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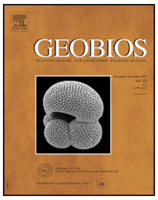

Original article

First record of a Chironomidae pupa in amber☆

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ABSTRACT

A pupal exuviae of a non-biting midge (Chironomidae) is recorded in Eocene Rovno amber from Ukraine. The exuviae belongs to an Orthocladiinae species. It possesses several traits characteristic for terrestrial species including non-fringed anal lobes without macrosetae, tergites with only minute shagreen, bare conjunctives, and absence of thoracic horns and frontal warts. Up to now, this is the earliest record of a preimaginal stage of Orthocladiinae, and the only record of a chironomidae pupal exuviae from amber.

1. Introduction

Flies and midges (Diptera) are among the most abundant and important inhabitants of modern terrestrial and freshwater ecosystems, deeply influencing the structure and functioning of food webs and biogeochemical cycles (Armitage, 1995; Ferrington, 2008). Fossil records of Diptera provide a fascinating opportunity to reconstruct the evolution of this major insect order; actually one of the most valuable sources of information on Diptera evolutionary history is fossil resins – ambers *sensu lato* (Ansorge, 1999; Krzemiński and Jarzembowski, 1999; Krzemiński and Krzemińska, 2003).

Non-biting midges (Chironomidae) are common inhabitants of most modern aquatic, semiaquatic and some terrestrial habitats occurring on all continents including Antarctica. They often dominate freshwater benthic communities in both abundance and species richness. They are also the most abundant and diverse group of Diptera in ambers (Seredszus and Wichard, 2007; Wichard et al., 2009). Chironomidae have a well-documented evolutionary history starting with *Aenne liasina* Ansorge, 1999 and *Aenne triassica* Krzemiński and Jarzembowski, 1999 from the Lower Jurassic and Triassic, respectively (Krzemiński and Krzemińska, 2003). Numerous records of chironomid adults, pupae and larvae are known from various Jurassic deposits from Siberia (Kalugina and Kovalev, 1985; Kalugina, 1993; Lukashevich and Przhiboro, 2011; Lukashevich, 2012). Jurassic Chironomidae records are mostly attributed to the subfamilies Podonominae and Tanypodinae, with many species and genera described, mostly by Kalugina (1993) and co-authors and Lukashevich and Przhiboro (2012, 2015). From the Cretaceous, several rich Chironomidae faunas are known from amber laggerstätten – in particular Lebanese amber, Canadian amber, and Siberian amber from Taimyr – as well as rich records of compressions from lake deposits, e.g., from the Lower Cretaceous Manlay Lake in Mongolia (Boesel, 1937; Kalugina, 1976, 1980a, 1980b; Veltz et al., 2007; Jarzembowski et al., 2008; Azar and Nel, 2010). Representatives of the subfamilies Orthocladiinae and Prodiamesinae first appear in Cretaceous ambers (Veltz et al., 2007). So far, only a few genera of Chironomidae have been recognized based on Cretaceous preimaginal material (Kalugina, 1980a; Lukashevich and Przhiboro, 2012) including a recently erected tribe of the subfamily Diamesinae, based on a fossil pupa from the Lower Cretaceous of Mongolia (Lukashevich and Przhiboro, 2015). No preimaginal

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stages of Chironomidae subfamilies other than Podonominae and Tanypodinae are known with certainty from the Mesozoic (Veltz et al., 2007).

The richest chironomid fossil record come from Eocene ambers. Chironomidae constitute 34–41% of all Diptera inclusions in representative collections of Baltic amber (Perkovsky et al., 2012; Szwedo and Sontag, 2013), and more than 80% of Diptera inclusions in the Sakhalin amber (Baranov et al., 2015). When properly identified and described, they can provide valuable information about the climate and the type of water bodies available in the deposition site at the time of fossil formation (Seredszus and Wichard, 2007; Wichard et al., 2009; Zelentsov et al., 2012).

No record of Chironomidae pupae or larvae from amber has been published to date. Indeed, the immature stages of most chironomid species are associated with aquatic habitats and thus not easily trapped in amber (Seredszus and Wichard, 2007). However, some genera of the subfamily Orthocladiinae have terrestrial or semiterrestrial larvae (Seredszus and Wichard, 2007; Moller Pillot, 2008). Below we describe the first chironomid pupal exuviae ever recorded from amber. The pupal exuviae was found in a piece of Eocene Rovno amber and possesses all the traits characteristic of terrestrial Orthocladiinae pupae.

2. Geological context

Eocene Rovno amber, collected in the northwest of the Ukraine, represents a southern coeval analogue of the famous Baltic amber (Fig. 1; Perkovsky et al., 2010; Zelentsov et al., 2012). Rovno amber is found predominantly in the lower part of the Oligocene Mezhygorje Fm. (Perkovsky et al., 2010) and Upper Eocene Obukhov Fm. (Fig. 2). The age and geographic origin of the Rovno amber is a controversial issue discussed in numerous works (e.g., Sontag and Szadziewski, 2011; Perkovsky et al., 2012; Perkovsky and Rasnitsyn, 2013). Based on the ant species composition, the Rovno amber is usually dated to the Upper Eocene (ca. 36 Ma; Dlussky and Rasnitsyn, 2009). Recent works by Giłka, Zakrzewska and co-authors on the Tanytarsini (Chironomidae, Chironominae) from the Rovno amber suggest an age for the Rovno amber very close to the Gulf of Gdańsk, as three common species of Tanytarsini were recorded from both deposits (Giłka et al., 2013; Zakrzewska and Giłka, 2014). Considering how fast chironomids of the tribe Tanytarsini evolved, records of the same species in amber taken from the Gulf of Gdańsk and from Rovno clearly indicates that this amber was formed at least partly at the same time (Giłka et al., 2013; Zakrzewska and Giłka, 2014, 2015).

