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Two new species of *Eukiefferiella* Thienemann (Diptera, Chironomidae) from northern Finland

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Abstract

The genus *Eukiefferiella* is one of the most species-rich genera within Chironomidae, but its northern European diversity is comparatively low. In this study, we describe two new species of this genus, *Eukiefferiella paasivirtai* **sp. nov.** and *Eukiefferialla viljamii* **sp. nov.**, from northern Finland, based on molecular barcoding data and the morphology of adult males and pupal exuviae. Molecular analyses indicate that the closest known relatives of both species occur in North America (minimum K2P interspecific distances 8.6 % and 9.0 %). The morphology of the adult males and molecular analysis places both species in the *Eukiefferiella gracei* group. ASAP analysis supported morphological species delimitation, with a 4.5 % threshold separating the ten studied *Eukiefferiella* taxa. The restricted northern distribution of the two new species suggests ecological specialization to subarctic river ecosystems. Keys to pupal exuviae of northern European *Eukiefferiella* species and to adult males of the *E. gracei* group are provided.

Key words: Chironomidae, Orthocladiinae, river ecosystem, northern Finland, DNA barcoding

Introduction

The chironomid genus *Eukiefferiella* Thienemann is among the most species-rich genera within Chironomidae. Ashe & O'Connor (2012) recognized 85 valid species worldwide. Later additions have increased the figure to 95 (Qi *et al.* 2012, Ree 2012, Moubayed-Breil & Ashe 2015, Moubayed-Breil & Mary 2019, Makarchenko *et al*. 2019, Imada 2020, Moubayed & Langton 2024). Notably, 40 species have been recorded from Japan (Yamamoto & Yamamoto 2014). The genus is comparatively poorly represented in northern Europe, with a total of 14 species in Finland, Norway and Sweden (Suomen Lajitietokeskus 2026 based on Paasivirta 2014, Artsdatabanken Norway 2026, Artdatabanken Sweden 2026). Continental France hosts 22 of the 23 species known from Europe (Moubayed-Breil & Ashe 2015, Moubayed & Langton 2024). In the northern Nearctic, the presence of only nine species of *Eukiefferiella* is known, with seven of them having a Holarctic distribution (Namayandeh *et al*. 2024). The Nearctic *Eukiefferiella* likely includes many undescribed species and many unassociated immatures lacking species confirmation (Cranston *et al*. 1989, Namayandeh *et al*. 2024).

During a sampling trip to northern Finland in 2025 J. Raunio collected adult males and pupal exuviae of *Eukiefferiella* from two tributaries of the large subarctic River Teno (R. Utsjoki and R. Vetsijoki). Two *Eukiefferiella* taxa could not be identified using available keys or existing species descriptions. Their morphological distinctiveness, combined with DNA barcoding results, indicated that they represent two undescribed species.

In this paper, we describe *Eukiefferiella paasivirtai* **sp. nov.** and *Eukiefferialla viljamii* **sp. n** based on their adult male and pupal exuviae morphology, and the molecular analysis. We also compare the new species with their closest morphological and genetic relatives and discuss their biogeographical distribution in northern Fennoscandia.

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Material and Methods

Morphological terminology for males follows Sæther (1980) and Oliver & Dillon (1989), except that wing length is measured from the joint point with the thorax instead of from the tip of the arculus. Wing length when measured from arculus is approximately 12–14 % shorter. Morphological terminology for pupa follows Langton (1994) and Langton & Visser (2003). Measurements are given in µm unless stated otherwise. The number of specimens or structures measured (n) is indicated in parentheses. For structures with at least three different measurements, average and range (in parentheses) are provided.

Digital, multi-focus imaging and measurements were made using a Leica M205 C stereomicroscope, Leica DM2500 LED compound microscope, Leica K5C digital camera, and LAS X software. LAS X multi-focus images were stacked using Helicon Focus software and were subsequently edited with Adobe Photoshop.

The adult males and pupal exuviae were collected using a hand net at two tributaries of the River Teno (R. Utsjoki and R. Vetsijoki) in far northern Finland in late June 2025. Samples were preserved in ethanol. The adult males and pupal exuviae were separated under a dissecting microscope. Selected specimens were dissected, macerated in KOH, and mounted in Euparal. The holotypes, paratypes, and pupal exuviae are deposited in the collection of the Regional Museum of Lapland (LMM) in Rovaniemi.

DNA barcoding was performed at Bioname Oy. Molecular data have been uploaded to the BOLD database (Ratnasingham *et al*. 2024) and are publicly available. A taxon ID tree of the two new *Eukiefferiella* species (*Eukiefferiella viljamii*, BOLD:ADA1265 and *Eukiefferiella paasivirtai*, BOLD:AHF2600) was inferred together with other Nordic (specimens collected from Finland, Sweden and Norway) congenerics available in the BOLD (*Eukiefferiella brevicalcar* (Kieffer, 1911) (BOLD:AAY3164), *Eukiefferiella claripennis* (Lundbeck, 1898) (BOLD: AHC6526 and BOLD:AAE4568), *Eukiefferiella devonica* (Edwards, 1929) (BOLD:AAE4574), *Eukiefferiella dittmari* Lehmann, 1972 (BOLD:AAI5129), *Eukiefferiella gracei* (Edwards, 1929) (BOLD:ADG3574), *Eukiefferiella ilkleyensis* (Edwards, 1929) (BOLD:AAI5128), *Eukiefferiella minor* (Edwards, 1929) (BOLD:AAE3299), together with their closest genetic relatives (*Eukiefferiella* sp. from Canada, BOLD:ABA1245) using the Neighbor-Joining method (Saitou & Nei 1987). More than 400 barcoded specimens of the Canadian taxon and over 100 specimens of *E. minor* from northern Europe are available in the BOLD system, of which ten were included in our analysis to maintain readability of the phylogenetic tree. The data set BOLD ID's are provided as supplementary data. The sequences of the two new species are available at: dx.doi.org/10.5883/DS-EUKIEF. Pairwise genetic K2P distances (Kimura 1980) were computed using the Nearest Neighbor method, with results expressed as the number of base substitutions per site. The analysis encompassed 130 coding nucleotide sequences using 1st, 2nd, 3rd, and non-coding positions. Ambiguous positions were removed using the pairwise deletion option, resulting in a final alignment of 654 positions. The analyses were conducted in MEGA12 software (Kumar *et al*. 2024). In addition, ASAP species delimitation analysis available in SPART explorer (Puillandre *et al*. 2021, Miralles *et al*. 2022) was conducted to test for concordance of genetic and morphological species delineations in the data set.

Results

Eukiefferiella paasivirtai sp. nov.

Eukiefferiella sp., pr. *gracei,* Paasivirta (2014), 72 (checklist)

Etymology. The species is named after Lauri Paasivirta, honoring his significant contribution to chironomid research in Finland.

Type material. Holotype: male, FINLAND: Utsjoki county, River Utsjoki (Lat. N 69.892923611°; Long. E 27.018188889°, WGS84), 29. VI. 2025, leg. J. Raunio, museum sample id NVO.chiro541, deposited in LMM. Paratypes, same as holotype except: NVO.chiro542, 1 male (Euparal); NVO.chiro551, 1 male (Euparal); NVO. chiro549, 1 male (Euparal); Rovaniemi, Ounaskoski, Lat. N 66.497578056°; Long. E 25.740785000°, 17. VI. 2017, leg. L. Paasivirta, NVO.chiro550, 1 male (Euparal) and pupal exuviae, same as holotype except: NVO.chiro540, 3 males (Euparal); NVO.chiro538, 1 male (Euparal).

