Oropuella, a new genus of Orthocladiinae from the western Nearctic

Andrew Fasbender

Rhithron Associates Inc., 33 Fort Missoula Road, Missoula, MT 59804, USA
E-mail: afasbender@rhithron.com

http://zoobank.org/B1BF8C50-50DE-432B-BA3E-BC86F743481D

Abstract

A new genus and two species of the subfamily Orthocladiinae are described from western North America. *Oropuella* gen. n. shows affinities to *Parametriocnemus* Goetghebuer and *Paraphaenocladius* Thienemann, but can be distinguished by morphology for all life stages. Two novel species are placed in the new genus, *Oropuella eidolon* sp. n and *Oropuella pallida* sp. n. Amendments to current genus keys are given to incorporate the new genus, and the current state of Orthocladiinae species taxonomy in the western Nearctic is discussed.

Introduction

The Orthocladiinae fauna of temperate western North America (here defined as the region west of 100°W longitude, excluding the arctic and subarctic) is poorly understood when compared with the eastern Nearctic fauna. Water quality monitoring programs involving benthic macroinvertebrate bioassessment are administered by every U.S. state and many other entities (cities, counties, national parks, tribal organizations, etc.) (Roper *et al.* 2010). Chironomidae are a key component of the macroinvertebrate community (Ferrington *et al.* 2008), but classification beyond genus is impossible for the majority of Orthocladiinae from the region. Perusal of Oliver *et al.* (1990) demonstrates a large gap in faunistic records of Orthocladiinae in the west; this paucity of taxonomic information is not related to a lack orthoclads in the region, but rather the need for more taxonomic effort. Only preliminary faunistic work has been undertaken (Sæther 1969, Sublette & Sublette 1971, Sublette *et al.* 1998, Namayandeh & Culp 2016), although some material from the region has been treated in revisionary works of broader geographic scope (Sæther 1975, Sæther 1976, Soponis 1977, Oliver 1981, Oliver 1983, Sæther & Sublette 1983, Soponis 1990, Cranston 1999, Hestenes & Sæther 2000, Ferrington & Sæther 2011) and isolated descriptions have been produced (Roback 1957, Cranston & Judd 1987, Cranston & Oliver 1988b, Oliver 1977, Oliver 1984, Cranston *et al.* 2007).

One reason for this disconnect between field sampling effort and taxonomic description is most benthic monitoring programs preserve samples prior to sorting and identification for reasons of practicality. Association of the different life stages of an undescribed taxon is essential for establishing taxonomic novelty and placement, which is traditionally accomplished by rearing individual specimens to the adult stage. Preserving samples before sorting, killing the organisms present, renders this method nonviable. Another method of ascertaining life stage associations is the "ontogenetic method" (Hogue & Bedoya Ortiz 1989), where diagnostic features of a developing lifestage are observed under the cuticle of the preceding stage. Pharate associations have a long history of use for species descriptions in Chironomidae (e.g. Brundin 1966), but only occasionally has the ontogenetic method been used in the description of new genera (Harrison & Cranston 2007, Cranston & Krosch 2011).

A larva of Orthocladiinae difficult to place to genus regularly occurs in benthic macroinvertebrate samples from the Intermountain West and Pacific Coast region of North America. Resembling *Parametriocnemus* Goetghebuer and *Paraphaenocladius* Thienemann in the structure of the labrum and mentum, it is readily separable by the presence of a break in the second antennal segment, pale tan coloration of the mentum, and small head capsule with an elongate body. Discovery of larval specimens with pharate pupal features allowed association with another problematic taxon, a pupa keying to *Parametriocnemus* but with absence of pedes spurii A, shorter pedes spurii B, no polygonal sclerotization of the conjunctives and single pearl row. Pharate adult males from these pupae demonstrate a low antennal ratio (0.19–0.32), wedge shaped dorsomedial extension of the eye, last antennal segment weakly clavate with an apical emargination, wing membrane with macrotrichia, absence of tibial spurs, and a hypopygium with a strong anal point and without a virga. A cytochrome c oxidase subunit I (COI) barcode was sequenced, which is distinct from sequenced species of *Parametriocnemus* and *Paraphaenocladius* in the

BOLD database (Ratnasignham & Hebert 2007), allowing for recognition using molecular methods. This genus is here described as *Oropuella* gen. n., containing two species: *Oropuella eidolon* sp. n. and *Oropuella pallida* sp. n.

Materials and Methods

Specimens examined in this study were pulled from benthic macroinvertebrate samples processed by Rhithron Associates Inc. (RAI). Specimens were stored in 80% ethanol before being moved to 99% isopropanol in preparation for mounting. Specimens were then transferred to a vial containing 99% isopropanol layered over methyl salicylate. When the specimens absorbed sufficient methyl salicylate to clear soft tissue (shown by sinking to the bottom of the vial) the isopropanol layer was decanted via pipette and individual specimens transferred to Canada balsam on a slide, where they were dissected and cover slipped. Leica DM1000 and Olympus CH compound microscopes were used for this study; photomicrography was performed using an Amscope DM1000 camera, and calibrated measurements were recorded using the Amscope 3.7 software package. Illustrations were prepared using Inkscape 0.92. DNA extraction was performed on a larval specimen (from Cave Creek, Oregon Caves National Monument) using a Lifescanner Kit, with sequencing performed by Biolytica Inc. (Lifescanner 2019); the COI barcode was compared with the public barcode database from BOLD Systems and a neighbor-joining identification tree was generated using the Kimura 2-Parameter neighbor-joining species tree functionality from BOLD (Ratnasingham & Hebert 2007). The tree was pruned and visualized using Interactive Tree of Life (iTOL) 4.3.3 (Letunic & Bork 2006, 2019).

Terminology follows Sæther (1980), modified by Langton (1994) for "taeniae" to describe flattened pupal setae, "gonopod" for the male clasping structure subdivided into gonocoxite and gonostylus (Wood 1991; Cumming & Wood 2017), "ommatrichia" for the microtrichia between ommatidia in the adult eye (Cumming & Wood 2017) and wing venation derived from Cumming & Wood (2017) and Ekrem *et al.* (2017). Lifestage associations were made using an ontogenetic method by examining pharate pupae in last instar larvae and developing adults in mature pupae (Hogue & Bedoya Ortiz 1989). Descriptions of the larval stage are given only for the genus, because no characters currently separate larvae at the species level and the associations are insufficient to assign them to the named male species. Only significant mensural characters are described, due to the use of pharate adults and the limitations of these characters when allometric variation is considered (McKie & Cranston 2005; Gresens *et al.* 2012). Continuous measurements are given as ranges, with the median value following in parenthesis when more than two specimens were measured. Additionally, differentiation of setae between pharate adults and pupae was difficult; setal data for adults and pupae should thus be considered tentative. All setae are only counted on one side for bilaterally symmetrical structures based on the midline of the body. Holotypes and additional material are deposited in the Academy of Natural Sciences, Philadelphia [ANSP], while the remaining material is retained in the author's personal research collection for further investigations [AFPC].

Results

Oropuella new genus

http://zoobank.org/B6B591F4-49D4-4B28-9FD1-9F7F6F6CC59F

Type species: *Oropuella eidolon* sp. n.

Etymology: *Oro-* Greek for "mountain," as the known species is recorded from mountain streams; *-puella* the Latin for "maiden," in reference to the delicate appearance of the larva. The gender of the name is feminine.

Diagnostic characters: *Adult male.* Eye bare, with wedge shaped dorsomedial extension. Antenna with 13 flagellomeres, apical flagellomere weakly clavate with emarginate apex. Wing membrane with extensive macrotrichia at apex and along posterior margin. Anal point strongly developed, apex extending beyond inferior volsella.

