

Early adaptations of true flies (Diptera) to moist and aquatic continental environments

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Abstract: Insect colonization of continental aquatic ecosystems and their immediate surroundings was paramount for the establishment of complex trophic nets and organic-matter recycling in those environments. True flies and other insects such as mayflies developed crucial ecological roles in early continental aquatic ecosystems, as early as the Triassic. However, the mode and tempo of these processes remain poorly known, partly due to a critical fossil record gap before the Middle Triassic. Here we study the dipterans from the early Middle Triassic Konservat-Lagerstätte of Pedra Alta (Aegean, early Anisian, Spain), which yields the oldest records of the order. *Protoanisolarva juarezi* gen. et sp. nov., based on an exceptionally preserved larva, shares key features with the extant nematoceran family Anisopodidae. Developing in inferred moist terrestrial environments contiguous with pools inhabited by aquatic organisms, it represents the only known

Triassic dipteran larva with terrestrial affinities indicating that the amphipneustic respiratory system of insect larvae extends back to c. 247 Ma. Two nematoceran aquatic pupae are also described: one classified as *Voltziapupa* cf. *cornuta*, and the other as an indeterminate taxon. Finally, an egg cluster belonging to the ootaxon *Clavapartus latus* is likely to have been produced by chironomids. These eggs were included in a mucilaginous matrix, a probable adaptation against predation and/or changing conditions, including desiccation. These new findings provide key data on the early evolutionary history of the mega-diverse order Diptera, the ecology of their ancestral pre-adult forms, and the functioning of early Middle Triassic continental aquatic ecosystems.

Key words: insect evolution, palaeoecology, taphonomy, egg-laying, Diptera, Mesozoic.

STUDIES on insect diversity are traditionally biased towards adult forms (Smith 1989). However, fossils representing larval, nymphal or pupal stages are not rare in compression deposit assemblages. These fossils of pre-adult stages have an aquatic origin and were produced under a set of circumstances often conducive to an abundant record of aquatic insects with exceptional preservation, mostly body remains or products of activity or life cycle such as exuviae (e.g. Gall 1972; Peñalver 2002; Martínez-Delclòs *et al.* 2004). Aside from a few exceptional cases (e.g. Fleck *et al.* 2002; Wang *et al.* 2015), recognizing conspecific pre-adult and adult forms in the fossil record is challenging, if not virtually impossible, and will always remain conjectural to a certain extent, even if the two forms from a given taxonomic group are recorded in the same deposit. In addition, formal taxonomic description of pre-adult stages of insects is generally desirable due to

the key palaeoecological and/or phylogenetic data that it can provide (e.g. Lukashevich *et al.* 2010; Chen *et al.* 2014). This is particularly true in insect groups, in which adults play a limited role in trophic nets, and might not even feed at all. However, the study of larval stages is one of the most overlooked topics in palaeoentomology (Peñalver & Pérez-de la Fuente 2014). Knowledge on these pre-adult stages is crucial, for example, to reconstruct the organic matter recycling in continental aquatic ecosystems and surrounding areas (e.g. Sinitshenkova 2003; Peñalver *et al.* in press) and the structure of trophic nets (e.g. Peñalver & Gaudant 2010). The study of fossil pre-adult forms is especially useful for phylogenetic analysis in some groups (Lukashevich 2008).

Triassic dipterans, both pre-adults and adults, are of particular interest in palaeoentomology because they are the oldest representatives of one of the four megadiverse insect

orders; in fact, arguably the most ecologically disparate insect order (Grimaldi & Engel 2005). Nevertheless, dipterans are not abundant in the few Triassic outcrops yielding insects that have been discovered to date. The Konservat-Lagerstätte of 'Grès à Voltzia' from the Vosges (France) provided a first view of the earliest insect groups to inhabit continental aquatic ecosystems, including the oldest dipterans hitherto known. A detailed study of these specimens enabled the first comprehensive view of early Triassic Diptera as pre-adult stages (Lukashevich *et al.* 2010). Although aquatic dipteran pupae were found abundantly and well preserved in this locality, larvae were scarce and did not provide sound morphological characters to enable a reliable placement at familial level, or even at infraordinal level, and were considered as Culicomorpha indet. (*Chironomoidea incertae familiae*) (Lukashevich *et al.* 2010). This circumstance prevented critical palaeoecological inferences based on the pre-adult forms of dipterans from Grès à Voltzia. Several adult dipteran taxa were also described from this locality (Krzemiński *et al.* 1994; Krzemiński & Krzemińska 2003). Moreover, a new species assigned to the Mesozoic dipteran family Nadipteridae was recently described based on an isolated wing from the Röt Formation of Lower Franconia (Germany), roughly coetaneous to Grès à Voltzia (Bashkuev *et al.* 2012; Lukashevich 2021). Triassic Diptera have also been found elsewhere, such as in Great Britain or Argentina (Krzemiński & Krzemińska 2002; Lara & Lukashevich 2013).

Outside of the Central European Basin, some authors (Calafat 1988; Shcherbakov *et al.* 1995) noted the possible presence of an adult dipteran specimen from the Pedra Alta locality (Cala d'Estellencs, Mallorca, Balearic Islands, Spain), Anisian in age. That specimen is in a historical private collection (Tomeu Sáez collection in Binissalem, Mallorca) and was obtained during the early 1980s, before the establishment of laws regulating the fossil collection in Spain. However, a recently published review (Matamalas-Andreu *et al.* 2021) identified it as a badly preserved mayfly with strongly folded wings. A small dipteran wing portion from Cala d'Estellencs, currently kept in a private collection in Germany, was figured by Zessin (2008). This specimen was collected under irregular circumstances together with other insect remains in 2007 and has not been assessed for the present work.

Based on material extracted during legal palaeontological excavations in 2019 and 2021, we herein describe terrestrial and aquatic pre-adult forms of dipterans from

Pedra Alta: a larva and two pupae, respectively, as well as an egg cluster. These dipteran fossils represent the oldest known records for the entire order, and provide key insights into the early evolution of Diptera and the ecology of early Middle Triassic ecosystems.

