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Alaskacladius gen. nov., (Diptera: Chironomidae), a unique new orthoclad from Alaska

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Abstract

We describe a new genus *Alaskacladius* **gen. nov.**, based on the adult stages collected from Alaska, USA, and British Columbia, Canada. Molecular and morphological assessment of adult specimens supports the presence of a new genus. *Alaskacladius* **gen. nov.**, is related to the genera *Doithrix* Sæther & Sublette, 1983; *Georthocladius* Strenzke, 1941; *Parachaetocladius* Wülker, 1959; and *Pseudorthocladius* Goetghebuer, 1932. Based on the molecular analysis result and intergeneric K2P distance obtained from Cytochrome Oxidase I (COI) genes, *Alaskacladius* is closest and forms a sister group with *Doithrix*.

Key words: Orthocladiinae, new genus, Alaska, British Columbia, western Nearctic

Introduction

The subfamily Orthocladiinae is the largest subfamily of Chironomidae worldwide (Ashe and O'Connor, 2012). These authors reported 174 valid genera and 2275 valid species within this subfamily. Since then, this number has increased to nearly 184 genera, with new genera mainly described from the Neotropical region compared to the Nearctic and the Palearctic (Andersen *et al.* 2015a, b, c; Andersen *et al.* 2016; Fasbender 2020; Krasheninnikov 2019; Makarchenko *et al.* 2020; Mendes & Andersen 2013; Mohammadi *et al.* 2021; Namayandeh & Hudson 2022). Only two recent genera have been discovered and described in the Nearctic: *Oropuella* Fasbender, 2020 and *Cedrimyia* Namayandeh & Hudson, 2022. The Nearctic is the second most diversity-rich geographical region for Chironomidae, with around 1092 species and probably another thousand species waiting to be discovered (Ferrington 2008). The extent of faunistic discovery and biodiversity of the Nearctic genera requires consistent taxonomic work and sampling combined with acquiring multiple sampling methods from understudied habitats and regions. Fasbender (2020) attributed the poorly understood Orthocladiinae fauna of temperate western North America to the scarcity of faunistic studies in the western Nearctic.

The discovery of the new genus in this study results from recurring collections of Chironomidae by Patrick Hudson and his son John Hudson since 1994 in Juneau and various parts of the southeast and interior of Alaska. In the present study, we describe a new genus *Alaskacladius* **gen. nov.**, based on the adult males of *Alaskacladius*

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johnhudsoni **sp. nov.**, collected from Mount Roberts in Juneau and Revillagigedo Island, Alaska, and two adult females collected in Vancouver and Haida Gwaii, British Columbia. Our morphological assessment of adult specimens supports the presence of a new genus. Also, the new genus is similar and related to the genera *Doithrix* Sæther & Sublette, 1983; *Georthocladius* Strenzke, 1941; *Parachaetocladius* Wülker, 1959; and *Pseudorthocladius* Goetghebuer, 1932. Based on the molecular analysis result and intergeneric K2P distance obtained from Cytochrome Oxidase I (COI) genes, *Alaskacladius* is closest and forms a sister group with *Doithrix*.

Material and Methods

Sampling collection, preparation, and imagery

The specimens of *A. johnhudsoni* **sp. nov.,** were collected using a sweep net and were preserved in 70% ethanol. The microscope slides were prepared following a procedure outlined in Namayandeh and Hudson (2022). The imagery of *A. johnhudsoni* was produced using a Diagnostic Instruments Inc. Spot 5.1 camera mounted on an Olympus BX51 compound scope. The illustrations for this species were produced based on the obtained images using Inkscape 1.2.2(2022): Draw Freely software. Measurements are given as ranges, followed by a mean when more than three specimens are measured. Morphological terminology and measurements follow those of Sæther (1977, 1980). We used Sæther and Sublette's (1983) key to some genera of Orthocladiinae, with some modifications to construct our key. The holotype and six male paratype specimens of *A. johnhudsoni* **sp. nov.,** are deposited at the Michigan State University, the Albert J. Cook Arthropod Research Collection (ARC), and two female paratypes are deposited at the Centre for Biodiversity Genomics, University of Guelph, Canada (CBG).

Molecular analysis

We extracted, amplified, and sequenced the cytochrome oxidase 1 barcode as described previously by Failla *et al.* (2016) and Vasquez *et al.* (2022). Briefly, the genomic DNA was extracted from the tissues of an adult male (whole specimen) using the Qiagen DNA Blood and Tissue Kit (Qiagen, Inc., Germantown, MD). We amplified the COI's 587 base pair fragment using the universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994). We performed the amplification cycles using iCycler iQ™ Realtime thermocycler (BioRad, Irvine, CA), initiated by heating to 95 °C for 5 min, followed by 40 cycles of 95 °C for 30 s, 51 °C for 30 s, 72 °C for 1 min, and then a final extension of 72 °C for 7 min followed by a hold at 15 °C. The Genewiz (subsidiary of Azenta Life Sciences, South Plainfield, NJ, USA) sequenced the PCR products using Sanger sequencing. We trimmed and assembled the reverse and forward sequences using Bioedit 7.2.5 (Hall 1999). We submitted all newly obtained sequences to the GenBank and BOLD databases.

