is arranged in five pairs, situated along and behind the anal cleft (Fig. 1B) (Rozkošný 1981).

Summary of main 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. 2 A-C, 3 A-C, 4A-C, Supplementary figures 1,2, 3, 4, 5, 6, 7). Syninclusions: see Table 1.

Description:

Habitus. ~~Medium~~ Medium-sized larva with slightly dorsoventrally flattened, ~~spindle~~ spindle-shaped body, fully covered with oval pellets or scales (Figs. 2 A-C, 4A).

Body length 2.3-1.1 mm (n=9). ~~Body~~ The body differentiated into presumably 20 segments, ocular segment plus 19 post-ocular segments (Figs. 2 A-C, 4A). Anterior segments are forming a distinct head capsule.

The Head capsule sclerotized anteriorly, and the posterior part was 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 (Figs. 4A-C).

Ocular segment recognizable by its appendage derivative, clypeo-labrum complex. Clypeus (clypeal sclerite) longer than wide. Labrum was roughly triangular, much longer than wide, strongly sclerotized (Figs. 3A-C, 4B, C). ~~Segment~~ A segment with small apparent stemmata, posterolaterally.

Post-ocular segment 1 recognizable by its pair of appendages, antenna [antennula]. Antenna prominent, robust 25 µm long (n=1) (Figs. 4B, C)

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

Post-ocular segments 3 & 4 recognizable by its pair of appendages , mandibular-maxillary complex (Figs. 2A-C, 3A-C). ~~Proximal~~ The proximal part of fully sclerotized with strong, multi-branched setae on the dorsodistal surface, as well as laterally. Distal part, palp, conical, with two elements (palpomeres). Basal part of the complex bearing a large molar "grinder", which is occupying the almost entire ventral side of the head capsule and heavily sclerotized (Figs. 2A-C, 3B, C).

Post-ocular segment ~~5~~ five recognizable by its appendages, forming the labium, represented by a fleshy lobe.

Trunk (thorax+abdomen) with 11 visible units, interpreted as ~~3~~ three thorax segments, ~~7~~ seven abdomen segments, and a trunk end (abdomen unit 8). The trunk is yellowish-brown, except for the very first unit, which is light-yellow. ~~Cuticle~~ The cuticle is covered with oval pellets or scales. Units of the posterior trunk do, however, bear complex armament on dorsal and ventral sclerites (tergites and sternites; Figs. 1A-C).

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

~~Prothorax~~ The prothorax is 450-770 µm long (n=2), without protrusions. ~~Armament represented by oval pellets or scales~~Oval pellets or scales represents armament, and large rhombic sclerite on the sternite, with two rows of small, flat spikes arranged anteriorly on sternite. Distinct spiracle (anterior spiracle) on shallow depression at the posterolateral 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 oval pellets or scales.

Metathorax yellowish-brown, 400-660 µm long (n=2), bears two rows of triangular, flat spines on the anterior edge of sternite, as well as numerous oval pellets or scales.

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

Abdomen unit 1 rectangular in dorsoventral plain, 440-760 µm long (n=2). Bearing numerous oval pellets or scales, 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~~robust, dorsoventrally triangular spines.

Abdomen units 2-7 rectangular (370-920 µm long). Bearing numerous oval pellets or scales. Posterior edge of dorsal sclerites, tergites, each with a row of ~~very strong~~robust 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 dorsoventral view, 620-750 µm long (n=2). With three pairs of small lateral setae, two pairs of strong black setae on two mounds at the middle of the tergite; 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 a transversal cleft, ventrally. Large, transversal anal cleft, surrounded by an elevated oval sclerotized area, of a markedly darker color than the rest of the cuticle visible at the trunk end.

Morphotype 2 (Stratiomyomorpha: Stratiomyidae)

Material examined: LACM ENT 366281(5 specimens in a single piece). Most measurements are based on a single specimen, well preserved and visible in dorsal aspect. (Figs. 5A-B, 6 A-D, Supplementary figure 8 A-B).
Syninclusions: NA

Description:

Habitus. ~~Medium~~ Medium-sized larva with somewhat dorsoventrally flattened, ~~spindle~~ spindle-shaped body, covered with oval pellets of the calcium carbonate (Figs. 5 A-B).