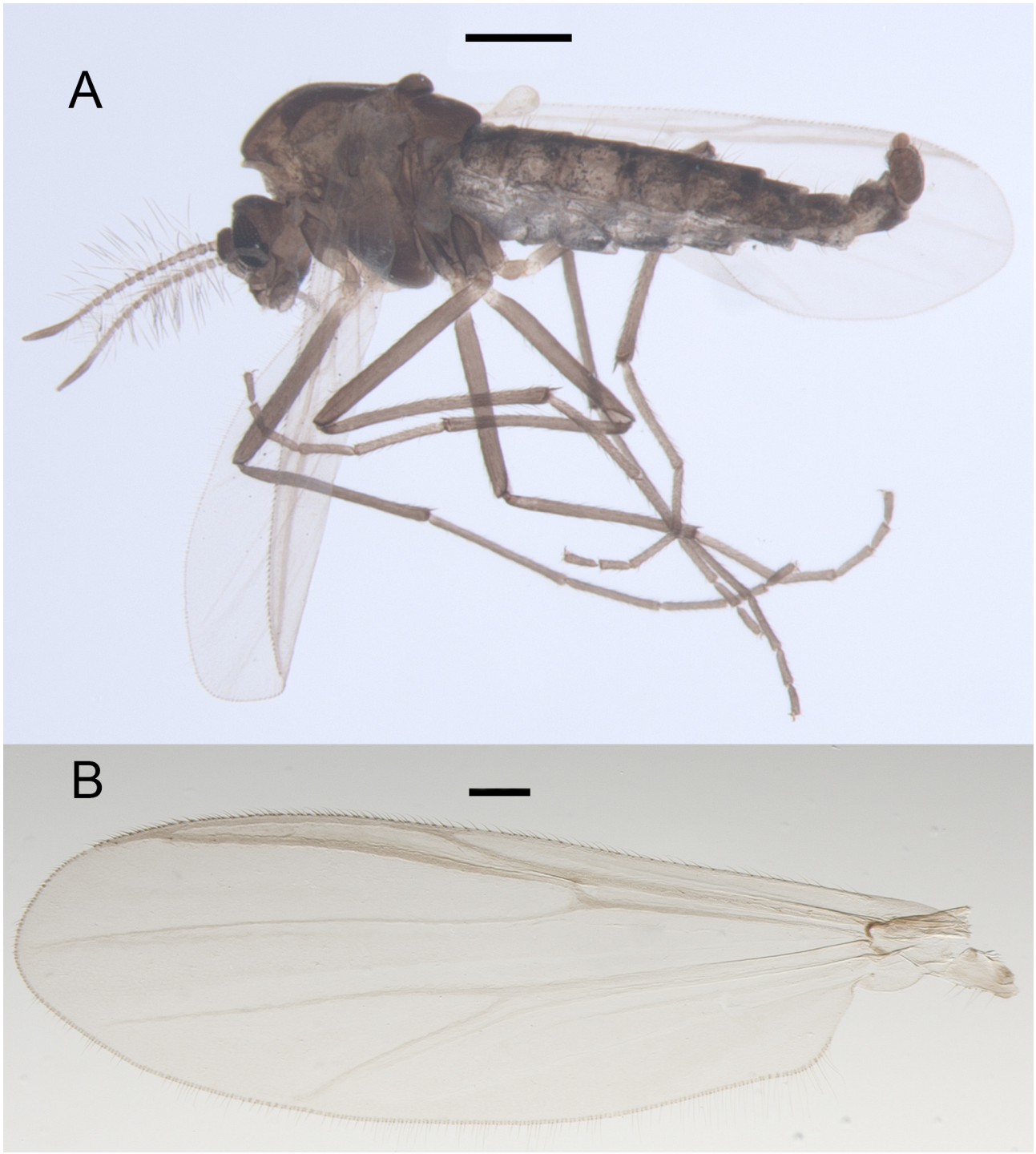

FIGURE 1. *Eukiefferiella paasivirtai* **sp. nov.**, adult male (A–B). A. Habitus, lateral view, scale bar 250 µm (paratype, NVO. chiro557). B. Wing, scale bar 100 µm (holotype).

Diagnostic characters

The male belongs to the *Eukiefferiella gracei*-group characterized by a gonostylus which in a dorsal view is broadest before midsection and with inner section densely covered with short setae. *E. paasivirtai* can be separated from all other members of the group by the following combination of characters: inferior volsella digitiform and distinctly curved, antenna AR 0.57–0.6, antenna with short plume and eyes bare. In addition, *E. paasivirtai* can be distinguished from the morphologically similar *E. gracei* by its smaller size (wing length 1.4–1.6 versus 2.0–3.5 mm) and by the much longer extension of vein costa beyond R_{4+5} (57–80 µm versus 0–5 µm).

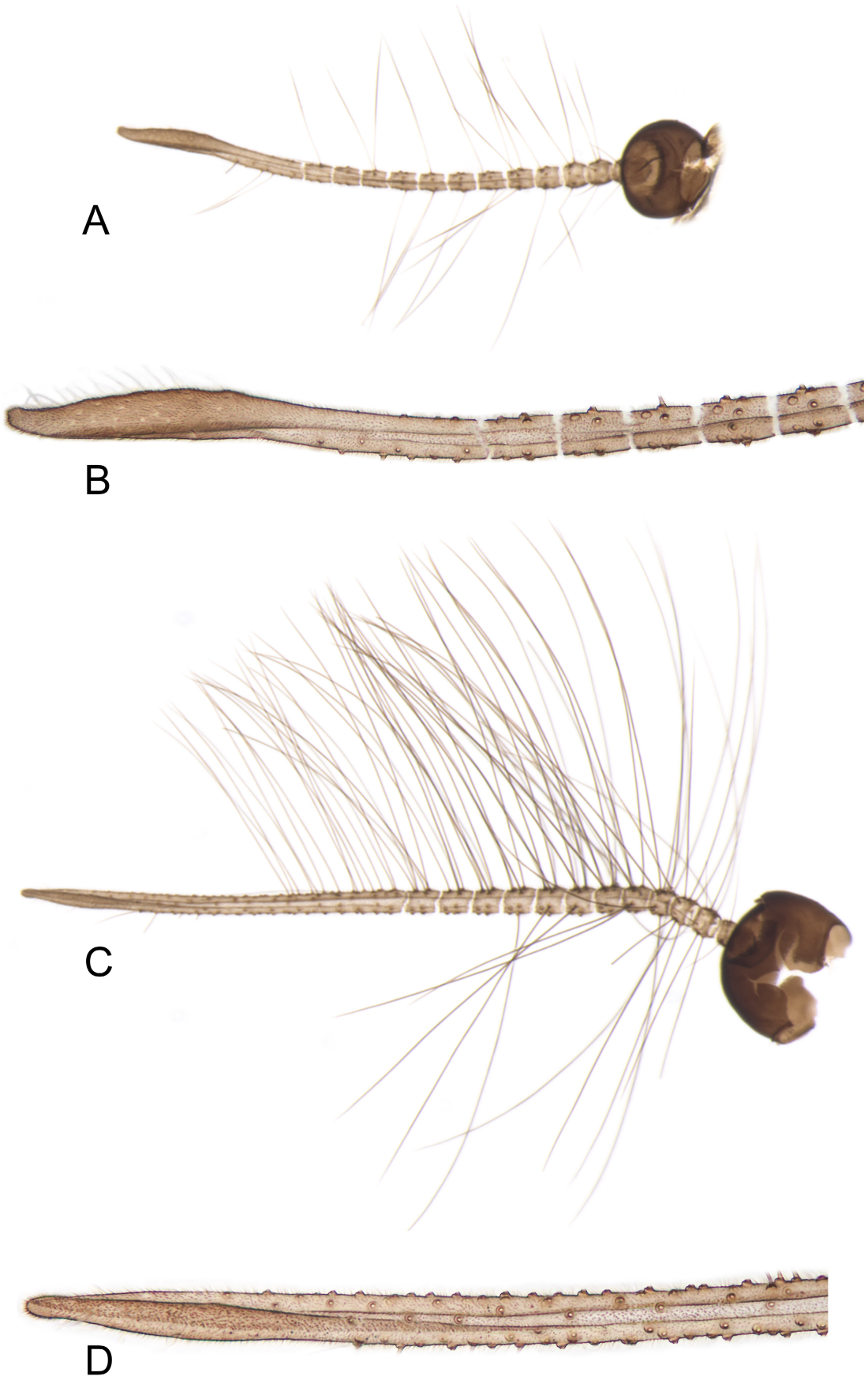

FIGURE 2. *Eukiefferiella paasivirtai* (A–B) and *Eukiefferiella viljamii* (C–D). A and C. Antenna. B and D. Tips of antenna. *E. paasivirtai* NVO.chiro542, paratype; *E. viljamii* NVO.chiro548, paratype. A–D in the same scale. Antennal length of *Eukiefferiella paasivirtai* 525 um and *Eukiefferiella viljamii* 750 um.

