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Pupae of the Nearctic species of *Culicoides* Latreille subgenus *Monoculicoides* Khalaf (Diptera: Ceratopogonidae)

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Abstract

There are 160 valid species of *Culicoides* Latreille described from the Nearctic Region, but many are unknown as immatures or, although partially described, cannot be readily identified. We provide pupal diagnoses, descriptions, and a key to all seven known species of *Culicoides* subgenus *Monoculicoides* Khalaf occurring in the Nearctic biogeographic region. A diagnosis of several characters unique to this subgenus is provided to allow for the recognition of the included species from those of other subgeneric groupings. Microphotography of important morphological characters for both the key and descriptions are provided. This study highlights the usefulness of examining the pupae of *Culicoides* as a tool for species identification. The pupae of *Culicoides grandensis* Grogan & Phillips, *C. occidentalis* Wirth & Jones, and *C. shemanchuki* Grogan & Lysyk are newly described and those of *C. riethi* Kieffer, *C. stigma* (Meigen), and *C. variipennis* Wirth & Jones are redescribed in detail. The seventh species, *C. sonorensis*, was fully described recently.

Key words: Immature, aquatic, pupal key, Bluetongue, Epizootic Hemorrhagic Disease, vector

Introduction

The distribution of *Culicoides* Latreille is nearly worldwide (it is absent from New Zealand and Antarctica), with 1,368 valid species, 32 subgenera, 38 species groups, and 176 unplaced species (Borkent 2016). Commonly referred to as biting midges, most female members of this genus feed on a multitude of vertebrate hosts including fish, turtles, lizards, birds, and mammals, and many are ecologically important as disease pathogen vectors (Borkent 2004). Immature *Culicoides* can be found in a variety of aquatic or semiaquatic habitats, including tree holes, ponds, marshes, streams, various muddy and saturated organic materials, damp or rotting vegetation, and manure (Kettle & Lawson 1952; Jamnback 1965; Borkent 2014). Beyond the general biology and habitats of the larvae and pupae, not much is known about these stages of *Culicoides*. Of the identified species, immature descriptions do not exist for 87% of larvae and 83% of pupae of the group (Borkent 2014).

Study of pupal characters can aid in the interpretation of some of the more morphologically challenging species groups within *Culicoides*. Some studies have shown distinctive variation in the pupae of certain species with otherwise very similar adults (Kettle & Elson 1976; Nevill *et al*. 2007; Nevill *et al*. 2009). Aside from their value in distinguishing species, the character states of pupae may be useful in cladistic analyses of these taxa. Recent studies have produced very thorough pupal descriptions of *Culicoides* pupae and have provided a template for future descriptions (Nevill *et al.* 2009; Ronderos *et al.* 2013; Anjos-Santos *et al.* 2014; Shults *et al.* 2016; Harsha *et al.* 2017).

The most economically important and thoroughly studied species of *Culicoides* in North America is *Culicoides sonorensis* Wirth & Jones which is a known vector of the Bluetongue (BT) and Epizootic Hemorrhagic Disease (EHD) viruses. This species of biting midge is a member of the subgenus *Monoculicoides* Khalaf, which includes 23 species that are primarily distributed in the Palearctic and Nearctic regions, with one Oriental species and one South African species (Shults 2015). Since the turn of the last millennium, the taxonomy of Nearctic species of this

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subgenus has seen an influx of new information. The five subspecies of the *C. variipennis* complex proposed by Wirth & Jones (1957) were electrophoretically and morphologically re-examined by Holbrook *et al*. (2000) who recognized three distinct species: *C. occidentalis* Wirth & Jones, *C. sonorensis*, and *C. variipennis* (Coquillett), with the remaining two subspecies becoming synonyms of *C. sonorensis*. Two additional species have been recently described, *Culicoides grandensis* Grogan & Phillips and *Culicoides shemanchuki* Grogan & Lysyk (Grogan & Phillips 2008; Grogan & Lysyk 2015). The North American species *C. gigas* Root & Hoffman was sunk as a junior synonym of the Palearctic species, *C. riethi* Kieffer, and *C. stigma* Meigen, previously restricted to the Palearctic, was newly recognized in the Nearctic (Grogan & Lysyk 2015). There are, therefore, currently seven species of *C*. (*Monoculicoides*) recognized in the Nearctic Region but the differences between the pupae of these have been uncertain. Based on the detailed recent study of the pupa of *C. sonorensis* (Shults *et al*. 2016), we provide the first descriptions of the pupae of *C. grandensis*, *C. occidentalis*, and *C. shemanchuki*, and those of *C. riethi*, *C. stigma*, and *C. variipennis* are redescribed.

Materials and methods

Slide mounted exuviae of all known Nearctic species of *C*. (*Monoculicoides*), except *C. sonorensis*, which was described in detail by Shults *et al*. (2016), were borrowed from the following collections:

CNCI	Canadian National Collection of Insects, Ottawa, Ontario, Canada.
NYSM	New York State Museum, Albany, New York, USA.
TAMU	Texas A&M University Entomology Museum, College Station, Texas, USA.
USNM	National Museum of Natural History, Washington, D.C., USA.
ZIN	Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia.

The diagnosis and description of *C*. (*Monoculicoides*) was determined through examination of specimens of all those species noted in bold, as well study of all previously published literature worldwide describing pupae in Borkent (2014: Table 2). In addition, 35 Nearctic species of *Culicoides* in 9 of the 12 other subgenera from North America (Borkent and Grogan, 2009) were studied in further detail to ensure the validity of the diagnosis. As such, the Nearctic subgenera compared to the diagnosis of *Monoculicoides* were *Amossovia* Glukhova, *Beltranmyia* Vargas, *Culicoides* Latreille, *Diphaomyia* Vargas, *Haematomyidium* Goeldi, *Hoffmania* Fox, *Oecacta* Poey, *Selfia* Khalaf, and *Silvaticulicoides* Glukhova. We were unable to obtain specimens of any North American pupae of *Avaritia* Fox or *Drymodesmyia* Vargas, and no Nearctic pupa of *Wirthomyia* Vargas has been described. We did however examine the pupal descriptions of species of *C*. (*Avaritia*) and *C*. (*Drymodesmyia*), including those from other regions (Jamnback 1965; Lamberson 1992; Nevill *et al*. 2007). Pupal exuviae and their associated adults were slide-mounted in Canada balsam (Borkent & Spinelli 2007) and observed with a Nikon Alphashot-2 YS2 compound microscope and a Leica S6D dissecting microscope.

Measurements and statistics are reported as: range followed by the following in parentheses: mean, standard deviation (SD), number measured (n). Analysis of Variance (ANOVA), Students-t, posthoc analyses, and graphs were completed using the software program JMP Pro 13.

Terms and abbreviations follow those used by Borkent (2014) and Shults *et al*. (2016). We examined additional characters to those previously noted as important diagnostic features for the identification of *Culicoides* pupae (Jones 1961; Jamnback 1965; Atchley 1970; Lamberson 1992; Nevill *et al*. 2007). Descriptions follow an anterior to posterior and dorsal to ventral organization. Features present in all *Culicoides* (Borkent 2014) are not repeated here. The use of the term *C. variipennis* complex denotes the grouping of three species, *C. occidentalis*, *C. sonorensis*, and *C. variipennis* previously regarded as subspecies of *C. variipennis* (Wirth & Jones 1957).

Results

Species descriptions are arranged alphabetically. An overview of the morphometric data can be found in tables 1 and 2.

TABLE 1. Combined male and female morphometric data of total length, cephalothorax length, respiratory organ (RO) length, and respiratory organ (RO) width for all species. The measurements are given as: range (mean) and are in millimeters.

Species	n	Total Length	Cephalothorax Length	RO Length	RO Width
occidentalis	11	2.71–3.40 (3.05)	1.30–1.37 (1.33)	0.36–0.45 (0.41)	0.04–0.05 (0.04)
sonorensis	24	1.68–2.25 (2.05)	0.94–1.07 (1.00)	0.28–0.35 (0.32)	0.03–0.03 (0.03)
variipennis	11	2.60–4.15 (3.43)	1.25–1.65 (1.48)	0.36–0.43 (0.39)	0.04–0.06 (0.05)
grandensis	1	2.77	1.05	0.30	0.04
riethi	5	3.30–3.94 (3.60)	1.24–1.40 (1.33)	0.37–0.41 (0.39)	0.05–0.06 (0.06)
shemanchuki	2	2.95–3.57 (3.26)	1.26–1.35 (1.31)	0.34–0.36 (0.35)	0.04–0.04 (0.04)
stigma	32	1.94–2.86 (2.39)	1.00–1.20 (1.08)	0.21–0.26 (0.24)	0.03–0.04 (0.03)

TABLE 2. Combined male and female morphometric data of respiratory organ (RO) ratio (width/length), dorsal apotome (DA) length, dorsal apotome (DA) width, and dorsal apotome (DA) ratio (width/length) for all species. The measurements are given as: range (mean) and are in millimeters.

Species	n	RO Ratio	DA Length	DA Width	DA Ratio
occidentalis	11	0.09–0.14 (0.11)	0.29–0.37 (0.32)	0.21–0.25 (0.22)	0.59–0.79 (0.70)
sonorensis	24	0.09–0.11 (0.09)	0.23–0.30 (0.26)	0.18–0.21 (0.19)	0.62–0.89 (0.75)
variipennis	11	0.10–0.16 (0.12)	0.27–0.38 (0.31)	0.20–0.25 (0.22)	0.59–0.86 (0.73)
grandensis	1	0.13	0.23	0.20	0.87
riethi	5	0.12–0.16 (0.14)	0.31–0.34 (0.32)	0.21–0.23 (0.22)	0.64–0.74 (0.68)
shemanchuki	2	0.11–0.12 (0.12)	0.27–0.32 (0.30)	0.18–0.21 (0.20)	0.56–0.78 (0.67)
stigma	32	0.12–0.19 (0.13)	0.23–0.32 (0.28)	0.17–0.22 (0.19)	0.56–0.80 (0.66)

Subgenus *Culicoides* (*Monoculicoides*)

Diagnosis. The only group of species of Nearctic *Culicoides* with the following combination of pupal characters: Dorsal apotome without a dorsomedial tubercle, with dark banding on the apex of the pedicel and the base of the respiratory organ (Fig. 1); respiratory organ elongate and slender, with a dark apex; midlength portion bearing scales (Fig. 2A–B), and 2–5 subbasal pores arranged along its length (Fig. 2A–B); shagreen present on the anterior and posterior margins of the abdominal segments with varying amounts of shagreen laterally (Figs 6A–B & 7A–B); and terminal processes extending posterolaterally and with a dark apex (Fig. 8A–B).

Description. Male. Total length = 2.05–3.70 mm. Light to medium brown coloration throughout (Fig. 1). **Head:** Dorsal apotome (DA) with or without spicules, without dorsomedial tubercle, dorsal margin rounded, lateral margins with distinct points (Figs 3A–D & 4A–C), DA length (DAL) = 0.26–0.37 mm; DA width (DAW) = 0.17–0.22 mm; DAW/DAL= 0.56–0.75. DA–1–H medium long, thick seta on well–developed tubercle, DA–2–H dorsal to tubercle base. Dorsolateral cephalic sclerite with one long, one short seta. Palpus extending posteriorly to posterolateral margin of labium, CL–1–H about twice length of CL–2–H. **Thorax:** Cephalothorax length = 0.96–1.60 mm. Pedicel of respiratory organ short with dark banding at apex (Fig. 1). Respiratory organ (RO) elongate, slender, apex dark brown, mid-length portion with scales, without annulations, with short membranous base, medium brown coloration on posterior margin, with pores closely abutting in single row at apex, 2–5 subbasal pores (Fig. 2A–B), RO length = 0.27–0.45 mm; RO width = 0.03–0.06 mm; ROW/ROL = 0.08–0.15, tracheal tube with reticulations. Anterolateral sensilla (one long, one short seta) on well–developed tubercle. Mesonotum with small bumps anterior to D–5–T, smooth posteriorly, D–1–T, D–2–T short, stout, each on rounded tubercle, D–3–T posterior to small rounded tubercle bearing long, slender D–4–T, D–5–T short or long, on small rounded tubercle, D–1–T, D–2–T, D–5–T in longitudinal row (Fig. 5A–B). Wing with apical tubercle; halter apex slightly separated from hind leg. Metathorax completely separated medially, with long, thin M–1–T near anterior margin, M–2–T, M–3–T more posterior. **Abdomen:** Tergites 2–7 each with darker pigmentation as medial group of