Body covered by the white film, precluding observation of many fine details. Length 3.3-3.7 mm (n=3). ~~Body~~ The body differentiated into presumably 20 segments, ocular segment plus 19 post-ocular segments (Figs. 5 A-B, Supplementary figure 8 A, B). Anterior segments are forming a distinct head capsule.

The Head capsule sclerotized anteriorly, posterior part reduced to several longitudinal structures (unpaired metacephalic rod, paired tentorial arms), retracted into prothorax. Dimensions of head capsule: 480 µm long, 340 µm wide (n=1).

~~Surface~~ The surface of the head capsule is covered with pellets of calcium carbonate (Figs. 6 A-D).

Ocular segment recognizable by its appendage derivative, clypeo- labral complex. Clypeus (clypeal sclerite) fused with the frontal sclerite. Labrum roughly beak-like (100 µm long, 70 µm wide), much longer than wide, strongly sclerotized (Figs. 6 A-D). ~~Segment~~ A segment with small apparent stemmata, anterolaterally.

Post-ocular segment 1: not externally recognizable, possible structures (antennae) not apparent.

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

Post-ocular segments 3 & 4 recognizable by their pairs of appendages forming a mandibular-maxillary complex~~,~~ (Figs. 6 A-D). Distal lobe brown in color, distal ends chisel-like. Palp (distal part) not visible on any of the specimens available. (Figs. 5A-D).

Post-ocular segment ~~5~~ five not recognizable, its appendages, presumably forming the labium~~-~~, not visible in any of the specimens available (Figs. 6A-D).

Trunk (thorax+abdomen) with 11 visible units, interpreted as ~~3~~ three thorax segments, ~~7~~ seven abdomen segments, and trunk end (abdomen unit 8). The trunk is yellowish-brown, except for the very first unit, which is light-yellow. ~~Cuticle~~ The cuticle is covered with round deposits of calcium carbonate pellets. Trunk dorsoventrally flattened, spindle-shaped, total length 1.9-2.7 (n=3) mm long; densely covered with oval pellets or scales (Figs. 5 A-B, Supplementary figure 8 A, B).

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

Prothorax, ring-like, 240µm long, 630 µm (n=1), ~~wide~~broad, with ventral excision at the place of head capsule insertion. Small spiracles on the posterolateral surface. Prothorax bears no protrusions. Oval pellets or scales represent the aArmament~~Armament represented by oval pellets or scales~~. ~~Anterior~~ The anterior spiracle sits on a conical protrusion, ca. 35 µm long, spiracle itself with a single longitudinal opening (Figs. 5 A-B, Supplementary figure 8 A, B).

Mesothorax is 110 µm long, 780 µm wide (n=1), ring~~-~~-shaped, with no visible protrusion, bearing numerous oval pellets or scales.

Metathorax is 180 µm long, 820µm wide (n=1), ~~ring~~ ring-shaped, with one pair of the long, wavy setae.

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

Abdomen units were 1-7 wider than long (200-260 µm long; 900-1000 µm wide). All units were bearing several wavy lateral setae; unit 7 additionally bears two lateral wavy setae.

Trunk end (abdomen unit 8, undifferentiated abdomen segments 8-11?) roughly square shaped in dorsal or ventral view, (502 µm long, 525 µm wide); with two pairs of the large, wavy setae. Anal cleft sitting on large elevated mounds posteriorly on tergite (Figs. 5 A-B, Supplementary figure 8 A, B).

Morphotype 3 (Stratiomyomorpha: Stratiomyidae)

Material examined: Piece of Dominican amber with a single fossil larva from the PED collection (collection number PED-0001; Figs. 7 A-B, 8 A-D, 9, Supplementary figures 9 A-B, 10 A-B). The larva is well preserved, anterior trunk obscured ventrally by a large air bubble. Head capsule details inaccessible.

Description:

Habitus. Medium -sized larva with dorsoventrally flattened body, and triangular posterior end in the dorsoventral plain) (Figs. 7 A-B, 8 A-B, 9). Body armored with oval pellets or scales . Total length 9.5 mm. Body differentiated into presumably 20 segments, ocular segment plus 19 post-ocular segments (Figs. 7 A-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 oval pellets or scales (Fig. 7A-B).

