Zootaxa 5419 (2): 245–264
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5419.2.4
http://zoobank.org/urn:lsid:zoobank.org:pub:2091D14E-FA61-417B-8FD9-FAA4994C0563

Parachaetocladius lenferringtoni (Diptera: Chironomidae), a new orthoclad from North America, with keys to adult males and pupae of the Nearctic *Parachaetocladius* (Wülker, 1959)

R. WILLIAM, BOUCHARD, JR.[1,*], ARMIN NAMAYANDEH[2,3] & PATRICK L. HUDSON[4]
[1]*Department of Entomology, University of Minnesota, 1980 Folwell Avenue, St. Paul, MN 55108, USA*
bouc0048@umn.edu; https://orcid.org/0000-0001-9073-8364
[2]*Department of Environmental and Life Sciences, Trent University, 1600 West Bank Drive, Peterborough, ON, Canada*
a.namayan@taxanama.com, arminnamayandeh@trentu.ca; https://orcid.org/0000-0003-2136-0497
[3]*Department of Physiology, School of Medicine, Wayne State University, Detroit, MI, USA, 4820*
[4]*5597 New Meadow Drive, Ypsilanti, MI, USA, 48197*
patrick_hudson@sbcglobal.net; https://orcid.org/0000-0002-7646-443X
**Corresponding author*

Abstract

In this study, we describe *Parachaetocladius lenferringtoni* **sp. nov.**, based on all its life stages, and provide keys to adult males and pupae of the Nearctic *Parachaetocladius*. The new species was originally placed in *Parachaetocladius abnobaeus* (Wülker 1959) due to the similarity in the hypopygium of these species. However, a detailed examination of adults and associated immature specimens demonstrates that *P. lenferringtoni* **sp. nov.** represents a single species that is different from *P. abnobaeus*. Diagnostic adult and pupal characters were identified to separate this new species from *P. abnobaeus* and other species of *Parachaetocladius*. The separation of the larva of *P. lenferringtoni* **sp. nov.** and *P. abnobaeus* is currently tenuous, but some possible diagnostic characters are provided. Material of *P. lenferringtoni* **sp. nov.** was obtained for this study from Canada (Manitoba) and the United States of America (Kansas, Minnesota, Nebraska, and Wisconsin), indicating this species is largely limited to central North America. The habitat of this new species matches that of other *Parachaetocladius* species in that it has been collected from sandy substrates in springs and groundwater-dominated streams.

Key words: *Parachaetocladius*, Nearctic, new species, life stages, Kansas, Manitoba

Introduction

The genus *Parachaetocladius* Wülker, 1959 was reviewed and revised by Sæther & Sublette (1983). They placed this genus along with *Doithrix* Sæther & Sublette, 1983, *Georthocladius* Strenzke, 1941, and *Pseudorthocladius* Goetghebuer, 1943 in a monophyletic group. Namayandeh *et al.* (2020) reviewed *Parachaetocladius* and examined the type and voucher specimens (i.e., P.L. Hudson personal collection and Canadian National Collection (CNC) materials) originally assigned as *Parachaetocladius hudsoni* (Sæther 1977a), now a junior synonym of *Parachaetocladius abnobaeus* (Wülker 1959; see Cranston & Oliver 1988). Among these, they examined a specimen of an adult male from Kansas and an adult male specimen from Manitoba, Canada, which was noticeably different from *P. abnobaeus*. At the time, the authors considered the most distinguishing characteristic of these adult males to be the absence of an inferior volsella and the shape of the sternapodeme. However, Namayandeh *et al.* (2020) did not describe these specimens as a new species because they noted that additional material should be examined. Independently, collections of pupal exuviae by L. C. Ferrington, Jr. and R. W. Bouchard Jr. in Minnesota produced a pupa that, based on several characteristics, was a separate species from *P. abnobaeus*. Based on a detailed examination of adults and associated immature specimens from Canada (Manitoba), and the United States of America (Kansas and Minnesota), we concluded that this material represents a single species that is different from *P. abnobaeus*. A further examination of additional adults and immatures from Nebraska and Wisconsin (USA) in comparison to *P.*

Accepted by B. Rossaro: 30 Jan. 2024; published: 6 Mar. 2024

abnobaeus specimens (including the Holotype of *Habrobaenus hudsoni* = *P. abnobaeus*) from Alabama, Michigan, Pennsylvania, Tennessee, and South Carolina (USA), and Ontario and Nova Scotia (Canada) revealed additional distinguishing characteristics between the two species. We here describe *Parachaetocladius lenferringtoni* **sp. nov.**, based on all its life stages, and provide keys to adult males and pupae of the Nearctic *Parachaetocladius*.

Methods, materials, and terminology

Specimens collected by the authors were preserved in 75% ethanol and slide mounted in Euparal following the procedures described by Pinder (1986), Langton & Pinder (2007), and Andersen *et al.* (2013). Specimens obtained from other collections were preserved and slide-mounted using various methods. We photographed adult specimens using a Spot 5.1 camera (Diagnostic Instruments Inc., USA) and focus stacking was performed using CombineZP (Hadley 2010). Illustrations for the adults were produced in Inkscape 1.2.2 (Inkscape 2022) from specimen photographs. The pupal and larval illustrations were created using a drawing tube and inked using technical pens. We photographed pupal and larval specimens of *P. lenferringtoni* **sp. nov.** using a Leica ICC50W digital microscope camera (Leica Microsystems, Germany) and focus stacking was performed using Zerene Stacker 1.04 (Zerene Systems LLC, USA).

Morphological nomenclature follows that of Sæther (1977b, 1980a). The following abbreviations were used: Adults: AR (antennal ratio) = length of 11th flagellomere/length of flagellomeres 1–10; VR (venarum ratio) = length of cubitus (Cu)/length of media (M); BV = length of (femur + tibia + ta_1)/length of (ta_2 + ta_3 + ta_4 + ta_5); LR (leg ratio) = length of ta_1/length of tibia; SV = length of (femur + tibia)/length of ta_1; HR (hypopygium ratio) = length of gonocoxite/length of gonostylus; HV (hypopygium value) = total length (TL)/length of gonostylus times ten; P_1 = fore leg; P_2 = mid leg; P_3 = hind leg; fe = femur; ti = tibia; ta_1–ta_n = 1st tarsus–nth tarsus; R = radius; R_1 = radius 1 vein; R_{4+5} = radius 4+5 vein; WL (adult wing length) = length from arculus to apex of wing. Pupae: Pc = precorneal setae; Dc = dorsocentral setae; AM = anal macrosetae. Larvae: AR (antennal ratio) = length of antennal segment 1/length of flagellum (remaining antennal segments). Measurements and counts are provided in the following form: minimum-maximum, mean.

Locations and depositories of specimens are as follows: Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Canada (CNC); Snow Entomological Museum Collection (SEMC); University of Minnesota Insect Collection, St. Paul, Minnesota, USA (UMSP); Zoological Museum University of Oulu, Finland (ZMUO); private collection of Dean C. Hansen, Stillwater, Minnesota, USA (DCH); private collection of Patrick, L. Hudson, Ypsilanti, Michigan, USA (PLH); private collection of R.W. Bouchard, Jr., Circle Pines, Minnesota, USA (RWB); private collection of Thomas Bendt, Heyerhütte, Germany (TB); and private collection of Peter H. Langton (PHL). We used the keys provided by Namayandeh *et al.* (2020), Sæther & Sublette (1983), and Makarchenko and Yavorskaya (2021) with modifications and corrections to produce our keys.

Taxonomy

Genus *Parachaetocladius* Wülker

Adults and immatures as diagnosed by Wülker (1959), Sæther & Sublette (1983), and Namayandeh *et al.* (2020), with the following corrections: Adult male without virga; the posteromedial meeting point of the gonocoxites (i.e., penis cavity) with strong sclerotization; the apex of aedeagal lobes with well-developed sclerotization appearing as lateral sclerites below sternapodeme; inferior volsella blunt, lobed, or digitiform. Pupa with sensillum coeloconica located on a low mound with the lateral antepronotal; 3 short anal macrosetae (AM) which are subequal or one AM shorter than the other 2, AM may or may not bear denticles.

Parachaetocladius lenferringtoni sp. nov., Bouchard & Namayandeh

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Figs. 1–4; 6A, C, G, K; 7A–D; 8A–B; 9A, C, E–F

Type material. *Holotype:* Male; USA, Kansas, Kiowa County, Rezeau Ranch, channel of spring that feeds pond #6, R16W, T295, Sec 15, 5 ½, 19.iii.1982, leg. J.K. Gelhaus, deposited at SEMC.

Paratypes: 1 male and 1 female; Canada, Manitoba, Duck Mountains, South Duck River, 16.v.1980, leg. F.R.B. Winnipeg, Det. DRO 86, CH 5056, deposited at CNC. *Paratype*: 1 pupa, USA, Kansas, Kiowa County, Rezeau Ranch, channel of spring that feeds pond #6, R16W, T295, Sec 15, 5 ½, 19.iii.1982, leg. B.G. Coler and J.K. Gelhaus, deposited at SEMC. *Paratype*: 1 male; USA, Kansas, Scott Co., State Lake, Big Springs at County State Lake, 13.3 min N., 0.6 min W of Fort Scott, 14.iii.1984, leg. L.C. Ferrington Jr., deposited at CNC. *Paratypes*: 2 females, 2 pupae, and 1 larva, same data as previous specimen except deposited at SEMC. *Paratypes*: 2 males; USA, Minnesota, Dakota County, Pine Creek; Upstream @80 St. E ~ 2 min N of Cannon Falls, 44.5435º, -92.9003º, 24.iv.2003, leg. R.W. Bouchard Jr., deposited at UMSP. *Paratype*s: 6 males, 1 female; USA, Minnesota, Dakota County, Trout Brook, Miesville Ravine Park Reserve, upstream of 280th Avenue, 9.2 km northeast of Cannon Falls, 44.5453º, -92.8057º, 8.v.2008; leg. R.W. Bouchard Jr., deposited at UMSP. *Paratypes*: 1 female, 1 pupae, same data as previous specimen except collection date is 10.iv.2023. *Paratypes*: 2 pupae and 2 larvae, same data as previous specimens except collection date is 10.iv.2023 and deposited at RWB. *Paratype*: 1 pupa, same data as previous specimens except collection date is 10.iv.2003 and deposited at RWB. *Paratype*: 1 pupa, USA, Minnesota, Scott County, Eagle Creek, upstream of 125th Street, ~2 mi East of Savage, 44.7752º, -92.3855º, 27.iii.2003, leg. R.W. Bouchard Jr., deposited at RWB. *Paratype*: 1 male; USA, Minnesota, Washington Co., Valley Creek, Belwin Preserve, 300 m downstream of Stagecoach Trail; 2 km SW of Lakeland; elevation 344 m, 44.9192º, -92.7988º, 8.v.2008, leg. L.C. Ferrington Jr., deposited at UMSP. *Paratype*: 2 pupae, USA; Minnesota, Washington County, Valley Creek, Belwin Conservancy, 44.9188º, -92.8006º, 24.iv.2002, leg. L.C. Ferrington Jr., deposited at RWB. *Paratype*: 2 pupae, USA, Nebraska, Garden County, headwater spring of Blue Creek, upper end, 18 mi North and 1 mi West of Oskosh, 23.iv.1991, M.A. Blackwood and L.C. Ferrington Jr., deposited at UMSP. *Paratype*: 2 pupae, USA, Wisconsin, Burnett Co., Spring Brook, small first-order, summer-cool stream, UTM 15T 0566695E 5063330N, rift net, 11.v.2014, leg. D.C. Hansen, deposited at DCH. *Paratype*: 3 larvae, USA, Wisconsin, Buffalo County, unnamed tributary (1781700) to Traverse Valley Creek, Pape Valley Road, 10 m upstream from the confluence with Traverse Valley Creek, 2-xi-2021, leg. K. Rasmussen, deposited at UMSP.

Etymology. The new species is named in honor of the late Leonard C. Ferrington Jr., who originally collected the species in Kansas, USA, and who also collected specimens from Minnesota, USA.

