

Wanted, tracked down and identified: Mesozoic non-biting midges of the subfamily Chironominae (Chironomidae, Diptera)

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Here we provide evidence for the presence of non-biting midges of the subfamily Chironominae in the Mesozoic, based on descriptions of exceptionally rare amber inclusions. The subfamily has already been reported, but based on a single and unspecified record from Late Cretaceous Taimyr amber (~84 Mya). That record is here revised and confirmed. Moreover, a new find in Burmese amber locates the temporal boundary of the subfamily back to the mid-Cretaceous (probably ~100 Mya). We describe two new genera assigned to the tribe Pseudochironomini: **Mesoacentron gen. nov.** with the species **Mesoacentron kaluginae sp. nov.** (Taimyr amber) and **Palaeocentron gen. nov.** with the species **Palaeocentron krzeminskii sp. nov.** (Burmese amber), the oldest known representative of the subfamily. The systematic position of the new taxa is discussed, and a key to the identification of adult males of extinct and extant Pseudochironomini genera is presented. Insights in the phylogeny and diversification tempo of the Chironominae and Pseudochironomini in the past are also provided.

ADDITIONAL KEYWORDS: amber – Cretaceous – evolution – new taxa – Pseudochironomini – systematics.

INTRODUCTION

The Chironomidae (non-biting midges) are a large family of dipterans with nearly 7500 species and 550 genera described (Pape *et al.*, 2011; plus data published thereafter). Although their larvae may be terrestrial or semi-aquatic, the majority are

strictly aquatic developing in freshwater habitats, both lotic and lentic (Armitage *et al.*, 1995). Many species have narrow tolerances for temperature, trophic state, salinity or acidity, which renders them important bioindicators of environmental changes and water quality. This allows fossil chironomids to be used in reconstructing histories of environmental changes from the near to more distant past (e.g. Velle *et al.*, 2005; Verschuren & Eggermont, 2006; Stebner *et al.*, 2017).

Aenne triassica Krzemiński & Jarzembowski, 1999 from the Upper Triassic deposits (~202 Mya;

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Krzemiński & Jarzembowski, 1999) is the oldest known chironomid species. Subsequent Mesozoic representatives of some chironomid subfamilies, but not the Chironominae, have been described based on Jurassic and Cretaceous compressions (e.g. Kalugina, 1980a, 1985, 1986, 1993; Zhang, 1991; Lukashevich & Przhiboro, 2011, 2015, 2018; Lukashevich, 2020) and Cretaceous amber inclusions from Lebanon (early Barremian, ~128 Mya; e.g. Brundin, 1976; Veltz *et al.*, 2007; Azar *et al.*, 2008; Maksoud *et al.*, 2017; Maksoud & Azar, 2020), United Kingdom (early Barremian, ~128 Mya; Jarzembowski *et al.*, 2008; Baranov *et al.*, 2019), Myanmar (Albian–Cenomanian boundary, ~100 Mya; Baranov *et al.*, 2017; Giłka & Zakrzewska, 2017; Giłka *et al.*, 2019, 2020) and Russia (Santonian, ~84 Mya; Kalugina, 1976, 1980b).

Evidence for dating chironomids of the subfamily Chironominae back to the Mesozoic have been sought in the fossil record for decades. In contrast to the well-documented Cretaceous members of the subfamily Orthocladiinae (Boesel, 1937; Veltz *et al.*, 2007; Giłka *et al.*, 2019), which is regarded as a sister-group to the Chironominae, to date no Mesozoic descriptions of the latter have been published. The oldest Chironominae have been described solely from younger Palaeogene (Eocene) resins, i.e. Cambay (India; Zakrzewska *et al.*, 2018, 2020), Oise (France; Doitteau & Nel, 2007), Fushun (China; Giłka *et al.*, 2016) and amber from Rovno and the Baltic region (Seredusz & Wichard, 2007; Giłka, 2010, 2011; Giłka *et al.*, 2013; Zakrzewska *et al.*, 2016). The challenge to find and identify a Mesozoic representative of the Chironominae has thus become significant to test hypotheses of diversification tempo based on molecular markers derived from extant Chironominae (e.g. Cranston *et al.*, 2012; Cranston, 2019a; Krosch *et al.*, 2020).

Cretaceous Chironominae have been recognized to the present only from a note by Kalugina listing a single specimen (Kalugina, 1974: 48, table 2), but with no further data. Fifty years later we describe two genera: one based on Kalugina's original material, here re-examined in detail, and the second based on a recently obtained specimen. Both are assigned to the tribe Pseudochironomini.

MATERIAL AND METHODS

GEOLOGICAL CONTEXT

The specimens studied are adult male inclusions found in Cretaceous ambers from Asia, in Russia (Taimyr amber from Yantardakh; Upper Cretaceous) and in Myanmar (Burmese amber from Hukawng Valley; mid-Cretaceous).

Yantardakh is the largest and best-studied insect-bearing site of the Kheta Formation [Taimyr Peninsula, the right bank of River Maimecha (a tributary of the Kheta), 3 km upstream of its mouth, at Yantardakh Hill (71°18'26"N, 99°33'46"E)]. The deposits date back to the Santonian (Zherikhin & Sukatsheva, 1973; Rasnitsyn *et al.*, 2016). The Chironomidae described herein were found among about 3000 arthropod inclusions by the expedition of the Paleontological Institute of the Academy of Science of the USSR in 1970–71. All the Chironomidae collected in Yantardakh at that time were sorted and identified by N. S. Kalugina in subfamilies as follows: Orthocladiinae s.s. – 599, Aphroteniinae – 50, Tanyptodinae – 22, Diamesinae – three, Podonominae – one and Chironominae – one; but only Aphroteniinae and Diamesinae were described (Kalugina, 1974, 1976, 1980b). Although a single Chironominae specimen was mentioned, actually two individuals of this subfamily were collected and marked by Kalugina, including one identified tentatively because of its poor state of preservation. Both specimens are described here for the first time.

Burmese amber, or Burmite, is predominantly mined in the Hukawng Valley, in the district of Myitkyina, State of Kachin in the north of Myanmar (Xing & Qui, 2020). Due to redepositions, its geological age has long been debated, but a dating of Burmite from Kachin to near the Albian–Cenomanian boundary is now well-justified (Rasnitsyn *et al.*, 2016). The number of insect inclusions described from Burmite has increased greatly over the past 20 years. About 400 insect families have been recorded, rendering the Burmite fauna the richest Cretaceous fossil resin arthropod assemblage known to date (Rasnitsyn *et al.*, 2016; Ross, 2019, 2020). As usual in Cretaceous ambers, chironomids are numerous in Burmite, although only several members of the subfamilies Podonominae, Buchonomyiinae and Orthocladiinae have been described (Baranov *et al.*, 2017; Giłka & Zakrzewska, 2017; Giłka *et al.*, 2019, 2020). In contrast, to date no Chironominae have been found in Burmite. The specimen described here is thus the oldest known representative of the subfamily.

TECHNIQUES USED AND REPOSITORY DATA

Amber pieces were ground and polished following the protocols of Sidorchuk (2011) and Sidorchuk & Vorontsov (2018) (Taimyr amber) or manually (Burmese amber), so that the inclusions and their diagnostic structures could be examined and photographed at high magnification. Owing to the exceptionally fragile nature of the Taimyr amber, the pieces examined were embedded in Buehler's EpoThin (an epoxy resin). The epoxy embedding of amber

inclusions, routinely practiced for more than 20 years (Nascimbene & Silverstein, 2000), is believed to be the safest way to deal with brittle amber pieces and to protect them during prolonged storage. However, epoxy resin readily fills not only fissures in the amber, which would be one of the purposes of embedding, but also those parts of the inclusion that are open to the surface of the piece. The resin-filled parts of the inclusion become less visible, sometimes almost transparent, yet can be imaged with optical contrasting methods. For this reason, the Taimyr amber pieces were imaged both before and after the embedding.

Specimen dimensions are given in micrometres, except for the total length (in millimetres, rounded off to the first decimal place). The body and wing lengths were measured from the antennal pedicel to the end of the gonostylus and from the arculus to the tip, respectively. The lengths of leg segments and palpomeres were rounded to the nearest 5 and 1 μm , respectively. The antennal, leg and venarum ratios (AR, LR and VR) were calculated to the second digit after the decimal point. The morphological terminology and abbreviations follow Sæther (1980), with additions for hypopygial structures proposed by Cranston (2019b). Photographs were taken using a PZO Biolar SK14 microscope with a Sony NEX-3N digital camera (Burmese amber) and a Nikon E-800 microscope, with dry ($\times 4$, $\times 10$) and water immersion optics ($\times 40$ and $\times 60$) and an Olympus OM-D E-M10II digital camera (Taimyr amber). Brightfield, differential interference contrast and incident illumination were used. The stacks were corrected for colour, brightness and noise with Adobe Lightroom. The images were compiled using the Helicon Focus 6 stacking software.