Ocular segment recognizable by its appendage derivative, clypeo- labral complex. With 2 pairs of setae, 2 labral setae and 2 frontoclypeal setae. Clypeus continuous with labrum, clypeus narrow, labrum expanding distally (Figs. 8 A, C). Segment with pair of apparent stemmata (larval eyes).

Post-ocular segment 1 recognizable by its pair of appendages, antennae [antennula], inserting ventrolaterally at the anterior end of the head capsule (Figs. 8 B). Antenna short, consists of two elements.

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

Post-ocular segments 3 & 4 recognizable by its pair of appendages, mandibular-maxillary complex. Proximal heavily sclerotized, with basal plates. Main part lobe hook-shaped, continuous with appendages of the following post-ocular segment, integrated into the mandibular-maxillary complex. Inner surface forms longitudinal striated "molar" area (Figs. 8 A, B). Distal lobe fleshy, with numerous maxillary setae (Figs. 8 A, B).

Post-ocular segment 5 recognizable by its appendages, forming the labium . Labium bearing 3 pairs setae (2 ventral setae and 4 ventrolateral). Proximal part of labium forms a funnel connected to oral cavity. Labium distally with 2 projections, probably palps. Labium highly modified, connected to cibarial (pharyngeal) skeleton of the head capsule (Fig. 8A, B).

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

Anterior trunk, thorax with three segments, pro-, meso- and metathorax. Thoracic “leg” setae groups seemingly with 2 setae in each group (Figs. 7 A-B, 9, 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 ventrolateral and 6 ventral (Figs. 7 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 ventrolateral and 6 ventral.

Metathorax 500 µm long, with numerous setae: 6 dorsal, 2 dorsolateral, 4 lateral, 2 ventrolateral and 6 ventral setae (Figs. 6 A-B, Supplementary figures 9 A-B, 10 A-B). Posterior trunk (abdomen) with 8 apparent units flattened dorsoventrally, mostly oval in the dorsal plain, with triangular posterior hind-end, (Figs. 7 A-B, 9). Abdomen units 1-7 with numerous setae: 6 dorsal setae, 2 dorsolateral setae, 4 lateral setae, 2 ventrolateral setae 4 ventral, on each segment (Figs. 7 A-B, Supplementary figures 9 A-B, 10 A-B).

Trunk end (abdomen unit 8, undifferentiated abdomen segments 8-11?) triangular in general shape, dorsoventrally, bears well visible anus on the ventroterminal part. Carries numerous setae: ventral setae pairs v1 through v4, two pairs of anal setae and 8 dorsolateral setae. Terminal end elongated into the two rod-shaped protrusions, each carrying anal setae. No cuticular “teeth” along anal opening (Fig. 7 A, B).

Morphotype 4 (Stratiomyomorpha: Stratiomyidae)

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 posterior trunk (units 5–8) are folded upon the tergites of the more anterior ones (1–4). Coloration of specimen very well preserved (Figs. 10 A-B, 11 A-D, Supplementary figure 12 A-B, 13 A-D).
Description:

Habitus. Medium sized larva with dorsoventrally flattened body and rounded posterior end. Body armored with oval pellets or scales. Total length 6.4 mm. Body differentiated into presumably 20 segments, ocular segment plus 19 post-ocular segments (Figs. 10 A-B). Anterior segments forming distinct head capsule.

Anterior body visible in ventral perspective only, of the posterior body also the dorsal region can be seen. Body with distinct 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 (Figs. 10 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. 11 A-D, Supplementary figure 13 B).

Ocular segment Ocular segment recognizable by its appendage derivative, clypeo- labral complex. Clypeus continuous with labrum, clypeus narrow, blade-shaped (Figs. 11 A-D). With pair of apparent hemispherical stemmata (larval eyes), at about mid length of the head capsule, dorsolaterally. Segment surface bears multiple small setae.

Post-ocular segment 1 recognizable by its pair of appendages, antennae [antennula]. Antenna inserted dorsolaterally at the distal end of the head capsule (Figs. 11 A-D, Supplementary figure 13 B). Antenna short, with two elements.

