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New or little-known species of *Chaetocladius* s. str. Kieffer, 1911 (Diptera: Chironomidae: Orthocladiinae) from the Amur River basin (Russian Far East)

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Abstract

Chironomids of the subgenus *Chaetocladius* s. str. from the Amur River basin are revised using both morphological characters and molecular data. Three new species, *C. egorych* **sp. nov.**, *C. lopatinskiy* **sp. nov.** and *C. yavorskayae* **sp. nov.**, are described and figured. The pupa of *C. fedotkin* is described for the first time. Adult males of *C. ligni* and *C. piger*, little-known in the Far East, are redescribed and annotated, and key to males of the *Chaetocladius* s. str. from the Amur River basin is provided. A reference 658 bp barcode sequence from a fragment of the mitochondrial gene cytochrome oxidase I (COI) was used as a tool for species delimitation. Comparisons with corresponding regions of COI between 5 species in the subgenus produced K2P genetic distances of 8.3–12.6%, values well associated with interspecific variation. Molecular data were also used for the reconstruction of the phylogenetic relationships within the subgenus *Chaetocladius* s. str.

Key words: Chironomidae, Orthocladiinae, *Chaetocladius*, new species, key, barcoding, Russian Far East

Introduction

The genus *Chaetocladius* Kieffer, 1911 is divided into two subgenera—*Amblycladius* Kieffer, 1923, including two species—*A. subplumosus* Kieffer, 1923 and *A. franzjosephi* Krasheninnikov, 2013 (Krasheninnikov & Gavrilo 2013) from the North of Russia, and *Chaetocladius* s. str., with 56 Palaearctic species (Ashe & O'Connor 2012; Kobayashi 2012; Wang *et al.* 2012; Makarchenko & Makarchenko 2013a–c), 5 Nearctic species, 7 Oriental species and 2 Afrotropical species (Ashe & O'Connor 2012). Twenty two species of *Chaetocladius* s. str. were known for Russia (Zelentsov & Shilova 1996; Ashe & O'Connor 2012), of which 15 species were described as new from the Russian Far East and bordering territories—*C. amnunnycta* Makarchenko *et* Makarchenko, 2011; *C. amurensis* Makarchenko *et* Makarchenko, 2006; *C. antipovae* Makarchenko *et* Makarchenko, 2011; *C. autumnalis* Makarchenko *et* Makarchenko, 2004; *C. elegans* Makarchenko *et* Makarchenko, 2002; *C. elenae* Makarchenko *et* Makarchenko, 2013; *C. fedotkin* Makarchenko et Makarchenko, 2013; *C. insularis* Makarchenko *et* Makarchenko, 2004; *C. khrulevae* Makarchenko *et* Makarchenko, 2013; *C. ketoiensis* Makarchenko *et* Makarchenko, 2004; *C. magnalobus* Makarchenko *et* Makarchenko, 2009; *C. nudisquamus* Makarchenko *et* Makarchenko, 2003; *C. pseudoligni* Makarchenko *et* Makarchenko, 2002; *C. unicus* Makarchenko *et* Makarchenko, 2002; *C. variabilis* Makarchenko *et* Makarchenko, 2003 (Makarchenko & Makarchenko 2001, 2003, 2004, 2006a,b, 2009, 2011a,b, 2012).

In this paper, the subgenus *Chaetocladius* s. str. is revised in part on the basis of materials from the Amur River basin, the greatest one in the East Siberia and the Russian Far East. As a result of our study, three new species, *Chaetocladius egorych* **sp. nov.**, *C. lopatinskiy* **sp. nov.** and *C. yavorskayae* **sp. nov.**, as well as *C. fedotkin* pupa are described. Adult males of *C. ligni* and *C. piger*, the little-known species in the Far East, are redescibed and annotated. A key to adult males of the *Chaetocladius* s. str. species from the Amur River basin is provided.

In the present study we have also evaluated a fragment of mitochondrial Cytochrome Oxidase I (COI), which

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represents the barcode region, as described by Hebert *et al.* (2003, 2004). Standardized mitochondrial fragments from COI (DNA barcodes) have been used to identification (Montagna *et al.* 2016; Ekrem *et al.* 2010; Carew *et al.* 2005), as well as Orthocladiinae of Chironomidae (Silva & Wiedenbrug 2014, Makarchenko *et al.* 2015). K2P genetic distances between DNA barcodes of the subgenus *Chaetocladius* s. str. have also been used to evaluate interspecific variability. Moreover, we have constructed the ML tree using obtained DNA barcodes and other species of the genus from GenBank.

Material and methods

The adult specimens were collected mainly with a sweep net, near rivers and streams; a few specimens were taken in emergence traps and some pupae with exuviae in drift net. The larvae were associated with pupae based on larval heads sticking to the mature pupae; the males were associated with pupae based on hypopygia of pharate specimens extracted from exuviae. Association of preimaginal and imaginal stages of most species studied was supported by results of barcoding.

For morphological study the material was preserved in 70% ethanol and Oudemans' solution, and mounted on slides following the procedure outlined by Makarchenko (1985). The terminology follows Sæther (1980).

For barcoding the material was fixed in 96% ethanol and deposited in refrigerator. Total DNA was extracted from each sample using the Invitrogen PureLink Genomic DNA Mini Kit and Qiagen DNeasy Blood & Tissue kit in accordance with the protocol in a final elution volume of 50 µL. A 650–700 bp fragment of the COI barcode region was amplified with the primers COIF-ALT (5'-ACAAATCAYAARGAYATYGG-3') and COIR-ALT (5'-TTCAGGRTGNCCRAARAAYCA-3') described in Mikkelsen *et al.* (2006). PCR amplification was carried out in a final volume of 10 µL, including 5 µL of Go Taq Green Master Mix (Promega), 0.5 µL of each primer, 3 µl nuclease-free water and 1 µL of purified DNA. All PCR amplifications were verified using electrophoresis on a 1,5% TBE agarose by visualizing on GelDoc XR+ imaging systems (Bio-Rad), only positive PCR products were purified for cycle sequencing using Exonuclease I (ExoI) and Thermosensitive Alkaline Phosphatase (FastAP) by ThermoFisher Scientific. DNA sequencing was performed on both strands using BigDye 3.1® sequencing kit (ThermoFisher Scientific), in a 10 µL reaction including 1.25 µL ABI 5X dilution buffer, 1 µL Big Dye, 0.5 µL of primer and 1.2 µL of PCR product. Each sequencing reaction was purified by ethanol precipitation described in the protocol. Finally, all samples were analyzed in an ABI 3130XL® (Applied Biosystems) automated capillary sequencer. Consensus sequences and interspecies genetic distances based on the K2P were obtained with MEGA 7 (Kumar *et al.* 2016). Sequence divergences among species were quantified by using the GTR+I distance model (Tavare 1986) and graphically displayed in a maximum likelihood (ML) tree. All sequences of investigated species of *Chaetocladius* were deposited in GenBank (KT285301–KT285306, KY175213–KY175224).

Holotypes and paratypes of the new species are deposited in the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia (FSCEATB FEB RAS).

Genus: *Chaetocladius* Kieffer

Subgenus: *Chaetocladius* s. str. Kieffer, 1911: 182.

Type species: *Dactylocladius setiger* Kieffer, 1908 [= *Chironomus perennis* Meigen, 1830], by subsequent designation of Goetghebuer 1940–50: 57.

Key to males of *Chaetocladius* s. str. Kieffer from the Amur River basin

1. Hypopygium large and chitinized stronger than preceeding tergites. Tergite IX and laterosternite IX fused. Inferior volsella flat, with long setae. Gonostylus intricate, wide in the basal two thirds, with subapical rounded crista dorsalis (Makarchenko & Makarchenko 2006a: Figs. 1–2) . . . . . *C. amurensis* Makarchenko *et* Makarchenko
- Hypopygium small and chitinized as preceeding tergites. Tergite IX and laterosternite IX separated. Gonostylus never as above 2