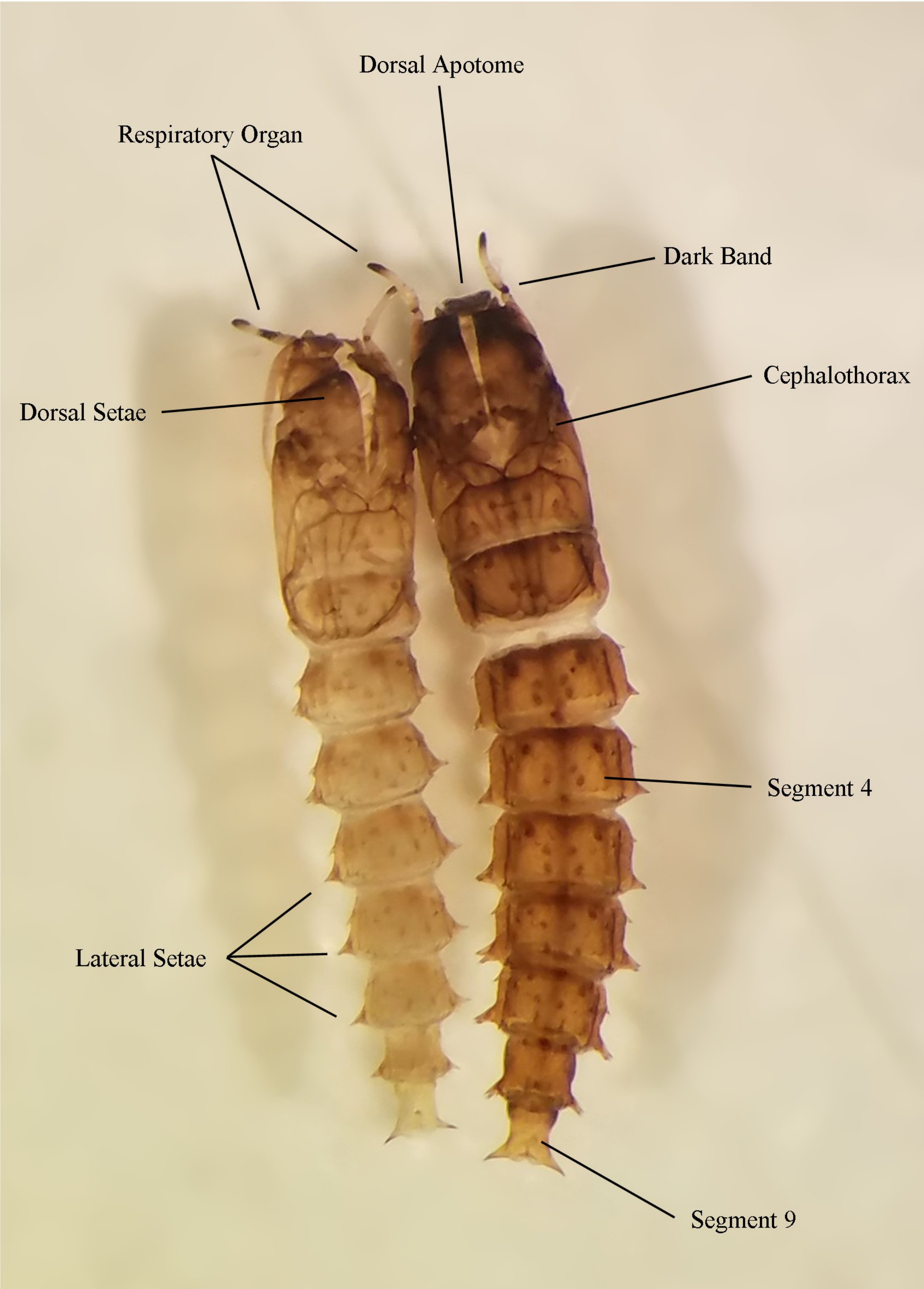

FIGURE 1. Habitus of the pupae of *Culicoides occidentalis* (left) and *C. riethi* (right) in dorsal view.

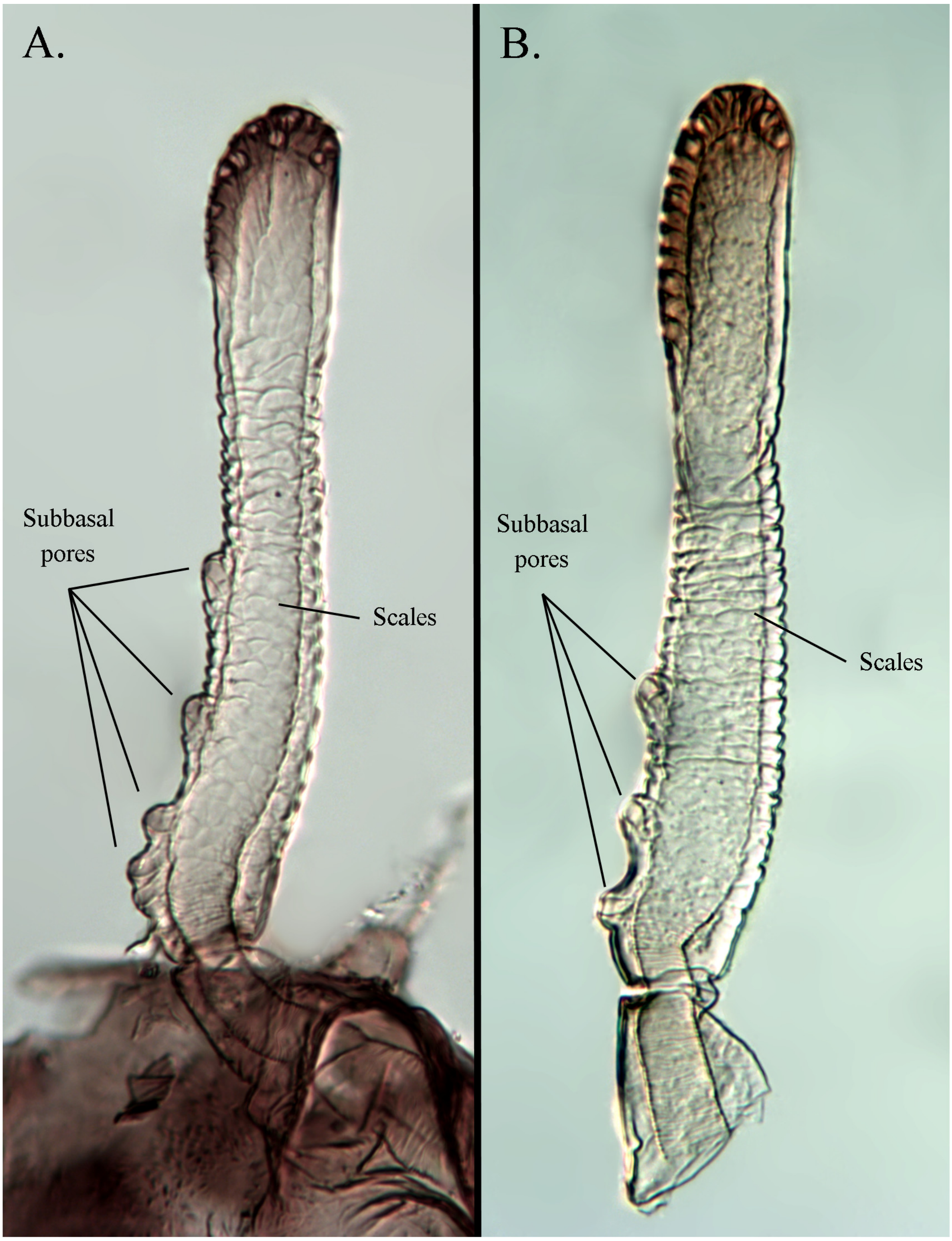

FIGURE 2. Respiratory organs, in lateral view. (A) *Culicoides shemanchuki*. (B) *C. occidentalis*.

three patches, with anterolateral pair, sternites 3–7 each with two medial patches, with anterolateral pair (Figs 1, 6A–B, & 7A–B). Distribution of shagreen of abdominal segments variable, though never excessive. Tergite 1 with long D–3–I, short D–2–I, D–7–I anterior, campaniform D–4–I, short D–8–I, long, thin D–9–I on short tubercle posteriorly, L–2–I, L–3–I short separated medially by long, thin L–1–I. Tergite 2 without elongate tubercles, minute L–2–II, L–4–II separated medially by long, thin L–3–II on anterolateral margin. Chaetotaxy of tergite 2, 3, 5–7 similar to that of tergite 4. Tergite 4 with short D–2–IV on short tubercle, thin D–3–IV on elongate, pointed tubercle, D–5–IV, D–4–IV, D–7–IV, D–8–IV, D–9–IV in transverse row, arranged medially to laterally, minute D–5–IV on slightly formed tubercle, D–4–IV on small tubercle, D–7–IV on small rounded tubercle, D–8–IV, D–9–IV each long on pointed tubercle, D–8–IV seta thicker than D–9–IV (Fig. 7A–B); lateral tubercles with elongate, slender apices, L–1–IV thick seta on bifurcate tubercle, L–2–IV, L–4–IV thick setae, each on elongate bifurcate tubercle, separated by thin L–3–IV on elongate bifurcate tubercle (Figs 6A–B & 7A–B); sternite 4 with minute V–5–IV on small rounded tubercle, V–6–IV thin on pointed tubercle, small V–7–IV on pointed tubercle, ventral setae in transverse row, shagreen along anterior margins (Fig. 6A–B). Segment 8 chaetotaxy with seven sensilla: D–8–VIII, D–9–VIII, L–2–VIII, L–3–VIII, L–4–VIII, V–6–VIII, V–7–VIII. Segment 9 with genital lobes extending to posterior margin, terminal process projecting posterodorsolaterally to laterally, tapered to pointed, dark apex (Fig. 8A–B).

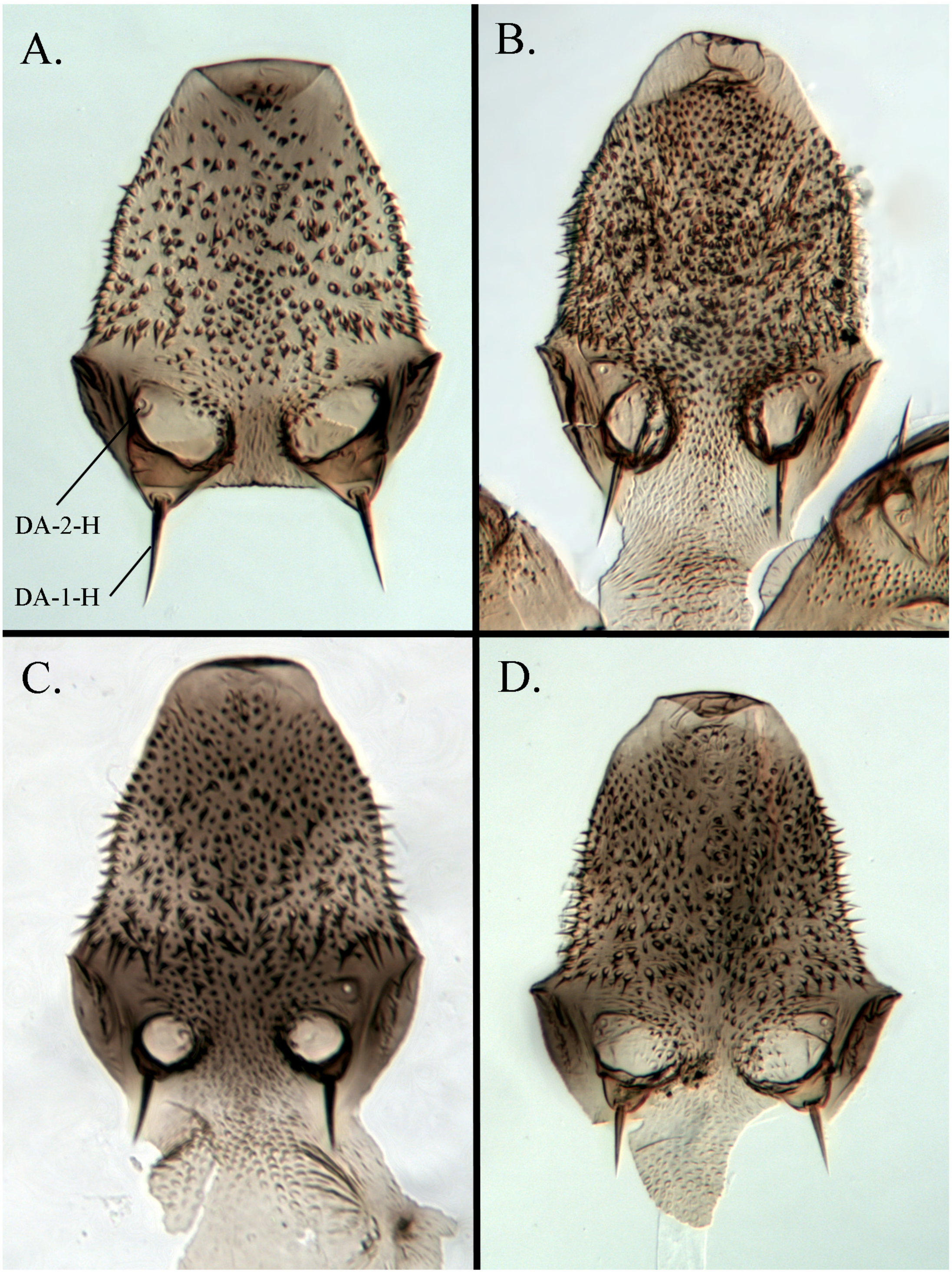

FIGURE 3. Female, dorsal apotomes, in anterior view. (A) *Culicoides occidentalis*. (B) *C. riethi*. (C) *C. sonorensis*. (D) *C. variipennis*.