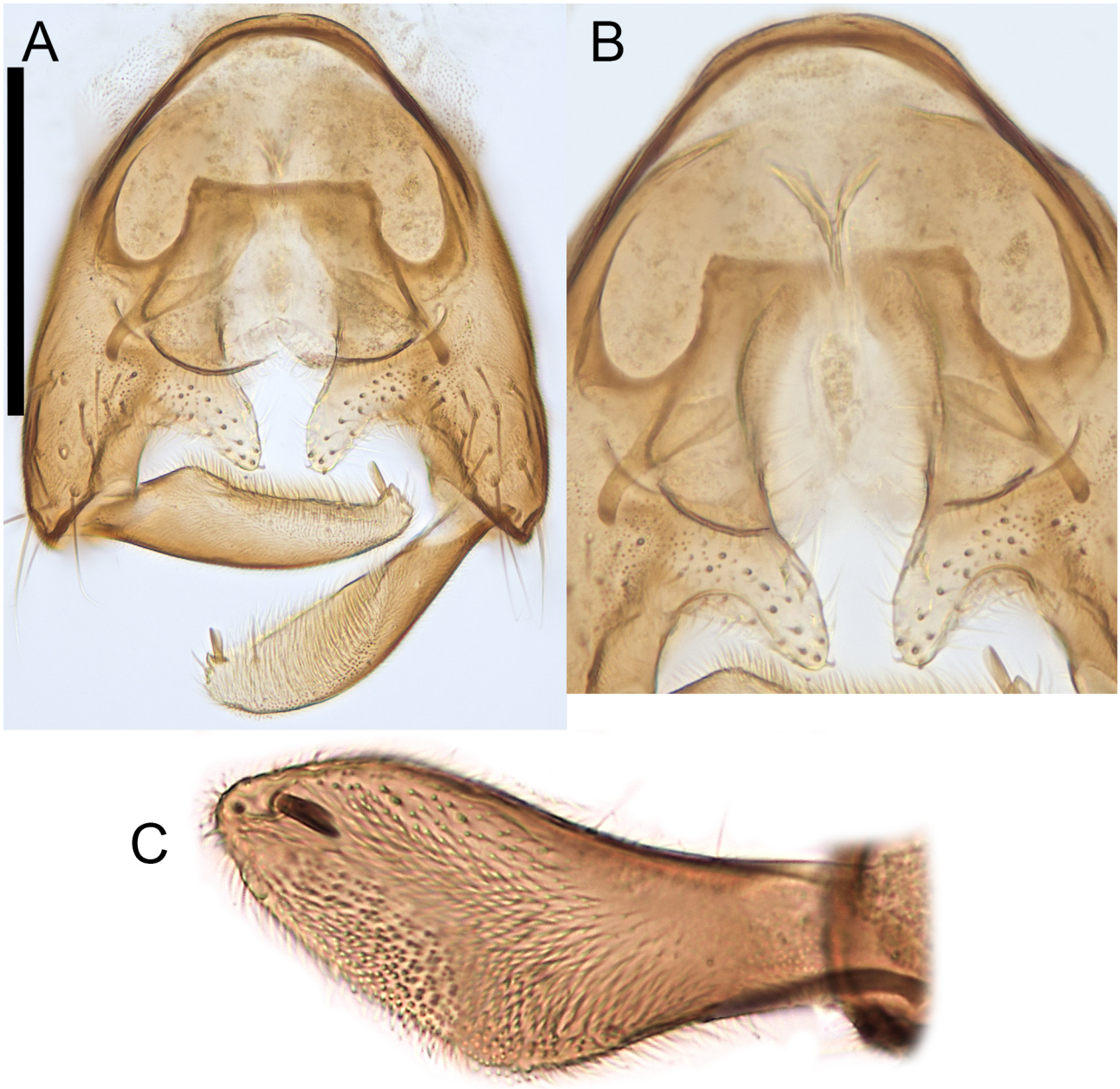

FIGURE 3. *Eukiefferiella paasivirtai* **sp. nov.**, adult male (A–C). A. Hypopygium, dorsal view (holotype). B. Hypopygium, internal structures, dorsal view (holotype). C. Gonostylus, mesial view (paratype, NVO.chiro551). Scale bar 100 µm.

Pupal exuviae are similar to those of *E. claripennis*, but separable by a larger thoracic horn ratio (length of apical filament / length of the base), longer posterior teeth on tergites III–IV (III 20–27; IV 20–30 µm versus III 15–20; IV 13–18 µm) and paratergites II–VI covered with shagreen.

Description

Male (n = 3, Fig. 1). Relatively small species. Total length: 1.8 (1.8–1.9) mm, abdomen length 1.2 (1.1–1.3) mm. Overall coloration mostly brown, legs without bands; wings unmarked with pale veins (Fig. 1B).

Head. Temporals consist of 4 (3–5) setae. Maxillary palp 5–segmented; lengths (µm) of palpomeres 29 (28–30); 41 (38–43); 63 (55–68); 75 (64–81); 87 (85–89). Antenna 15 segmented, antennal groove clearly visible, beginning on segment 5 and reaching ultimate flagellomere that is expanded preapically and slightly nippled at tip, overall length 545 (537–572) µm long, last flagellomere 202 (190–215) µm, AR 0.59 (0.57–0.6), antenna with short plume (Fig. 2A–B).

Thorax. Setae: lateral antepronotals 1; dorsocentrals 3 (2–4); prealars 2 (2–3); scutellars 4 (4).

Wing. Length 1.51 (1.4–1.6) mm and width 0.46 (0.42–0.48) mm, VR 3.33 (3.29–3.35), anal lobe weak (Fig. 1B). Squama with 5 (4–6) setae. Wing without vein r_{2+3}, costa extention about 70 (57–80) µm beyond end of R_{4+5} and ending opposite or slightly proximal to the tip of vein M_{3+4}.

Legs. Lengths of leg segments are shown in Table 1. Leg ratios are as follows: LR_1: 0.73 (0.72–0.73), LR_2: 0.46 (0.45–0.47), LR_3: 0.52 (0.52–0.53).

Hypopygium. Tergite IX without macrosetae; laterosternite without macrosetae (Figs. 3–4). Anal point absent. Virga triangular. Superior volsella absent. Inferior volsella digitiform and distinctly curved, dorsally with short setae from base to the tip. Gonostylus 104 (103–104) µm long, distinctly expanded proximally, inner surface densely covered with short setae, megaseta 10 (10–11) µm.

Pupal exuviae (n = 2, males). Exuviae 2.31–2.57 mm long (Fig. 5).

Cephalothorax. Thoracic horn 223–252 µm. Length of horn base relative to horn total length: 0.34–0.36. Median antepronotal setae 1,2; 127–132; 71–80 µm long; precorneal setae 1–3: 116–125; 65–72; 62–67µm long; dorsocentral setae 1–4: 62–73; 23–31; 19–28; 24–29 µm long. Pearl row absent on the wing sheets.

Abdomen. Hook rows on tergites broken medially, hooks III: 18–23, IV: 19–22, V: 16–21. Tergite I smooth; tergites II–VIII with anterior band of shagreen. Paratergites II–VIII covered with shagreen. Tergites II–VI with a posterior band of teeth. Posterior teeth on tergites III 20–27; IV 20–30 µm long. Segment VIII with two strong setae posterolaterally (Fig. 5).

TABLE 1. Lengths (in µm) and ratios of leg segments of adult male of *Eukiefferiella paasivirtai* **sp. nov.**

	Fe	Ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV
P_1	493 (450–515)	359 (329–371)	244 (240–246)	166 (154–174)	104 (96–110)	88 (82–96)	88 (82–96)	0.73 (0.72–0.73)	2.20 (2.11–2.26)	2.69 (2.67–2.70)
P_2	485 (422–522)	488 (463–503	225 (213–239)	139 (135–148)	106 (103–110)	67 (60–74)	75 (73–77)	0.46 (0.45–0.47)	3.09 (2.95–3.16)	4.32 (4.15–4.51)
P_3	541 (487–522)	554 (531–571)	290 (277–299)	169 (163–180)	140 (135–144)	73 (69–75)	74 (68–78)	0.52 (0.52–0.53)	3.03 (2.91–3.13)	3.77 (3.68–3.84)