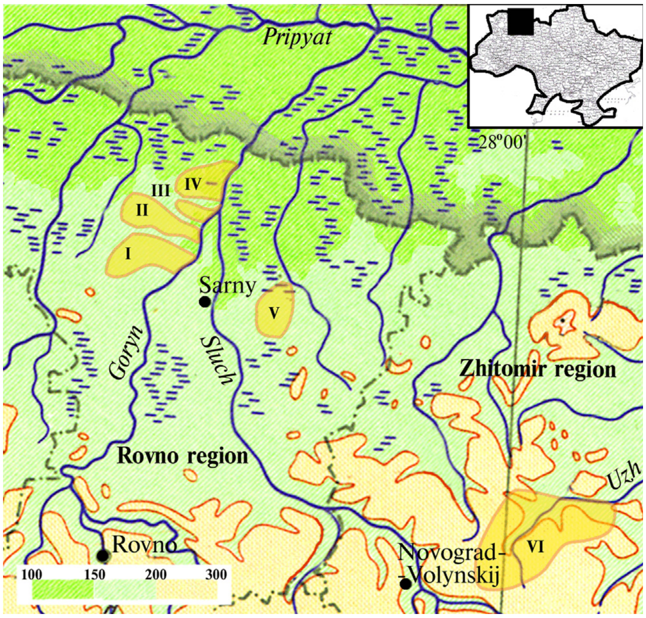

Fig. 1. Map of the amber-bearing zones in the Rovno and Zhitomir regions (Polesje): I–IV: Dubrovitsa and Vladimirets; V: Klesov; VI: Barash. Scale in the lower left corner represents elevation (m a.s.l.).

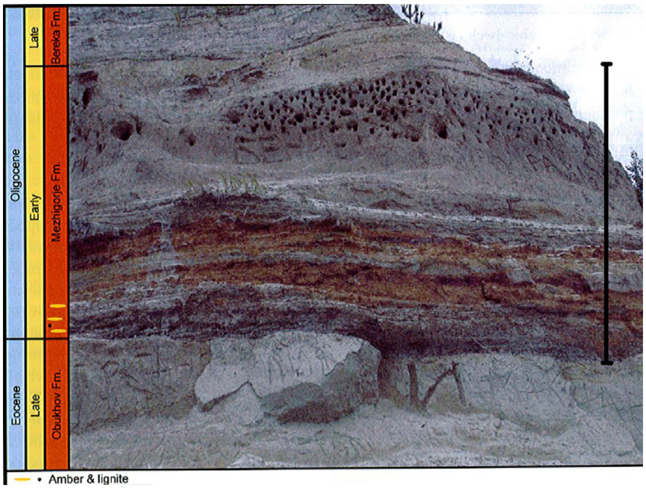

Fig. 2. Amber-bearing part of the Mezhygorje Formation (stratotype). Scale bar: 6 m.

3. Material and methods

The studied exuviae (pupal skin which remains after the adult midge has emerged) belongs to a female based on the structure of the antennae and the genital sac. It is housed in the I.I. Schmalhausen Institute of Zoology, National Academy of Science of Ukraine (SIZK), Kiev, Ukraine. The specimen was imaged using micro-CT scan and Confocal Laser Scanning Microscopy (CLSM) at The Natural History Museum, London. Micro-CT was carried out using a Nikon HMX ST 225 (Nikon Metrology, Tring, UK) with a molybdenum target, at 140 kV and yielding a resolution of 5 μm. The scan was reconstructed using CT Pro (Nikon Metrology); the picture provided good contrasts between the specimen and surrounding amber, but lacked the resolution necessary to resolve fine details. Confocal images were acquired with a Nikon A1-Si confocal microscope using 10× and 20× objectives (numerical apertures of 0.3 or 0.5, respectively).

We refrained from formally naming the species as most Chironomidae species described from amber are based on adult males and it is impossible to associate with certainty the female pupal exuviae with any adult male.

4. Systematic Palaeontology

Order DIPTERA Linnaeus, 1758
Family CHIRONOMIDAE Newman, 1834
Subfamily ORTHOCLADIINAE Kieffer, 1911
Figs. 3, 4

Material: A female pupal exuviae found in a piece of Rovno amber and housed in the collection of the I.I. Schmalhausen Institute of Zoology (SIZK – ref. No. K-7447), Kiev, Ukraine. The amber piece was collected at Klesov, Rovno region, Ukraine. The pupal exuviae contains several air bubbles (Fig. 3(A)).

Measurements: Entire body length, 1.10 mm; Cephalothorax length (to the tip of the wing sheaths), 541 μm; Abdominal segments II–IX length, 588 μm.