We used 53 sequences, including one from *Alaskacladius*; one related sequence from British Columbia, Canada, obtained from GenBank (MG141390.1); one sequence from a Chinese species of *Doithrix* Obtained from BOLD (TIBCH403-22); two outgroup sequences (*Procladius denticulatus* MG448792 and GBDPC382-14 *Tanypus neopunctipennis* AB838641); and 48 other sequences from other genera in Orthocladiinae, including available sequences of related genera in BOLD and GenBank, *Parachaetocladius* and *Pseudorthocladius*. The list of sequences, codes, GenBank, or BOLD accessions is provided in Supplementary file 1-Tables S1. We obtained the phylogenetic trees based on two methods, Neighbour-Joining (NJ) and Maximum Likelihood (ML). The NJ phylogenetic tree was constructed using Kimura's 2-parameter (K2P) model in MEGA X with 10,000 bootstrap replications (Kumar *et al.* 2018). To construct the tree using ML, we aligned sequences using Clustal X version 2.1 software (Larkin *et al.* 2007). We constructed the ML trees using RAxML-HPC BlackBox (8.2.12) software (Stamatakis 2014) in the CIPRES Scientific Gateway v.3.3 XSED (Miller *et al.* 2012) and with 10,000 Bootstrap repeats. We visualized the ML tree in FigTree v. 1.4.2 (Rambaut 2014).

The K2P model calculated intraspecific between our sequence and sequence number MG141390.1, using MEGA X software (Kumar *et al.* 2018). The K2P model was also used to calculate the intergeneric genetic distances between the 51 Orthocladiinae sequences used in this study (Supplementary file 1-Table S1).

Results

Taxonomy

Genus *Alaskacladius* gen. nov.
Figs. 1–3
urn:lsid:zoobank.org:act:E9C0F7BE-C2AF-4555-9DDC-875CB5566B97

Type species. *Alaskacladius johnhudsoni* **sp. nov.**, by present designation.

Diagnostic characters. The new genus can be separated from other Orthocladiinae by a combination of the following characteristics: Imago medium size species; antennal last flagellomere with a stiff, straight apical seta; acrostichals present starting close to antepronotum and extending to mid-section of the scutum; humeral pit absent; wing bare; squama of the wing with setae; R_{4+5} setae starting on apical 2/3rd in male; femur, tibia, and tarsus of all legs with long and strong beard; pseudospurs absent; male hypopygium with prominent anal point, cone-shaped, broad basally, bearing simple moderately thick setae; virga a cluster of four extremely thin and compacted long spines placed vertically close to the sternapodeme, two hyaline lamellae adjacent to virga; inferior volsella trapezoidal, located halfway along the gonocoxite; gonostylus prominent, expanded from basal 1/3rd to the apex, outer corner (projection) well-developed; female genitalia with gonocoxite well-reduced, not extending beyond the anterior of segment IX; Gonapophysis VIII with large ventrolateral lobe well separated basally and apically from dorsomesal lobe and with distinct apodeme lobe between them; tergite IX not divided, large, and crescent-shaped.

Description.

Imago.

Medium size species with wings 4 × as long as wide.

Head (Figs. 1A, 2A). Male antenna with 13 flagellomeres and a stiff, straight apical seta. Eyes bare, male with very short dorsomedial extension, female no extension, temporal setae present with postoculars, uniserial orbitals, and outer verticals. Female tentorium narrower than male. Clypeus nearly square in male, in female wider than long. Palp 5 segmented, the apex of the third segment with few medial, lateral, and ventral sensilla clavata.

Thorax (Figs. 1B, 2B). Decumbent acrostichals present; dorsocentrals uni to biserial; prealars present in a semicircular pattern in male, in female less so; scutellars present in two rows. Antepronotal lobes bearing setae basally. Humeral pit absent.

Wing (Figs. 1C, 2C). Wing bare with fine punctation. Squama setose with around 19 setae in male and around 6 in female. R, R_1 and R_{4+5} setose; other veins bare. Costa with short extension. Cu_1 moderately curved. Anal lobe rounded in male, reduced in female.

Legs. Femur, tibia and tarsus of all legs with long and strong beard. Pulvilli well-developed. Spurs present on fore, mid and hind tibias; hind tibia comb well-developed. Pseudospurs absent.

Hypopygium (Fig. 1D). Anal point prominent, cone-shaped, broad basally, bearing simple, moderately thick setae. Virga with extremely thin and compacted long spines placed vertically close to sternapodeme. Hyaline lamellae with striation adjacent to virga. Sternapodeme slightly arched, oral projections developed. Inferior volsella trapezoidal. Gonostylus prominent, long, expanded from basal 1/3rd to the apex, outer corner (projection) well-developed, megaseta present.