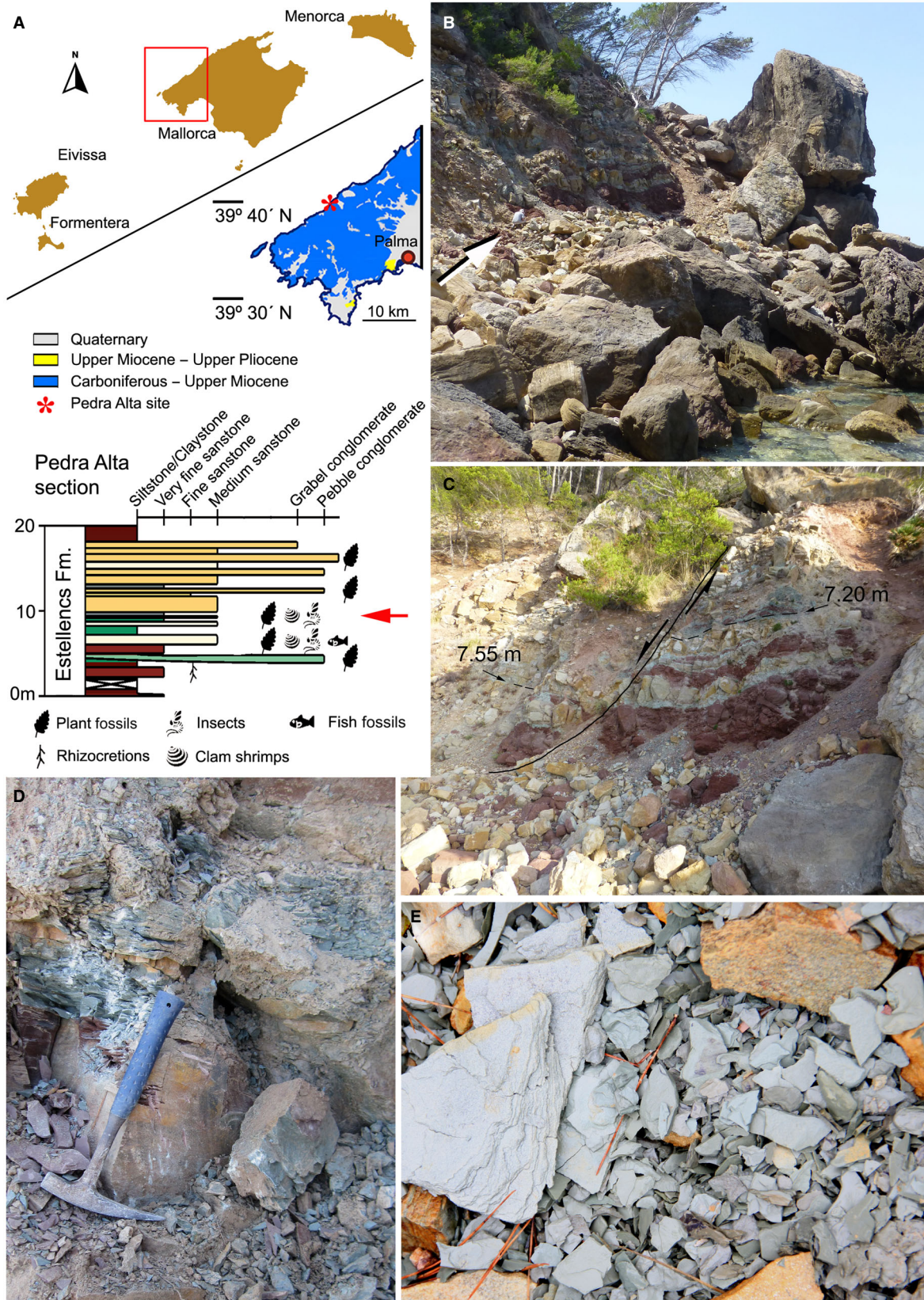
GEOGRAPHICAL AND GEOLOGICAL CONTEXT

Mallorca is the largest of the Balearic Islands (Fig. 1), an archipelago located in the western Mediterranean that was uplifted during the Alpine Orogeny. It is essentially structured in several horsts and grabens of broad south-west–northeast orientation. The horsts correspond to the main three mountain ranges of the islands, with rocks that are Carboniferous to Middle Miocene in age, and the grabens are the main Cenozoic basins, with rocks and sediments that range from the Upper Miocene to the Holocene. Triassic outcrops of continental origin are scarce and limited to some cliffs in the northwest margin of the Serra de Tramuntana, between Cala d'Estellencs and Punta de son Serralta (Estellencs municipality). Matamalas-Andreu *et al.* (2021) recently described four lithostratigraphic formations that encompass this succession (from stratigraphically lower to upper): Punta Roja Formation, Estellencs Formation, Pedra Alta Formation and Son Serralta Formation. These were dated as ranging from the upper Olenekian to the lower Anisian (Matamalas-Andreu *et al.* 2021; Scholze & Matamalas-Andreu 2021).

The dipteran fossils herein studied were collected from several horizons in the Pedra Alta site (Fig. 1; see Matamalas-Andreu *et al.* 2021; Scholze & Matamalas-Andreu 2021, for further details). This site has been correlated to the upper part of the Estellencs Formation (Fig. 1A). The fossils have been found in green siltstone beds (sometimes slightly sandy) with sub-horizontal lamination. These have been interpreted as deposits formed in environments of low-energy to still waters, corresponding to backswamps and pools among sand bars of abandoned channels of the braided river system during the low-water phase (Matamalas-Andreu *et al.* 2021).

The fossiliferous beds of the Estellencs Formation of Mallorca are slightly older than the Grès à Voltzia

FIG. 1. The Pedra Alta site and section, lower Anisian. A, simplified geological map of the west end of the island of Mallorca (Balearic Islands), with the position of the study area and Pedra Alta site, and simplified stratigraphic log of the Pedra Alta section (simplified from Matamalas-Andreu *et al.* 2021); red arrow indicates the level at which the fossil dipterans were found, and colours in the stratigraphic log indicate variable rock colouration. B, panoramic view of the outcrop of Pedra Alta (arrow indicates a member of excavation team). C, detail of fossiliferous levels with indication of a normal fault and the 7.20 m level at which the anisopodoid larva was found. D–E, detail of level of green claystones with sub-horizontal lamination, and aspect of fossiliferous slabs of green claystones after weathering, respectively.



deposits (Vosges), which are well known for their rich record of Anisian fossil insects. The age of the Estellens Formation is essentially constrained by the biostratigraphic data obtained from sporomorphs and clam shrimps. The sporomorphs were studied by Diez *et al.* (2010), who revised some samples collected at the base of the overlying Pedra de s'Ase Formation (Diez 2000; Matamales-Andreu *et al.* 2021) and considered them Aegean in age. The clam shrimps were studied by Scholze & Matamales-Andreu (2021), who identified the species *Hornestheria* aff. *sollingensis* from the same beds as the insects, suggesting an uppermost Olenekian to lowermost Anisian age (Sollingensis Zone) for those beds. In contrast, the age of Grès à Voltzia is more challenging to determine. It is widely accepted that it represents a diachronic facies, younger towards the west, and it has been broadly considered 'lower Anisian' (Durand & Jurain 1969; Gall 1971; Bourquin *et al.* 2006). The most precise age attribution was provided by Kozur & Weems (2010), who considered that the Grès à Voltzia in the Vosges Mountains could be dated as lower Bithynian because of the presence of the clam shrimp *Euestheria albertii albertii* (Albertii albertii Zone). Given that this species appears in the same localities as the insects (cf. Gall 1971; Sinitshenkova *et al.* 2005; Lukashevich *et al.* 2010), the insects can also be considered lower Bithynian in age. All of the above indicates that the specimens from Mallorca are slightly older than those from the Vosges, up to c. 1.9 Ma, based on the absolute age attributions for the boundaries of these substages given by Ogg *et al.* (2020). The Röt Formation of Lower Franconia, from which a nadipterid species has been recently described (Bashkuev *et al.* 2012; Lukashevich 2021), is correlated with Grès à Voltzia following the presence of the bivalve *Myophoria vulgaris*, and thus it is also dated as Bithynian (Kozur & Weems 2010; Bashkuev *et al.* 2012).

MATERIAL AND METHOD

The specimens studied herein were found during two palaeontological excavations (further details in Matamales-Andreu *et al.* 2021) and are housed at the Museu de Mallorca (Palma, Mallorca, Balearic Islands, Spain), with an abbreviation in the form: DA21/[campaign number]-[horizon number]-[specimen number].

All of the specimens were moistened with a mixture of alcohol and water for examination, photography and drawing, to enhance the contrast between the carbonaceous parts and the light-coloured rock matrix and thus facilitate the observation of their minute morphological details. It is important to note that moistening caused damage to some of the tiny carbonaceous structures such as the spiracles in the larval specimen, which underwent fracturing and

shrinkage, and therefore its use was strongly limited. After examination, the head and spiracles of the part of the larval specimen were consolidated using PRIMAL™ SF-016 ER Acrylic Emulsion Polymer. Photographs (habitus) were taken with the digital camera Canon EOS 650D using Macrofotografía version 1.1.0.5 (IGME-CSIC, Madrid); the software created composite images by integrating photographs obtained at sequential focal planes. Microphotographs of anatomical details were taken with a ColorView IIIu Soft Imaging System digital camera attached to an Olympus BX51 microscope (IGME-CSIC, Madrid). An Olympus BX53 compound microscope equipped with a camera lucida tube was used to create the drawings (IGME-CSIC, Valencia). Photography was enhanced in Photoshop CS2 v9.0 to increase contrast; composite figures were prepared using the same software.

The literature followed for the general description of the larval specimen was Fuller (1935), Keilin & Tate (1940), Peterson (1981), Smith (1989) and Hancock & Amorim (2009). We follow the divisions of the superfamily Anisopodoidea by Krzemiński & Krzemińska (2003) and the conservative approach for the family Anisopodidae by Amorim *et al.* (2016). Description of the pupae follows Lukashevich *et al.* (2010), and that of the egg clusters follows Gall & Grauvogel (1966).

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SYSTEMATIC PALAEONTOLOGY

by Enrique Peñalver, André Nel & Ricardo Pérez-de la Fuente

Order DIPTERA Linnaeus, 1758

Suborder NEMATOCERA Schiner, 1862

Infraorder BIBIONOMORPHA Hennig, 1948

Superfamily ANISOPODOIDEA Knab, 1912

Stem-group ANISOPODIDAE Knab, 1912

Genus PROTOANISOLARVA nov.

LSID. <https://zoobank.org/NomenclaturalActs/69BBB680-8083-46F8-9AFA-AC2D8CD2269F>

Derivation of name. *Protoanisolarva* refers to the oldest known larval stage of anisopodoid flies, using the prefix 'proto', meaning 'first' or 'foremost'. Gender: feminine.

Type species. *Protoanisolarva juarezi* sp. nov. by monotypy.

Diagnosis. Larva. Body glabrous, lacking conspicuous processes. Abdomen 8-segmented, with anterior intercalary segments; segment VIII the widest, longer than the two previous combined.

Anterior spiracles circular in shape, with a curved row of about six primary openings, circular or subcircular in shape. Segment VIII barely divided, its perianal shield covering the complete ventral surface. Posterior spiracles terminal, subcircular to circular in shape, with over 30 primary circular openings, arranged around the entire perimeter of an inner, heavily sclerotized disc. Posterior spiracles present on a short and broad cuticular process, in a distal position on that process.

Protoanisolarva juarezi sp. nov.

Figures 2, 3, S1

LSID. <https://zoobank.org/NomenclaturalActs/7F029393-98E2-422D-A473-9C721C7B6B05>

Derivation of name. The specific epithet honours Mr Josep Juárez, from Mallorca, an expert on Mallorcan palaeontology and finder of the holotype specimen.

Holotype. Virtually complete, exceptionally preserved larva as part (preserving the ventral side of the head) and counterpart (preserving the dorsal side of the head), DA21/03-02-91a,b, in small slabs of claystone (part 5.0 × 3.8 cm, counterpart 4.1 × 3.8 cm). The fossil larva in the part was accidentally damaged in the margin of the last abdominal segments in an attempt to prepare the specimen (the same area is not preserved in the counterpart due to imperfect rock exfoliation). The virtually complete posterior spiracle was also damaged as a result of the alcohol–water wetting cycles applied during observation and photography. The holotype, in two slabs, is housed at the Museu de Mallorca (Palma, Mallorca, Balearic Islands, Spain).

Diagnosis. As for the genus, with the following additional character: abdominal segments broader than long.

