

## Mosquitoes (Diptera: Culicidae) in Eocene amber from the Rovno region, Ukraine

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### Abstract

*Culex ekaterinae* sp. nov. is described based on an adult male embedded in amber from the Rovno region, northwestern Ukraine. The new species is compared with its presumed closest known relative, i.e. *Culex erikae* Szadziewski et Szadziewska, 1985 described from adults in Baltic amber. The two species exhibit distinct differences in the size of the main body parts, including the antenna, maxillary palpus and proboscis, as well as in the venation of the wing, the structure of the unguis and male genitalia—characters crucial in the diagnosis of adult mosquitoes. A redescription of *Culiseta gedanica* Szadziewski et Giłka, 2011, to date known from a single adult male, is presented based on a male in amber from Voronki, Rovno region. A tabulation of fossil Culicidae with their geological ages is provided.

**Key words:** *Culex*, *Culiseta*, fossils, new species, systematics

### Introduction

Rovno amber has been dated to the Priabonian Age (33.9–37.8 Mya) of the Eocene Epoch (Sokoloff *et al.* 2018; Radchenko & Perkovsky 2021; Radchenko *et al.* 2021). Most Rovno amber containing nematoceran Diptera, and most other inclusions studied thus far, was mined in Klesov (Sarny district, Rovno region) (Perkovsky *et al.* 2010; Mitov *et al.* 2021). The new findings, including mosquitoes, come from the former Zarechnoye and Vladimirets districts of the northwestern part of the Rovno region (Perkovsky & Nel 2021 and references therein).

The nematoceran Diptera constitute more than half (58%) of all Rovno amber insect inclusions, but only 144 species have been recorded so far (Azar *et al.* 2013; Perkovsky & Fedotova 2016 and references therein; Fedotova & Perkovsky 2017; Sontag & Szadziewski 2011; Zakrzewska & Gilka 2014; Perkovsky & Sukhomlin 2015; Baranov *et al.* 2016; Pielowska *et al.* 2018; Kopeć *et al.* 2019; Wojtoń *et al.* 2019; Skartveit 2021; Wagner 2021), and 110 (76%) of these species are not known from coeval Baltic amber. Fossil representatives of four families of the infraorder Culicomorpha have been described from Rovno amber, including Ceratopogonidae (Sontag & Szadziewski 2011; Perkovsky & Rasnitsyn 2013; Perkovsky 2013, 2017), Chironomidae (Zelentsov *et al.* 2012; Gilka *et al.* 2013; Baranov & Perkovsky 2014; Baranov *et al.* 2014; Zakrzewska & Gilka 2014; Andersen *et al.* 2015; Zakrzewska *et al.* 2016; Dietrich & Perkovsky 2020), Corethrellidae (Baranov *et al.* 2016) and Simuliidae (Perkovsky *et al.* 2013; Perkovsky & Sukhomlin 2015, 2016); however, information on mosquitoes (Culicidae) from this amber has not been published to date.

Twenty-seven extinct fossil species of Culicidae have been described. Three species are representatives of three extinct genera that existed in the Late Cretaceous Epoch or at the turn of the Early and Late Cretaceous (today called the “mid-Cretaceous”) of the Mesozoic Era. Twenty-four species of the extant genera *Aedes*, *Anopheles*, *Coquillettidia*, *Culex*, *Culiseta* and *Toxorhynchites*, and three extinct genera are known from the more recent Cenozoic Era.

The 27 species and their geological ages are listed in Table 1 (see also for their authorship, which is not included at first mention in the text).

## Material and methods

The specimens studied here are adult male inclusions found in amber from the Rovno region, Ukraine. The new *Culex* species was found in the Veselukha river valley [see Lyubarsky & Perkovsky (2020); Kuchotskaya Volya is the most well-known locality there] in a big piece of amber LKV-102 (27.2 g before primary treatment, length of piece 68 mm, width 44 mm, height 19 mm), and the *Culiseta* species was found in a large piece of amber (174 g after primary treatment, length of piece 128 mm, width 46 mm, height 20 mm) from Voronki, Vladimirets district (local fauna is discussed in Perkovsky *et al.* 2020).

Except for the unguis and genitalia, for which lengths are given in micrometres ( $\mu\text{m}$ ), measurements are in millimetres (mm) rounded to the first decimal place (total body lengths) or to the second digit after the decimal point (remaining measurements, including calculated ratios). The body and wing lengths were measured from the antennal pedicel to the apex of the genitalia and from the arculus of the wing to the wing tip, respectively. The morphological terminology and abbreviations follow Harbach & Knight (1980, 1982), revised and updated in the Anatomical Glossary of the Mosquito Taxonomic Inventory (<http://mosquito-taxonomic-inventory.info/>), except for crossveins of the wing which follow the usage of Belkin (1962). Photographs were taken using a PZO Biolar SK14 microscope with a Sony NEX-3N digital camera, a Leica M205A stereomicroscope with a DCF 495 camera (*Culex*) and a Leica Z16 APO stereomicroscope with a DFC 450 camera (*Culiseta*). The images were compiled using the LAS Montage multifocus and the Helicon Focus 6 stacking software. The type specimen of the new species designated here is the property of the I.I. Schmalhausen Institute of Zoology, National Academy of Sciences of the Ukraine, Kiev (SIZK); the specimen L-800 (1DNCCC2021) from the Victor Gusakov collection (Zviodnyi gorodok, Russia; GVGM) is on long-term loan in the SIZK.

## Results

### *Culex ekaterinae* sp. nov.

LSID: urn:lsid:zoobank.org:act:74519104-A47C-4A85-98BD-80C2269524A7

Figs 1A–D; 2A, B, D, E, H, I; 3A, B, D

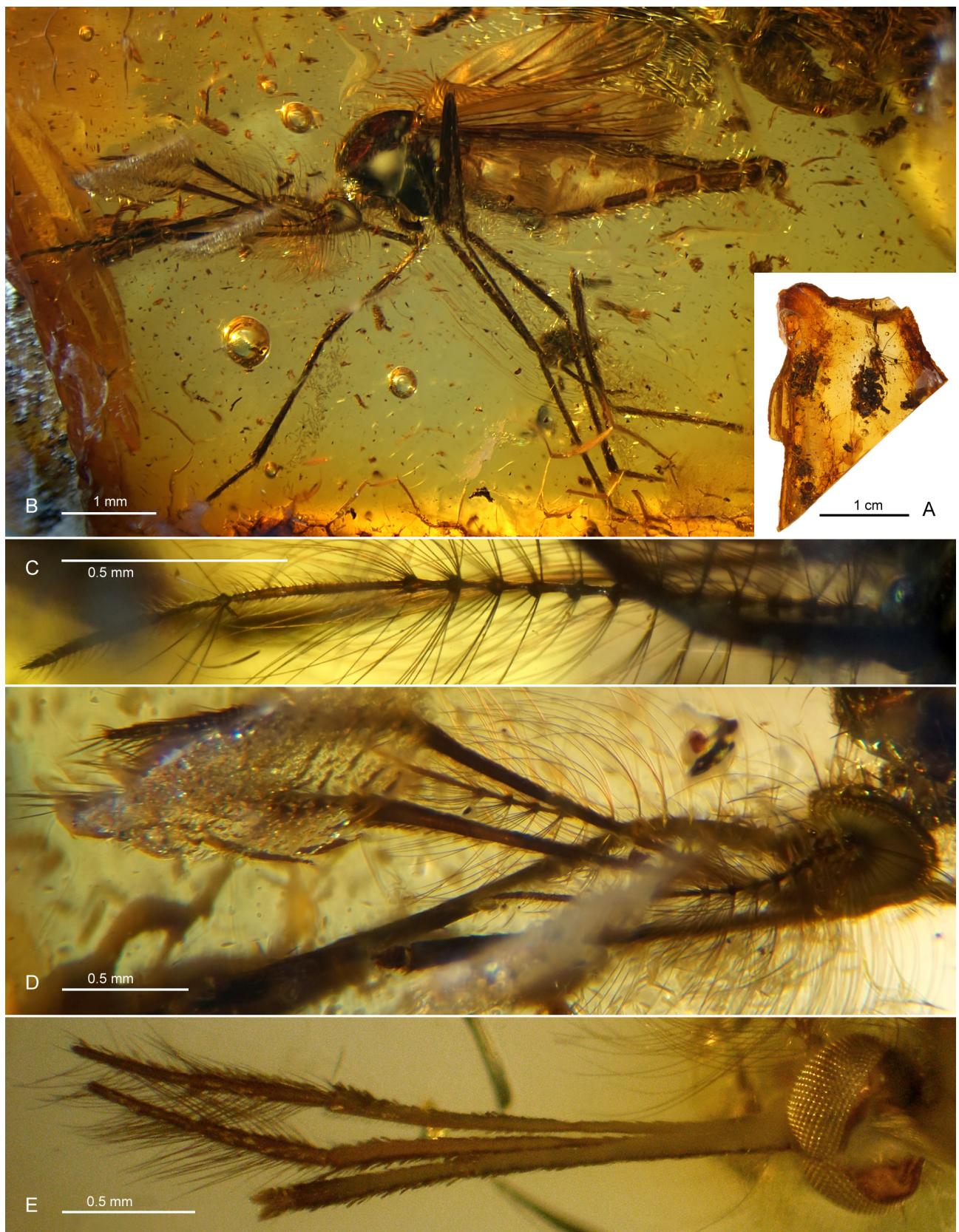
**Derivation of the name.** The specific name is a feminine patronym commemorating the Russian palaeontologist Ekaterina Alekseevna Sidorchuk (1981–2019).