For the amber not embedded in epoxy resin, bought on the open market, the Fourier transform infrared spectroscopy (FT-IR) spectrum [attenuated total reflectance (ATR)] was obtained to support identification of the Myanmar inclusion examined (see Discussion: infrared (IR) spectrum). A Nicolet iS10 FT-IR spectrometer was used to record the ATR (diamond crystal) FT-IR spectrum. The resolution was 4 cm^{-1} ; 16 scans were performed. The spectrum with reference curve numbers obtained is registered and stored in the archives of the Laboratory of the International Amber Association (IAA), Gdańsk, Poland and at the Laboratory of Systematic Zoology, Department of Invertebrate Zoology & Parasitology (LSZ DIZP), University of Gdańsk, Poland.

The Yantardakh amber inclusions studied are housed in the collection of the Borissiak Paleontological Institute, Russian Academy of Sciences, Moscow (PIN). The specimen preserved in the Burmese amber is the property of the Natural History Museum, Institute of Systematic and Evolution of Animals, Polish Academy

of Sciences, Kraków (ISEZ PAN) enriched with a collection of amber inclusions from Myanmar in 2015.

RESULTS

SYSTEMATICS

FAMILY: CHIRONOMIDAE NEWMAN, 1834

SUBFAMILY: CHIRONOMINAE NEWMAN, 1834

TRIBE: PSEUDOCHIRONOMINI SÆTHER, 1977

In adult Chironomidae, the lack of the wing vein MCu is the synapomorphy for Chironominae + Orthocladiinae, a clade comprising the two largest, most diverse and probably youngest chironomid subfamilies, considered sister to the remaining Chironomidae combined (e.g. Sæther, 2000; Cranston *et al.*, 2012). A character state common to all the Chironominae concerns the gonostylus that is backward-directed and connected rigidly with the gonocoxite in most Chironominae, although able to flex slightly in a few taxa (Cranston *et al.*, 1989; Tang & Cranston, 2019). Within four Chironominae tribes (Chironomini, Pseudochironomini, Tanytarsini and the recently erected Xiaomyiini), the character state best separating the tribe Pseudochironomini is the dark comb on the apex of the foreleg tibia, similar to that on mid- and hindlegs (a probable tribal synapomorphy) and the pars ventralis, the structure being, however, weakly developed or absent in most genera.

Following reports on a distinct heteromorphy within the Pseudochironomini, especially with respect to the adult genital morphology, as highlighted recently by Krosch *et al.* (2020), the significance of diagnostic characters or character combinations of a tribe should be treated with the highest caution. The wing anal lobe usually well-developed (vs. anal lobe weak or absent in the Tanytarsini, but also in some Pseudochironomini genera), the vein RM oblique (vs. RM parallel to M and R_{4+5} in the Tanytarsini or transverse to M and R_{4+5} in most other Chironominae) and the foreleg ratio ($LR_1 = ta_1/ti$) usually close to, or less than 1 (vs. higher than 1 in most Chironominae) are the character states in Pseudochironomini that may support the tribe definition. However, these features, and the presence of the pars ventralis, rarely appear as a full set, hence their proper interpretation usually requires a reference to taxa of other Chironominae that may display character combinations or some character states similar to those listed above. Owing to the difficulty in establishing a clear diagnosis based on a set of synapomorphies for the tribe (in the current taxa composition), its monophyly is still being tested but not confirmed (Cranston *et al.*, 2012; Cranston 2019a, b; Tang & Cranston, 2019; Krosch *et al.*, 2020).

To date, the following six extant genera are included in the tribe Pseudochironomini (Andersen, 2016; Krosch et al., 2020): *Aedokritus* Roback, 1958, *Manoa* Fittkau, 1963, *Madachironomus* Andersen, 2016, *Pseudochironomus* Malloch, 1915, *Riethia* Kieffer, 1917, and *Megacentron* Freeman, 1961, the latter with *Megacentron eocenicus* Doitteau & Nel, 2007 – the sole Pseudochironomini known from the fossil record (Eocene Oise amber) (Doitteau & Nel, 2007). Descriptions of two further genera, based on unique characters found in specimens preserved in the Cretaceous ambers, are presented below.

MESOACENTRON GILKA, ZAKRZEWSKA, LUKASHEVICH AND CRANSTON, GEN. NOV.

(FIGS 1, 2)

Zoobank registration: urn:lsid:zoobank.org:act:3BF03CD1-3197-4688-BD6E-F5D107968060.

Type species: *Mesoacentron kaluginae* Gilka et al., sp. nov. (by present designation).

Derivation of name: This first described Mesozoic (*Meso-*) representative of the subfamily Chironominae is named with reference to the genus it was compared with, *Megacentron* (*-centron*), with the prefix *a*, to emphasize the lack of true median volsellae.

Diagnostic description: Eyes bare, with short dorsomedian extensions. Antenna with 14 flagellomeres, plume fully developed. Proboscis short. Scutum strongly developed, protruding; head deeply embedded between scutum and sternum. Wing squama with setae. Anal lobe well developed, round. RM oblique relative to M and R_{4+5} . Midleg tibia with single, straight spur and narrow comb; hindleg tibia with two spurs and fan-shaped combs. Gonostylus straight, seemingly rigidly connected to gonocoxite, slightly shorter than gonocoxite. Anal point narrow, elongate. Two pairs of volsellae, digitus, pseudovolsella and pars ventralis present. Superior volsella well developed, roundish. Inferior volsella small. Digitus minute. Pseudovolsella in a form of aggregation of linearly merged tubercles. Median volsella absent. Pars ventralis well developed, bearing setae distally.

MESOACENTRON KALUGINAE GILKA, ZAKRZEWSKA, LUKASHEVICH AND CRANSTON, SP. NOV.

(FIGS 1A–E, 2A–H)

Zoobank registration: urn:lsid:zoobank.org:act:008459CE-58FD-474F-AB20-EF63844900A9.

Derivation of the name: The specific epithet is dedicated to Nadezhda Sergeevna Kalugina (1930–90), eminent

Russian palaeoentomologist and dipterologist, who first recognized the specimen to the subfamily level.

Type material: *Holotype*, PIN No. 3130/224: adult male (both fore- and left midleg tarsi missing; distal part of antennal flagellum damaged, in a separate amber fragment) preserved in a 6.2 × 3.8 × 1.8-mm piece of the Taimyr amber (Yantardakh, Taimyr Peninsula, Russia; Kheta Formation, Santonian, Late Cretaceous; Fig. 1A). Four smaller (less than 0.5 mm), fragments of the same piece embedded together in epoxy resin. *Syninclusions:* Psocoptera(?), one specimen; Acari (Hydrachnidia), one specimen attached to the abdomen of the chironomid.

Diagnosis: Adult male. Small species, total length ~2.1 mm; wing length: 1045 µm. FCu placed well distally of RM, VR_{Cu} 1.31. Superior volsella ellipsoidal, with distinct longitudinal incision, directed medially. Inferior volsella hook-shaped. Digitus minute, finger-like, placed at base of inferior volsella. Pseudovolsella in a form of trifold tubercle. Pars ventralis broadly fused with gonocoxite, subtriangular, with tubercles bearing setae. Other diagnostic characters as for the genus.

Description [adult male (N = 1, holotype)]

Total length: ~2.1 mm (head: ~220 µm, thorax: ~450 µm, abdomen: ~1410 µm); wing length: 1045 µm.

Head (Fig. 1B–E): Eyes bare, with short wedge-shaped dorsomedian extensions. Frontal tubercles not observed. Antenna with 14 flagellomeres, AR unmeasurable (apices of distal flagellomeres damaged), plume fully developed. Mouthparts shortened, poorly preserved.

Thorax (Fig. 1B, E): Scutum strongly developed, protruding; head deeply embedded between scutum and sternum. Ac at least 6; Dc at least 8 on each side; Scts at least 6, Pa not observed.

Wing (Fig. 2A, B): Broadest at two-thirds of the length, width: 360 µm, length/width ratio 2.90. Squama bearing several long setae. Anal lobe well developed, round. Subcosta fading above RM area; R_{2+3} running closely to R_{4+5} , fading just before C; R_{4+5} straight, M_{1+2}/R_{4+5} length ratio 1.16; RM oblique; FCu placed well distally of RM, VR_{Cu} 1.31. Macrotrichia observed only on wing margin.