Diagnosis. *Parachaetocladius lenferringtoni* **sp. nov.** can be distinguished from other *Parachaetocladius* species based on a combination of the following characteristics. Adult male with AR 0.82–0.98, 0.91; clypeus appearing hexagonal with lateral edges and the base curved; wing's squama with 11–15, 14 setae, R_{4+5} with 0–7, 2 setae; anal point shallow and cone-shaped; sternapodeme slightly arched, oral projections thick; inferior volsella blunt, gonostylus well expanded in mid to anterior section with crista dorsalis developed, stretching from mid to anterior sections, but visible at certain orientations. Adult female with AR 0.42–0.48, 0.46; clypeus appears hexagonal with lateral edges and the base curved; the apodeme lobe indistinct, however, large; notum 127–134, 130 µm long; tergite IX not divided, though a moderate mid-section notch is present, crescent-shaped. Pupa with 10–18 well-developed spines in posterior spine row on sternite III; anal lobes posteriorly extended into slender tips; a row of blunt spines posterolaterally on anal lobes and extending onto tips; inner macrosetae smaller than other two, denticles on anal macrosetae usually absent. Fourth instar larva with postmentum 167–185, 179µm long; antenna 6 segmented with last segment vestigial, AR 1.1–1.5, 1.3, blade 1.4–1.7, 1.5 × the length of antennal segments; anal tubules with one or two constrictions 0.6–0.8, 0.6 × the length of the posterior parapods.

Description

Fig. 1A–G; 6A, C, G, K

Male (n = 12, unless otherwise stated)

Total length 3.1–4.1, 3.6 mm. Wing 1.6–2.1, 1.9 mm long, and 0.43–0.60, 5.3 mm wide (n = 11).

Coloration of mounted specimen. Head, thorax, legs, and abdomen dark brown. Wing and halters light to golden-brown

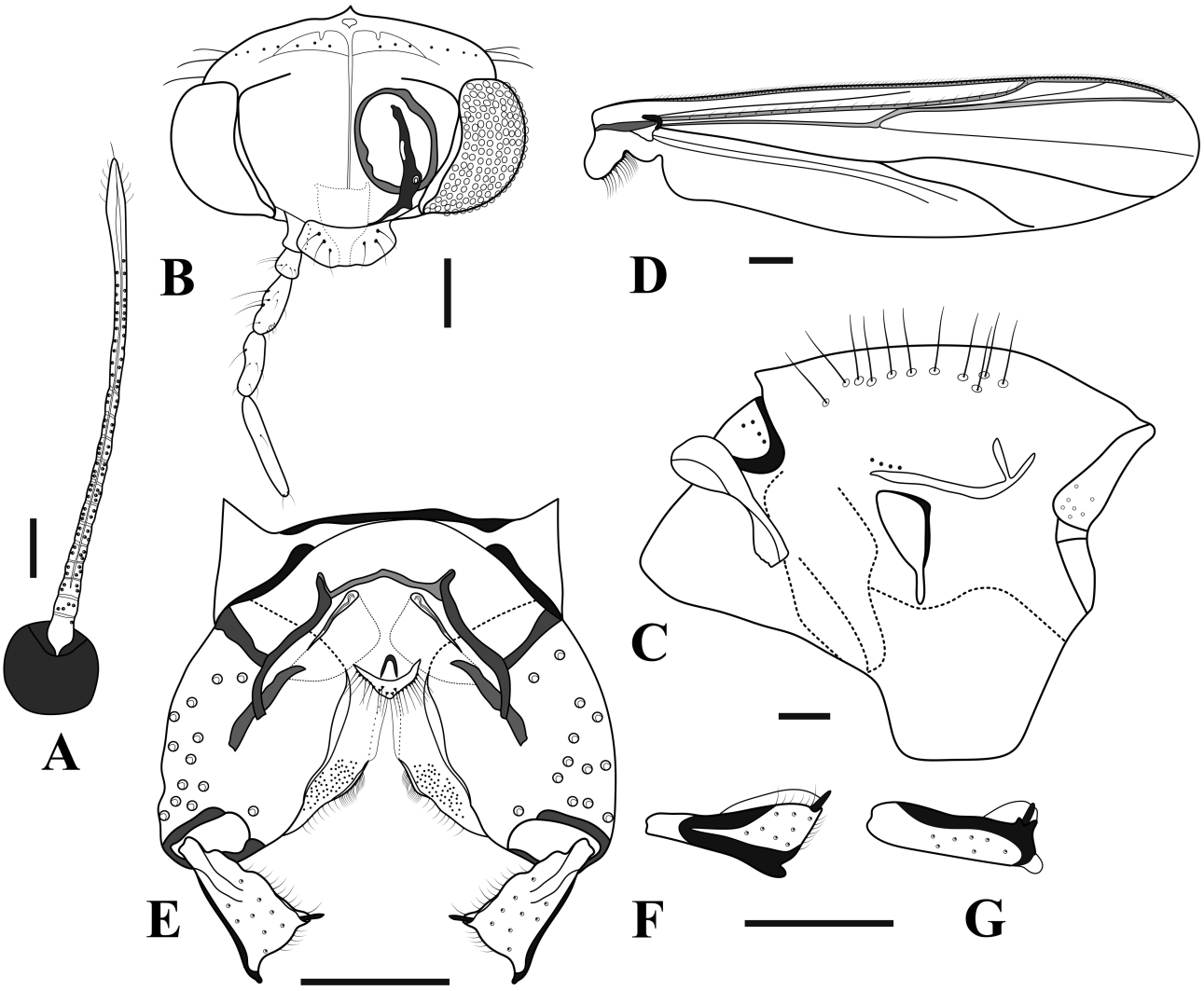

FIGURE 1. *Parachaetocladius lenferringtoni* **sp. nov.**, male (A–G). A. antenna. B. head; C. thorax; D. wing; E. hypopygium. F. gonostylus variation. G. gonostylus variation. Scale bars are 100 µm.

Head. Antenna with 13 flagellomeres, last flagellomere with 6–10, 8 sensilla chaetica, 2nd–3rd segments each with 2 sensilla chaetica (n = 10), groove starts at 3rd segment (Figs. 1A; 6A); AR 0.82–0.98, 0.91 (n = 10). Eyes bare, with very weak wedge-shaped dorsomedial extension. Temporal setae 7–11, 10, including 6–8, 7 inner verticals, 1–4, 3 outer verticals (n = 10). Tentorium with large tentorial pit close to midsection (Fig. 1B), tentorium 150–203, 181 µm long (n = 11). Clypeus appears hexagonal with lateral edges and the base curved (Figs. 1B; 6K), 58–72, 67 µm long and 125–150, 141 µm wide, bearing 4–8, 6 setae, setae 49–85, 60 µm long (n = 10). Palpal segments lengths (in µm, n = 9): 51–65, 56; 33–55, 45; 79–114, 93; 90–107, 93; 117–175, 137. Third palpomere with 1 sensilla clavata.

Thorax (Fig. 1C). Dorsocentrals 8–19, 13 in a mostly single row, prealars 4–5, scutellars 8 in double rows. Humeral pit absent. Antepronotal lobes with gap, bearing 5–11, 7 setae ventrally; antepronotal lobe L/W 2.2–5.6, 3.6.

Wing (Fig. 1D). Brachiolum with 1–2 setae. Squama with 11–15, 14 setae. R with 12–18, 14 setae; R_1 with 5–11, 8 setae; R_{4+5} with 0–7, 2 setae; other veins bare. Costa not extended. VR 1.0–1.2. Microtrichia visible at 10 x magnification.

Legs. Mid and hind femur with keel. Mid and hind tibia with long sparse beard. Pulvilli present. Fore tibia spur 45–59, 53 µm long; mid tibia spurs 35–45, 40 and 30–46, 36 µm long; hind tibia spurs 55–69, 61 and 27–50, 35 µm long; hind tibia comb with around 11–17, 14 spines, hind comb 34–66, 52 µm long. Pseudospurs present on tarsal segments 1–2 of the mid and hind legs. Lengths and proportions of legs as in Table 1.

TABLE 1. Male leg lengths (μm) and proportions of *Parachaetocladius lenferringtoni* **sp. nov.**

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV
P_1	784	920	565	331	225	134	107	0.61	2.9	3.0
P_2	798	875	310	191	132	92	96	0.35	3.9	5.4
P_3	883	1008	528	318	216	113	99	0.52	3.2	3.5

Hypopygium (Fig. 1E). Anal point short and cone-shaped, wide at the base, bearing 20 moderately thick setae; anal point 13–23, 20 μm long, 43–77, 61 μm wide at the base and 18–40, 30 μm wide medially. Virga absent. Two diagonal, lateral sclerites present at the apex of the aedeagal region, just below the sternapodeme. Penis cavity well-sclerotized and appearing as a bifid arched sclerite. Sternapodeme slightly arched, appearing narrow, oral projections thick (Figs. 1E; 6C), sternapodeme 90–127, 109 μm long. Phallapodeme 74–93, 86 μm long. Inferior volsella blunt (Figs. 1E; 6G). Gonostylus well expanded in mid to anterior section with outer corner or projection; crista dorsalis developed, stretching from mid to anterior of gonostylus but only visible at certain orientations (Fig. 1F–G); gonostylus 100–134, 118 μm long. Gonocoxite 202–230, 220 μm long. HR 1.6–2.1, 1.9, HV 2.6–3.6, 3.1.

Female (n = 4, unless otherwise stated)

Fig. 2A–E

Total length 3.5–4.0, 3.7 mm. Wing 1.9–2.2, 2.0 mm long and 0.62–0.74, 0.69 mm wide.

The coloration of mounted specimen. Head, thorax, halter, and abdominal tergites dark brown. Legs, wings and abdominal sternites lighter.

Head (Fig. 2A). Antenna with 6 flagellomeres, last flagellomere with 8 sensilla chaetica, 3rd–5th segments each with 2 long sensilla chaetica (Fig. 2A); antennal segments in μm: 48–73, 62; 58–67, 60; 65–71, 67; 60–72, 65; 55–64, 59; 129–162, 141, AR 0.42–0.48, 0.46. Eyes bare, with short dorsomedial extension. Temporal setae 11–15, 12, uniserial, including 7–11, 8 inner verticals and 3–4 outer verticals (Fig. 2B). Tentorium 133 μm long (n = 3), with small tentorial pit closer to mid-section. Clypeus appears hexagonal with lateral edges and the base curved, 41–78, 61 μm long and 133–151, 140 μm wide, bearing 8–12, 9 setae, setae 35–87, 51 μm long. Palpal segments lengths (in μm, n = 2): 56; 40–41; 83–95, 89; 91–98, 94; 105–129, 117; third palpomere with 1 sensilla clavata.

Thorax (Fig. 2B). Dorsocentrals 8–20, 15 mainly in a single row, prealars 5, scutellars 10–14, 11 in two rows. Antepronotal lobes with gap, bearing 4–5 setae ventrally; antepronotal lobe L/W 4–5.

Wing (Fig. 2C). Brachiolum with 1 seta. Squama with 8–22, 15 setae. R with 19–26, 22, R_1 with 11–16, 13 setae, R_{4+5} with 18–24, 20 setae, other veins bare. Costa not-extended, Anal lobe squared. VR 0.9–1.2, 1.1. Microtrichia visible at 10 x magnification.

Legs. Mid and hind femur with keel. Mid and hind tibia with long sparse beard. Pulvilli absent. Fore tibia spur 47–55, 51 μm long; mid tibia spurs 43–53, 47 and 36–46, 40 μm long; hind tibia spurs 57–77, 69 and 27–49, 37 μm long; hind tibia comb with around 16–18, 17 spines. Lengths and proportions of legs as in Table 2.

Genitalia (Fig. 2D–E). Gonocoxite very long, slightly passing the posterior part of segment IX, bearing close to 20 setae. Seminal capsules large and nearly circular without detectable neck, spermathecal ducts without loops (Fig. 2D), seminal capsule 105–136, 125 μm long, 82–118, 103 μm wide. Notum 127–134, 130 μm long, notum and ramus 206–234, 218 μm long. Gonapophysis VIII divided into large ventrolateral lobe covering the base of smaller dorsomesal lobe. The apodeme lobe indistinct, however, large. Tergite IX not divided, though a slight mid-section notch is present, crescent-shaped, bearing 20–26, 24 setae posteriorly (Fig. 2E). Sternite IX with 13 setae posteriorly. Cercus small (Fig. 2E), 72–107, 92 μm long and 55–87, 67 μm wide.