Type locality. Pedra Alta site, Cala d'Estellencs, Mallorca, Balearic Islands, Spain; Pedra Alta section (7.20 m), upper part of Estellencs Formation (Fig. 1A), Aegean (lower Anisian).

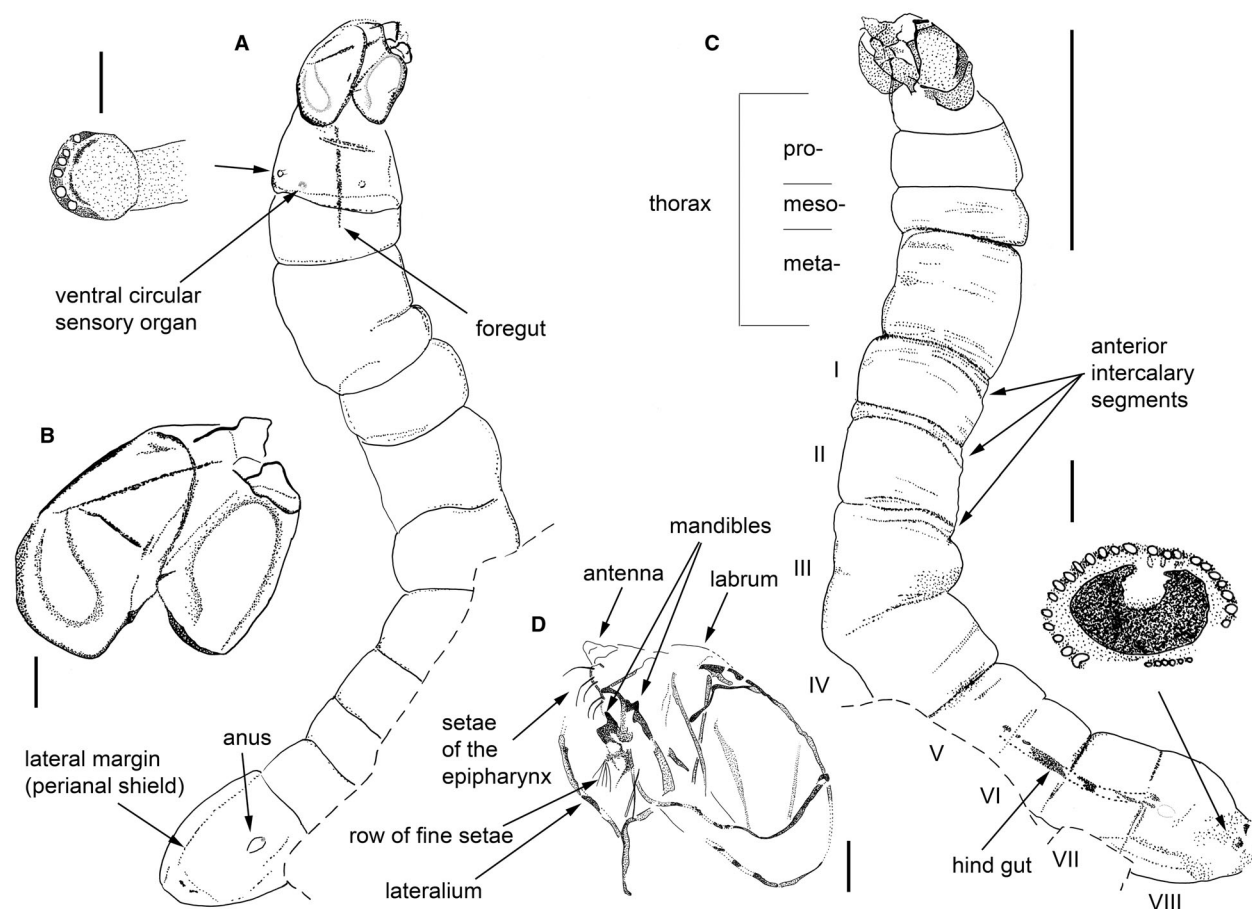


FIG. 2. Triassic terrestrial larva *Protoanisolarva juarezi* gen. et sp. nov. (Diptera, Anisopodoidea, stem-group Anisopodidae), holotype DA21/03-02-91, from the Pedra Alta site, lower Anisian. Camera lucida drawings. A–B, habitus, counterpart (DA21/03-02-91b): A, one of the anterior spiracles enlarged; B, detail of head, showing the dorsal surface. C–D, habitus, part (DA21/03-02-91a): C, one of the posterior spiracles enlarged (one preserved spiracle is complete but some small portions are present in the counterpart); D, detail of head, showing ventral and inner structures. I–VIII indicate abdominal segments. Scale bars represent: 0.02 mm (detail in A, detail in C); 0.1 mm (B, D); 1 mm (C).

Description. Body long and narrow, 4.8 mm long, 0.6 mm greatest width. Body slender, glabrous, and lacking minute spicules, projections, tubercles or conspicuous fleshy processes. Body segments weakly sclerotized, without evident colour patterns.

Head capsule well developed (eucephalic larva), probably not capable of retraction into thorax due to its shape and size (Fig. 2), 0.6×0.5 mm, subconical, heavily sclerotized (Figs 2D, 3B, S2B), more sclerotized dorsally than ventrally as evidenced by differential fossilization of head in part (ventral body surface) and counterpart (dorsal surface). Antennae allegedly the visible anterior pair of sclerotized processes, short and without sensory structures. Eye spots probably arranged in a way preventing their observation in the position of fossilization of the carcass. Sensory pits not visible. Labrum apparently without setae. Lateralities with strongly sclerotized margins. Some preserved tentorial rods running longitudinally, their connections to other structures unclear. Head with a preserved row of setae in a ventroanterior position, most probably corresponding to the epipharynx. Mandibles strongly sclerotized and apparently with a single tooth, having a basal piece with a row of fine setae or at least this row very close to mandible base. Maxillae not visible. Labium apparently not preserved.

Thoracic segments not fused and lacking prolegs. Prothoracic segment slightly broader than long, with a narrow anterior intercalary segment (pseudo-segmentation), bearing the anterior spiracles and a ventral circular structure lacking setae tentatively interpreted as a sensory organ (Fig. 3C). Anterior spiracles circular in shape (small, 0.03 mm in diameter), with a distal curved row of around six primary openings circular/subcircular in shape (Figs 2A, 3C); remains of two spiracles close to the posterior limit of prothorax (visible only in counterpart, mostly preserving dorsal features). Mesothoracic segment distinctly short. Metathoracic segment as long as wide. Thorax and first abdominal segment well separated.

Eight abdominal segments, broader than long, lacking prolegs and setae. Abdominal segments I–VII lacking spiracles. Each abdominal segment with an anterior intercalary segment (Fig. 2C). Remains of foregut (part) and hind gut (counterpart; visible in abdominal segments V–VIII, although portion in segments V and VI may correspond to the midgut) present. Segment VIII barely divided (apparently with only a weak terminal division), with a perianal shield on the complete ventral segment surface as evidenced by a markedly thick lateral margin on the segment (Fig. 2A). Segment VIII the widest and the longest (longer than two previous segments combined). Anus anteroposteriorly elongated, wide in anterior apex and acute in posterior one, 0.11×0.05 mm (Fig. 3D). Posterior spiracles terminal (one exceptionally preserved and showing its features, the other preserved in a very lateral position, thus with its features strongly distorted and not well discernible), close together, subcircular to circular in shape (not crescent-shaped or oval-shaped as observed in extant Anisopodidae); with 34 primary openings preserved (counted in both part and counterpart), but c. 37 estimated, circular in shape, visible around an inner heavily sclerotized disc in its entire perimeter as noted by superposition of its preserved remains in part and counterpart (Fig. 2C). Posterior spiracle area lacking fleshy lobes or setae, but posterior spiracles

located distally on a cuticular process, apparently in the shape of a conical frustum (Fig. 3F). Entire abdominal segment VIII devoid of setae. Body apparently terminating in a short conical process (Fig. 2C) as in the extant anisopodid genus *Olbiogaster* (Keilin & Tate 1940).

Remarks. This fossil corresponds to a holometabolous insect due to the presence of three well-separated tagma (head, thorax and abdomen). The vermiform body lacking thoracic legs and subcylindrical in shape is typical of dipteran larvae (Stehr 1991). The absence of thoracic legs allows separation from many holometabolous larval types such as the eruciform larvae of Lepidoptera, Mecoptera, or 'symphytan' Hymenoptera (Teskey 1981). Coleoptera larvae lacking thoracic legs have body shapes deviating from the subcylindrical shape, often with enlarged thoraces or more grub-like bodies (Stehr 1991). Although larvae of apocritan Hymenoptera also lack thoracic legs, these are unknown in the Triassic and live in specialized environments (parasitoids on or in other insects, in galls, in nests etc.). They are quite unlikely to be found in a Triassic lacustrine environment, given that they are also unknown as compression fossils of more recent periods.

Following the key to the fly families of Afrotropical larvae of Borkent & Sinclair (2017), the present fossil would fall in the Anisopodidae because of the following characters: well-differentiated mouthparts, moving against one another obliquely or in horizontal plane (not parallel to one another); head capsule complete and eucephalic, not longitudinally excised dorsally; head, thorax and first abdominal segments distinct; amphipneustic respiratory system (spiracles on prothorax and abdominal segment VIII only); no elongate respiratory siphon at apex of abdomen; thoracic segments individually distinguishable, equal in diameter to widest abdominal segments; prolegs absent on thorax and abdominal segments; terminal abdominal segments without any processes; prothorax and abdominal segments with distinct annulus anteriorly.