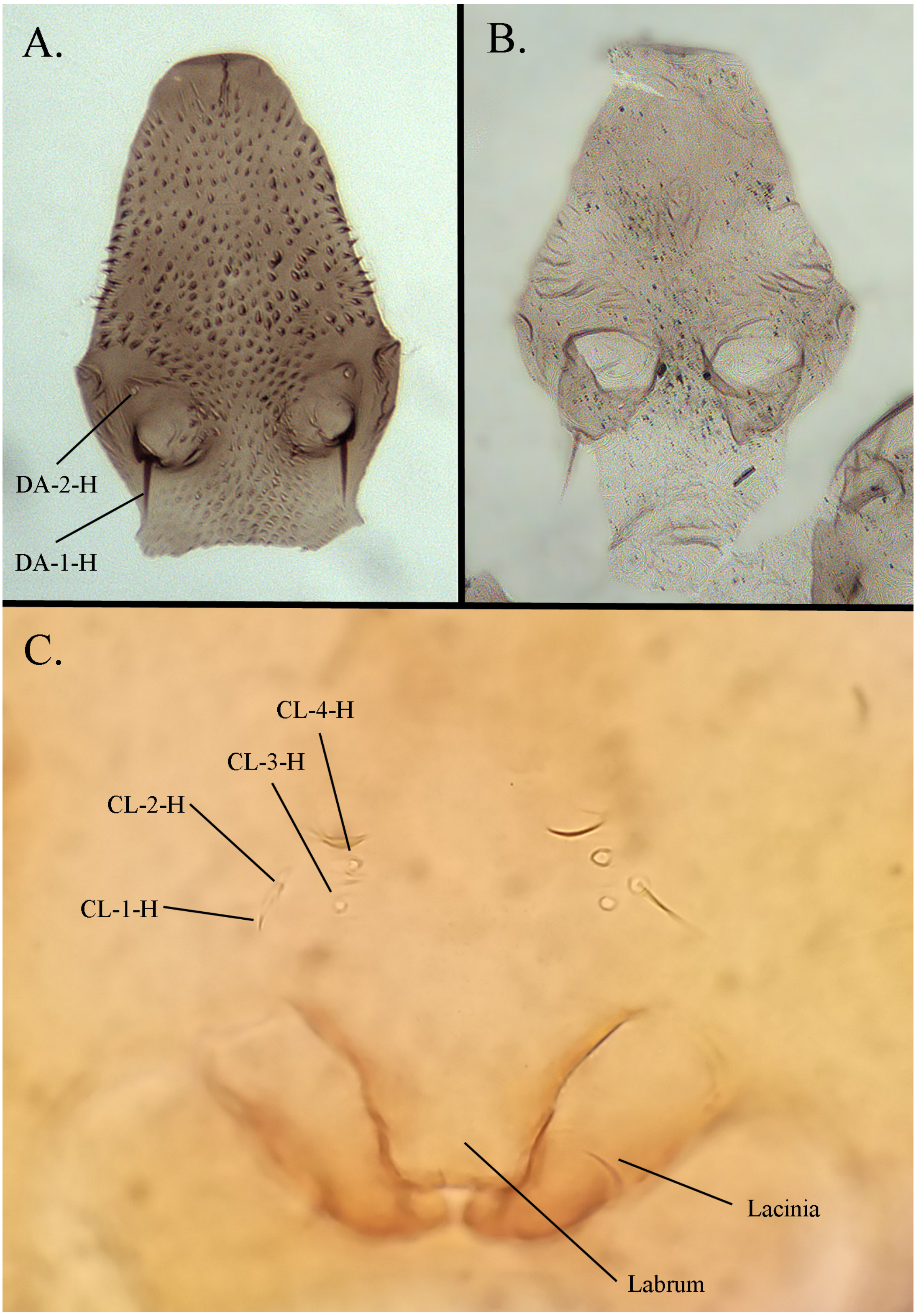

FIGURE 4. Female, dorsal apotomes, in anterior view. (A) *Culicoides stigma*. (B) *C. grandensis*. (C) Female mouthparts, in anterior view of *C. occidentalis*.

Female. Similar to male other than sexual differences on segment 9 and the following: total length = 1.81–3.94 mm, DA length = 0.23–0.34 mm, DA width = 0.18–0.24 mm, DAW/DAL = 0.64–0.85, Cephalothorax length = 0.96–1.55 mm, Ro Length = 0.21–0.43 Mm, Ro Width = 0.03–0.06 Mm, Row/rol = 0.08–0.16.

Taxonomic discussion. Comparison with the pupae of other species of *C*. (*Monoculicoides*) outside of the

Nearctic Region was difficult due to limited material or because they remain undescribed. Of these species, we examined pupae of *C. helveticus* Callot, *C. longicollis* Glukhova, *C. puncticollis* (Becker), and *C. nubeculosus* (Meigen), and these were found to be consistent with our diagnosis for *C.* (*Monoculicoides*). Additionally we observed clear morphological differences between these species and the seven Nearctic species treated herein. Though this subgenus includes 23 species, only 13 have pupal descriptions, and we examined the pupae of ten of those species. The species of *C.* (*Monoculicoides*) with previously described pupae not examined here were those of *C. cornutus* de Meillon (Afrotropical), *C. homotomus* Kieffer (Oriental), and *C. parroti* Kieffer (Palearctic). Although descriptions of these species include fewer pupal characters than the current study, we found no contradictory information to either the diagnosis or description here.

Of the 12 other subgenera of *Culicoides* that occur in North America, we have tested our subgeneric diagnosis against all of them. We re-examined Nearctic pupal exuviae from nine subgenera and compared literature descriptions of two subgenera (*C.* (*Avaritia*) and *C.* (*Drymodesmyia*)). We also tested our diagnosis against previously described pupae of an array of species groups and those considered miscellaneous (Borkent 2016) as listed by Borkent (2014: Table 2), for some based only on descriptions of species outside the Nearctic Region.

This is the first diagnosis of the pupa of any subgenus within *Culicoides* other than that of *C.* (*Selfia*) by Atchley (1970) but which included errors (see below).

Culicoides grandensis **Grogan & Phillips**

(Fig. 4B)

Diagnosis. The only species of *C.* (*Monoculicoides*) lacking spicules on the dorsal apotome (Fig. 4B).

Description. Male. Unknown.

Female. Total length = 2.77 mm (n=1). Light brown coloration throughout. **Head:** Dorsal apotome (DA) without spicules (Fig. 4B), DA length = 0.23 mm (n=1), ventral margin of the DA not extending past the DA–1–H apex; DA width = 0.20 mm (n=1); DAW/DAL = 0.87 (n=1). CL–1–H long, thin, CL–2–H not visible, O–4–H not visible. **Thorax:** 1.05 mm (n=1). Respiratory organ (RO), elongate, slender, with 3–4 subbasal pores, RO length = 0.30 mm (n=1); RO width = 0.04 mm (n=1); ROW/ROL = 0.13 (n=1), D–1–T, D–2–T stout, each on elongate, rounded tubercle, , D–5–T short, on small rounded tubercle **Abdomen:** Tergite 1 with long D–3–I and D–2–I, D–7–I anterior, campaniform D–4–I, thin D–8–I, and long, thin D–9–I posteriorly, L–2–I, L–3–I short separated medially by long, thin L–1–I on lateral margin. Shagreen along the anterior margins of segments 2–9, without lateral shagreen. Chaetotaxy of tergite 2 difficult to discern. Chaetotaxy of segment 3 similar to that of segment 4. Tergite 4 with D–2–IV on small tubercle, thin D–3–IV on elongate, pointed tubercle, D–5–IV, D–4–IV, D–7–IV, D–8–IV, D–9–IV in transverse row, arranged medially to laterally, minute D–5–IV on small tubercle, D–4–IV on small rounded tubercle, D–7–IV on small rounded tubercle, D–8–IV, D–9–IV each long on elongate pointed tubercle, D–8–IV seta thicker than D–9–IV; lateral tubercles elongate with short, blunt apices, L–1–IV long, stout seta on small rounded tubercle, L–2–IV, L–4–IV stout setae, each on elongate bifurcate tubercle, separated by thin L–3–IV on elongate pointed tubercle,; sternite 4 with minute V–5–IV on small rounded tubercle, V–6–IV thin on small tubercle, V–7–IV on small tubercle, ventral setae in transverse row. Chaetotaxy of segments 5–7 similar to that of segment 4. Segment 8 chaetotaxy with seven sensilla on reduced tubercle, without lateral shagreen, Segment 9 with anterior margin not strongly modified

Taxonomic discussion. With only one pupal exuviae to examine, we cannot report any variation. For the available specimen, CL–2–H was not visible, and it was difficult to discern the shagreen and chaetotaxy of abdominal segment 2. There was a lack of overall shagreen compared to the other members of this subgenus, and it is the only member without any spicules on the dorsal apotome. The tubercles of L–1 on each segment are rounded whereas in all other species of *C.* (*Monoculicoides*) examined; these tubercles are pointed or bifurcate. The tubercles of L–2 and L–4 of each segment are similar to most members of the subgenus, contrasting the most with those of *C. sonorensis* whose lateral tubercle apices are elongate and slender.

This species is known only from southeastern Utah.

Material examined: ALLOTYPE female #3660 (USNM), USA, Utah, Grand Co, near Cisco, margin of alkaline stream, VI.30.1958, R.H. Jones.

***Culicoides occidentalis* Wirth & Jones**
(Figs. 3A, 4C, 5B, 6A, 8B)

Diagnosis. The only species of *C.* (*Monoculicoides*) with no more than 70% of the dorsal apotome covered in uniform spicules (Fig. 3A) and bearing three to four clypeal setae (Fig. 4C).

Description. Male. Total length = 2.71–3.15 (2.99, 0.196 SD, n=4) mm. Light brown coloration throughout (Fig. 1). **Head:** Dorsal apotome (DA) roughly 70% covered in uniform spicules decreasing in abundance dorsally (Fig. 3A), DA length = 0.33–0.37 (0.35, 0.021 SD, n=4) mm; DA width = 0.21–0.22 (0.216, 0.004 SD, n=4) mm; DAW/DAL= 0.59–0.65 (0.615, 0.027 SD, n=4), CL–1–H about twice length of CL–2–H, with CL-3-H, with or without campaniform CL-4-H (Fig. 4C); O–2–H, O–4–H separated medially by O–1–H and O–3–H. **Thorax:** Cephalothorax length = 1.30–1.37 (1.33, 0.031 SD, n =4) mm. Respiratory organ (RO) elongate, slender, 3–4 subbasal pores (Fig. 2B), RO length = 0.39–0.45 (0.42, 0.035 SD, n=4) mm; RO width = 0.04–0.05 (0.043, 0.005 SD, n=4) mm; ROW/ROL = 0.08–0.12 (0.102, 0.019 SD, n=4), D–5–T short. **Abdomen:** Tergite 1 with long D–3–I, short D–2–I, D–7–I anterior, campaniform D–4–I, short D–8–I, and long, thin D–9–I on short tubercle posteriorly, L–2–I, L–3–I short separated medially by long, thin L–1–I on lateral margin. Chaetotaxy, shagreen of tergite 2 similar to tergite 4, without elongate tubercles, minute L–2–II, L–4–II separated medially by long, thin L–3–II on anterolateral margin. Chaetotaxy, shagreen of segment 3 similar to that of segment 4. Tergite 4 (Fig. 6A) with short D–2–IV on short tubercle, thin D–3–IV on elongate, pointed tubercle, D–5–IV, D–4–IV, D–7–IV, D–8–IV, D–9–IV in transverse row, arranged medially to laterally, minute D–5–IV on slightly formed tubercle, D–4–IV on small tubercle, D–7–IV on small rounded tubercle, D–8–IV, D–9–IV each long on pointed tubercle, D–8–IV seta thicker than D–9–IV; lateral tubercles with elongate, apices, L–1–IV thick seta on small bifurcate tubercle, L–2–IV, L–4–IV thick setae, each on elongate bifurcate tubercle, separated by thin L–3–IV on elongate bifurcate tubercle; sternite 4 with minute V–5–IV on small rounded tubercle, V–6–IV thin on pointed tubercle, small V–7–IV on pointed tubercle, ventral setae in transverse row, shagreen along anterior margins, lateral portion with scattered shagreen. Segments 5 and 6 with similar chaetotaxy, shagreen to that of segment 4. Segment 7 with similar chaetotaxy to segment 4, with reduced lateral shagreen. Segment 8 chaetotaxy with seven sensilla, without lateral shagreen. Segment 9 with anterior shagreen, anterior margin not strongly modified (Fig. 8B).

Female. Similar to male other than sexual differences on segment 9 and the following: total length = 2.76–3.20 (2.93, 0.234 SD, n=3) mm, DA–1–H apex extending past ventral margin of DA, DA length = 0.29–0.32 (0.30, 0.015 SD, n=3) mm, DA width = 0.23–0.25 (0.237, 0.012 SD, n=3) mm, DAW/DAL = 0.77–0.79 (0.78, 0.013 SD, n=3), Cephalothorax length = 1.30–1.37 (1.34, 0.035 SD, n=3) mm, RO length = 0.42–0.43 (0.423, 0.006 SD, n=3) mm, RO width = 0.04–0.05 (0.043, 0.006 SD, n=3) mm, ROW/ROL = 0.09–0.11 (0.102, 0.012 SD, n=3).