Female genitalia (Fig. 2D–E). Gonocoxite well-reduced, not extending beyond the anterior of segment IX, bearing moderately long setae. Seminal capsules semi-circular, longer than wide. Spermathecal ducts with loop. Gonapophysis VIII with large ventrolateral lobe well separated basally and apically from dorsomesal lobe; apodeme lobe distinct between these lobes. Tergite IX undivided, large, and crescent-shaped, bearing numerous setae (Fig. 2E). Cercus semicircular, about the same size as seminal capsule.

Etymology. The new genus is named after its type locality, the state of Alaska in the USA. The suffix "*cladius*" is Greek for the clade or branch.

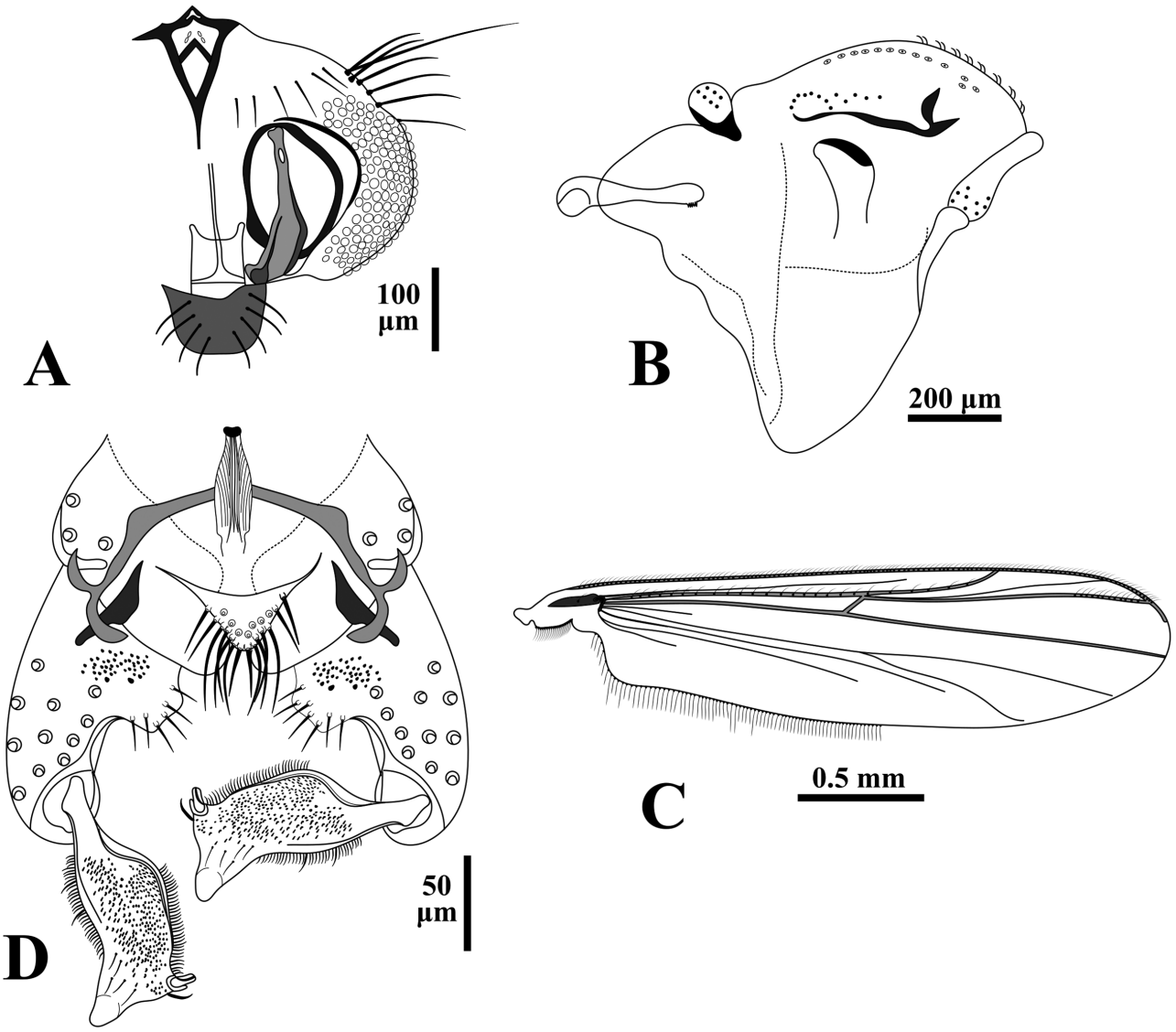

FIGURE 1. *Alaskacladius johnhudsoni* **gen. nov.**, **sp. nov.**, male. A. head; B. thorax; C. wing; D. hypopygium.