Although the anatomical characters of *Protoanisolarva juarezi* generally agree with those of extant anisopodid larvae (Keilin & Tate 1940; Peterson 1981; Smith 1989; Hancock & Amorim 2009), it possesses some peculiarities such as the features of the posterior spiracles, the anus morphology, and some proportions of the body segments. The inner heavily sclerotized disc of the posterior spiracles could be related to the 'dense black external scar near the middle of the concave side of the spiracular tubercle' described by Keilin & Tate (1940) in the extant anisopodid species *Olbiogaster africanus*. It is important to note that the quantities of primary openings in both anterior and posterior spiracles fall within the variability observed in extant species of the family Anisopodidae. The anterior spiracles of *P. juarezi* have around six primary openings. In extant species of the family these spiracles usually have between three and 19 primary openings (Peterson 1981), although Fuller (1935) figured 24 openings in *Olbiogaster insularis*. The posterior spiracles of the fossil have 37 estimated primary openings, but in the extant genera *Olbiogaster*, *Sylvicola* and *Mycetobia* there are only 12–25 (Peterson 1981). Nevertheless, Fuller (1935) figured 35 and 36 primary openings in the posterior spiracles of *Anisopus dubius* and *Olbiogaster insularis*, respectively. A placement in the crown

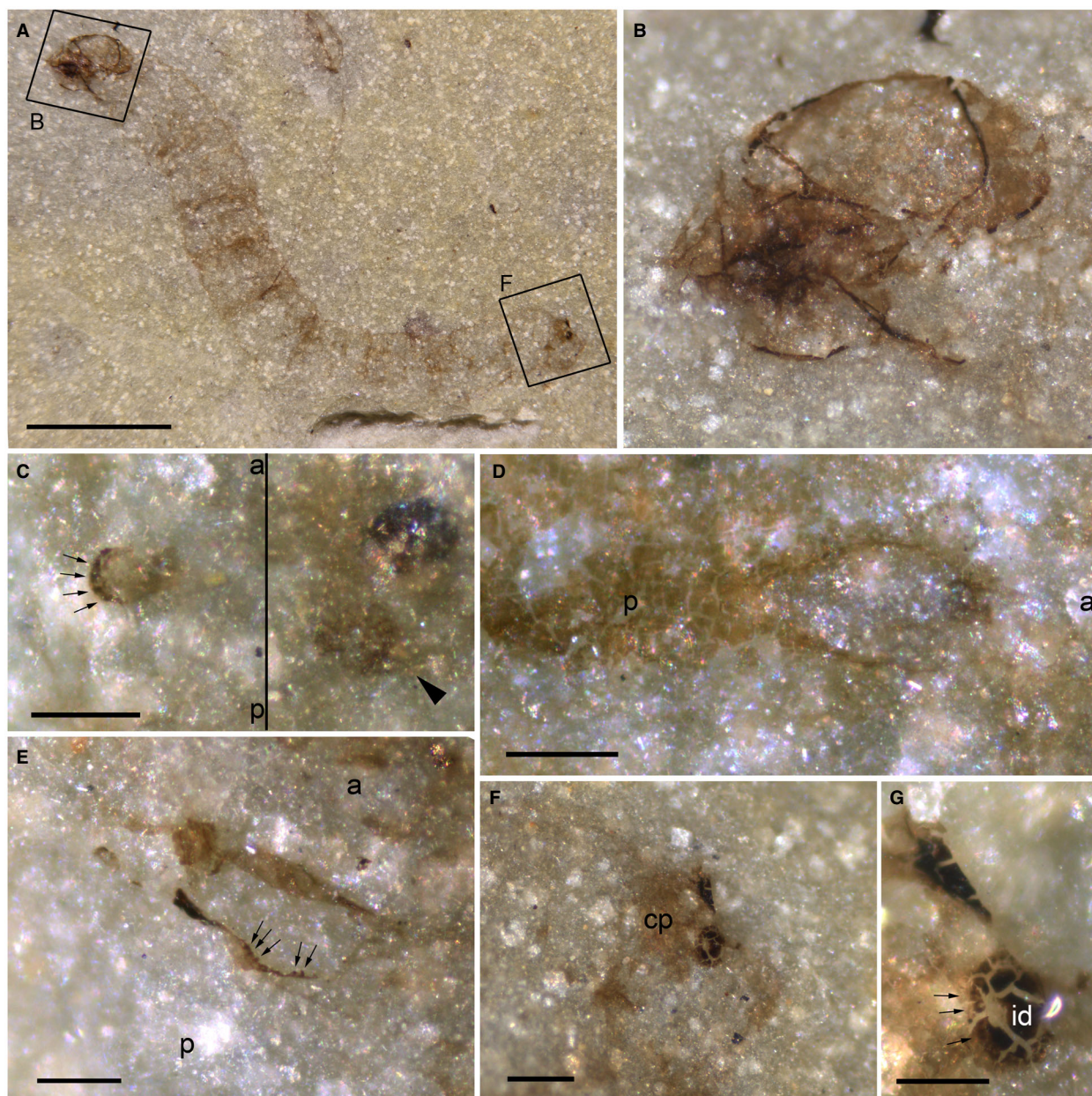


FIG. 3. Triassic terrestrial larva *Protoanisolarva juarezi* gen. et sp. nov. (Diptera, Anisopodoidea, stem-group Anisopodidae), holotype DA21/03-02-91a-b, from the Pedra Alta site, lower Anisian. A–B, habitus and detail of head (part, DA21/03-02-91a). C–E, details of structures preserved in counterpart (DA21/03-02-91b); C, anterior spiracle (left) and a ventral circular structure tentatively interpreted as a sensory organ (arrowhead, right) (vertical line separates two pictures of the same region taken in different focal planes); D, anus; E, preserved remains of posterior spiracles (small portions of posterior margins of both). F–G, details of posterior spiracles (part), showing a spiracle-bearing cuticular process, apparently in the shape of a conical frustum. Arrows indicate some of the preserved circular primary openings. Photographs made with consecutive pictures taken at successive focal planes (except C and E–G) and specimens photographed moistened with a mixture of alcohol and water (except G). Microphotograph in G taken after hardening of spiracles using PRIMAL™; see its appearance before damage in Figure 2C. Abbreviations: a, anterior part; cp, cuticular process; id, inner disc; p, posterior part. Scale bars represent: 1 mm (A); 0.05 mm (C–E, G); 0.1 mm (F).

group Anisopodidae cannot be justified on the sole basis of the characters of this larva. Therefore, regarding *P. juarezi* as a stem-group Anisopodidae is the most plausible stance based on

the current data. Also, a closer affiliation of *P. juarezi* with the two known extinct families of Anisopodoidea, Protorhyphidae Handlirsch, 1906 and Siberhyphidae Kovalev, 1985 (also in

the stem group of the Anisopodidae, based on adults) cannot be ruled out. Larvae of these two Mesozoic families remain unknown. The oldest fossil of the stem group Anisopodidae that does not belong to the Protorhyphidae or the Siberhyphidae is Early Jurassic (Sinemurian) (Krzemińska *et al.* 2010). The oldest Protorhyphidae is Middle Triassic, and the unique Siberhyphidae (*Siberhyphus lebedevi* Kovalev, 1985) is Jurassic (Kovalev 1985; Krzemiński & Krzemińska 2003).

The body length of the larva is only 4.8 mm, which is small compared with the common sizes of mature larvae of extant Anisopodidae species (Keilin & Tate 1940; Peterson 1981). The body is long and narrow, but less gracile than typical fully grown extant anisopodid larvae. Given that early instars of the extant anisopodid *Anisopus fenestralis* are metapneustic (Keilin & Tate 1940), the size and the presence of an amphipneustic respiratory system suggest that *P. juarezi* does not represent an early instar or a fully grown one.

The excellent preservation of the holotype enables visualization of the gut tube, possessing a straight and narrow hind gut as observed in the extant anisopodid species *Mycetobia pallipes* (Keilin & Tate 1940). Despite the excellent preservation of *P. juarezi*, several interesting head features are not evident, such as eye spots, labium and maxillae. The head is fossilized in a ventrolateral position (part) implying a certain deformation aside from the fossil diagenetic flattening. Even though the head preserves internal characters that can be observed due to transparency, the interpretation of the different parts is challenging and incomplete. Note that the limits between body segments cannot be exactly matched between part and counterpart (Fig. 2) given that each part preferentially preserves one of the body sides. Moreover, a slight difference and/or displacement in the segment limits occurred in one side of the body in relation to the other. A larval morphotype similar to the present Pedra Alta taxon has not been found at Grès à Voltzia.

Infraorder ?PSYCHODOMORPHA Hennig, 1968
Superfamily ?GRAUVOGELIOIDEA Lukashevich &
Shcherbakov, 1999
Family ?GRAUVOGELIIDAE Krzemiński, Krzemińska &
Papier, 1994
Genus VOLTZIAPUPA Lukashevich *et al.*, 2010
Voltziapupa cornuta Lukashevich *et al.*, 2010

Voltziapupa cf. cornuta
Figure 4A–D

Material. An almost complete pupal specimen preserved in lateral position, DA21/14-02-06, from the Pedra Alta site, in Pedra Alta section (7.55 m), upper part of the Estellencs Formation, Aegean (lower Anisian). The specimen lacks fine detail, and the distal abdominal segments are lost.