Taxonomic discussion. This species has 3-4 subbasal pores on the respiratory organ and along with *C. shemanchuki,* are the only members of *C.* (*Monoculicoides*) with four subbasal pores on the respiratory organ. We have not observed any specimens of *C. sonorensis* or *C. variipennis*, the other members of the *C. variipennis* complex, with more than three subbasal pores. This could potentially be a useful identifying character within this complex, however, more specimens are needed to confirm this as high variation has been observed in the number of subbasal pores within other species (Lawson 1951). This species and *C. sonorensis* are the only members of *C.* (*Monoculicoides*) examined where some specimens had a 4th ocular seta, O–4–H (Shults *et al.* 2016). Of the members of the subgenus with spicules on the dorsal apotome, *C. occidentalis* has the lowest spicule density (Fig. 3A). Pupae of this species had 1 or 2 extra campaniform clypeal sensilla, CL–3–H and CL–4–H (Fig. 4C). One extra CL sensilla is also present in *C. riethi* but *C. occidentalis* is the only species observed with a CL–4–H.

Culicoides occidentalis ranges from British Columbia to Baja California, east to Nevada, southeast eastern New Mexico and west Texas. Immatures have been reported from aquatic sites with highly salinity (Wirth & Jones 1957, Holbrook *et al*. 2000).

Material examined: 1 male, 2 females (CNCI), Canada, B.C., Kamloops, alkaline lake, 21.VII.1994, Grogan & McKinnon. 4 males, 4 females, (1 male, 1 female TAMU, voucher series #732; 3 males, 3 females CNCI), Canada, B.C., White Lake, 5 km SW Okanagan Falls, 49°18’27.51"N, 119°138’00.23"W, 4.V.2014, A. Borkent.

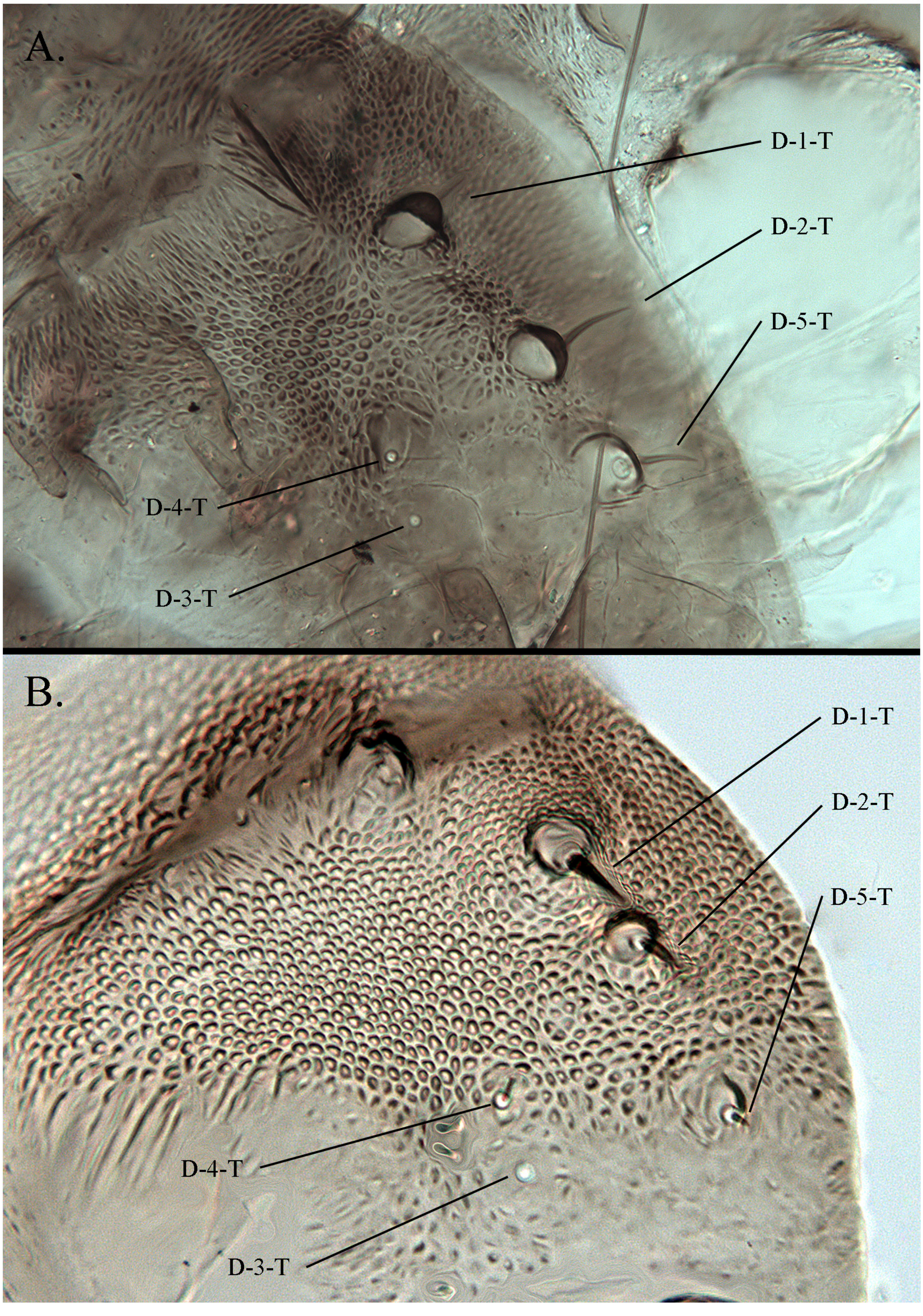

FIGURE 5. Thoracic dorsal setae, left side, dorsal view. (A) *Culicoides shemanchuki*. (B). *C. occidentalis*.

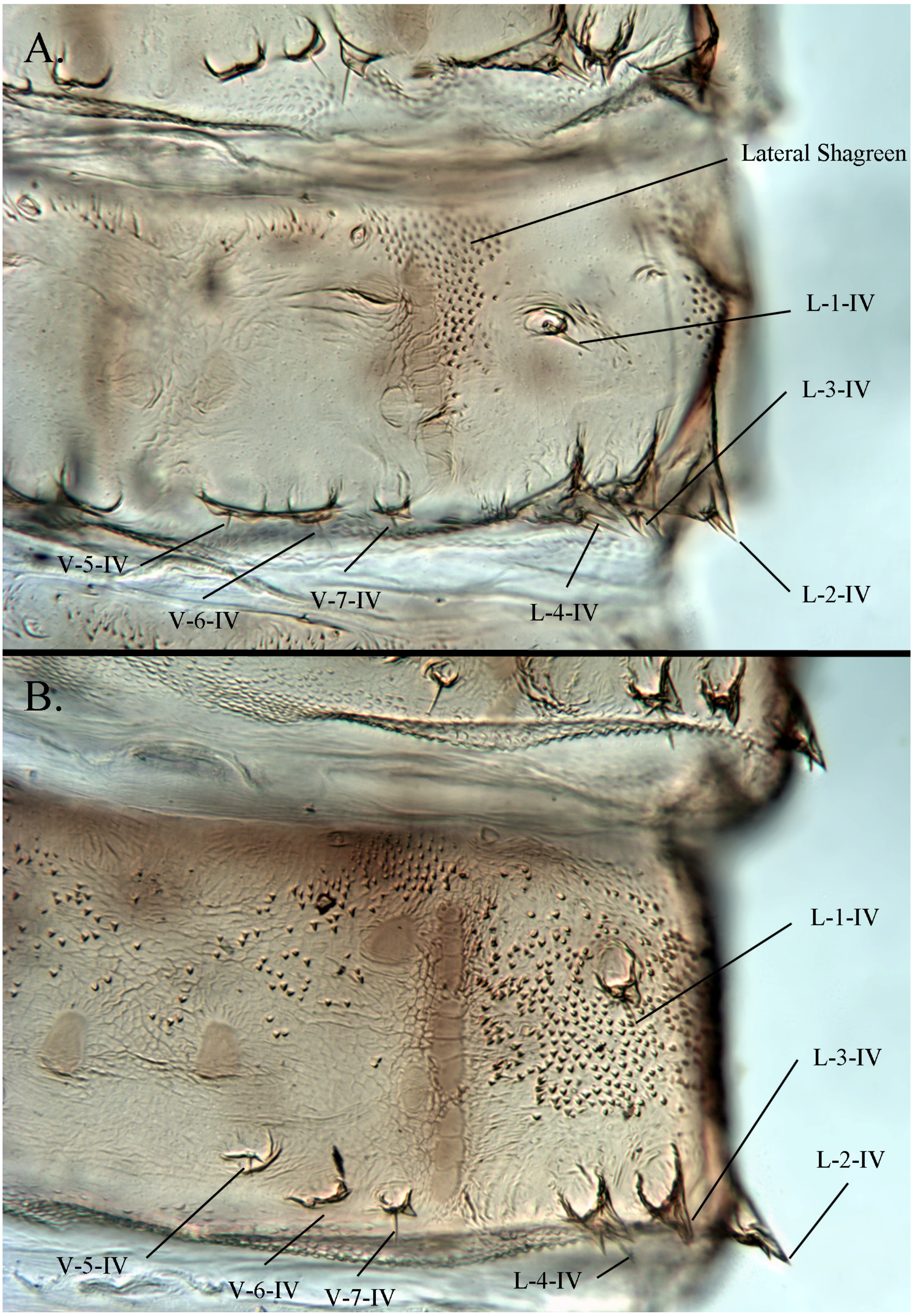

FIGURE 6. Male, lateral and ventral tubercles of abdominal segments 4, in dorsal view (A) *Culicoides occidentalis*. (B) *C. riethi*.

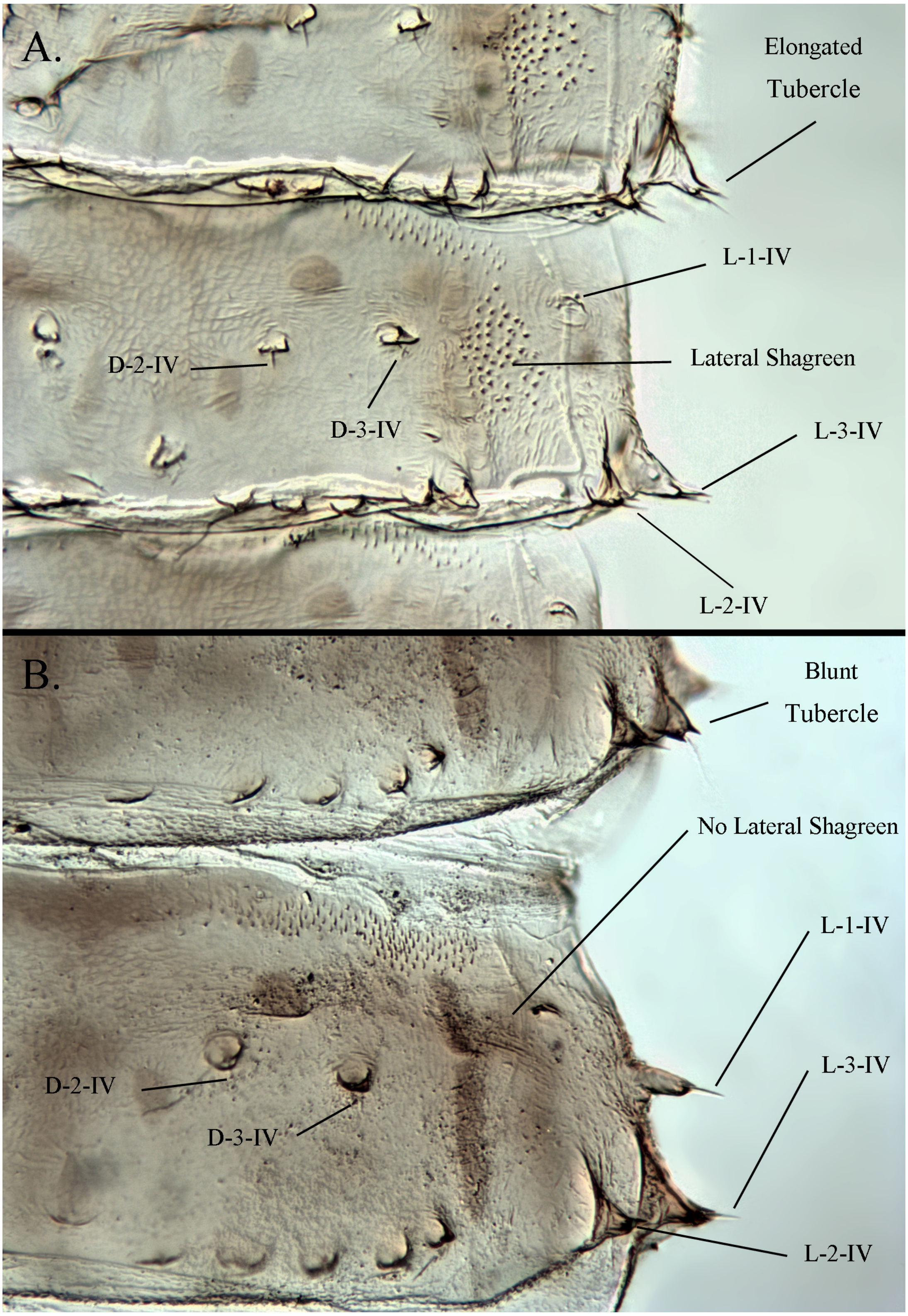

FIGURE 7. Male, lateral and dorsal tubercles of abdominal segments 4, in dorsal view (A) *Culicoides sonorensis*. (B) *C. variipennis*.

