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A new fossil *Stempellinella* from Eocene Baltic amber, with systematic notes and amendment of the generic diagnosis (Diptera: Chironomidae)

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Abstract

Stempellinella gilkai **sp. nov.**, an Eocene non-biting midge of the subfamily Chironominae, tribe Tanytarsini is described on the basis of the adult male. This is the sixth fossil representative of the genus and the fifth described from Baltic amber (~40–45 Ma). The species displays a set of morphological characters typical of the genus. To facilitate a delimitation of fossil *Stempellinella*, a comparison of the most significant characters is provided. The key features of antenna and hypopygium are discussed in detail. A comparative analysis of both extinct and extant *Stempellinella* representatives allowed to perform an amendment of the current generic diagnosis. Notes on the genus' palaeoecology and species richness during the Eocene are also included.

Key words: Chironominae, new species, non-biting midges, palaeoecology, Tanytarsini, taxonomy

Introduction

When it comes to an inventory of fossil chironomids, including those of the tribe Tanytarsini, the taxa known from Eocene ambers are undoubtedly the best studied ones, with the vast majority of specimens identified and species described. So far, 26 Eocene Tanytarsini species were assigned to both extant (*Nandeva* Wiedenbrug, Reiss *et* Fittkau, 1998, *Rheotanytarsus* Thienemann *et* Bause, 1913, *Stempellina* Thienemann *et* Bause, 1913, *Stempellinella* Brundin, 1947 and *Tanytarsus* van der Wulp, 1874) and extinct Tanytarsini genera (*Archistempellina* Giłka *et* Zakrzewska, 2013, *Corneliola* Giłka *et* Zakrzewska, 2013, *Eonandeva* Giłka *et* Zakrzewska, 2015 and *Gujaratomyia* Giłka *et* Zakrzewska, 2018), and thus provide a much wanted insight into the tribe's evolution course (Seredszus & Wichard 2007, Giłka 2010, 2011a, Giłka *et al.* 2013, 2016, Zakrzewska & Giłka 2013, 2014, 2015a, 2015b, Zakrzewska *et al.* 2016, 2018, 2020, Stebner *et al.* 2017). One of the most diverse representatives belong to the genus *Stempellinella*.

Today, the genus *Stempellinella* (subtribe Zavreliina Sæther, 1977) is represented by 20 extant species distributed worldwide (Ekrem 2007, Guo & Wang 2007, Fusari & Lamas 2014), and five fossil species known from Eocene resins, mainly from ambers of the Baltic region (called Baltic amber, ~40–45 Ma): *S. bicorna* Seredszus *et* Wichard, 2007, *S. ivanovae* Giłka *et* Zakrzewska, 2014, *S. electra* Giłka *et* Zakrzewska, 2015 and *S. fibra* Giłka, Zakrzewska *et* Krzemiński, 2016 (Seredszus & Wichard 2007, Zakrzewska & Giłka 2014, 2015a, Zakrzewska *et al.* 2016); *S. pollex* Giłka *et* Zakrzewska, 2020, is the only Eocene representative described from outside the Baltic region (early Eocene Cambay amber, ~54 Ma, Gujarat, India), what makes this species both the oldest known in the genus and one of the oldest Tanytarsini (Zakrzewska *et al.* 2020).

In this paper we present the sixth fossil representative of the genus, which is also the fifth *Stempellinella* species found in Baltic amber deposits.

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Material and methods

The specimen examined is an adult male preserved in Baltic amber. The amber piece was ground and polished manually to enable the highest possible visibility to study and illustrate the inclusion and its diagnostic structures.

Measurements of specimen are in micrometres, except for the total length (in millimetres, rounded off to the second decimal place). The body length was measured from the antennal pedicel to the end of the gonostylus, while the wing length—from the arculus to the tip. Lengths of leg segments and palpomeres were rounded off to the nearest 5 and 1 μm, respectively. The antennal, leg, and venarum ratios (AR, LR, VR) were calculated to the second decimal place. The morphological terminology and abbreviations follow Sæther (1980). In this paper we introduce a median volsella ratio (MVoR), which is a ratio of the longest lamella length to the stem length. Photographs were taken using the classic microscope PZO Biolar SK14 and Helicon Focus 6 image stacking software.