Description. Pupa subcylindrical, at least *c.* 4.3 mm long as preserved. Head with large frontal horns (at least one preserved). Mesothoracic respiratory horns and antennae not preserved. Dorsum of cephalothorax without large setae. Wing sheaths well preserved, *c.* 1.7 mm long and 0.6 mm wide, with rounded tip, without marks of venation observed, and reaching approximately body mid-length. Mid-dorsal cleft partially preserved. Leg sheaths poorly preserved (only two small

remains present). Abdominal segments relatively well preserved, apparently subequal and lacking secondary segmentation, with tergites more sclerotized than sternites and with small spines in posterior margin (a few spines preserved, not arranged in obvious rows).

Remarks. Lukashevich *et al.* (2010) described the genus *Voltzia-pupa* and the two species *V. tentata* and *V. cornuta* from the aquatic insect assemblage of Grès à Voltzia. These authors considered that these pupae possibly belonged to the extinct family Grauvogeliidae, described from adult remains preserved in Grès à Voltzia, and, if belonging to this family, then they would be assigned to Psychodomorpha in the superfamily Grauvogelioidea close to the Ptychopteroidea (Lukashevich & Shcherbakov 1999). The preserved characters of the new pupa from Pedra Alta agree with those in the diagnosis of the genus *Voltziapupa* but the specimen shows only a few characters. The habitus and measurements are very similar. The presence of one large frontal horn (the only character in the diagnosis of the species) that is identical to those in the holotype and paratype of *V. cornuta* enables the identification of this incomplete specimen. However, we prefer to use open nomenclature until more complete material is discovered, given that the frontal horn is not sufficiently well preserved. The wing sheaths from the Pedra Alta pupa are smaller, but a high degree of intraspecific variation was observed in this character within the genus (i.e. the 15 specimens of *V. tentata* and the 3 specimens of *V. cornuta*). The rows of small spines present along the posterior abdominal margins in *Voltziapupa* have not been observed in the present specimen, which bears only a few spines, two of them close to one another (see Fig. 4D), but this might be due to poor preservation.

NEMATOCERA indet. (pupa) Figure 4E

Material. One incomplete, poorly preserved pupal specimen preserved in slightly lateral position, DA21/14-02-07, from the Pedra Alta site, in the Pedra Alta section (7.55 m), upper part of the Estellencs Formation, Aegean (lower Anisian). The specimen's distal abdominal segments are lost.

Description. Pupa subcylindrical, at least 4.3 mm long as preserved. Head structures, leg sheaths and wing sheaths not preserved. Mid-dorsal cleft partially preserved. The well-preserved abdominal segments seem subequal and without secondary segmentation; rows of spines along posterior abdominal margins not preserved.

Remarks. This specimen is poorly preserved but it is herein figured due to the sparse record of dipteran pupae in this outcrop and in most Triassic localities, except for Grès à Voltzia (Lukashevich *et al.* 2010). The present specimen could in fact represent another *V. cornuta* specimen, although very poorly preserved.

Ootaxon *Clavapartus latus* Gall & Grauvogel, 1966 Figures 5, S2

Material. One incomplete egg cluster in side b of the slab DA21/14-02-01 from the Pedra Alta site, in the Pedra Alta

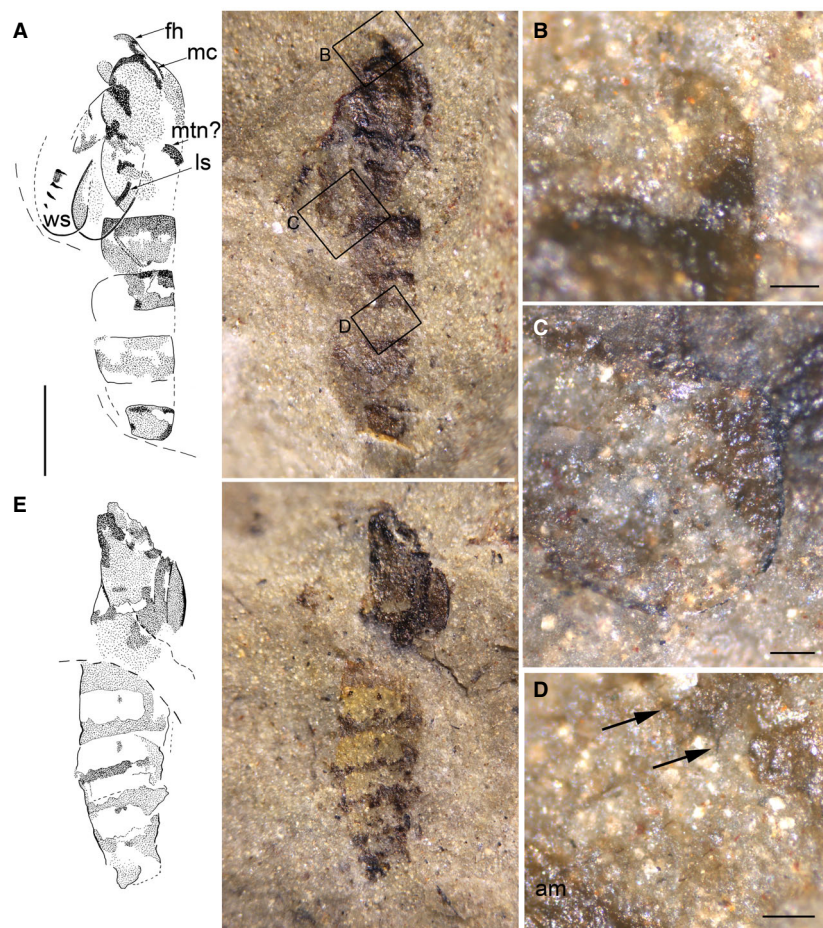


FIG. 4. Triassic aquatic dipteran pupae from the Pedra Alta site, lower Anisian. A–D, habitus of *Voltziapupa* cf. *cornuta* (?Grauvogeliidae), DA21/14-02-06, drawing and photograph, and details of the frontal horn (B), rounded tip of one of the forewing sheaths (C), and two abdominal small spines (D; arrows). E, habitus of incomplete pupa of *Nematocera* indet., DA21/14-02-07, drawing and photograph. A shares the scale with E. Photographs made with consecutive pictures taken at successive focal planes (except for C and D) and specimens photographed moistened with a mixture of alcohol and water. *Abbreviations:* am, abdominal margin; fh, frontal horn; ls, leg sheath; mc, mid-dorsal cleft; mtn, metanotum remain; ws, forewing sheath. Scale bars represent: 1 mm (A, E); 0.1 mm (B–D).

section (stratigraphic location uncertain, but most likely from 7.2–7.55 m in the section), upper part of the Estellencs Formation, Aegean (lower Anisian). The egg cluster is incomplete due to the limits of the claystone surface that preserved it. Most eggs are badly preserved due to weathering or sampling damage to the slab surface.

Description. Incomplete egg cluster elongated in shape, 21 mm long as preserved, 4 mm wide. Preserved portion containing 250–300 eggs. Eggs oval-shaped, c. 0.3×0.2 mm (length/width ratio = 1.5), slight brown in colour due to slight sclerotization. Eggs unhatched, some showing two-lobed tips. Eggs randomly distributed but generally distanced from each other; a few appear to be in contact (Fig. 5C) but this is most likely to have resulted from compression; the eggs were probably separated in the vertical plane prior to fossilization.

Remarks. The distribution of the eggs in the cluster indicates that these were almost certainly originally included in a mucilaginous matrix, although the latter is not evident in the fossil. The features of this egg cluster are identical to those of the very abundant *Clavapartus latus* described by Gall & Grauvogel (1966) from Grès à Voltzia, often associated to the similar ootaxon *Monilipartus tenuis* described in the same publication. The present partial specimen contains only 250–300 eggs, but between 500 and 2500 were described from the complete specimens of *C. latus* from Grès à Voltzia. Without doubt, these egg clusters covered by a mucilaginous matrix were directly laid in a water pool. Extant anisopodids lay egg clusters included in mucilaginous matrices in terrestrial substrates (Keilin & Tate 1940; Smith 1989). However, the Grès à Voltzia fossils probably do not correspond to such clusters due to their taphonomic features, which show evidence of absence of transport by