***Alaskacladius johnhudsoni* sp. nov.**
Figs. 1A–D, 2A–E, 3A–C
urn:lsid:zoobank.org:act:A2C04A6A-4981-4BC5-A6FE-F31C243C8D38

Type material: *Holotype:* male, USA, Alaska, Revillagigedo Island, Margaret Creek; 55.696, -131.629; 5.v.1994; sweep net, leg. J. Hudson; deposited at ARC. *Paratypes:* 3 males, USA, Alaska, Juneau, Mount Roberts Trail; 58.2961, -134.7117; 5.vi.2019; leg. K. Frangos; deposited at ARC. *Paratypes:* 3 males, USA, Alaska, Juneau, Mount Roberts Trail; 58.2961, -134.7117; 18.vi.2022; leg. K. Frangos and J. Hudson; deposited at ARC. *Paratype:* 1 female, Canada, British Columbia, Vancouver, Stanley Park, 49.301, -123.14; leg. B. Titaro; deposited at CBG. *Paratype:* 1 female, Canada, British Columbia, Queen Charlotte Islands, Haida Gwaii; 53.257, -132.089; leg. S. Querengesser; deposited at CBG.

Etymology. The new species is named after John Hudson, who has enormously contributed to our Chironomidae collection from Alaska since 1994.

Diagnostic characters. Same as the genus.

Description.

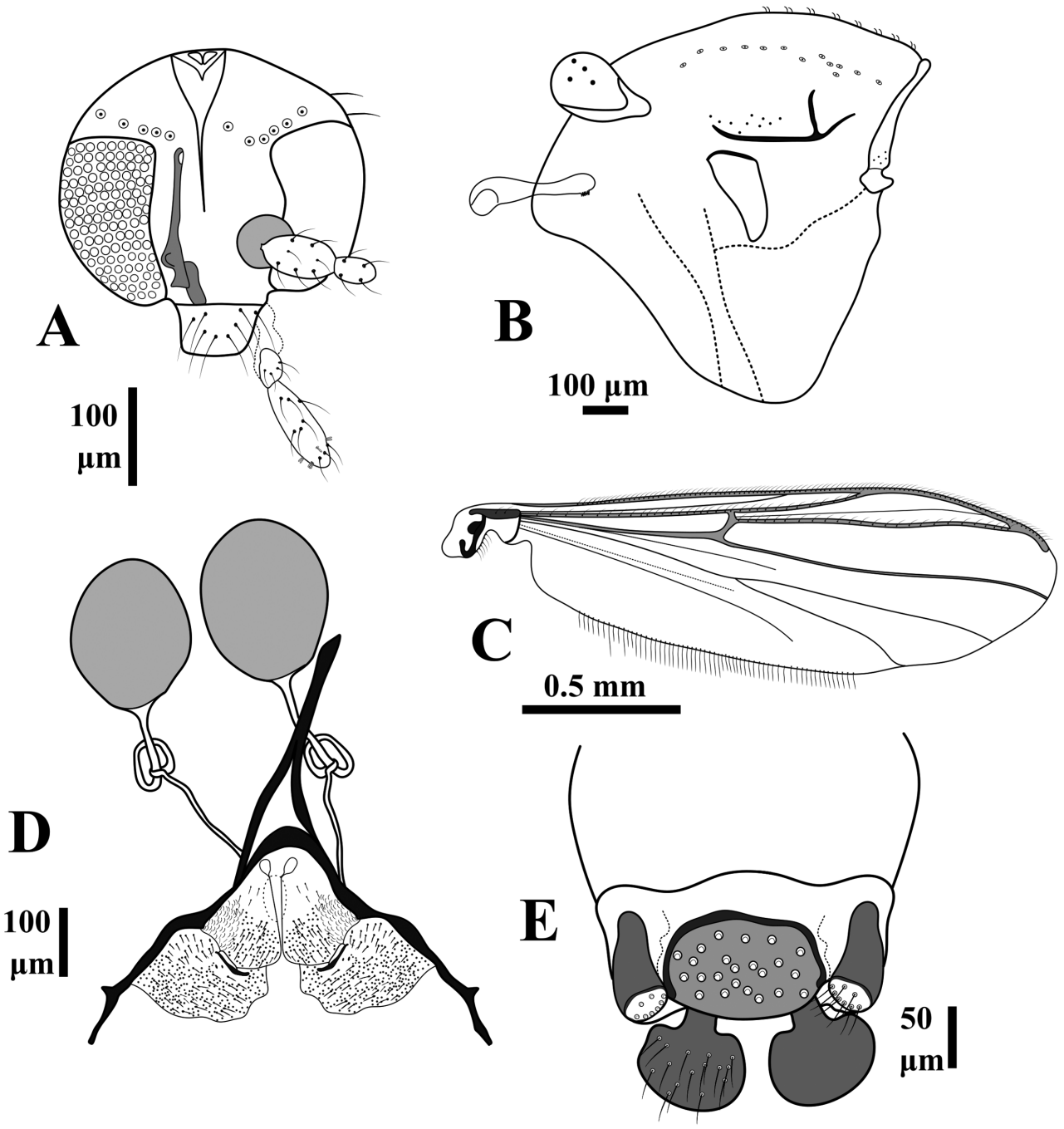

FIGURE 2. *Alaskacladius johnhudsoni* gen. nov., sp. nov., female. A. head; B. thorax; C. wing; D. genitalia ventral; E. genitalia dorsal.