Legs (Figs 1B, 2C, D): Both forelegs damaged, only femora and proximal parts of tibiae preserved. Midleg tibia bearing single, straight spur, ~45 µm long, comb narrow, teeth up to 30 µm long (Fig. 2C). Hindleg tibia with two spurs, longer ~40 µm, shorter ~35 µm

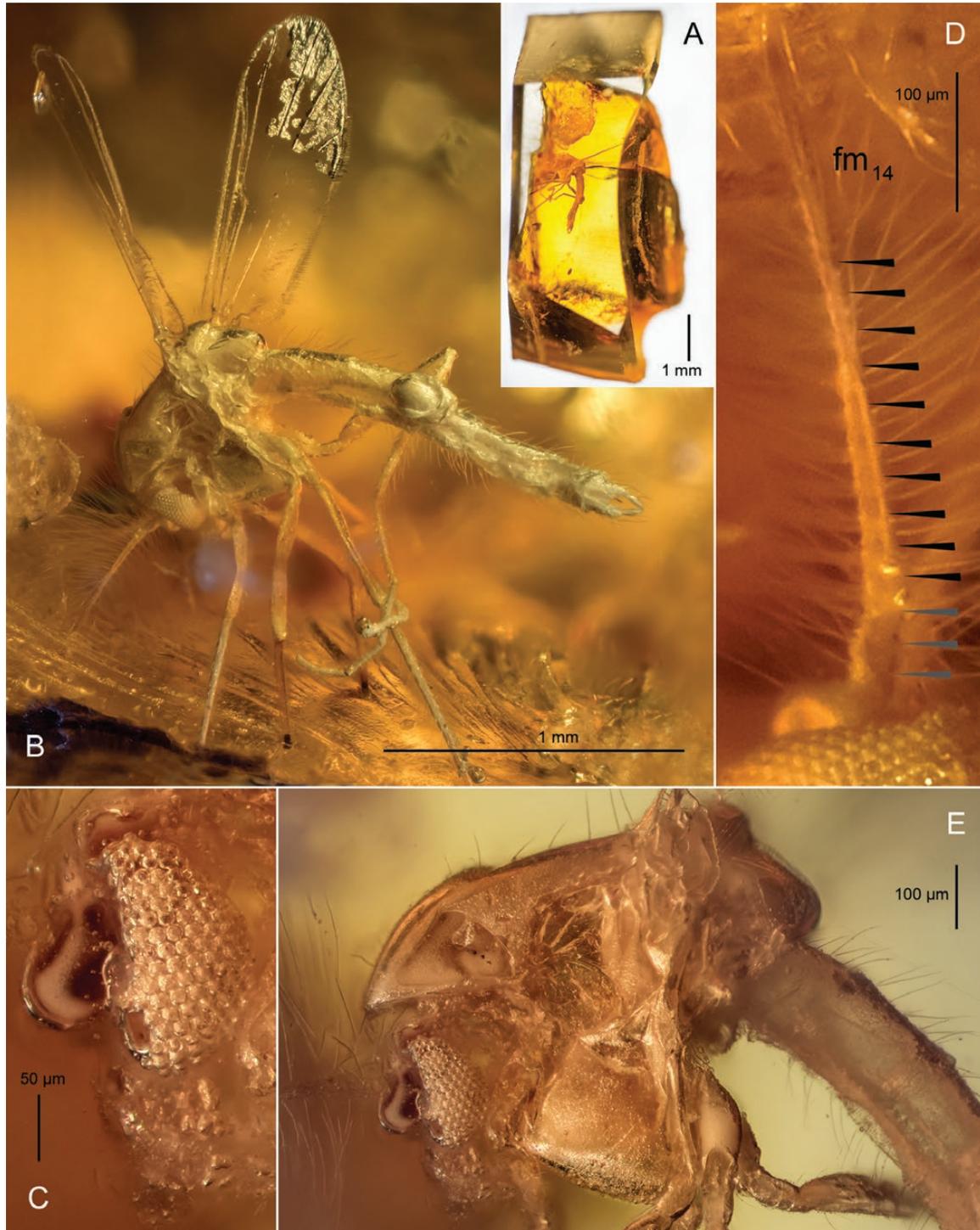


Figure 1. *Mesoacentron kaluginae*, adult male, PIN 3130/224; Late Cretaceous, Santonian; Yantardakh, Taimyr Peninsula, Russia. A, inclusion in amber embedded in epoxy resin. B, habitus. C, head. D, antenna (arrowheads indicate borders between flagellomeres fm 1–14: black – distinctly separated, grey – weakly separated or partially fused). E, head and thorax in lateral aspect.

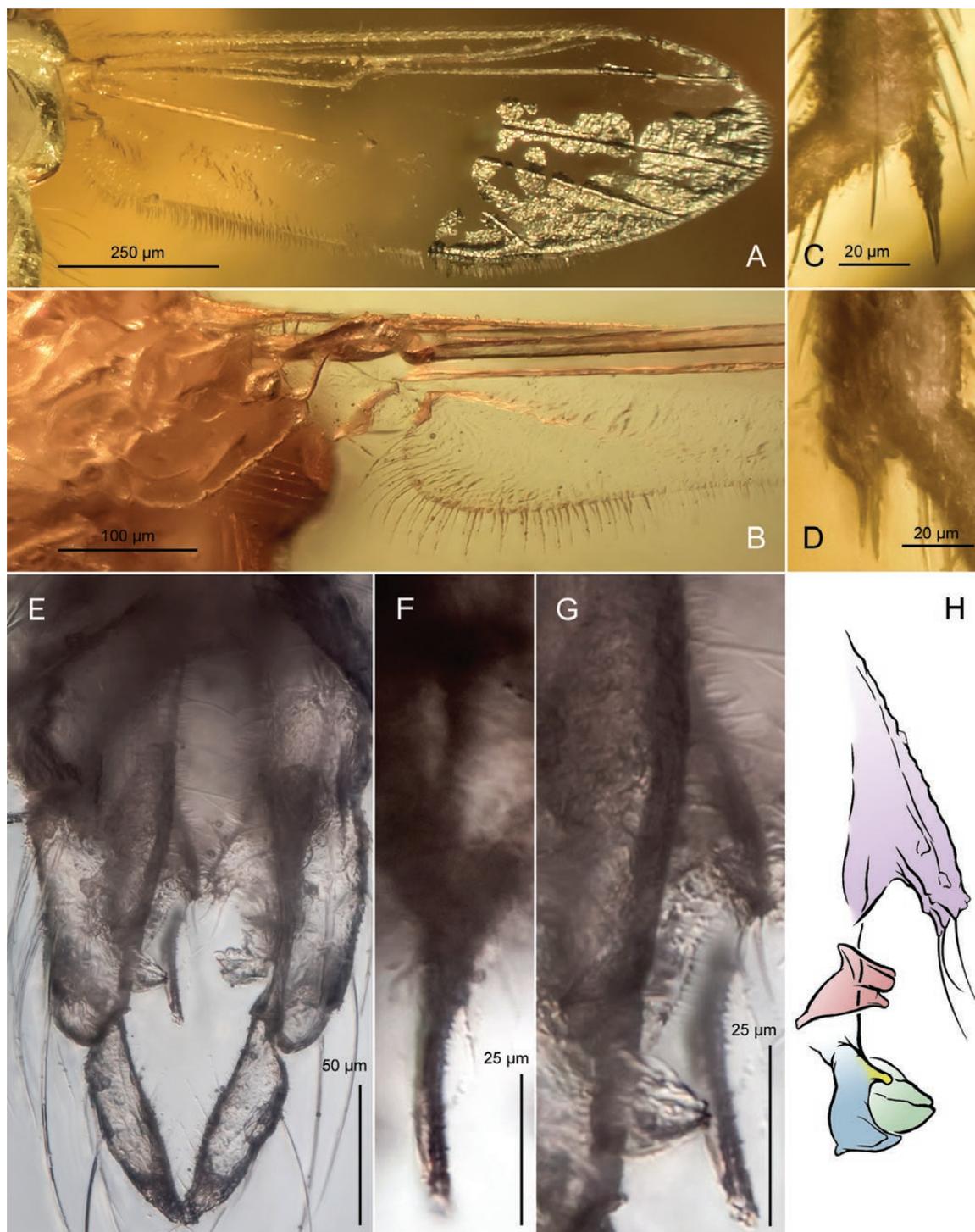


Figure 2. *Mesoacatron kaluginae*, adult male, PIN 3130/224; Late Cretaceous, Santonian; Yantardakh, Taimyr Peninsula, Russia. A, B, wing, with anal lobe and squama magnified (B). C, D, tibial armature of mid (C) and hindleg (D). E, hypopygium, ventral aspect. F, anal point. G, H, hypopygial volsellae: superior (green), inferior (blue), digitus (yellow), pseudovolsella (red) and pars ventralis (purple) photographed (G) and drawn (H).

long, each spur with ~15 µm long, fan-shaped comb (Fig. 2D). Length of hindleg segments and leg ratio in Table 1.

Hypopygium (Fig. 2E–H): Gonostylus straight, constricted at base, broadest at midlength, tapering towards blunt apex, ~80 µm long, but shorter than elongated gonocoxite (~95 µm long) (Fig. 2E). Anal point long, narrow, tapering towards blunt apex (Fig. 2E, F). Superior volsella ellipsoidal, with distinct longitudinal incision (bilobed), directed medially. Inferior volsella hook-shaped, directed medially. Digitus minute, finger-like, placed at base of inferior volsella, ventral to superior volsella. Pseudovolsella in a form of aggregation of linearly merged tubercles forming a distinct trifid protrusion; setae not observed. Pars ventralis well developed, broadly fused with gonocoxite, subtriangular, with distinct tubercles and setae in distal part (Fig. 2G, H).