TABLE 2. Female leg lengths (μm) and proportions of *Parachaetocladius lenferringtoni* **sp. nov.**

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV
P_1	790	865	509	329	222	129	118	0.59	2.7	3.2
P_2	804	865	305	192	136	90	109	0.35	3.7	5.5
P_3	930	1033	528	331	228	112	109	0.51	3.2	3.7

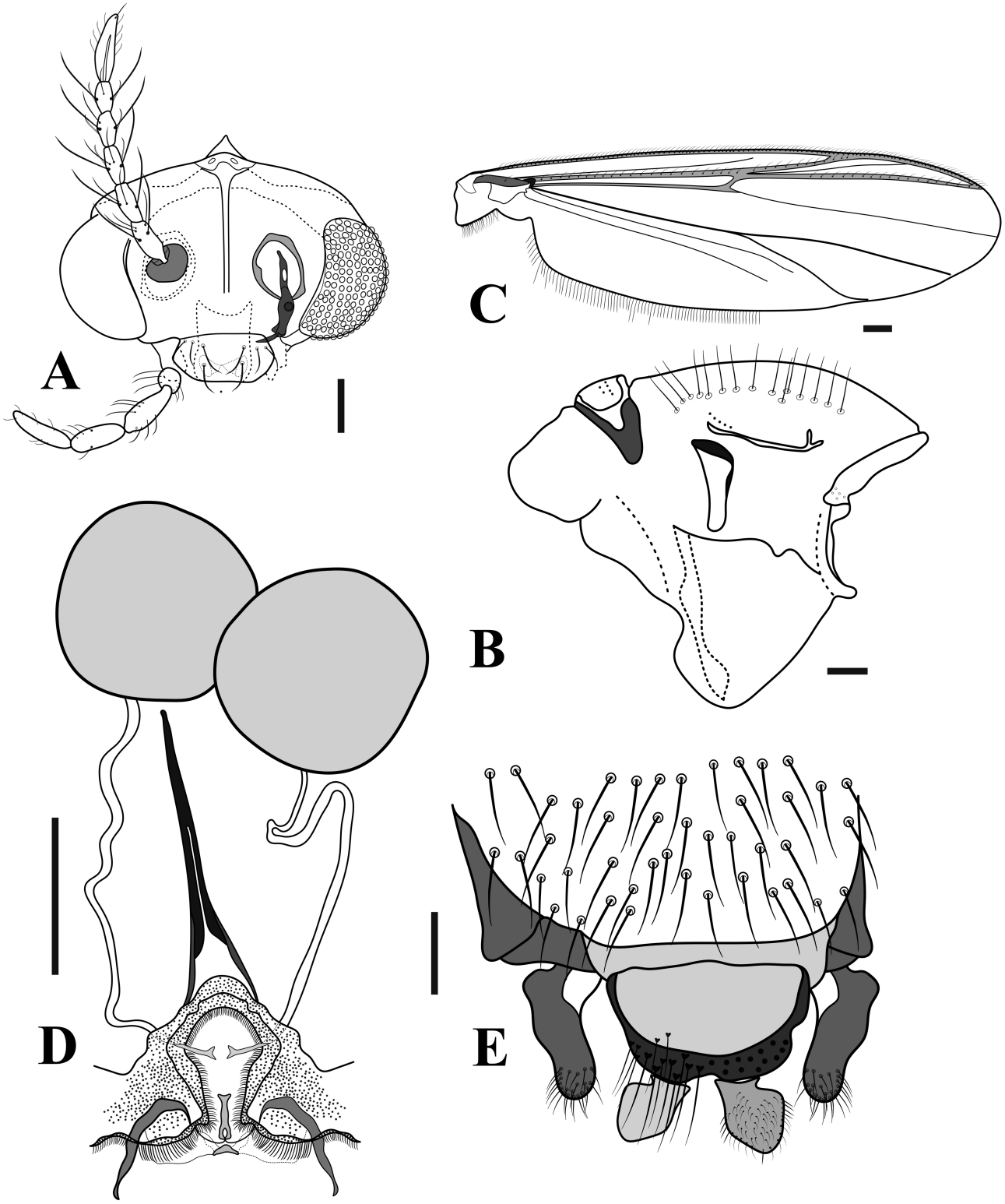

FIGURE 2. *Parachaetocladius lenferringtoni* **sp. nov.**, female (A–E). A. head; B. thorax; C. wing; D. genitalia ventral view; E. genitalia, dorsal view. Scale bars are 100 µm.

Pupa (n = 12, unless otherwise stated)
Figs. 3A–E; 7A–D; 8A–B

Total length 4.4–5.9, 5.3 mm.

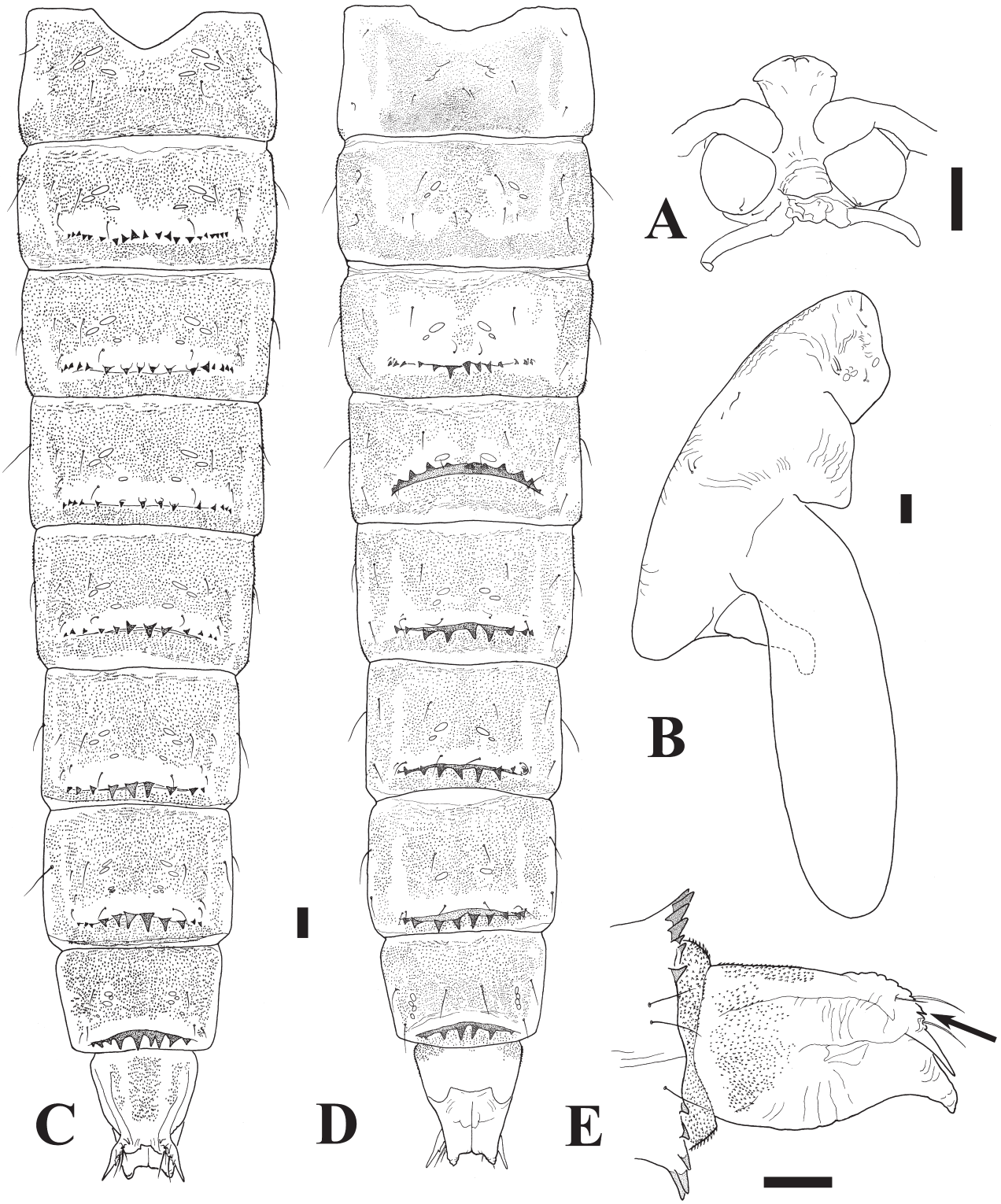

FIGURE 3. *Parachaetocladius lenferringtoni* **sp. nov.**, pupa (A–E), A. frontal apotome; B. thorax; C. abdominal tergites; D. abdominal sternites; E. posterior of tergite VIII and tergite IX, lateral view, arrow indicates the posterolateral blunt spines. Scale bars are 100 μm.

The coloration of the exuvia. Golden-brown

Cephalothorax (Fig. 3A–B). Frontal setae absent. Frontal apotome largely smooth with some wrinkles and low tubercles anteriorly. Ocular field with 2 moderately developed postorbitals; vertical setae absent. Thoracic horn absent. Thorax wrinkled, stronger anteriorly and along ecdysial margin; prealar tubercle asymmetrically quadrate

and wrinkled. Two median antepronotals and 1 lateral antepronotal; sensillum coeloconica located on a low mound with the lateral antepronotal. Three precorneals, in a triangular pattern, Pc_1 50–76, 63 μm long (n = 11), Pc_2 49–86, 64 μm long, Pc_3 39–78, 64 μm long; Dc setae with Dc_1 and Dc2 paired or with Dc_2 equidistant between Dc_1 and Dc_3, Dc_3 and Dc_4 paired, Dc_1 66–85, 78 μm long (n = 10), Dc_2 25–84, 55 μm long, Dc_3 28–79, 60 μm long (n = 11), Dc_4 36–70, 57 μm long (n = 10); supraalars, prealars, and metanotals absent.

Abdomen (Fig. 3C–E; 7A–D; 8A–B). Tergites, sternites, and pleurites smooth. Tergites I–VIII largely covered with well-developed shagreen medially, with bare areas anterior of posterior spine rows and around muscle scars, anterior shagreen fine and in short rows; tergite IX with 1 medial and 2 lateral shagreen patches. Sternites I–II with numerous, weak shagreen points covering much of the surface; sternite III bare posteriorly, with shagreen anteriorly and medially; sternites III–VIII largely covered with well-developed shagreen, although less so than on tergites, with small rows of fine points anteriorly and bare areas anterior of posterior spine rows and around muscle scars; sternite IX largely bare, with small anterolateral patches of shagreen. Pleurite I largely bare except posteriorly; pleurites II–VIII largely covered with shagreen although with some segments with posterior bare areas. All conjunctives bare. Tergites II–VIII and sternites III–VIII (III–VII in females) with a posterior, transverse row of thorn-like spines; posterior tergite and sternite spines triangular and evenly golden-brown throughout, spines becoming larger and generally less numerous on posterior tergites, sternite spines lighter in color than tergite spines. The number of spines in posterior spine rows on the tergites and sternites are as follows: tergite I 0–4, 1 (Fig. 7A), when present, spines consist of enlarged medial shagreen points (Fig. 7C); tergite II 12–23, 18; tergite III 13–24, 17; tergite IV 9–19, 15; tergite V 9–18, 13; tergite VI 6–16, 12; tergite VII 7–17, 11; tergite VIII 6–18, 11; total number of posterior spines on tergites 66–136, 97 (Fig. 3C); sternites I–II none; sternite III 10–18, 14 (Fig. 7B, D); sternite IV 9–15, 12; sternite V 6–13, 10; sternite VI 7–12, 10; sternite VII 6–13, 9; sternite VIII 6–10, 8 (males: n = 5), posterior spine row on sternite VIII absent in females; total number of posterior spines on sternites 49–66, 58 (males: 51–61, 55 (n = 5); females: 49–66, 60 (n = 7); Fig. 3D). Abdominal setation as follows: segment I with 5 D, 3 V, and 1 L setae, L seta strong and 93–121, 1106 μm in length (n = 11); segments II–VII with 5 D, 4 V, and 3 L setae, L setae pale golden, L1 and L3 setae ventral and moderately developed and L2 dorsal and well-developed, L2 71–129, 101 μm in length (n = 11); segment VIII with 3 D, 1 V, and 2 L setae, $L_{1–2}$ setae ventral and moderately developed; no O setae. Anal lobes posteriorly extended into slender tips, tips 31–127, 84 μm long (measured from the base of AM_3; n = 11;); a row of blunt spines posterolaterally on anal lobes and extending onto tips (Figs. 3E; 8A–B); 3 AM, AM_3 smaller than other two (often missing on specimens; Figs. 3C–E; 8A–B), $AM_{1–3}$ 48–61, 54 μm (n = 9); 65–108, 83 μm; 64–98, 77 μm; denticles on anal macrosetae usually absent, if present not abundant and consisting on only 1 or 2 isolated denticles. Male genital sacs subequal to the anal lobe, genital sac 378–449, 413 μm (n = 5) long, anal lobe 354–450, 406 μm long (n = 11).