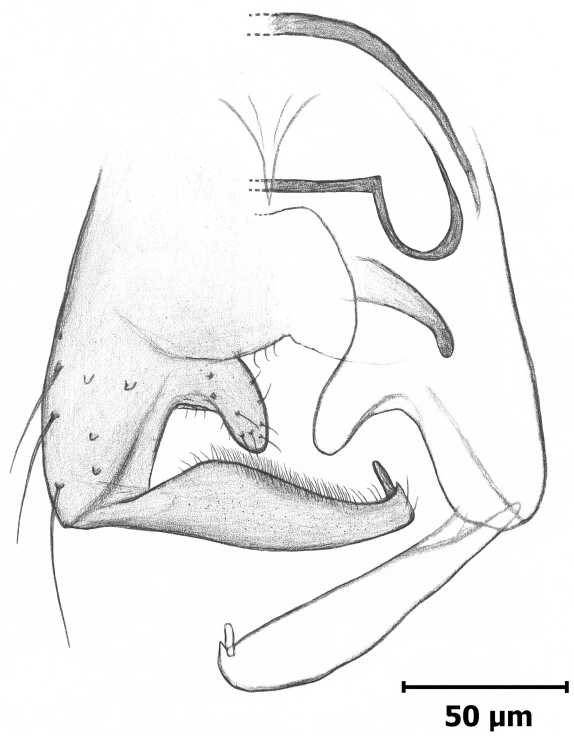

FIGURE 4. Drawing of the hypopygium of *Eukiefferiella paasivirtai* **sp. nov.**

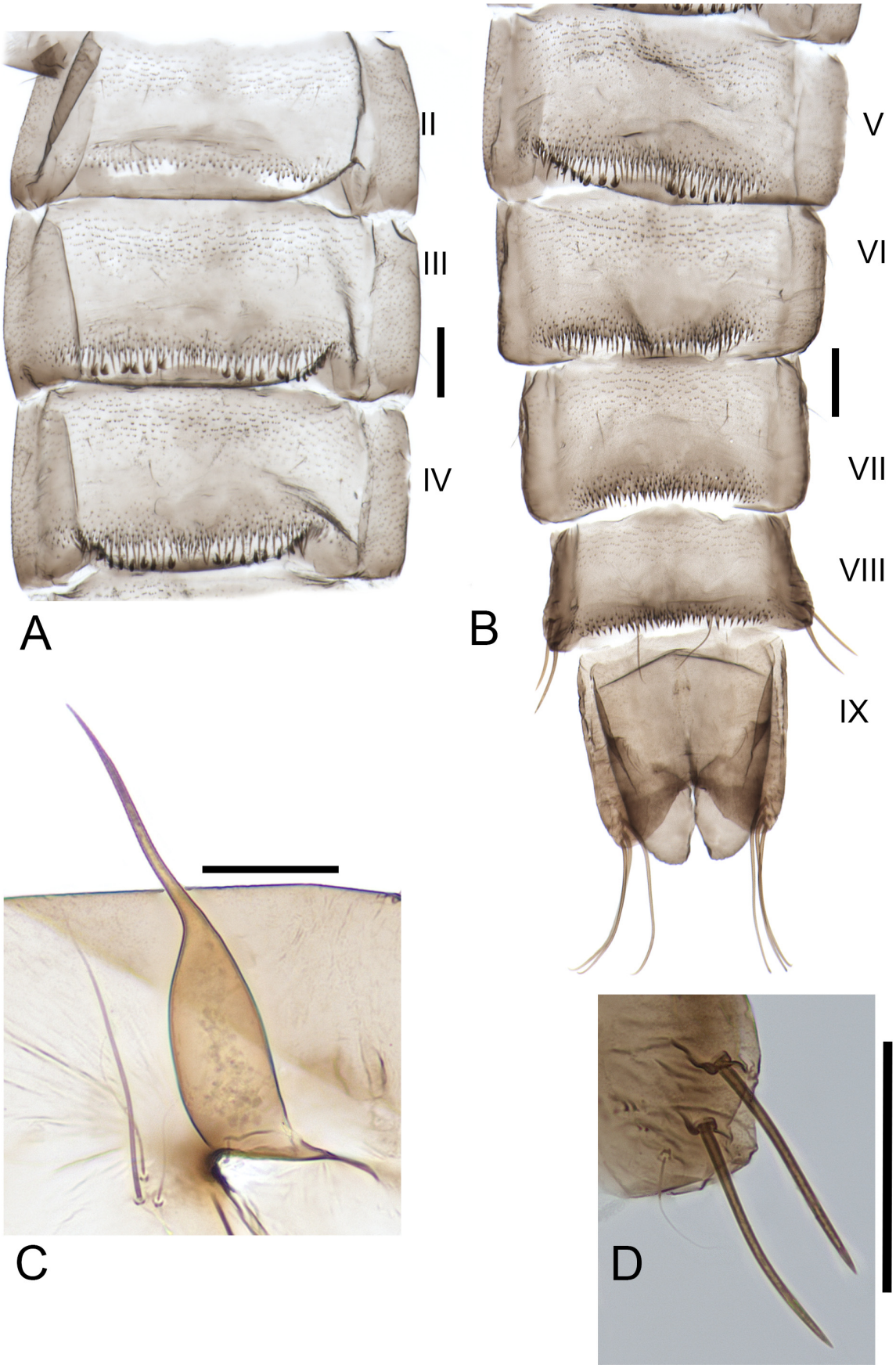

FIGURE 5. *Eukiefferiella paasivirtai* **sp. nov.**, male exuvia (A–D). A. Tergites II–IV. B. Tergites V–IX C. Thoracic horn. D. Lateral setae of tergite VIII (paratype NVO.chiro540, scale bars: A, B 75 µm, C 50 µm, D 90 µm).

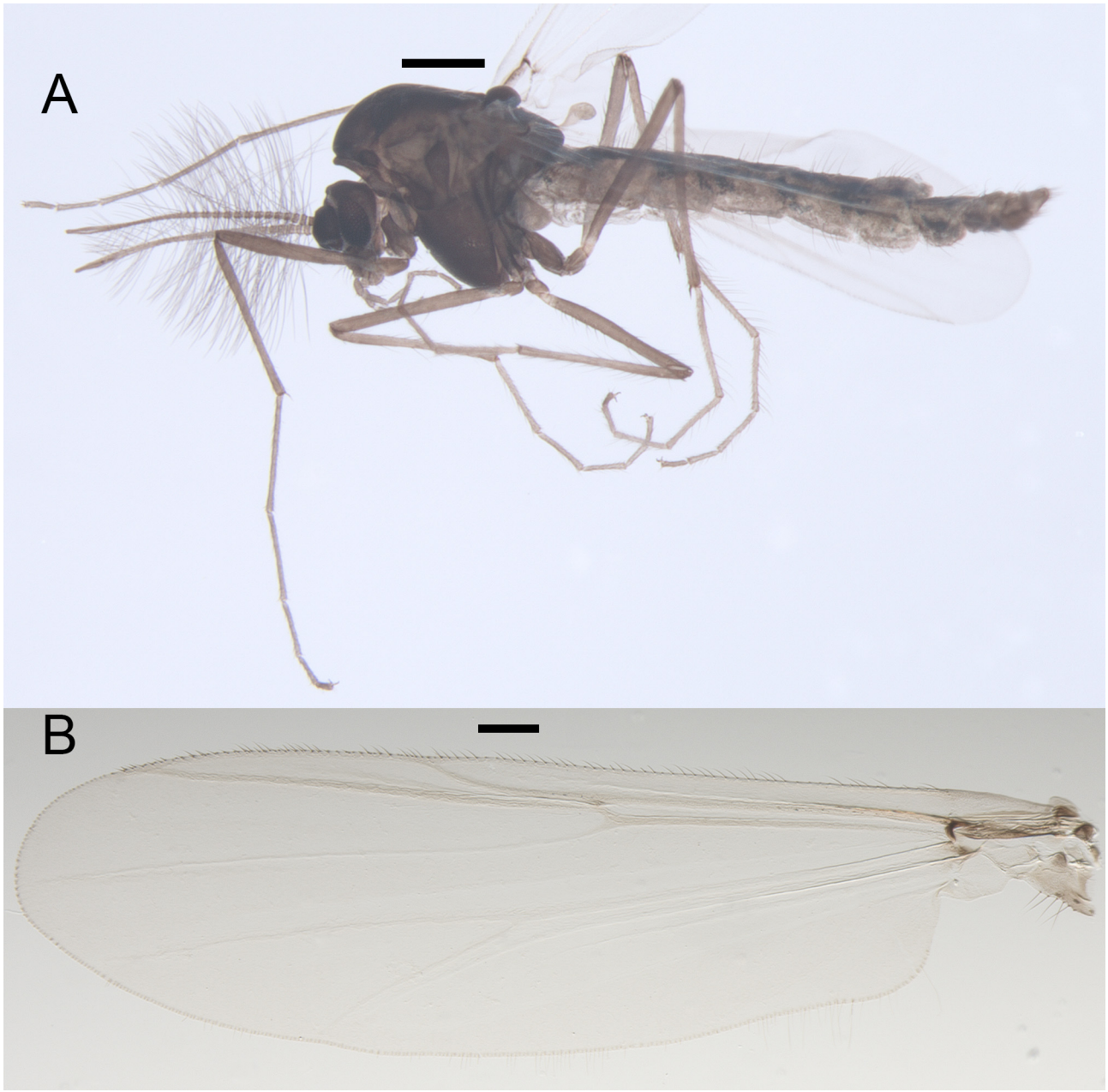

FIGURE 6. *Eukiefferiella viljamii* **sp. nov.**, adult male (A–B). A. Habitus, lateral view, scale bar 250 µm (paratype, NVO.chiro555). B. Wing, scale bar 100 µm (paratype, NVO.chiro548).

Eukiefferiella viljamii sp. nov.

Etymology. The species is named after the first author's son.