**Type material.** Holotype, adult male, right hindleg broken, tarsomeres incomplete or missing, preserved in a subtriangular piece of amber  $28.5 \times 20.5 \times 7.5$  mm (SIZK LKV-178, Fig. 1A), Veselukha river valley, Rovno region, Ukraine; Eocene, Priabonian (33.9–37.8 Mya). Syninclusions: LKV-178/I, Sciaridae; SIZK LKV-179, Dolichopodidae.

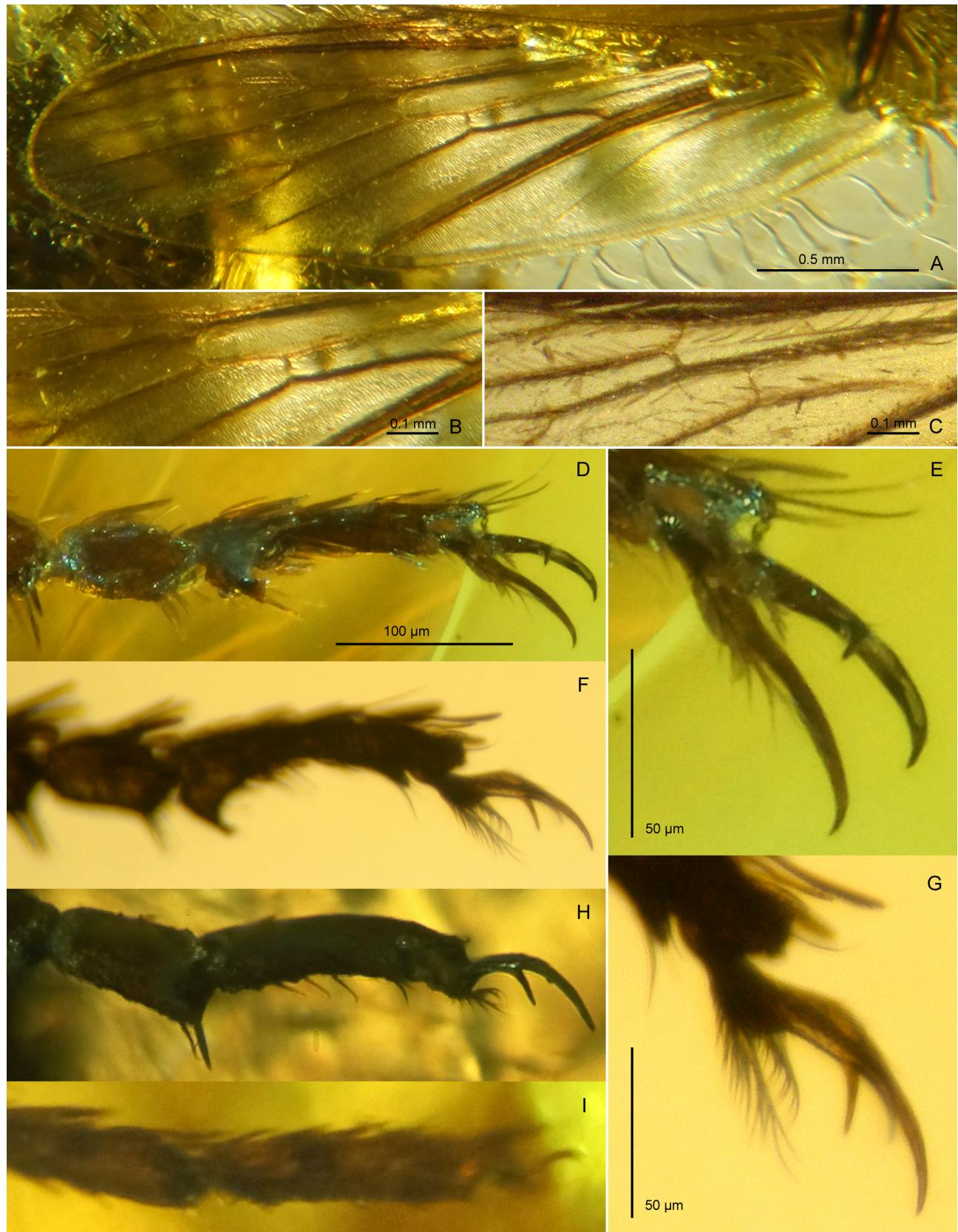
**Description.** Adult male (holotype). *Colour:* Dark brown to black, with proximal abdominal segments slightly lighter (Fig. 1B). *Size:* Body length 5.2 mm (excluding proboscis), body + proboscis 7.0 mm, thorax + abdomen 4.7 mm (Fig. 1B). *Antenna* (Fig. 1C): Length including pedicel 1.97 mm, flagellum 1.85 mm; flagellomeres 12 and 13 longest (0.44 and 0.47 mm, respectively), their combined length slightly less than total length of preceding flagellomeres (ratio 0.97); whorls well developed, flagellomeres 1–12 each with whorl of many long setae, flagellomere 13 with whorl of few shorter setae and short conical apical prolongation; antenna about 1.11 x length of proboscis. *Proboscis* (Fig. 1D): Length 1.77 mm, distinctly shorter than maxillary palpus (ratio 0.65), with a distinct constriction 0.67 from base; labella with well-defined proximal and distal sclerites; forefemur/proboscis ratio 0.84. *Maxillary palpus* (Fig. 1D): Length 2.72 mm, much longer than proboscis (ratio 1.54); palpomeres 1–3 ankylosed (combined length 1.65 mm), about 0.93 length of proboscis; palpomere 4 (0.57 mm) longer than palpomere 5 (0.50 mm), ratio 1.14; palpomeres 1–3 slender, broadening toward apex of palpomere 3; palpomere 4 broadest, palpomere 5 tapering toward narrow apex (cf. *Culex erikae*: Fig. 1E). *Wing* (Fig. 2A, B): Length 2.90 mm; veins  $R_2$  and  $R_3$  distinctly longer than vein  $R_{2+3}$  ( $R_2/R_{2+3} = 3.00$ ,  $R_3/R_{2+3} = 3.16$ ); arrangement of veins in area of radiomedial and mediocubital crossveins as shown in Fig. 2B; mediocubital crossvein [base of  $M_{3+4}$  of Harbach & Knight (1980)] unusually short, with media

**Table 1.** The 27 extinct species of Culicidae formally described prior to the present paper, known from a male (m), female (f) and/or a leg (l), wing (w); egg (e). For names/nominal species removed from Culicidae see Poinar *et al.* (2000) and Harbach (2021).