Fourth instar larva (n = 6, unless otherwise stated)

Figs. 4 A–F; 9A, C, E–F

Total length 6.5–8.4, 7.6 mm (n = 5). Head capsule length 405–455, 433 μm (n = 5); head capsule width 305–353, 332 μm (n = 5); post–mentum 167–185, 179 μm; head capsule dark golden.

Antenna (Fig. 4A, 9A). Antenna 6 segmented with 6th segment vestigial and often difficult to see (Figs. 4A; 9A). Lengths of antennal segments in μm: 43–56, 51; 14–20, 17; 5–9, 7; 7–10, 9; 5–8, 6; 2–2, 2. AR 1.1–1.5, 1.3; basal antennal segment 16–25, 20 μm wide; ring organ in basal 1/3rd of 1st antennal segment, distance from base ring organ 11–16, 13 μm, diameter of ring organ 6–7, 7 μm (n = 5). Blade 56–65, 61 μm long (n = 4), blade 1.4–1.7, 1.5 × the length of the flagellum; accessory blade length 5–11, 9 μm long (n = 3); style 7–10, 9 μm long; Lauterborn organs well developed, 5–10, 7 μm long, ending at the apex of segment 3.

Labro-epipharyngeal region (Fig. 4B). Labral seta SI weakly plumose, with lateral dissections; in some specimens, the SI appears to be simple, but this may be due to the orientation of the setae; labral setae SII and SIII simple, lamelliform; SIV well developed (Fig. 4B). Pecten epipharyngis consists of 3 peg-like scales, 8 pairs of chaetulae laterales in two rows or layers of 4 (Fig. 4B). Two pairs of bifid chaetulae basales. Premandible with a wide apical tooth and a broad inner tooth, 67–87, 76 μm long (Fig. 4B).

Mandible (Fig. C). Mandible distally dark brown; 103–128, 117 μm long; with 1 apical and 2 lateral teeth; seta interna divided into 5 serrate branches with branch dissections increasing in complexity in more basal branches, basal–most branch deeply dissected giving appearance of 3 branches; seta subdentalis wide with pointed apex (Fig. 4C).

Mentum (Fig. 4D). Mentum with dark brown teeth, median tooth slightly divided, 35–41, 39 μm wide, four lateral teeth with outermost lateral teeth with apical notch; ventromental plates 10–15, 12 μm wide (Fig. 4D); seta submenta aligned with the base of ventromental plates horizontally and 2nd lateral tooth vertically.

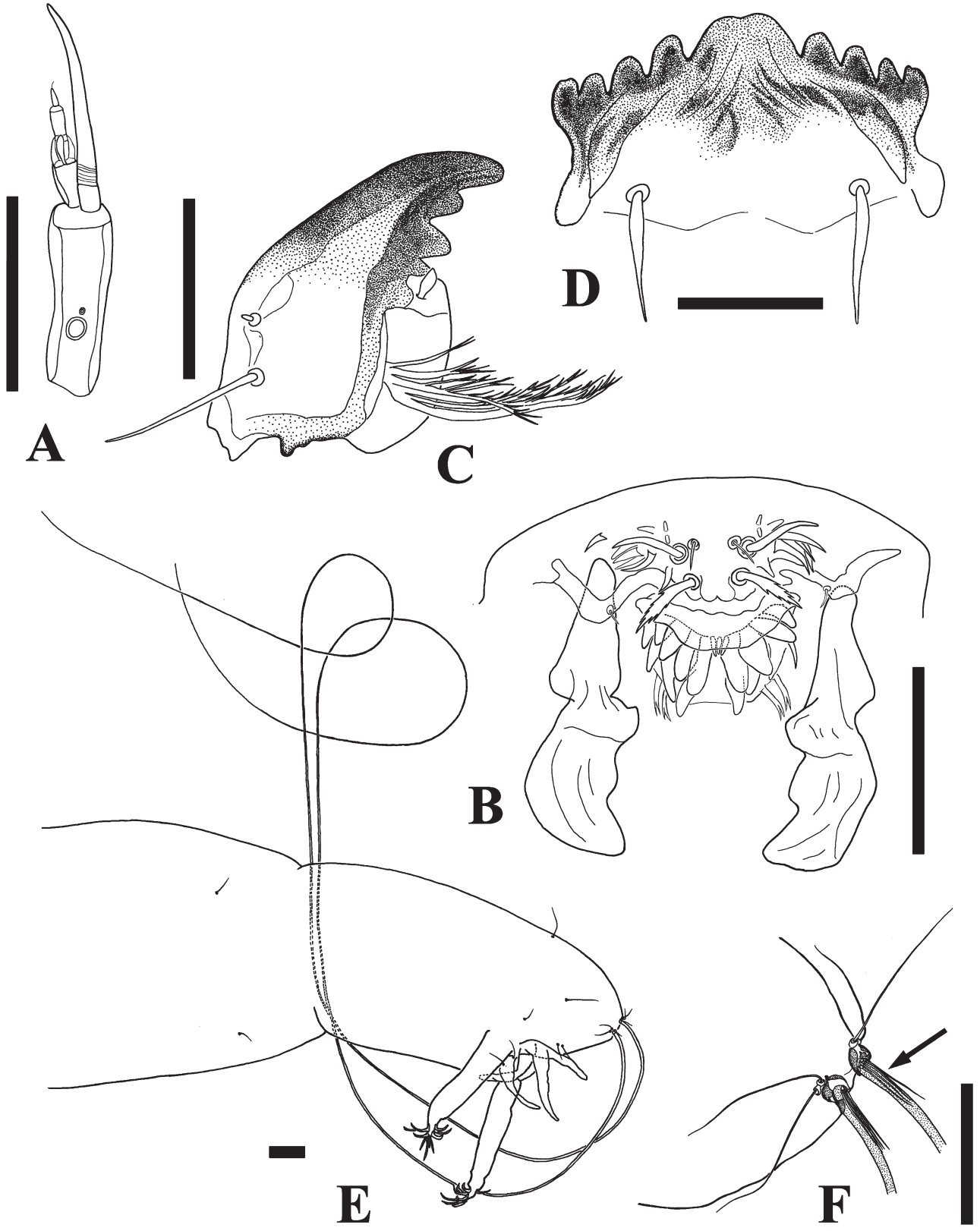

FIGURE 4. *Parachaetocladius lenferringtoni* **sp. nov.**, larva (A–F). A. antenna; B. labrum. C. mandible; D. mentum; E. posterior portion of the larva; F. procercus, arrow indicates the comb. Scale bars are 50 μm.

Maxilla. Maxillary palp short, with a long, stout a sensillum, short, slender b sensillum, and bisensillum and other maxillary palp sensilla short and peg-shaped; chaetulae of palpiger scale-like. Lamellae of galea finely pointed. Pectin galea absent. Lacinia with a short paraxial seta and long antaxial seta which is similar in length to dorsal chaetae of the lacinia; anterior lacinial chaeta leaf-shaped and much shorter than dorsal chaetae of the lacinia; 6 dorsal chaetae of the lacinia, all long, posterior chaeta longest and with one side serrated, other chaetae simple.

Abdomen (Fig. 4E–F; 9C, E–F). Procercus 8–14, 10 µm long and 8–14, 12 µm wide, 1 long apical procercal macroseta present, 1.6–2.2, 1.9 mm long, procercus with a comb or partial ring of 4 or more long spines on the procercus and closely appressed to the base of the procercal macroseta, this comb is only visible at 100 × magnification and not visible in all specimens (Fig. 4E–F; 9C,E); 2 lateral setae at the base of each procercus, 49–78, 66 µm long; supraanal setae 35–88, 65 µm long (n = 5); 8 or more additional setae located on posterior segment. Anal tubules 110–163, 136 µm long with one or two constrictions. Posterior parapods slender, 187–243, 215 µm long.

Distribution and ecology

Parachaetocladius lenferringtoni **sp. nov.** occurs in central North America from Kansas, USA, north to Manitoba, Canada. It has also been recorded in the USA from Nebraska, Minnesota, and Wisconsin. The larva of the new species has been collected from sand and sand/gravel substrates in springs and other groundwater-dominated streams (Fig. 5). Ferrington (1987) described the ecology of *P. lenferringtoni* **sp. nov.** under *P. hudsoni*. He noted that the larva inhabits the root systems of watercress *Nasturtium officinale* W.T. Aiton and monkeyflower *Erythranthe michiganensis* (Pennell) G.L. Nesom (= *Mimulus glabratus*) in Big Springs, Kansas, USA. In these spring habitats, the above plants were rooted in sandy habitats, indicating larvae can be mainly found in sandy substrates. Our collections of *P. lenferringtoni* **sp. nov.** from Minnesota confirmed that sand is the primary substrate in which the larva occurs. In Trout Brook (USA: Minnesota), most larval specimens were found in a mix of sand and gravel substrates in run habitats of the stream (Fig. 5). The larvae of the new species appear to prefer habitats with relatively stable sand substrates. In the Kansas springs studied by Ferrington (1987), macrophyte roots stabilize these substrates, and in the Minnesota streams studied, the mixed sand/gravel substrates are more stable than sand-only substrates. All pupal exuviae and adults of this new species were collected from March–May, indicating a spring emergence with a relatively synchronous emergence period. It is presumably univoltine based on this emergence pattern. This univoltine life history matches what is reported for another *Parachaetocladius* species (likely not *P. lenferringtoni* **sp. nov.** based on pupal description) in the Nearctic (Barton *et al.* 1987). There is some difference in the timing of emergence along the latitudinal gradient of this species' distribution, with the more southern Kansas and Nebraska specimens emerging in March, the Minnesota and Wisconsin specimens emerging in April and May, and the most northern Canadian specimens emerging in May.

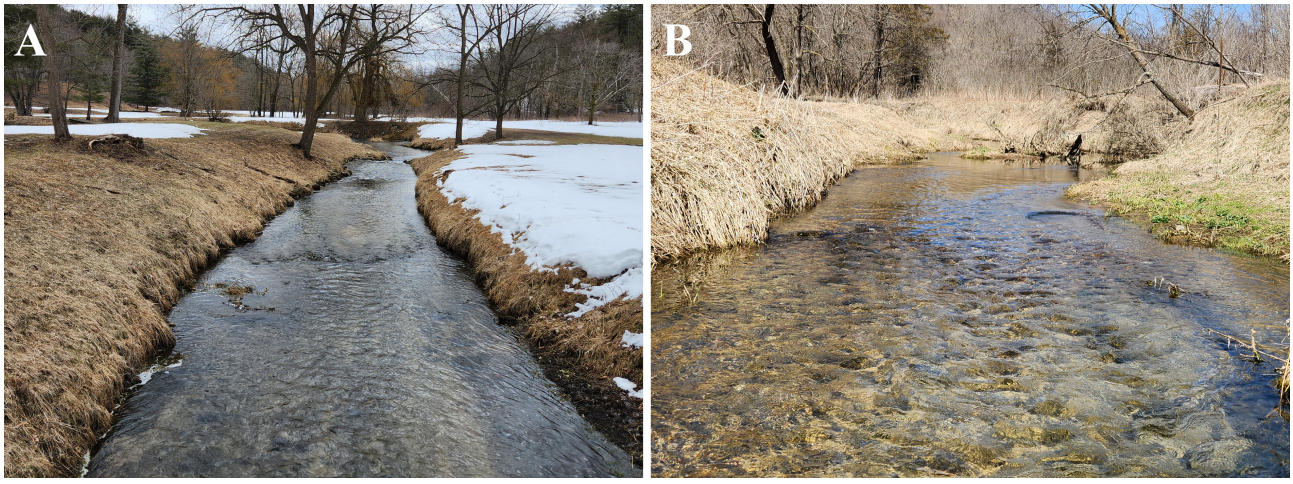

FIGURE 5. *Parachaetocladius lenferringtoni* **sp. nov.** collection sites and habitats in Minnesota, USA. A. Valley Creek; B. Trout Brook.