***Culicoides riethi* Kieffer**
(Figs. 1, 3B, 6B, 8A)

Diagnosis. The only species of *C*. (*Monoculicoides*) with anterior portion of segment 9 greatly swollen (Fig. 8A).

Description. Male. Total length = 3.30–3.70 (3.5, 0.283 SD, n=2) mm. Medium brown coloration throughout (Fig. 1). **Head:** Dorsal apotome (DA) roughly 90% covered in uniform spicules (Fig. 3B). DA length = 0.32–0.33 (0.325, 0.007 SD, n=2) mm; DA width = 0.21–0.22 (0.215, 0.007 SD, n=2) mm; DAW/DAL= 0.636–0.688 (0.662, 0.036 SD, n=2). DA–1–H apex extending past ventral margin of DA. CL–1–H about twice length of CL–2–H, CL–3–H campaniform sensilla medial. **Thorax:** Cephalothorax length = 1.00–1.13 (1.08, 0.057 SD, n =2) mm. Respiratory organ (RO) elongate, slender, 1–2 subbasal pores, RO length = 0.21–0.26 (0.23, 0.016 SD, n=2) mm; RO width = 0.03–0.035 (0.030, 0.001 SD, n=2) mm; ROW/ROL = 0.11–0.15 (0.13, 0.01 SD, n=2), D–5–T short. **Abdomen:** Tergite 1 with long D–3–I, short D–2–I, D–7–I anterior, short D–4–I, and long, thin D–9–I separated medially by campaniform D–8–I on short tubercle posteriorly, L–2–I, L–3–I short separated medially by long, thin L–1–I on lateral margin. Chaetotaxy, shagreen of tergite 2 similar to tergite 4, without elongate tubercles, minute L–2–II, L–4–II separated medially by long, thin L–3–II on anterolateral margin. Chaetotaxy, shagreen of segment 3 similar to that of segment 4. Tergite 4 (Fig. 6C) with short D–2–IV on short tubercle, thin D–3–IV on elongate, pointed tubercle, D–5–IV, D–4–IV, D–7–IV, D–8–IV, D–9–IV in transverse row, arranged medially to laterally, minute D–5–IV on slightly formed tubercle, D–4–IV on small tubercle, D–7–IV on small rounded tubercle, D–8–IV, D–9–IV each long on pointed tubercle, D–8–IV seta thicker than D–9–IV; lateral tubercles with elongated apices, L–1–IV thick seta on small bifurcate tubercle, L–2–IV, L–4–IV thick setae, each on elongate, bifurcate tubercle, separated by thin L–3–IV on elongate, bifurcate tubercle; sternite 4 with minute V–5–IV on small rounded tubercle, V–6–IV thin on pointed tubercle, small V–7–IV on pointed tubercle, ventral setae in transverse row, shagreen along anterior margins, with lateral shagreen. Segments 5–7 with similar chaetotaxy, shagreen to that of segment 4. Segment 8 slightly swollen, with seven sensilla, with lateral shagreen. Segment 9 with anterior shagreen, anterior margin greatly swollen.

Female. Similar to male other than sexual differences on segment 9 and the following: total length = 3.50–3.94 (3.66, 0.241 SD, n=3) mm, DA length = 0.31–0.34 (0.32, 0.017 SD, n=3) mm, DA width = 0.21–0.23 (0.22, 0.01 SD, n=3) mm, DAW/DAL = 0.65–0.74 (0.689, 0.048 SD, n=3), Cephalothorax length = 1.35–1.40 (1.38, 0.025 SD, n=3) mm, RO length = 0.37–0.41 (0.387, 0.021 SD, n=3) mm, RO width = 0.05–0.06 (0.057, 0.006 SD, n=3) mm, ROW/ROL = 0.13–0.16 (0.147, 0.015 SD, n=3).

Taxonomic discussion. Pupae of *C. riethi* are medium brown in coloration (Fig. 1), similar to that of *C. shemanchuki*, but have much more lateral shagreen than that species. The density of the spines on the DA (Fig. 3B), abundance of lateral shagreen on all abdominal segments, and the swollen anterior most region of segment 9 (Fig. 8A) make this the most distinctive species of *C.* (*Monoculicoides*). Like *C. occidentalis,* this species has an additional clypeal seta not seen in other members of the subgenus, although no specimen has been observed with a CL–4–H. The pupa of *C. riethi* has been redescribed by many authors since its original description by Kieffer (1914) (Rieth 1915, Thienemann 1928, Mayer 1934, Kettle & Lawson 1952, Dzhafarov 1964, Damian-Georgescu & Spătaru 1971, and Glukhova 1989) and we found no contradictions in those papers to our description.

This species is Holarctic in distribution. In North America it ranges from Alaska to Manitoba, south to British Columbia, Wyoming and Nebraska (Borkent & Grogan 2009 (as *C. gigas*), Grogan & Lysyk 2015).

Material examined: 2 males, 3 females, (1 female TAMU, voucher series #732; 2 males, 2 females CNCI), Canada, B.C., White Lake, 5 km SW Okanagan Falls, 49°18’27.51"N, 119°138’00.23"W, 4.V.2014, A. Borkent.

***Culicoides shemanchuki* Grogan & Lysyk**
(Figs. 2A, 5A)

Diagnosis. The only species of *C*. (*Monoculicoides*) with 5 pores on the subbasal portion of the respiratory organ (Fig. 2A) and D–5–T equal in length to D–1–T and D–2–T (Fig. 5A).

Description. Male. Total length = 2.95 mm (n=1). Medium brown coloration throughout. **Head:** Dorsal apotome (DA) covered in short spicules. DA length = 0.32 mm (n=1); DA width = 0.18 mm (n=1); DAW/DAL= 0.563 (n=1).**Thorax:** Cephalothorax length = 1.26 mm (n=1). Respiratory organ (RO) elongate, slender, 5 subbasal

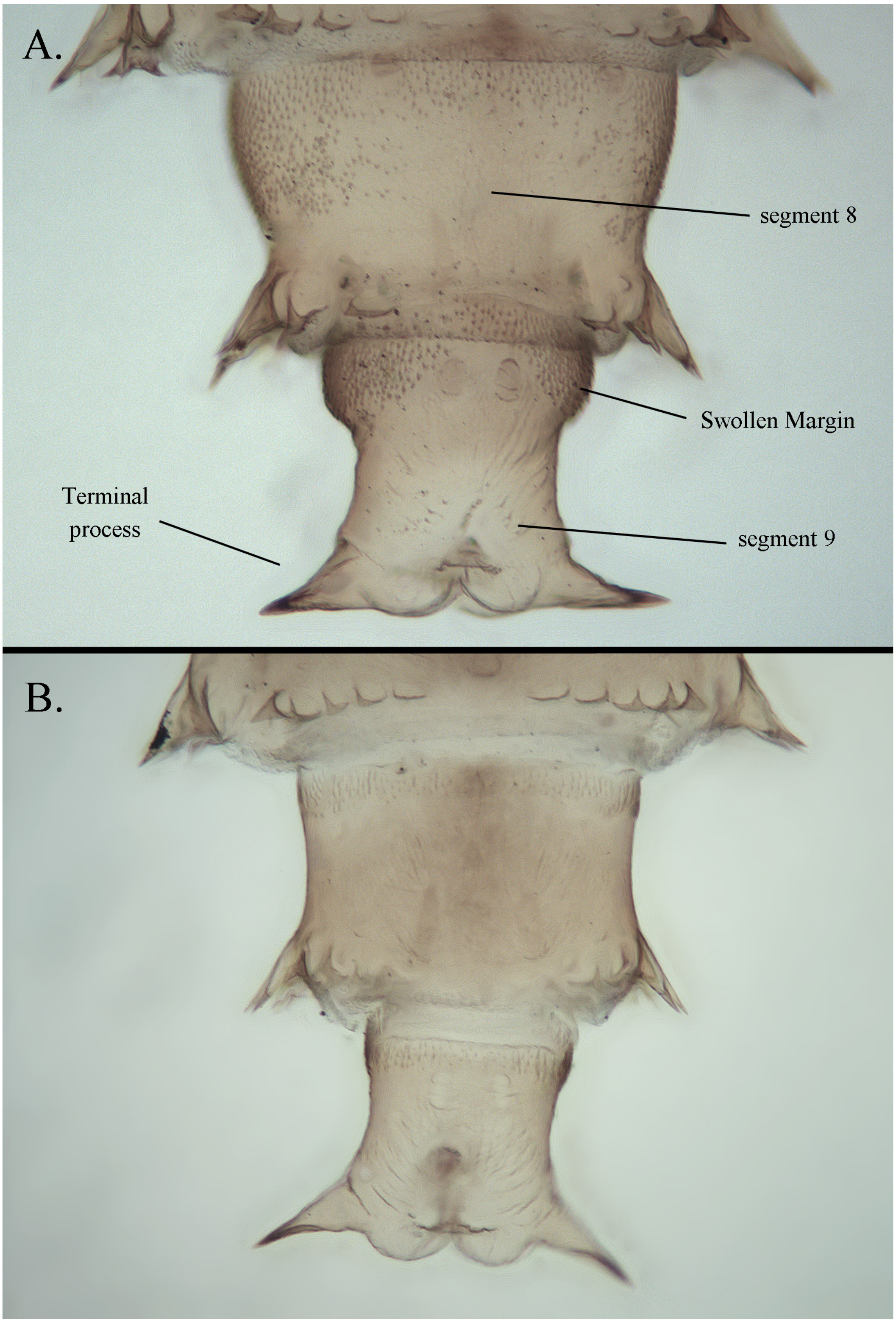

FIGURE 8. Female abdominal segments 8–9, in dorsal view. (A) *Culicoides riethi*. (B) *C. occidentalis*.

pores (Fig. 2A). RO length = 0.34 mm (n=1); RO width = 0.04 mm (n=1); ROW/ROL = 0.118 (n=1). D–1–T, D–2–T, D–5–T long, stout, equal in length, each on rounded tubercle in longitudinal row (Fig. 5A). **Abdomen:** Tergite 1 with long D–3–I, short D–2–I, D–7–I anterior, short D–4–I, and long, thin D–9–I separated medially by campaniform D–8–I on short tubercle posteriorly, L–2–I, L–3–I short separated medially by long, thin L–1–I on lateral margin. Chaetotaxy, shagreen of tergite 2 similar to tergite 4, without elongate tubercles, small L–2–II, L–4–II separated medially by thin L–3–II on anterolateral margin. Chaetotaxy, shagreen of segment 3 similar to that of segment 4. Tergite 4 with short D–2–IV, thin D–3–IV on short tubercle, D–5–IV, D–4–IV, D–7–IV, D–8–IV, D–9–IV in transverse row, arranged medially to laterally, minute D–5–IV on slightly formed tubercle, D–4–IV on small tubercle, D–7–IV on small rounded tubercle, D–8–IV, D–9–IV each long on rounded tubercle, D–8–IV seta thicker than D–9–IV; lateral tubercles with elongate, slender apices, L–1–IV thick seta on bifurcate tubercle, L–2–IV, L–4–IV thick setae, each on elongate bifurcate tubercle, separated by thin L–3–IV on elongate bifurcate tubercle; sternite 4 with minute V–5–IV on small rounded tubercle, V–6–IV thin on rounded tubercle, V–7–IV on rounded tubercle, ventral setae in transverse row, shagreen along anterior margins, no lateral shagreen. Segments 5–7 with similar chaetotaxy, shagreen to that of segment 4, D–9 tubercles becoming more pointed with subsequent segments. Segment 8 chaetotaxy with seven sensilla, without lateral shagreen. Segment 9 with anterior shagreen, anterior margin not strongly modified.

Female. Similar to male other than sexual differences on segment 9 and the following: total length = 3.57 mm (n=1), DA length = 0.27 mm (n=1), DA width = 0.21 mm (n=1), DAW/DAL = 0.77 (n=1), Cephalothorax length = 1.35 mm (n=1), RO length = 0.36 (n=1), RO width = 0.04 mm (n=1), ROW/ROL = 0.11 (n=1).

Taxonomic discussion. The coloration of the pupa of *C. shemanchuki* is similar to that of *C. riethi* (Fig. 1) but it has much less lateral shagreen on its abdominal segments. This species has 4-5 subbasal pores on the respiratory organ, and is the only *C.* (*Monoculicoides*) we examined to have 5. However, we only examined two specimens and there is a significant possibility that there could be greater variation in the number of subbasal pores (Lawson 1951). Both specimens examined had folded dorsal apotomes, which made it difficult to obtain accurate measurements, descriptions, and photographs of this structure.

This species is known only from southern Alberta and north-central North Dakota (Grogan & Lysyk 2015).