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

Post-ocular segments 3 & 4 recognizable by its pair of appendages, forming the mandibular-maxillary complex. Complex with a proximal lobe, heavily sclerotized, with basal plates : the main part is a lobe hook-shaped, continuous with appendages of the following post-ocular segment. Inner surface forms longitudinal striated "molar" area (Figs. 11 A-D, Supplementary figure 13 B). Distally with fleshy lobe, with numerous setae (Fig. 11 A).

Post-ocular segment 5 recognizable by its pair of appendages, forming the labium . Labium bears 3 pairs setae (2 ventral setae and 4 ventrolateral), on the ventral and lateral surface respectively. Proximal part of labium forms a three-pronged structure, adjacent to the oral cavity (Figs. 10 A-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) (Figs. 10 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 oval pellets or scales.

Prothorax 300 µm long. Bears a pair of large spiracles (100 µm in diameter at the opening). Prothorax with several small setae on the dorsal surface (Figs. 10 A-B, Supplementary figure 12 A-B , 13 A, B)).

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

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

Posterior trunk, abdomen with 8 distinct units. Anterior seven units representing true segments. Posterior trunk mostly oval with rounded posterior hind-end (Figs. 10 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 in dorsoventral view; bears anus on ventroterminal part. No cuticular “teeth” along anal opening (Figs. 10 A-B, Supplementary figure 13 C).

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. 12 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 in dorsoventral view, 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. 12 A-B).

Trunk (thorax+abdomen) spindle shaped, parallel sided, rounded at the hind-end in dorsoventral view. Anterior part of the trunk entirely obscured by cracks. Subdivision of units unclear. Posterior trunk bear densely arranged strong setae. Trunk end (undifferentiated abdominal segments 8-11?) rounded in general shape, carries strong coronet formed by 19 unbranched setae (Figs. 12 A-B). Additionally, bears a pair of large spiracles, surrounded by this coronet of setae and upper and lower sclerotized "lips".

Morphotype 6 (possibly Stratiomyomorpha: Stratiomyidae)

Material examined: two fossil larvae on one slab from the Grube Messel, stored in the S.F. collection (collection number SF-MeI 4666; Figs. 13 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. 13 A-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 capsules more heavily sclerotized (Figs. 13 A-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 recognizable by an internally located pharyngeal grinding mill (visible in both fossil specimens; Fig. 13 C).

Trunk (thorax+abdomen) spindle-shaped, parallel-sided, rounded at the hind-end. With eleven units: three thorax segments , 7 abdomen segments plus trunk end. Units of the trunk are much wider than long. No setae preserved. No traces of spiracles or a distal coronet of setae present (Fig. 13 C).

Anterior trunk, thorax, consisting of pro-, meso-, and metathorax.

Prothorax with general outlines visible; heavily sclerotized posterior part of the head capsule can be seen retracted into prothorax (Fig. 13 A-C).

Mesothorax bears two distinct pigment dots at the hind edge (Fig. 13 A, C).

Metathorax bearing no spiracles (Fig. 13 A-C).

Posterior trunk (abdomen) with 8 units (Fig. 13 A-C).

Abdomen units 1-6 bearing distinct lines of pigmentation, 2 medially on all tergites, and 2 laterally on most tergites (Fig. 13 A-C).

Abdomen unit 7 preserves no details, only general outlines can be seen (Fig. 13 A-C).

Trunk end (Abdominal unit 8) only general outline can be seen; square in general shape, with a rounded posterior edge, when viewed in the dorsoventral view (Fig. 13 A-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 walking (“ambulatory”) legs on the thorax, as well as the spiracle arrangement. The six morphotypes differ in numerous characters; 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. 1 A-C, 2 A-C, 3 A-C, Supplementary figures 1,2, 3, 4, 5, 6, 7): larva elongated and flattened, with head, thorax and 8 abdominal units; body with oval pellets or scales, resembling calcium carbonate scales ; presence of such scales is a synapomorphy of Stratiomyidae+Xylomyidae . (Figs. 2 A-C, 3 A-C, 4 A-C, Supplementary figures 1, 2, 3, 4, 5, 6, 7). The thorax of these specimens bears oval pellets or scales, rather than hardened sclerites as in Xylomyidae (Fig. 2 B). Mandibles and maxillae are conjoined into a mandibular-maxillary complex (Fig, 4 B, C). Larvae possess a large molar grinder and a setal brush at this mandibular-maxillary complex (Figs. 4 B, C). The brush of the mandibular-maxillary complex, as indeed complex itself, is substantially reduced and simplified (Figs. 4 B, C). This condition is, however, not uncommon among extant representatives of Stratiomyidae, for example in mature larvae of Hermeteiinae and Sarginae (Rozkošný 1981).