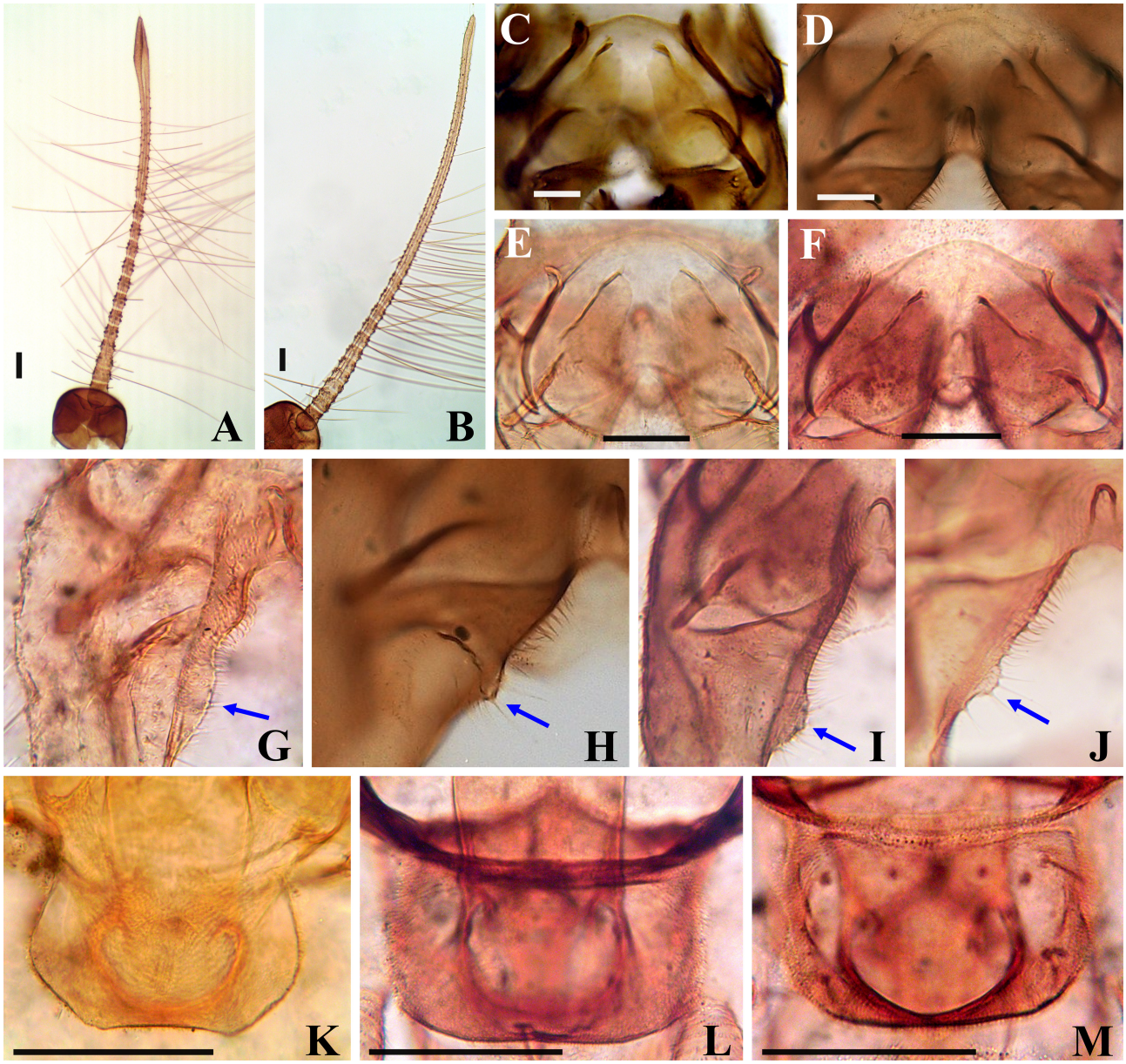

FIGURE 6. Comparison of some characteristics of *Parachaetocladius lenferringtoni* **sp. nov.** and *Parachaetocladius abnobaeus* (Wülker, 1959) adult males. *P. lenferringtoni* **sp. nov.**, (A, C, G, K); *P. abnobaeus* voucher, Alabama (B); *P. abnobaeus* paratype (D, H); *P. abnobaeus* voucher, Michigan (E, M); *P. abnobaeus* holotype of *H. abnobaeus* (F, I); *P. abnobaeus* voucher, Nova Scotia (J); *P. abnobaeus* voucher, Pennsylvania (L). A–B. antenna. C–F. sternapodeme; G–J. inferior volsella (blue arrows); K–M. clypeus. Scale bars are 50 µm. Images D and H courtesy of Mr. M. Spies and Dr. M. Kotrba of ZSM.

Other *Parachaetocladius* species examined

Parachaetocladius abnobaeus (Wülker, 1959)

Figs. 6B, D–F, H–J, L–M; 7G–L; 8C–E; 9B, D, G

Chaetocladius (Parachaetocladius) abnobaeus Wülker, 1959
Habrobaenus hudsoni Sæther, 1977
Parachaetocladius hudsoni Sæther & Sublette, 1983

Description. As described by Wülker (1959), Sæther (1977a, 1980b), and Sæther & Sublette (1983), with the following additions and corrections: Larva with 5–6 segments, 6th segment hair-like an only visible at ≥ 100 ×

magnification (Fig. 9B), antennal blade 1.2–1.7 × the combined length of flagellum; blade 43–63 µm long; base of the blade with annulation (Fig. 9B). Postmentum 102–165 µm long. Two short and thin anal setae, 50–71 µm long; one very long and robust apical setae on the procercus; one short and thin lateral seta present on the procercus (Fig. 9G). Anal tubules with constriction (Fig. 9G).

Material examined. Vouchers, 2 larvae; Mountains of Hesse, in the Black Forest and the Berchtesgadener Land in the German Alps; leg. T. Bendt, det. T. Bendt; deposited at TB.

Additional material examined. 1 male; CANADA, Nova Scotia, Cape Breton Highlands National Park, PG789882, 31.v.1984; leg. M.E. Dillon Rempel, det. PSC, deposited at CNC, CH9422.3. 1 male; CANADA, Ontario, Algonquin Park, Canisby Creek, 11.vi.1982; leg. R. Rempel, det. DRO, deposited at CNC, CH5458. 1 male; same data as previous specimen except 5.v.1981, CH5457. 1 pharate female pupa; FINLAND, Mantta-Vilppulan, Mantta-Vilppulan Iahde, 24.iv.2015; leg. L. Paasivirta, deposited at ZMUO. 1 pupa; FRANCE, Gorg Estelat, Nohèdes Reserve Naturelle Pyrénées, 1240m asl, 30.v.1995; leg. P.H. Langton, deposited at PHL. 1 male; USA, Alabama, Lauderdale County, Bush Creek, Co. Highway 133, 34.926º, -87.979º, 1.v.1985; leg. P.L. Hudson, deposited at PLH. 1 male; USA, Michigan, Lake Michigan, Hog Island State Forest, 46.074º, -85.286º, 30.iv.1990; leg. P.L. Hudson, deposited at PLH. 1 male; USA, Michigan, Mackinac County, Lake Huron, among hemlocks, 45.981, -84.216, 12.v.2001; leg. P.L. Hudson, deposited at PLH. 2 males; USA, Pennsylvania, Crawford County, Kiowa County,

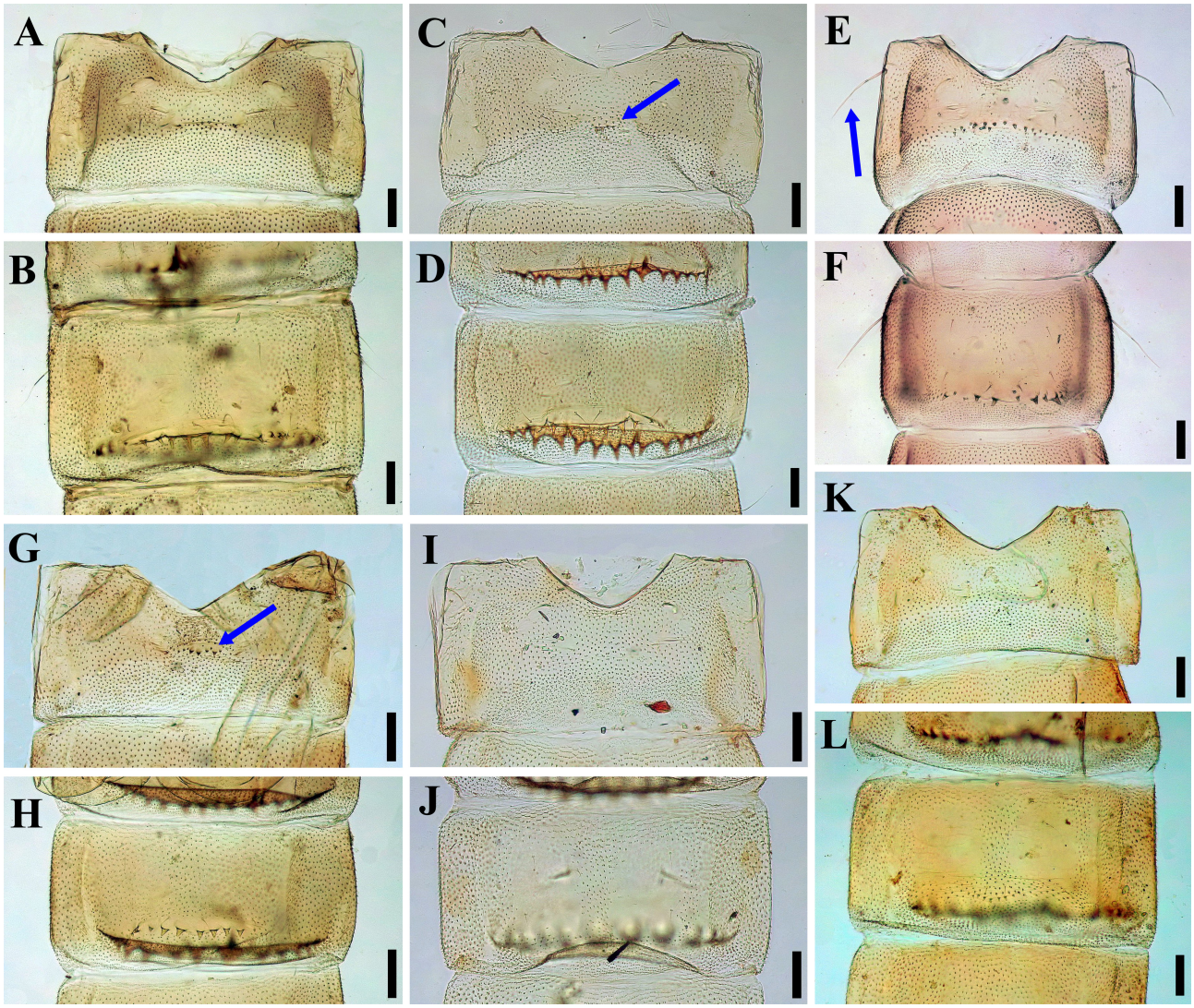

FIGURE 7. Comparison of pupal tergite I (A, C, E, G, I, K) and sternite III (B, D, F, H, J, L). *Parachaetocladius lenferringtoni* sp. nov., USA: Minnesota(A–B); USA: Wisconsin (C–D, blue arrow indicates weak spine row on tergite I). *Parachaetocladius hirtipectus* Sæther, 1969, Holotype, CANADA: British Columbia (E–F, blue arrow indicates large L seta in segment I). *Parachaetocladius abnobaeus* (Wülker, 1959), USA: South Carolina (G–J, blue arrow indicates weak spine row on tergite I); FRANCE: West Pyrenees (K–L). Scale bars are 100 µm.