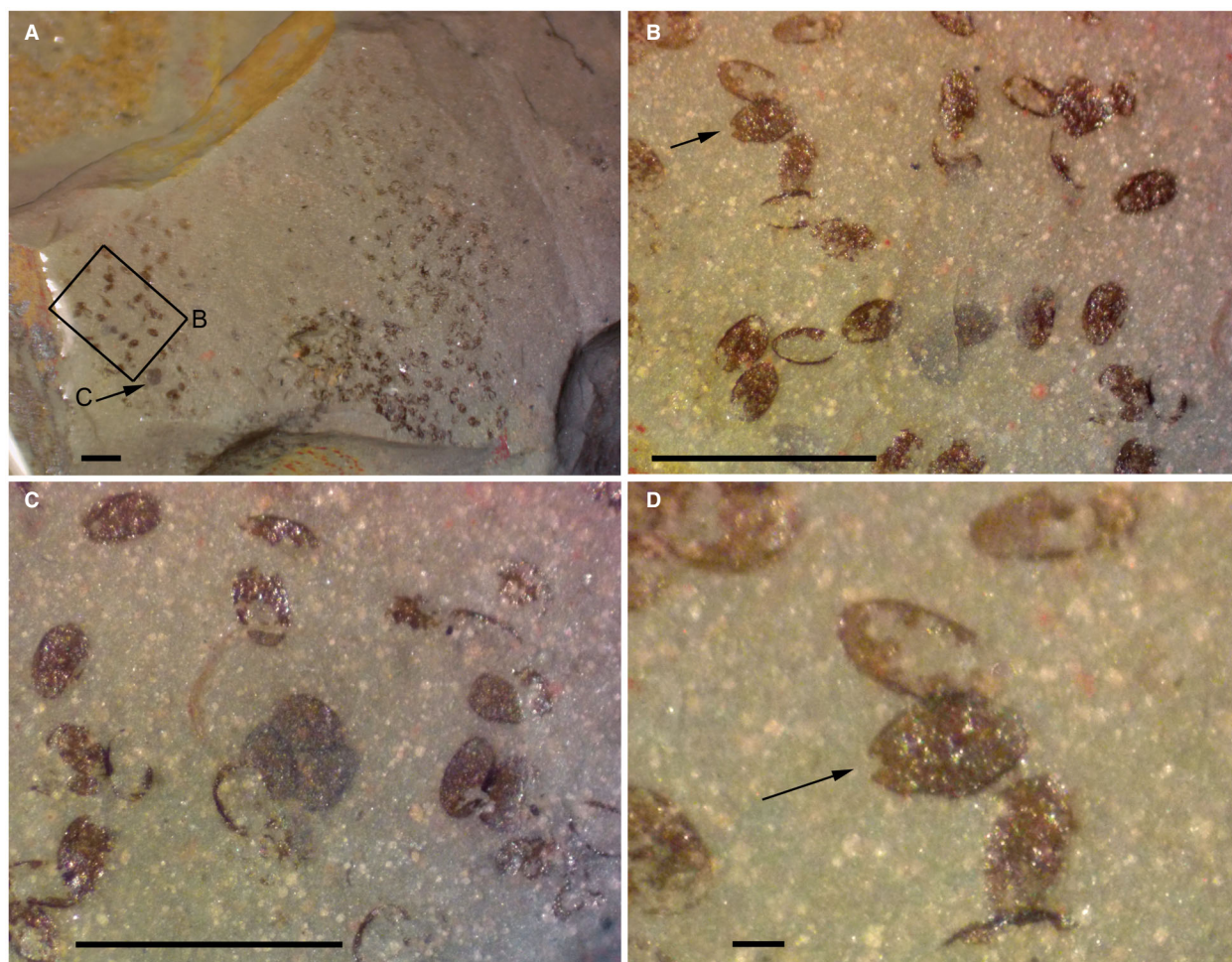


FIG. 5. Elongated, incomplete egg cluster originally in a mucilaginous matrix of the ootaxon *Clavapartus latus* Gall & Grauvogel, most likely produced by a nematoceran, present on the other side of one of the DA21/14-02-01 slab parts from the Pedra Alta site, lower Anisian. A, general view. B, detail from A (arrow indicates two-lobed tip from one oval egg (enlarged in D)). C, detail showing some eggs close together as preserved (three eggs together indicated with an arrow in A). D, detail of B. Specimen photographed moistened with a mixture of alcohol and water. Scale bars represent: 1 mm (A–C); 0.1 mm (D).

strong water currents towards the aquatic environments of burial. This explains the perfect integrity of abundant egg clusters despite their original delicate nature, as well as the lack of debris attached to the sticky mucilaginous matrix.

Gall & Grauvogel (1966) noted that nematocerans of the family Chironomidae were the most probable producers of *C. latus*, which resembles the egg clusters in mucilaginous matrices from extant *Chironomus* species; *Monilipartus tenuis* was also related to chironomids by these authors. Indeed, some Late Triassic representatives of the stem group Chironomidae are known (Krzemiński & Jarzembowski 1999). Gall & Grauvogel (1966) also considered the possibility that those egg clusters were laid by caddisflies (Trichoptera). Because trichopterans were most likely to have been present in running waters, the probability of any potential egg clusters to arrive in the quiet waters of the pools was low; this contradicts the abundance of intact clusters in Grès à Voltzia. Later, Grauvogel-Stamm & Kelber (1996) also

suggested anisopteran odonates as possible producers, given the resemblance of the egg clusters to those of some extant Corduliidae as well as the description of a few adult remains of Odonatoptera (Triadophlebiomorpha and Protozygoptera, but no Odonata s.s.) in Grès à Voltzia (Nel *et al.* 1996). However, nothing is known on the egg laying of these Triassic Odonatoptera, which are phylogenetically quite distant from the extant Anisoptera. In any case, the herein described egg cluster is similar to those from extant Chironomidae, as well as to egg clusters from the Cenozoic fossil record (Fig. S2) tentatively assigned to Chironomidae and Chaoboridae (see below).

Although here we follow the most plausible attribution indicated in the original publication (Gall & Grauvogel 1966), in a more conservative manner we consider that these egg clusters were most likely produced by nematoceran dipterans. Extant chironomids lay their eggs in the water together with a transparent mucilaginous material produced by a female gland, which

expands in the water and is adhesive, acting as a protection for the eggs against aquatic mites and fishes (Oliver 1981; Pinder 1995). These chironomid egg clusters can be cylindrical and very elongated or spherical. The eggs vary in form and in number depending on each cluster, thus showing some features that are species specific (Richards & Davies 1977), but are usually elliptical in shape, although they can be deltoid in two subfamilies (Nolte 1993; Pinder 1995). The length of the eggs varies between 170 and 612 μm and the length/width ratio of chironomid unhatched eggs ranges from 1.6 to 4.5 (Pinder 1995). Note that the herein unhatched eggs of *Clavapartus latus* have a ratio of 1.5, close to the smallest ratio for extant chironomid eggs, and that hatching slightly deforms the eggs. Extant chaoborid eggs are arranged in the form of a floating disc, which can contain mucilaginous material surrounding the eggs to prevent sinking (Saether 1997), and thus are very different to both Triassic and extant chironomid egg clusters.

Although descriptions of fossil nematoceran egg clusters are scarce, this is probably due to specimen misinterpretation and the paucity of studies, given that their potential for fossilization in lacustrine compression deposits is not as low as expected (Peñalver 2002). Despite the age difference, the Triassic egg clusters noted above share an original protective mucilaginous matrix with some ovoid clusters found in Konservat-Lagerstätten in the USA (Eocene) and Spain (Miocene). The shape of *Clavapartus* is very similar to the eggs found in the middle Eocene of the Kishenehn outcrop (Baranov *et al.* 2022), which are attributed to the genus *Chaoborus* (Chaoboridae), but smaller (Fig. S2); the Eocene egg clusters are 4.6×2.3 mm in size and the eggs are 0.47×0.20 mm (length/width ratio = 2.35) (from Baranov *et al.* 2022, fig. 22). These Eocene eggs were probably originated by chironomids instead of chaoborids, because the only cluster figured (Baranov *et al.* 2022, fig. 22A) does not have the eggs arranged in a spiral pattern as indicated by these authors in the main text, but instead are arranged without a pattern in a globular mass similar to what has been observed in some extant chironomid egg clusters. The Spanish Miocene clusters were found in Rubielos de Mora and Bicornb outcrops, Burdigalian (Lower Miocene) and Vallesian (Upper Miocene) in age, respectively, and attributed to the family Chironomidae (Peñalver 2002; Peñalver & Gaudant 2010). The mucilaginous matrix can be seen as a clear halo around the egg clusters from these two localities in relation to the rock matrix during the first seconds after moistening the specimens with alcohol (Peñalver 2002). In both Miocene outcrops the egg clusters with evidence of original mucilaginous matrices are very abundant, and the chironomids (pupae, exuviae, and adults) are the most abundant fossils. Trichopteran and odonatan fossils are, on the contrary, very scarce or absent. In these egg clusters, evidence of significant pre-burial transport is present: some organic structures, such as legs and other disarticulated parts from adult and pupal chironomids, were commonly ensnared by the mucilaginous matrices, which are mostly preserved broken (Peñalver 2002). The egg clusters from Rubielos de Mora are small, for instance 4.2×2.4 mm in a typical specimen, ovoid-shaped (spherical–globular type), and contain up to 75 fusiform eggs, 0.46×0.16 mm in a typical hatched egg (length/width ratio = 2.88); the egg clusters and egg size from Bicornb are very similar: 5.5×2.2 mm and

0.37×0.16 mm (length/width ratio = 2.31) in typical cluster and hatched egg specimens, respectively (Peñalver 2002; Peñalver & Gaudant 2010). In contrast, the Pedra Alta clusters are of the cylindrical and very elongated type. All of these Mesozoic and Cenozoic fossil eggs fall within the size and length/width ratio ranges observed in eggs of extant chironomids (Pinder 1995).

DISCUSSION

Palaeobiological implications of the new anisopodoid fossil larva

The dipteran fossils described herein represent the earliest known record of the megadiverse order Diptera. *Protoanisolarva juarezi* also represents the oldest anisopodoid dipteran known, and is evidence of the presence of stem-group Anisopodidae back to c. 247 Ma. The major lineages of the dipteran infraorder Bibionomorpha *s.l.* (i.e. Sciaroidea, Bibionoidea, Anisopodoidea and Scatopsoidea), as well as most of their families, including Anisopodidae, were supported as monophyletic by a molecular phylogeny combining nuclear and mitochondrial markers (Ševčík *et al.* 2016), and also by morphological data (e.g. Lambkin *et al.* 2013). Wojtoń *et al.* (2019) proposed the following phylogenetic hypothesis for the Anisopodoidea = (Protorhyphidae + (Siberhyphidae + Anisopodidae)) based on adult morphology. Prior to the present study, the oldest representative of the superfamily Anisopodoidea was *Vymrhyphus blagoderovi* Krzemiński & Krzemińska, 2003, of the extinct family Protorhyphidae, found in Grès à Voltzia.

