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Stictohironomus sticticus from the River Kemzelka, Perm Province, Russia. Male reared from larva on 19 June 2017. Photo: Andrey Przhiboro.

CHIRONOMUS Journal of Chironomidae Research

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Would you like to see your picture on the front page? Please send us your favourite midge photograph or drawing (torbjorn.ekrem@ntnu.no).

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Front page layout: Chironomid in title from photograph by Steve Marshall, Graphic design by Kolbjørn Skarpnæs, NTNU Information Division.

Front page photo: *Stictohironomus sticticus* from the River Kemzelka, Perm Province, Russia. Male reared from larva on 19 June 2017. Photo: Andrey Przhiboro.

Editorial

Describing the Undiscovered

Over 1.7 million species have been described; approximately one million (60%) of these species are insects (Roskov et al. 2018). That's remarkable diversity (and an impressive amount of work completed by insect taxonomists!). But again, these numbers represent only the *described* species. As we know, there are many, many more species awaiting description. When considering all species on earth, some of the most recent estimates indicate that there could be somewhere between 8.7 million (Mora et al. 2011) or even up to 6 BILLION species, if symbionts and cryptic species are considered (Larsen et al. 2017). When considering insect richness alone, estimates indicate that approximately 6 million species exist (e.g. Basset et al. 2012, Stork et al. 2015). If we narrow down our scope further, and just consider the Chironomidae, we have not yet described half of the projected 15,000 species (Armitage et al. 1995) that exist worldwide. No matter how you look at it, there are an incredible number of organisms that we know virtually nothing about.

I regularly share these statistics with students in my Biodiversity courses, emphasizing that we have barely scratched the surface in terms of what we know about life on Earth. To emphasize the point, I share my story of a short series of sampling sessions in four Minnesota, USA streams. For this particular study, I spent only perhaps 20-30 minutes in each stream, searching specifically for *Micropsectra* larvae and ignoring the rest of the stream biota. I walked away with three new species (Anderson et al. 2013). Many of you have similar stories, and ones that are even more impressive than mine. But, I find that stories like this always hook my students, prompting many good questions for discussion, such as: *How many new species might I have found if I did a comprehensive analysis of these streams? What if a stream in a more diverse ecosystem had been studied?* And the kicker: *Why has no one discovered these species yet?* In a world where taxonomy and systematics has taken a backseat in recent decades, we need to spend more time convincing people of the importance of our work. *Why do we do what we do, and why should others care?*

With climate change predictions looming in front of us, and species extinctions on the rise, how will the world look without these species? What cascading consequences lurk if there are mass extinctions caused by rising temperatures, habitat changes and loss, competition with invasive species etc., etc.? How will these changes impact interactions with other species? And thinking again about the millions of undescribed species – how many of them will we simply never get the chance to learn about?

Like many of you, I believe it is essential to learn all we can about this diversity and how certain species impact others via competition, predation/parasitism, mutualistic relationships, and so on, and also how the actions of *our* species might impact global diversity. Equally important is sharing these stories with the broader community and relaying the importance of understanding the diversity of life and what could happen if our actions (or inactions) result in loss of species.

This edition of *CHIRONOMUS* highlights what our relatively small community is doing to address some of these previously unknown questions. From descriptions of new species (Mouybyayed-Breil and Ashe, 2018), new records of genera (Hamerlik and da Silva 2018), previously unknown life stages (Egan and Langton 2018) and species range updates (Namayandeh and Beresford 2018) to notes on the unique oviposition behavior of a midge that parasitizes unionid mussels, there are clearly new and important findings to share. We hope you enjoy learning of these discoveries, and continue to relay the importance of your own findings, not only with us and other colleagues in the scientific community, but with the broader community as well.