Male (n = 7).

Total length 3.0–3.8, 3.3 mm. Wing 2.4–2.8, 2.6 mm long and 0.58–0.66, 0.62 mm wide.

General coloration of the mounted specimens. Head, thorax, halter, legs and tergites brown. Sternites slightly lighter. Wing greyish brown.

Head (Fig. 1A). Antenna with 13 flagellomere, last flagellomere with 10 sensilla chaetica, 2nd–3rd segments each with 2 sensilla chaetica, groove starts at 4th segment, AR 1.5–1.6. Eyes bare, with very short dorsomedial extension, temporal setae 7–8 with 1–2 postoculars, 6 uniserial orbitals, and 4–5 outer verticals (Fig. 1A). Tentorium 187–202, 196 µm long, narrow and long apically, base slightly expanded, large tentorial pit close to apex (Fig. 2A). Clypeus nearly squared, 67–100, 88 µm long and 98–131, 112 µm wide, bearing 8 setae, setae 254–355 µm long. Palpal

segments lengths (in µm): 73–99, 82; 52–79, 65; 114–169, 145; 109–135,121; 145–193, 163. Third palpomere with 1 sensilla clavata medially, 4 laterally, and 3 ventrally.

Thorax (Fig. 1B). Acrostichals 14–16; dorsocentrals 12; prealars 9–13, 8 in a semicircular pattern; scutellars 12–14 in two rows. Antepronotal lobes bearing 8–10 setae basally.

Wing (Fig. 1C). Wing bare with fine punctation visible at 40 x magnification. Brachiolum with 1 seta. Squama with 19 setae. R with 19 setae, and R_1 with 6 setae. R_{4+5} with 11 setae, starting on apical 2/3rd of the vein; other veins bare. Costa extension 71 µm. Cu1 moderately curved. Anal lobe rounded.

Legs. Femur, tibia and tarsus of all legs with long and strong beard. Pulvilli well-developed. Fore tibia spur 78–83, 80 µm long, mid tibia spurs 37–38 µm, and 29–37 µm long, hind tibia spurs 72–77, 74 and 28 µm long, hind tibia comb with around 17 bristles. Pseudospurs absent. Lengths and proportions of legs as in Table 1.

TABLE 1. Male leg lengths (µm) and proportions of *Alaskacladius johnhudsoni* **gen. nov.**, **sp. nov.**.

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV
P_1	838	911	575	359	237	157	117	0.63	2.7	3.0
P_2	879	881	396	221	180	109	110	0.45	3.5	4.4
P_3	940	1071	639	350	231	127	104	0.60	3.3	3.1