As a support for the identity of the amber piece examined, a Fourier transform infrared spectroscopy (FT-IR) spectrum [attenuated total reflectance (ATR)] was obtained (spectrometer type: Nicolet iS10 FT-IR, resolution: 4 cm^{-1}, number of scans: 16) and subjected to advanced ATR correction (baseline correction and spectral operations using a standard spectrophotometer software) (Fig. 1). The spectrum is registered and stored in the archives of the Laboratory of the International Amber Association (IAA), Gdańsk, Poland. The inclusion is part of the collection of the Laboratory of Systematic Zoology at the Department of Invertebrate Zoology & Parasitology (LSZ DIZP), University of Gdańsk, Poland. New nomenclatural acts and the paper were registered in Zoobank, in accordance with ICZN requirements (LSID: urn:lsid:zoobank.org:pub:A6ECE1FA-92E3-4EAE-BB87-288B8752AD38).

Systematics

Family: Chironomidae Newman, 1834

Subfamily: Chironominae Newman, 1834

Tribe: Tanytarsini Zavřel, 1917

Genus: *Stempellinella* Brundin, 1947

Type species: *Stempellinella saltuum* (Goetghebuer, 1921).

Stempellinella gilkai sp. nov.

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Type material. Holotype. Adult male (tarsi of both fore, left mid and right hind leg missing) preserved in 30 x 24 x 5 mm piece of amber (Fig. 2A, B) (Eocene Baltic amber, ~40–45 Ma, inventory no. F28a LSZ DIZP; IR spectrum no. 16218 IAA; Fig. 1). Syninclusions: Chironominae, 1 adult male (F28b LSZ DIZP), Sternorrhyncha, 1 specimen (F28c LSZ DIZP).

Derivatio nominis. Authors dedicate the specific epithet to their mentor, Prof. Wojciech Giłka, a Chironomidae specialist.

Diagnosis. Antennal flagellum composed of 13 flagellomeres. Sc ending far distal of RM; RM long, slightly oblique relative to M. Vein M_{3+4} ending opposite to R_{4+5}. Gonostylus slightly shorter than gonocoxite. Anal point stout, with strong crests framing numerous irregularly dispersed spinulae. Superior volsella elongate, peanut-shaped. Stem of median volsella long, bearing setiform and foliate lamellae, distal lamella with elongated filiform tip reaching apex of inferior volsella. Inferior volsella with spherical bulge bearing 4 medially directed distinct setae, apical part bent medially.

Description. Adult male (n = 1).

Total length ~1.55 mm; wing length 945 μm.