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***CRICOTOPUS* (S. STR.) *LATELLAI* SP. N., A NEW RHEOPHILIC SPECIES OF THE *TREMULUS*-GROUP FROM THE ITALIAN AND FRENCH MARITIME ALPS (DIPTERA: CHIRONOMIDAE)**

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<http://zoobank.org/4B55C4D5-9ADC-4BF9-B836-57DC3CB27F89>

Abstract

Cricotopus (*Cricotopus*) *latellai* sp. n. is described and diagnosed based on material comprising male and female pharate adults, pupal exuviae and larvae recently collected in some glacial streams in both the Italian and French Maritime Alps. Taxonomic notes provided in this paper include: description of *C. latellai* sp. n. as male and female adults, pupal exuviae and last instar larvae. The diagnosis of the *Cricotopus tremulus*-group is supplemented with additional characters in the male adult and pupal exuviae. *Cricotopus latellai* sp. n. keys near *C. mantetanus* Moubayed-Breil, 2016 and *C. storozhenkoi* Makarchenko & Makarchenko, 2016. Geographical distribution of *C. latellai* sp. n. is currently restricted to the Italian and French Maritime Alps. It belongs to the Tyrrhenian community of glacial relic species, which are considered to be indicators of climate change in this biogeographical region. Remarks, taxonomic position, and ecology of the new described species are given.

Introduction

Data on the taxonomy, geographical distribution and ecology of the *tremulus*-group of the genus *Cricotopus* v. d. Wulp, 1874 from the Palaearctic (Hirvenoja 1973; Sæther 1977; Coffman et al. 1986; Moubayed & Hirvenoja 1986; Cranston et al. 1989; Casas & Vilchez-Quero 1992; Ashe & O'Connor 2012; Lencioni et al. 2012; Andersen et al. 2013; Sæther & Spies 2013; Makarchenko & Makarchenko 2016; Moubayed-Breil & Ashe 2011, 2016) shows that six valid species are currently known including two from the Russian Far East (*C. leleji* Makarchenko & Makarchenko, 2016 and *C. storozhenkoi* Makarchenko & Makarchenko, 2016) and four from Europe: *C. mantetanus* Moubayed-Breil, 2016; *C. nevadensis* Casas & Vilchez-Quero, 1992; *C. royanus* Moubayed-Breil, 2016 and *C. tremulus* (Linnaeus, 1758). In this paper, a seventh new species (*C. latellai* sp. n.) is described based on associated pharate material

comprising male and female adults, pupal exuviae and larvae collected in glacial streams located in northwest Italy in the dolomitic upstream area of the Po River Basin in the Alpi Marittime at an altitude 1500-1750 m and in southeast France in the karstic upstream area of the Roya River Basin in the Maritime Alps at an altitude of 1500-1750 m.

Methods

Morphological terminology and measurements follow that of Sæther (1980) for the imagines and pupal exuviae. For a better examination of the specific features and more accurate description of the various taxonomic details of the pupa, the pupal abdomen was mounted not only in dorsal and ventral view but separately in lateral view, which facilitates proper examination and illustration of all the relevant taxonomic characters. Remarks and discussion on some related species and comments on the ecology and geographical distribution of the new species are given.

Results

Cricotopus latellai sp. n. belongs to the *tremulus*-group based on characters found in the male adult (distribution pattern of median setae on tergites II-V; shape of tergite IX, inferior volsella and gonostylus) and pupal exuviae (chaetotaxy and granulation of thorax; armament of abdominal segments, shape and size of anal macrosetae) and keys near *C. mantetanus* and *C. storozhenkoi*. The new species is only known from high and middle mountain streams located in both the Italian and French Maritime Alps. The 766 valid species known from continental France (Moubayed-Breil & Ashe own observations, Moubayed-Breil 2016) increases to 767 with the description of a new species of *Propilosocerus* Kieffer from eastern France (Moubayed-Breil, in press). Consequently, the description of *C. latellai* sp. n. increases the total number to **768** species known from continental France.