Pupa. Habitus pale (alcohol preserved specimens). Thoracic horn present, broadly flattened, with numerous triangular spinules. Wing sheath with single pearl row. Abdominal tergites II–VIII with rows of prominent triangular spinules along posterior margin. Pedes spurii B present on II, large and triangular without fingerlike apex, extending over ¼ length of segment. Posterior margin of sternite VIII with sexual dimorphism: row of spinules in male, simple in female. Anal lobes with broad rounded apex, three apical macrosetae with length subequal to anal lobe.

Larva. Habitus pale, cranium very light (alcohol preserved specimens). Antennae with six segments, apical segment minute and hair-like, desclerotized break at base of second segment. SI setae plumose. Mentum pale tan, with single broad median tooth and five lateral teeth; ventromental plates double, extending well beyond setae submenti. Posterior abdominal segment not directed ventrally.

Generic description: *Adult male (from pharate material)*: Habitus pale to brown.

Antenna. Figs 1a, b. 13 flagellomeres, groove beginning at flagellomeres 3–4. Antennal ratio (AR 0.19–0.32. Apical segment of antenna weakly clavate, apex emarginate without apical seta. Plume fully developed.

Head. Eye bare, with wedge shaped posteromedial extension. ~9 temporal setae, uniserial. With 5 palpomeres; 3 & 4 subequal, 5 slightly longer than 4.

Thorax. Antepronotum not narrowed medially, lateral setae present. Acrostichals strong, decumbent, extending from anterior of prescutum beyond midpoint of scutum; 19–24 dorsocentrals, irregularly staggered in two rows; 8 prealars, uniserial; supraalars absent; 4 scutellars, uniserial. Postnotum bare.

Wing. Setae present on all veins; membrane with macrotrichia in apical third and along posterior margin. Squama with several setae.

Legs. Sensilla chaetica absent. Tibial spurs absent, hind tibial comb present. Pseudospurs absent; tarsomere IV cylindrical, 1.3x length of V. Pulvilli weakly developed.

Abdomen. Tergites I–VII with setae in anterior and posterior rows medially, irregularly placed laterally; tergite VIII with 3 irregular rows of setae. Sternites with setae medially.

Genitalia. Fig. 2. Anal point strongly developed, extending beyond inferior volsella, base of anal point pentagonal, rapidly tapering to straight sided stylus, 6 scattered setae at free base of stylus, apex rounded, glabrous and hyaline. Sternapodeme without oral projections. Coxapodeme curved, apex acute. Virga absent. Gonocoxite without superior volsella, inferior volsella well developed, apex rounded. Gonostylus with crista dorsalis weak or well developed; gonocoxite/gonostylus ratio approximately 2.0.

Adult female (from pharate material). Description covers characters differing from the male.

Antenna. Figs 1c–e. Five flagellomeres; AR 0.24–0.35.

Head. Dorsomedial eye extension weak. Five temporal setae.

Thorax. Acrostichals ending just before midpoint of scutum.

Abdomen. Tergite I with a single row of setae, tergites II–VII with anterior, posterior, and uniserial lateral rows of setae.

Genitalia. Fig. 3. Tergite IX plate-like, undivided. Gonapophysis VIII with dorsomedial lobes divided, broadly separated or nearly touching; ventrolateral lobes nearly touching or fused medially. Labia simple, membranous. Two spermathecae subequal, necks symmetrical. Tergite X weakly developed; cerci large, pendulant.

Pupa. Fig. 4. Habitus pale.

Cephalothorax. Cephalic tubercles and frontal warts absent. Frontal setae absent. Antennal sheath smooth. Postorbitals absent. Antepronotum with one pair simple median setae. Thoracic horn long, flattened with broad apex, densely covered in strong, acute spinules. Two precorneal setae, three prealars present. Thorax smooth, wing sheath with a single pearl row, nose absent.

Abdomen. Tergite I without shagreen, tergites II–VIII with triangular shagreen, sparse anteriorly becoming denser toward posterior margin. Tergite I without spinules, tergites II–VIII with a strong band of 2–4 rows of dark spinules on posterior margin. Sternites VI–VII with similar posterior band of spinules. Conjunctives without polygonal sclerotization. Pedes spurii A absent; pedes spurii B conspicuous, triangular, length subequal to basal width. Posterior margin of sternite VIII sexually dimorphic, male with band of spinules similar to preceding sternite, female smooth. Abdominal setation (tentative, some setae obscured): tergite I with 0 D, 0 L; tergites II–VI 5 D, 1 L; tergite VII–VIII 5 D, 2 L; D 2–5 and L setae with adjacent sensillum chaetica. No taeniae. Anal lobe with rounded apex, apical spinules ventrally, without fringe, 3 subequal macrosetae apical, subequal to anal lobe length. Male genital sac subequal to anal lobes.

Larva (4th instar). Fig. 5. Measurements (n=5): total length 3.8–4.4 (4.1) mm; body/head length ratio 18–21 (19.5); head capsule length 191–230 (196) μm; head capsule width 144–178 (165) μm; antennal ratio 1.13–1.31(1.15). Habitus pale, head capsule light colored.

Head. Antenna with 6 flagellomeres; 6th segment hair like; 2nd segment divided near its base by a weakly sclerotized break; blade shorter than flagellum, reaching apex of 4th segment. SI plumose; SII and SIII with a few apical serrations. Pecten epipharyngis 3 short simple spines. Premandible with 4 teeth, brush absent. Mandible with 1 apical tooth and 4 inner teeth, apical tooth shorter than combined width of inner teeth, all teeth and apical portion of mandible pale tan. Mentum with simple median tooth and 5 pairs of lateral teeth, very pale tan. Ventromental plates well developed, extend-

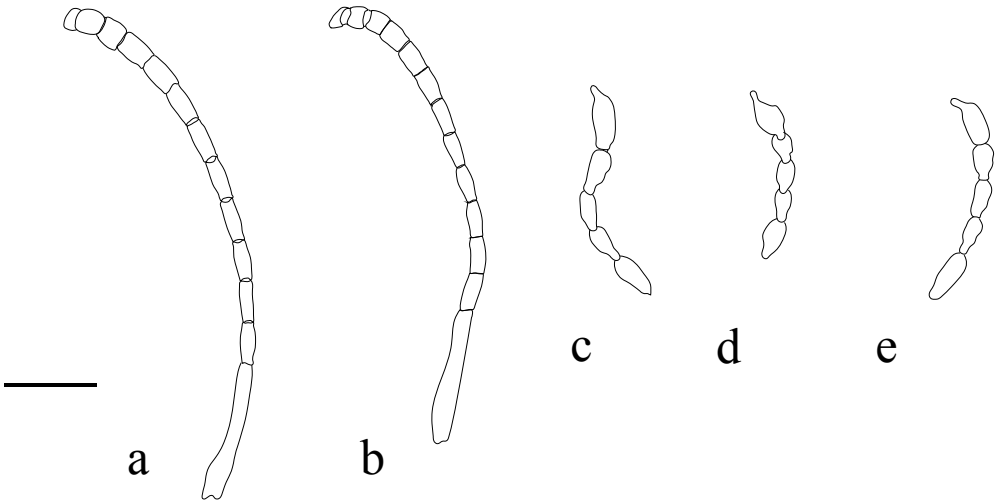

Figure 1. Adult antennae. Male antennae: a) *Oropuella eidolon* n. sp.; b) *Oropuella pallida* n. sp. Female antennae: c) *Oropuella* sp. F1; d) *Oropuella* sp. F2; e) *Oropuella* sp. F3. a), b) and d) exhibit some foreshortening due to the position of the antenna on the specimen; setae omitted; scale bar = 100 μm.