Type material. Holotype: male, FINLAND: Utsjoki county, River Vetsijoki (Lat. N 69.961322500°; Long. E 27.314297500°, WGS84), June 29. VI. 2025, leg. J. Raunio, museum sample id NVO.chiro545, deposited in LMM. Paratypes, 2 males, same as holotype except: NVO.chiro548, 1 male (Euparal); NVO.chiro561, 1 male (Euparal). 1 male exuviae. NVO.chiro544, 1 male (Euparal).

Other material, same as holotype except: NVO.chiro546, 7 female exuviae (ethanol); NVO.chiro547, 2 males (ethanol); NVO.chiro555, 3 males (ethanol); NVO.chiro556, 3 male exuviae (ethanol).

Diagnostic characters

The male belongs to the *Eukiefferiella gracei*-group characterized by a gonostylus which in a dorsal view is broadest before midsection and with inner section densely covered with short setae. *E. viljamii* can be separated

from all other members of the group by the following combination of characters: inferior volsella broadly conical not curved and length/width 1.0–1.1, antenna AR 1.12–1.16 and eyes bare. The morphologically most similar species *Eukiefferiella brevinervis* (Malloch, 1915), North America, has narrower inferior volsella with length/width 1.2–1.4 and a much higher antenna AR (2.2–2.4).

Pupal exuviae are similar to those of *Eukiefferiella pseudomontana* Goetghebuer, 1935, but separable by a larger number of hooks on abdominal tergites III–V, absence of long (well over 10 µm) posterior teeth on tergites III–IV and larger overall length of the exuviae compared to *E. pseudomontana*.

Description

Male (n = 3, Fig. 6). Relatively small species. Total length: 2.1 (1.9–2.3) mm, abdomen length 1.4 (1.3–1.5) mm. Overall coloration mostly brown; legs without markings; wings unmarked with pale veins (Fig. 6B).

Head. Temporals consist of 3 (3–4) setae. Maxillary palp with 5 segments; lengths (µm) of palpomeres 24 (20–28); 39 (34–43); 61 (53–68); 71 (70–82); 94 (75–120). Antenna 15 segmented, antennal groove clearly visible, beginning on segment 3 and reaching ultimate flagellomere that is nippled at apex, overall length 746 (737–751) µm long, last flagellomere 380 (349–403) µm, AR 1.14 (1.12–1.16) (Fig. 2C–D).

Thorax. Setae: lateral antepronotals 2 (1–2); dorsocentrals 6 (6); prealars 3 (2–3); scutellars 4 (4).

Wing. Length 1.73 (1.67–1.8) mm and width 0.47 (0.44–0.51) mm, VR 3.7 (3.5–3.9). Wing without vein r_{2+3}, costa extension about 48 (40–59) µm beyond end of R_{4+5} and ending above or slightly proximal to the tip of vein M_{3+4}. Wing anal lobe weak (Fig. 6B). Squama with 7 (6–7) setae.

Legs. Lengths of leg segments are indicated in Table 2. Leg ratios are as follows: LR_1: 0.68 (0.67–0.69), LR_2: 0.50 (0.47–0.52), LR_3: 0.56 (0.55–0.57) (Table 2).

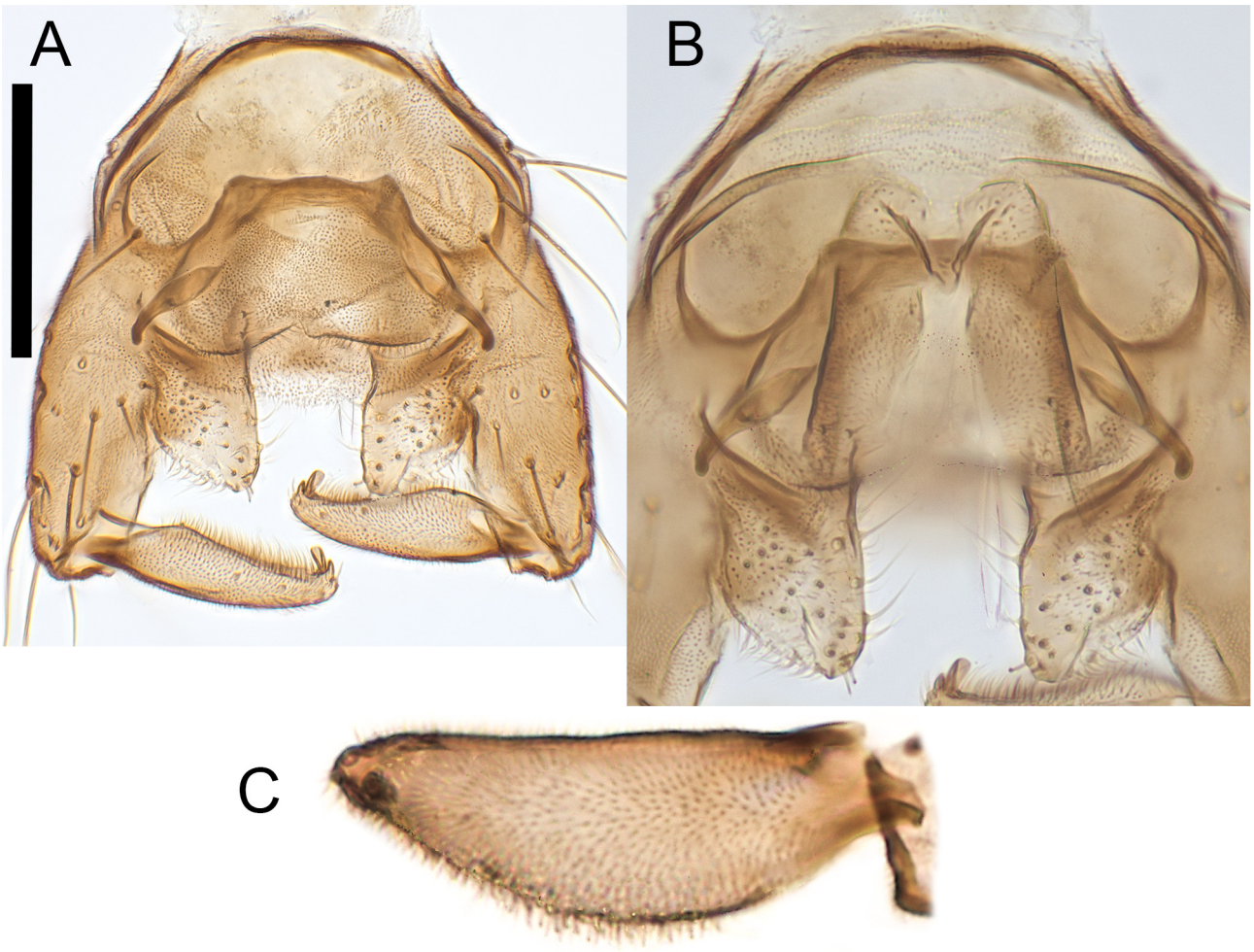

FIGURE 7. *Eukiefferiella viljamii* **sp. nov.**, adult male (A–C). A. Hypopygium, dorsal view (holotype). B. Hypopygium, internal structures, dorsal view (holotype). C. Gonostylus, mesial view (paratype, NVO.chiro548). Scale bar 90 µm.

TABLE 2. Lengths (in µm) and ratios of leg segments of adult male of *Eukiefferiella viljamii* **sp. nov.**

	Fe	Ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV
P_1	488 (473–497)	528 (515–544)	358 (349–363)	255 (246–263)	179 (169–186)	116 (113–118)	84 (82–85)	0.68 (0.67–0.69)	2.17 (2.16–2.17)	2.84 (2.81–2.87)
P_2	530 (515–544)	498 (484–520)	247 (243–256)	170 (162–178)	131 (122–138)	85 (83–90)	81 (78–83)	0.5 (0.47–0.52)	2.73 (2.68–2.76)	4.16 (4.04–4.32)
P_3	587 (547–610)	608 (591–619)	340 (325–353)	225 (219–236)	169 (157–178)	97 (93–102)	91 (88–95)	0.56 (0.55–0.57)	2.64 (2.59–2.70)	3.52 (3.48–3.57)

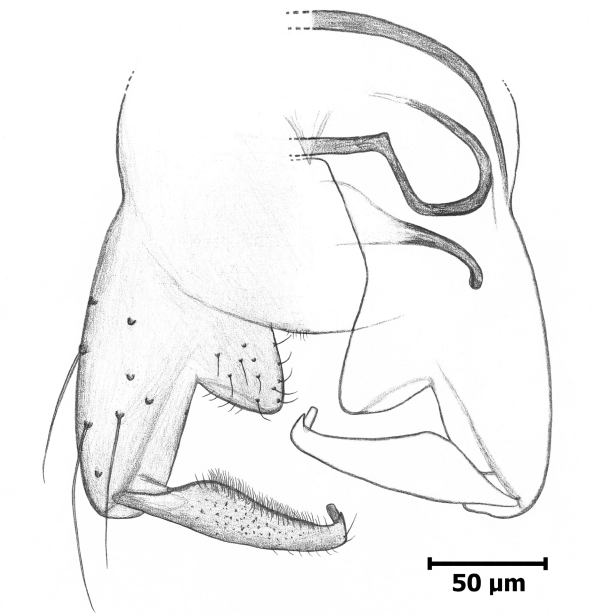

FIGURE 8. Drawing of the hypopygium of *Eukiefferiella viljamii* **sp. nov.**

Hypopygium. Tergite IX without macrosetae; laterosternites with four setae (Figs. 7–8). Anal point absent. Virga triangular. Superior volsella absent. Inferior volsella broadly conical with length/width 1.0–1.1, dorsally with short setae from base to the tip. Gonostylus 88 (77–103) µm long, distinctly expanded proximally, inner surface densely covered with short setae, megaseta 9 (9–10) µm long.