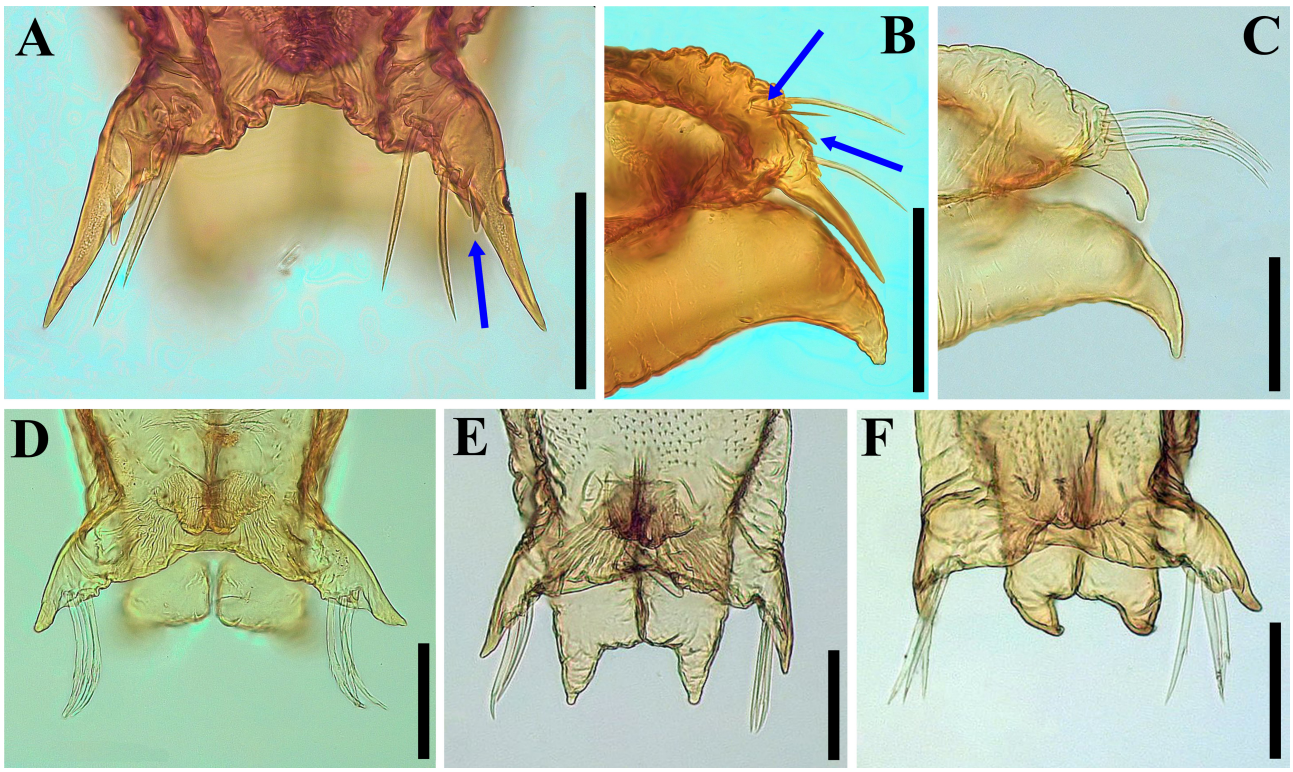

FIGURE 8. Comparison of dorsal (A, D, E, F) and lateral (B, C) views of pupal anal lobes of *Parachaetocladius lenferringtoni* **sp. nov.**, USA: Minnesota (A– B, blue arrows indicate anal lobe spines and small anal macrosetae) *Parachaetocladius abnobaeus* (Wülker, 1959), USA: South Carolina (C–D), FRANCE: West Pyrenees (E), and *P. hirtipectus,* (Holotype, CANADA: British Columbia (F). Scale bars are 100 µm.

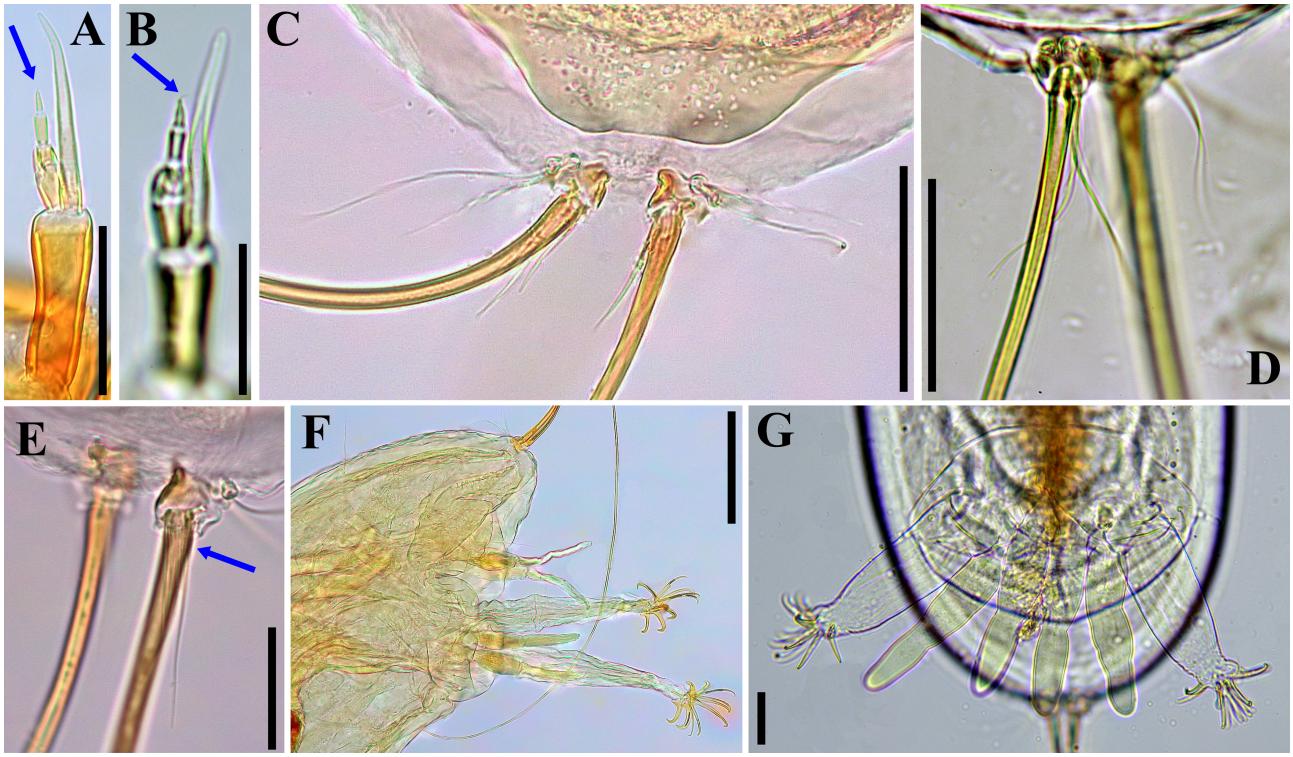

FIGURE 9. Comparison of some larval characteristics of *Parachaetocladius lenferringtoni* **sp. nov.** USA, Kansas (A), USA, Minnesota (C), USA, Wisconsin (E, F), and *Parachaetocladius abnobaeus* (Wülker, 1959), GERMANY: German Alps (B, D, G). A–B. antenna (blue arrow indicates the 6th antennal segment); C–D. procercus; E. procercus showing comb of setae (blue arrow indicates comb); F–G. posterior portion of the larva, posterior parapods, and anal tubules. Scale bars are 50 µm for A, B, E, and F and 25 µm for C and D. Images of larval *P. abnobaeus* are courtesy of Mr. Thomas Bendt.

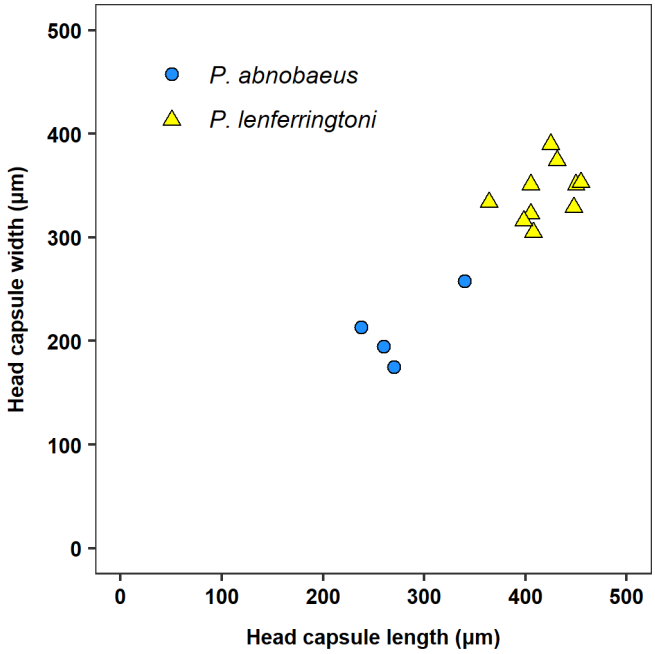

FIGURE 10. Larval head capsule lengths and widths for *Parachaetocladius lenferringtoni* **sp. nov.** (USA: Kansas, Minnesota, and Wisconsin) and *P. abnobaeus* (USA: South Carolina). Plot created using ggplot2 package (Wickham 2016) in R (R Core Team 2023).

Linesville Creek, 41.67º, -80.43º, 01.iv.1979; leg. P.L. Hudson, deposited at PLH. 1 male; *Habrobaenus hudsoni* Holotype, O.A. Sæther, USA, South Carolina, Oconee County, Walhalla National Fish Hatchery, Chattooga River, 15.ii.1976; leg. P.L. Hudson, deposited at CNC, No. 15277. 1 male; USA, South Carolina, Aiken County, Savannah River Plant, upper Three Runs Creek Co. Highway 133, 33.3519º, -81.6311º, 19.xi.1976; leg. P.L. Hudson, det. J.E. Sublette, deposited at PLH. 1 male, same data as previous specimen except 33.3519º, -81.6311º and 14.ix.1976. 1 male, same data as previous specimen except 33.3711º, -81.629º and 11.ii.1976. 1 larva; USA, South Carolina, Aiken County, Savannah River Plant, upper Three Runs Creek, Site D, 33.371º, -81.611º, no date; leg. Pritchard, deposited at PLH. 1 male; USA, South Carolina, Aiken County, Savannah River Plant, Tinker Creek, 33.3378º, -81.6042º, 19.xi.1976; leg. P.L. Hudson, deposited at PLH. 1 larva; USA, South Carolina, Orangeburg County, Santee State Park, 33.561º, -80.501º, 25.ii.1983; leg. P.L. Hudson, deposited at PLH. 1 male; USA, South Carolina, Pickens County, Issaqueena Forest, Indian Creek, 34.742º, -82.859º, 20.i.1980; leg. P.L. Hudson, deposited at PLH. 1 pupa, same data as previous specimen except collection date is 20.vi.1980. 1 pupa; USA, South Carolina, Pickens County, Six Mile Creek, 34.77948º, -82.84855º, 4.ii.1976; leg. P.L. Hudson, deposited at PLH. 1 pupa; USA, South Carolina, Clemson County, spring, Booker Springs Road, 34.6639º, -82.8018º, 28.xi.1982; leg. P.L. Hudson, deposited at PLH. 1 pupa; USA, South Carolina, York County, Wildcat Creek, 34.9112º, -81.0652º, 1.v.1976; leg. P.L. Hudson, deposited at PLH. 1 pupa; USA, South Carolina, Pickens County, Issaqueena Forest, Clemson University, Spring A, 34.742º, -82.854º, 15.iv.1982; leg. P.L. Hudson, deposited at PLH. 2 larvae; USA, South Carolina, Pickens County, Issaqueena Forest, Clemson University, Spring B, 34.741º, -82.854º, 10.iv.1982; leg. P.L. Hudson, deposited at PLH. 1 pupa, same data as previous specimen except collection date is 15.iv.1982. 1 male; USA, South Carolina, Barnwell County, Mill Creek site E, 33.317º, -81.584º, 19.ix.1976; leg. P.L. Hudson, deposited at PLH. 1 male; USA, South Carolina, Barnwell County, lower Three Runs Creek, 33.20º, -81.49º, 3.iii.1977; leg. P.L. Hudson, deposited at PLH. 1 male; USA, Tennessee, Sevier Spring, 12 Miles southeast of Gatlinburg of Route 441, 35.62º, -83.43º, 29.iv.2018; leg. P.L. Hudson, deposited at PLH.

***Parachaetocladius hirtipectus* Sæther, 1969**
Figs. 7E–F, 8F

Material examined. Holotype male with pupal exuviae; Canada, British Columbia, University of British Columbia Forestry Farm, Haney, Alloette River, at old crossing ¼ mile south of Marion Lake, 15.vii.1967, leg. A.L. Hamilton and O.A. Sæther, deposited at CNC, no. 9992.

Remarks

On the erection of the new species

Sæther (1977a) described *P. hudsoni* as *Habrobaenus hudsoni*. Sæther & Sublette (1983) placed this species in *Parachaetocladius* based on finding the correct associated pupa. Cranston & Oliver (1988) synonymized this species with *P. abnobaeus* (Wülker, 1959) because no hypopygial variations were found among the type and voucher specimens of the two species examined. However, their examined voucher specimens from Duck Mountains, Manitoba, Canada, and those from Big Spring, Kansas, USA showed small hypopygial differences from *P. abnobaeus* (Namayandeh *et al.* 2020). Further examination of this and additional material demonstrates that the antennal ratio of the adults, the shape and size of the adults' clypeus, blunt inferior volsella, the shape of sternapodeme of the adult males, the presence of posterolateral spines on anal lobe of the pupa, and the absence of denticles on anal macrosetae of the pupa separates *P. lenferringtoni* **sp. nov.** from *P. abnobaeus*. These morphological characteristics supported the erection of a new species (see also Table 3). The adult male and pupa of *P. lenferringtoni* **sp. nov.**, can be separated from other *Parachaetocladius* species based on the provided key and diagnosis and the female based on the diagnosis.