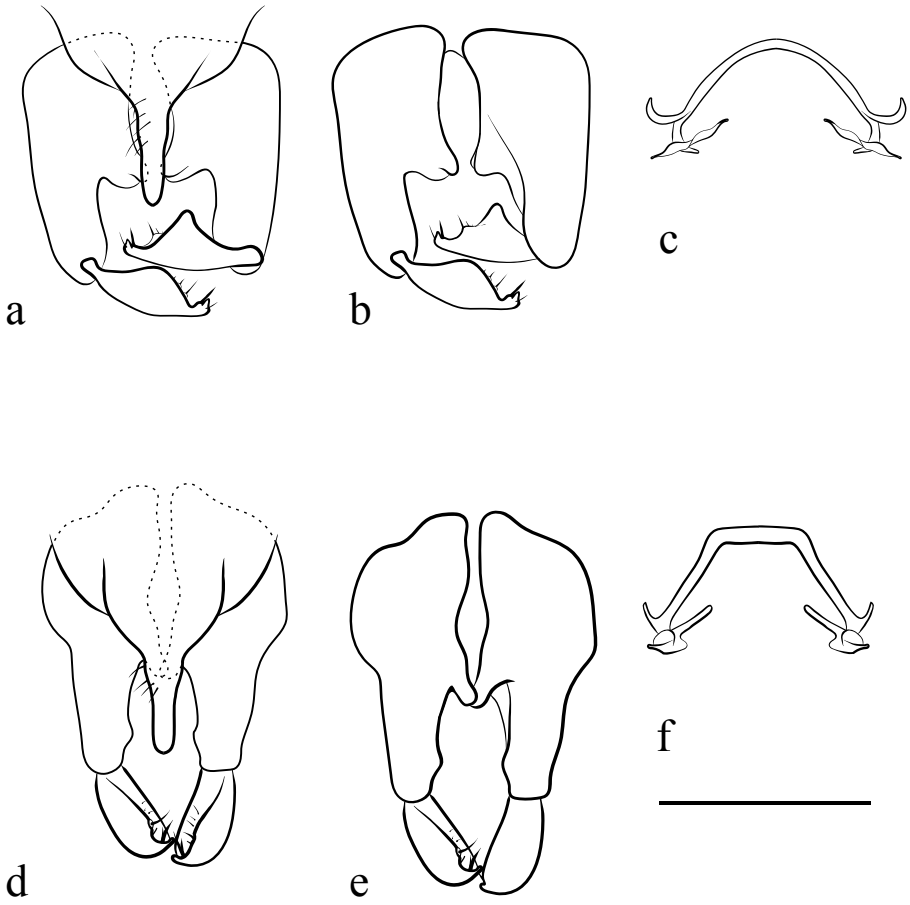

Figure 2. Male genitalia. a–c *Oropuella eidolon* n. sp.: a) anal point and gonopods; b) left gonopod in dorsal view, right gonopod in ventral view; c) sternapodeme and phallapodemes. d–f) *Oropuella pallida* n. sp.: d) anal point and gonopods; e) left gonopod in dorsal view, right gonopod in ventral view; f) sternapodeme and phallapodemes. Only significant setae illustrated; scale bars = 100 μm.

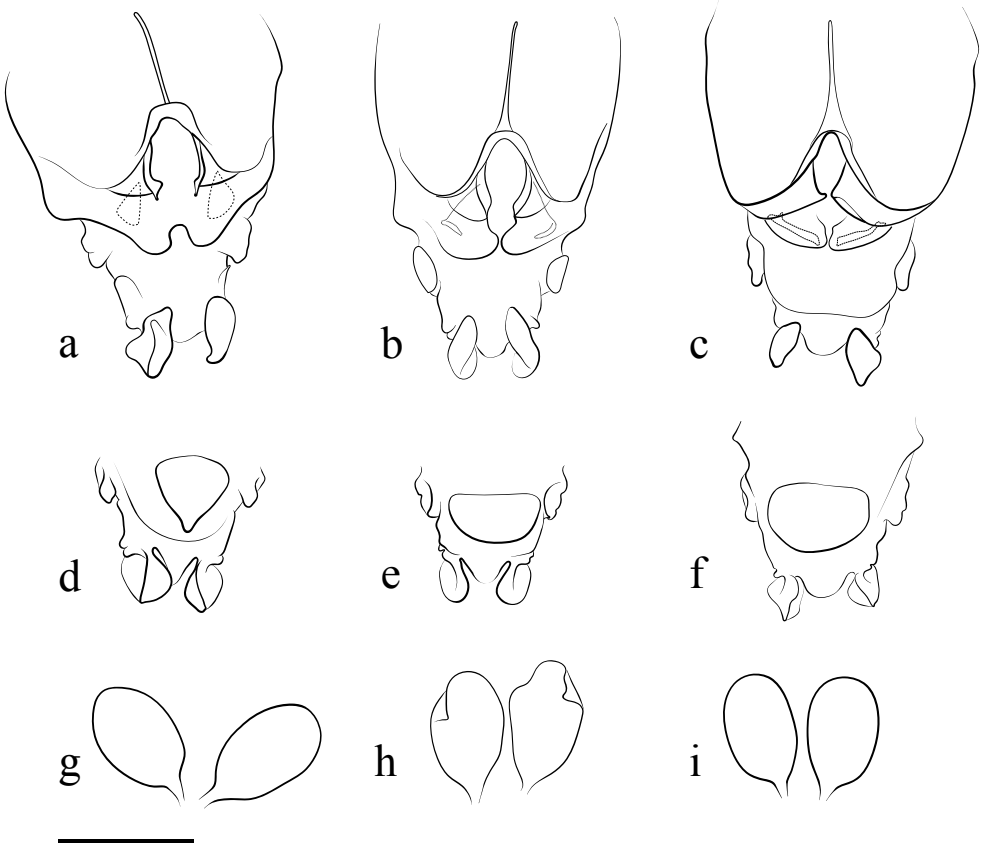

Figure 3. Female genitalia. a–c overall dorsal view: a) *Oropuella* sp. F1; b) *Oropuella* sp. F2; *Oropuella* sp. F3. d–f overall ventral view: d) *Oropuella* sp. F1; e) *Oropuella* sp. F2; f) *Oropuella* sp. F3. g–i spermathecae: g) *Oropuella* sp. F1; h) *Oropuella* sp. F2; i) *Oropuella* sp. F3. Scale bar = 100 μm.

ing beyond outer margin of mentum, and second set of smaller plates lying inside the main plate, directed perpendicular to postoccipital margin, posterior apex rounded. Ventromental plates extend well beyond setae submenti. Beard absent.

Thorax and abdomen. Body segments much longer than wide resulting in an elongate habitus. Body setae absent. Anterior parapods with serrated claws. Posterior parapods with simple claws. Procercus small, slightly wider than long, weakly sclerotized, with 4 anal setae. Anal tubules shorter than posterior parapods.

Remarks

A suite of morphological features from all life stages distinguish *Oropuella*. Males can be separated from similar genera (without ommatrichia, with acrostichals reaching the antepronotum, macrotrichia on the wing membrane, and simple gonostylus) by the following features: dorsomedial eye extension wedge shaped (parallel sided in *Parametriocnemus*), tibial spurs absent (present in *Paraphaenocladius*), tarsi without pseudospurs (present on tarsomeres 1 & 2 in *Metriocnemus* Wulp), pulvilli weak (well developed in *Pseudorthocladius* Goetghebuer), anal point elongate (absent in *Apometriocnemus* Sæther, short in *Pseudorthocladius*), virga absent (present in *Heterotrissocladius* Spärck, *Parametriocnemus,* and *Thienemannia* Kieffer). It is challenging to separate the males of *Oropuella* from *Paraphaenocladius*, with the most reliable features being the combination of a long, parallel sided anal point and lack of a virga (not found in any of the described *Paraphaenocladius*), and the absence of tibial spurs (Sæther & Wang 1995). Another potential feature to separate this genus from *Paraphaenocladius* is that C and R_{4+5} appear to terminate distal to M_{3+4} based on the pattern of vein macrotrichia in the developing wings, but it will require examination of eclosed specimens to confirm this character. Pupae are similar to *Parametriocnemus*, but have a less elongate pedes spurii B (length subequal to basal width). The configuration of the larval mentum (weakly convex, with double ventromental plates) is close to *Parametriocnemus* and *Paraphaenocladius*, but the pale tan mentum and desclerotized break in the second antennal flagellomere distinguish it from these taxa.