2. Tergite IX without anal point, trapezoidal, and densely covered with microtrichia, its free edge tucked down, with middle or long setae on inner part and on the edge. Gonostylus bilobate (Figs. 29–32) . . . . . *C. yavorskayae* **sp. nov.**
- Tergite IX with anal point. Gonostylus simple 3
3. Virga long (160–176 μm), begins in posterior half of segment VIII. Gonostylus short, with 1–3 megasetae (Makarchenko & Makarchenko 2004: Figs. 4–8) . . . . . *C. variabilis* Makarchenko *et* Makarchenko
- Virga much shorter if present. Gonostylus of another shape 4
4. Mesonotum yellowish, with 3 dark stripes. Fore leg with yellow *fe*, *ti* and black ta_1–ta_5. Mid and hind legs with yellow *fe*, *ti,* ta_1 and black ta_2–ta_5. Acrostichals more than 50. Inferior volsella small, tubercle-like, bare (Makarchenko & Makarchenko 2011a: Fig. 4) . . . . . *C. antipovae* Makarchenko *et* Makarchenko
- Mesonotum and legs uniformly brown. Acrostichals significantly less numerous. Inferior volsella never as above 5
5. Gonostylus triangular/angular 6
- Gonostylus never as above 7
6. Gonostylus expanding toward edge (angular) (Makarchenko & Makarchenko 2007: Fig. 6) . . . . . *C. holmgreni* (Jacobson)
- Gonostylus expanding in middle part (triangular) (Figs. 19–22) . . . . . *C. lopatinskiy* **sp. nov.**
7. Inferior volsella double 8
- Inferior volsella simple 10
8. Anal point wide (48 μm); gonocoxite with two parts well-separated into inferior volsella; gonostylus narrow, with small triangular preapical crista dorsalis (Makarchenko & Makarchenko 2009: Figs. 7–10) . . . . . *C. magnalobus* Makarchenko *et* Makarchenko
- Anal point not so wide; inferior volsella and gonostylus never as above 9
9. Ventral part of inferior volsella placed behind its dorsal part. Virga consists of tuft with dark setae (Figs. 16–17) . . . . . *C. ligni* Cranston *et* Oliver
- Ventral part of inferior volsella placed below its dorsal part. Virga weak, consists of several pale setae (Langton & Pinder 2007: Fig. 142, C) . . . . . *C. perennis* (Meigen)
10. Anal point nearly parallel-sided, with rounded apex; virga short (20–24 μm); gonostylus rounded and slightly broadened at outer margin (Makarchenko & Makarchenko 2013a: Figs. 1–3) . . . . . *C. fedotkin* Makarchenko *et* Makarchenko
- Anal point triangular; virga longer; gonostylus more slender and long 11
11. Anal point narrowly triangular and long (60–72 μm); virga with tuft of setae 72–80 μm long; inferior volsella with finger-shaped projection (Figs. 1–2); AR 1.04–1.18 . . . . . *C. egorych* **sp. nov.**
- Anal point broadly triangular and shorter (48 μm); virga weak; inferior volsella without finger-shaped projection (Fig. 18); AR 1.54 . . . . . *C. piger* (Goetghebuer)

Descriptions

Chaetocladius egorych Makarchenko *et* Makarchenko, sp. nov.

(Figs. 1–13, 34–38)

Chaetocladius sp.2 Makarchenko *et al.* 2014: 425.

Material. Holotype: adult male, Russian Far East, Jewish Autonomous Region, Obluchie District, Lopatinskyi Spring of Bidzhan River basin (Amur River basin), N 48º37'810", E 131º39'114", 7.IV. 2014, leg. E. Makarchenko. Paratypes: 4 males, 1 pupa, same data as holotype; 7 mature pupae, same data as holotype except 27.III. 2015; 1 pupa, same data as holotype except 31.III. 2015; 10 larvae, same data as holotype except 2–6.IV. 2016; leg. E. Makarchenko.

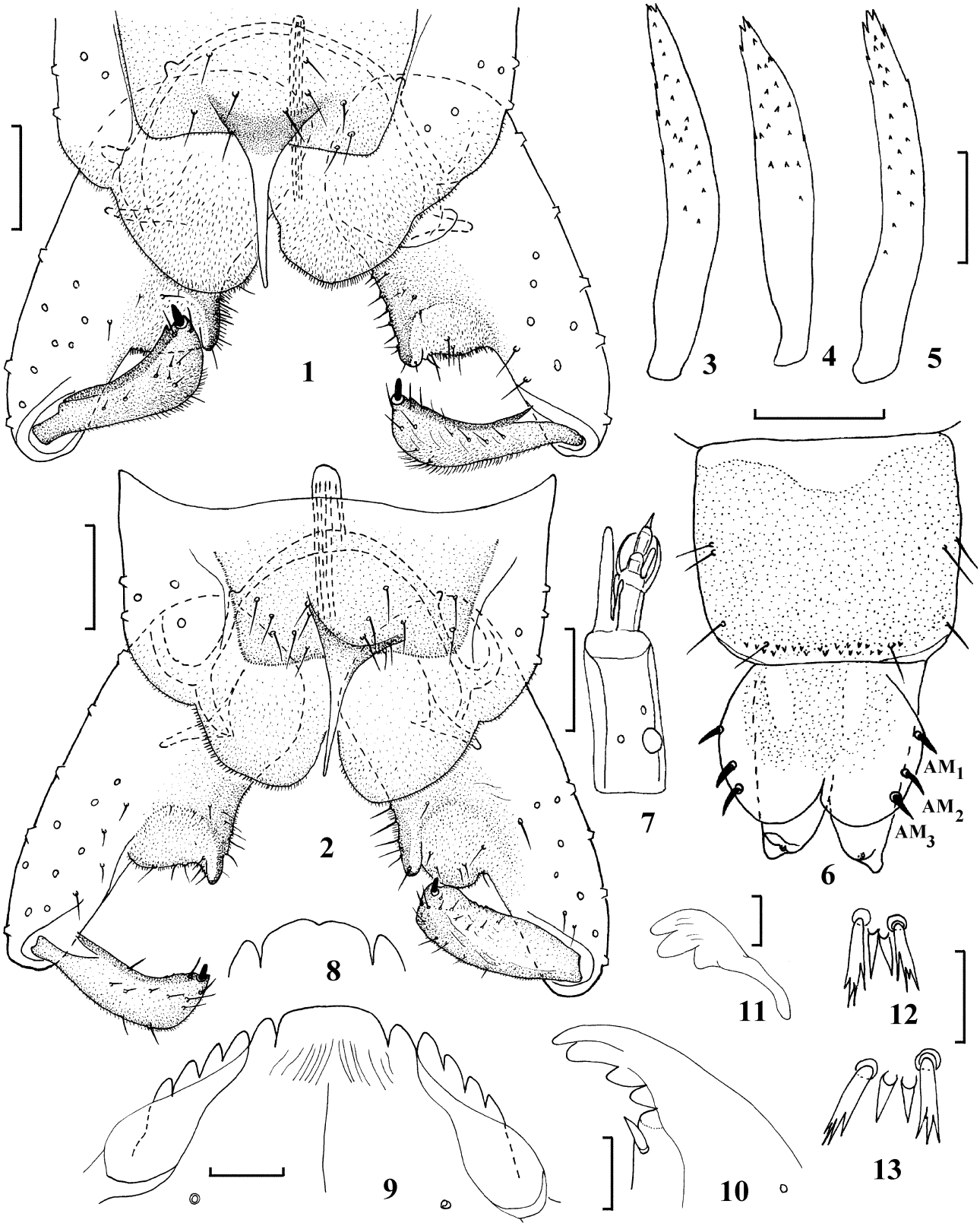
Larvae were associated with pupae and adults by DNA.

Adult male (n=3). Total length 2.0–2.9 mm. Wing length 1.96–2.20 mm. Total length/wing length 1.02–1.32. Colouration brown to dark brown.

Head. Eyes pubescent, without dorsomedian prolongations or sometimes only with weak extensions. Temporal setae (from one side): 7 verticals and 2–5 postorbitals. Clypeus with 4–6 setae. Antenna with 13 flagellomeres and well developed plume; AR 1.04–1.18. Lengths of palpomeres 2–5 (in μm): 48–60 : 124–132 : 112–120 : 156–200. Third palpomere with 5–6 sensilla clavata subapically.

Thorax chaetotaxy. Antepronotum with 5–7 lateral setae. Ac 14–18, beginning close to antepronotum; Dc 7–11; Pa 4–5; Scts 6–8, in 1 row.

Wing. Halteres dark. R with 13–17 setae, R_1 with 2 setae, R_{4+5} with 0–1 seta subapically. Costa extension 48–64 μm. Apex of R_{4+5} distal of apex M_{3+4}. Cu_1 curved in apical quarter. Anal lobe developed, rectangular-rounded. Squama with 6–8 setae.



FIGURES 1–13. *Chaetocladius egorych* **sp. nov.**, adult male (1–2), pupa (3–6) and fourth instar larva (7–13). **1–2,** hypopygium in dorsal view; **3–5,** thoracic horn; **6,** tergite VIII and anal segment of male; **7,** antenna; **8,** median teeth of mentum; **9,** mentum; **10,** mandible; **11,** premandible; **12–13,** S_1 and labral lamellae. AM_1—anterior anal macroseta, AM_2—middle anal macroseta, AM_3—posterior anal macroseta. Scale bars—Figs. 1–5—50 µm; Fig. 6—200 µm; Figs. 7–13—20 µm.