On the taxonomy of *Parachaetocladius*

The separation of *P. abnobaeus* from other *Parachaetocladius* species may be complicated by differences between populations of *P. abnobaeus* from the Palearctic and those from the Nearctic. There are some differences in the size and shape of immatures and adults of *P. abnobaeus* from the Nearctic and those from the Palearctic, which may suggest that they represent two different subspecies or even species. Wülker (1959) described the pupa and female of *P. abnobaeus* without providing detailed measurements. So, for now, we must rely on the description of the Nearctic female given as *P. hudsoni* by Sæther (1980b) and pupa by Sæther & Sublette (1983) to distinguish *P. abnobaeus* from other *Parachaetocladius* (Table 3). Concerning adults, Mr. Martin Spies of ZSM has kindly provided us with additional information and measurements of the type specimens of *P. abnobaeus* (i.e., from the Palearctic), which may be used in addition to those provided by previous authors. These are as follows. Clypeus with the main dorsal part transversely rectangular with rounded corners, anteromedial margin with a semi-circular extension to anteroventral that is slightly wider than half the clypeus width. Some of the clypei of the male-type specimens could be called trapezoidal rather than rectangular, as the distal margin can be a little wider than the proximal one. The only difference between the adult male and female is the number of setae, which in the male is around 8–10 and in the female is 5–6. Concerning the wing size, the holotype, reared from pupal exuviae, and a semi-pharate male from Baden-Württemberg, Germany, have standard wing lengths (i.e., arculus to tip) of approximately 2.3 mm, noting that the pharate wing is not fully unfolded. The paratype, possibly a semi-pharate male, has a standard wing length of approximately 2.6 mm. Finally, two paratypes, adult males, have standard wing lengths of 2.5 mm, respectively (M. Spies personal communication July 20–22, 2023).

With the synonymy of *P. abnobaeus* and *P. hudsoni* by Cranston & Oliver (1988), there are four species of *Parachaetocladius* with described pupae: *P. abnobaeus*, *Parachaetocladius akanoctavus* Sasa & Kamimura, 1987, *Parachaetocladius hirtipectus* Sæther, 1969, and *P. lenferringtoni* **sp. nov.** (Wülker 1959, Sæther 1969, Makarchenko & Yavorskaya 2021). Currently, the pupae of the Nearctic *Parachaetocladius broankerothurrie* Namayandeh & Beresford, 2020 and *Parachaetocladius imberbus* Sæther & Sublette, 1983 and the Palearctic *Parachaetocladius pyrenaeus* Moubayed, 2020 and *Parachaetocladius squamula* Liu, Cao, Zhao & Yan, 2020 are unknown. Sæther & Sublette (1983) noted an additional pupal morphospecies, *Parachaetocladius* sp. A (described as *P.* cf. *abnobaeus*

in Sæther (1969)). Langton (2023) also includes two additional pupal morphospecies (*Parachaetocladius* NA1 and *Parachaetocladius* NA2) from the Nearctic. The identity and status of these three morphospecies is unclear and they are not included in the key to Nearctic pupae.

TABLE 3. Comparison of some relevant characters of *Parachaetocladius abnobaeus* (Wülker) and *Parachaetocladius lenferringtoni* **sp. nov.** Abbreviations and description of values: AL = anal lobe; AM = anal macrosetae; AR = Antennal Ratio; L = length; LR = leg ratio; W = width; measurements and counts are provided in the following form: minimum-maximum, mean.

Characters	*P. abnobaeus* (Nearctic)	*P. abnobaeus* (Palearctic)	*P. lenferringtoni* sp. nov.
Male AR	1.2–1.6, 1.4	1.4–1.7	0.82–0.98, 0.91
Female AR	0.29	-	0.42–0.48, 0.46
Male wing L (mm)	1.7–2.5, 2.1	2.3–2.6	1.6–2.1, 1.8
Female wing L (mm)	1.9–2.3, 2.1	-	1.9–2.2, 2.0
Male clypeus L (µm)	52–86, 69	-	58–72, 67
Male clypeus W (µm)	102–143, 117	-	125–150, 141
Male No. setae on clypeus	4–10, 7	8–10	4–8, 6
Female No. setae on clypeus	-	5–6	8–12, 9
Male LR_1	0.57–0.71, 64	0.62	0.58–0.64, 0.61
Male LR_2	0.36–0.42, 0.4	0.39	0.32–0.40, 0.35
Male LR_3	0.55–0.63, 0.59	0.61	0.49–0.54, 0.52
Female notum L (µm)	121	-	127–134, 130
Female seminal capsule L (µm)	116	-	105–136, 125
Female seminal capsule W (µm)	93	-	82–118, 103
Pupa largest L-seta on segment I L (µm)	50–174, 103	115–160, 138	93–121, 106
Pupa posterolateral spine row on AL	absent	absent	present
Pupa AM_{1-3} L (µm)	81–119, 96; 79–111, 94; 75–116, 93	111–120, 116; 100–104,102; 104–130, 117	48–61, 54; 65–108, 83; 64–98, 77
Pupa relative size of AM	similar	similar	Most distal seta smaller than other two
Pupa AM denticles	usually present	usually present	usually absent, when present sparse
Larva AR	0.9–1.1, 1.0	1.4–1.5	1.1–1.5, 1.3
Larva antennal blade L (µm)	43–51, 46	60–63	56–65, 61
Larva postmentum L (µm)	102–119, 112	155–165	167–185, 179
Larva anal tubules L/ posterior parapods L	subequal	subequal	0.6–0.8, 0.6

Separation of the known pupae of *Parachaetocladius* species based on the posteromedial spines of the tergites and sternites may be tenuous due to the overlap of these characters among the known species. Sæther (1969) described the pupa of *P. hirtipectus* with an extended row of posteromedian spines on tergite I. This character may separate it from *P. abnobaeus* and *P. lenferringtoni* **sp. nov**. However, Sæther (1969) noted that no other significant dissimilarities could be found between the exuvia of *P. hirtipectus* and the description of the pupa of *P. abnobaeus* by Wülker (1959). We observed variation in pupae of different populations of *P. abnobaeus* from southeastern USA (Fig. 7G–J). In some pupae, tergite I and sternite III have a row of posteromedian spines (Fig. 7G–H), but in others these spine rows are lacking (Fig. 7I–J). It is possible that the variability in the pupa of *P. abnobaeus* in the

southeastern USA indicates the presence of two separate species because the specimens with a spine row on tergite I and sternite III have a smaller body size (3.2–3.6 mm, n = 3) and shorter L setae on segment I (50–96 μm, n = 3). In contrast, larger specimens (4.2–5.2 mm, n = 2) lack spine rows on both tergite I and sternite III and have long L setae on segment I (134–174, μm, n = 2). However, the available material and evidence are insufficient to determine whether these are separate species.

We also observed variation in the tergal spines on segment I in *P. lenferringtoni* **sp. nov.** Most specimens lacked tergal spines on tergite I, but some had a small number of enlarged posteromedian shagreen spines (Fig. 7C). However, *P. lenferringtoni* **sp. nov.** consistently has a well-developed, posterior spine row on sternite III (Fig. 7B, D). When present, the spines on tergite I in *P. lenferringtoni* **sp. nov.** and some *P. abnobaeus* were smaller and more closely resembled enlarged shagreen spines. This differed from *P. hirtipectus* which possesses better developed spines on tergite I (Fig. 7E), although they are still distinctly smaller than those on tergites II–VI/VII. The presence or absence of spine rows on tergites and sternites in the pupae is used to separate some species in the keys provided by Sæther & Sublette (1983), Makarchenko & Yavorskaya (2021), and Langton (2023). These characters may prove to be useful for separating the pupae of some species of *Parachaetocladius* (i.e., *P. abnobaeus* and *P. hirtipectus*); however, we have minimized the use of these characters in the provided key to Nearctic species due to the variability observed in *P. abnobaeus* and *P. lenferringtoni* **sp. nov**.

The length of the largest L seta on segments I–VII of the pupa appears to be a useful character for separating some species. Makarchenko & Yavorskaya (2021) used the size of the largest L seta on segment I in a key to separate *P. akanoctavus* from *P. abnobaeus*. In *P. hirtipectus* and *P. akanoctavus*, one L seta on segments I–VII is distinctly enlarged (Fig. 7E–F) whereas in *P. abnobaeus* and *P. lenferringtoni* **sp. nov.**, one L seta is larger than the other two, but not distinctly so (Fig. 7A–D, G–L). In the *P. abnobaeus* specimens we examined, the length of the longest L seta is variable, but still shorter than *P. hirtipectus*. The length of the longest L seta provides an unambiguous character for separating some species and here we use the length of the L seta on segment I to separate *P. hirtipectus* from other Nearctic species. The addition of the L seta character is useful due to the variability observed in the spine row on tergite I. The Palearctic species *P. akanoctavus* could potentially occur in the northwest of the Nearctic due to its presence in the Russian Far East (Makarchenko & Yavorskaya 2021). If this proven to be so, it is separable from other known Nearctic species by the presence of a long L seta (140–224 μm) on segment I, presence of posterior spine rows on sternite III, absence of posterolateral spine row on the anal lobe, and subequal anal macrosetae.

Currently, the larvae of only two of the described Nearctic species of *Parachaetocladius* are known: *P. abnobaeus* (= *P. hudsoni*) (Sæther & Sublette 1983) and *P. lenferringtoni* **sp. nov.** Sæther & Sublette (1983) included an additional larval morphotype, *Parachaetocladius* sp. B, based on a specimen collected from the same locality as *P. abnobaeus*. This larva differed from *P. abnobaeus* in possessing only one inner tooth on the mandible and the median tooth on the mentum that was not weakly subdivided. This specimen may be an anomalous *P. abnobaeus.* Mr. Thomas Bendt has kindly provided an assessment of two larvae of *P. abnobaeus* from the Black Forest and the Berchtesgadener Land of the German Alps (GERMANY). We also examined six larvae of Nearctic *P. abnobaeus* from some of the same localities in South Carolina (USA) which Sæther & Sublette (1983) based their description of the larvae of *P. abnobaeus* (= *P. hudsoni*). Based on the images and measurements provided, the larva of *P. lenferringtoni* **sp. nov.** is very similar and difficult to separate from that of *P. abnobaeus*, particularly those from the Palearctic (Fig. 9). The Palearctic *P. abnobaeus* larvae and *P. lenferringtoni* **sp. nov.**, both have a long blade which is approximately 1.5 × longer than the flagellum (Figs. 4A; 9A–B) in contrast to the shorter blade of *P. abnobaeus* from the Nearctic described and illustrated by Sæther & Sublette (1983) in Fig. 24A. The larvae from some of the same localities in South Carolina which Sæther & Sublette (1983) based their description of the larvae of *P. abnobaeus* (= *P. hudsoni*) also had antennal blades that were only slightly longer than the flagellum. The anal tubules are shorter than posterior parapods in the larva of *P. lenferringtoni* **sp. nov.** and are 0.6–0.8 x the length of the posterior parapods (Figs. 4E, 9F). In contrast, anal tubules in *P. abnobaeus* (both the Nearctic and Palearctic specimens; Sæther & Sublette (1983), Fig. 24H and T. Bendt, personal communication, September 11, 2023) are subequal in length to the posterior parapods (Fig. 9G). This character could also be useful in separating *P. lenferringtoni* **sp. nov.** from *P. abnobaeus*, although additional material should be examined.

The length of the postmentum and the head, along with head width, are other characters that could possibly separate the larvae of the two species. In *P. lenferringtoni* **sp. nov.**, the length of the postmentum is 167–185, 179 μm whereas in *P. abnobaeus* it is 102–119, 112 μm (Nearctic; USA: South Carolina) and 155–165 μm (n = 2; Palearctic; T. Bendt, personal communication, September 11, 2023). Plotting the head capsule length and width of *P. lenferringtoni* **sp. nov.** and Nearctic *P. abnobaeus* results in a clear separation of the two larvae (Fig. 10). The *P.*

abnobaeus larvae from South Carolina had smaller head capsule size and because we had small number of suitable larvae available for measurement, they did not group tightly in the plot. Also, we cannot with certainty confirm that the smaller larvae from South Carolina are 4th instar larvae. Therefore, due to the limited number of specimens available for examination at this time and the variability of characteristics discussed above in the larval specimens of *P. abnobaeus*, separation of *P. abnobaeus* and *P. lenferringtoni* **sp. nov.** in the larval stage remains tenuous. As a result, we do not provide a key for larval stages.