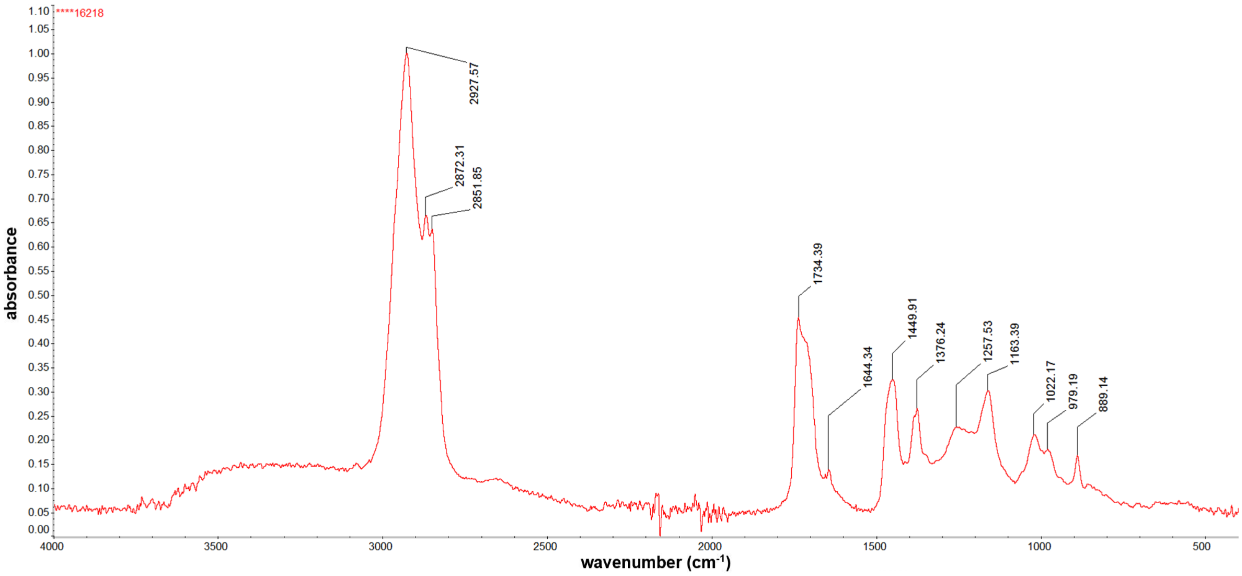

FIGURE 1. Fourier transform infrared spectroscopy (ATR) spectrum no. 16218 IAA obtained from the piece of Baltic amber (Eocene, ~40–45 Ma) with the examined inclusion.

Head (Fig. 2B, C). Eyes bare, without dorsomedian extension, broadly separated by frons. Frontal tubercles conical, length 8 µm, width 8 µm. Antenna with 13 flagellomeres (borders between flagellomeres 12 and 13 faintly visible but present) (Fig. 2C), AR 0.45, plume fully developed. Length of palpomeres 2–5 (in µm): 28, 60, 76, 117. Clypeus with at least 5 setae.

Thorax (Fig. 2B). Ac at least 7, Dc at least 7 on each side, Scts at least 3, Pa unobservable.

Wing (Fig. 3). Slender, with anal lobe weak, broadest at 2/3 length; width: 285 µm, length/width ratio: 3.32. Sc ending well distal of RM and slightly distal of FCu, R$_{2+3}$ not visible. RM oblique relative to M. FCu placed distally of RM; VR$_{Cu}$ 1.36. Veins ending as follows (in order from base to tip): An, Sc, Cu$_1$, R$_1$, R$_{4+5}$ and M$_{3+4}$, M$_{1+2}$. Wing membrane and most veins (except subcosta and media) covered with macrotrichia.

Legs (Fig. 2B). Tibial apices with their armature faintly visible. Foreleg tibia with fine spur, up to 10 µm long. Mid- and hindleg tibia with small combs ~10 µm long. One midleg comb bearing ~20 µm long spur, both hindleg combs with spurs (~15-25 µm long). Sensilla chaetica on ta$_1$ of p$_2$ not observed. Pulvilli absent. Lengths of leg segments and leg ratios as in Table 1.

TABLE 1. Lengths (µm) of leg segments and leg ratios of male *Stempellinella gilkai* **sp. nov.,** p$_1$–p$_3$ = pair of legs 1–3, fe=femur, ti=tibia, ta$_1$–ta$_5$ = tarsomeres 1–5, LR = leg ratio.

	fe	ti	ta$_1$	ta$_2$	ta$_3$	ta$_4$	ta$_5$	LR
p$_1$	445	250	-	-	-	-	-	-
p$_2$	490	360	230	115	90	60	45	0.64
p$_3$	475	415	295	155	135	90	50	0.71

Hypopygium (Fig. 4A–E). Gonostylus short, ~65 µm, slightly shorter than gonocoxite, gently tapering to blunt apex armed with both strong setae and microtrichia. Anal tergite subtriangular, with 5–6 posterolateral setae on each side of anal point, several irregularly dispersed fine median setae present. Anal point long and stout, reaching posterior margin of superior volsellae apices, tapering to blunt apex, with strong crests framing 21 irregularly dispersed spinulae (Fig. 4A, C). Superior volsella elongate, peanut-shaped, apex with protuberances bearing up to 4 setae. Digitus not observed. Stem of median volsella long (~30 µm), more or less cylindrical with 2 subtle conical processes on apex, bearing two setiform lamellae on the proximal part and 5 to 6 foliate lamellae, distal lamella with elongated filiform tip reaching apex of inferior volsella (Fig. 4B, D, E). Inferior volsella reaching 2/3 length of gonostylus, with spherical bulge bearing 4 medially directed distinct setae at 1/3 of its length, with apical part bent medially, bearing several strong setae (Fig. 4A–C).