The family Anisopodidae, containing only 120 extant species in seven genera (Grimaldi & Engel 2005), has been proposed as the closest living relative of the diverse sub-order Brachycera based on characters from both their essentially terrestrial larvae and adults (Woodley 1989; Oosterbroek & Courtney 1995; Shcherbakov *et al.* 1995; Grimaldi & Engel 2005). Although the clade Anisopodidae + Brachycera was found to be sister to the clade Tipulidae + Trichoceridae based on morphological characters (Oosterbroek & Courtney 1995), Anisopodidae was recovered as sister to the clade Bibionomorpha *s.s.* + Scatopsoidea by Ševčík *et al.* (2016). The oldest certain representatives of crown-group Anisopodidae are Early Jurassic in age (Wojtoń *et al.* 2019), all of them being adult forms. Hennig (1973) used both adult and larval characters in his classic analysis on the evolution and classification of the order Diptera. Larval cephalic features of Bibionomorpha have been found to be very informative from the phylogenetic standpoint (Bauernfeind *et al.* 2015). The discovery of *P. juarezi* illuminates important phylogenetic aspects of Bibionomorpha. The preserved cephalic features also shed light on the palaeobiology of the oldest bibionomorphans *s.l.* in the moist

surroundings of the first continental water bodies colonized with certainty by dipterans.

Protoanisolarva juarezi is interpreted as a terrestrial form based on its morphology and taxonomic affinities. It probably developed in pool marginal areas rich in organic matter. The excellent preservation and completeness of this larva, originally very delicate and with scarcely sclerotized cuticle, indicate that it is para-autochthonous. Thus, after a phase of biostratinomic transport that must have been short, the carcass went from the pool margin areas to the dysoxic or completely anoxic waters of the deepest pool parts.

The amphipneustic respiratory system exhibited by *P. juarezi* is externally evident thanks to the exceptional preservation of only anterior and posterior spiracles (Fig. 6). The new fossil adds this type of respiratory mode (typical of dipteran larvae) to the Pedra Alta Triassic insect assemblage, and represents its earliest known occurrence in the fossil record. In addition, the aquatic mayfly nymphs described from Pedra Alta preserved evidence of abdominal tracheal gills, chiefly observed as conspicuous rounded plates densely fringed on the borders, indicating an apneustic respiratory system as expected for this group (Peñalver *et al.* [in press](#)). Last, *Voltziapupa* bears elongate, cylindrical mesothoracic respiratory horns (not preserved in the Pedra Alta specimen), as evidenced by its rich and well-preserved record in Grès à Voltzia. This propneustic respiratory system is in accordance with the suggested semiaquatic mode of life for this taxon (Lukashevich *et al.* [2010](#)).

The great morphological similarities between *P. juarezi* and the larvae of extant Anisopodidae suggest that they had a similar biology. Extant anisopodid larvae are saprophagous and develop in areas with moist to wet decaying and fermenting organic matter of multiple origins (Peterson [1981](#); Hancock & Amorim [2009](#)). More specifically, the fossil larva possibly fed on fungal mycelia and/or fungal fruiting bodies, as is common among the extant relatives. For Zherikhin ([2002](#)), mycetophagy was probably widespread in the Mesozoic and was likely to have been an important condition in insect evolution and diversification. The fossil record of mycetophagy is sparse and has been poorly studied. In the *Protoanisolarva juarezi* specimen the hind gut is partially preserved, with possible gut contents corresponding to a black carbonaceous film that contrasts with the light brown of the preserved foregut, showing that the latter was empty when the larva died. Preparation of a hind gut sample could confirm mycelia and/or other identifiable fungal structures, but the minute size and the exceptional relevance of this holotype precludes partially destructive sampling.

Palaeobiology of the aquatic insect community

The aquatic assemblage of Pedra Alta developed in backswamps or among abandoned sand bars in channel margins

(Matamales-Andreu *et al.* [2021](#)) (Fig. 6). Aside from the dipteran taxa described herein, the Pedra Alta palaeoecosystem was inhabited by fishes (only one complete juvenile found) and clam shrimps ('conchostracans'; very abundant) of the species *Hornestheria* aff. *sollingsensis* Kozur & Lepper *in* Kozur & Weems, [2010](#) (Matamales-Andreu *et al.* [2021](#)). Other insects present in Pedra Alta were mayfly nymphs of an indeterminate detritivorous morphotype preserving cololites (abundant) and the species *Triassopemphera punctata* Sinitshenkova & Marchal-Papier *in* Sinitshenkova *et al.*, [2005](#), *Triassonurus doliiformis* Sinitshenkova & Marchal-Papier *in* Sinitshenkova *et al.*, [2005](#) and cf. *Voltziaepemphera fossoria* Sinitshenkova & Marchal-Papier *in* Sinitshenkova *et al.*, [2005](#) (all of these rare) (Matamales-Andreu *et al.* [2021](#); Peñalver *et al.* [in press](#)).

All of the organisms found in the Pedra Alta section are considered to be virtually coeval. With regard to the insects, the holotype and only specimen of *Protoanisolarva juarezi* was found at 7.20 m of the Pedra Alta section, whereas the aquatic pupae were recovered from 7.55 m of the same section. An abundant record of mayfly nymphs and adults is present in both of these horizons as well as at 4.75 m (Matamales-Andreu *et al.* [2021](#); Peñalver *et al.* [in press](#)).

Based on their completeness despite their original frailty, all of the pre-adult dipterans and the mayfly nymphs found in this deposit must have undergone relatively little disturbance during transport to the deepest areas of the water pool (Martínez-Delclòs *et al.* [2004](#)). The sedimentivorous mayfly nymphs from this locality were considered as inhabiting lentic habitats, which virtually were those of burial (Peñalver *et al.* [in press](#)). However, the other pre-adult forms found in Pedra Alta, such as those described herein, are very sparsely represented and could be considered allochthonous regarding the water pools. Despite the exceptional preservation and abundance of the fossils in Grès à Voltzia, palaeoecological inferences based on the material from that locality must be considered tentative. For Pedra Alta, with a lower number of specimens found and generally more poorly preserved, inferences and conclusions need to be at least equally cautious. The river shorelines with slow currents were deemed as the most plausible habitat for the Grès à Voltzia mayfly nymphs, namely *Voltziaepemphera* and *Triassonurus* (Sinitshenkova *et al.* [2005](#)). The same habitat was suggested for the dipteran *Voltziapupa* from Grès à Voltzia, recorded in the same beds as the mayflies, and aquatic or semiaquatic habitats for their unknown larvae (Sinitshenkova *et al.* [2005](#); Lukashevich *et al.* [2010](#)). Nevertheless, it is not clear from the taphonomic standpoint whether the fossil pupae from Pedra Alta can be attributed to lotic habitats.

The body fossils and invertebrate traces from Pedra Alta provide several insights into the early Middle Triassic