The *tremulus*-group was first emended by Hirvenoja (1973) then modified by Moubayed & Hirvenoja (1986), Casas & Vilchez-Quero (1992) and most recently by Moubayed-Breil (2016). The diagnosis of the *tremulus*-group is refined by including features found in the male adult and pupal exuviae:

Adult male. Palpomere 3 with 2-3 sensilla coeloconica placed distally; presence of lateral and occasionally median anteprenotals; apex of femur and base of tibia of fore-, mid-, and hind leg blackish; tergites I-II whitish, remaining tergites blackish; chaetotaxy and distribution pattern of setae on median part of tergites II-V; gonocoxite with rounded or truncated apex, bearing or lacking a sub-triangular to rounded setiferous projection placed dorsally close to base of inferior volsella; inferior volsella: (i) pointing downwards at an acute angle, narrow and finger-like (*C. nevadensis*), (ii) a projecting lobe with outer margin gradually bent downwards and lacking strong dorsal setae on outer edge (*C. latellai*, *C. royanus* and *C. tremulus*), (iii) a projecting lobe with outer margin abruptly bent downwards at a right angle and with 2 strong dorsal setae on outer edge (*C. mantetanus*); crista dorsalis medium to large, tooth-like, with pointed or rounded apex, moderately to strongly projecting orally.

Pupal exuviae. Colouration brownish or contrasting whitish to blackish; frontal apotome with wrinkles and granulation, occasionally domed, frontal setae inserted on prefrons, ventral to antennal sheaths; cephalothorax with moderate to dense wrinkles and granulation, bearing or lacking a transverse posteromedian blackish shading placed between the thoracic suture (Dc_3 and Dc_4) and base of wing sheath, dorsocentrals are all setae-like or include 1-2 bristle-like setae; thoracic horn tube-like, foliate to ellipse-shaped with or without narrowing apex, toothed in general, occasionally smooth, teeth are pointed or occasionally smooth; anterior and posterior fields of spines on tergites III-VI clearly separated; median field of spines on tergite VI circular, diamond-like to sub-oval, posterior area of sternites V-VI with or without a faint transverse median patch of spines which, when present, are more concentrated and markedly larger on VI; anal lobe with distal part narrowing or distinctly swollen, apical rows of small spines restricted to apical area or extending well above insertion of anal macrosetae; macrosetae short and pin-like with a slightly curved apices.

Description

Cricotopus latellai Moubayed-Breil & Ashe, sp. n.

<http://zoobank.org/125F054A-DCD5-4F0A-8AF3-5553C1A32B0A>

Material examined

Holotype. Italy, Alpi Marittime. Po River Basin, upstream, rapid to moderate rhithral and waterfalls, altitude 1500-1700 m, 44° 42' 05" N, 7° 05' 37" E, 11.VII.2017; 1 male pharate adult, leg. J. Moubayed-Breil and P. Ashe. Environmental data of water: moderately crystalline, conductivity about 20-30 μ S/cm; temperature 8-12 °C.

Paratypes. Italy, Alpi Marittime. Adults (5 males, 2 females); pharate adults (2 males, 2 females); pupal exuviae (10 males, 15 females); 3 larvae; same locality and date as for holotype. Continental France, Maritime Alps, Casterino stream, a tributary of the Roya River, alt. 1500-1700 m, 44° 4' 34" N, 7° 26' 18" E, 22.VI.2016; pupal exuviae (1 male and 2 females); leg. J. Moubayed-Breil. Environmental data of water are: calcareous water, conductivity 95-100 μ S/cm; temperature 8-12 °C during late spring and summer.

Holotype (on 2 slides, including the male adult and its pupal exuviae) with 1 additional paratype are deposited in the collections of the Zoologische Staatssammlung München (ZSM), Munich, Germany. Remaining paratypes are deposited in the senior author's collection. Type material was preserved in 80-85% alcohol, and later mounted in polyvinyl lactophenol. For each adult, the head, thorax and abdomen were cleared in 90% lactic acid then washed in about 60% ethanol before mounting on slides.