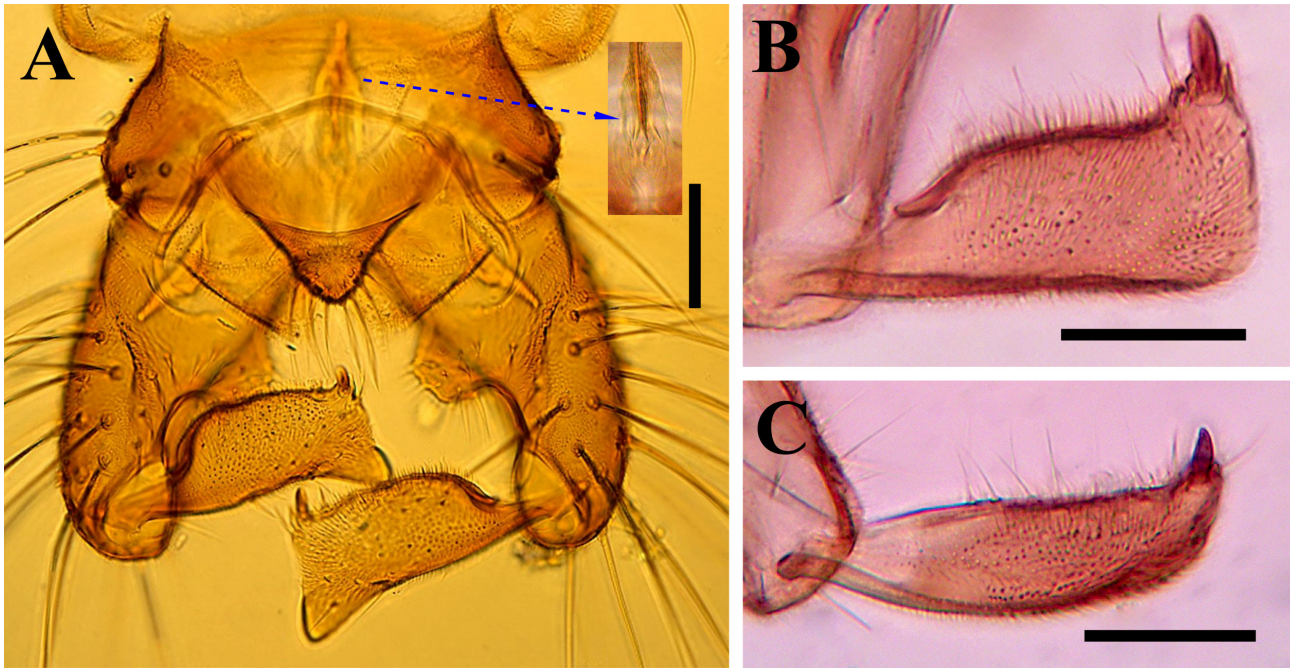

FIGURE 3. *Alaskacladius johnhudsoni* **gen. nov.**, **sp. nov.**, male. A. hypopygium, arrow indicates the virga; B. variation in the shape of turned gonostylus C. variation in the shape of turned gonostylus. The scale bars represent 50 µm.

Hypopygium (Figs. 1D & 2A). Anal point prominent, cone-shaped, broad basally, 32–42, 36 µm long and 78–111, 90 µm wide, bearing around 24 simple moderately thick setae. Virga with 4 thin and compacted long spines placed vertically close to sternapodeme, 76–92, 84 µm long, and two hyaline lamellae adjacent to them, lamella with striation. Sternapodeme 117–148, 124 µm long, slightly arched, oral projections developed. Phallapodeme 42–56, 48 µm long. Gonocoxite 163–202, 184 µm long. Inferior volsella trapezoid shape, located halfway along the gonocoxite. Gonostylus prominent, 117–143, 133 µm long, expanded from basal 1/3rd to the apex, outer corner (projection) well-developed, megaseta 15–19, 16 µm long. HR 1.4, HV 2.4.

Female (n = 2).

Total length 2.8 mm. Wing 1.9 mm long and 0.55 mm wide.

General coloration of the mounted specimens. Head, thorax, halter, legs and tergites brown. Sternites slightly lighter. Wing greyish brown.

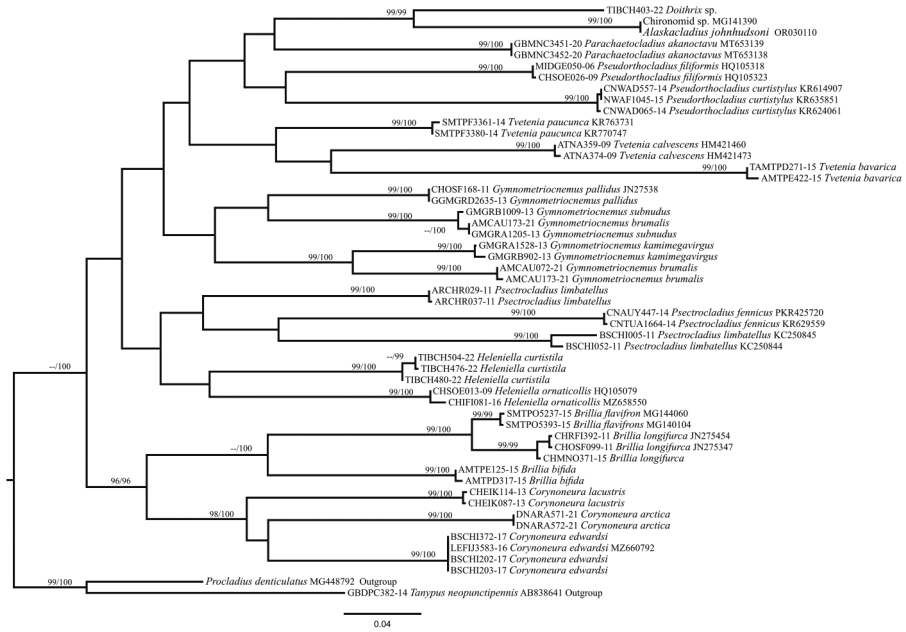

FIGURE 4. Neighbor-Joining (NJ) and Maximum Likelihood (ML) trees of the Orthocladiinae genera and two outgroups *Procladius denticulatus* Sublette, 1964 and *Tanypus neopunctipennis* Sublette, 1964 inferred from the COI nucleotide sequence data (567 bp). Numbers on branches represent the bootstrap value for Neighbor-Joining (NJ) and Maximum Likelihood (ML) (10000 replicates). Clades without numbers are supported with bootstrap support of ≤ 95.

Head (Fig. 2A). Antenna segment one 77–87, 82 µm long; second segment 51 µm long, other segments missing. Eyes bare, with no dorsomedial extension, temporal setae 8–10 with 6–7 uniserial orbitals, and 2–3 outer verticals (Fig. 2A). Tentorium 192–197, 195 µm long, narrow and long apically, base slightly expanded, large tentorial pit close to apex (Fig. 2A). Clypeus 57–66, 62 µm long and 97 µm wide, bearing 8 setae, setae 54–58, 56 µm long. Palpal segments lengths (in µm; n = 1): 67, 45, 98, 103, 139. Third palpomere with 1 sensilla clavata medially, 4 laterally, and 3 ventrally.