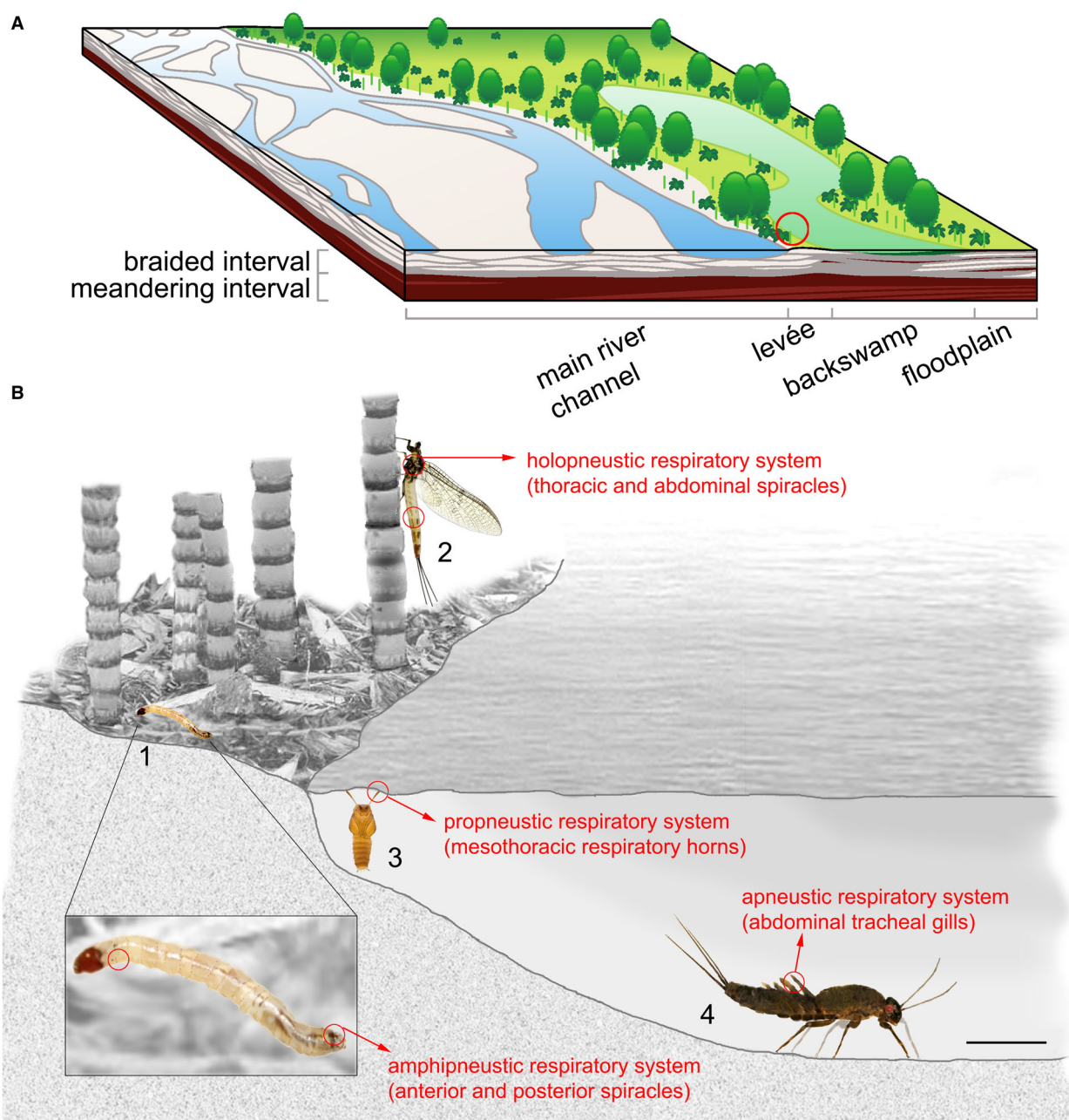


FIG. 6. Reconstruction of the moist and aquatic continental environment represented in the Pedra Alta site (Estellencs Fm., early Anisian), showcasing insect taxa, with different respiratory systems, that inhabited the palaeoecosystem. A, schematic diagram of the different environments of the Estellencs Fm. braided interval, and some of the plants found in the same levels as the insects: *Equisetites* sp., *Pelourdea* sp., *Voltzia* sp. B, schematic representation of a backswamp zone according to the interpretation of the Pedra Alta fossiliferous horizons (red circle in A): 1, dipteran larva *Protoanisolarva juarezi* gen. et sp. nov.; 2, mayfly adult *Hammephemera* cf. *pulchra*; 3, dipteran pupa *Voltziapupa* cf. *cornuta*, reconstructed based on more complete congeneric specimens found at Grès à Voltzia (Lukashevich *et al.* 2010); and 4, mayfly nymph *Triassonurus doliiformis*. All reconstructed insects to the same scale (scale bar applies to the insects only and represents 5 mm).

aquatic trophic net (Matamales-Andreu *et al.* 2021). Phytoplankton and photosynthetic bacteria could have been the primary producers in the palaeoecosystem. Although

no direct evidence from the rock of these fossiliferous beds is currently available, it seems that microbial films (not mats) developed in the water–sediment interphase

based on the exfoliation of the Pedra Alta fossiliferous rock following pure planes, where the insects are preserved as carbonaceous films. We infer that the growth of microbial mats on the pond bottom after each sedimentary input formed relatively clean limits separating these layers (Peñalver 2002). Moreover, it is likely that a hygrophytic flora developed around the freshwater body, providing abundant plant organic matter to the ponds and their surrounding terrestrial wet habitats (Matamalas-Andreu *et al.* 2021). Clam shrimps and mayfly nymphs were the primary consumers and/or saprophagous. The clam shrimps were most likely filter feeders, consuming plankton and organic detritus suspended in the water, as do most of their extant counterparts (Dodson & Frey 2001). The detritivore mayfly nymph morphotypes from Pedra Alta were sedimentivorous based on the content of their 3D cololites (Peñalver *et al.* *in press*). The rest of the mayfly nymph forms, also recorded in Grès à Voltzia with at least one burrowing species (cf. *Voltzia-ephemera fossoria*), were most likely detritivores (although not of the sedimentivorous type), grazers and filter-feeders (Sinitshenkova *et al.* 2005). Based on the currently known record, the only predators or secondary consumers were fishes, but direct evidence from the single fish specimen found in Pedra Alta to date is lacking. This palaeoecological scenario is complemented by the data presented herein on early dipterans. *Protoanisolarva juarezi* is interpreted as saprophagous, most likely mycetophagous, and probably developed in moist to wet decaying and fermenting organic matter contiguous to the water pools (Fig. 6). There is no evidence of feeding habits for the pre-adult nematocerans represented by the semiaquatic *Voltziapupa*, but these probably were primary consumers or saprophagous, with food intake by filtering or chewing.

According to the established regional palaeoenvironment and the presence of mass mortality records of mayfly nymphs, it seems clear that the Pedra Alta aquatic ecosystem underwent important shifts in environmental conditions, such as changes in water dynamics, turbidity and bathymetry, salinity, dissolved oxygen, and/or temperature (Matamalas-Andreu *et al.* 2021). Under that scenario, the fossil nematoceran egg clusters protected with a mucilaginous matrix present in Pedra Alta and Grès à Voltzia appear to have been an adaptation that minimized the mechanical damage and potential desiccating conditions (Gall & Grauvogel-Stamm 2005), and maybe even predation as well.

CONCLUSION

Following the remarkable gap in the terrestrial fossil record of the Early Triassic, including that of insects, Middle Triassic fossil insect localities remain very scarce globally. Pedra

Alta (early Anisian), almost 2 myr older than the well-studied locality of Grès à Voltzia, has yielded the earliest dipterans currently known. Those correspond to the pre-adult stages herein described, which offer valuable palaeoecological data. Moreover, Pedra Alta is, together with Grès à Voltzia, the only Triassic locality that has provided pre-adult dipterans, at both larval and pupal stages. These deposits provide evidence of the role of insects such as dipterans and mayflies in the colonization of the first continental aquatic ecosystems after/during the recovery period from the Permo-Triassic mass extinction event.

Pedra Alta has provided insights into some key putative adaptations in Triassic insects. Now these include specializations of the megadiverse insect order Diptera related to respiratory systems, possible trophic habits, and strategies to protect the eggs; some of these presumed adaptations represent the oldest known direct evidence, and are extended back to *c.* 247 Ma. Although Pedra Alta is generally poor in fossil insects and has only a few productive levels, the assemblage described herein has an exceptional degree of preservation. *Protoanisolarva juarezi* gen. et sp. nov. has an exquisitely preserved body, including external characters and some internal cephalic ones. This terrestrial larva is key to improving the understanding of the evolution and early ecology of true flies.

The new records described herein provide additional faunistic similarities between the Anisian localities of Pedra Alta and Grès à Voltzia. Taking into account differences in preservation and biases between these two localities, our data increase the knowledge on the early Middle Triassic entomofauna and its palaeobiology, with regard to aquatic environments. Furthermore, the presence of the same (or very similar) aquatic insect species in the two deposits indicates that the difference in their ages must be low. The maximum age difference between the two localities of up to *c.* 2 myr is compatible with the 3–10 myr inferred life span of insect species (Grimaldi & Engel 2005).

The study of the palaeoentomofauna from Pedra Alta has just begun. More excavations are necessary to recover new exceptionally preserved specimens. Likewise, increasing the sampling of more aquatic and terrestrial insect taxa from this locality is crucial to complete the current palaeoecological reconstruction. Greater sampling will also increase the taphonomic knowledge and shed light on the proportion of forms from diverse aquatic habitats such as lentic and lotic.

The pre-adult dipterans presently described are small and challenging to detect during palaeontological excavations. Most of the important records described herein were found during laboratory stereomicroscopy of slabs from which other specimens had already been found, namely clam shrimps and mayflies. Therefore, the collections from Pedra Alta would clearly be increased in specimen number and diversity if slabs from productive levels were able to be closely inspected in the laboratory.

According to the preservation observed in this Konservat-Lagerstätte, it is likely that some small insects, both aquatic and terrestrial, and belonging to uncommon groups in the Triassic fossil record, such as Thysanoptera, would be found following this approach.

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Author contributions. **Conceptualization** Enrique Peñalver (EP), Ricardo Pérez-de la Fuente (RPF); **Formal Analysis** EP, Rafel Matamalas-Andreu (RM-A), André Nel (AN), RPF; **Investigation** EP, RM-A, AN, RPF; **Methodology** EP, RM-A; **Supervision** EP; **Visualization** EP; **Writing – Review & Editing** EP, RM-A, AN, RPF.

DATA ARCHIVING STATEMENT

This published work and the nomenclatural acts it contains, have been registered in ZooBank: <http://zoobank.org/References/B668BD02-B77B-4BE5-BF80-136961F62874>

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SUPPORTING INFORMATION

Additional Supporting Information can be found online (<https://doi.org/10.1002/spp2.1472>):

Figure S1. Additional microphotographs of the Triassic terrestrial larva *Protoanisolarva juarezi* gen. et sp. nov. (Diptera, Anisopodoidea, stem-group Anisopodidae), holotype DA21/03-02-9b, from Pedra Alta site, lower Anisian.

Figure S2. Putative Mesozoic and Cenozoic nematoceran eggs from egg clusters present in a mucilaginous matrix.

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