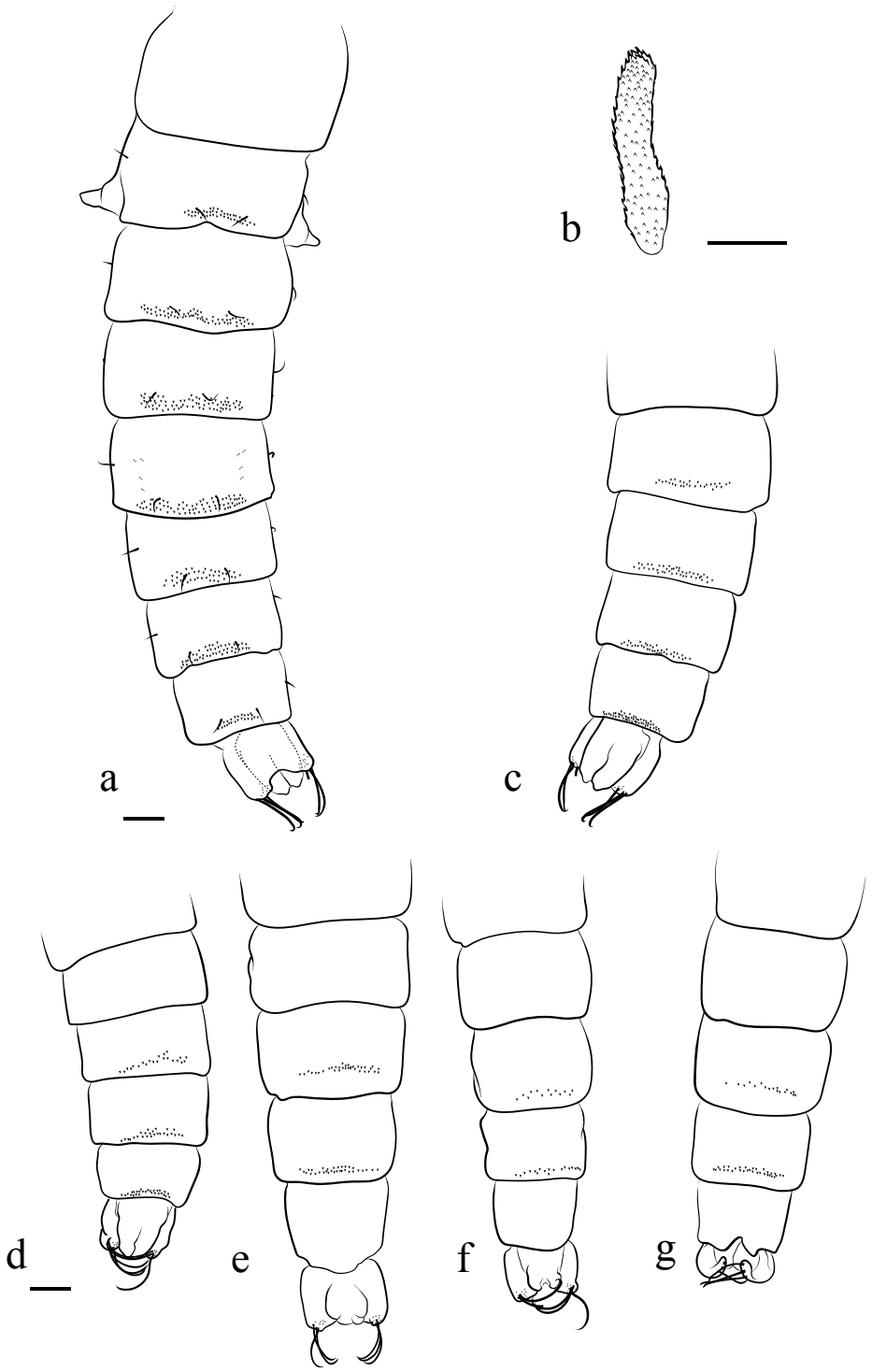

Figure 4. Pupae. a) *Oropuella eidolon* n. sp. complete abdomen, dorsal view; b) *Oropuella* sp. F2 thoracic horn; c) *Oropuella eidolon* n. sp. apical abdominal segments, ventral view; d) *Oropuella pallida* n. sp. apical abdominal segments, ventral view; e) *Oropuella* sp. F1 apical abdominal segments, ventral view; f) *Oropuella* sp. F2 apical abdominal segments, ventral view; g) *Oropuella* sp. F3 apical abdominal segments, ventral view. Setae omitted except in 4a, setae D2–5 illustrated only on tergite V; scale bars = 100 μm.

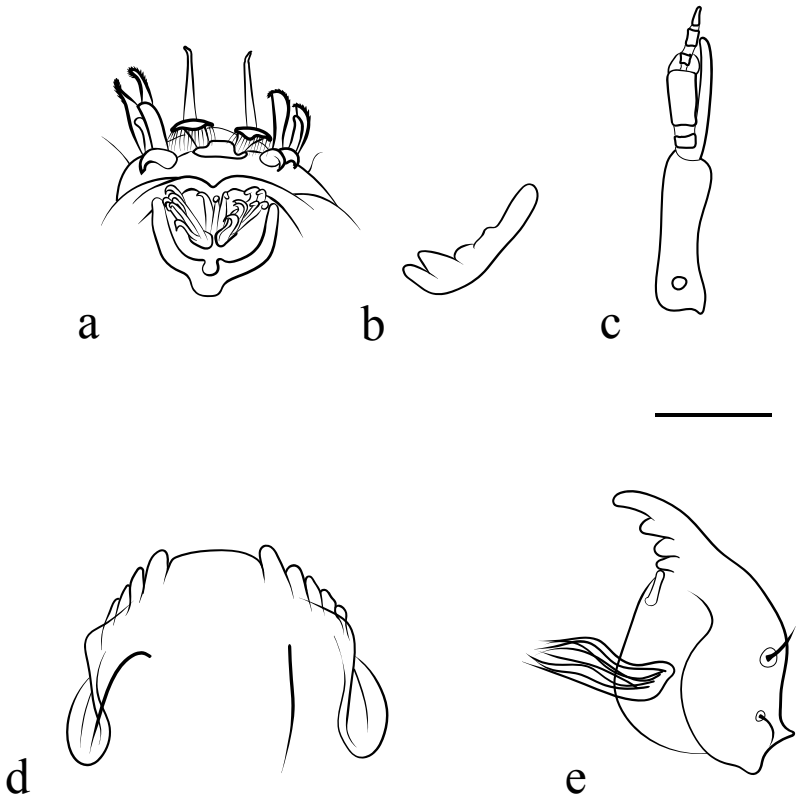

Figure 5. Larval head. A) labral setae; b) premandible; c) antenna; d) mentum and ventromental plates; e) mandible. Scale bar = 25 µm.

Key to the adult males of *Oropuella*

Gonostylus with prominent triangular crista dorsalis (Fig. 2b) ..... *Oropuella eidolon* sp. n.

Gonostylus with weak, straight crista dorsalis (Fig. 2e) ..... *Oropuella pallida* sp. n.

Key to the adult females of *Oropuella*

Tergite IX triangular (Fig. 3d) … *Oropuella* sp. F1

Tergite IX semicircular (Figs 3e, f) ……..…… 2

Dorsomedial lobe of gonapophysis VIII broadly separated medially (Fig. 3b) ..… *Oropuella* sp. F2

Dorsomedial lobe of gonapophysis VIII nearly touching medially (Fig. 3c) …… *Oropuella* sp. F3

Key to the pupae of *Oropuella*

Sternite VIII with posterior spinule band (Figs 4c, d) (male) ……..... 2

Sternite VIII without posterior spinule band (Figs 4 e–g) (female) …… *Oropuella* spp. F1, F2 and F3

Sternite V with posterior spinule band (Fig. 4c) ……..... *Oropuella eidolon* sp. n.