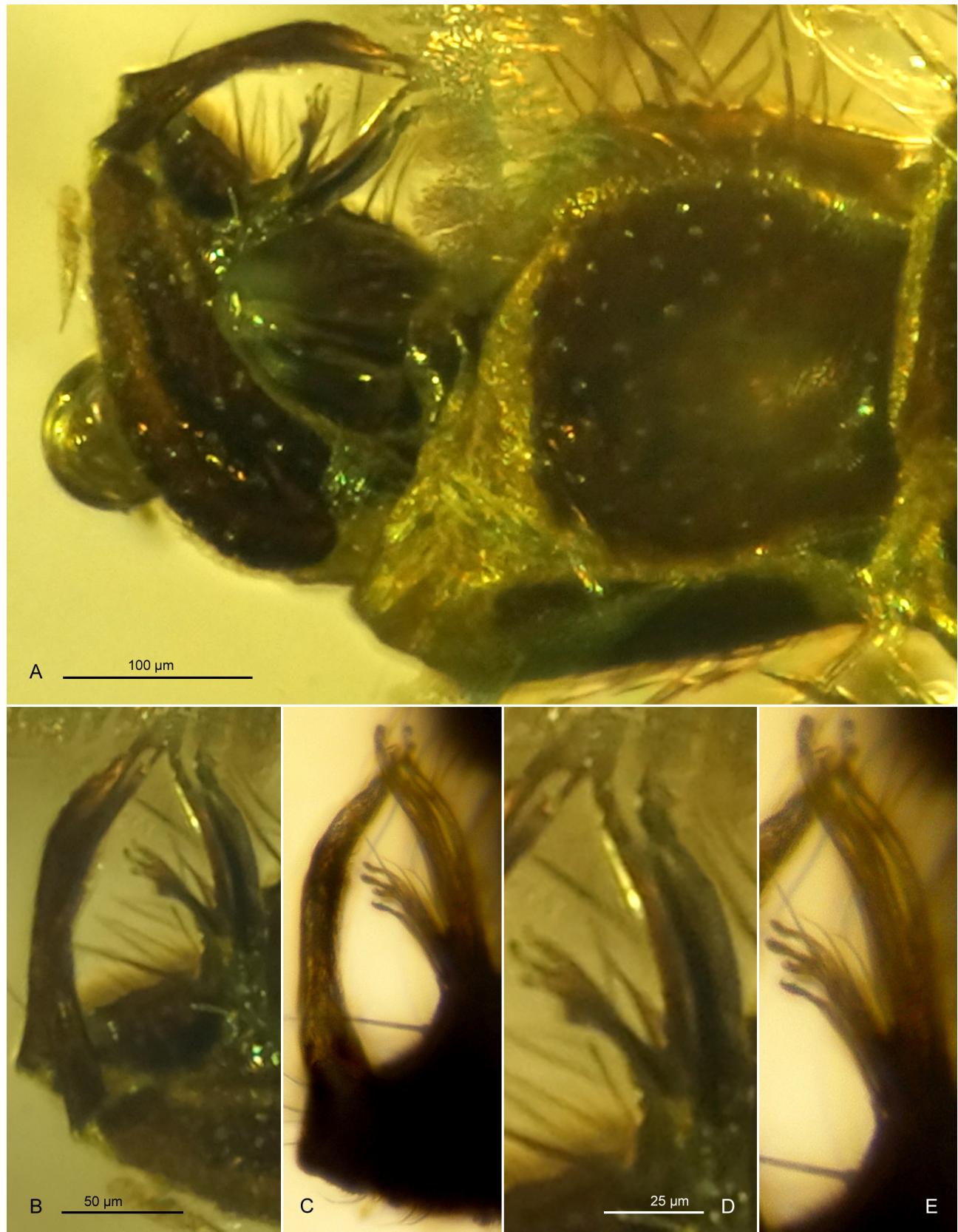
Genus	Species	Sex	Period (epoch)	Reference (taxonomy)
<i>Aedes</i> Meigen, 1818 (extant)	<i>protolepis</i> (Cockerell, 1916) (originally <i>Culex</i> , doubtful combination) <i>serafini</i> Szadziewski, 1998	w m	Eocene Eocene	Cockerell (1916), Krzeminski <i>et al.</i> (2019, as species of the genus <i>Ochlerotatus</i> Lynch Arribalzaga, 1891) Szadziewski (1998), Szadziewski & Gilka (2011)
<i>Aerhegapnomyia</i> Harbach <i>et</i> Greenwalt, 2012 (extinct)	<i>hoffeinsorum</i> (Szadziewski, 1998) (originally <i>Aedes</i> )	m f?	Eocene	Szadziewski (1998), Harbach & Greenwalt (2012)
<i>Anopheles</i> Meigen, 1818 (extinct)	<i>dominicanus</i> Zavortink <i>et</i> Poinar, 2000 <i>rottensis</i> Statz, 1944	f m	Miocene Oligocene	Zavortink & Poinar (2000) Statz (1944)
<i>Burnaculex</i> Borkent <i>et</i> Grimaldi, 2004 (extinct)	<i>antiquus</i> Borkent <i>et</i> Grimaldi, 2004	f	mid-Cretaceous	Borkent & Grimaldi (2004)
<i>Coquillettidia</i> Dyar, 1905 (extinct)	<i>adamowiczi</i> Szadziewski, Sontag <i>et</i> Szwed, 2019 <i>cockerelli</i> (Edwards, 1923) (originally <i>Taeniorhynchus</i> ) Lynch Arribalzaga, 1891) <i>gedanica</i> Szadziewski, Sontag <i>et</i> Szwed, 2019 <i>martinii</i> (Statz, 1944) (originally <i>Mansonia</i> Blanchard, 1901) <i>varivestita</i> (Statz, 1944) (originally <i>Mansonia</i> )	m f m f f	Eocene Eocene Oligocene	Szadziewski <i>et al.</i> (2019) Edwards (1923), Poinar <i>et al.</i> (2000), Krzeminski <i>et al.</i> (2019) Szadziewski <i>et al.</i> (2019) Statz (1944), Krzeminski <i>et al.</i> (2019)
<i>Culex</i> Linnaeus, 1758 (extinct)	<i>damnatorium</i> Scudder, 1890 <i>erikae</i> Szadziewski <i>et</i> Szadziewska, 1985	f fm	Oligocene Eocene	Statz (1944), Krzeminski <i>et al.</i> (2019) Scudder (1890) Szadziewski & Szadziewska (1985), Szadziewski & Gilka (2011)
	<i>malariae</i> Poinar, 2005 <i>protorhinus</i> Cockerell, 1916 <i>rectensis</i> Edwards, 1923 <i>winchesteri</i> Cockerell, 1919	f m f f	Miocene Eocene Eocene Eocene	Poinar (2005) Cockerell (1916) Edwards (1923) Cockerell (1919)
<i>Culiseta</i> Felt, 1904 (extinct)	<i>gedanica</i> Szadziewski <i>et</i> Gilka, 2011 <i>kishinehni</i> Harbach <i>et</i> Greenwalt, 2012 <i>lemniscata</i> Harbach <i>et</i> Greenwalt, 2012	m m f f	Eocene Eocene	Szadziewski & Gilka (2011) Harbach & Greenwalt (2012) Harbach & Greenwalt (2012)
<i>Eoededes</i> Harbach <i>et</i> Greenwalt, 2012 (extinct)	<i>damzeni</i> (Szadziewski, 1998) (originally <i>Aedes</i> )	m	Eocene	Szadziewski (1998), Harbach & Greenwalt (2012)
<i>Neoculicites</i> Evenhuis, 1994 (extinct)	<i>arrvernensis</i> (Piton, 1936) (originally <i>Culicites</i> ) <i>ceyx</i> (von Heyden, 1870) (originally <i>Culex</i> ) <i>deperi</i> (Meunier, 1915) (originally <i>Culicites</i> )	f f f	Pleistocene Oligocene Oligocene	Piton (1936), Evenhuis (1994) von Heyden (1870), Meunier (1915), Evenhuis (1994) Meunier (1915), Evenhuis (1994)
<i>Paleoculicites</i> Poinar, Zavortink, Pike <i>et</i> Johnston, 2000 (extinct)	<i>minutus</i> Poinar, Zavortink, Pike <i>et</i> Johnston, 2000	m	Late Cretaceous	Poinar <i>et al.</i> (2000)
<i>Priscoculex</i> Poinar, Zavortink <i>et</i> Brown, 2019	<i>burmamicus</i> Poinar, Zavortink <i>et</i> Brown, 2019	f	mid-Cretaceous	Poinar <i>et al.</i> (2020)
Brown, 2019 (extinct)				
<i>Toxorhynchites</i> Theobald, 1901 (extant)	<i>mexicanus</i> Zavortink <i>et</i> Poinar, 2008	f	Miocene	Zavortink & Poinar (2008)



**FIGURE 1.** Males of *Culex ekaterinae* sp. nov. (A–D) and *Culex erikae* Szadziewski et Szadziewska, 1985 (E). A, Inclusion in amber; B, habitus; C, antenna; D, E, head.