Notes

The wing with an extensive, round anal lobe, the squama bearing a fringe of setae, the RM vein oblique relative to M and R_{4+5} and the genital apparatus with two pairs of volsellae, digitus, pseudovolsella and a well-developed pars ventralis form a set of characters testifying to the position of the new species within the Pseudochironomini, yet are unique in the tribe. The arrangement of hypopygial appendages in a set typical of Pseudochironomini, but with a pseudovolsella instead of the median volsella (see below), combined with a strongly elongated, narrow anal point have not been found in any extant/extinct chironomid and thus support our decision to erect the new genus, *Mesoacentron*.

The antenna of previously described Pseudochironomini males consists of 13 flagellomeres, the only exception being 14 flagellomeres found in the extinct *Megacentron eocenicus* (Doitteau & Nel, 2007).

Table 1. Leg segment lengths (in micrometres) and leg ratios in the examined specimens

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR
<i>Mesoacentron kaluginae</i>								
p ₃	520	600	290	220	155	95	90	0.48
Pseudochironomini? <i>incertae sedis</i>								
p ₂	-	-	240	150	110	85	-	-
p ₃	495	615	305	-	-	-	-	0.50
<i>Palaeocentron krzeminskii</i>								
p ₁	610	790	575	305	250	180	115	0.73
p ₂	630	640	340	195	155	110	80	0.53
p ₃	~595	765	425	230	210	125	90	0.56

Abbreviations: fe, femur; LR, leg ratio; p1–p3, pair of legs 1–3; ta1–ta5, tarsomeres 1–5; ti, tibia.

The species was placed in *Megacentron* based on the reference to the main generic characters, including the number of antennal segments, as defined by Freeman (1961) and Cranston *et al.* (1989). However, Freeman's diagnoses relied on the old style of antennal segment counting, whereby the pedicel was included into the total count. Therefore, the *Megacentron* described by Freeman have an antenna with a pedicel and 13 flagellomeres, the number (13) resulting also from the modern way of description (Cranston *et al.*, 1989). The inconsistency in referring to this feature and a definition of the volsellae arrangement (including the lack of data on the superior volsella) may call into question the generic assignment of the species described by Doitteau & Nel (2007). However, it cannot be ruled out that the number of flagellomeres in *Megacentron eocenicus*, higher than that in extant congeners, follows a trend similar to that known from other Chironominae genera, in which a higher number is interpreted as plesiomorphy (Zakrzewska & Głka, 2014, 2015; Zakrzewska *et al.*, 2016, 2020; Zakrzewska & Jankowska, 2021). This contention seems to be supported by the antenna bearing 14 flagellomeres, recorded here in Cretaceous Pseudochironomini. Interestingly, a partial fusion of flagellomeres 1–4, observed in *Mesoacentron* (Fig. 1D), may be regarded as a transitional phase between the oldest known Pseudochironomini (bearing 14 flagellomeres, cf. Fig. 5B) and the extant ones (13 flagellomeres).

The hypopygial pars ventralis is a lobe between the bases of gonocoxites (Sæther, 1980). It is a structure known from the Pseudochironomini, but vestigial or absent in most genera/species, i.e. in *Aedokritus* (e.g. Pinho, 2018; Pinho *et al.*, 2019), *Madachironomus* (Andersen, 2016), *Riethia* (e.g. Trivinho-Strixino *et al.*, 2009; Trivinho-Strixino & Shimabukuro, 2018; Cranston, 2019b), *Megacentron* (Freeman, 1961; Doitteau & Nel, 2007) and in most *Manoa* (Andersen & Sæther, 1997; Jacobsen & Perry, 2002; Qi *et al.*, 2017). Until now, the pars ventralis has been recognized as distinct mostly in species of *Pseudochironomus*. It is now also reported from *Mesoacentron* (Fig. 2E, G, H). With its elongation, it resembles the pars ventralis known from *Pseudochironomus*, while the basolateral fusion with the gonocoxite is similar to that in *Manoa tangae* (cf. Andersen & Sæther, 1997: fig. 1H).

Mesoacentron lacks a median volsella, while a trifid protrusion or aggregation of tubercles with linearly merged bases is observed in the male hypopygium (Fig. 2G, H). Recently, some hypopygial structures in the Chironominae have been redefined by Cranston (2019b). According to that approach, a structure treated so far as the median volsella in some *Riethia*, cannot – due to its morphology and location – be regarded as homologous with the true median volsella, as it is more a cluster of several setal tubercles that lacks a stem and is located on

the inner part of the gonocoxite. It is proposed to call the structure a pseudovolsella. On account of a considerable spatial depth of the hypopygium, the position of its appendages along the vertical axis is particularly difficult to determine in fossil specimens, even in amber inclusions mounted using modern methods. However, the linearly merged aggregation of tubercles observed in *Mesoacentron kaluginae* corresponds well with Cranston's description, thus it is identified here as the pseudovolsella. Cranston's suggestions regarding the terminology of the hypopygium appendage structures concern also a lobe of a variable size that is either separated from the inferior volsella or fused with it. A minute finger-like lobe placed at the base of the inferior volsella, under the superior volsella, was also observed in *Mesoacentron* (Fig. 2G, H). Following Cranston (2019b), we call the structure the digitus. The recent key to the identification of Pseudochironomini males (Strixino & Shimabukuro, 2018) uses the presence/absence of the digitus as a character that delimits two closely related genera, *Riethia* and *Manoa*. However, the digitus was later observed in both genera (Cranston, 2019b), thus, the only characters that enable separation of these taxa appear to be those of the immature stages. Consequently, our key to adult males treats the two genera jointly (see below).

PSEUDOCHIRONOMINI? INCERTAE SEDIS

(FIG. 3A–H)

Material examined: PIN No. 3130/223: adult male (severely damaged, head and both forelegs missing) preserved in a 2.9 × 2.4 × 0.9-mm piece of the Taimyr amber (Yantardakh, Taimyr Peninsula, Russia; Kheta Formation, Santonian, Late Cretaceous; Fig. 3A).

Description [adult male (N = 1)]

Total length: Only thorax (~610 µm long) and abdomen preserved (~1095 mm long); wing length: ~1000 µm (arculus not visible).

Head: Missing.

Thorax (Fig. 3B): Poorly preserved; at least three scutellars.

Wing (Fig. 3C, D): Broadest at two-thirds of the length, width: 330 µm, length/width ratio ~3.0. Anal lobe well developed, round. Subcosta long, fading far distally of RM; R_{2+3} ending at one-third of the length between apices of R_1 and R_{4+5} ; R_{4+5} curved up distally just before reaching end of C, M_{1+2}/R_{4+5} length ratio 1.21; RM oblique (Fig. 3D); FCu placed well distally of RM. Macrotrichia observed only on wing margin.

Legs (Fig. 3E, F): Forelegs not preserved. Midleg tibia with single, straight spur, ~28 µm long, comb consisted

of several teeth, the longest tooth ~19 µm. Hindleg tibia with two spurs, longer ~40 µm, shorter ~30 µm long, each spur with comb (teeth up to ~18 µm long). Length of leg segments and leg ratios in Table 1.

Hypopygium (Fig. 3G, H): Gonostylus ~65 µm long, crescent, with apex blunt, as long as gonocoxite. Anal point slender, tapering towards tip. Remaining structures poorly observable or not visible.

Notes

The male of the species has the gonostyli directed backward, which justifies its assignment to the subfamily Chironominae. Placement in the Pseudochironomini is tentative on the basis of several character states known from the tribe (a well-developed and round anal lobe of the wing, and the RM vein oblique relative to M and R_{4+5}), as well as several features similar to those of *Mesoacentron kaluginae*, i.e. the tibial spurs and comb structure/arrangement and the wing venation pattern (with slight differences in the length of Sc and the length ratio/arrangement of R_{4+5} and M_{1+2}). However, the two Taimyr specimens most likely represent different species. They differ distinctly in the size/length proportions of the main body parts (thorax and abdomen) and the presence/lack(?) of the pars ventralis, well developed in *M. kaluginae* but not observed in the 'Pseudochironomini? incertae sedis'. Unfortunately, the severely damaged specimen precludes its identification or accurate description as a potential new species. Nevertheless, the record is a valuable confirmation of the Chironominae being present in the Cretaceous Taimyr amber.

PALAEOCENTRON GILKA, ZAKRZEWSKA,
LUKASHEVICH AND CRANSTON, GEN. NOV.

(FIGS 4–7)

Zoobank registration: urn:lsid:zoobank.org:act:9F1A8453-8622-45EA-AC51-917ED2B4B674.

Type species: *Palaeocentron krzeminskii* Gilka et al., sp. nov. (by present designation).