Thorax (Fig. 2B). Acrostichals 14; dorsocentrals 18; prealars 10; scutellars 8 in two rows. Antepronotal lobes bearing 5 setae basally.

Wing (Fig. 2C). Wing bare with fine punctation visible at 40 x magnification. Brachiolum with 1–2 setae. Squama with 3–6, 5 setae. R with 17–21, 19 setae, and R_1 with 12–14, 13 setae. R_{4+5} with 25–31, 28 setae; other veins bare. Costa extension 86 µm. Cu_1 moderately curved. Anal lobe reduced.

Legs. Pulvilli well-developed. Fore tibia spur 32 µm long, mid tibia spurs 24 µm, hind tibia spurs 62–64, 63 and 21 µm long, hind tibia comb with around 12 bristles. Pseudospurs absent. Lengths and proportions of legs as in Table 2.

TABLE 2. Female leg lengths (µm) and proportions of *Alaskacladius johnhudsoni* **gen. nov.**, **sp. nov.**.

	fe	**ti**	**ta_1**	**ta_2**	**ta_3**	**ta_4**	**ta_5**	**LR**	**BV**	**SV**
P_1	596	685	354	197	126	94	94	0.52	3.2	3.6
P_2	627	589	269	137	87	54	48	0.46	4.6	4.5
P_3	701	750	424	229	184	98	95	0.56	3.1	3.4

Genitalia (Fig. 2D–E). Gonocoxite well-reduced, not extending beyond the anterior of tergite IX, bearing around 8 setae. Seminal capsules semi-circular, 71–79, 76 µm long, and 91–103, 96 µm wide. Spermathecal ducts with loop (Fig. 2D). Notum 100 µm long, ramus 91 µm long. Gonapophysis VIII divided into large ventrolateral lobe well separated basally and apically from dorsomesal lobe; apodeme lobe distinct between these lobes. Tergite

IX not divided, large, and crescent-shaped, bearing around 25 setae (Fig. 2E). Cercus semi-circular (Fig. 2E), 63–75, 69 µm long and 73–75, 74 µm wide.

Immatures. Unknown.

Remarks

The *Alaskacladius* **gen. nov.** is a very unusual and unique Orthocladiinae possessing some similar characteristics to related genera *Georthocladius* and *Pseudorthocladius*. However, the new genus is more related and forms the sister group with *Doithrix* (Fig. 4). The presence of a vertical virga close to the sternapodeme along with the lack of pseudospurs resembles some *Pseudorthocladius* species. The outer projection of the male gonostylus, the shape of the anal point, and its dorsal setae resemble those of *Georthocladius* species. However, the trapezoid inferior volsella and the presence of longitudinal virga with hyaline striated lamellae separate it from *Georthocladius* and *Pseudorthocladius*. The adult male of the new genus differs from *Doithrix* by the character of virga, the presence of a cone-shaped broad anal point with moderately thick setae, and the shape of inferior volsella and gonostylus. Species of *Doithrix* have long, triangular anal points with strong lamellate setae, and inferior volsella is either reduced or well-developed but, in either case, are more triangular or pointed; the virga of *Doithrix* is not long and lacks hyaline lamellae. Additionally, the gonostylus of *Doithrix* is medially expanded and lacks an outer corner or projection. We examined the images of the adult male of the Chinese species *Doithrix* sp. TIBCH403-22, sent to us kindly by Dr. Hongqu Tang. The Chinese species fall within the genus *Doithrix* with defined morphological characters of the genus and, therefore, differs from *Alaskacladius* based on the aforementioned characters. The adult male of the new genus differs from *Parachaetocladius* based on the presence of acrostichals, the shape of inferior volsella, and the thickness of the anal point setae. The female of *Alaskacladius* differs from *Georthocladius*, *Parachaetocladius*, and *Pseudorthocladius* by the presence of well-separated ventrolateral lobe and dorsomesal lobe, distinguished apically and basally and by well-reduced gonocoxite. The female of *Doithrix* has not been described. The structure of virga in *Alaskacladius* may resemble some *Gymnometriocnemus* species, particularly the structures of the lamellae. Bare wing, setose squama, and a prominent anal point separate *Alaskacladius* from the species of *Gymnometriocnemus*. In some mounted specimens, the outer projection of the gonostylus can turn and may not be visible (Fig. 3B–C).