Legs. Spur of fore tibia 56–60 μm. Spurs of mid tibia 28–32 μm and 24 μm long, of hind tibia 56–64 μm and 20–24 μm long. Hind tibial comb with 14 setae. Pulvilli small. Length (in μm) and proportions of leg segments as in Table 1.

TABLE 1. Lengths (in μm) and proportions of leg segments of *Chaetocladius egorych* **sp. nov.,** male (n=3).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	768–832	928–1040	560–640	320–368	240	144–160	112–120
P_2	784–896	848–960	384–432	224–256	176–192	112–144	112–120
P_3	896–992	992–1152	576–656	304–368	264–288	160–176	112–128

continued.

	LR	BV	SV	BR
P_1	0.60–0.63	2.76–2.84	2.87–3.0	2.2–2.4
P_2	0.45–0.46	3.12–3.40	4.15–4.30	2.2–2.6
P_3	0.57–0.58	2.91–2.94	3.22–3.28	3.2–4.0

Hypopygium (Figs. 1–2). Tergite IX with 18–21 long setae and narrowly triangular anal point 60–72 μm long and 16–20 μm wide in base. Anal point in base with microtrichia, the remaining part naked. Laterosternite IX with 7–9 setae on each side. Transverse sternapodeme rounded, 108–112 μm long, with short rounded oral projections. Virga 72–80 μm long, consisted of several setae. Gonocoxite 212 μm long; inferior volsella as in Figs. 1–2. Gonostylus 88–92 μm long, with small subapical crista dorsalis in form of tooth, megaseta 8–12 μm long.

Pupa (n=2). Total length 3.0–3,75 mm.

Cephalothorax. Frontal apotome with 2 setae 36–60 μm long. Thorax light brown and smooth. Antepronotum with 2 median and 1 lateral antepronotals 72–80 μm long. Thoracic horn 104 μm (females) or 108–160 μm long (males) and 16–20 μm wide, tapering to pointed apex, with small spinules in distal part (Figs. 3–5). Precorneal setae hair-like, arranged as triangle or one after the other, 60–84 μm long. Distance between Pc_1 and Pc_2 8–12 μm; distance between Pc_2 and Pc_3 8 μm. Dorsocentrals hair-like, 48–60 μm long. Distance between Dc_1 and Dc_2 44–64 μm; between Dc_2 and Dc_3 108 μm; between Dc_3 and Dc_4 24–28 μm.

Abdomen. Tergite I without shagreen and only with not numerous spinules on each side along posterior edge. Tergites II–VIII uniformly covered with shagreen spinules of one size and with 1–2 rows of larger spines near posterior edge; anal lobes with not numerous spinules (Fig. 6). Sternites I–III without shagreen of spinules; sternite IV with small spinules in distal half; sternites V–VIII covered with small spinules of one size; sternite IX without shagreen. Segment I with 2 pair of hair-like lateral setae 60–100 μm long. Segments II–VII with 4 pairs of lateral setae: 2 pairs of seta-like setae on small tubercles (LS_1 and LS_3) and 2 pairs of hair-like setae (LS_2 and LS_4). Segment VIII with 3 pairs of hair-like lateral setae. Anal lobe 228–240 μm long, with 3 spine-like macrosetae 40–56 μm long and *ca* 8 μm wide. Distance from base of anal lobe to anterior macroseta (AM_1) 0.45–0.50 (males) or 0.57 (female) of anal lobe length. Location of anal megasetae illustrated in Figs. 6, 35. Male genital sac extending 64–72 μm beyond anal lobe.

Forth instar larva (n=3). Total length 4.9–5.6 mm.

Head. Head capsule brown, more light in lateral parts; 270 μm long and 230–240 μm wide. S_I of labrum branched into 3–4 short and pointed branches, sometimes serrated laterally (Figs. 12–13), S_{II}–S_{IV} simple. Labral lamella paired, high triangular shape (Fig. 12–13). Pecten epipharyngis consisted of 3 scales which sometimes stick together and look like a plate. Premandible with two apical teeth and one inner tooth, with brush (Fig. 11). Antenna 56–60 μm long, with 5 segments; one large and one small ring organs in proximal half of basal segment, third ring organ little distal of large organ; segment 2 on apex with large lauterborn organs and its tip reaches middle of segment 4; longest branch of blade ending about apex of segment 4 (Fig. 7); AR 1.33–1.50. Mandible with 4 teeth, apical tooth shorter to combined width of 3 inner teeth; seta subdentalis tapering to pointed apex (Fig. 10); seta interna with 7 simple plumose branches. Pecten galearis weakly developed and very often invisible. Mentum with 1 median tooth and 5 pairs of lateral teeth; middle tooth almost 4 times wider than first lateral tooth; middle tooth of one larva with notch in middle (Fig. 8). Ventromental plate large and elongate, extends beyond the lateral teeth

(Fig. 9). Anal tubules shorter of posterior parapods. Procercus 28 µm long and 20 µm wide, bearing 7 apical anal setae of different length, namely 3 setae 180–240 µm long and 4 setae 108–160 µm long; 2 lateral setae short and thin.

Diagnostic characters. Adult male of the new species is close related to C. *longivirgatus* Stur et Spies but virga of hypopygium *C. egorych* **sp. nov.** consisted of several not fused setae about the same length and gonostylus slightly expanded distally. Virga of C. *longivirgatus* with up to 5 long, thin spines and 6–16 shorter spines near base; gonostylus without significant expansions (Stur & Spies 2011). Also see the key and results of barcoding (Fig. 45).

Etymology. The species is named in memory of our friend, Russian hydrobiologist Sergey Egorovitch Sirotsky, often called by us Egorych. The name is a noun in apposition.

Chaetocladius fedotkin Makarchenko *et* Makarchenko
(Figs. 14–15, 39)

Chaetocladius fedotkin Makarchenko *et* Makarchenko, 2013: 390, Figs. 1–3; Makarchenko *et al.* 2014: 424.

Remarks. This species was described from the Amur River basin for adult male only. Below we describe pupa of this species.

Material. 1 mature pupa of male, Russian Far East, Jewish Autonomous Region, Obluchie District, Lopatinskyi Spring of Bidzhan River basin (Amur River basin), N 48º37'810", E 131º39'114", 2.IV. 2016, leg. E. Makarchenko.

Pupa (n=1). Total length 4.7 mm.

Cephalothorax. Frontal apotome with 2 setae 84 µm long. Antepronotum with 2 median and 1 lateral antepronotals *ca* 80 µm long. Thoracic horn 224–240 µm long and 24–28 µm wide in basal 1/4, with large spinules, namely in distal 3/4; in basal 1/4 spinules located in lesser degree (Figs. 14–15). Precorneal setae hair-like, arranged as triangle, 60–64 µm long. Dorsal surface of thorax little roughened. Dorsocentrals broken off. Distance between Dc_1 and Dc_2 84 µm; between Dc_2 and Dc_3 96 µm; between Dc_3 and Dc_4 12 µm.

Abdomen. Tergite I without shagreen. Tergites II–VIII uniformly covered with shagreen spinules of one size and with 1–2 rows of larger spines near posterior edge, longest of which 4.4–5.3 µm; anal lobes with not numerous spinules (Fig. 39). Sternites I–III without shagreen of spinules; sternite IV with small spinules along posterior edge in middle part; sternites V–VII covered with small spinules, size of which large near posterior edge; sternite VIII uniformly covered with shagreen spinules of one size; sternite IX without shagreen. Segment I with 2 pair of hair-like lateral setae. Segments II–VII with 3 pairs of strong lateral setae on small tubercles and 1 pair weak hair-like setae. Segment VIII with 2 pairs of strong lateral setae on small tubercles and 1 pair with weak hair-like lateral setae. Anal lobe 320 µm long, with 3 spine-like macrosetae; anterior macroseta (AM_1)112 µm long and 8 µm wide; middle macroseta (AM_2) 132 µm long and 10 µm wide; posterior macroseta (AM_3) 116–124 µm long and 8 µm wide. Location of anal megasetae illustrated in Fig. 39. Male genital sac extending 40 µm beyond anal lobe.