Sternite V without posterior spinule band (Fig. 4d) ……..... *Oropuella pallida* sp. n.

***Oropuella eidolon* new species**

http://zoobank.org/00B5DC58-372D-4A9A-AB8C-D4B38DE2B5C9

Type material: Holotype male pupa (slide): USA. OREGON: Crater Lake National Park, Unnamed trib. to Vidae Creek, 42.8798°N, 122.0970°W, 6.viii.2018, NPS18CRLA004; ANSP-ENT-124822 [ANSP]. Paratype 1 male pupa (slide): same data as holotype; ANSP-ENT-124823 [ANSP].

Etymology: *Eidolon* is Greek for a specter or phantom, a reference to the difficulty in establishing life stage associations within this genus. This epithet is treated as a noun in apposition.

Diagnostic characters: *Adult male*. Gonostylus with crista dorsalis triangular, large and protruding.

Pupa. Sternites V–VIII with posterior spinule band.

Description: *Adult male (pharate).* Figs 1a, 2a–c. Measurements (n = 2): flagellomeres (µm): 28–35, 20–34, 25–36, 27–34, 34–38, 36–43, 32–42, 34–45, 42–49, 35–42, 40–44, 40–45, 126–155; AR 0.31–0.32; anal point 50–55 µm; gonocoxite 120–124 µm; gonostylus 58–62 µm. Habitus brown,

with lighter maculations around abdominal setae.

Genitalia. ~6 setae on each side of anal point. Lateral margin of gonocoxite straight; prominent triangular crista dorsalis extending nearly the entire length of the gonostylus, wider than remainder of gonostylus, apex of gonostylus with small subacute spine; megaseta adjactent and proximal to spine, small seta on dorsal surface distal to spine, three setae on carina on ventral surface of crista dorsalis. Sternapodeme smoothly arched; lateral apex of phallapodeme width subequal to medial portion.

Pupa. Figs 4a, c. Measurements (n = 2): length 2.7–2.8 mm. Sternites V–VIII with posterior spinule band.

***Oropuella pallida* new species**

http://zoobank.org/340A973D-1804-46BB-BEFA-1750FF294B23

Type material: Holotype male pupa (slide): USA. WASHINGTON: King Co., Boise Creek at Enumclaw golf course. 47.1951°N, 121.9533°W, 30.viii.2017, KC17BOC002; ANSP-ENT-124824 [ANSP].

Etymology: The Latin adjective *pallida* refers to the pale adult male.

Diagnostic characters: *Adult male*. Gonostylus with crista dorsalis weakly curved, not protruding.

Pupa. Sternites VI–VIII with posterior spinule band.

Description: *Adult male (pharate)*. Figs 1b, 2d–f. Measurements (n = 1): flagellomeres (µm): 34, 22, 23, 29, 38, 34, 40, 35, 34, 44, 39, 42, 80; AR: 0.19; anal point 57 µm; gonocoxite 134 µm; gonostylus 44 µm. Habitus uniformly pale.

Genitalia. ~5 setae on each side of anal point. Lateral margin of gonocoxite bulging anteriorly; crista dorsalis parallel to inner margin of gonostylus, weakly curved; megaseta subapical, with closely associated seta at base, large seta subequal in size to megaseta located ¼ length from apex of gonostylus ventral to crista dorsalis, one smaller seta located between megaseta and the large seta and three small setae proximal to large crista dorsalis seta. Medial portion of sternapodeme quadrate; lateral apex of phallapodeme expanded.

Pupa. Fig. 4d. Measurements (n=1): length 2.1 mm. Sternites VI–VIII with posterior spinule band.

***Oropuella* sp. F1**

Material examined: USA. CALIFORNIA: Lassen Volcanic National Park, King's Creek, 40.4664°N, 121.4142°W 16.viii.2017, NPS17LAS007 (1 female pupa (slide) ANSP-ENT-124825; 1 larva with pharate pupal features (slide); ANSP-ENT-124827 [ANSP]).

Diagnostic characters: *Adult female.* Tergite IX triangular, with ~14 setae. Gonapophysis VIII dorsomedial lobes broadly separated.

Description: *Adult female (pharate)*. Figs 1c, 3a, d, g. Measurements (n = 1): flagellomeres (µm): 65, 44, 44, 41, 50; AR 0.25; spermathecae 169–200 x 106–118. Tergite IX triangular. Gonapophysis VIII dorsomedial lobe weakly convergent at apex, coming to distinct point; ventrolateral lobes fused medially with U shaped notch.

Pupa. Fig. 4e. Measurements (n=1): length 2.5 mm. Sternites VI–VII with posterior spinule band.

***Oropuella* sp. F2**

Material examined: USA. WASHINGTON: King Co., Vashon Island, Judd Creek, 28A_17, 47.4034°N, 122.4688°W, 3.viii.2017, KC17VAS003 (1 female pupa (slide) ANSP-ENT-124826 [ANSP], 1 female pupa (slide) [AFPC]).

Diagnostic characters: *Adult female*. Tergite IX semicircular. Gonapophysis VIII dorsomedial lobes broadly separated.

Description: *Adult female (pharate)*. Figs 1d, 3b,e,h. Measurements (n=1): flagellomeres(µm): 58, 34, 39, 38, 42; AR 0.24; spermathecae 164–182 x 92–108. Tergite IX semicircular, with ~11 setae. Gonapophysis VIII dorsomedial lobe weakly convergent at apex, coming to distinct point; ventrolateral lobes separate.

Pupa. Fig. 4f. Measurements (n=2): length 2.1–2.2mm. Sternites VI–VII with posterior spinule band.

***Oropuella* sp. F3**

Material examined: USA. WASHINGTON: Pend Oreille Co., North Fork Sullivan Creek, 48.8607°N, 117.3272°W, 19.vii.2017, WADO-E17SE004 (1 female pupa (slide) [AFPC]).

Diagnostic characters: *Adult female.* Tergite IX semicircular, with ~11 setae. Gonapophysis VIII dorsomedial lobes nearly touching at apex.

Description: *Adult female (pharate)*. Figs 1d, 3c, f, i. Measurements (n = 1): flagellomeres (µm): 51, 41, 40, 41, 62; AR 0.35; spermathecae 157–159 x 102. Tergite IX semicircular. Gonapophysis VIII dorsomedial lobes nearly touching medially, apex broadly pointed; ventrolateral lobes separate.

Pupa. Fig. 4g. Measurements (n = 1): length 2.5 mm. Sternites VI–VII with posterior spinule band.

***Oropuella* unassociated larval material:** USA. CALIFORNIA: Lassen Volcanic National Park, Manzanita Creek, 40.5373°N,121.5926°W, 21.viii.2017, NPS17LAS010 (1 larva (slide) ANSP-ENT-124838 [ANSP]); Redwood National Park, Godwood Creek, 41.3704°N, 124.026°W, 11.vi.2018, NPS18REDW002 (2 larvae (slide) ANSP-ENT-124836–124837 [ANSP]); Redwood National Park, Unnamed Streelow Creek Trail, 41.3465°N, 124.0387°W, 6.vi.2018, NPS18REDW003 (1 larva (alcohol) ANSP-ENT-124839 [ANSP]). MONTANA: Granite Co., Lolo National Forest, Butte Cabin Creek, 46.512°N, 113.745°W, 29.v.2016, leg. A Fasbender (1 larva (slide) [AFPC]); Lewis and Clark Co., Dearborn River Middle Fork downhill from Hwy 200, 47.0914°N, 112.3618°W 27.ix.2017. MTDEQ17REF4005 (8 larvae (alcohol) [AFPC]). OREGON: Crater Lake National Park, Sun Creek, 42.8656°N, 122.08647°W 12.ix.2018, NPS18CRLA026 (3 larvae (slide) ANSP-ENT-124833–124835 [ANSP]); Josephine Co., Booze Creek at RM 0.2, 42.6596°N, 123.6597°W 24.viii.2017, ODEQ17SH031 (1 larva (alcohol) [AFPC]); Oregon Caves National Monument, Cave Creek, 42.098°N, 123.411°W, 2.vi.2015, NPS15ORCA001 (1 larva with pharate pupa ANSP-ENT-124832, 4 larvae (slide) ANSP-ENT-124828–124831 [ANSP]; 1 larva sequenced). WASHINGTON: King Co., Vashon Island, Judd Creek, 28A_17, 47.4034°N, 122.4688°W, 3.viii.2017, KC17VAS003 (1 larva (slide) [AFPC]); Pierce Co., Huckleberry Creek, 47.0085°N, 121.6173°W 2.viii.2017, WADOE17AM006 (3 larvae (alcohol) [AFPC]).