Diagnostic characters

Based on similarity of characters found in the male adult and pupal exuviae of the *tremulus*-group, three species (*C. latellai* sp. n., *C. mantetanus* and *C. storozhenkoi*) are considered to be sister species. However *C. latellai* sp. n. can be easily separated with the following characters:

Male adult: palpomere 3 with 2 sensilla coeloconica (tubule-like) placed distally; tergites III-IV with 3 median setae placed distally; laterosternite VIII not lobe-like; inferior volsella consists of a projecting lobe-like, with rounded outer margin gradually bent downwards, posterior area bearing two minute lobes, with a distinct rounded setiferous lobe placed close to its base; crista dorsalis large, tooth-like and orally projecting, apex rounded in dorsal view and pointed in lateral view;

Pupal exuviae: thoracic horn foliate to ellipse-like, narrowing distally with pointed to smooth ending apically, toothed, teeth are smooth in general or occasionally weakly pointed; tergite VI with median dorsal field of spines semi-circular to diamond-like; posterior area of sternite VI with a distinct median patch of spines; distal part of anal lobe narrowing, apical rows of small spines extending well above insertion of anal macrosetae; macrosetae short with markedly curved apices.

Etymology: The new species is named '*latellai*' after our colleague Dr. Leonardo Latella, curator of zoology at the Museum of Natural History of Verona (Italy), who is contributing to preserve the biological and ecological quality of water and environment in Verona and surrounding areas.

Male adult

(n = 7: 2 pharate adults + 5 adults; Figs 1-4, 7-11, 13-15)

Medium to large sized *Cricotopus* species. Total length 3.50-4.00 mm. Wing length 2.20-2.40 mm; TL/WL = 1.60-1.70. General colouration contrasting blackish to brownish to yellowish with black mesonotal stripes. Head dark brown, antennae pale brown, thorax brown to dark brown, mesonotal stripes distinctly blackish; wing pale to pale brown. Legs mostly yellowish brown to dark brown, only base of femur and base and apex of tibia of PI-PIII blackish. Tergites I-II whitish, tergites III-VIII entirely brownish to blackish, anal segment contrasting brown to dark brown.