Despite the overall similarity with larvae of Stratiomyidae, the fossil larvae of morphotype 1 exhibit several traits unknown among any modern forms of 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. 3 B). 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. 3C).

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. 2 A-C).

While the combination the features is, so far, unknown for Stratiomyomorpha; some of the characters are similar to the other Diptera larvae. Extremely elongated head capsules and large tergal spines are known in larvae of Asiloidea, especially in the groups Mydidae and Bombyliidae (Marshall, 2012). An elongated metacephalic rod is in particular common in larvae of Mydidae, Xylophagidae, Thervidae and Scenopidae (Hennig 1968, James 1981; Irwin and Leneborg 1981; Kelse 1981; Wilcox 1981). This makes the interpretation of morphotype 1 larvae relatively 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 hyper diverse groups lead to a number of “experimental” morphologies (Budd and Mann 2018 ; Haug and Haug 2019). In total we have found 13 larvae of this morphotype, 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 featuring prominent oval pellets or scales, similar to calcium carbonate nodules of modern larvae of Stratiomyidae. Therefore, we consider this morphotype as a likely ingroup within Stratiomyidae (Figs. 5 A-B, 6 A-D, Supplementary figure 9 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). Yet such autecological generalizations should be approached with caution. There are species with terrestrial larvae in groups that otherwise have mostly aquatic larvae (e.g. *Oxycera* (*Oxycera*) *leonina* (Panzer, 1798); Rozkošný 1987)). Also, the other way round, there are species with aquatic larvae in groups that generally have terrestrial larvae (e.g. *Ptecticus*; Jung et al. 2012). Therefore, morphology of the fossil alone can be an indication, but never a proof of the autecological affinities of an animal.

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 head capsule (in relation to the body), absence of the any spines on the tergites, as well as by the absence of long setae on the trunk .

Morphotype 3: This morphotype clearly has closer relationships with Stratiomyidae based on the presence of a honeycomb pattern formed by oval pellets or scales, presence of a mandibular-maxillary complex and presence of brushes on this complex. Additionally, the habitus of the larva is highly reminiscent of extant terrestrial larvae of the group Stratiomyidae (see below).

Within Stratiomyidae, the specimen can be interpreted as an ingroup of Pachygastrinae based on the following combination of characters: absence of a coronet of so-called 'hydrofuge' setae on the trunk end; larva uniformly colored; trunk tergites with transversal rows of 6 setae each; labium not sclerotized and weakly developed; dorsal part of the mandibular-maxillary complex sclerotized; small larva , less than 10 mm (Rozkošný 1981, 1982). 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. 7 A, B, 9).

In general, the larva is relatively unusual 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 Rozkošný 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 (Supplementary 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). It is important to note however, that the specimen is rather poorly preserved, and identification should be seen rather as approximation of the phylogenetic affinity rather than final conclusion.

Morphotype 4: This morphotype clearly has closer relationships with Stratiomyidae based on the presence of a honeycomb pattern formed by oval pellets or scales, presence of a mandibular-maxillary complex and presence of brushes on this complex. Additionally, the habitus of the larva is highly reminiscent of extant terrestrial larvae of the group Stratiomyidae (see below). Within Stratiomyidae, further identification is impossible, due to the insufficient preservation of the specimen. The habitus in general, is reminiscent of terrestrial larvae of Stratiomyidae, i.e. from the ingroup Pachygastrinae (Rozkošný 1981).