**FIGURE 2.** Males of *Culex ekaterinae* sp. nov. (A, B, D, E, H, I) and *Culex erikae* Szadziewski et Szadziewska, 1985 (C, F, G). A, Wing; B, C, area of radiomedial and mediocubital crossveins; D–I, ultimate tarsomeres with unguis of fore- (D–G), mid- (H) and hindleg (I) and unguis magnified (E, G).



**FIGURE 3.** Males of *Culex ekaterinae* sp. nov. (A, B, D) and *Culex erikae* Szadziewski et Szadziewska, 1985 (C, E). Genitalia in lateral aspect and setae of gonocoxite (C and E magnified proportionally relative to B and D, respectively).

and vein  $M_{3+4}$  both indented at point of attachment with the crossvein (cf. *Culex erikae*: Fig. 2C); cubitus posterior [plical vein of Belkin (1962)] developed as strongly as cubitus anterior, with scales on at least proximal part; anal vein (1A) ending 0.67 of distance between intersection of mediocubital crossvein and cubitus and base of  $M_{3+4}$  toward wing apex. Legs (Fig. 2D, E, H, I; length of segments in Table 2): Entirely dark-scaled; foreleg with ungues strongly curved apically; ungues equally long on foreleg (110  $\mu\text{m}$ ), unequal on midleg (90 and 50  $\mu\text{m}$ ), equal on hindleg (50  $\mu\text{m}$ ); anterior unguis of fore- and midlegs with medial tooth: minute on foreleg (Fig. 2D, E; cf. *Culex erikae*: Fig. 2F, G) and stout on midleg (Fig. 2H), posterior unguis of fore- and midlegs without teeth; ungues of hindleg simple (for length of leg segments see Table 2). Genitalia (Fig. 3A, B, D): Observed in lateral view; length of gonocoxite 300  $\mu\text{m}$ , subapical lobe prominent, undivided, with 2 groups of setae: proximal group comprised of 3 long, stout closely appressed setae (setae *a*, *b*, *c*), narrowed distally with retrorse or hooked apices; distal group of partially fused shorter setae (setae *d*–*g*) with spoon-shaped apices (Fig. 3B, D; cf. *Culex erikae*: Fig. 3C, E); length of gonostylus 190  $\mu\text{m}$ , evenly curved, slightly enlarged distally, with long apical gonostyilar claw.

**TABLE 2.** Lengths (mm) of leg segments of the holotype male of *Culex ekaterinae* sp. nov.

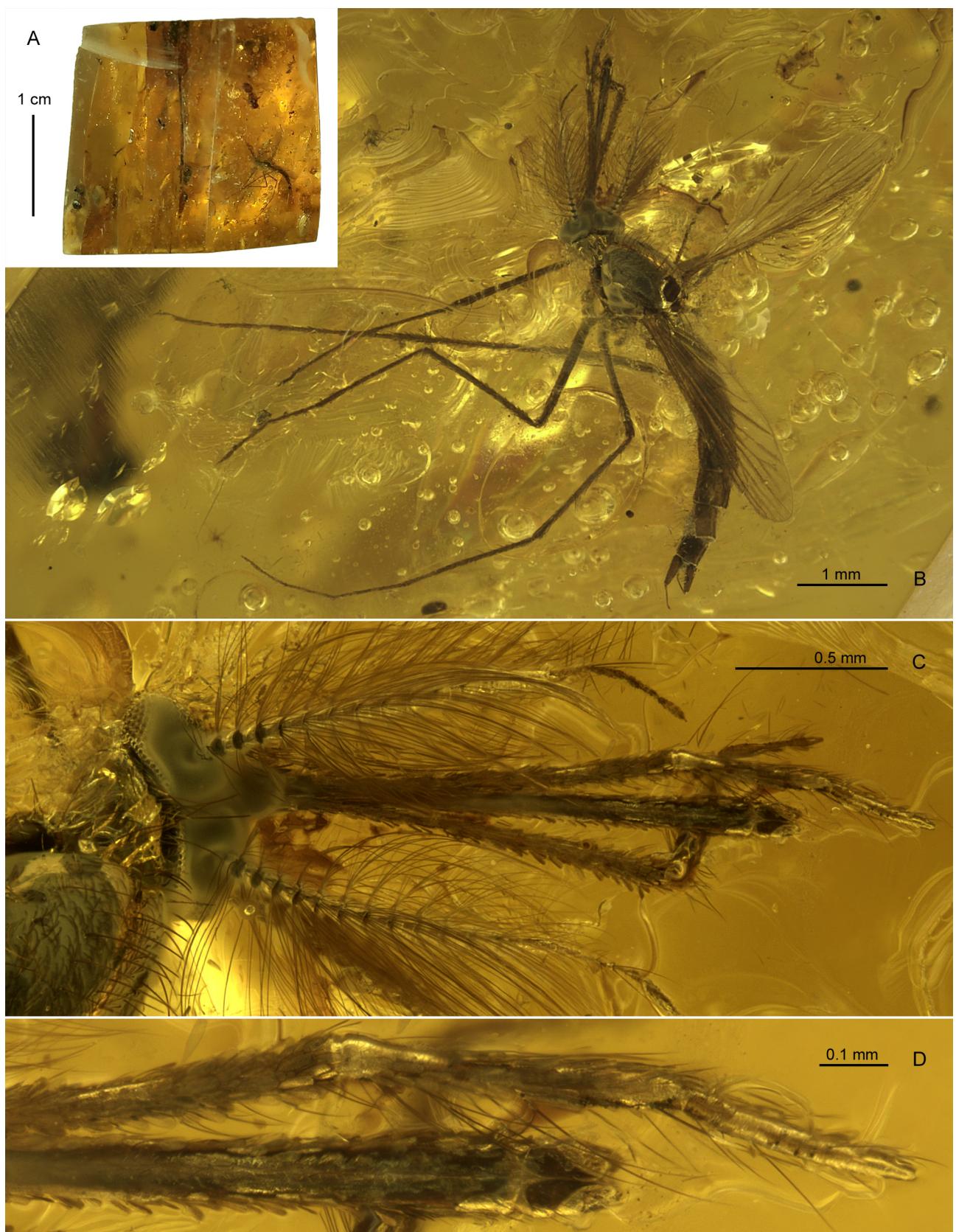
Leg	Femur	Tibia	Tarsomeres				
			1	2	3	4	5
Foreleg	1.49	1.59	1.03	0.29	0.18	0.09	0.19
Midleg	1.56	1.81	1.54	0.50	0.33	0.09	0.15
Hindleg	1.46	1.90	1.65	0.91	0.71	0.36	0.19