Derivation of the name: This is the oldest known representative of the subfamily Chironominae, named with reference to the genera *Megacentron* and *Mesoacentron*, to which it is compared.

Diagnostic description: Eyes bare, with triangular dorsomedian extensions. Frontal tubercles absent. Antenna with 14 flagellomeres, plume fully developed. Proboscis short. Spur of foreleg tibia stout, with narrow dark comb at base; mid and hindleg tibia with two well-separated combs, each with a spur; all combs and

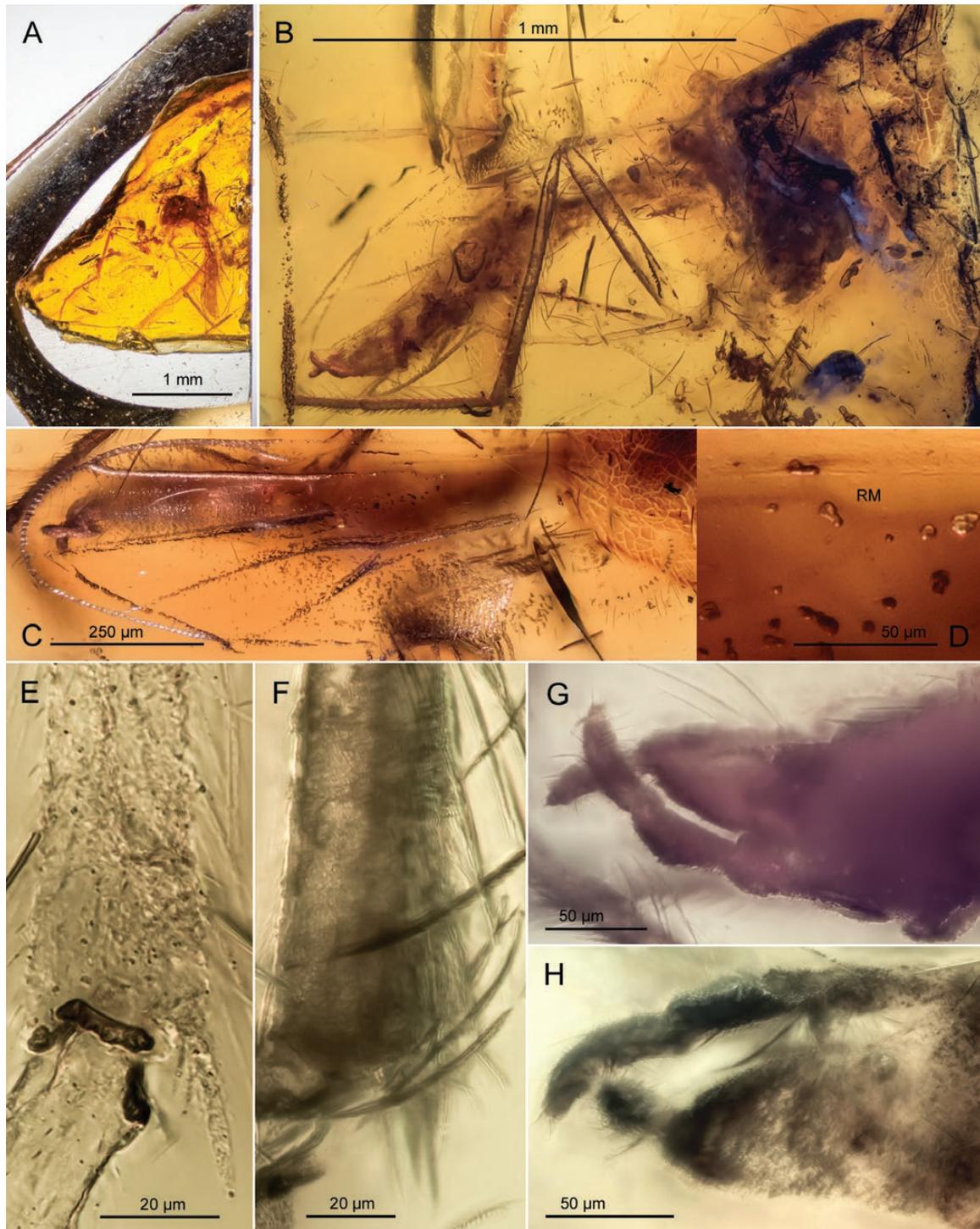


Figure 3. *Pseudochironomini? incertae sedis*, adult male, PIN 3130/223; Late Cretaceous, Santonian; Yantardakh, Taimyr Peninsula, Russia. A, inclusion in amber embedded in epoxy resin. B, habitus. C, D, wing, with RM vein area magnified (D). E, F, tibial armature of mid (E) and hindleg (F). G, H, hypopygium in dorsolateral (G) and ventrolateral aspect (H).

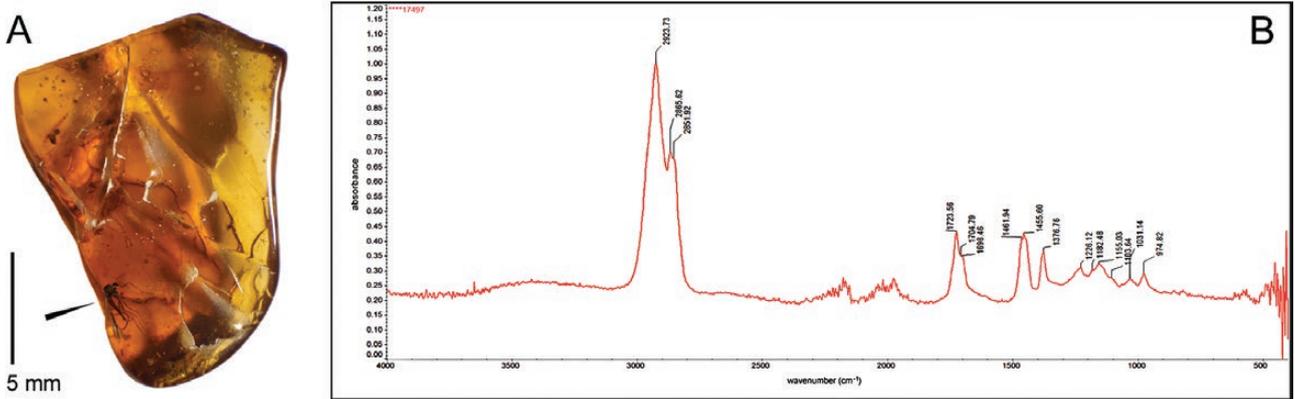


Figure 4. *Palaeocentron krzeminskii*, adult male, MP/4020 (ISEZ PAN); mid-Cretaceous, probably Albian–Cenomanian; Hukawng Valley, Kachin State, Myanmar. A, inclusion in amber. B, Fourier transform infrared spectroscopy (attenuated total reflectance) spectrum no. 17497 IAA, obtained from amber piece with the examined inclusion.

spurs dark brown to black. Hindleg tibia with strong thorn-like bristles arranged in a longitudinal row and subapical fan. LR < 1. Gonostylus likely rigidly connected with gonocoxite, both strongly elongate. Anal point well developed. Two pairs of volsellae: superior volsella narrow, finger-shaped; inferior volsella fin-shaped. Median volsella, pseudovolsella and pars ventralis absent.

PALAEOCENTRON KRZEMINSKII GILKA,
ZAKRZEWSKA, LUKASHEVICH AND CRANSTON,
SP. NOV.

(FIGS 4A–B, 5A–B, 6A–G, 7A–G)

Zoobank registration: urn:lsid:zoobank.org:act:CA8F04BC-3897-45E8-98E8-A881A239E5CC.

Derivation of the name: The specific epithet honours Wiesław Krzemiński, Polish palaeoentomologist and the discoverer of the oldest known chironomid.

Type material: *Holotype*, MP/4020 (ISEZ PAN): adult male (wings missing) preserved in a 17 × 13 × 3-mm piece of the Burmese amber (Hukawng Valley, Kachin State, Myanmar; probably Albian–Cenomanian, mid-Cretaceous, Fig. 4A). IR spectrum no. 17497 IAA, Fig. 4B).

Diagnosis: Adult male. Total length: ~2.5 mm. AR ~1.25. Second palpomere longer than third and fourth. LR low, 0.73. Gonostylus straight, broadened in distal half, with rounded apex. Anal point stout, parallel-sided, distally rounded and excavated, with narrow spike-shaped prolongation. Superior volsella with setae-bearing roundish swelling at midlength, distal part swollen, directed posteromedially. Inferior volsella narrowed at base, broadest at midlength, tapering to

pointed apex, with swollen ventral margin and semi-transparent dorsal edge. Other diagnostic characters as for the genus.

Description [adult male (N = 1, holotype)]

Total length: ~2.5 mm (Fig. 5A).