Considering the exceptional preservation of this cuticle fossil, it is important to remember the possibility of contamination of the geological record by modern day holometabolan larvae, in particular fly larvae (Rasnitsyn 2008). Fly larvae are known to crawl into 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 from a fresh split rock sample and an imprint was observed on the negative (unfortunately not collected). Still, we cannot entirely rule out that this larva is an extant contamination of the shale (Gašparič and Hyžný 2015).

Morphotype 5: This morphotype seems to be a representative of Stratiomyidae , probably of the ingroups Stratiomyinae, Raphiocerinae or Nemotelinae, based on the presence of a coronet of “hydrofuge “setae (Rozkošný 1981; Pujol-luz et al 2004). 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).

Morphotype 6: This morphotype is represented by two very poorly preserved fossils; therefore, no definitive statement on its phylogenetic affinity can be made. Nevertheless, we decided to include it here, due to the overall similarities in the body shape and presence of the coloration patterns of cuticle, like those in i.e. larvae of the group *Odontomyia* or other representatives of Stratiomyidae (Supplementary figure 14). For these reasons we think that it is prudent to consider this as a probable fossil of the Stratiomyidae, though there are no definite ways to further support this. This morphotype is too poorly preserved for any detailed systematic interpretation. Not much more information could be gained from the larvae, since the poorly preserved body falls into the “unknown” habitat category of the morphospace.

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 (Baranov et al., 2019), their fossil records can provide a wealth of paleo-ecological information. Hence these new larval stratiomyomorphan specimens widen our understanding of the respective paleo-ecosystems from which they originated. Even on the adult side, representatives of Stratiomyidae are rare in the fossil record, with only 73 occurrences (specimens) having ever been recorded (according to PBDB, for the search parameters see Methods). This number is however excluding representatives of a unique, extinct group of flies with the long proboscides (Zhangsolvidae), known from the Early Cretaceous of China and Brazil, as well as Late Cretaceous of Myanmar (Peñalver et al. 2015). These flies have emerged as important pollinators of the gymnosperm plants in Cretaceous (Peñalver et al. 2015).

It is common for many organisms living in water to leave traces of their activity. Hence it should not be surprising that in the deep past ichnofossils provide most of the geological record of larval activity of Stratiomyidae, rather than body fossils. The most common example is the Jurassic "ichnogenus" *Helminthopsis* Heer 1877. It was interpreted as originally caused by larvae of soldier flies of the group *Stratiomys* Geoffroy, 1762 or at least a closely related species (Mángano, Buatois, and Claps 1996). This expands the potential range of the geological record of the group from the Barremian (Cretaceous) to the mid Jurassic (Mángano, Buatois, and Claps 1996; Pickerill, Han, and Jiang 1998). Body fossils of stratiomyomorphans, as mentioned, are rare. All known larval fossil records are listed in Table 1, together with the material used in this contribution. Myanmar 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 this amber . Both morphotypes are characterized by features intermediate between two stratiomyomorphan ingroups , Stratiomyidae and Xylomyidae (Liu, Hakim, and Huang 2020). Such chimera-like characteristics are also apparent in the one of the new morphotypes, namely morphotype 1. It can be interpreted as a result of the "Push of the past" phenomenon (Budd and Mann 2018). In contrast to morphotype 1, morphotype 2 from Myanmar amber has a much less conspicuous morphology, and seemingly is a representative Stratiomyidae s. str. as characterized by Hauser, Woodley, and Fachin (2017).

The record from Dominican amber, morphotype 3, is only the third record of the group Stratiomyomorpha from this otherwise very productive deposit (Grund and Hauser 2005). Only two specimens 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 precluding 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 another ingroup of Diptera: Chironomidae (non-biting midges). For

Chironomidae , there are more fossil species known from Dominican amber then there are extant species on the entire island of Hispaniola (Grund 2006). The situation of 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- sized 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).

The cuticle fossil from Činžat (morphotype 4) originates from deep-water, low energy sedimentary environment (Gašparič and Hyžný 2015). It is difficult to explain how a larva of seemingly terrestrial Stratiomyidae ended up there. One possible explanation could be that the specimen drowned with driftwood and other terrestrial debris (which are present in the deposit) after a storm event.