### *Culiseta gedanica* Szadziewski et Gilka, 2011

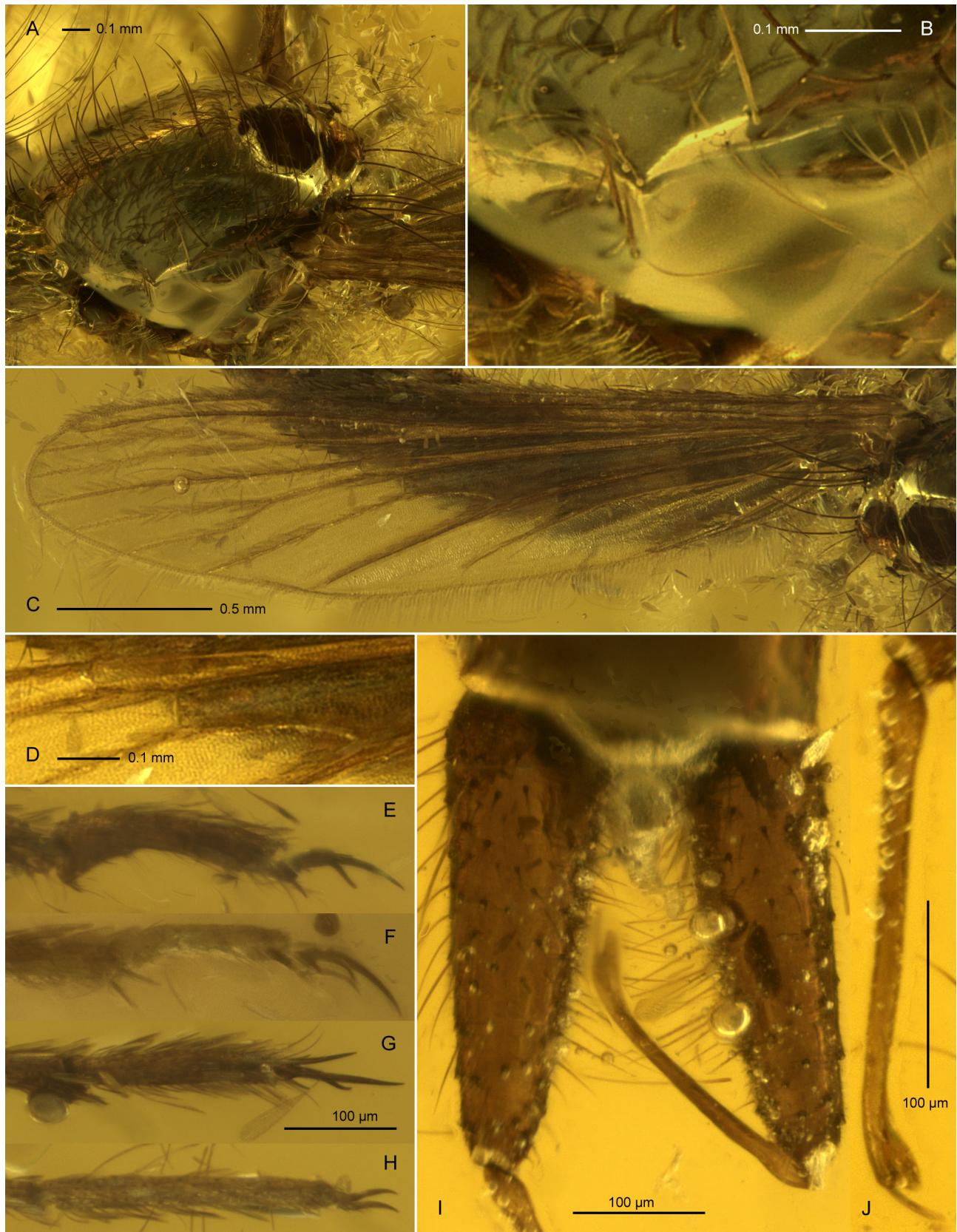
Figs 4–6

**Material examined.** Adult male (left foreleg missing) preserved in a sub-rectangular piece of amber 25.5  $\times$  23  $\times$  13.5 mm (SIZK L-800, Fig. 4A; Voronki, Rovno region, Ukraine; Eocene, Priabonian (33.9–37.8 Mya). Syninclusions. SIZK L-800: stellate hairs, 2 Formicidae [male of *Plagiolepis solitaria* Mayr and worker of *Ctenobethylus goepperti* (Mayr)], Aphidinea (*Germaraphis* sp.), Dolichopodidae, Ceratopogonidae, Aranei; SIZK L-801: Arctomyiidae (*Electribius* sp.); SIZK L-802: Acari, mammalian hair; SIZK L-803: *incertae sedis*; SIZK L-804: Aranei; SIZK L-805: Dolichopodidae, Acari, Collembola; SIZK L-806: 3 Dolichopodidae, Sciaridae, 2 Aranei, Acari, *incertae sedis*. Holotype male (cf. Szadziewski & Gilka 2011).

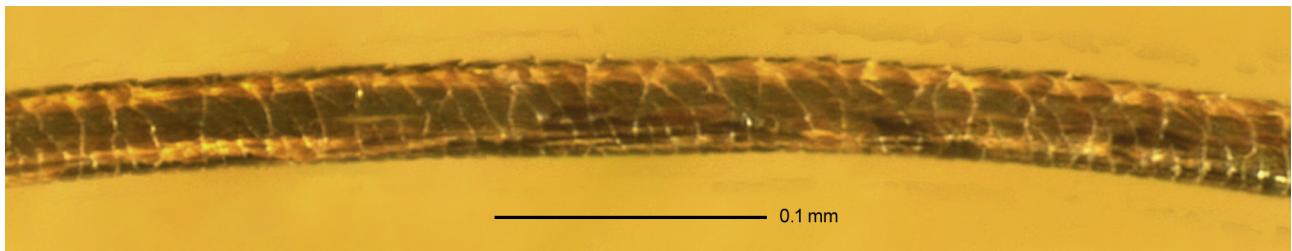
**Description.** Adult male ( $n = 2$ , measurements of holotype in boldface). *Colour:* Greyish-brown, with thorax and genitalia slightly darker (Fig. 4B). *Size:* Body length **4.6**–5.0 mm, body + proboscis **6.7**–6.8 mm, thorax + abdomen **4.2**–4.6 mm (Fig. 4B). *Antenna* (Fig. 4B, C): Length including pedicel  $\sim$ 1.75 mm, flagellum 1.68 mm, flagellomeres 12 and 13 0.38 and 0.40 mm, respectively, their combined length less than total length of preceding flagellomeres (ratio 0.87); whorls well developed, flagellomeres 1–11 each with whorl of many long setae, flagellomeres 12 and 13 with whorls of fewer weaker setae; antenna about 0.95 length of proboscis. *Proboscis* (Fig. 4C, D): Length **1.80**–**2.06** mm, shorter than maxillary palpus (ratio **0.78**–0.83). *Maxillary palpus* (Fig. 4C, D): Length **2.16**–**2.63** mm, longer than proboscis (ratio **1.20**–**1.28**); palpomeres 1–3 ankylosed (combined length 1.30 mm), about 0.72 length of proboscis; palpomere 4 (0.50–**0.59** mm), longer than palpomere 5 (0.36–**0.48** mm), ratio **1.23**–1.39; palpomeres 2–4 stout, parallel-sided; palpomere 5 smaller, slightly swollen medially, tapering towards narrow apex. *Thorax* (Fig. 5A, B): Setae as shown in Fig. 5A; mesothoracic prespiracular area with several setae, postspiracular setae not observed. *Wing* (Fig. 5C, D): Length 2.81–**2.88** mm; distal 0.5 of wing with slender lanceolate and spatulate scales on most veins; veins  $R_2$  and  $R_3$  distinctly longer than vein  $R_{2+3}$  ( $R_2/R_{2+3} = 2.25–**2.40**,  $R_3/R_{2+3} = 2.40–**2.50**); arrangement of veins in area of radiomedial and mediocubital crossveins as shown in Fig. 5D; anal vein (1A) ending 0.5 of distance between intersection of mediocubital crossvein and cubitus and base of  $M_{3+4}$  towards wing apex. *Legs* (Fig. 5E–H): Ungues of unequal length on fore- (~120 and 80  $\mu\text{m}$ ) and midleg (~120 and 85  $\mu\text{m}$ ), equally long on hindleg (~50  $\mu\text{m}$  long); larger anterior unguis of fore- and midleg each with stout proximal and medial teeth, smaller unguis of fore- and midlegs each with proximal tooth, medial tooth absent; hindungues simple (for length of leg segments see Table 3). *Genitalia* (Fig. 5I, J): Gonocoxite long (~360  $\mu\text{m}$ ), tapering toward rounded apex; gonostylus slender (~310  $\mu\text{m}$ ), hockey-stick-shaped, distinctly curved subapically, with apico-medial lobe and long apical tooth; claspette short, curved, with darkly pigmented, serrate margins and pointed apex (cf. Szadziewski & Gilka 2011: fig. 3).$$



**FIGURE 4.** Male of *Culiseta gedanica* Szadziewski et Gilka, 2011. **A**, Inclusion in amber; **B**, habitus; **C**, head; **D**, maxillary palpus and proboscis.



**FIGURE 5.** Male of *Culiseta gedanica* Szadziewski et Gilka, 2011. **A**, Thorax in dorsolateral aspect; **B**, area of mesothoracic spiracle; **C**, wing; **D**, area of radiomedial and mediocubital crossveins; **E–H**, ultimate tarsomeres with unguis of fore- (**E**), mid- (**F, G**) and hindleg (**H**); **I**, genitalia; **J**, gonostylus.