COI Barcoding

Oropuella was not closely associated with *Parametriocnemus, Paraphaenocladius* or other genera based on publicly available chironomid COI sequences in the BOLD database (Fig. 6). Although a neighbor-joining analysis does not provide a cladistic interpretation of relationships, *Oropuella* shows greater genetic distance from *Parametriocnemus* and *Paraphaenocladius* than specimens of either of those genera from the other. Since the specimen sequenced was a larva, the barcode is not currently assignable to a named species. The COI barcode sequence is provided in supplemental material.

Discussion

Identification and taxonomic placement. As genus keys are regularly used by non-specialists to identify chironomids the following amendments for the keys in common use for the western Nearctic are proposed. The pupae of *Oropuella* key directly to *Parametriocnemus* in Coffman *et al.* (1986) couplet 45 and Ferrington *et al.* (2008) couplet 188. In both instances adding the following couplet will separate the genera:

Pedes spurii B digitiform, length >1.5 width at base; wing sheath with 2–4 pearl rows ….....
.....…..… *Parametriocnemus* Goetghebuer

Pedes spurii B triangular, length subequal to width at base; wing sheath with single pearl row ……....
.....… *Oropuella* gen. n.

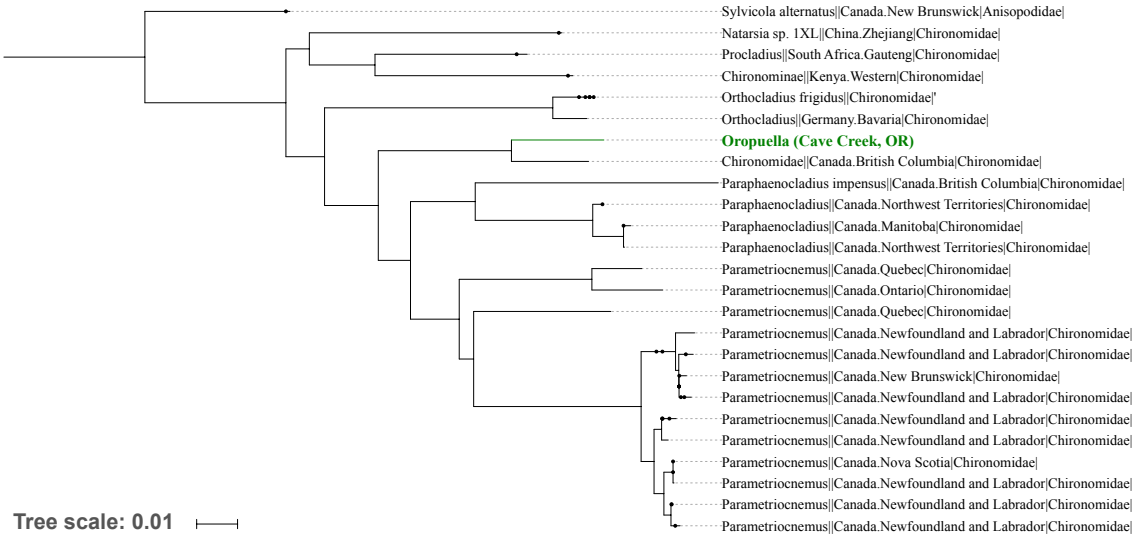

Figure 6. Kimura 2 Parameter neighbor-joining tree of COI barcode sequences for *Oropuella* and related genera. Circles denote nodes with multiple sequences pruned for clarity.

Larvae of *Oropuella* do not key readily in Andersen *et al.* (2013), as the antennal ratio (median 1.15) falls usually falls into the gap between <1.0 (*Paraphaenocladius* and *Aagardia*) and >1.25 *(Parametriocnemus*) in couplet 61. The antenna structure and single median tooth of the mentum are incorrect for *Parametriocnemus*, though the character of antennal blade shorter than flagellum is correct. If forced to couplet 62 the genus will key to *Paraphaenocladius*, though the antenna ratio and structure is also different and the terminal abdominal segment is not bent ventrally. A suggested solution would be to add the following couplet before couplet 61:

Antenna with break in second flagellomere .... …. *Oropuella* gen. n.

Antenna without break in second flagellomere ….. 61

Ferrington *et al*. (2008) also keys this species to *Parametriocnemus* in couplet 54, provided conflicting character states are ignored (such as the double median tooth of the mentum in couplet 52). If the number of medial teeth in couplet 52 is amended the extra couplet provided above could be inserted immediately following it, leading into couplet 53. Due to the usage of pharate adults it is problematic to update Cranston *et al.* (1989), because of the wing venation character in couplet 35.

Oropuella shows strong affinities to *Parametriocnemus* and *Paraphaenocladius* in all life stages, suggesting it should be included in a clade with these genera. Synapomorphies which support this grouping are: weakly convex mentum with double ventromental plates directed perpendicular to the occipital margin (larva), pearl rows on the wing sheath (not found in all *Paraphaenocladius*), and spinules or spines at the apex of the anal lobes (pupa). The following characters are hypothesized synapomorphies of each genus in this clade: *Oropuella* larval antenna with desclerotized break in second flagellomere, mentum pale; *Parametriocnemus* pupa with digitiform pedes spurii B, adult male with parallel sided posteromedial eye extension; *Paraphaenocladius* larva with antennal ratio <1.0, terminal segment of abdomen bent ventrally, pupa with reduced anal lobe and 0–2 anal setae. The structure of the larval antenna in *Oropuella* is quite unlike that found in either of the other genera in this clade, being similar to *Heleniella* Gowin with the break in the second segment. However, the significance of this character is difficult to interpret, as a similar break in the second antennal segment is found in the distantly related genus *Brillia* Kieffer.

The distribution, habitat and species taxonomy of Oropuella. Only a fraction of the material and records available for this study were able to be associated with the male pharate pupae upon which the named species are based. The limited number of pharate male specimens make confident statements about species diversity or distribution in the genus impossible. Both named species are from the Pacific Coast region, on the western edge of the range of the genus; only further associated material can clarify the range of each species, or if additional species are present. *O. eidolon* and *O. pallida* are delineated primarily by features of the male genitalia and the presence/absence of a posterior spinule row on sternite V in the pupa, but additional collections may uncover further diagnostic characters.

Female material examined in this study could be separated into three morphospecies based on adult genitalia characters. Unfortunately, none of these female morphospecies could be associated with either of the male species based on locality data. This is compounded because the female pupa morphology differs from the male species in the number of sternites with spinule bands. The lack of a spinule band on sternite VIII is hypothesized to be a sexual dimorphism based on similar features in related genera (*Parametriocnemus* and *Heterotrissocladius*). The female pupae could perhaps be distinguished by meristic characters, but the number of specimens in this study is insufficient for such inferences. From these considerations I have refrained from either giving tentative associations or formally naming species from the female material.

Larvae are currently inseparable at the species level, due to the paucity of associated larval material. Despite this limitation, larval records from the RAI project database show *Oropuella* (reported as RAI Orthocladiinae #0001) to be widely distributed in the Pacific drainage and western Great Basin of North America, from the states of California, Idaho, Nevada, Oregon, Washington, and the Clark Fork and Kootenai drainages of Montana. Additionally, larvae were sampled from the Missouri River drainage on the east side of the Continental Divide in Montana in the Little Belt Mountains and Rocky Mountain Front. As these eastern ranges are contiguous or adjacent to ranges contiguous with the continental divide, adult dispersal of *Oropuella* eastward from the Pacific drainage seems the likely mode of colonization.