Pupal exuviae (n = 2, males). Exuviae 2.67–2.71 mm long (Fig. 9).

Cephalothorax. Thoracic horn 161–186 µm. Length of horn base relative to horn total length: 0.37–0.40. Median antepronotal setae 1,2: 77–85; 55–62 µm long; precorneal setae 1–3: 86–88; 56–67; 36–45 µm long; dorsocentral setae 1–4: 33–38; 24–30; 19–27; 24–31 µm long. Pearl row absent.

Abdomen. Hook rows on tergites broken medially, hooks III: 16–18, IV: 18, V: 15–18. Tergite I smooth, tergites II–VIII with anterior band of shagreen, medially also with shagreen but to lesser extent than anteriorly. Posterior teeth short on tergites II–VI (III–IV 2–5 µm long). Segment VIII with all posterolateral setae short and fine (Fig. 9D).

Molecular analysis

According to molecular analysis *E. paasivirtai* (BOLD:AHF2600) has an average interspecific distance (K2P distance) of 9.6 % (minimum interspecific distance of 8.6 %) to its nearest sequence (*Eukiefferiella* sp., BOLD: ABA1245), collected from Ontario, Canada (Fig. 10, Table 3). *E. viljamii* (BOLD:ADG3574) shares its closest relative with *E. paasivirtai* and (*Eukiefferiella* sp., BOLD:ABA1245), with an average interspecific distance of 10.0 % (minimum interspecific distance of 9.0 %) (Fig. 10, Table 3). Average interspecific distance between the new species is 12.1 %.

The two new species together with morphologically (*E. gracei*) and genetically (*Eukiefferiella* sp. from Canada) form a distinct cluster in the phylogenetic tree (Fig. 10). *E. minor*, that is close relative of *E. gracei* is also in this group. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the genetic distances used to infer the phylogenetic tree.

ASAP analysis showed that the data that were originally partitioned into ten morphologically distinct taxa received support from molecular data. ASAP had a threshold distance of 4.5 % with the number subsets (i.e. taxa) of ten.

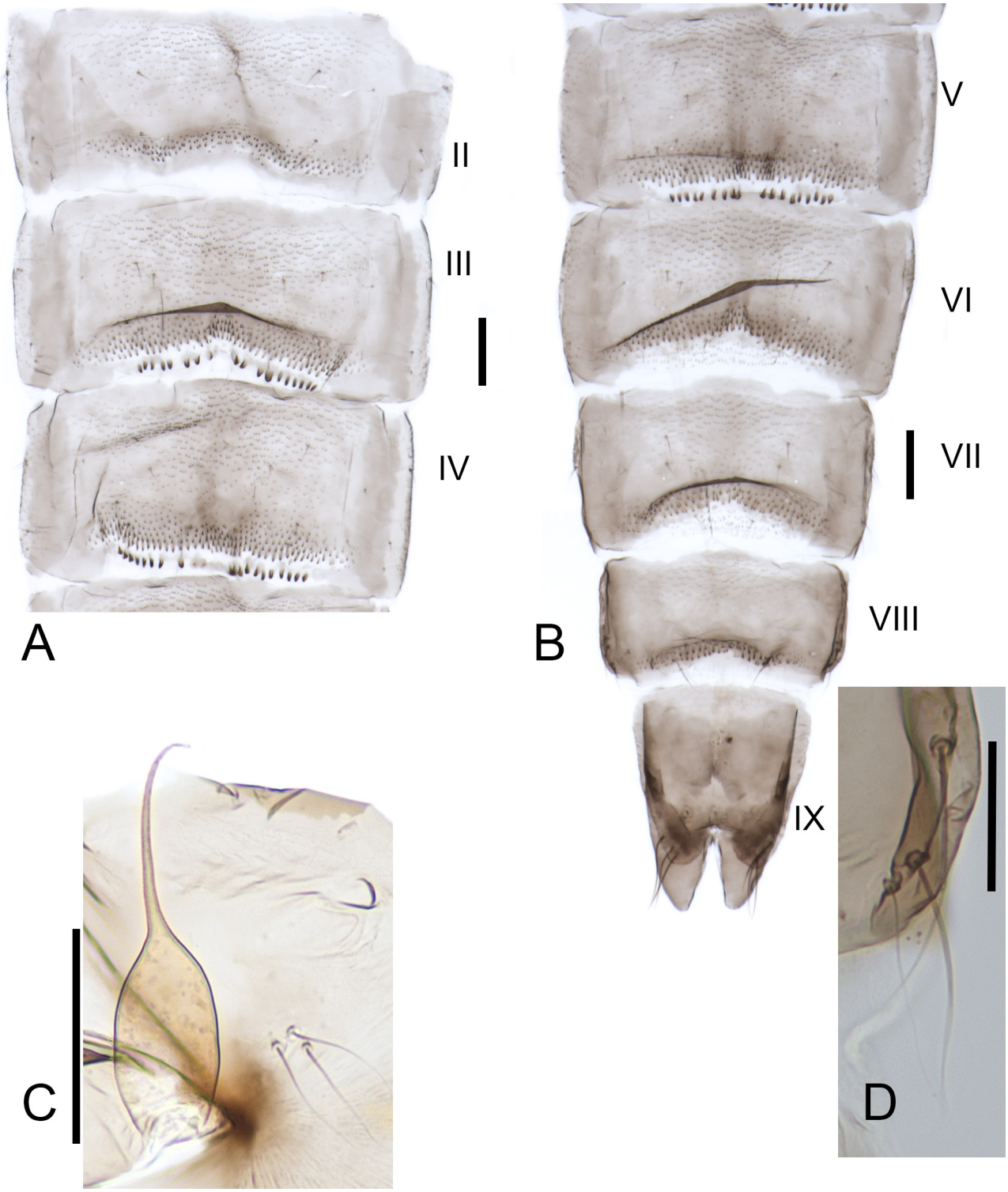

FIGURE 9. *Eukiefferiella viljamii* **sp. nov.**, male exuvia (A–D). A. Tergites II–IV. B. Tergites V–IX. C. Thoracic horn. D. Lateral setae of tergite VIII (paratype NVO.chiro544, scale bars: A, B 75 µm, C 70 µm, D 30 µm).

TABLE 3. Ten *Eukiefferiella* taxa and their average intraspecific distances, and average and minimum interspecific distances (K2P) to their nearest genetic relatives.

Taxon	Average intraspecific distance (%)	Nearest relative	Average interspecific distance (%)	Minimum interspecific distance (%)
E. brevicalcar	0.56	*E. dittmari*	17.2	15.0
E. claripennis	0.56	*E. dittmari*	15.6	14.1
E. devonica	0.00	*E. ilkleyensis*	13.0	12.8
E. dittmari	0.63	*E. devonica*	11.4	10.3
E. gracei	0.13	*E. viljamii* **sp. nov.**	17.9	17.5
E. ilkleyensis	0.01	*E. devonica*	13.0	12.8
E. minor	0.45	*E. paasivirtai* **sp. nov.**	15.4	14.8
E. paasivirtai	0.07	*E.* sp.	9.6	8.6
E. sp.	0.17	*E. paasivirtai* **sp. nov.**	9.6	8.6
E. viljamii **sp. nov.**	0.15	*E.* sp.	10.1	9.1