**FIGURE 6.** The mammalian hair—the syninclusion of the examined specimen of *Culiseta gedanica*.

**TABLE 3.** Lengths (mm) of leg segments of the male of *Culiseta gedanica* Szadziewski et Gilka, 2011. ? Unobservable; measurements of holotype in boldface.

Leg	Femur	Tibia	Tarsomeres				
			1	2	3	4	5
Foreleg	?	1.45	<b>1.03</b> –1.09	<b>0.37</b> –0.41	0.22– <b>0.24</b>	<b>0.12</b> –0.14	<b>0.20</b> –0.21
Midleg	1.37– <b>1.77</b>	1.55– <b>1.89</b>	1.39– <b>1.42</b>	0.59– <b>0.63</b>	0.37– <b>0.40</b>	0.14– <b>0.15</b>	0.19– <b>0.21</b>
Hindleg	1.28	1.59– <b>1.89</b>	1.57	0.82	0.58	0.33	0.25

## Discussion

### *Culex*

*Culex ekaterinae* is the seventh extinct species of the genus *Culex* described to date. Three of the seven species are known from inclusions in amber (*Cx. erikae*, *Cx. ekaterinae* and *Cx. malariager*) and four are known from compressions/impressions in sedimentary rock (*Cx. damnatorum*, *Cx. protorhinus*, *Cx. vectensis* and *Cx. winchesteri*). The geological ages of the six previously described species are given in Table 1. Three of the fossils are known from North America: *Cx. damnatorum* from deposits in Wyoming, *Cx. malariager* from Dominican amber (Dominican Republic) and *Cx. winchesteri* from deposits in Colorado. The other four species are known from Europe: *Cx. protorhinus* and *Cx. vectensis* from deposits in England, *Cx. erikae* from Baltic amber (Gulf of Gdańsk) and *Cx. ekaterinae* from Rovno amber of Ukraine. The four compression fossils and the inclusion fossil *Cx. malariager* are all females, all of which are morphologically distinct from and obviously not conspecific with the males of *Cx. erikae* and *Cx. ekaterinae*.

Szadziewski & Szadziewska (1985) described *Cx. erikae* as a species of the nominate subgenus. However, the placement of *Cx. ekaterinae*, and also *Cx. erikae* due to close similarity, in the subgenus *Culex* is doubtful in view of the following unique anatomical features: the distinct constriction beyond mid-length of the proboscis, the very short mediocubital crossvein with the adjoining veins distinctly indented and the strongly developed posterior cubitus. It is also noted that the agglutination of setae on the subapical lobe of the male gonocoxite is unusual, and seta *g*, which is most often foliform in species of the subgenus *Culex*, is not leaf-like in *Cx. ekaterinae*. These features may justify the recognition of a new subgenus, but until the phylogenetic relationships among extant species of the subgenus *Culex* are better understood, we refrain from introducing a new genus-group taxon at this time.

The adult males of *Cx. ekaterinae* and *Cx. erikae*, compared here, are presumably the most closely related fossil species known so far. They distinctly differ, however, in characters of the head, wing, legs and genitalia. Detailed character comparisons are shown in Figs 1–3 and listed in Table 4.

### *Culiseta*

Interestingly, among a number of arthropod syninclusions in the large piece of amber with the specimen of *Culiseta gedanica*, a mammalian hair was found (Fig. 6). The presence of adults of both sexes of the studied mosquito, including a hematophagous female in the same place and time seems to be highly probable, as does the co-occurrence of the mosquito with the hair of a mammal, the potential host. Although the structure of the hair is not sufficiently specific to determine a particular mammal (being similar to that known from different systematic groups of these animals), the shape of the scales and their arrangement are the characters that may be interpreted as advanced, typical of several extant taxa (J. N. Izdebska, pers. comm.).

**TABLE 4.** Comparison of diagnostic characters of the males of *Culex ekaterinae* sp. nov. and *Culex erikae* Szadziewski et Szadziewska, 1985. <sup>1</sup>Present data, <sup>2</sup>Szadziewski & Gilka (2011); a, body without proboscis; b, proboscis; c, thorax + abdomen.

Character	<i>Culex ekaterinae</i> <sup>1</sup>	<i>Culex erikae</i> <sup>1,2</sup>
Colour	Dark brown to black	Yellowish brown to dark brown
Main body parts (a, b, c)	5.2, 1.77, 4.7 mm	5.0, 2.06, 4.5 mm
Antennal flagellum	1.85 mm	1.77 mm
Flagellomeres 12 and 13	0.44 and 0.47 mm	0.48 and 0.48 mm
Flagellomeres 12+13 / 1–11 ratio	0.97	1.19
Maxillary palpus	2.72 mm	2.74 mm
Palpomeres 4 and 5	0.57 and 0.50 mm	0.53 and 0.45 mm
Palpomeres 4+5 / 1–3 ratio	0.65	0.56
Antennal flagellum/proboscis ratio	1.05	0.86
Proboscis/maxillary palpus ratio	0.65	0.75
Wing	2.90 mm	2.98 mm
Veins R <sub>2</sub> /R <sub>2+3</sub> and R <sub>3</sub> /R <sub>2+3</sub> ratios	3.00 and 3.16	3.1 and 3.2
Base of M <sub>3+4</sub> /rm ratio	< 1	> 1
Foreungues	Equally long, apically curved	Unequal, evenly curved
Teeth of foreungues (anterior   posterior)	Proximal absent, medial minute   proximal absent, medial absent	Proximal absent, medial stout   proximal small, medial absent
Teeth of midungues (anterior   posterior)	Proximal absent, medial stout   proximal absent, medial absent	Proximal absent, medial stout   proximal small, medial absent
Proximal setae <i>a–c</i> of subapical lobe of gonocoxite	Closely appressed, stout	Separated, narrow
Distal setae of subapical lobe	Partially fused, apices spoon-shaped	Separated, apices spoon-shaped

## Conclusions

Mosquitoes, as hematophagous insects and vectors of pathogens of diseases in humans, have been of special interest to zoologists since the discovery of their involvement in the epidemiology of malaria and yellow fever at the turn of the nineteenth century. At the time of this writing, 3,585 extant species classified in 41 genera have been described. As indicated in the Introduction, prior to the new species described above, the mosquito fossil record consisted of 27 species ascribed to 12 genera (six extinct; six extant); however, the morphology of each new fossil species provides useful information for understanding the phylogeny of the Culicidae. Three of the fossil species lived in the Cretaceous; the others existed in the Cenozoic, with 16 in the Eocene, including *Culex ekaterinae* described here. This indicates that the diversity of mosquitoes in the Mesozoic was still relatively meagre and increased significantly in the Paleogene, concomitant with the evolution of their avian and mammalian hosts.