FIGURE 2. *Stempellinella gilkai* **sp. nov.**, holotype, adult male (F28a LSZ DIZP, Eocene Baltic amber, ~40–45 Ma): **A** inclusion in amber, **B** habitus, **C** head and antenna (white arrows: borders between flagellomeres). Scale bars: 1 cm (A), 500 µm (B), 200 µm (C).

Discussion

1. Diagnostic remarks

The characters of the adult male of *Stempellinella gilkai* are in accordance with generic diagnosis by Ekrem (2007): the specimen is relatively small, with bare ovoid eyes (Fig. 2B, C) and conical frontal tubercles, the wing is covered in macrotrichia except veins Sc and M, the vein R_{4+5} ends opposite to the M_{3+4} (Fig. 3), the hypopygium with well-developed anal point, spinulae and crests present, the median volsella with elongated stem and the gonostylus shorter than the gonocoxite (Fig. 4A–E).

In order to enable an effective delimitation of Eocene *Stempellinella*, a comparison of the most significant characters are shown in Table 2. The key features are discussed in more detail below.

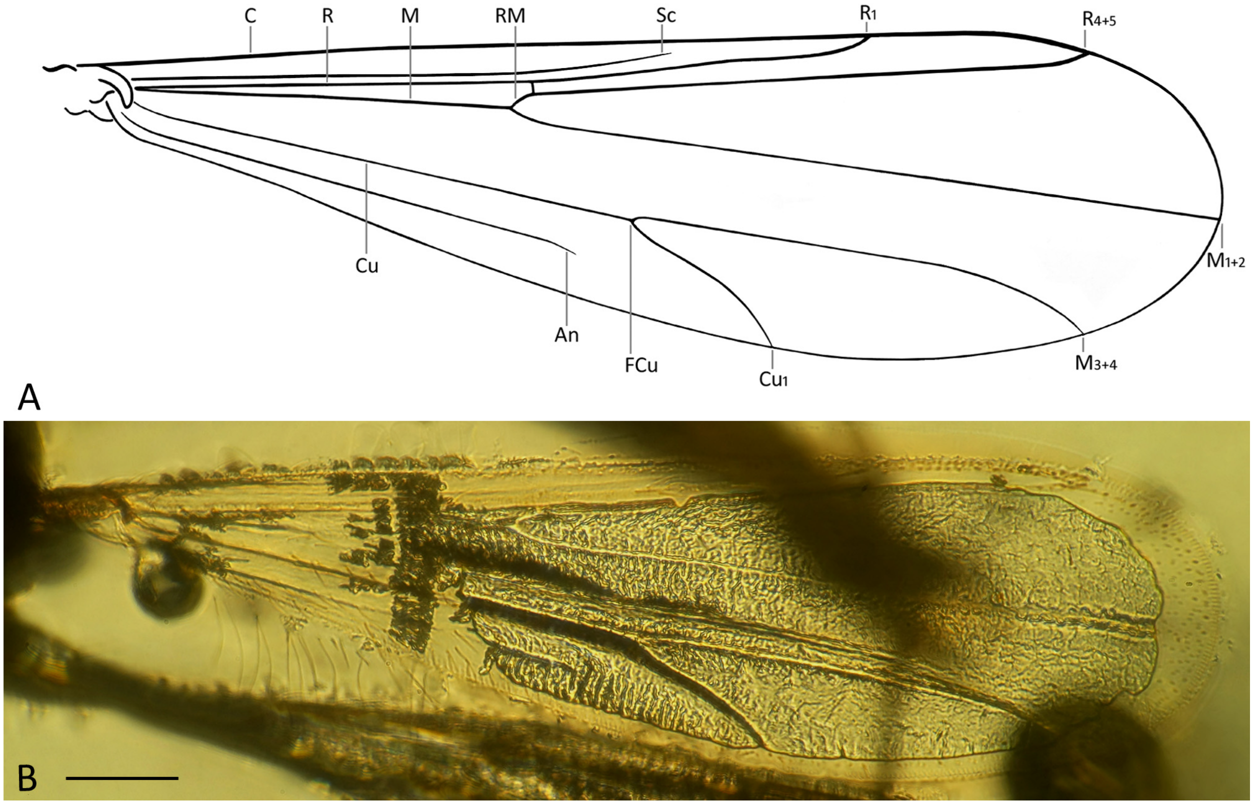

FIGURE 3. *Stempellinella gilkai* **sp. nov.**, holotype, adult male (F28a LSZ DIZP, Eocene Baltic amber, ~40–45 Ma): **A, B** wing. Scale bar: 100 µm. C—costa, Sc—subcosta, R, R_1, R_{4+5}—radial veins, RM—radial-median crossvein, M, M_{1+2}, M_{3+4}—median veins, Cu, Cu_1—cubital veins, FCu—cubital crossvein, An—anal vein.

Antennal flagellum

At present, the majority of adult Tanytarsini males tend to have the antenna consisted of 13 flagellomeres, although in some taxa distal or proximal ones may be partially or even completely fused, what result in their lower number. All extant *Stempellinella* species have the antenna consisted of only 10 discernible flagellomeres—the state considered a generic apomorphy (Ekrem 2007, Sæther & Andersen 1998, Sæther & Roque 2004).

The character in more plesiomorphic, transitional phase has been observed in Eocene *Stempellinella* species, albeit with varying degrees of fusion/partial fusion of flagellomeres 11 to 13 (Zakrzewska & Giłka 2014, 2015a, Zakrzewska *et al.* 2016, 2020).