As mentioned above, *P. akanoctavus* could potentially occur in the northwest Nearctic The larvae of *P. akanoctavus*, has been described by Makarchenko & Yavorskaya (2021). This larva appears to be relatively distinct from *P. lenferringtoni* **sp. nov.** in that the SI is simple and the antennal blade is 1.9–2.0 × the length of antennal segments 2–5 in *P. akanoctavus*. Makarchenko and Yavorskaya (2021) described the blade as "1.9–2.0 times of the antennal segments 1–5," but it appears this should be segments 2–5. The simple SI in *P. akanoctavus* differs from the other known *Parachaetocladius* larvae and would alter the generic description of the larva. The SI in *P. lenferringtoni* **sp. nov.** is weakly plumose, although in some specimens examined, the SI appears to be simple which may be an artifact of the orientation of the SI. As a result, this character may need to be used with caution. The long blade of *P. akanoctavus* does appear to be a useful character as the blade in *P. lenferringtoni* **sp. nov.** is 1.4–1.7 × the length of antennal segments 2–5. Although we do not include a key to the larvae, this character could be used to separate *P. akanoctavus* from other known larvae of *Parachaetocladius*.

We also observed a procercus comb or ring of spines appressed to the large anal seta of *P. lenferringtoni* **sp. nov.** at 100 × magnification (Figs. 4F, 9E). This structure has not been documented in other *Parachaetocladius* larvae; however, it is often closely appressed to the large anal seta and is difficult to see in many *P. lenferringtoni* **sp. nov.** specimens. Specimens of *Parachaetocladius* from New Brunswick, Canada, which are likely not *P. lenferringtoni* **sp. nov.**, indicate that this character is present in other species of *Parachaetocladius* (B. Bilyj, personal communication, October 13, 2023). This suggests that this comb may be a generic character that has been previously overlooked. However, material from additional species needs to be examined to determine if it is a generic character or if it has some utility for separating *Parachaetocladius* species.

A key to the adult males of the Nearctic *Parachaetocladius* (Wülker, 1959)

1. Gonostylus with blunt to slightly curved outer projection and no inner or anterior projection (Namayandeh *et al.* 2020: Fig. 5B) . . . . . *P. broankerothurrie* Namayandeh & Beresford (*broankerothurrie* group)
- Gonostylus with inner or anterior projection well-developed. Outer projection well-developed or absent 2
2. Sternapodeme highly arched (Sæther 1969: Fig. 51; Namayandeh *et al.*, 2020: Fig. 3D). Preepsiternum and anepisternum with several setae. Inferior volsella large and triangular (Sæther 1969: Fig. 59). . . . . *P. hirtipectus* Sæther (*hirtipectus* group)
- Not with the above combination of characters 3
3. Gonostylus with well-developed anterior and outer projections. Inferior volsella blunt, slightly digitiform or digitiform and bent downward (Namayandeh *et al.*, 2020: Fig 3A–C). Pseudospurs present . . . . . *abnobaeus* group 4
- Gonostylus without well-developed anterior projection; outer projections low to slightly developed (Sæther & Sublette 1983: Figs. 25D–F; Namayandeh *et al.* 2020: Fig. 3E). Inferior volsella prominently digitiform, bent downward (Sæther & Sublette 1983: Figs. 25D–E). Pseudospurs absent . . . . . *P. imberbus* Sæther & Sublette (*imberbus* group)
4. AR 1.2–1.7, 1.5. Sternapodeme more arched, oral projections small (Sæther, 1977a: Fig. 1F; Wülker, 1959: Fig. 2; Fig. 6D–F). Inferior volsella present, small digitiform or lobed (Sæther, 1977a: Fig. 1E–F; Wülker, 1959: Fig. 2; Fig. 6H–J). Clypeus appears rectangular (Sæther, 1977a: Fig. 1A; Wülker, 1959: Fig. 2; Fig. 6L–M) . . . . . *P. abnobaeus* (Wülker)
- AR 0.82–0.98, 0.91. Sternapodeme less arched to straight, oral projections large (Fig. 6C). Inferior volsella blunt (Fig. 6G). Clypeus appears hexagonal (Fig. 1B; Fig. 6K) . . . . . *P. lenferringtoni* **sp. nov.**

A key to the known pupae of the Nearctic *Parachaetocladius* (Wülker, 1959)

1. Anal lobe with a row of blunt spines posterolaterally which extend onto anal lobe tips (Figs. 3E; 8A–B). One anal macroseta distinctly shorter than the other 2 (note: the smaller macroseta is commonly missing in this species; Figs. 3E, 8A–B). Denticles on anal macrosetae usually absent, if present only 1–2 (Figs. 3E, A–B) . . . . . *P. lenferringtoni* **sp. nov.**
- Anal lobe lacking posterolateral spines (Fig. 8C–F). Anal macrosetae similar in size (Fig. 8C–F). Denticles on anal macrosetae usually present, but may be absent or only 1–2 are present (Fig. 8C–F) 2
2. Largest L seta on segments I–VII distinctly larger than other L setae, > 175 μm long (Fig. 7E–F). Posteromedian spines present on Tergite I (Fig. 7E, Sæther 1969: Fig. 52). . . . . *P. hirtipectus* Sæther
- Largest L seta not distinctly larger than other L setae, < 175 μm (Figs. 7G–L). Posteromedian spines present or absent on Tergite I, if present very short (Fig. 7G–L; Sæther and Sublette 1983: Fig. 23A, B) . . . . . *P. abnobaeus* (Wülker)

Acknowledgments

We gratefully acknowledge the help and cooperation we received from many dedicated researchers and express our sincere thanks to all of these, without whom this work would not have been possible: the Belwin Conservancy (Valley Creek) and Dakota County Parks (Trout Brook) for permission to sample the study streams; the following individuals who provided *Parachaetocladius* specimens for this paper including: Dr. Dean Hansen (University of Minnesota, St. Paul, Minnesota, USA), Mr. Jeffrey J. Dimick (University of Wisconsin-Stevens Point, Wisconsin, USA), Dr. Peter Langton (University of Cambridge, United Kingdom), Dr. Andrew Fasbender (Rhithron Associates Inc., Missoula, Montana, USA), Dr. Scott Brooks and Diptera researchers at the Canadian National Collection of Insects, Arachnids and Nematodes, and Dr. Marko Mutanen (University of Oulu, Finland); Mr. Bohdan Bilyj (Biotax, Ontario, Canada) for providing feedback on the morphology of the immature stages; Mr. Martin Spies (Zoologische Staatssammlung Munich (ZSM), Germany) for providing us with literature, comments on adults morphology; Mr. Thomas Bendt (Heyerhütte, Germany) for larval imagery and morphology; and Dr. Marion Kotrba (ZSM, Germany) for the imagery.

References

Andersen, T., Cranston, P.S. & Epler, J.H. (2013) Chironomidae of the Holarctic Region: keys and diagnoses: larvae. *Scandinavian Society of Entomology Supplement*, 66, 1–573.

Barton, D.R., Pugsley, C.W. & Hynes, H.B.N. (1987) The life history and occurrence of *Parachaetocladius abnobaeus* (Diptera: Chironomidae). *Aquatic Insects*, 9, 189–194. https://doi.org/10.1080/01650428709361295

Cranston, P.S. & Oliver, D.R. (1988) Additions and corrections to the Nearctic Orthocladiinae (Diptera: Chironomidae). *The Canadian Entomologist*, 120, 425–462. https://doi.org/10.4039/Ent120425-5

Ferrington Jr., L.C. (1987) Microhabitat preference of larvae of three Orthocladiinae species (Diptera, Chironomidae) in Big Springs a sandbottom spring in the high plains of western Kansas. *Entomologica Scandinavica*, 29, 361–368.

Hadley, A. (2010) CombineZP Image Stacking Software. Available from: https://combinezp.software.informer.com/ (accessed 30 January 2024)

Inkscape Project (2023) Inkscape. Available from: https://inkscape.org (accessed 30 January 2024)

Langton, P.E. (2023) *A key to pupal exuviae of Nearctic Chironomidae based on the collections of William P. Coffman*. Published by the author, Coleraine, (4) + 9 + 478 pp.

Langton, P.H. & Pinder, L.C.V. (2007) *Keys to the adult male Chironomidae of Britain and Ireland. Vols. 1 & 2.* Freshwater Biological Association (FBA), The Ferry House, Far Sawrey, Ambleside, Cumbria, 168 & 239 pp.

Liu, W., Cao, W., Zhao, C. & Yan, C. (2020) *Parachaetocladius squamula* (Diptera, Chironomidae, Orthocladiinae), a new species from China. *Annales Zoologici Fennici*, 57, 145–149. https://doi.org/10.5735/086.057.0116

Makarchenko, E.A., & Yavorskaya, N.M. (2021) Redescription of the little-known chironomid species *Parachaetocladius akanoctavus* Sasa *et* Kamimura, 1987 (Diptera: Chironomidae: Orthocladiinae) from the Russian Far East. *Euroasian Entomological Journal*, 20, 119–124. https://doi.org/10.15298/euroasentj.20.3.01

Namayandeh, A., Moubayed, J., Ghaderi, E. & Beresford, D.V. (2020) A review of the genera *Georthocladius* Strenzke, 1941 and *Parachaetocladius* Wülker, 1959 (Chironomidae, Orthocladiinae): new species descriptions, and keys based on the morphological characters of adult male. *Polish Journal of Entomology*, 89, 59–80. https://doi.org/10.5604/01.3001.0014.1517

Pinder, LC.V. (1986) The pupae of Chironomidae (Diptera) of the Holarctic region— Introduction. *In*: Wiederholm, T. (Ed.), *Chironomidae of the Holarctic region: Keys and diagnoses. Part 2. Pupae*. *Entomologica Scandinavica*, *Supplement 28.* Borgströms Tryckeri, Lund, pp. 5–7.

R Core Team (2023) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. Available from: https://www.R-project.org/ (accessed 30 January 2024)

Sasa M. & Kamimura, A. (1987) Chironomid midges collected on the shores of lakes in the Akan National Park, Hokkaido (Diptera, Chironomidae). *Research Report from the National Institute for Environmental Studies Japan*, 104, 9–61.

Sæther, O.A. (1969) Some Nearctic Podonominae, Diamesinae and Orthocladiinae. *Bulletin of Fisheries Research Board of Canada*, 170, 154.

Sæther, O.A. (1977a) *Habrobaenus hudsoni* n. gen., n. sp. and the immatures of *Baeoctenus bicolor* Sæther (Diptera: Chironomidae). *Journal of the Fisheries Board of Canada*, 34, 2354–2361. https://doi.org/10.1139/f77-315

Sæther, O.A. (1977b) Female genitalia in Chironomidae and other Nematocera: morphology, phylogenies, keys. *Bulletin of the*

Fisheries Research Board of Canada, 197, 1–220.
Sæther, O.A. (1980a) Glossary of chironomid morphology terminology (Diptera: Chironomidae). *Entomologica Scandinavica*, 14, 1–51.
Sæther, O.A. (1980b) Three Female Chironomid Genitalia (Diptera). *In*: Murray, D.A. (Ed.), *Chironomidae Ecology, Systematics Cytology and Physiology: Proceedings of the 7th International Symposium on Chironomidae, Dublin, August 1979.* Pergamon Press, Oxford, pp. 115–121.
https://doi.org/10.1016/B978-0-08-025889-8.50023-4
Sæther, O.A. & Sublette, J.E. (1983) A review of the genera *Doithrix* n. gen., *Georthocladius* Strenzke, *Parachaetocladius* Wülker and *Pseudorthocladius* Goetghebuer (Diptera: Chironomidae, Orthocladiinae). *Entomologica Scandinavica*, Supplement 20, 100, SEK 80.
Wülker, W. (1959) Drei neue Chironomiden - Arten (Dipt.) und ihre Bedeutung fur das Konvergenzproblem bei Imagines und Puppen. *Archiv für Hydrobiologie Supplement*, 25, 44–49.
Wickham, H. (2016) *ggplot2: Elegant Graphics for Data Analysis*. Springer, Cham, XVI + 260 pp.
https://doi.org/10.1007/978-3-319-24277-4