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## References

- Andersen, T., Baranov, V., Goral, T., Langton, P., Perkovsky, E. & Sykes, D. (2015) First record of a Chironomidae pupa in amber. *Geobios*, 48 (4), 281–286.  
<https://doi.org/10.1016/j.geobios.2015.06.004>
- Azar, D., Nel, A. & Perkovsky, E. (2013) A new *Sycorax* species from Eocene Ukrainian Rovno Amber (Diptera: Psychodidae: Sycoracinae). In: Azar, D., Engel, M.S., Jarzembski, E., Krogmann, L. & Santiago-Blay, J. (Eds.), *Insect evolution in an amberiferous and stone alphabet*. Proceedings of the 6th International Congress on Fossil Insects, Arthropods and Amber, Leiden, Boston, Brill, pp. 27–46.  
[https://doi.org/10.1163/9789004210714\\_004](https://doi.org/10.1163/9789004210714_004)
- Baranov, V., Andersen, T. & Perkovsky, E. (2014) A new genus of Podonominae (Diptera: Chironomidae) in Late Eocene Rovno amber from Ukraine. *Zootaxa*, 3794, 581–586.  
<https://doi.org/10.11646/zootaxa.3794.4.9>
- Baranov, V.A., Kvifte, G.M. & Perkovsky, E.E. (2016) Two new species of fossil *Corethrella* Coquillett from Late Eocene Rovno amber, with a species-level phylogeny for the family based on morphological traits (Diptera: Corethrellidae). *Systematic Entomology*, 41 (2), 531–540.  
<https://doi.org/10.1111/syen.12172>
- Baranov, V.A. & Perkovsky, E.E. (2014) First record of gynandromorphy in fossil Chironomidae (Diptera) from Late Eocene Rovno amber. *Chironomus*, 27, 55–57.
- Belkin, J.N. (1962) *The mosquitoes of the South Pacific (Diptera, Culicidae)*. Vols 1 & 2. University of California Press, Berkeley and Los Angeles, xii + 608 and 412 pp.
- Blanchard, R. (1901) Observations sur quelques moustiques. *Comptes Rendus Hebdomadaires des Séances et Mémoires de la Société de Biologie*, 53, 1045–1046.
- Borkent, A. & Grimaldi, D.A. (2004) The earliest fossil mosquito (Diptera: Culicidae), in mid-Cretaceous amber. *Annals of the Entomological Society of America*, 97 (5), 882–888.  
[https://doi.org/10.1603/0013-8746\(2004\)097\[0882:TEFMDC\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2004)097[0882:TEFMDC]2.0.CO;2)
- Cockerell, T.D.A. (1916) British fossil insects. *Proceedings of the United States National Museum*, 49 (2119), 469–499, 6 pls.  
<https://doi.org/10.5479/si.00963801.49-2119.469>
- Cockerell, T.D.A. (1919) The oldest mosquitoes. *Nature*, 103 (2577), 43–46.  
<https://doi.org/10.1038/103044b0>
- Dietrich, C.H. & Perkovsky, E.E. (2020) A new genus and species representing the first leafhopper (Hemiptera: Cicadellidae) from Eocene Rovno amber. *Palaeoentomology*, 3 (2), 180–187.  
<https://doi.org/10.11646/palaeoentomology.3.2.7>
- Dyar, H.G. (1905) Remarks on genitalic genera in the Culicidae. *Proceedings of the Entomological Society of Washington*, 7 (1), 42–49.
- Edwards, F.W. (1923) Oligocene mosquitoes in the British Museum; with a summary of our present knowledge concerning fossil Culicidae. *Quarterly Journal of the Geological Society of London*, 79 (2), 139–155, 1 pl.  
<https://doi.org/10.1144/GSL.JGS.1923.079.01-04.10>
- Evenhuis, N.L. (1994) *Catalogue of the fossil flies of the world (Insecta: Diptera)*. Backhuys Publishers, Leiden, 600 pp.
- Fedotova, Z.A. & Perkovsky, E.E. (2017) New genus and species of gall midges (Diptera, Cecidomyiidae, Porricondylinae, Holoneuriini) from the Late Eocene amber of Olevsk (Zhitomir Region, Ukraine). *Vestnik Zoologii*, 51 (1), 23–30.  
<https://doi.org/10.1515/vzoo-2017-0004>
- Felt, E.P. (1904) Mosquitos or Culicidae of New York State. *New York State Museum Bulletin*, 79 (323), 241–391, 57 pls, 391a–391f+ 393–400.
- Gilka, W., Zakrzewska, M., Dominiak, P. & Urbanek, A. (2013) Non-biting midges of the tribe Tanytarsini in Eocene amber from the Rovno region (Ukraine): a pioneer systematic study with notes on the phylogeny (Diptera: Chironomidae). *Zootaxa*, 3736 (5), 569–586.  
<https://doi.org/10.11646/zootaxa.3736.5.8>
- Harbach, R.E. (2021) Mosquito Taxonomic Inventory. <http://mosquito-taxonomic-inventory.info> (accessed 29 April 2021).
- Harbach, R. & Greenwalt, D. (2012) Two Eocene species of *Culiseta* (Diptera: Culicidae) from the Kishenehn Formation in Montana. *Zootaxa*, 3530 (1), 25–34.  
<https://doi.org/10.11646/zootaxa.3530.1.2>
- Harbach, R.E. & Knight, K.L. (1980) *Taxonomists' glossary of mosquito anatomy*. Plexus Publishing, Marlton, NJ, xi + 415 pp.
- Harbach, R.E. & Knight, K.L. (1982) Corrections and additions to *Taxonomists' Glossary of Mosquito Anatomy*. *Mosquito Systematics*, 13 (2), 201–217. [for 1981]
- Kopeć, K., Perkovsky, E. & Skibińska, K. (2019) A new species of a genus *Cheilotrichia* (Diptera: Limoniidae) from Baltic and Ukrainian amber. *Annales Zoologici*, 69 (2), 423–426.  
<https://doi.org/10.3161/00034541ANZ2019.69.2.009>
- Krzemiński, W., Blagoderov, V., Azar, D., Lukashevich, E., Szadziewski, R., Wedmann, S., Nel, A., Collomb, F.-M., Waller, A. & Nicholson, D. (2019) True flies (Insecta: Diptera) from the late Eocene insect limestone (Bembridge Marls) of

- the Isle of Wight, England, UK. *Earth and Environmental Science Transactions of the Royal Society of Edinburgh*, 110 (3–4), 495–554.  
<https://doi.org/10.1017/S1755691018000464>
- Linnaeus, C. (1758) *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis*. Tomus 1, Editio Decima. Impensis Direct, Laurentii Salvii, Holmiae, 824 pp.  
<https://doi.org/10.5962/bhl.title.542>
- Lynch Arribálzaga, F. (1891) Dipterología argentina. *Revista del Museo de La Plata*, 1, 345–377.
- Lyubarsky, G.Y. & Perkovsky, E.E. (2020) First Rovno amber species of the genus *Telmatophilus* (Coleoptera: Clavicornia: Cryptophagidae) from Veselukha floodplain. *Invertebrate Zoology*, 17 (1), 25–35.  
<https://doi.org/10.15298/invertzool.17.1.03>
- Meigen, J.W. (1818) *Systematische Beschreibung der bekannten europäischen zweiflügeligen Insekten*. Vol. 1. Aachen, xxxvi + 332 pp., errata (1 p.), 11 pls.  
<https://doi.org/10.5962/bhl.title.12464>
- Meunier, F. (1915) Nouvelles recherches sur quelques insectes des plâtrières d'Aix en Provence. *Verhandelingen der Koninklijke Akademie van Wetenschappen le Amsterdam*, Tweede Sectie, 18 (5), 1–17, 5 pls.
- Mitov, P.G., Perkovsky, E.E. & Dunlop, J.A. (2021) Harvestmen (Arachnida: Opiliones) in Eocene Rovno amber (Ukraine). *Zootaxa*, 4984 (1), 43–72.  
<https://doi.org/10.11646/zootaxa.4984.1.6>
- Perkovsky, E.E. (2013) *Eohelea sinuosa* (Meunier, 1904) (Diptera, Ceratopogonidae) in Late Eocene Ambers of Europe. *Paleontological Journal*, 47 (5), 503–512.  
<https://doi.org/10.1134/S0031030113040163>
- Perkovsky, E.E. (2017) Comparison of biting midges of the Early Eocene Cambay amber (India) and Late Eocene European ambers supports the independent origin of European ambers. *Vestnik Zoologii*, 51 (4), 275–284.  
<https://doi.org/10.1515/vzoo-2017-0033>
- Perkovsky, E.E. & Fedotova, Z.A. (2016) *Rovnodiplosis eduardi* gen. et sp. nov., the first record of a fossil gall midge of the supertribe Mycodiplosidi (Diptera, Cecidomyioidea, Cecidomyiidae) in the Late Eocene of the Rovno amber. *Paleontological Journal*, 50 (9), 1027–1032.  
<https://doi.org/10.1134/S0031030116090124>
- Perkovsky, E.E. & Nel, A. (2021) New Rovno amber termite genus (Isoptera, Rhinotermitidae) from Styr River basin. *Palaeontology Electronica*, 24 (1), a05.  
<https://doi.org/10.26879/1127>
- Perkovsky, E.E., Olmi, M., Vasilenko, D.V., Capradossi, L. & Guglielmino, A. (2020) First *Bocchus* Ashmead (Hymenoptera: Dryinidae) from Upper Eocene Rovno amber: *B. schmalhauseni* sp. nov. *Zootaxa*, 4819 (3), 544–556.  
<https://doi.org/10.11646/zootaxa.4819.3.6>
- Perkovsky, E.E. & Rasnitsyn, A.P. (2013) Biting midges (Diptera, Ceratopogonidae) in amber forest communities based on analysis of syninclusions in Late Eocene Rovno amber. *Terrestrial Arthropod Reviews*, 6, 71–80.  
<https://doi.org/10.1163/18749836-06021059>
- Perkovsky, E.E. & Sukhomlin, E.B. (2015) New Late Eocene blackflies (Diptera, Simuliidae) from the Rovno Amber (Ukraine). *Paleontological Journal*, 49 (6), 608–614.  
<https://doi.org/10.1134/S0031030115060106>
- Perkovsky, E.E. & Sukhomlin, E.B. (2016) A new species of *Hellichiella* (Diptera: Simuliidae) with 11-segmented antenna from the Eocene. *Israel Journal of Entomology*, 46, 79–86.  
<https://zenodo.org/record/59292#.YF9c599KhhE>
- Perkovsky, E.E., Sukhomlin, E.B. & Panchenko, A.A. (2013) The modern distribution of blackflies genera from Baltic and Rovno amber. In: *Proceedings, VIII Congress of the Ukrainian Entomological Society*, Kiev, 26–30 August 2013, pp. 121–122.
- Perkovsky, E.E., Zosimovich, V.Y. & Vlaskin, A.P. (2010) Rovno amber. In: Penney, D. (Ed.) *Biodiversity of fossils in amber from the major world deposits*. Siri Scientific Press, Manchester, pp. 116–136.
- Pielowska, A., Sontag, E. & Szadziewski, R. (2018) Haematophagous arthropods in Baltic amber. *Annales Zoologici*, 68 (2), 237–249.  
<https://doi.org/10.3161/00034541ANZ2018.68.2.003>
- Piton, L. (1936) Addition à la faune entomologique des cinérites du Lac Chambon (Puy-de-Dôme). *Revue Scientifique du Bourbonnais*, 1936, 16–24.
- Poinar, G. Jr. (2005) *Culex malariager*, n. sp. (Diptera: Culicidae) from Dominican amber: the first fossil mosquito vector of *Plasmodium*. *Proceedings of the Entomological Society of Washington*, 107 (3), 548–553.
- Poinar, G., Zavortink, T.J. & Brown, A. (2020) *Priscoculex burmanicus* n. gen. et sp. (Diptera: Culicidae: Anophelinae) from mid-Cretaceous Myanmar amber. *Historical Biology*, 32 (9), 1157–1162.  
<https://doi.org/10.1080/08912963.2019.1570185>
- Poinar, G.O. Jr., Zavortink, T.J., Pike, T. & Johnston, P.A. (2000) *Paleoculicis minutus* (Diptera: Culicidae) n. gen., n. sp., from Cretaceous Canadian amber, with a summary of described fossil mosquitoes. *Acta Geologica Hispanica*, 35 (1–2), 119–128.
- Radchenko, A.G. & Perkovsky, E.E. (2021) Wheeler's dilemma revisited: first *Oecophylla – Lasius* syninclusion and other ants