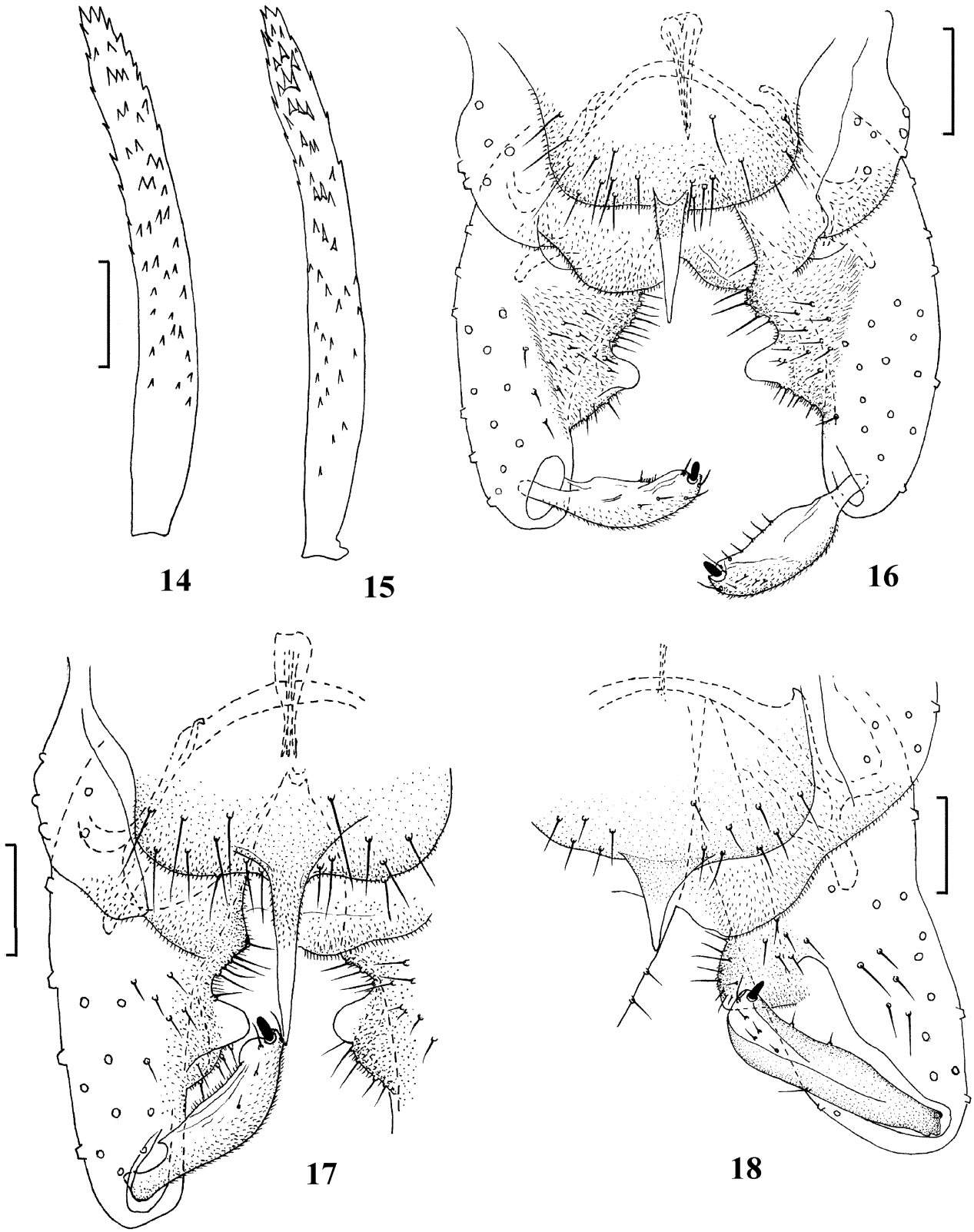
Larva unknown.

Distribution. Known only from type locality in the Amur River basin.

Chaetocladius ligni Cranston *et* Oliver
(Figs. 16–17)

Chaetocladius ligni Cranston *et* Oliver, 1988: 144, Figs. 1–13; Makarchenko & Makarchenko 2011: 111; Makarchenko *et al.* 2014: 424; Ashe & O'Connor 2012: 171.

Remarks. This species was described as adult male, female, pupa and larva from North America (Cranston & Oliver 1988), and later was recorded by us for the Russian Far East (Makarchenko & Makarchenko 2011b). The present revision of *Chaetocladius* s. str. from the Amur River basin indicates differences in the structure of the males from the Nearctic and East Palearctic regions. In this regard, we have found it appropriate to redescribe the adult male on the basis of our material and to present the results of DNA analysis that will allow a detailed comparing the known populations of *C. ligni* in the future.



FIGURES 14–18. *Chaetocladius fedotkin* Makarchenko et Makarchenko (14–15), *Chaetocladius ligni* Cranston et Oliver (16–17) and *Chaetocladius piger* Brundin (18), pupa (14–15) and adult male (16–18). **14–15,** thoracic horn; **16–18,** hypopygium in dorsal view. Scale bar: 50 µm.

Material. 3 males, Russian Far East, Jewish Autonomous Region, Obluchie District, Fedotkin Spring of Bidzhan River basin (Amur River basin), N 48º38'409", E 131º37'217", 6.IV. 2014, leg. E. Makarchenko.

Adult male (n=2–3). Total length 3.0–3.6 mm. Wing length 2.16–2.48 mm. Total length/wing length 1.33–1.45. Colouration brown.

Head. Eyes bare, with rounded short dorsomedian. Temporal setae include (from one side) 5–6 verticals and 5–7 postorbitals. Clypeus with 6–14 setae. Antenna with 13 flagellomeres and well developed plume; flagellomere 13 subapical with seta; AR 0.94–1.06. Lengths of palpomeres 2–5 (in μm): 52 : 160 : 148 : 260. Third palpomere in subapical part with 7 sensilla clavata.

Thorax. Brown, with dark brown stripes; humeral pit with light spot. Antepronotum with 9–11 lateral setae. Acrostichals 16–19; dorsocentrals 11–21 (in 1–2 rows); prealars 4–7; scutellum with 7–8 setae in 1 row.

Wing. Halteres light. R with 20–21 setae, R_1 with 3–8 setae, R_{4+5} with 20–25 setae. Costa extension *ca* 60 μm. Apex of R_{4+5} distal of apex M_{3+4}. Cu_1 curved in apical quarter. Anal lobe developed, wide rectangular-rounded. Squama with 7–8 setae.

Legs. Spur of fore tibia 80 μm. Spurs of mid tibia 24–28 μm long, of hind tibia 84 μm and 20 μm long; $ta_1$and ta_2 of mid and hind legs with 2 pseudospurs. Hind tibial comb with 13–15 setae. Length (in μm) and proportions of leg segments as in Table 2.

TABLE 2. Lengths (in μm) and proportions of leg segments of *Chaetocladius ligni* Cranston et Oliver, male (n=2).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	928–1024	1024–1184	768–896	384–416	272–304	192–224	128–144
P_2	912–1056	944–1104	448–544	272–304	208–224	144–160	112–144
P_3	1040–1168	1152–1344	720–832	352–400	304–320	192–208	144–160

continued.

	LR	BV	SV	BR
P_1	0.75–0.76	2.79–2.85	2.46–2.54	2.1
P_2	0.47–0.49	3.13–3.25	3.97–4.14	2.5
P_3	0.62–0.64	2.93–3.07	3.02–3.04	2.6

Hypopygium (Figs. 16–17). Tergite IX with 18–21 long setae and long triangular anal point 56–64 μm long (n=2) (Fig. 16) and 92 μm long (n=1) (Fig. 17). Anal point in basal 1/3–1/4 with microtrichia, the remaining part naked. Laterosternite IX with 6–7 setae on each side. Transverse sternapodeme rounded, 92 μm long, with weak oral projections. Virga 56 μm long, consists of some setae. Gonocoxite 200–215 μm long; inferior volsella as in Figs. 16–17. Gonostylus 84–88 μm long, with rounded preapical crista dorsalis, megaseta 10–12 μm long.

Pupa and ***larva*** are described by Cranston & Oliver (1988), but absent in our material.

Distribution. *C. ligni* is known from North America (U.S.A., Oregon) and the Russian Far East.