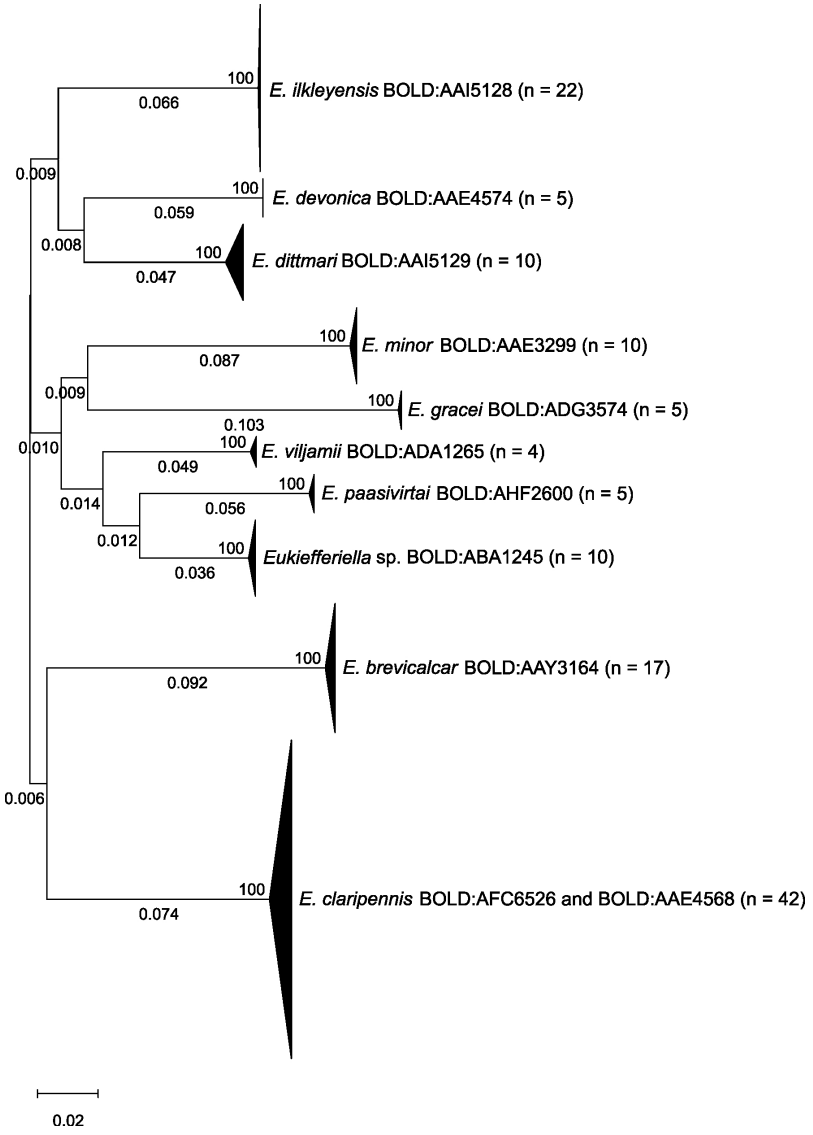

FIGURE 10. *Eukiefferiella* taxon tree (test: Neighbor-Joining, NJ), based on specimens (n = 130) of ten different taxa, inferred from the COI nucleotide sequence data (654 base pairs). Numbers on branches represent the bootstrap value for NJ, respectively (values < 95 omitted). Taxon tree nodes with < 1 % distance are collapsed to make the tree more readable.

A key to the pupal exuviae of the northern European *Eukiefferiella* species[1]

1. Thoracic horn absent; lateral setae on VIII short and thin; posterior teeth of tergite III 20–30 µm long . . . . . *E. dittmari* Lehmann, 1972

— Thoracic horn present (but see note in couplet 5); if all lateral setae on VIII short and thin then posterior teeth of tergite III 2–13 µm long 2

2. Hook rows on tergites continuous. Lateral setae of segment VIII of either all narrow (Fig. 9D) or one of them long, filamentous or stiff 3

— Hook rows on tergites broken medially; if continuous, then two lateral setae of segment VIII sturdy (Fig. 5) 8

3. Thoracic horn short (40–165 µm); apical filament, if present, shorter than or as long as the base 4

— Thoracic horn long (230–680 µm); apical filament longer than the base 6

4. Thoracic horn shorter (40–45 µm long); smooth, thin-walled and sack-like; without apical filament. Lateral seta of VIII short and narrow (Lehmann 1972: Fig. 29) . . . . . *E. coerulescens* (Kieffer, 1926)

— Thoracic horn longer (93–165 µm long), thicker-walled and not sack-like; with small apical filament. Segment VIII with one long, thick lateral seta and another that is finer and shorter 5

5. Thoracic horn distally with obvious short spinules. Apical filament of thoracic horn shorter than the base (Lehmann 1972: Fig. 37). Sternite VII without a posterior transverse row of hooks . . . . . *E. ilkleyensis* (Edwards, 1929)

— Thoracic horn distally without obvious short spinules. Apical filament of thoracic horn longer than or as long as the base (Lehmann 1972: Fig. 32). Sternite VII usually with a posterior transverse row of hooks. . . . *E. devonica* (Edwards, 1929)[2]

6. Thoracic horn base swollen, obviously distinct from the apical filament (Lehmann 1972: Fig. 44; as *E. potthasti*); tergites III–VI medially bare. . . . . *E. gracei* (Edwards, 1929)

— Thoracic horn base contracted, not so obviously distinct from the apical filament; tergites III–VI medially with shagreen points 7

7. Tergites III–IV with long posterior spines of 35–53 µm long (Lehmann 1972: Figs. 41–42). . . . *E. minor* (Edwards, 1929)

— Tergites III–IV with short posterior teeth of 7–10 µm long (Lehmann 1972: Figs. 18–19) . . . *E. cyanea* Thienemann, 1936

8. Segment VIII with all posterolateral setae short and fine (Fig. 9D) 9

— Segment VIII with two strong posterolateral setae (Fig. 5D) 10

9. Anal macroseta of anal lobes absent (Langton & Visser 2003: Fig. 50); thoracic horn less than 150 µm long . . . . . *E. ancyla* Svensson, 1986

— Anal macroseta of anal lobes present (Fig. 9B); thoracic horn > 150 µm long. . . . . ***E. viljamii* sp. nov.**[3]

10. Tergites II–VIII with a single row of dark strong teeth (III–IV 23–25 µm long) posteriorly. Thorax granulate (Lehmann 1972: Fig. 17). . . . . *E. clypeata* (Kieffer, 1923)

— Tergites II–VIII without a single row of dark strong teeth posteriorly. Thorax smooth 11

11. Thoracic horn long (290–400 µm), often with dark apical filament. Hook rows usually continuous (Lehmann 1972: Fig. 5) . . . . . *E. brevicalcar* (Kieffer, 1911)

— Thoracic horn short (160–290 µm). Hook rows with median gap. 12

12. Thoracic horn with apical filament shorter than or as long as the base (ratio of filament / base ≤ 1.0) (Lehmann 1972: Fig. 9) . . . . . *E. claripennis* (Lundbeck, 1898)

— Thoracic horn with apical filament longer than the base (ratio of filament / base > 1.2). 13

13. Tergites II–V with shagreen restricted to an anterior transverse band; thoracic horn base obviously expanded (Fig. 5) . . . . . ***E. paasivirtai* sp. nov.**[4]

— Tergites II–V nearly covered with small points; thoracic horn base not very expanded, narrowed to the origin of the apical filament (Lehmann 1972: Fig. 13) . . . . . *E. lobifera* Goetghebuer, 1934

A key for adult males of *Eukiefferiella gracei* group of species worldwide

1. Gonostylus in dorsal view broadest before midsection[5]. Inner section of gonostylus densely with short setae (Fig. 3–4, 7–8, 11) . . . . . *gracei* group 2

— Gonostylus in dorsal view broadest at mid or beyond midsection. Inner section of gonostylus not densely with short setae . . . . . other *Eukiefferiella* species

1 Pupal exuviae of *E. boevrensis* Brundin, 1956 is unknown

2 Thoracic horn of *E. devonica* is easily broken off and usually missing, whereas that of *E. ilkleyensis* is rarely lost. *E. devonica* may thus be confused with *E. dittmari* but is separable by having one long, thick lateral seta and another that is finer and shorter on VIII. Lateral setae of segment VIII of *E. dittmari* are all short and thin.

3 *E. viljamii* is similar to *E. pseudomontana* Goetghebuer, 1935 (central and southern Europe) as pupae but can be distinguished from this species by the smaller number of hooks on tergites (III–V: 10–13, 10–12 and 11–12 vs.16–19, 18 and 15–18 of *E. viljamii*) and by the presence of longer posterior teeth (13–17 µm vs. 2–5 µm of *E. viljamii*) on abdominal tergites III–IV.

4 Female exuviae of *E. paasivirtai* usually have longer apical filaments of thoracic horn than males.

5 Dorsal view of gonostylus means that it is oriented towards the inferior volsella or at a right angle in relation to the longitudinal axis of the specimen cf. Figs. 3–4.