Material examined: 1 male, 1 female (CNCI), USA, North Dakota, Pierce Co, Pleasant Lake, June 1969, W.W. Wirth, alkaline lake.

Culicoides stigma Meigen

(Fig. 4A)

Diagnosis. The only species of *C.* (*Monoculicoides*) with short respiratory organ (≤ 0.26 mm in length) (Fig. 9) and short uniform spicules on the dorsal apotome (Fig. 4A).

Description. Male. Total length = 2.05–2.60 (2.39, 0.137 SD, n=17) mm. Light brown coloration throughout. **Head:** Dorsal apotome (DA) roughly 90% covered in short, uniform spicules (Fig. 4A), DA length = 0.28–0.32 (0.299, 0.014 SD, n=17) mm; DA width = 0.17–0.19 (0.179, 0.004 SD, n=17) mm; DAW/DAL= 0.58–0.64 (0.601, 0.024 SD, n=17). **Thorax:** Cephalothorax length = 1.00–1.13 (1.08, 0.057 SD, n =17) mm. Respiratory organ (RO) slender, 1–2 subbasal pores, RO length = 0.21–0.26 (0.23, 0.016 SD, n=17) mm; RO width = 0.030–0.035 (0.03, 0.001 SD, n=17) mm; ROW/ROL = 0.11–0.15 (0.13, 0.01 SD, n=17). **Abdomen:** Tergite 1 with long D–3–I, short D–2–I, D–7–I anterior, short D–4–I, and long, thin D–9–I separated medially by campaniform D–8–I on short tubercle posteriorly, L–2–I, L–3–I short separated medially by long, thin L–1–I on lateral margin. Chaetotaxy, shagreen of tergite 2 similar to tergite 4, without elongate tubercles, minute L–2–II, L–4–II separated medially by long, thin L–3–II on anterolateral margin. Chaetotaxy, shagreen of segment 3 similar to that of segment 4. Tergite 4 with short D–2–IV on short tubercle, thin D–3–IV on elongate, pointed tubercle, D–5–IV, D–4–IV, D–7–IV, D–8–IV, D–9–IV in transverse row, arranged medially to laterally, minute D–5–IV on slightly formed tubercle, D–4–IV on small tubercle, D–7–IV on small rounded tubercle, D–8–IV, D–9–IV each long on pointed tubercle, D–8–IV seta thicker than D–9–IV; lateral tubercles with short, blunt apices, L–1–IV thick seta on small bifurcate tubercle, L–2–IV, L–4–IV thick setae, each on elongate, bifurcate tubercle, separated by thin L–3–IV on elongate, bifurcate tubercle; sternite 4 with minute V–5–IV on small rounded tubercle, V–6–IV thin on pointed tubercle, small V–7–IV on pointed tubercle, ventral setae in transverse row, shagreen along anterior margins, lateral shagreen reduced or absent. Segments 5–7 with similar chaetotaxy, shagreen to that of segment 4. Segment 8 chaetotaxy with seven sensilla, without lateral shagreen. Segment 9 with anterior shagreen, anterior margin not strongly modified.

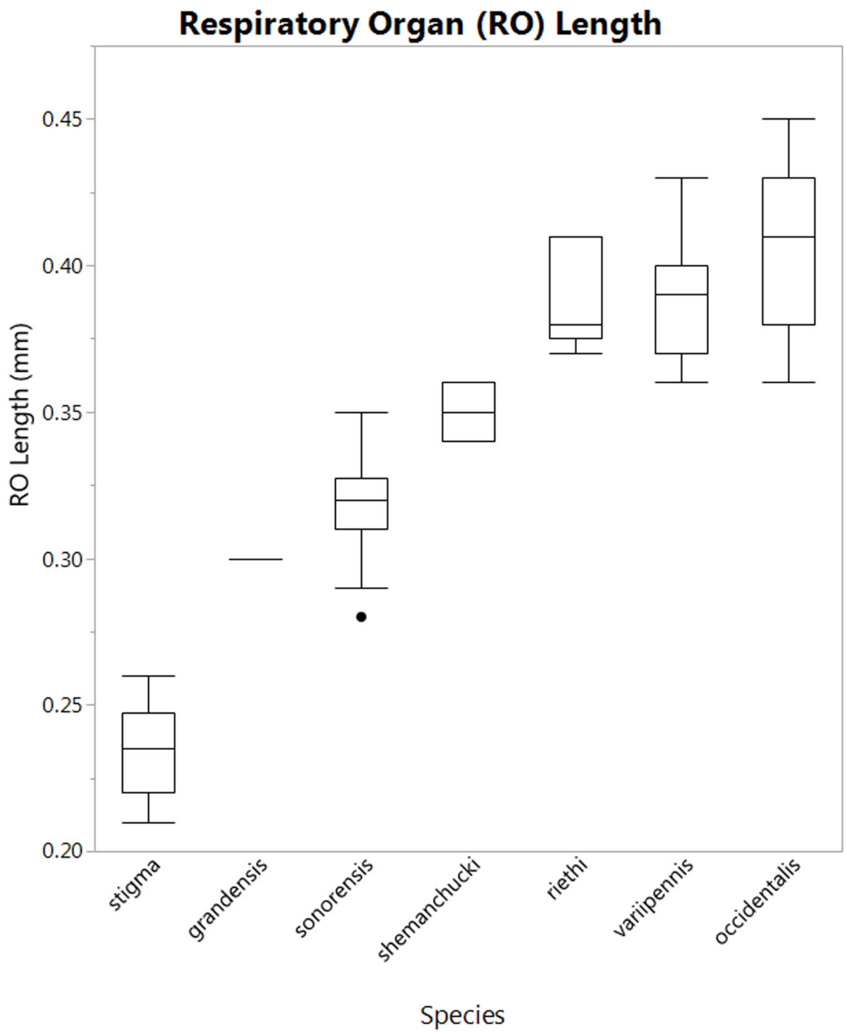

FIGURE 9. Box plots of measurements of the length of respiratory organs by species. Species ordered by ascending values.

Female. Similar to male other than sexual differences on segment 9 and the following: total length = 1.94–2.86 (2.04, 0.248 SD, n=15) mm, DA–1–H apex extending past ventral margin of DA, DA length = 0.23–0.3 (0.267, 0.018 SD, n=15) mm, DA width = 0.17–0.215 (0.197, 0.016 SD, n=15) mm, DAW/DAL = 0.65–0.79 (0.737, 0.035 SD, n=15), Cephalothorax length = 1.00–1.15 (1.07, 0.059 SD, n=15) mm, RO length = 0.21–0.26 (0.237, 0.015 SD, n=15) mm, RO width = 0.03–0.035 (0.031, 0.003 SD, n=15) mm, ROW/ROL = 0.11–0.15 (0.133, 0.019 SD, n=15).

Taxonomic discussion. This species is one of the smaller members of *C.* (*Monoculicoides*), along with *C. sonorensis* and *C. grandensis*, but has a proportionally smaller respiratory organ (Fig. 9) in comparison to its body size. The total range of the ratio of respiratory organ length to cephalothorax length of all other *C.* (*Monoculicoides*) examined was 0.27–0.31 (n=54), whereas in *C. stigma*, it is significantly lower 0.19–0.22 (n=32).

This species is Holarctic. However, in North America it is currently known only from central Alberta. The pupa of this species was originally described by Kettle & Lawson (1952) and redescribed in two other studies (Dzhafarov 1964; Spătaru 1971), and our study did not find any contradictions with these studies. Although this species is reported as Holarctic, our description of the pupa is based solely on Palearctic material. It is possible that there are morphological differences between populations from the two regions.

Material examined: 12 males, 7 females (ZIN), Russia, Kaliningrad Province, Rybachii village, Curonian Spit National Park [at the Baltic Sea coast], 16–17.07.1989, Glukhova. 1 female (ZIN), Russia, Leningrad Province, Luga district, forest ditch with water, 27.07.1979, Brodskaya. 1 female (ZIN), Russia, Leningrad Province, environs of Luga town, ditch with moss, 13.08.1979, Brodskaya. 1 female (ZIN), Russia, Leningrad Province, Luga district, floodplain of river Obla, 24.08.197, Brodskaya. 3 males, 3 females (ZIN), Luga district, in ditch, 12–14.VIII.1979. 1 male, 1 female (ZIN), Russia, Republic of Karelia, 1966. 1 male, 1 female (ZIN), Russia, Republic of Karelia, 2–4.VII.54.

***Culicoides variipennis* Wirth & Jones**
(Figs. 3D, 7B)

Diagnosis. The only species of *C.* (*Monoculicoides*) with lateral areas of all abdominal segments mostly bare or without any shagreen (Fig. 7B), and lateral tubercles with blunt apices (Fig. 7B).

Description. Male. Total length = 2.60–4.15 (3.49, 0.621 SD, n=7) mm. Light brown coloration throughout. **Head:** Dorsal apotome (DA) roughly 90% covered in spicules of various sizes decreasing in abundance dorsally (Fig. 3D), DA length = 0.29–0.38 (0.32, 0.031 SD, n=7) mm; DA width = 0.20–0.25 (0.21, 0.018 SD, n=7) mm; DAW/DAL= 0.58–0.75 (0.663, 0.051 SD, n=7). **Thorax:** Cephalothorax length = 1.25–1.65 (1.51, 0.150 SD, n =7) mm. Respiratory organ (RO) elongate, slender with 2–3 subbasal pores, RO length = 0.36–0.43 (0.39, 0.023 SD, n=7) mm; RO width = 0.04–0.06 (0.051, 0.009 SD, n=7) mm; ROW/ROL = 0.10–0.15 (0.13, 0.019 SD, n=7). **Abdomen:** Tergite 1 with long D–3–I, short D–2–I, D–7–I anterior, campaniform D–4–I, short D–8–I, and long, thin D–9–I on short tubercle posteriorly, L–2–I, L–3–I short separated medially by long, thin L–1–I on lateral margin. Chaetotaxy, shagreen of tergite 2 similar to tergite 4, without moderate tubercles, minute L–2–II, L–4–II separated medially by long, thin L–3–II on anterolateral margin. Chaetotaxy, shagreen of segment 3 similar to that of segment 4. Tergite 4 (Fig. 7D) with short D–2–IV on short tubercle, thin D–3–IV on elongate, pointed tubercle, D–5–IV, D–4–IV, D–7–IV, D–8–IV, D–9–IV in transverse row, arranged medially to laterally, minute D–5–IV on slightly formed tubercle, D–4–IV on small tubercle, D–7–IV on small rounded tubercle, D–8–IV, D–9–IV each long on pointed tubercle, D–8–IV seta thicker than D–9–IV; lateral tubercles short with, blunt apices (Fig. 7B), L–1–IV thick seta on small bifurcate tubercle, L–2–IV, L–4–IV thick setae, each on elongate, bifurcate tubercle, separated by thin L–3–IV on elongate, bifurcate tubercle; sternite 4 with minute V–5–IV on small rounded tubercle, V–6–IV thin on pointed tubercle, small V–7–IV on pointed tubercle, ventral setae in transverse row, shagreen along anterior margins, lateral shagreen reduced or absent. Segments 5–7 with similar chaetotaxy, shagreen to that of segment 4. Segment 8 chaetotaxy with seven sensilla, without lateral shagreen. Segment 9 with anterior shagreen, anterior margin not strongly modified.

Female. Similar to male other than sexual differences on segment 9 and the following: total length = 2.96–3.75 (3.33, 0.404 SD, n=4) mm, DA–1–H apex extending past ventral margin of DA, DA length = 0.27–0.28 (0.279, 0.006 SD, n=4) mm, DA width = 0.22–0.24 (0.233, 0.009 SD, n=4) mm, DAW/DAL = 0.80–0.85 (0.834, 0.029 SD, n=4), Cephalothorax length = 1.32–1.55 (1.413, 0.122 SD, n=4) mm, RO length = 0.36–0.42 (0.388, 0.025 SD, n=4) mm, RO width = 0.04–0.05 (0.043, 0.005 SD, n=4) mm, ROW/ROL = 0.10–0.11 (0.109, 0.007 SD, n=4).

Taxonomic discussion. Shults *et al*. (2016) reported distinguishing features between *C. sonorensis* and *C. variipennis* based on the shape of the apices of the lateral tubercles on the abdominal segments, and further illustration of these characters can be found in that study. Those of *C. sonorensis* are long and slender (Fig. 7A), whereas in *C. variipennis*, they are short and blunt (Fig. 7B). In comparison to the other Nearctic members of *C.* (*Monoculicoides*), these two species seem to represent the extremes of the variation, with the bluntness/elongation of the lateral tubercle apices of the other species falling between these two species. Here we also report the overall reduced shagreen on all abdominal segments in *C. variipennis* and in particular the lack of lateral shagreen on abdominal segments 4–7 as a diagnostic character state.