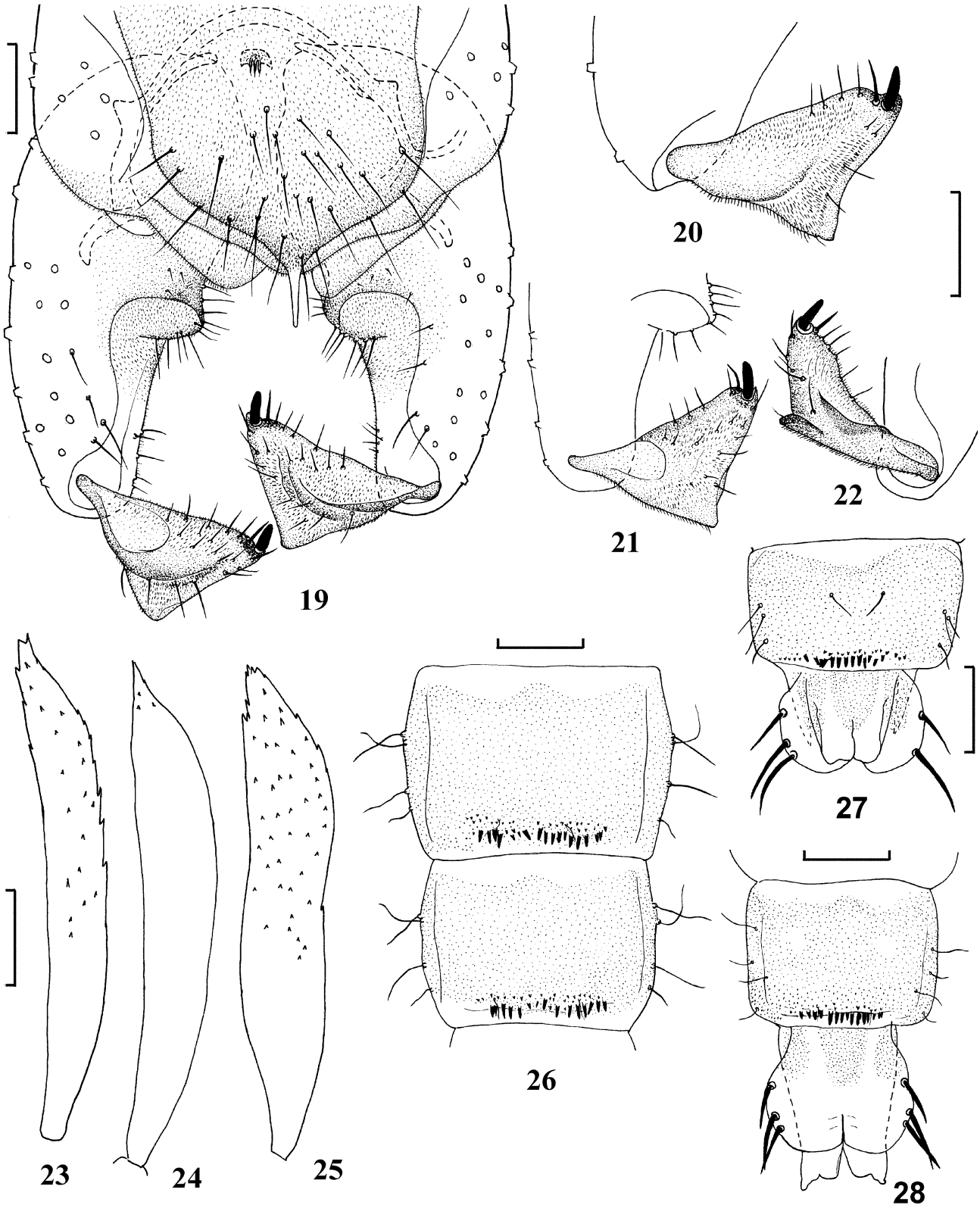
Chaetocladius lopatinskiy Makarchenko *et* Makarchenko, sp. nov.

(Figs. 19–28, 40–41)

Chaetocladius laminatus Brundin, 1947; Makarchenko *et al.* 2014: 424, misidentification.

Material. Holotype: adult male, Russian Far East, Jewish Autonomous Region, Obluchie District, Lopatinskyi Spring of Bidzhan River basin (Amur River basin), N 48º37'810", E 131º39'114", 31.III. 2015, leg. E. Makarchenko. Paratypes: 1 mature pupa of male, the same data as holotype, 31.III. 2015; 2 males, 1 mature pupa of male, the same data as holotype except , Fedotkin Spring of Bidzhan River, N 48º38'409", E 131º37'217", 5.IV. 2014, leg. E. Makarchenko.

Adult male (n=3). Total length 3.5–4.0 mm. Wing length 2.28–2.76 mm. Total length/wing length 1.27–1.62. Colouration brown to dark brown.



FIGURES 19–28. *Chaetocladius lopatinskiy* **sp. nov.**, adult male (19–22) and pupa (23–28). **19,** hypopygium in dorsal view; **20–22,** gonostylus in varies positions; **23–25,** thoracic horn; 2**6,** tergites VI–VII; **27,** tergite VIII and anal segment of female; **28,** the same of male. Scale bars—Figs. 19–25—50 μm; Figs. 26–28—200 μm.

Head. Eyes bare, with dorsomedian prolongation. Temporal setae include (from one side) 6–11 verticals and 5–10 postorbitals. Clypeus with 8–10 setae. Antenna with 13 flagellomeres and well developed plume; top of

flagellomere 13 pointed; AR 2.14–2.33. Lengths of palpomeres 2–5 (in μm): 60–64 : 164–182 : 164–172 : 244–264. Third palpomere in distal part with 10 sensilla clavata.

Thorax. Dark brown. Antepronotum with 2–7 lateral setae. Acrostichals 16–23, beginning close to antepronotum; dorsocentrals 11–15; prealars 5–6; scutellum with 6–8 setae in 1 row.

Wing. Halteres dark brown. R with 9–13 setae, R_1 with 1–2 setae, R_{4+5} with 0–2 setae subapically. Costa extension 60–96 μm. Apex of R_{4+5} distal of apex M_{3+4}. Cu_1 curved in apical quarter. Anal lobe developed, rectangular-rounded. Squama with 13–16 setae.

Legs. Spur of fore tibia 64–84 μm. Spurs of mid tibia 28–32 μm and 24–28 μm long, of hind tibia 64–72 μm and 22–28 μm long; ta_1 of fore leg with 0–2 pseudospurs; ta_1– ta_3 of mid and hind legs 2 pseudospurs. Hind tibial comb with 15–18 setae. Pulvilli small. Length (in μm) and proportions of leg segments as in Table 3.

TABLE 3. Lengths (in μm) and proportions of leg segments of *Chaetocladius lopatinskiy* **sp. nov.,** male (n=3).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	912–1024	1040–1200	704–800	400–480	320–352	192–240	144–160
P_2	944–1088	1008–1136	464–512	280–320	224–256	144–176	128–144
P_3	1008–1216	1184–1360	688–768	400–448	304–352	192–208	144–160

continued.

	LR	BV	SV	BR
P_1	0.66–0.68	2.45–2.62	2.77–2.84	2.3–2.7
P_2	0.45–0.46	3.04–3.11	4.21–4.31	2.2–2.3
P_3	0.56–0.58	2.77–2.90	3.19–3.41	2.9–3.5

Hypopygium (Figs. 19–22, 40). Tergite IX with 24–35 long setae and narrow naked anal point 28–40. Laterosternite IX with 6–8 setae on each side. Transverse sternapodeme rounded, 144–156 μm long, with oral projections like tubercles. Virga 16–32 μm long, consists of 2–3 small spines. Gonocoxite 268–304 μm long; inferior volsella as a rounded projection covered with microtrichia and setae (Figs. 19, 40). Gonostylus 112–120 μm long, triangular shape, with megaseta 16 μm long and strong long seta close (Figs. 19–21). In some positions gonostylus might look like with projection (Fig. 22).

Pupa (n=2). Total length 4.0–4.65 mm.

Cephalothorax. Frontal apotome with 2 setae 56–88 μm long. Antepronotum with 2 median and 1 lateral antepronotals 80–96 μm long. Thorax yellowish-brown and smooth. Thoracic horn 232–280 μm long, with pointed apex and covered with small spinules in distal 2/3, but sometimes spinules only in subapical part (Figs. 23–25). Precorneal setae hair-like, 64–112 μm long. Dorsocentrals hair-like, 44–120 μm long. Distance between Dc_1 and Dc_2 96–136 μm; between Dc_2 and Dc_3 28–32 μm; between Dc_3 and Dc_4 20–30 μm.

Abdomen. Tergite I with shagreen of not numerous spinules. Tergites II–VIII uniformly covered with shagreen spinules of one size and with large spines (20–44 μm long) along posterior edge (Figs 26–28); anal lobes with not numerous spinules in proximal part (Figs. 27–28). Sternites I–II without shagreen of spinules; sternite III with group of 7–16 spinules in middle part near posterior edge; sternites IV–VIII with shagreen of spinules as in tergites but spines near posterior edge not so large. Segment I with 0–1 pair of hair-like lateral setae. Segments II–VII with 2 pairs of seta-like lateral setae on tubercles and 2 pairs of hair-like setae. Length of lateral setae of segment IV: LS_1 (seta-like on tubercle) 116 μm; LS_2 (hair-like) 136 μm; LS_3 (seta-like on tubercle) 116 μm; LS_4 (hair-like) 80 μm. Segment VIII with 3–4 pairs of hair-like lateral setae 60–88 μm long. Anal lobe 280–320 μm long, with 3 spine-like macrosetae; anterior macroseta (AM_1)104–136 μm long and 8 μm wide; middle macroseta (AM_2) 148–168 μm long and 8 μm wide; posterior macroseta (AM_3) 112–164 μm long and 8 μm wide. Distance from base of anal lobe to AM_1 0.44–0.52 of anal lobe length. Distance between AM_1 and AM_2 64–80 μm; between AM_2 and AM_3 24–32 μm. Location of anal megasetae illustrated in Figs. 27–28, 41. Male genital sac extending 40 μm beyond anal lobe.