The larva from Baltic amber is poorly preserved, and only identifiable as a larva of a soldier fly by the presence of the coronet of setae on the rear end. Overall, the fossil resembles extant aquatic larvae of the group *Odontomyia* (Supplementary figure 14 A, B), however, there are not enough diagnostic characters for a conclusive identification (also see discussion above). It is still conceivable that this specimen represents a species of Stratiomyidae with an aquatic larva. There are several larval forms of Insecta that have an aquatic lifestyle and have been recorded from Baltic amber. This includes immature of Odonata (damselflies), Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) (Wichard, Gröhn, and Seredszus 2009). Martínez-Delclòs, Briggs, and Peñalver (2004) suggested that aquatic larvae 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 represent a rare find of aquatic insect larvae from this deposit. Unfortunately, the larvae are too poorly preserved for the detailed interpretation. Aquatic insects are generally rare in the oil shale of Messel and in other maar lake deposits, because the fossil-bearing sediments (the so-called oil shale) formed only in the deeper parts of the former maar lake, not in its shore-region (Wedmann 2018). Aquatic insects, such as some larvae of Stratiomyidae 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 the oil shale 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 (Haug et al. 2020b). 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 accessing the diversity of ecomorphotypes of a group of organisms by examining body outlines. Here we used all available fossil and extant Stratiomyomorpha 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 analyze 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 are occupying three main types of habitats: 1) aquatic, 2) terrestrial, mostly upper soil, leaf litter, and lower vegetation, and 3) living in wood, hence a saproxylic lifestyle. Many of the extant larvae of Stratiomyomorpha, in particular larvae of Pachygastrinae, are terrestrial saprotrophic and live under the bark of the dead wood (McFadden 1967; Marshall 2012).

Larvae of Pantophthalmidae are saproxylic, inhabiting living wood (Marshall 2012). Many other larvae of Stratiomyidae (i.e. not those of Pachygastrinae), are occupying aquatic habitats. 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 are less clear (Whalley and Jarzembowski 1985; Liu, Hakim, and Huang 2020).

We have attempted to compare ecomorphotypes of the extant aquatic , 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 larvae, roughly circular to oval in the cross-section, as larvae of *Stratiomyia*, *Oxycera*, *Odontomyi*a, 2) terrestrial and saproxylic larvae with spindle-shaped or cylindrical bodies (Figs. 14 A, B). Analyses of the shape distribution in morphospace have shown that thickness of the body and shape of the body at the ends are determining separation of the morphotypes. These two characteristics of shape are of the predominant importance, as they are making major contributions into the Principal components (P.C.) 1 and 2. These two P.C.s are explaining 36.1% and 21.2% of the shape variability respectively (Figs. 14 A, B and Supplementary figure 15). It is important to note however, that no significant separation between the morphotypes exist, as ascertained by a MANOVA test. P.C.1 and P.C.2 components have $p>0.05$, when the type of the habitat is used as an independent variable for morphotypes separation. This is also not surprising as “aquatic” and “terrestrial” groups of the larvae are overlapping broadly in the general shape, and the “saproxylic” larvae morphotype is deeply nested in the “terrestrial” morphospace (Fig. 14 A, B).

Ecomorphology of fossil stratiomyomorphan larvae.

The fossil larvae are widely distributed in the stratiomyomorphan morphospace (Figs. 14 A, B). Most Cenozoic larvae (from Slovenian shale, 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 .

Morphotypes 2 , 3, 4 as well as the larvae from Liu et al. (2020) firmly fell into the part of the morphospace occupied by modern terrestrial ecomorphotypes (Figs. 14 A). The larva from Baltic amber plots into the “aquatic ” type habitats, as did the specimens from morphotype 1, due to their elongated body(Fig. 14 A). Despite that, we are hesitant to claim that morphotype 1 larvae are aquatic. Specimens of this morphotype are lacking the tell-tale characteristics of (most) extant aquatic stratiomyomorphan larvae, the coronet of “hydrofuge” setae (Rozkošný 1991). Additionally, the extremely high abundance of morphotype 1 larvae (at least by the standards of the dipteran larvae in an amber deposit) can be explained by a possible close association with tree trunks . It is possible that these larvae lived under the bark of trees, as seen in many extant larvae of Stratiomyidae (McFadden, 1967, Marshall, 2012). It is well known, that organisms associated with tree trunks in the amber forests had higher chance of being preserved in amber (Solorzano-Kraemer et al 2018). On top of that, a rich set of the syninclusions present in the amber piece PED-0031 together with morphotype 1 larvae is pointing towards the terrestrial environment (Supplementary figures 2, 3). Such syninclusions include: a mite, a possible scale insect, parts of other representatives of Insecta, a fly of the group Bibionomorpha, a beetle larva, a spider and a millipede. This strongly indicates a terrestrial environment for morphotype 1 larvae .