Head (Fig. 5B): Eyes bare, with triangular dorsomedian extensions, slightly separated by frons. Frontal tubercles absent. Antenna with 14 distinct flagellomeres, proximal flagellomere distinctly paler, AR ~1.25, plume fully developed. Second palpomere relatively long, third palpomere shorter than second and fourth; length of palpomeres 2–5 (µm): ~100, 85, ~95, ~135. Clypeus with ten short setae at least.

Thorax chaetotaxy: Ac at least 13; Dc at least nine on each side; Scts and Pa unobservable.

Legs (Fig. 6): Spur of foreleg tibia dark brown to black, stout (~30 µm long), pointed, with narrow dark comb at base (Fig. 6A, B). Mid- and hindleg tibia with two well-separated combs, teeth up to 32 µm long on midleg and 34 µm long on hindleg, each comb bearing a straight or slightly curved spur, 32 and 42 µm long on midleg and 38 and 44 µm long on hindleg, spur and combs dark brown to black (Fig. 6C, D); hindleg tibia subapically bearing fan composed of five to six strong thorn-like bristles arranged as in Fig. 6E; 15–19 bristles of similar shape arranged in a row at two-thirds the length of the hindleg tibia (Fig. 6F, G). Length of leg segments and leg ratios in Table 1.

Hypopygium (Fig. 7): Gonostylus straight, broadened in distal half, with apex rounded,

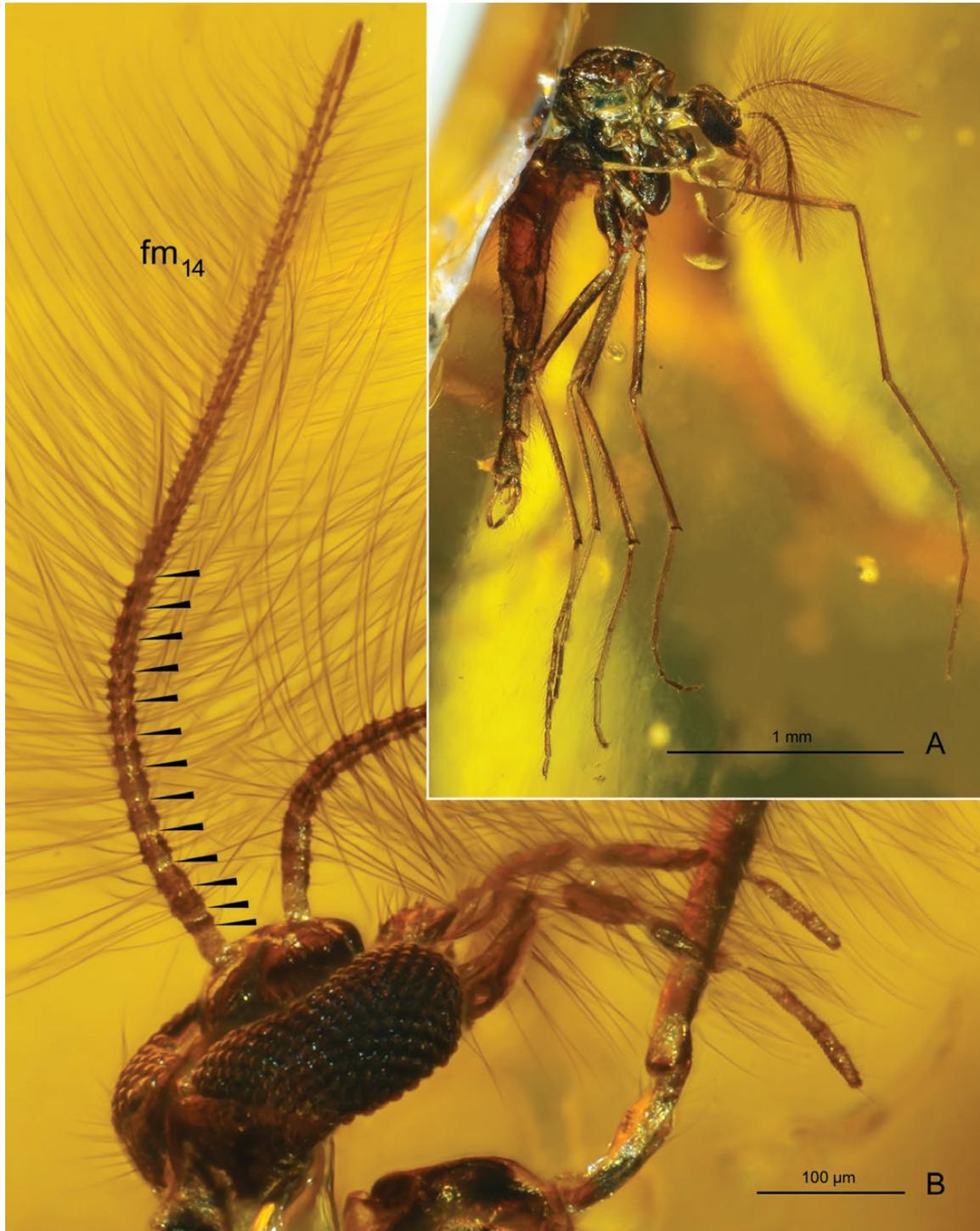


Figure 5. *Palaeocentron krzeminskii*, adult male, MP/4020 (ISEZ PAN); mid-Cretaceous, probably Albian–Cenomanian; Hukawng Valley, Kachin State, Myanmar. A, habitus. B, head and antenna (arrowheads indicate borders between flagellomeres fm 1–14).

~100 µm long, similar in length to gonocoxite (Fig. 7A, B). Anal point stout, parallel-sided, distally rounded and excavated, with narrow spike-shaped

median prolongation (Fig. 7C, D). Superior volsella narrow, finger-like, with distinct roundish swelling at midlength bearing one or two setae, distal part

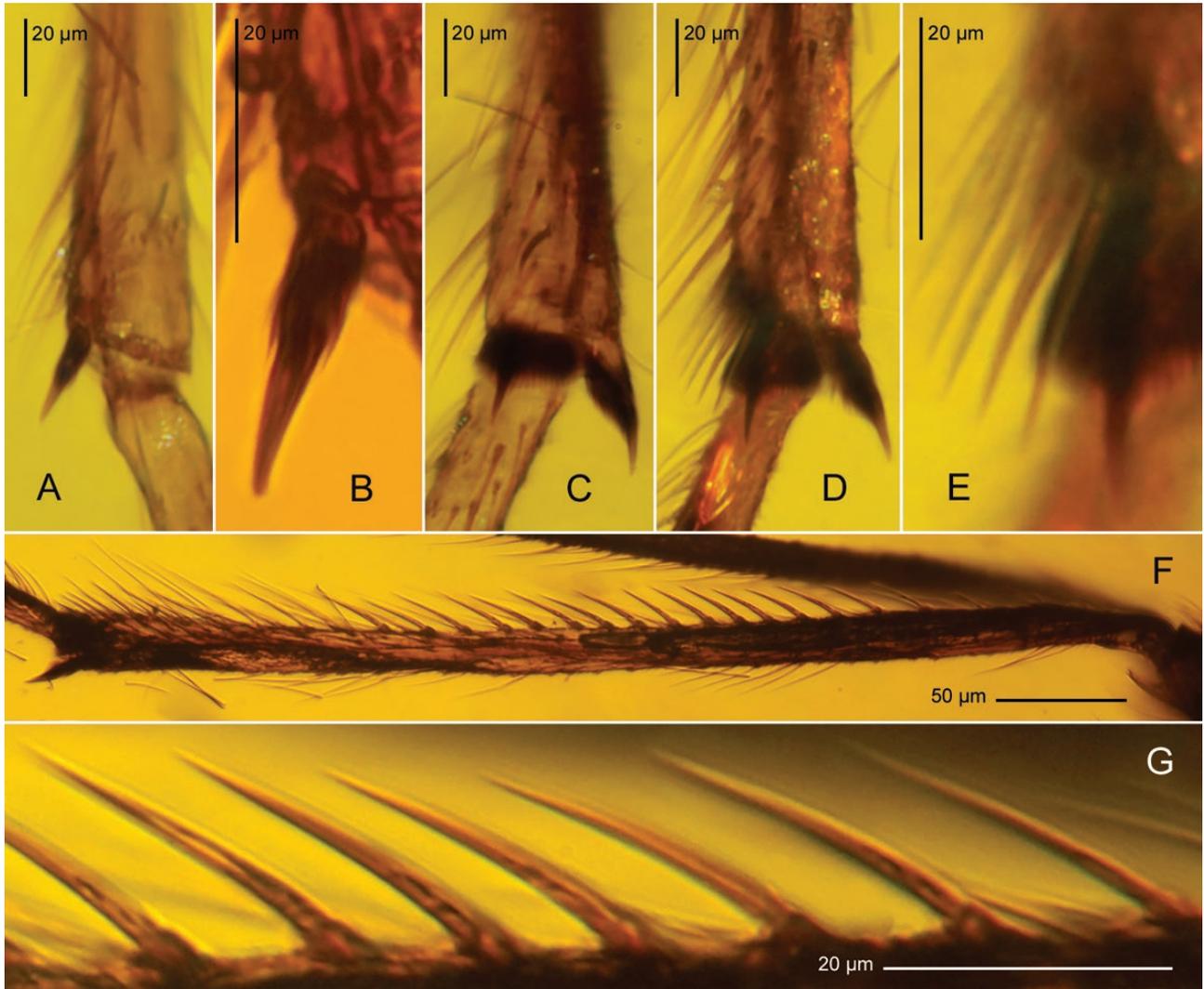


Figure 6. *Palaeocentron krzemiński*, adult male, MP/4020 (ISEZ PAN); mid-Cretaceous, probably Albian–Cenomanian; Hukawng Valley, Kachin State, Myanmar. A–E, tibial apices of fore (A, B) mid- (C) and hindleg (D, E), with bristle fan magnified (E). F, G, thorn-like bristles on hindleg tibia: arrangement (F) and shape (G).

swollen and directed posteromedially (Fig. 7E). Inferior volsella fin-shaped, narrowed at base, broadest at midlength, tapering to pointed apex, with curved swollen ventral margin, and straight semi-transparent dorsal edge, covered with setae and microtrichia (Fig. 7F, G).