- syninclusions in the Bitterfeld amber (late Eocene). *Invertebrate Zoology*, 18 (1), 47–65.  
<https://doi.org/10.15298/invertzool.18.1.05>
- Radchenko, A.G., Perkovsky, E.E. & Vasilenko, D.V. (2021) *Formica* species (Hymenoptera, Formicidae, Formicinae) in late Eocene Rovno amber. *Journal of Hymenoptera Research*, 82, 237–251.  
<https://doi.org/10.3897/jhr.82.64599>
- Scudder, S.H. (1890) *The tertiary insects of North America. United States Geological Survey of the Territories*. Vol. 13. Government Printing Office, Washington, D.C., 734 pp.  
<https://doi.org/10.5962/bhl.title.44698>
- Skartveit, J. (2021) A new fossil species of the genus *Bibio*, with an update on bibionid flies from Baltic and Rovno amber (Diptera, Bibionidae). *Deutsche Entomologische Zeitschrift*, 68 (1), 81–99.  
<https://doi.org/10.3897/dez.68.60611>
- Sokoloff, D.D., Ignatov, M.S., Remizowa, M.V., Nuraliev, M.S., Blagoderov, V., Garbout, A. & Perkovsky, E.E. (2018) Staminate flower of *Prunus s. l.* (Rosaceae) from Eocene Rovno amber (Ukraine). *Journal of Plant Research*, 131 (6), 925–943.  
<https://doi.org/10.1007/s10265-018-1057-2>
- Sontag, E. & Szadziewski, R. (2011) Biting midges (Diptera: Ceratopogonidae) in Eocene Baltic amber from the Rovno region (Ukraine). *Polish Journal of Entomology*, 80 (4), 779–800.  
<https://doi.org/10.2478/v10200-011-0058-4>
- Statz, G. (1944) Neue Dipteren (Nematocera) aus dem Oberoligocän von Rott. II. Teil. V. Familie Culicidae (Stechmücken). *Palaeontographica*, 95 (A), 108–120, 6 pls.
- Szadziewski, R. (1998) New mosquitoes from Baltic amber (Diptera: Culicidae). *Polskie Pismo Entomologiczne*, 67 (3–4), 233–244.
- Szadziewski, R. & Gilka, W. (2011) A new fossil mosquito, with notes on the morphology and taxonomy of other species reported from Eocene Baltic amber (Diptera: Culicidae). *Polish Journal of Entomology*, 80 (4), 765–777.  
<https://doi.org/10.2478/v10200-011-0057-5>
- Szadziewski, R., Sontag, E. & Szwedo, J. (2019) Mosquitoes of the extant avian malaria vector *Coquillettidia* Dyar, 1905 from Eocene Baltic amber (Diptera: Culicidae). *Palaeoentomology*, 2 (6), 650–656.  
<https://doi.org/10.11646/palaeoentomology.2.6.16>
- Szadziewski, R. & Szadziewska, M. (1985) New mosquitoes from Baltic amber (Diptera: Culicidae). *Polish Journal of Entomology*, 55, 513–518.
- Theobald, F.V. (1901) The classification of mosquitoes. *Journal of Tropical Medicine*, 4, 229–235.
- von Heyden, L. (1870) Fossile Dipteren aus der Braunkohle von Rott im Siebengebirge. *Palaeontographica*, 17 (6), 237–266, 2 pls.
- Wagner, R. (2021) *Neoarisemus groehni* sp. nov., a notable moth fly (Diptera, Psychodidae, Psychodinae) from Ukrainian amber. *Palaeoentomology*, 4 (1), 19–22.  
<https://doi.org/10.11646/palaeoentomology.4.1.3>
- Wojtoń, M., Kania, I. & Krzeminski, W. (2019) Review of *Mycetobia* Meigen, 1818 (Diptera, Anisopodidae) in the Eocene ambers. *Zootaxa*, 4544 (1), 1–40.  
<https://doi.org/10.11646/zootaxa.4544.1.1>
- Zakrzewska, M. & Gilka, W. (2014) The oldest known chironomids of the tribe Tanytarsini (Diptera: Chironomidae) indicate plesiomorphic character states. *Geobios*, 47, 335–343.  
<https://doi.org/10.1016/j.geobios.2014.07.004>
- Zakrzewska, M., Krzeminski, W. & Gilka, W. (2016) Towards the diversity of non-biting midges of the tribe Tanytarsini from Eocene Baltic amber (Diptera: Chironomidae). *Palaeontologia Electronica*, 19.2.18A, 1–21.  
<https://doi.org/10.26879/621>
- Zavortink, T.J. & Poinar, G.O. Jr. (2000) *Anopheles (Nyssorhynchus) dominicanus* sp. n. (Diptera: Culicidae) from Dominican Amber. *Annals of the Entomological Society of America*, 93 (6), 1230–1235.  
[https://doi.org/10.1603/0013-8746\(2000\)093\[1230:ANDSND\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2000)093[1230:ANDSND]2.0.CO;2)
- Zavortink, T.J. & Poinar, G.O. Jr. (2008) *Toxorhynchites (Toxorhynchites) mexicanus*, n. sp. (Diptera: Culicidae) from Mexican amber: a New World species with Old World affinities. *Proceedings of the Entomological Society of Washington*, 110 (1), 116–125.  
<https://doi.org/10.4289/0013-8797-110.1.116>
- Zelentsov, N.I., Baranov, V.A., Perkovsky, E.E. & Shobanov, N.A. (2012) First records on non-biting midges (Diptera: Chironomidae) from the Rovno amber. *Russian Entomological Journal*, 21 (1), 79–87.  
<https://doi.org/10.15298/rusentj.21.1.10>