This species is known from British Columbia, Alberta, Washington, and Montana to Nova Scotia, and the United States primarily east of the Mississippi river, Texas and Louisiana (Wirth & Jones 1957; Holbrook *et al*. 2000; Borkent & Grogan 2009; Grogan & Lysyk 2015; Shults 2015; Jewiss-Gaines *et al*. 2016). Moderate to large numbers of pupae have been reared and collected from fresh water polluted with manure (Wirth & Jones 1957, Jamnback 1965). Additionally, specimens have been collected from saltmarshes (Blanton & Wirth 1979). The pupa of *C. variipennis* was briefly described by several authors (Malloch 1915, Thomsen 1937, Fox 1942, Jones 1955, Jamnback 1965, Blanton & Wirth 1979, Weber 2001) but it is difficult to determine whether these descriptions agree with ours as we are uncertain which species of the *C. variipennis* complex were actually studied by these authors.

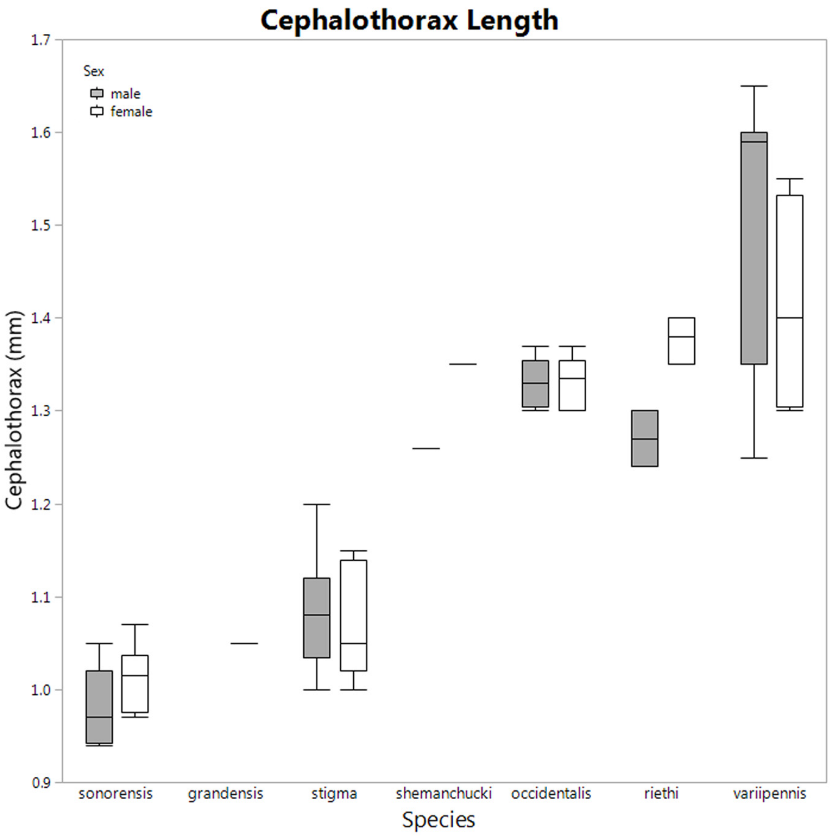

FIGURE 10. Box plots of measurements of the length of cephalothorax by species, separated by sex. Species ordered by ascending values.

The specimens we examined from Vernon, B.C. account for the large amount of size variation seen in fig. 10, as they are much smaller (roughly the size of *C. occidentalis*) than the other specimens of *C. variipennis* examined. The pupae from Vernon key to *C. variipennis* but have slightly longer lateral tubercles. It is unclear if these measurements and features fall within the normal geographic variation of this species, represent intermediate character states, or even infer a new cryptic species.

Material examined: 3 males, 2 female (CNCI), Canada, Ontario, Alfred, 30–VI–1985, Dillion & Oliver. 2 males, 2 females (CNCI), Canada, British Columbia, Vernon, Coldstream Ranch, 19.VII.1994, Grogan & McKinnon. 2 males (NYSM), USA, Missouri, Petersburg, Boone's Lick salt spring, 11.III.1955, P.J. Spangler.

Morphometric results

(Figs. 9, 10, 11, Tables 1 & 2)

Morphometric analyses of the Nearctic species of *C.* (*Monoculicoides*) show general trends among the size of each species (Figs. 9 & 10) as well as sexual dimorphism within the species examined (Fig. 11). *Culicoides stigma* has a statistically significant smaller respiratory organ in comparison to the length of the cephalothorax (RO length/cephalothorax length) than all other species of *C.* (*Monoculicoides*) (ANOVA with Tukey's, $p < 0.001$). There was no significant difference in this ratio between any other species.

The dorsal apotome of male pupae tend to be longer and narrower than those of female pupae. This is most easily observable in the DA ratio (DAW/DAL) (Fig. 11). *Culicoides riethi* is the only species examined to show no statistically significant difference in the DA ratio between males and females (Fig. 11) (Student's t, $p = 0.57$). All other species examined showed a sexual dimorphism (Student's t, $p < 0.05$). There is however a sexual dimorphism in the length of the cephalothorax in *C. riethi* (Student's t, $p < 0.05$) that does not exist in any other species examined (Fig. 10).

Key to Pupae of *Culicoides* (*Monoculicoides*) of North America

1 Dorsal apotome without dorsomedial tubercle (Figs. 3A–D, 4A–B), with dark banding on apex of pedicel and base of respiratory organ (Fig. 1), respiratory organ elongate, slender with black apex, midlength with scales, 2–5 subbasal pores (Figs. 2A–B), shagreen restricted to the anterior, posterior, and lateral margins of the abdominal segments 3–7 or absent (Figs. 6 & 7), terminal process extending posterolaterally to laterally, with dark apex (Figs. 8A–B) . . . . *C.* (*Monoculicoides*) (2)

- Specimens without above combination of features. . . . . other species of *Culicoides*

2 Dorsal apotome bare of spicules (Fig. 4B) . . . . *C. grandensis*

- Dorsal apotome covered in spicules (Figs. 3A–D) 3

3 D–5–T longer than half the length of either D–1–T or D–2–T (Fig. 5A). . . . . *C. shemanchuki*

- D–5–T less than half the length of either D–1–T or D–2–T (Fig. 5B) 4

4 Dorsal apotome with spicules of uniform size (Fig. 4A) 5

- Dorsal apotome with spicules of varying size, with the longest spicules on the lateral margins (Figs. 3B–D) 6

5 Less than 3/4 of dorsal apotome covered in spicules (Fig. 3A); 3–4 subbasal pores on respiratory organ (Fig. 2B); CL–3–H present (Fig. 4C) . . . . *C. occidentalis*

- Almost all of the dorsal apotome covered in spicules (Fig. 4A); 1–2 subbasal pores; CL–3–H absent. . . . . *C. stigma*

6 Lateral shagreen of segment 8 of the abdomen abundant (Fig. 6B); anterior 1/3 margin of abdominal segment 9 markedly swollen (Fig. 8A); CL–3–H present (as in Fig. 4C) . . . . *C. riethi*

- Segment 8 of the abdomen with little to no lateral shagreen (Fig. 7); anterior margin of abdominal segment 9 at most slightly swollen (Fig. 8B); CL–3–H absent 7

7 L–2, L–3, and L–4 of abdominal segments 4–7 each on a long bifid tubercle with elongate and slender apices, with lateral shagreen on segments 4–7 (Fig. 7A) . . . . *C. sonorensis*

- L–2, L–3, and L–4 of abdominal segments 4–7 each on a short bifid tubercle with short and blunt apices, without lateral shagreen on segments 4–7 (Fig. 7B) . . . .*C. variipennis*

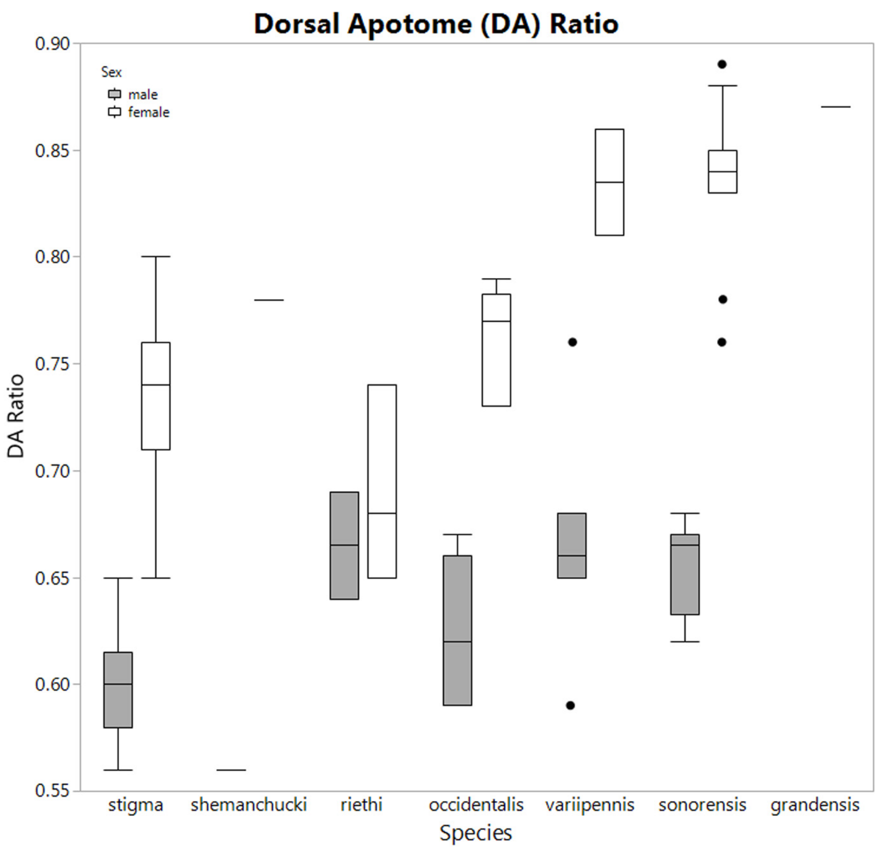

FIGURE 11. Box plots of dorsal apotome ratios by species, separated by sex. Species ordered by ascending values.

Discussion

One of the main goals of this study was to identify unique diagnostic characters, independent of sex and location, to distinguish the pupal characters of *C. (Monoculicoides)*, and to describe these in detail. We report that the pupae of all Nearctic *C.* (*Monoculicoides*) can be identified to species. Considering this, it is likely that the presently undiagnosed Palearctic, Oriental, and Afrotropical species will also have significant interspecific differences. This suggests that as taxonomists continue to describe the immature stages of *Culicoides*, we will be able to develop more complete keys and improve our understanding of the biology, morphology, and phylogenetic relationships within the genus.

We have categorized pupae as being light brown or medium brown in color (Fig. 1). It is worth noting that the specimens of *C. occidentalis* and *C. riethi* used to illustrate this color difference in figure 1 were collected from the same lake margin on the same day, suggesting that degree of pigmentation is not due to environmental causes.

Previous studies concluded that members of *C.* (*Monoculicoides*) share the following suite of characters: large larval pharyngeal complex (Kettle & Lawson 1952), a dark ring near the base of the respiratory organ of the pupa (Lawson 1951 & Shults *et al*. 2016), a single spermatheca in the adult female (generally with an enlarged spermathecal duct opening), and posteriorly fused parameres (Khalaf 1954 and Shults 2015) and a bifid aedeagus (Fox 1955) in adult males. These characters were considered by Shults (2015) to be synapomorphies for the subgenus and evidence for the group's monophyly. Our study here provides the first subgeneric diagnosis for pupae of any subgenus of *Culicoides* and examines additional pupal character states that will likely be useful in future phylogenetic studies (in prep.).

Grogan & Lysyk (2015) recognized two species groups within the Nearctic species of *C.* (*Monoculicoides*): the *C. variipennis* complex and the *C. nubeculosus-stigma* complex. The latter complex is not based on morphological similarity and there is no cladistic evidence to support its validity. Using adult morphological data, Shults (2015) reported that *C. nubeculosus* belongs to a clade which includes the *C. variipennis* complex, and assigned *C. stigma* to an entirely different clade. We were unable to find any pupal character states that show support for either complex, and the phylogenetic relationship of *C. grandensis*, *C. riethi*, and *C. shemanchuki* remain unresolved.

The exact phylogenetic relationship between species of the *C. variipennis* complex also remains unresolved despite studies using both genetic and morphological data (Shults 2015, Hopken 2016, and Jewiss-Gaines *et al*. 2017), though some of these studies suggest that *C. occidentalis* is the sister taxa to the other two species. Some of the uncertainty stems from the morphological variation within this complex which led Wirth & Jones (1957) to originally describe five subspecies within the *C. variipennis* complex (three of which are now considered valid species). It could be worthwhile examining the pupae of the species we currently recognized to help clarify their taxonomy and potentially explain some of the morphological variation seen within the *C. variipennis* complex. The ability to identify a specimen from the pupal exuviae alone would allow for the adult to be used in genetic analysis while still maintaining a voucher for species identification. However, one factor that could be potentially complicating morphological analyses is hybridization. Velten & Mullens (1997) were able to show under laboratory conditions that *C. occidentalis* and *C. sonorensis* will hybridize and produce viable offspring for at least 6 generations. These hybrids showed intermediate characters states from the parents. Future studies are needed to investigate if this phenomenon is happening in nature, and if so, determine the consequences it has on morphology and potentially even their species status.