Diagnostic characters. This new species is closely related to *C. laminatus* Brundin, but the pupa of *C. lopatinskiy* **sp. nov.** has a longer body (3.95–4.1 mm vs. 3.0–3.5 mm), thoracic horn (232–280 μm vs 120–138 μm)

and anal macrosetae (108–168 μm vs. 25–30 μm) (cf. Langton & Visser 2003). See also the results of barcoding (Fig. 45) and the key.

Etymology. The species is named after the type locality: the Lopatinskiy Spring; the name is a noun in apposition.

Chaetocladius piger (Goetghebuer)

(Fig. 18)

Dactylocladius piger Goetghebuer, 1913: 157, Figs. 8–9.
Chaetocladius piger (Goetghebuer); Goetghebuer 1940-50: 62 (as subgenus of *Orthocladius*); Pankratova 1970: 234; Caldwell 1997: 118; Langton & Pinder 2007: 34, Fig. 143, A; Ashe & O'Connor 2012: 174.
Hydrobaenus (*Chaetocladius*) *stamfordi* Johannsen, 1947: 171; Cranston *et al.* 1989: 267, Fig. 9.13, A.
Chaetocladius oliveri Sæther, 1969: 93.

Remarks. *Chaetocladius piger,* known from many sites in the Palaearctic and Nearctic regions, but is recorded for the first time for the Amur River basin and for the Russian Far East. The male from our material fits the description of this species and has following main features. Total length 3.9 mm. Wing length 2.32 mm. Total length/wing length 1.68. AR 1.54. Thorax brown. Acrostichals 19, dorsocentrals 9, prealars 6, scutellum with 11 setae. LR_1 0.67. Hypopygium as in Fig. 18. Anal point length 48 μm. Gonostylus length 116 μm.

Material. 1 adult male, Russian Far East, Jewish Autonomous Region, Obluchie District, Lopatinskyi Spring of Bidzhan River basin (Amur River basin), N 48º37'810", E 131º39'114", 7.IV. 2014, leg. E. Makarchenko.

Distribution. Holarctic species.

Chaetocladius yavorskayae Makarchenko *et* Makarchenko, sp. nov.

(Figs. 29–33, 42–44)

Chaetocladius sp.1 Makarchenko *et al.* 2014: 424.

Material. Holotype: adult male, Russian Far East, Jewish Autonomous Region, Obluchie District, Lopatinskyi Spring of Bidzhan River basin (Amur River basin), N 48º37'810", E 131º39'114", 31.III. 2015, leg. E. Makarchenko. Paratypes: 1 mature pupa of male, the same data as holotype, 31.III. 2015, leg. E. Makarchenko; 3 males, the same data as holotype except, Fedotkin Spring of Bidzhan River basin, N 48º38'409", E 131º37'217", 6.IV. 2014, leg. E. Makarchenko; 2 males, the same data as holotype except, Kolgaevskiy Spring of Bidzhan River basin, N 48º38'522", E 131º54'788", 6.IV. 2014, leg. E. Makarchenko.

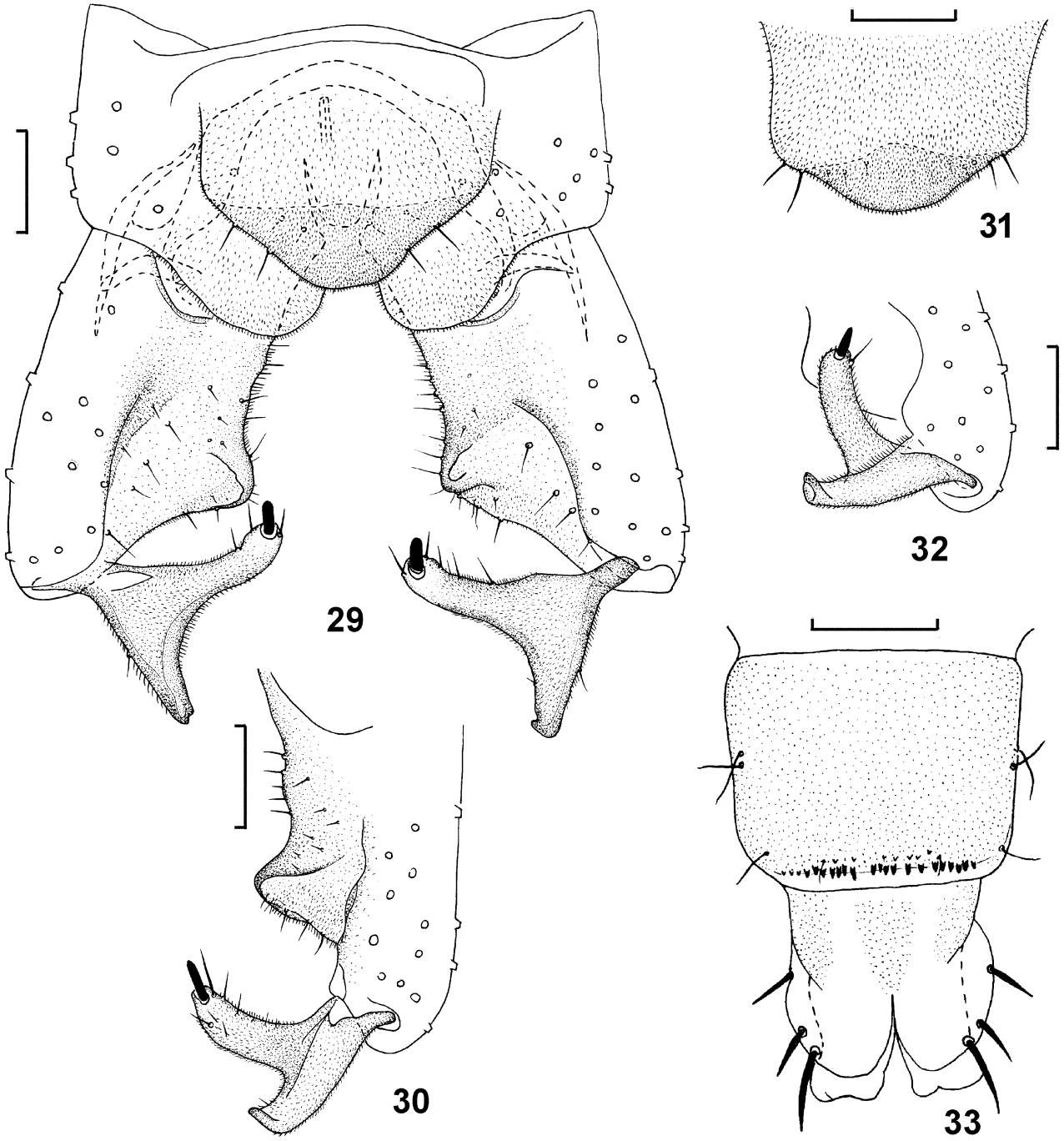
Adult male (n=3). Total length 3.2–3.7 mm. Wing length 2.32–2.56 mm. Total length/wing length 1.38–1.45. Colouration brown to dark brown.

TABLE 4. Lengths (in μm) and proportions of leg segments of *Chaetocladius yavorskayae* **sp. nov.**, male (n=3).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	944–1024	1088–1200	720–752	304–400	272–288	192	128–144
P_2	976–1056	992–1088	448–512	280–288	192–208	128–144	128
P_3	1088–1168	1232–1344	720–768	384–416	320–336	176–192	144

continued.