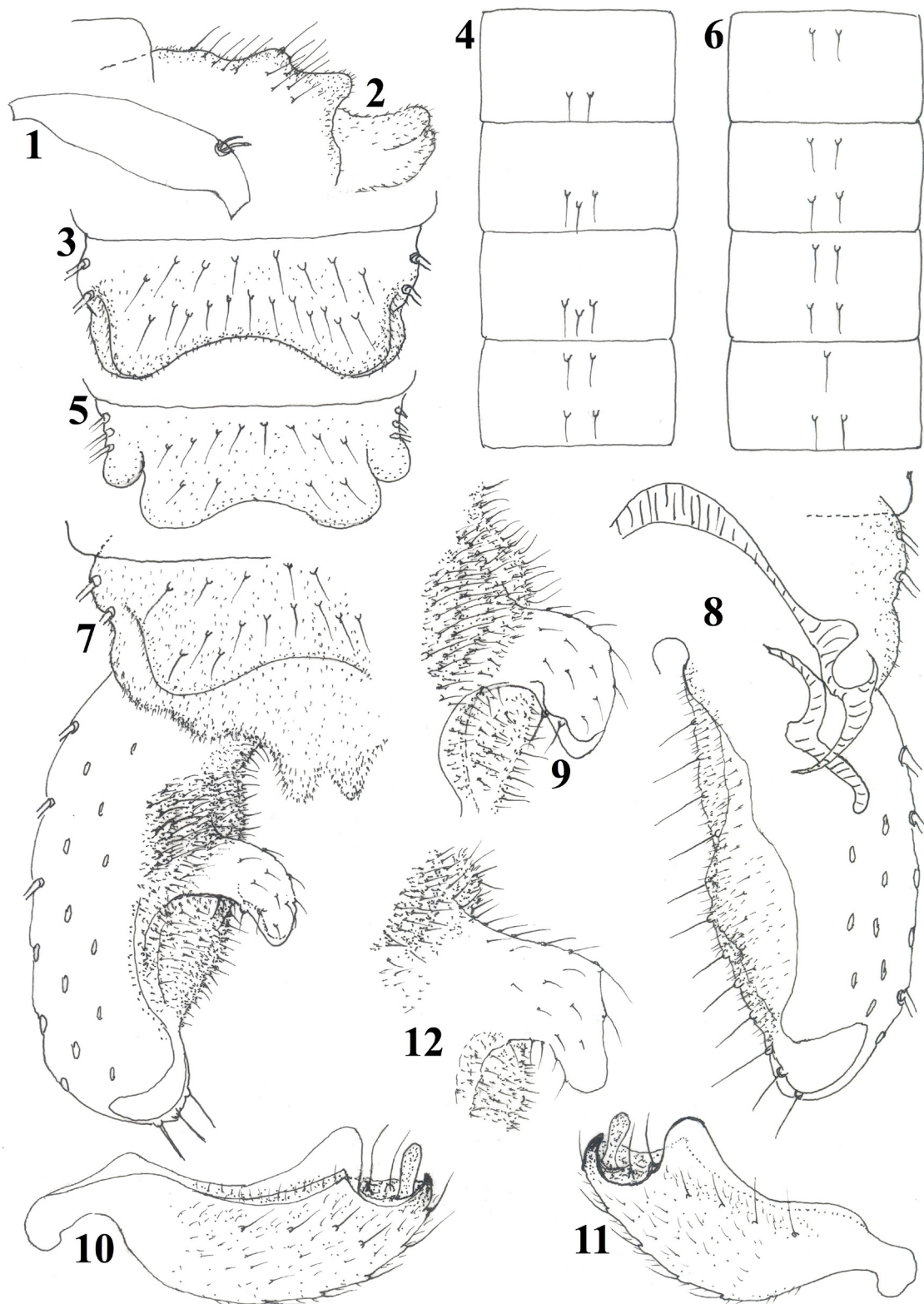
Head. Eyes hairy, inner eye margin bare. Temporals consist of 8 uniserial setae including 5 inner and 3 verticals. Clypeus trapezoidal to sub-square shaped with 14-16 setae placed in 4 rows. Palp 5-segmented; first and second palpomeres fused; length (µm) of segments 40, 70, 120, 135, 195; palpomere 3 with 2 sensilla coeloconica (tubule-like) inserted in a circular depression placed on distal part (Fig. 1). Antenna 900-930 µm long, 13-segmented; antennal groove beginning on segments 3-4 and reaching ultimate flagellomere; ultimate flagellomere 400-430 µm long, distinctly clubbed and bearing a brush of curved sensilla chaetica apically. AR 0.80-0.85.

Thorax. Lobes of antepnotum gaping, with 3 median and 3-4 antepnotals which are slightly indistinct; acrostichals 29-31 uni-biserial, dorso-centrals, 16-18 multiserial and converging medially, prealars 3-4. Scutellum with 10 setae in a single row. Wing. Brachiolum with 1 seta. Number of setae on veins: R, 3-5; remaining veins bare. Squama with 14-16 setae in a single row. Legs. Tarsomere ta_5 of PI, PII and PIII distinctly shorter than ta_4 . Sensilla chaetica densely present on tarsomeres ta_1 to ta_5 of PI, PII and PIII. Length (µm) and proportions of legs (Table 1).