2. Wing vein C not or only slightly extended beyond R_{4+5} end (0–5 µm). Wing vein R_{4+5} end opposite or beyond M_{1+2} end. Wing length 2.0–3.5 mm. . . . . *E. gracei* (Edwards, 1929)

— Wing vein C distinctly extended beyond R_{4+5} end (40–115 µm, Fig. 1B). Wing vein R_{4+5} end before M_{1+2} end. Wing length 1.4–2.0 mm. 3

3. Eyes pubescent or hairy. Wing FCu fork much distal of RM (VRCu 1.47–1.50; Brundin 1956: Fig. 7) . . . . . *E. boevrensis* Brundin, 1956

— Eyes bare. Wing FCu fork moderately distal of RM (VRCu 1.25–1.36). 4

4. Antenna AR 0.5–0.6. Inferior volsella apical length/gonostylus length 0.31–0.34. Inferior volsella distinctly curved (Figs. 3–4) . . . . . ***E. paasivirtai* sp. nov.**

— Antenna AR 1.1–2.4. Inferior volsella apical length/gonostylus length 0.43–0.50. Inferior volsella conical not or only slightly curved. 5

5. Antenna AR 2.2–2.4. Inferior volsella length/width 1.2–1.4. . . . . *E. brevinervis* (Malloch, 1915)

— Antenna AR 1.1–1.2. Inferior volsella length/width 1.0–1.2 (Figs. 7–8) . . . . . ***E. viljamii* sp. nov.**

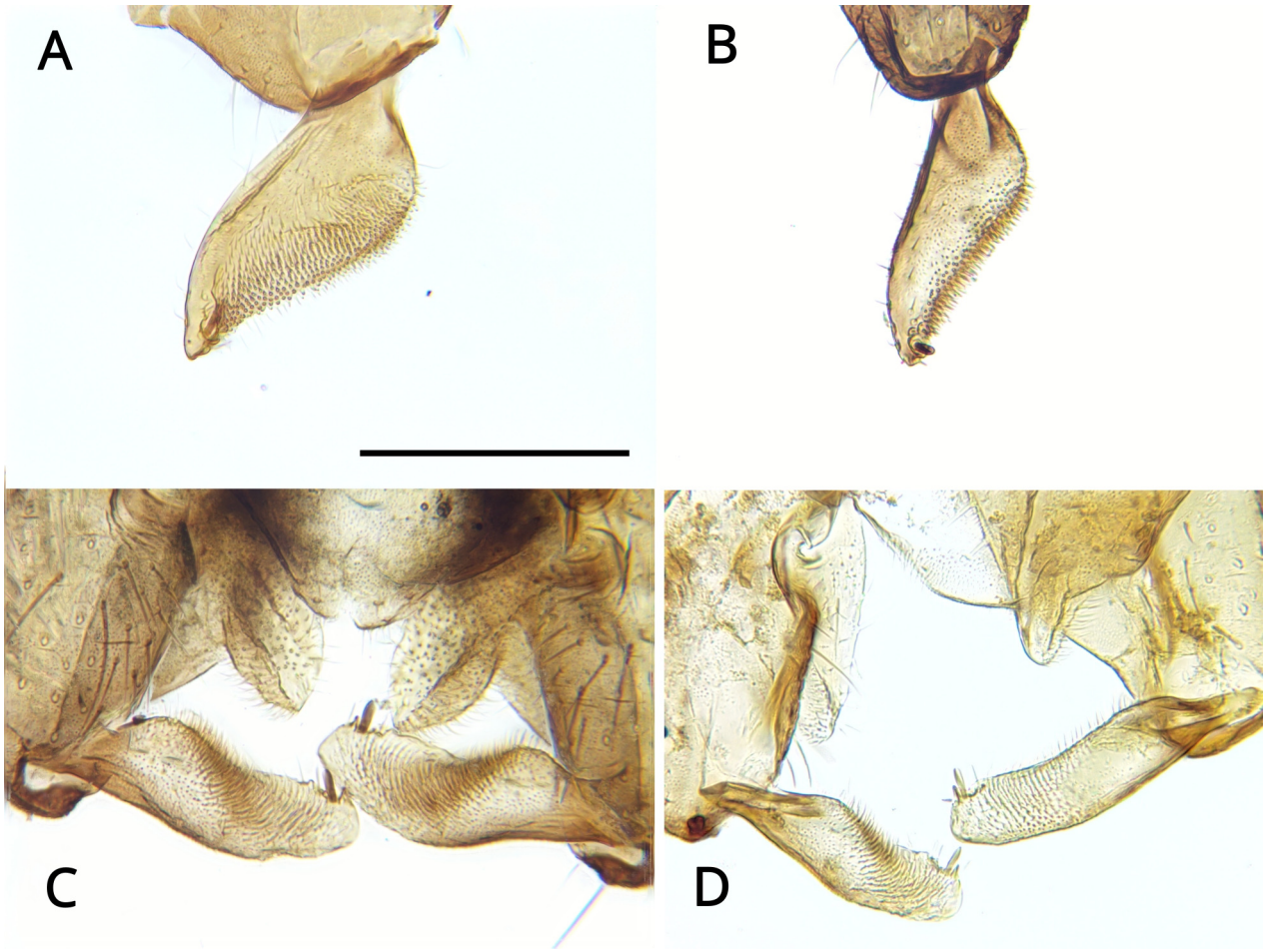

FIGURE 11. Gonostylus of *Eukiefferiella gracei* (A–D). A and B. Lateral view. C and D. Dorsal view. Scale bar: A–D 100 µm.

Discussion

Prior to this study, nine species of *Eukiefferiella* were known from Finland (Paasivirta 2014). Our findings raise this number to 11 in Finland and to 16 in the Nordic countries Finland, Norway and Sweden. At the European scale, the known diversity increases from 23 to 25 species.

Pupal exuviae and the adult male wings of the two new species place them in the *claripennis* group sensu Lehmann (1972), whereas the morphology of the adult male gonostylus places them in the *gracei* group. Barcoding results supported the latter view, as the new species were grouped into the same cluster as *E. gracei*. However, both new species have their closest genetic relative in North America, although even the minimum interspecific distance to the nearest neighbor species was as high as 8.6 % and 9.0 %, respectively. ASAP analysis supported

the morphological species delimitations, with a 4.5 % threshold distance separating the ten *Eukiefferiella* taxa, thus being clearly lower than any of the minimum pairwise interspecific distances between the taxa. There is no generally agreed upon barcode distance for species separation of *Eukiefferiella*, or Chironomidae or other Diptera, but it has been suggested e.g. by Lin *et al*. (2015) and Brodin (2025) that 3–5 % interspecific distance might be enough to separate species of Chironomidae. Our results are thus in accordance with the previous studies discussing the threshold distance among Chironomidae species.

Morphological species identification of Chironomidae is often challenging, particularly in the species-rich Orthocladiinae genera such as *Eukiefferiella* (Lehmann 1972), *Orthocladius* (Soponis 1977) and *Cricotopus* (Hirvenoja 1973). Male hypopygium characters, particularly the anal point, inferior volsella and gonostylus, are usually the most useful for species identification (e.g. Pinder 1978, Sasa & Kikuchi 1995, Langton & Pinder 2007). In *Eukiefferiella* all species lack an anal point (Cranston *et al*. 1989, Sæther *et al*. 2000), making the inferior volsella and gonostylus crucial characters. The naturally highly variable orientation of the gonostylus may cause difficulties in interpretation. In the key to the adult males above, we rely on the gonostylus in a dorsal view, oriented towards the inferior volsella or at a right angle in relation to the longitudinal axis of the specimen. This gonostylus orientation is usually adopted to distinguish species of *Eukiefferiella* in taxonomic literature (Lehmann 1972, Sasa & Kikuchi 1995, Langton & Pinder 2007), not denying that other orientations of the gonostylus with the lateral side turned upwards might render interesting morphological information (Prat 1979, Moubayed-Breil & Ashe 2015).

Eukiefferiella paasivirtai is currently known only from Finland, occurring in three geographical regions in the northern part of the country (*E.* probably *gracei* in Paasivirta 2014). *E. viljamii*, on the other hand, has only been recorded from the River Vetsijoki in far northern Finland and from Finnmark in northernmost Norway. Both species appear to inhabit flowing water, as other species of *Eukiefferiella* (Cranston *et al*. 1989). *E. paasivirtai* was found in both slow-flowing and riffle sections, whereas *E. viljamii* was collected from a shallow riffle with relatively high flow, suggesting more rheophilic habitat preferences. Swarming of *E. viljamii* was observed above exposed river boulders near the shoreline during period of low summer water levels. At present, the known distribution of both species is restricted to northern Fennoscandia, and the available evidence suggests that *E. viljamii* in particular is confined to subarctic river ecosystems.

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Supplementary Materials. The following supporting information can be downloaded at the DOI landing page of this paper:

Supplementary Data. *Eukiefferiella* taxa and the sequences used in molecular analysis.