Stempellinella gilkai, herein described, confirms the trend by having 13 flagellomeres, what indicate the most plesiomorphic state noted and highlights the transitional steps of the character within the genus, observed within other Eocene species (Fig. 2C).

Stempellinella bicorna with 10 discernible flagellomeres seems to be an only exception that may suggest that differentiation in evolutionary pace within the genus was already evident in the Eocene (Seredszus & Wichard 2007, fig. 19); however, the accuracy of species description is debatable as it lacks many details for effective comparison. A re-examination of the holotype in order to clarify the doubts is suggested as a possible solution.

Hypopygium

The long anal point with distinct crests and dispersed spinulae found in *Stempellinella gilkai* is the character typical of the genus. A similar set can be observed in several extant species, e.g. *S. ciliaris* (Goetghebuer, 1944), *S. fimbriata* Ekrem, 2007, *S. flavidula* (Edwards, 1929), *S. sublettorum* Ekrem, 2007, *S. tamaseptima* (Sasa, 1980) (Ekrem, 2007), as well as in Eocene *S. ivanovae*.

The median volsella in *Stempellinella gilkai* resembles that known from another Eocene species described from Baltic amber—*S. fibra* Giłka, Zakrzewska *et* Krzemiński, 2016, although they can be easily distinguished by comparing the shape of the stem (club-shaped in *S. fibra* vs more cylindrical in *S. gilkai*) as well as distribution and proportions of foliate lamellae—in *S. fibra* only one lamella bears extraordinarily elongated filiform tip while other lamellae are grouped on the stem apex, whereas in *S. gilkai* the tip of distal lamella is only slightly longer than tips of other lamellae which are evenly distributed over distal 2/3 of the stem (cf. Fig. 4D, E & Zakrzewska *et al*. 2016, fig. 8).

Although an overall shape of the inferior volsella also resembles *S. fibra*, the characteristic bulge bearing four setae is a distinctive diagnostic feature, not observed in any fossil or extant *Stempellinella* species (op. cit.).