Collections of *Oropuella* have been from small mountain streams, usually <10m wide. I was able

Figure 7. Larval habitat. USA, Montana, Granite Co., Lolo National Forest, Butte Cabin Creek, 46.512°N, 133.745°W, 29.v.2016.

to collect larvae of *Oropuella* from Butte Cabin Creek, Granite Co., Montana on 29.v.2016. The stream at the collection site was 3–4m wide, with a canopy of *Alnus* sp. extending from the riparian zone (Fig. 7). The current was swift, with the substrate consisting of cobble. Specimens were collected via kick sample using a D-net, with one larva containing a pharate pupa found. Phenology of the genus or individual species is not yet established, but pharate pupae have been collected in both late May and August from different localities, with apparent last instar larvae present in June, July and September. Whether this represents a bivoltine lifecycle or is an artifact of local climatic conditions merits investigation.

Orthocladiinae taxonomy in western North America. Oropuella is not the sole new genus in the western Nearctic, as there are a number of undescribed larval and pupal taxa that appear in aquatic macroinvertebrate sampling (Ferrington *et al.* 2008, A. Fasbender pers. obs.), currently waiting on associations with adult males for formal description. Species taxonomy in almost all of the established genera remains in the early stages of discovery. For example, *Cricotopus* Wulp and *Eukiefferiella* Thienemann have numerous distinguishable morphospecies in western North America (A. Fasbender pers. obs.), yet the Nearctic species taxonomy for both of these genera remains deficient or confused. While there are 68 valid Nearctic *Cricotopus* species (Oliver *et al.* 1990; Sublette *et al.* 1998), many were identified assuming that the western Palaearctic keys in Hirvenoja (1973) would be applicable to the Nearctic fauna (Simpson *et al.* 1983). Only limited attempts have been to verify the Nearctic identifications with comparison to type material or molecular vouchers of the European species (Epler 2001; Gresens *et al.* 2012); furthermore, western *Cricotopus* immatures have been found which challenge the current pupal and larval diagnoses or exhibit novel characters for the genus (A. Fasbender pers. obs.). On the other hand, the knowledge of *Eukiefferiella* (one of the most speciose orthoclad genera globally) is so rudimentary that there are six named Nearctic species (Oliver *et al.* 1990; Sublette *et al*. 1998) while eight species groups recorded from the bioregion (Bode 1983).

Smaller genera are no better known, for example *Parorthocladius* has been recorded from the region for over 30 years yet no species have been described (Coffman *et al.* 1986; Cranston *et al.* 1989;

Oliver *et al.* 1990; Andersen *et al.* 2013). Examination of "species poor" taxa such as *Psilometriocnemus* Sæther (Cranston & Oliver 1988a) from the region has also revealed undescribed species diversity (A. Fasbender pers. obs.). Based on the above observations, and considering the climate, geography and geology of the region, high species diversity of Orthocladiinae is to be expected.

Conclusions

Oropuella is a distinctive member of the Nearctic orthoclad fauna, readily diagnosable in the immature stages, and the adult males are also distinguishable from similar Orthocladiinae genera via a combination of body and genitalia characters. Locality records and field observations show the taxon is rheophilic, inhabiting cool, montane streams through much of northwestern North America.

Acknowledgements

Eric Dinger (National Park Service), Shannon Hubler (Oregon Department of Environmental Quality), Chad Larson (Washington Department of Ecology) and Kate Macneale (King County Department of Natural Resources) provided access to material for this study. Rebecca Spring surveyed the RAI database for records, and assisted in retrieving information from BOLD. Torbjørn Ekrem, Barbara Hayford, Jeff Webb and two anonymous reviewers provided useful suggestions on previous drafts of this manuscript.

References

Andersen, T., Sæther, O.A., Cranston, P.S. and Epler, J.H. 2013. The larvae of Orthocladiinae (Diptera: Chironomidae) of the Holarctic region – keys and diagnoses. *In*: Andersen, T., Cranston P.S., and Epler, J.H. Chironomidae of the Holarctic region – keys and diagnoses - larvae. - *Insect Systematics and Evolution Supplement* 66: 189–385.

Bode, R.W. 1983. Larvae of North American *Eukiefferiella* and *Tvetenia*. - *New York State Museum Bulletin* 452: 1–40.

Brundin, L. 1966. Transantarctic relationships and their significance, as evidenced by chironomid midges with a monograph of the subfamilies Podonominae and Aphroteniinae and the austral Heptagiae. - *Kungliga Svenska Vetenskapsakademiens Handlingar* 11: 1–472

Coffman, W.P., Cranston, P.S., Oliver, D.R. and Sæther, O.A. The pupae of Orthocladiinae (Diptera: Chironomidae) of the Holarctic region – keys and diagnoses. *In*: Wiederholm, T. Chironomidae of the Holarctic region keys and diagnoses. Part 2. Pupae. - *Entomologica Scandinavica Supplement* 28: 147–296.

Cranston, P.S. 1999. Nearctic *Orthocladius* subgenus *Eudactylocladius* revised (Diptera: Chironomidae). - *Journal of the Kansas Entomological Society* 71: 272–295.

Cranston, P.S., Benigno, G.M. and Dominguez, M. 2007. *Hydrobaenus saetheri* Cranston, new species, an aestivating, winter emerging chironomid (Diptera: Chironomidae) from California. *In*: Andersen, T. *Contributions to the systematics and ecology of aquatic Diptera*. - *A tribute to Ole Sæther*: 73–79.

Cranston, P.S. and Judd, D.D. 1987. *Metriocnemus* (Diptera: Chironomidae) – an ecological survey and description of a new species. - *Journal of the New York Entomological Society* 95(4): 534–546.

Cranston, P.S. and Krosch, M. 2011. *Barbadocladius* Cranston & Krosch, a new genus of Orthocladiinae (Diptera: Chironomidae) from South America. - *Neotropical Entomology* 40: 560–567. DOI: http://dx.doi.org/10.1590/S1519-566X2011000500007

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

Cranston, P.S. and Oliver, D.R. 1988b. Aquatic xylophagous Orthocladiinae – systematics and ecology. - *Spixiana Supplement* 14: 143–154.

Cranston, P.S., Oliver, D.R. and Sæther, O.A. The adult males of Orthocladiinae (Diptera: Chironomidae) of the Holarctic region – keys and diagnoses. *In*: Wiederholm, T. Chironomidae of the Holarctic region keys and diagnoses. Part 3. Adult males. - *Entomologica Scandinavica* 34: 165–352.

Cumming, J.M. and Wood, D.M. 2017. Adult morphology and terminology. *In*: Kirk-Spriggs, A.H. and Sinclair, B.J. Manual of Afrotropical Diptera. Volume 1. Introductory Chapters and keys to Diptera families. - *Suricata* 4: 89–133.

Ekrem, T., Ashe, P., Andersen, T. and Stur, E. 2017. Chironomidae. *In*: Kirk-Spriggs, A.H. and Sinclair, B.J. Manual of Afrotropical Diptera. Volume 2. Nematocerous Diptera and lower Brachycera. - *Suricata* 5: 813–863.

Epler, J.H. 2001. Identification Manual for the larval Chironomidae (Diptera) of North and

South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida. - Special Publication SJ2001-SP13. North Carolina Department of Environment and Natural Resources, Raleigh, NC, and St. Johns River Water Management District, Palatka, FL. 1–526.

Ferrington, L.C., Berg, M.B. and Coffman, W.P. 2008. Chironomidae. In Merritt, R.W., Cummins, K.W. and Berg, M.B. (Ed.) *An Introduction to the Aquatic Insects of North America*, 4th ed. Kendall/Hunt Publishing Company, pp. 847-989.