Notes

As mentioned above, the dark comb on the foreleg tibia is regarded as the key character (tribal synapomorphy) placing the new species in the Pseudochironomini. The comb at the base of the spur, observable in lateral view (Fig. 6A, B) and

similar to combs on mid- and hindlegs (Fig. 6C, D) but slightly narrower, in the new species is nearly identical to that known from, for example, *Manoa* (cf. Qi et al., 2017: fig. 5d–f). Hereby, we consider assigning the new species to the tribe Pseudochironomini legitimate. However, in the present understanding of the importance and interpretation of other morphological diagnostic characters defining the Pseudochironomini, we see no justification in assigning the new species to any of the genera of the tribe known so far. Consequently, we propose that the species be included in the new genus, *Palaeocentron*, peculiar in having several distinct features discussed below.

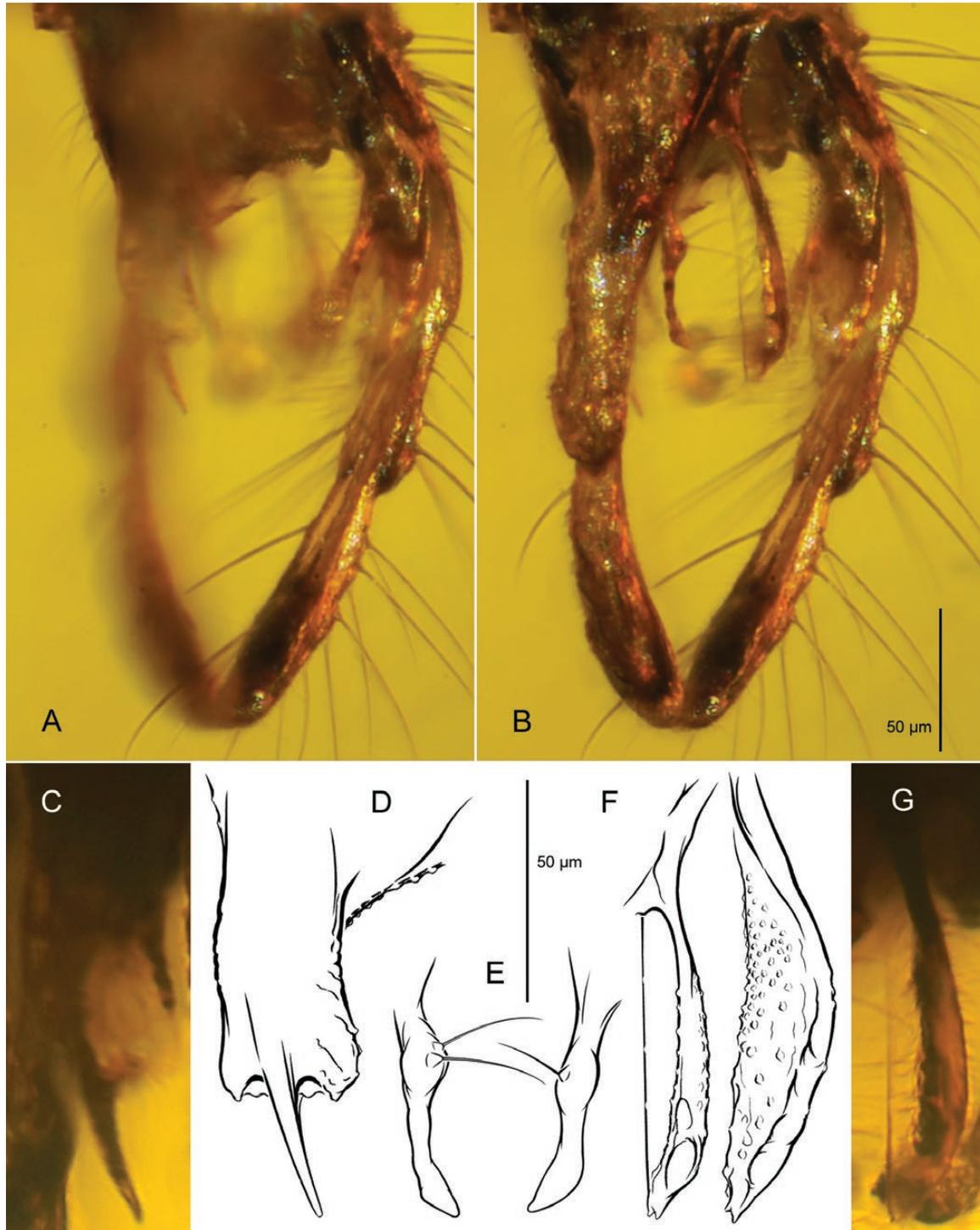


Figure 7. *Palaeocentron krzeminskii*, adult male, MP/4020 (ISEZ PAN); mid-Cretaceous, probably Albian–Cenomanian; Hukawng Valley, Kachin State, Myanmar. A, B, hypopygium in dorsolateral (A) and ventrolateral aspect (B). C, D, anal point photographed (C) and drawn (D). E, superior volsellae. F, G, inferior volsellae drawn (F) and photographed (G).

Palaeocentron lacks the pars ventralis, since no trace of this lobe was observed. The relatively simple structure of the hypopygium in *Palaeocentron*

(without the median volsella or pseudovolsella) is tentatively defined as plesiomorphic in relation to other Pseudochironomini, i.e. the Late Cretaceous

Mesoacentron and the remaining extant genera that display combinations of more complex and probably more advanced structures/character states.

In *Megacentron eocenicus*, the sole previously described extinct Pseudochironomini species, the general structure of the hypopygium (distinctly elongated, with the gonostylus broadened and rounded apically and the well-developed but narrow anal point) resembles that of *Palaeocentron* (cf. Doitteau & Nel, 2007: figs 49, 50). However, the three pairs of volsellae in *Megacentron* vs. two pairs in *Palaeocentron* clearly discriminate between the two genera.

The stout, parallel-sided hypopygial anal point with a spike-like extension (Fig. 7A, C, D), observed in the adult male of *Palaeocentron*, is unique within the Pseudochironomini. The anal point is absent in *Manoa*, *Pseudochironomus* and *Riethia*. Doitteau & Nel (2007) and Andersen (2016) gave a brief overview of shapes of the structure in other Pseudochironomini genera, including *Madachironomus* (a strong and nearly parallel-sided anal point, but with a rounded apex), *Aedokritus* (a relatively short triangular or subtriangular anal point) and *Megacentron* (a narrow anal point, slightly similar to that described in *Mesoacentron*; see above).

An unusual structure found in the *Palaeocentron* male is the hindleg armature: a row of strong, thorn-like bristles placed along the tibia and forming a fan near its apex (Fig. 6D–G). Similar leg structures are known from closely related families (mostly in females), especially the predatory/haematophagous midges (e.g. Downes, 1978; Szadziowski et al., 2007; Urbanek et al., 2015). In these flies, the mouthparts usually are elongated and/or provided with structures used in biting and intake of liquid nourishment, blood or haemolymph. However, no predatory/haematophagous species have been so far recorded in the subfamily Chironominae that as adults feed on honeydew or are considered aphygous. Thus, the function of these thorn-like bristles in *Palaeocentron*, which features a shortened proboscis and mouthparts reduced as in a typical Chironominae, remains an open question (cf. Sivinski, 1997).