2. Adult males of *Stempellinella*—generic diagnosis amended and annotated

An analysis and comparison of characters of extinct and extant *Stempellinella* representatives enabled us to clarify the generic diagnosis given by Ekrem (2007). Therefore, we present an amended diagnosis of adult *Stempellinella* males. The changes are given in bold, some of the features are explained in detail in comments below:

Adult male. Small, wing length 0.7–1.7 mm. Body green, yellow, or brown with darker vittae.

Head: **antenna with 10–13 flagellomeres in fossil species, only 10 are easily discernible in extant species. Antennal ratio 0.4–1.5.** Eye ovoid, bare, without dorsomedian extension. Frontal tubercles present as small dots or well-developed cones; 5–12 temporal setae in one row. Mouthparts and apodemes normal.

Thorax: antepronotum widely divided dorsally, bare. Scutum overhanging pronotum, with or without weak scutal tubercle. Acrosticals 8–17; dorsocentrals 5–12; prealars 1–2; scutellars 4–11, humerals 0–3; thorax otherwise bare.

Wing: cuneiform. Membrane and most veins extensively covered with macrotrichiae, denser distally; subcosta, media, and squama bare. Costa not produced; **R_{4+5} ending opposite or proximal to apex of M_{3+4}—feature excluded (see Comment 1).**

Legs: fore leg tibia with short, slender, apical spur. Combs of mid and hind legs tibiae narrow, well separated, with one or two spurs. Pulvilli absent or minute.

Hypopygium: anal tergite with transverse or slightly T-shaped anal tergite band. Median tergite setae long anteriorly on tergite or short close to anal point or both. Anal point well-developed, slender to broad at base, often narrower and pointed towards apex, with or without well-developed anal crests and anal point spinulae. Some species with groups of microtrichia near anal point base. Setiger of superior volsella oval to almost rectangular, straight to strongly bent medially, with three to six dorsal and two (*Stempellinella truncate* three) median setae. Superior volsella otherwise bare. Digitus absent, but contour of superior volsella stem on setiger often prominent. Median volsella with well-developed simple or branched stem with or without microtrichia, **lamellae simple or setiform, in extinct species also falciform, pectinate or foliate**. Inferior volsella long, straight or club-shaped with concave median margin, reaching past apex of gonocoxite; with apical setae and microtrichia, dorsal surface otherwise bare. **Some species with the additional, variously formed process on the inferior volsella (see Comment 2). Gonostylus at least slightly shorter than gonocoxite**, often with concave median margin and medially directed apex; median margin with several strong setae, dorsomedian margin usually without microtrichiae.

Comment 1: As the R_{4+5} and M_{3+4} endings setup occur not only as was given by Ekrem (2007), but also in configuration where R_{4+5} ends distal to apex of M_{3+4} (see: *S. flavidula* (Edwards, 1929), *S. saltuum* (Goetghebuer, 1921) or *S. depilisa* Guo *et* Wang, 2007), we hereby propose to exclude that feature from the generic diagnosis.

Comment 2: Process on the inferior volsella: as the character is present in several species, both extant (*S. ciliaris*, *S. flavidula*, *S. saltuum*, *S. sublettorum*) and extinct (*S. pollex*) (see Zakrzewska *et al*. 2020), we propose to treat the feature as an underlying synapomorphy (see Sæther 1979, 1983), unify the morphological terminology and add the feature to generic diagnosis.

3. Palaeoecology

The studies on the Tanytarsini fauna in Eocene ambers, although conducted for a relatively short period (over the last several years) and certainly not closing the list of species occurring at that time, resulted in the discovery and description of as many as six *Stempellinella* species. The five of them, obtained from the Baltic amber account for 24% of all Tanytarsini from this best-researched fossil source. By comparing these data with the number of extant representatives occurring in Europe, i.e. six *Stempellinella* species count as ~3% of nearly 200 European Tanytarsini species (Langton & McBean 2010, Giłka 2011b, Giłka & Jażdżewska 2012, Moubayed-Breil *et al*. 2012 + works/specific names published afterwards, Giłka *et al*. 2018, Puchalski *et al*. 2018), it can be assumed that back at the Eocene the speciation within the genus was already advanced and *Stempellinella* share within the Eocene Tanytarsini fauna was much greater than at Present. The state of affairs can be explained by ecological conditions prevailing at that time.