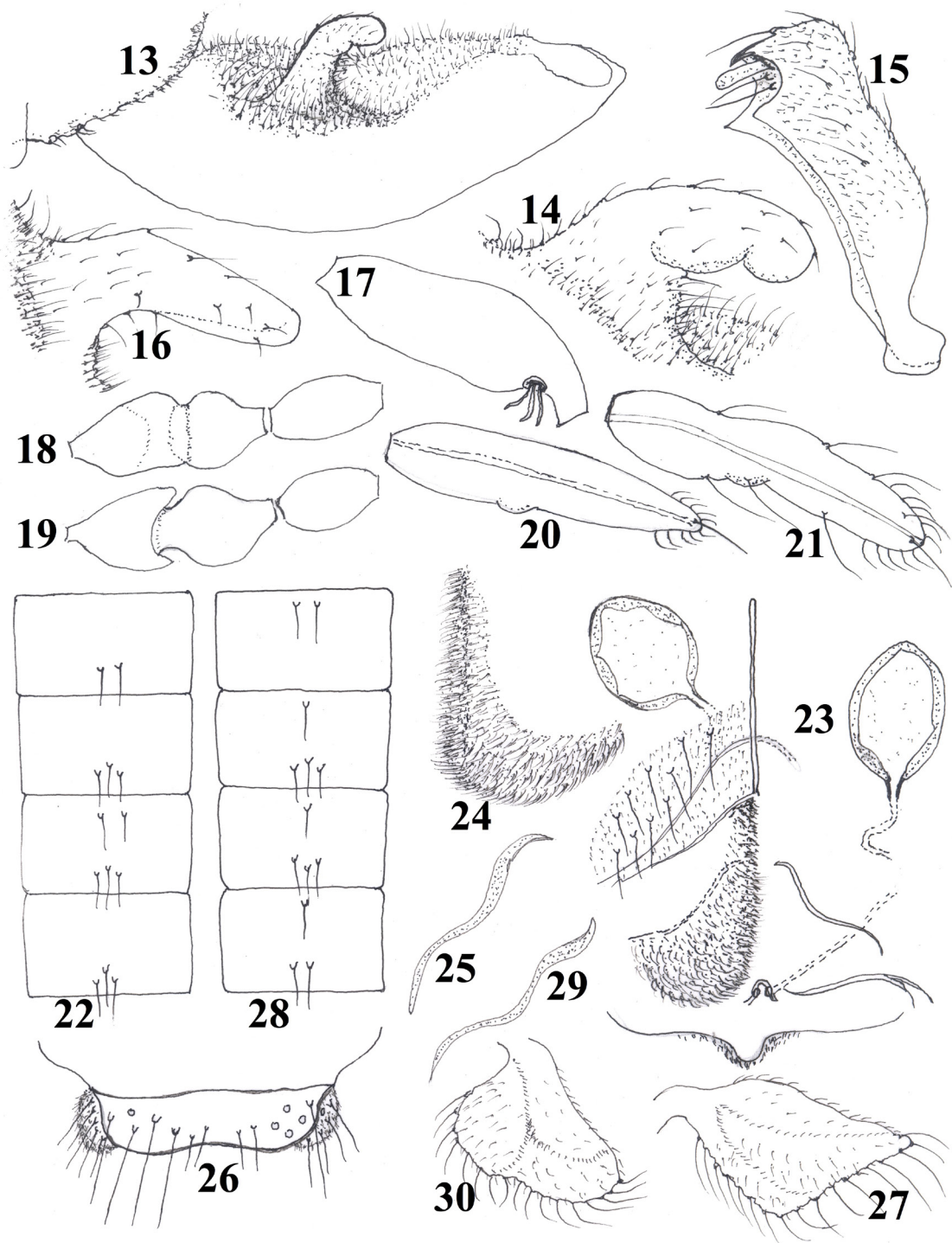
Abdomen. Dorsal margin of tergite IX sinuous with a distinct median lobe (Fig. 2); posterior margin broadly bilobed (Figs 3, 7); presence of 17-20 dorsal setae placed in 2 rows (7-8 anteriorly, 10-12 posteriorly). Laterosternite IX with 4 setae (2 on each side), extended vertically and lacking lateral lobe-like expansions on each side. Anal point absent. Sternapodeme and phallapodeme as in Fig. 8, transverse sternapodeme orally produced and arc-like; phallapodeme markedly sickle shaped. Distribution pattern of setae on median area of tergites II-V as illustrated (Fig. 4): tergite II (2 setae distally); III-IV (3 setae distally); V (4 setae, 2 anteriorly and 2 distally). Hypopygium in dorsal, ventral and lateral view as in Figs 7-11, 13-15, ventral view (Fig. 8) with tergite IX removed. Gonocoxite 250-260 µm long, apex rounded in dorsal view (Fig. 7) and distinctly truncate in lateral view (Fig. 13); inferior volsella hyaline, long lobe-like, projecting with rounded outer margin gradually bent downwards, wider at base and broadly narrowing distally to a rounded apex, presence of two minute lobes on posterior part which are clearly visible in lateral view (Fig. 13), presence of 5-6 small setae on dorsal area. Gonostylus (Figs 10-11, 15) 120-130 µm long, narrowing distally to a pointed apex; posterior margin rounded medially in dorsal view (Figs 10-11), distinctly sinuous in lateral view (Fig. 15), anterior margin concave medially with an orally directed triangular projection clearly visible in lateral view (Fig. 15); 2 orally directed strong setae are placed close to the megaseta. Crista dorsalis (Figs 10-11, 15) large, tooth-like and orally projecting, apex rounded in dorsal view (Figs 11-12) and sharply pointed in lateral view (Fig. 15). Megaseta 18-21 µm long and slender.