	LR	BV	SV	BR
P_1	0.63–0.66	2.86–3.07	2.82–2.96	2.2
P_2	0.45–0.50	3.29–3.44	3.94–4.39	2.3–2.8
P_3	0.57–0.58	2.94–3.11	3.17–3.27	3.0



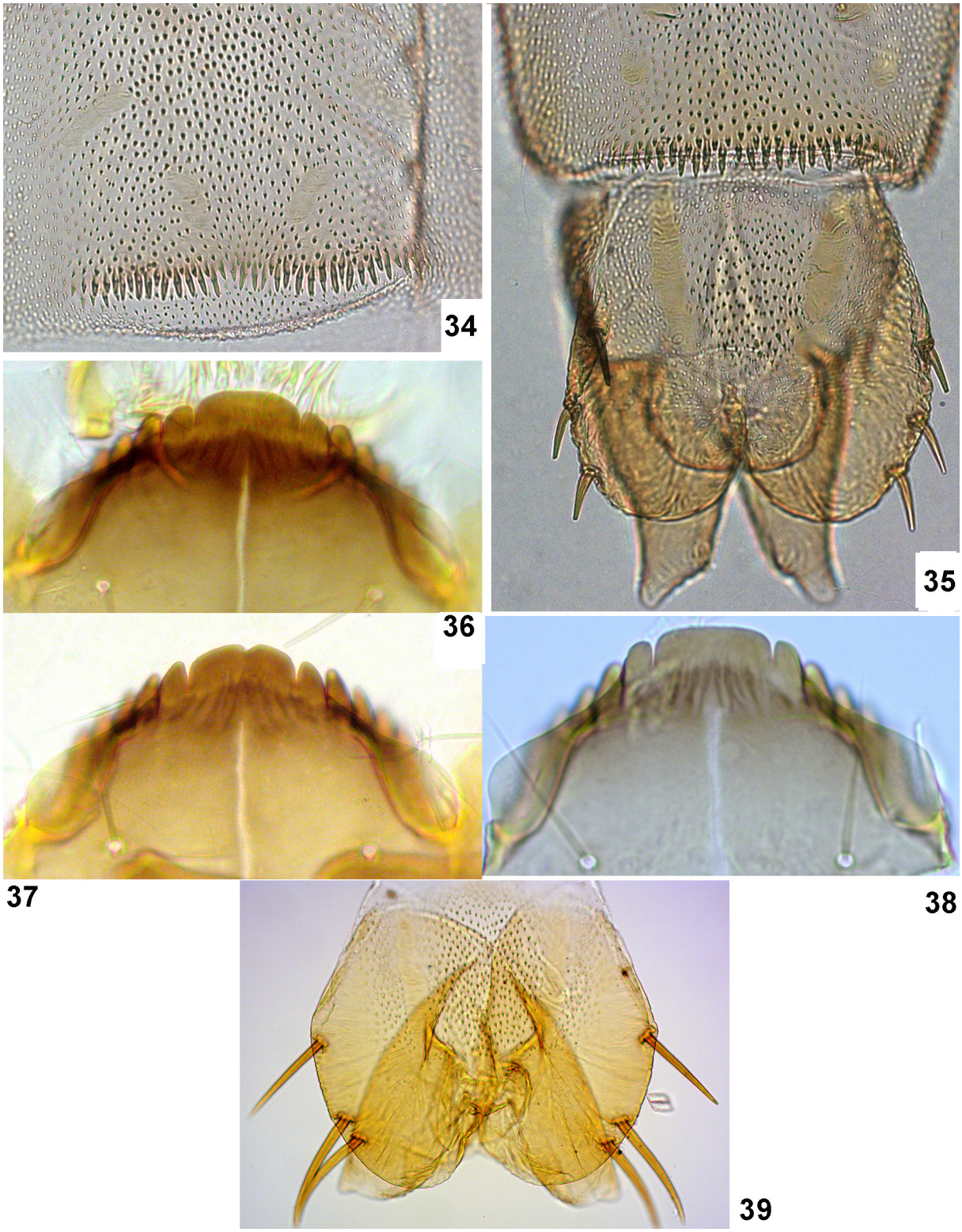
FIGURES 29–33. *Chaetocladius yavorskayae* **sp. nov.**, adult male (29–32) and pupa (33). **29,** hypopygium in dorsal view; **30,** gonocoxite and gonostylus; **31,** tergite IX; **32,** gonostylus; **33,** tergite VIII and anal segment of male. Scale bars: Figs. 29–32—50 μm; Fig. 33—200 μm.

Head. Eyes bare, without dorsomedian prolongations. Temporal setae include (from one side) 7–8 verticals and 5–6 postorbitals. Clypeus with 6–8 setae. Antenna with 13 flagellomeres and well developed plume; AR 1.91–2.38. Lengths of palpomeres 2–5 (in μm): 48–52 : 144–168 : 128–176 : 252–296. Third palpomere in distal part with 8–10 sensilla clavata.

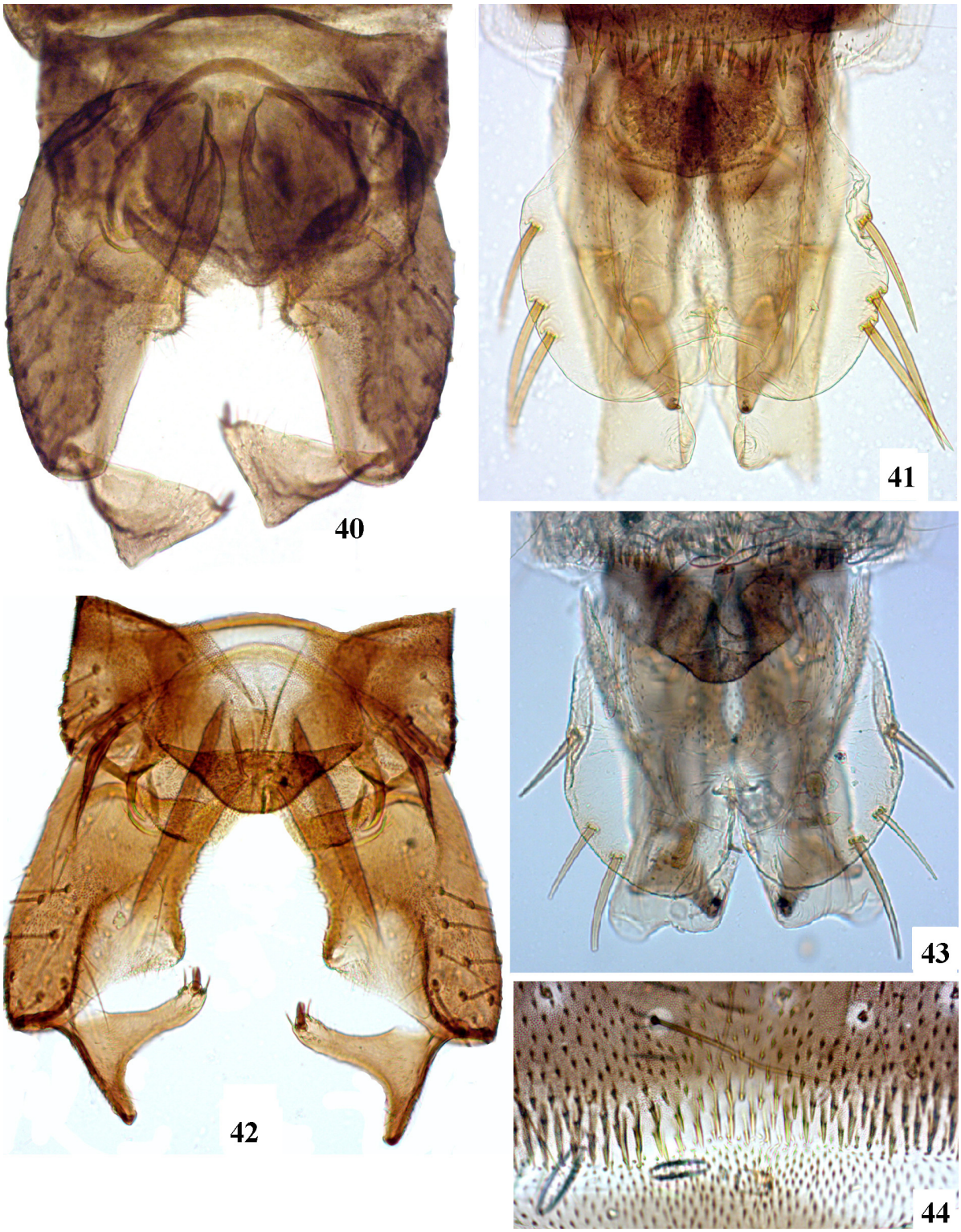
Thorax. Brown. Antepronotum with 5–6 lateral setae. Acrostichals 15–21, beginning close to antepronotum; dorsocentrals 11–16; prealars 3–4; scutellum with 7–8 setae in 1 row.

Wing. Greyish, with more dark veins and good visible microtrichia. Halteres brownish-yellow. R with 10–11 setae, R_1 and R_{4+5} without setae. Costa extension 56–80 μm. Apex of R_{4+5} distal of apex M_{3+4}. Cu_1 curved in apical part. Anal lobe developed, rectangular-rounded. Squama with 9–13 setae.

Legs. Spur of fore tibia 76–80 µm. Spurs of mid tibia 24–32 µm and 28–32 µm long, of hind tibia 60–68 µm and 16–28 µm long;ta_1 and ta_2 of mid leg 2 pseudospurs, ta_3 with 1–2 pseudospurs; ta_1–ta_3 of hind leg with 2 pseudospurs. Hind tibial comb with 15–19 setae. Pulvilli small. Length (in µm) and proportions of leg segments as in Table 4.



FIGURES 34–39. *Chaetocladius egorych* **sp. nov.** (34–38) and *Chaetocladius fedotkin* Makarchenko et Makarchenko (39), pupa (34–35, 39) and fourth instar larva (36–38). **34,** tergite IV; **35,** tergite VIII and anal segment of male; **36–38,** mentum; **39,** anal segment of male.