Ferrington, L.C. and Sæther, O.A. 2011. A revision of the genera *Pseudosmittia* Edwards, 1932, *Allocladius* Kieffer, 1913, and *Hydrosmittia* gen. n. (Diptera: Chironomidae, Orthocladiinae). - *Zootaxa* 2849: 1–314. DOI: http://dx.doi.org/10.11646/zootaxa.2849.1.1

Gresens, S.E., Stur, E. and Ekrem, T. 2012. Phenotypic and genetic variation within the *Cricotopus sylvestris* species-group (Diptera, Chironomidae), across a Nearctic – Palaearctic gradient. - *Fauna Norvegica* 31: 137–149. DOI: https://doi.org/10.5324/fn.v31i0.1417

Harrison, A.D. and Cranston, P.S. 2007. *Elpiscladius* Harrison and Cranston, a new orthoclad (Diptera: Chironomidae) in the Brillia-group from South Africa. - *Annals of the Eastern Cape Museums* 6: 1–11.

Hestenes, T.C. and Sæther, O.A. 2000. Three new Nearctic *Thienemanniella* Kieffer species with a review of the Nearctic species. *In*: Hoffrichter, O. - *Late 20th Century Research on Chironomidae: an Anthology from the 13th International Symposium on Chironomidae*: 103–127.

Hirvenoja, M. 1973. Revision der Gattung *Cricotopus* van der Wulp und ihrer Verwandten (Diptera, Chironomidae). - *Annales Zoologici Fennici* 10(1): 1–363.

Hogue, C.L. and Bedoya Ortiz, I. 1987. The net-winged midge fauna (Diptera: Blephariceridae) of Antioquia Department, Colombia. - *Contributions in Science, Natural History Museum of Los Angeles County* 413: 1–57.

Langton, P.H. 1994. If not "filaments" then what? - *Chironomus Newsletter on Chironomidae Research* 6: 9.

Letunic, I. and Bork, P. 2006. Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. - *Bioinformatics* 23(1): 127–128. DOI: https://doi.org/10.1093/bioinformatics/btl529

Letunic, I. and Bork, P. 2019. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. - *Nucleic Acids Research* gkz239. DOI: https://doi.org/10.1093/nar/gkz239

Lifescanner. 2019. http://lifescanner.net. Accessed 4/11/2019.

McKie, B.G. and Cranston, P.S. 2005. Size matters: systematic and ecological implications of allometry in the responses of chironomid midge morphological ratios to experimental temperature manipulations. - *Canadian Journal of Zoology* 83: 553–568. DOI: https://doi.org/10.1139/Z05-051

Namayandeh, A. and Culp, J.M. 2016. Chironomidae larvae from the lower Athabasca River, AB, Canada and its tributaries including macroscopic subfamily and tribe keys, indices for environmental tolerance, and trait based information for biomonitoring. - *Journal of Entomological and Acarological Reseach* 48: 201–232. DOI: https://doi.org/10.4081/jear.2016.6075

Oliver, D.R. 1977. *Bicinctus*-group of the genus *Cricotopus* van der Wulp (Diptera: Chironomidae) in the Nearctic with a description of a new species. - *Journal of the Fisheries Research Board of Canada* 34: 98–104. DOI: https://doi.org/10.1139/f77-011

Oliver, D.R. 1981. Description of *Euryhapsis* new genus, including three new species (Diptera: Chironomidae). - *The Canadian Entomologist* 133: 711–722. DOI: https://doi.org/10.4039/Ent113711-8

Oliver, D.R. 1983. Redescription of *Brillia* Kieffer (Diptera: Chironomidae) with descriptions of Nearctic species. - *The Canadian Entomologist* 115: 257–279. DOI: https://doi.org/10.4039/Ent115257-3

Oliver, D.R. 1984. Description of a new species of *Cricotopus* van der Wulp associated with *Myriophyllum spicatum*. - *The Canadian Entomologist* 116: 1287–1292. DOI: https://doi.org/10.4039/Ent1161287-10

Oliver, D.R., Dillon, M.E. and Cranston, P.S. 1990. A catalog of Nearctic Chironomidae. - *Agriculture Canada Publication* 1857/B: 1–89.

Ratnasingham, S. and Hebert, P.D.N. 2007. BOLD: The Barcode of Life Data System. - *Molecular Ecology Notes* 2007: 1–10. DOI: https://doi.org/10.1111/j.1471-8286.2007.01678.x

Roback, S.S. 1957. Some Tendipedidae from Utah. - *Proceedings of the Academy of Natural Sciences of Philadelphia* 109: 1–24.

Roper, B.B., Buffington, J.M., Bennett, S., Lanigan, S.H., Archer, E., Downie, S.T., Faustini, J., Hillman, T.W., Hubler, S., Jones, K., Jordan, C., Kaufmann, P.R., Merritt, G., Moyer, C. and Pleus, A. 2010. A comparison of the performance and compatibility of protocols used by seven monitoring groups to measure stream habitat in the Pacific Northwest. - *North American Journal of Fisheries Management* 30: 565–587. DOI: https://doi.org/10.1577/M09-061.1

Sæther, O.A. 1969. Some Nearctic Podonominae, Diamesinae, and Orthocladiinae. - *Bulletin of the Fisheries Research Board of Canada* 170: 1–154.

Sæther, O.A. 1975. Nearctic and Palaearctic *Heterotrissocladius* (Diptera: Chironomidae). - *Bulletin of the Fisheries Research Board of Canada* 193: 1–67.

Sæther, O.A. 1976. Revision of *Hydrobaenus, Trissocladius, Zalutschia, Paratrissocladius* and some related genera (Diptera: Chironomidae). - *Bulletin of the Fisheries Research Board of Canada* 195: 1–287.

Sæther, O.A. 1980. Glossary of chironomid morphology terminology (Diptera: Chironomidae). - *Entomologica Scandinavica Supplement* 14: 1–51.

Sæther, O.A. 1984. *Apometriocnemus fontinalis* gen. n. sp. n. (Diptera: Chironomidae) from Tennessee, USA. - *Entomologica Scandinavica* 15: 536–539.

Sæther, O.A. and 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: 1–100.

Sæther, O.A. and Wang, X. 1995. Revision of the genus *Paraphaenocladius* Thienemann, 1924 of the world (Diptera: Chironomidae, Orthocladiinae. - *Entomologica Scandinavica Supplement* 48: 1–69.

Simpson, K.W., Bode, R.W., Albu, P. 1983. Keys for the genus *Cricotopus* adapted from "Revision der Gattung *Cricotopus* van der Wulp und ihrer Verwandten (Diptera, Chironomidae)" by M. Hirvenoja. - *New York State Museum Bulletin* 450: 1–133.

Soponis, A.R. 1977. A revision of the Nearctic species of *Orthocladius* (*Orthocladius*) van der Wulp (Diptera: Chironomidae). - *Memoirs of the Entomological Society of Canada* 102: 1–187. DOI: https://doi.org/10.4039/entm109102fv

Soponis, A.R. 1990. A revision of the Holarctic species of *Orthocladius* (*Euorthocladius*). - *Spixiana Supplement* 13: 1–56.

Sublette, J.E., Stevens, L.E. and Shannon, J.P. 1998. Chironomidae (Diptera) of the Colorado River, Grand Canyon, Arizona, USA, I: systematics and ecology. - *Great Basin Naturalist* 58(2): 97–146.

Sublette, J.E. and Sublette, M.S. 1971. The Orthocladiinae (Chironomidae, Dipt.) of California. I. The *Cricotopus infuscatus* group. - *Entomological News* 82: 85–102.

Wood, D.M. 1991. Homology and phylogenetic implications of male genitalia in Diptera: the ground plan. *In*: Weismann, L., Orszagh, I. and Pont, A.C. - *Proceedings of the Second International Congress of Dipterology*: 255–284.

Article submitted 21. May 2019, accepted by Torbjørn Ekrem 31. January 2020, published 12. February 2020.