There are a few of keys to pupae of Nearctic species of *Culicoides* that provide useful tools for identifying this stage. Regional pupal keys exist for Wisconsin (Jones 1955), New York (Jamnback 1965), and Florida (Blanton & Wirth 1979). Jones (1961) and Lamberson *et al*. (1992) provide pupal descriptions with keys to 13 species from various subgenera. Keys also exist for the eastern Nearctic species of the *obsoletus* group (Jamnback & Wirth 1963), the *guttipennis* group (Kann 1980) and the subgenus *Selfia* (Atchley 1970), but these keys are of limited use because it is uncertain how these subgeneric groups may be recognized as such. Both Jones (1961) and Atchley (1970) reported that the presence of two anterolateral sensilla was diagnostic for *C.* (*Selfia*), as the pupae of other species of *Culicoides* have three of these sensilla. However, Borkent (2014) noted that the pupa of other species of *Culicoides* also have two anterolateral setae and one campaniform sensillum. The scanning electron microphotography in Shults *et al*. (2016) clearly shows that *C. sonorensis* have two anterolateral setae and no campaniform sensillum, and our current study further confirms this. As such, there are presently no diagnostic features for *C.* (*Selfia*).

When he proposed the subgenus *C.* (*Monoculicoides*), Khalaf (1954) included four species groups referred to as the *nubeculosus*, *fulvithorax*, *guttifer* and *crepuscularis* groups. The concept of this subgenus has been revised since that time and the summary of the current classification provided by Borkent (2016), shows that 3 of Khalaf's groups and 1 species of Khalaf's *nubeculosus* group have since been placed into other subgenera. Borkent (2016) however warns that the current subgeneric classification of *Culicoides* is not based on sound cladistic analyses so the monophyly of the subgenera have not been tested. If the groups formerly placed into *C*. (*Monoculicoides*) have diagnostic pupal characters which are synapomorphic, they could be used to validate their respective subgeneric classifications and help to define their relationships to *C*. (*Monoculicoides*). Ultimately, the examination of all life stages will no doubt contribute to a superior understanding of the genus *Culicoides*.

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References

Anjos-Santos, D., Funes, A., Spinelli, G.R. & Ronderos, M.M. (2014) The immatures of *Culicoides lacustris* Ronderos (Diptera: Ceratopogonidae). *Zootaxa*, 3881 (6), 591–596.
https://doi.org/10.11646/zootaxa.3881.6.7

Atchley, W.R. (1970) A biosystematic study of the subgenus *Selfia* of *Culicoides* (Diptera: Ceratopogonidae). *University of Kansas Science Bulletin*, 49, 181–336.

Blanton, F.S. & Wirth, W.W. (1979) The sand flies (*Culicoides*) of Florida (Diptera: Ceratopogonidae). *Arthropods of Florida and Neighboring Land Areas*. *Vol. 10*. Florida Department of Agriculture and Consumer Services, Gainesville, FL, xv + 204 pp.

Borkent, A. (2004) 10. The Biting Midges, the Ceratopogonidae (Diptera). *In*: Marquardt, W.C. (Ed.), *Biology of Disease Vectors*. *2nd Edition*. Elsevier Academic Press, Burlington, Massachusetts, pp. i–xxiii + 1–785.

Borkent, A. (2014) The pupae of the Biting Midges of the world (Diptera: Ceratopogonidae), with a generic key and analysis of the phylogenetic relationships between genera. *Zootaxa*, 3879, 1–327.
https://doi.org/10.11646/zootaxa.3879.1.1

Borkent, A. (2016) The subgeneric classification of species of *Culicoides* – thoughts and a warning. Available from: http://wwx.inhs.illinois.edu/research/flytree/borkent.html (accessed 8 December 2017)

Borkent, A. & Grogan, W.L. (2009) Catalog of the new world Biting Midges north of Mexico (Diptera: Ceratopogonidae). *Zootaxa*, 2273, 1–48.

Borkent, A. & Spinelli, G.R. (2007) Neotropical Ceratopogonidae (Diptera: Insecta). In: Adis, J., Arias, J.R., Rueda-Delgado, G. & Wnatzen, K.M. (Eds.), *Aquatic Biodiversity in Latin America (ABLA). Vol. 4*. Pensoft, Sofia-Moscow, pp. 1–198.

Damian-Georgescu, A. & Spătaru, P. (1971) Metamorfoza la *Culicoides riethi* Kieff, 1914 (Diptera—Ceratopogonidae). *Studii si cercetari de Biologie*, Seria Zoologie, 23, 531–536.

Dzhafarov, S.M. (1964) *Blood-sucking midges (Diptera, Heleidae) of the Transcaucasus*. Akademija Nauk Azerbaidzanskoi SSR, Instituta Zoologicheskiy, Sankt-Peterburg, 414 pp. [in Russian]

Fox, I. (1942) The respiratory trumpet and anal segment of the pupae of some species of *Culicoides* (Diptera: Ceratopogonidae). *Puerto Rico Journal of Public Health and Tropical Medicine*, 17, 412–425. [Spanish version, pp. 426–434]

Fox, I. (1955) A catalogue of the bloodsucking midges of the Americas (*Culicoides*, *Leptoconops* and *Lasiohelea*) with keys to the subgenera and Nearctic species, a geographic index, and bibliography. *Journal of Agriculture of University of Puerto Rico*, 39, 214–285.

Glukhova, V.M. (1989) Blood-sucking midges of the genera *Culicoides* and *Forcipomyia* (Ceratopogonidae). *Fauna of the USSR*, 139, 3 (5a), 1–408. [in Russian]

Grogan, W.L. & Lysyk, T. (2015) A revision of the biting midges in the *Culicoides* (*Monoculicoides*) *nubeculosus*-*stigma* complex in North America with the description of a new species (Diptera: Ceratopogonidae). *Insecta Mundi*, 441, 1–24.

Grogan, W.L. & Phillips, R.A. (2008) A new species of biting midge in the subgenus *Monoculicoides* of *Culicoides* from Utah (Diptera: Ceratopogonidae). *Proceedings of the Entomological Society of Washington*, 110, 196–203.
https://doi.org/10.4289/0013-8797-110.1.196

Harsha, R., Paul, N. & Mazumdar, A. (2017) Description of immature stages of *Culicoides peregrinus* (Diptera: Ceratopogonidae), a potent vector of bluetongue virus. *Turkish Journal of Zoology*, 41, 812–820.
https://doi.org/10.3906/zoo-1605-45

Holbrook, F.R., Tabachnick, W.J., Schmidtmann, E.T., McKinnon, C.N., Bobian, R.J. & Grogan, W.L. (2000) Sympatry in the *Culicoides variipennis* complex (Diptera: Ceratopogonidae): a taxonomic reassessment. *Journal of Medical Entomology*, 37, 65–

76.
https://doi.org/10.1603/0022-2585-37.1.65

Hopken, M.W. (2016) *Pathogen vectors at the wildlife-livestock interface: molecular approaches to elucidating Culicoides (Diptera: Ceratopogonidae) biology*. Ph.D. dissertation, University of Colorado, Fort Collins, CO, 134 pp.

Jamnback, H. (1965) The *Culicoides* of New York state (Diptera: Ceratopogonidae). *New York State Museum and Science Service Bulletin*, No 399, viii + 154 pp.

Jamnback, H. & Wirth, W.W. (1963) The species of *Culicoides* related to *obsoletus* in eastern North America (Diptera: Ceratopogonidae). *Annals of the Entomological Society of America*, 56, 185–198.
https://doi.org/10.1093/aesa/56.2.185

Jewiss-Gaines, A., Barelli, L. & Hunter, F.F. (2017) First records of *Culicoides sonorensis* (Diptera: Ceratopogonidae), a known vector of Bluetongue Virus, in southern Ontario. *Journal of Medical Entomology*, 54, 757–762.

Jones, R.H. (1955) *The Culicoides of Wisconsin*. Ph.D. dissertation, University of Wisconsin, vi + 204 pp., 3 tables.

Jones, R.H. (1961) Descriptions of pupae of thirteen North American species of *Culicoides* (Diptera: Ceratopogonidae). *Annals of the Entomological Society of America*, 54, 729–746.
https://doi.org/10.1093/aesa/54.5.729

Kann, K.A. (1980) A taxonomic study of the immature stages of the *Culicoides guttipennis* group *(Diptera: Ceratopogonidae)*. M.Sc. thesis, University of Wisconsin-Madison, Madison, WI, iv + 96 pp.

Kettle, D.S. & Elson, M.M. (1976) The immature stages of some Australian *Culicoides* Latreille (Diptera: Ceratopogonidae). *Journal of the Australian Entomological Society*, 15, 303–332.
https://doi.org/10.1111/j.1440-6055.1976.tb01711.x

Kettle, D.S. & Lawson, J.W.H. (1952) The early stages of British biting midges *Culicoides* Latreille (Diptera: Ceratopogonidae) and allied genera. *Bulletin of Entomological Research*, 43, 421–467, pls. 14–19.

Kieffer, J.J. (1914) Zwölf neue Culicoidinenarten. *Archiv für Hydrobiologie und Planktonkunde*, 2 (Supplement), 231–241.

Khalaf, K. (1954) The speciation of the genus *Culicoides*. *Annals of the Entomological Society of America*, 47, 34–51.
https://doi.org/10.1093/aesa/47.1.34

Lamberson, C., Pappas, C.D. & Pappas, L.G. (1992) Pupal taxonomy of the tree–hole *Culicoides* (Diptera: Ceratopogonidae) in eastern North America. *Annals of the Entomological Society of America*, 85, 111–120.
https://doi.org/10.1093/aesa/85.2.111

Lawson, J.W.H. (1951) The anatomy and morphology of the early stages of *Culicoides nubeculosus* Meigen (Diptera: Ceratopogonidae=Heleidae). *Proceedings of the Royal Entomological Society of London*, 102, 511–570, pl. 1.

Malloch, J.R. (1915) The Chironomidae, or midges, of Illinois, with particular reference to the species occurring in the Illinois River. B*ulletin of the Illinois State Laboratory of Natural History*, 10, 275–543, 24 pls.

Mayer, K. (1934a) Die Metamorphose der Ceratopogonidae (Dipt.). Ein Beitrag zur Morphologie, Systematik. Ökologie und Biologie der Jugendstadien dieser Dipterenfamilie. *Archiv für Naturgeschicthe*, 3, 205–288.

Nevill, H., Venter, G.J., Meiswinkel, R. & Nevill, E.M. (2007) Comparative descriptions of the pupae of five species of the *Culicoides imicola* complex (Diptera, Ceratopogonidae) from South Africa. *Onderstepoort Journal of Veterinary Research*, 74, 97–114.
https://doi.org/10.4102/ojvr.v74i2.130

Nevill, H., Nevill, E.M. & Venter, G.J. (2009) Description and comparison of the pupae of a further two *Culicoides* (*Avaritia*) species from the dung of large herbivores in South Africa (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 76, 277–284.
https://doi.org/10.4102/ojvr.v76i3.29

Rieth, J.T. (1915) Die Metamorphose der Culicoidinen (Ceratopogoninen). *Archiv für Hydrobiologie*, 2 (Supplement), 377–442.

Ronderos, M.M., Spinelli, G.R. & Keppler, R.L.F. (2013) Description of the pupa of *Culicoides crucifer* Clastrier (Diptera: Ceratopogonidae). *Neotropical Entomology*, 42, 492–497.
https://doi.org/10.1007/s13744-013-0142-4

Shults, P. (2015) *A study of the taxonomy, ecology, and systematics of Culicoides species (Diptera: Ceratopogonidae) including those associated with deer breeding facilities in southeast Texas*. M.S. Thesis, Texas A&M University, College Station, TX, 134 pp.

Shults, P., Borkent, A. & Gold, R. (2016) The pupa of *Culicoides sonorensis* Wirth and Jones (Diptera: Ceratopogonidae)—first detailed description of this stage of the Bluetongue Virus vector. *Annals of the Entomological Society of America*, 109, 280–318.
https://doi.org/10.1093/aesa/sav119

Spătaru, P. (1971) Variabilitatea unor Trasaturi Caracteristice la Larvele si Pupele de *Culicoides stigma* (Meigen) (Ceratopogonidae, Diptera). *Comunicari si Referate ale Muzeul de Stiintele Naturii Ploiesti*, 1971, 189–198.

Thienemann, A. (1928) Chironomiden—Metamorphosen I. *Archiv für Hydrobiologie*, 19, 585–623.

Thomsen, L.C. (1937) Aquatic Diptera Part V. Ceratopogonidae. *Cornell University Agricultural Experiment Station Memoir*, 210, 57–80, pls. 10–18.

Velten, R.K. & Mullens, B.A. (1997) Field morphological variation and laboratory hybridization of *Culicoides variipennis sonorensis* and *C. v. occidentalis* (Diptera: Ceratopogonidae) in southern California. *Journal of Medical Entomology*, 34, 277–284.
https://doi.org/10.1093/jmedent/34.3.277

Weber, L. (2001) What's eating you? Minnesota's biting flies. *Minnesota Conservation Volunteer*, 64, 32–43.

Wirth, W.W. & Jones, R.H. (1957) The North American subspecies of *Culicoides variipennis* (Diptera, Heleidae). *U.S. Department of Agriculture Technical Bulletin*, 1170, 1–35.