DISCUSSION

PSEUDOCHIRONOMINI – TEMPO OF DIVERSIFICATION

Studies of Chironomidae have been prominent in estimations of phylogeny and dating of the tempo of their evolution. Brundin (1966, 1976) demonstrated how a phylogenetic hypothesis could be melded with geological evidence, obtained from both fossil specimens and biogeography from earth history. The conclusions drawn by Brundin (1966) have found substantial support for both the evolutionary relationships and the timing (e.g. Cranston et al., 2010,

2012), although reconstruction methods differ now with more explicit analyses and additional sources of evidence. Foremost have been advances in molecular techniques to allow reconstruction of phylogenies with many more character states available than from morphology alone, even compared with data derived from all life stages – the gold standard for morphology. Allied to this has been development from early (naive) ideas concerning a ‘molecular clock’ from which the tempo of evolutionary events could be reconstructed and tested against earth history events. With Bayesian analyses to the fore, hypotheses of relationships could be supplemented by complex models of molecular evolution, with calibration points derived from fossils, to estimate the tempo of change across any well-sampled clade. ‘Well sampled’ evidently is critical, as material from which molecular phylogenies are derived ought to be freshly collected for study, in contrast to the more easily available historical slide-mounted morphological material: the inclusion of Pseudochironomini in contemporary studies is impeded by shortfall in the diversity sampled. It is worth noting that molecular data provide an independent timeline on major evolutionary events, but in no way can be argued to represent the ‘truth’. Inferences of stem and crown ages of clades are only as accurate as the models of molecular evolution and the appropriateness of the calibrations selected and their ages based on fossil records.

In testing Brundin’s ideas on Gondwanan-induced tempo using divergence time analysis, Cranston et al. (2010) estimated a sole species of *Riethia* to have separated from a solitary *Polypedilum* (representing Chironomini) at ~62 Mya, and these two combined (representing Chironominae) to have separated from *Brillia* (an Orthocladiinae) at ~100 Mya. Dates typically have wide statistical variances (HPD, highest posterior density intervals) of +/- 30%. Increased sampling in Cranston et al. (2012), but with Pseudochironomini still undersampled (with only *Riethia* included), was inconclusive on monophyly and tribal relationships. Most recently, with formal recognition of the tribe Xiaomyiini as sister to all other Chironominae (Tang & Cranston, 2019; Krosch et al., 2020), and with Pseudochironomini enhanced by inclusion of *Pseudochironomus*, Krosch et al. (2020) inferred stem-group origination of Chironominae in the earliest Cretaceous and tribe Pseudochironomini separation from tribes Chironomini + Tanytarsini at the end of Early Cretaceous (~108 Mya). Median dates for origin of crown diversification of Pseudochironomini (still comprising only *Riethia* and *Pseudochironomus*) ~87 Mya, and *Riethia* originated at either ~61 Mya or ~52 Mya, depending on the position of two problematic Neotropical species.

KEY TO THE IDENTIFICATION OF FOSSIL AND EXTANT PSEUDOCHIRONOMINI GENERA (ADULT MALES)

1. Gonostylus directed backward, usually rigidly connected with gonocoxite, with slight ability of flexion inwards at most (Figs 2E, 3G, H, 7A, B)..... Chironominae (2)
 - Gonostylus movable and usually folded inward..... other Chironomidae subfamilies (not keyed)
2. Pars ventralis present (Fig. 2E, G, H) and/or foreleg tibia with spur surrounded by darkly pigmented comb similar to those on mid- and hindlegs (Fig. 6A, B) Pseudochironomini (3)
 - Pars ventralis absent or represented by depressed oval area at most (possibly remnant of pars ventralis), foreleg tibia with bristle(s) or spur at most but comb never present ..other Chironominae tribes (not keyed)
3. Anal point of hypopygium present (Figs 2E–G, 7A, C, D) 4
 - Anal point of hypopygium absent, anal tergite with crenate apical extension at most (Cranston *et al.*, 1989: fig. 10.54e)..... 8
4. True median volsella absent (Fig. 7A, B), pseudovolsella as aggregation of linearly-merged tubercles at most (Fig. 2G, H); antenna with 14 flagellomeres (Figs 1D, 5B)..... 5
 - True median volsella present; antenna with 13 flagellomeres (exceptionally 14 flagellomeres present) (Freeman, 1961: fig. 18a; Oliveira & Messias, 1989: fig. 7; Doitteau & Nel, 2007: figs 48–50; Andersen, 2016: fig. 7; Pinho, 2018: fig. 1e; Pinho *et al.*, 2019: fig. 1d) 6
5. Hypopygium with two pairs of volsellae – digitus, pseudovolsella and pars ventralis absent; anal point stout, parallel-sided, with spike-shaped prolongation (Fig. 7); hindleg tibia with strong thorn-like bristles arranged in row and subapical fan (Fig. 6D–G)..... *Palaeocentron*
 - Hypopygium with two pairs of volsellae, digitus, pseudovolsella and pars ventralis; anal point narrow, without spike-shaped prolongation (Fig. 2E–H); hindleg tibia without thorn-like bristles *Mesoacentron*
6. Anal point slender or sharp; antenna with 13 flagellomeres (extant species), exceptionally with 14 flagellomeres (single known fossil species, *M. eocenicus*) (Freeman, 1961: fig. 18a; Doitteau & Nel, 2007: figs 48–50)..... *Megacentron*
 - Anal point broad, parallel-sided or triangular/subtriangular; antenna with 13 flagellomeres (Oliveira & Messias, 1989: fig. 4; Andersen, 2016: fig. 6; Pinho, 2018: fig. 1d; Pinho *et al.*, 2019: fig. 1b, c).....7
7. Wing membrane with shaded areas along radial, medial and cubital veins, but without distinctly outlined spots, anal lobe moderately developed, not protruding; median volsella medially directed, with stem split apically (Andersen, 2016: figs 7, 8) *Madachironomus*
 - Wing membrane with distinct colour spots and/or crossbands, anal lobe well-developed, strongly protruding; median volsella posteriorly directed, with stem single-lobed (Oliveira & Messias, 1989: figs 2, 7; Pinho, 2018: fig. 1c, e; Pinho *et al.*, 2019: fig. 1a, d) *Aedokritus*
8. Anal lobe of wing large, distinctly protruding; pars ventralis strongly developed (Paggi & Rodriguez-Garay, 2015: figs 3, 4, 6) *Pseudochironomus*
 - Anal lobe of wing moderately developed and only slightly protruding at most (Trivinho-Strixino & Shimabukuro, 2018: fig. 8b), but usually weak or absent; pars ventralis as small protrusion(s) at most or absent (Andersen & Sæther, 1997: fig. 1h; Qi *et al.*, 2017: fig. 6c, d) *Manoa* + *Riethia*

The addition of molecular data from *Pseudochironomus* in this study showed it to be strongly supported as sister to *Riethia*, yet the monophyly of the broader Pseudochironomini is tested only trivially, lacking the geographically disparate and rare additional proposed members of the tribe (see above) and likely to remain unattainable.

Regarding calibrations of molecular phylogenies with dated fossils, following discussions with those

studying the *Kalugina* fossil described here, Krosch *et al.* (2020) included it as calibration of Chironominae less Xiaomyiini. Confirmation here as a member of the Pseudochironomini consolidates and refines their analyses, in which the fossil date was set at a minimum age of 71.5 Mya with the crown radiation estimated as 86.8 Mya with HPD of 68–112 Mya (Krosch *et al.*, 2020) quite compatible with the fossil dates here.

INFRARED (IR) SPECTRUM

Amber (or amber-like materials) offered by private sellers on the free market may be often substituted by fakes (e.g. Eriksson & Poinar, 2015). Moreover, the provenance of amber can be mistakenly assigned to geological deposits other than those from which it actually originated. Clearly this serious problem may also affect specimens designated as types for new taxa. Recording and archiving the amber IR spectra is thus a highly recommended procedure for museum materials to confirm the validity of the newly described taxa (Zakrzewska *et al.*, 2020). One of the specimens examined in the present study was purchased on the free market, hence the analysis of its IR spectrum was vital. The FT-IR (ATR) spectrum obtained shows features typical of Burmite from Kachin, with the location and relative intensity of absorption peaks in fingerprint regions 1226, 1155, 1031 and 974 cm^{-1} (Fig. 4B) (E. Wagner-Wysiecka, personal communication).

CONCLUSIONS

The riddle of the presence of the Chironominae in the Cretaceous has been revisited and is now finally solved. The subfamily had been previously recorded only once, based on a single and unspecified record from Late Cretaceous Taimyr amber, the record that is now revised and identified. Finding a further member of the subfamily in Burmese amber demonstrates the group to have occurred as early as the mid-Cretaceous. All specimens studied have been assigned to the Pseudochironomini on the basis of a set of features putatively considered diagnostic for this tribe. Information provided by the specimens examined are in line with results of current research on the rate of diversification within the family/subfamily, based on molecular markers derived from extant representatives. All the data obtained so far support the hypothesis that the Chironominae appeared in the Cretaceous, diversifying subsequently throughout this period. Extremely rare findings of Chironominae in the ambers studied here and the lack of data on these dipterans in other Mesozoic fossiliferous deposits may suggest that their abundance/species richness in the Cretaceous was still relatively meagre, while it increased significantly in the Eocene (see Introduction).

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