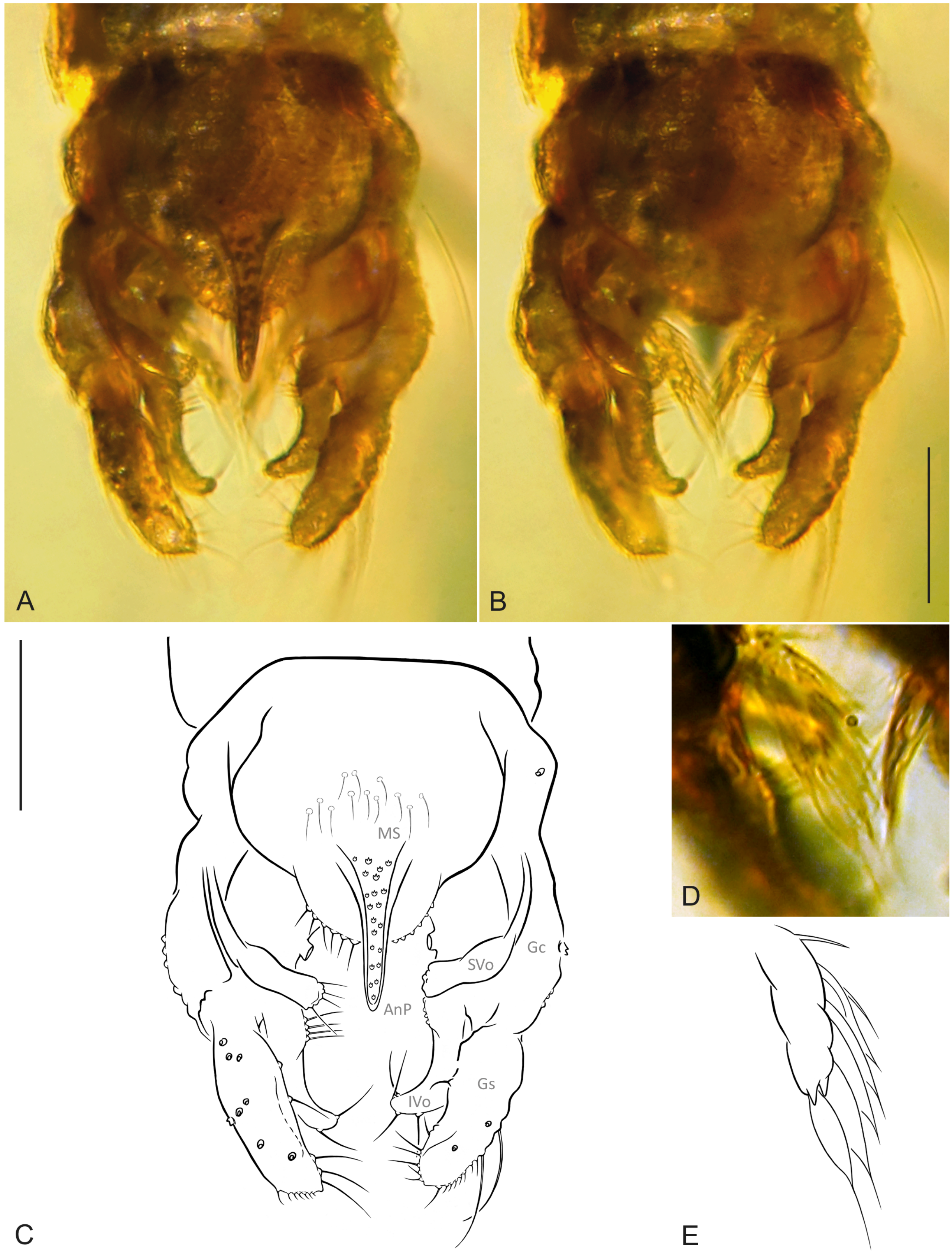

FIGURE 4. *Stempellinella gilkai* **sp. nov.**, holotype, adult male (F28a LSZ DIZP, Eocene Baltic amber, ~40–45 Ma): **A–C** hypopygium in dorsal aspect, stacked at different depth levels (A, B) and drawn (C), **D**, **E** median volsella (magnified c. 2 times relative to hypopygium photo). Scale bars: 50 µm. MS—median setae, AnP—anal point, SVo—superior volsella, IVo—inferior volsella, Gc—gonocoxite, Gs—gonostylus.