The characters of hyaline striated lamellae of the virga, and trapezoid inferior volsella of the male are autoapomorphic in *Alaskacladius*, missing in related genera. *Doithrix* and *Pseudorthocladius* either have a short-spined virga in close or scattered clusters or, in the case of *Pseudorthocladius (s.s.*) *macrovirgatus* Sæther & Sublette, 1983 with long thick virga without lamellae. Previously, Namayandeh *et al.* (2020), reporting on the structure of virga in some *Georthocladius* species, noticed the existence of a tripartite sclerite with an arched bifid median sclerite located at the posteromedial meeting point of the gonocoxites with two narrow diagonal lateral sclerites located close to sternapodeme or close to median sclerite, seen on most *Georthocladius* species and all *Parachaetocladius* species. Further, these authors reported an apparent trifid virga-like structure observed on *Georthocladius platystylus* Sæther & Sublette, 1983 (Namayandeh *et al.*, 2020: Fig. 2E). These sclerites are noteworthy morphological structures, varying in shape and size which are likely the thickening of the posteromedial point of gonocoxites and aedeagal regions. These sclerites are weakly developed in *Georthocladius fimbriosus* Sæther & Sublette, 1983, and are missing in Afrotropical *Georthocladius longicalcaneum* Sæther & Andersen, 1996. Sæther (1980) described virga as a terminal group of spines sometimes attached to the end of endophallus; when resting in the penis cavity, it is at the level of sternapodeme. Therefore, we should reconsider the previously reported structures by Namayandeh *et al*. (2020) as virga and consider the genera *Georthocladius* and *Parachaetocladius* without it.

A key to the adult male of some genera of Orthocladiinae

1. Antenna with a stiff and straight apical seta. Eyes naked. Acrostichals present or absent. If present long, starting in front of antepronotum. Squama of the wing setose; Cu_1 curved. Pulvilli is present and well-developed. 2
- Not with the above combination of characters . . . . . Not keyed here
2. Acrostichals present or absent. Preepisternum sometimes with seta(e). Pseudospurs present or sometimes absent. Gonostylus with outer corner. 3

- Acrostichals present. Preepisternum without seta. Pseudospurs absent. Gonostylus without outer corner 5
3. Acrostichals present. Inferior volsella mainly prominent, triangular, or trapezoid shape; if reduced, never digitiform 4
- Acrostichals absent. Inferior volsella small and digitiform . . . . . .*Parachaetocladius* Wülker
4. Virga absent. Pseudospurs present. Inferior volsella, mainly triangular or occasionally reduced . . . . . *Georthocladius* Strenzke
- Virga present. Pseudospurs absent. Inferior volsella trapezoid shape (Figs. 1D & 3A) . . . . . .*Alaskacladius* **gen. nov.**
5. Anal point well-developed and long; bearing strong lamellate setae. . . . . *Doithrix* Sæther & Sublette
- Anal point triangular or occasionally absent, never long; bearing moderately strong setae but never lamellate . . . . . *Pseudorthocladius* Goetghebuer

Results of molecular analysis

NJ and ML analyses produced the same tree topology. The sequence of *Alaskacladius johnhudsoni* clustered with a sequence identified as Chironomid sp., accession number MG141390, from British Columbia, Canada (leg. B. Titaro; J. R. Dewaard direct submission). This places this sequence in *Alaskacladius johnhudsoni* (Fig. 4). The results of the NJ and ML phylogenetic tree places the new genus among the related genera of *Doithrix*, *Georthocladius*, *Parachaetocladius*, and *Pseudorthocladius*. The ML phylogenetic tree shows *Alaskacladius* is closest and forms a sister group with the genus *Doithrix* (Fig. 4).

K2P distance between a sequence identified as Chironomid sp., accession number MG141390, and *Alaskacladius johnhudsoni* is 0%, which clearly shows that both sequences belong to the same species. The longest intergeneric K2P distance between *Alaskacladius* was with *Corynoneura*, 28.3 %, and the shortest was with *Doithrix*, 14.0 %. The average intergeneric K2P distance between ten Orthocladiinae genera was 21.6 %, with *Alaskacladius* only having lower than average distance with two genera (i.e., *Doithrix* and *Parachaetocladius*). The intergeneric K2P distances between *Alaskacladius*, *Parachaetocladius*, and *Pseudorthocladius* were 20.2 % and 22.6 %, respectively. The average intergeneric K2P distance of *Alaskacladius* with nine other genera of Orthocladiinae was 23.2 (Supplementary file 1-Table S2). The intergeneric K2P distance between *Alaskacladius* and *Doithrix* suggests a recent divergence, although in general, COI sequences are not optimal for the separation and determination of the genus (Ekrem *et al.* 2007; Makarchenko *et al.* 2020).

Ecology, habitat, and distribution. The holotype of *A. johnhudsoni* was collected from Revillagigedo Island (Prince of Wales Islands) at the mouth of Margaret Creek. The aquatic habitats nearby (in addition to the creek) are intertidal mudflats, supratidal ponds, and seeps flowing into the bay. The paratypes were collected along the Mount Roberts Trail above Juneau. The area of collection is subalpine with a few snow-melt ponds and seeps. Woody vegetation in this area is dominated by Sitka alder and Dwarf blueberries; typical heath with wildflowers is common in small meadows along the trail. The adults were observed in small tight swarms about 0.5 m off the ground in openings among the vegetation adjacent to the trail. Although the adults collected near various habitats, it is our sense that the larvae inhabit seep-like semiterrestrial habitats. *A. johnhudsoni* so far has only been found in Alaska (USA) and British Columbia (Canada).

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