Only one of the morphotypes described by Liu et al. (2019) falls outside of the morphospace occupied by the extant Stratiomyomorphan larvae (Fig. 14 B, Supplementary figure 15).

Our analysis has shown that the morphospace of stratiomyomorphan larvae has become significantly larger over time. Only a small part of the occupied area of the morphospace was lost, when we consider the general body shape . We think that increase in the morphospace size of the Stratiomyomorpha can be explained by the gradual diversification of the group from the Late Cretaceous onwards as it was shown by Wiegmann et al. (2011).

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 palaeoecology 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 Paleogenic ambers as well as in other types of fossil deposits . Further in-depth studies of amber and compression fossils collections will certainly lead to more new discoveries pertaining to larval biology of Stratiomyomorpha and other groups of Diptera.

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List of figures

Figure 1. Morphology of larva of the group Startiomyidae, exemplified by a larva of *Pachygaster atra*. A) Ventral view, marked; B) dorsal view, marked. Abbreviations: *a1-a7* – abdomen units one through seven, ap – anal setae, asl – anal slit, as anterior spiracle, D1-D3 – dorsal setae 1-3, DL –dorsolateral setae, ep – eye prominence, hc – head capsule, L – lateral setae (of abdomen unit), L1-L2 – lateral setae (of trunk end), mp – maxillary palp, mt – metathorax, pt – prothorax, sa – subapical setae, v1-v4 – ventral setae one – through four (of the trunk end), VL – ventrolateral setae (of the abdomen units 1-7).

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

Figure 3. Larva head of morphotype 1, specimen PED-0031-2. A) Lateral view; B) Lateral view, marked; C) Lateral view, line drawing. 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 4. 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 5. 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.

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

Figure 8. A-B) Fossil larvae of the group Pachygastrinae, morphotype 3, PED-0462 and C-D) head of larva of the extant species *Pachygaster atra* . A & B) Ventral view of the head capsule

unmarked and marked; C & D) Ventral view of the head capsule unmarked and marked; Abbreviations: *an* –antenne; *as* – anal setae; *pt* – prothorax, *ey* – eyes; *lb* – labrum; *mp* – maxillar palp; *mb* – base of mandibular-maxillar complex (“grinder”); *v1*-3 – ventral setae 1-3, *la* – labium.

Figure 9. Speculative reconstruction of the habitus and habitat of the fossil larvaof the group Pachygastrinae, morphotype 3. Onychophora *Tertiapatus* sp stalking at the background. Artwork by Christian Mccal, reproduced with permission.

Figure 10. 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 end, *ps* – posterior spiracle, *fc* – folded cuticle.

Figure 11. 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 12. Stratiomyinae, larva, morphotype 5 (PED-0464). A) Habitus; B) Close-up photo of coronet of the “hydrofuge” setae.

Figure 13. Stratiomyinae, larvae, morphotype 6 (SF-MeI 4666). A) compression fossil, habitus; B) compression fossil, marked; C) overview. Abbreviations: *hc* – head capsule; *phc* – pharyngeal grinding mill; *pt* –prothorax; mt – metathorax; *a1-a6* – abdominal units; *te* – trunk’s end.

Figure 14. Ecomorphospace occupied by extant and fossil larvae of the group Stratiomyomorpha . Both plots presenting the same morphospace, split by different grouping variables. Total captured variation = 57.3%; 36.1% at PC1 and 21.2 % at PC2. A) Morphospace split by larval habitat: green – saproxylic, violet – terrestrial, blue – “unknown” (fossils), red – aquatic; B) Morphospace split by the geological age/deposit of the larvae: blue – extant, magenta – Myanmar amber, the rest of the deposits are represented by the single labelled dots.