TABLE 2. Comparison of diagnostic characters of fossil *Stempellinella* species. Legend: “+” for the presence of the character, “-“ for the absence of the character, “x” for character not included in original description or unobservable, grey background of the cell for character measurement taken from original description figure.

	Stempellinella bicorna	***Stempellinella electra***	***Stempellinella fibra***	***Stempellinella gilkai***	***Stempellinella ivanovae***	***Stempellinella pollex***
origin	Baltic amber	Baltic amber	Baltic amber	Baltic amber	Baltic amber	Cambay amber
total length (mm)	1.4	1.50	1.55	1.50	1.18	1.10–1.20
FT length (µm)	present, not measured	10	15	8	-	x
number of flagellomeres	10	13 (11–13 partly fused)	13 (12–13 partly fused)	13	13 (12–13 partly fused)	13 (12–13 partly fused)
AR	AR 0.9 (as 10-segmented)	AR 0.76 (as 11-segmented) AR 0.62 (as 12-segmented) AR 0.50 (as 13-segmented)	AR 0.68 (as 12-segmented) AR 0.52 (as 13-segmented)	AR 0.45 (as 13-segmented)	AR 0.67 (as 12-segmented) AR 0.51 (as 13-segmented)	AR 0.61–0.66 (as 12-segmented) AR 0.46–0.52 (as 13-segmented)
wing length (µm)	1100	890	x	945	870	640–690
wing length/width ratio	3.18	3.12	x	3.32	3.90	3.20–3.21
VRCu	1.44	1.38	1.55	1.35	1.41	1.50
position of R_{4+5} vs M_{3+4} ending	$R_{4+5} > M_{3+4}$	$R_{4+5} = M_{3+4}$	-	$R_{4+5} = M_{3+4}$	$R_{4+5} = M_{3+4}$	$R_{4+5} = M_{3+4}$
p1 spur (µm)	x	-	x	~ 10 µm	x	~15 µm
LR1	x	2.13	-	-	2.24	1.97
LR2	x	0.66	-	0.64	-	0.62
LR3	x	0.71	0.76	0.71	0.81	-
gonostylus (µm)	45	50	60	65	45	50
process on gonostylus apex	-	-	-	-	nipple-like process	-
median setae	x	+	+	+	x	x
anal point spinulae	x	-	-	+	+	x
MVo stem length (µm)	x	20	30	30	10	5
longest lamella (µm)	x	20	25	30	~ 7	20
MVoR	x	= 1	< 1	= 1	< 1	>> 1
process on inferior volsella	-	-	-	-	-	+

As the Chironomidae are usually sensitive to both climate and environmental changes, they often serve as a great indicators that help both to monitor the changes occurring in waterbodies in the Present and also are widely used in reconstructions of aquatic habitats in the Past (e.g. Larocque *et al*. 2001, Stebner *et al*. 2017). Adults and immatures of extant *Stempellinella* inhabit fresh waters, mainly lotic: rivers, springs and streams (only a few species are limnophilous) (Ekrem 2007). By being nutrient-tolerant, they tend to thrive in eutrophicated waters (Raunio *et al*. 2007, Yang *et al*. 2017). The process of enriching water with nutrients began during Cretaceous, being a consequence of an intense terrestrial angiosperms' radiation at that time (extensive production of biomass led to the growth of hydrocoenosis trophicity through surface runoff), and is considered to be the starting point of Chironominae diversification (Kalugina 1974, Buatois *et al*. 2016; authors' unpublished data). The observed high diversification of fossil *Stempellinella* may be thus an evidence of a prevalence of eutrophicated freshwaters during the Eocene.

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