FIGURES 40–44. *Chaetocladius lopatinskiy* **sp. nov.** (40–41) and *Chaetocladius yavorskayae* **sp. nov.** (42–44), adult male (40, 42) and pupa (41, 43–44). **40, 42,** hypopygium in dorsal view; **41, 43,** anal segment of male; **44,** spinules of tergite IV.

Hypopygium (Figs. 29–33, 42). Tergite IX without anal point, trapezoidal,densely covered with microtrichia, its free edge as it is curled inwards, in inner side and on the edge with middle and long 11–19 setae (Fig. 31). Laterosternite IX with 8–9 setae on each side. Transverse sternapodeme rounded, 108–112 µm long, with weak oral projections. Virga 40–48 µm long, consists of 2–3 colorless setae. Gonocoxite 260 µm long; inferior volsella as a rounded projection located in distal half of gonocoxite, covered with microtrichia and setae (Figs. 29–30). Gonostylus bifurcate, inner branch 104–108 µm long, with megaseta 14–20 µm long (Figs. 29–30, 32, 42**).**

Pupa (n=1). Cephalothorax was destroyed and we had chance to study only abdomen.

Abdomen. Tergite I without shagreen. Tergites II–VIII uniformly covered with shagreen spinules of one size and with larger long spinules in 2–3 rows along posterior edge, behind long spinules on tergites III–VII located small spinules tops of which are directed forward (Fig. 44). Sternites I–III, VII–VIII without shagreen of spinules; sternite IV with row of small spinules near posterior edge; sternites V–VI with small spinules on surface. Segment I with 2 pairs of hair-like lateral setae. Segments II–VII with 2 pairs of seta-like lateral setae on tubercles and 2 pairs of hair-like setae. Segment VIII with 3 hair-like setae. Anal lobe 284 µm long, with 3 spine-like macrosetae; anterior macroseta (AM_1) 80 µm long and 8 µm wide; middle macroseta (AM_2) 76–80 µm long and 6 µm wide; posterior macroseta (AM_3) 116 µm long and 8 µm wide. Distance from base of anal lobe to AM_1 0.49 of anal lobe length. Distance between AM_1 and AM_2 68 µm; between AM_2 and AM_3 20 µm. Location of anal megasetae illustrated in Figs. 33, 43. Male genital sac extending 32–36 µm beyond anal lobe.

Diagnostic characters. A new species is separate from all known species of *Chaetocladius* s. str. by absence of anal point and bifurcate gonostylus. See also the results of barcoding (Fig. 45) and key.

Etymology. The species is named in honour of Dr. N.M. Yavorskaya from the Institute of Water and Ecological Problems FEB RAS (Khabarovsk), the hydrobiologist who collected the more specimens of chironomids in the Amur River basin for describing of a new species.

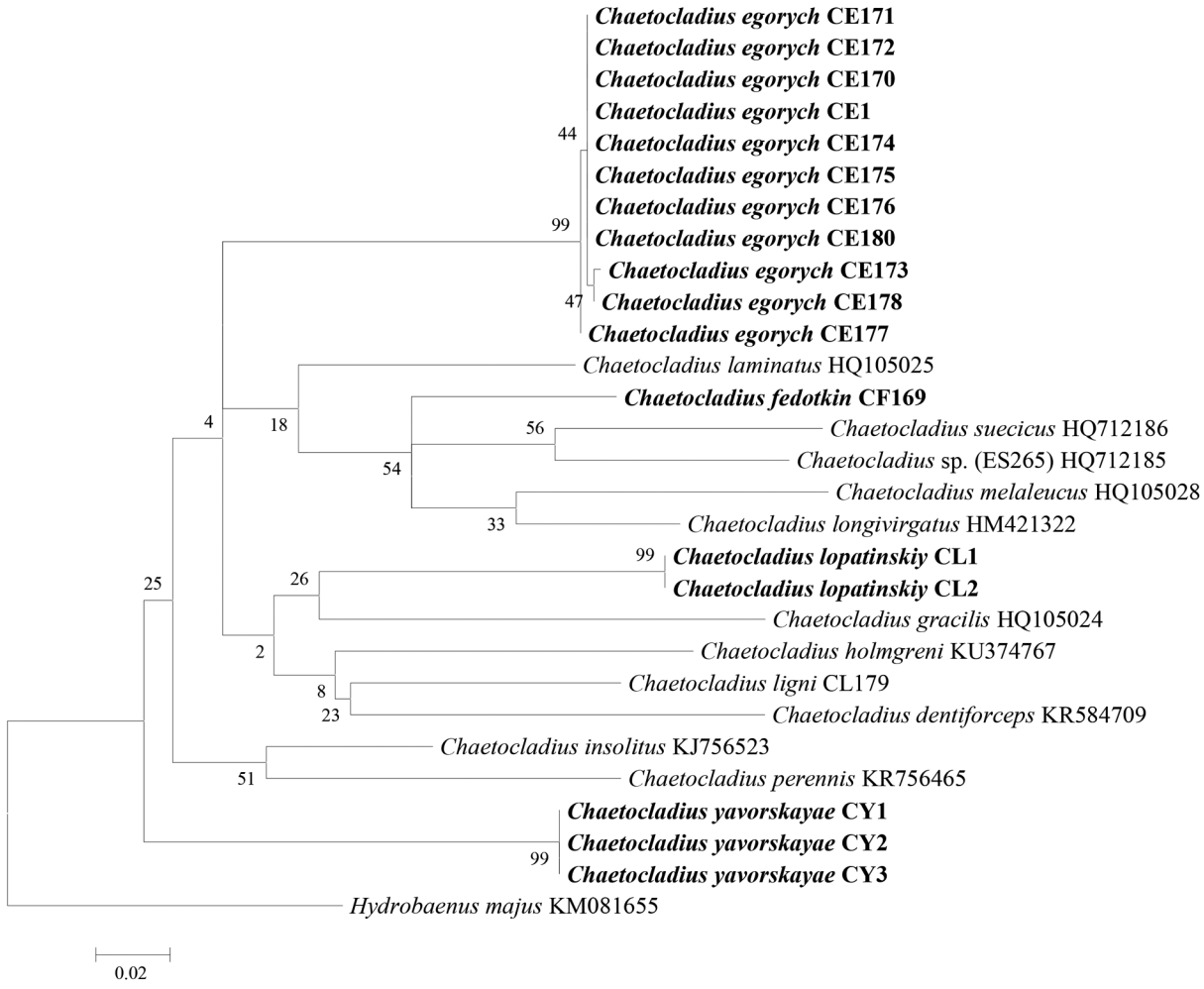


FIGURE 45. Phylogenetic tree of the fifteen *Chaetocladius* species and one out-group, *Hydrobaenus majus* Makarchenko et Makarchenko (Orthocladiinae), based on the mitochondrial cytochrome c oxidase I (COI) barcode gene sequences (658 bp). The numbers are support values for 500 bootstraps. The specimens obtained in this study are in bold.

Results of DNA barcoding

Genetic variability within the 658 base pair barcode region of COI was examined for 18 individuals belonging to 5 species of the subgenus *Chaetocladius* s. str.—*C. egorych* **sp. nov.**, *C. fedotkin*, *C. ligni*, *C. lopatinskiy* **sp. nov.** and *C. yavorskayae* **sp. nov.** Interspecific sequence divergence for five species based on K2P distance ranged from 13.9–16.5% (mean 15.3%). According to Montagna *et al.* (2016) and Ekrem *et al.* (2010) these values are sufficient to maintain the species level. Only one substitution was non-synonymous and changed in *C. fedotkin* glycine to serine in protein sequensis (position 119). For all barcodes of the subgenus *Chaetocladius* s. str. in GenBank identified to species level K2P distance ranged from 10.6–18.9% (mean 14.9%). Thus, 5 species described in this work have high interspecific distance that complies with other species of the subgenus *Chaetocladius* s. str.

Within the specimens of *C. egorych* (n=11), seven haplotypes were encountered, which are divergent by eight synonymous substitutions among which 7 transitions and one transversion. Two haplotypes detected in the sequence of *C. yavorskayae* are formed one synonymous transition substitution. Other species of the subgenus *Chaetocladius* s. str. had one haplotype, or represented by a single sequencing. Intraspecific distances for 5 analysed species varied between 0–0.29% which is lower than values shown by other authors (Ekrem *et al.* 2010; Montagna *et al.* 2016).

The monophyly of the species studied, *C. egorych* **sp. nov.**, *C. fedotkin, C. lopatinskiy* **sp. nov.**, *C. ligni* and *C. yavorskayae* **sp. nov.** is strongly supported (Fig. 45). All specimens are evenly distributed on a tree without the closest between different species.

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