Table 1. *Cricotopus latellai* sp. n. Length (µm) and proportions of legs.

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
PI	860	1045	540	350	245	175	120	0.52	2.75	3.53	2.00
PII	970	900	390	235	170	120	105	0.43	3.59	4.80	2.00
PIII	875	990	480	290	220	140	120	0.48	3.05	3.90	1.70



Figures 1-12. Male adult of *Cricotopus* spp. *C. latellai* sp. n.: 1, tarsomere 3 with sensilla coeloconica; 2, tergite IX, lateral; 3, tergite IX, dorsal; 4, distribution pattern of setae on median area of tergites II-V. *C. mantetanus*: 5, tergite IX, dorsal; 6, distribution pattern of setae on median area of tergites II-V. *C. latellai* sp. n.: 7-8, hypopygium in dorsal and ventral view; 9, inferior volsella, dorsal; 10, left gonostylus, dorsal, at right angle; 11, right gonostylus, dorsal, at acute angle. *C. mantetanus*: 12, inferior volsella, dorsal.



Figures 13-30. Male and female adults of *Cricotopus* spp. *C. latellai* sp. n., male adult: 13, gonocoxite and inferior volsella, lateral; 14, inferior volsella, lateral; 15, gonostylus, lateral. 16, *C. mantetanus*: inferior volsella, lateral. *C. latellai* sp. n., female adult: 17, palpomere 3 with sensilla coeloconica; 18-19, antenna, segments 1-3, two aspects; 20-21, last flagellomere, two aspects; 22, distribution pattern of setae on median area of tergites II-V; 23, genitalia, ventral and dorsal view with gonapophysis VIII, sternite VIII and seminal capsules; 24, dorsomesal and ventrolateral lobes; 25, apodeme lobe; 26, tergite IX, dorsal; 27, cercus. *C. mantetanus*: 29, apodeme lobe; 30, seminal capsule.

Female adult

(n = 3: 2 paratype adults + 1 adult; Figs 17-27)

Colouration as in the male adult except for the antennae, which are dark brown to blackish. Segments 1-5 of antenna brown with blackish apex, last flagellomere entirely blackish. Total length (TL) 4.20-4.50 mm. Wing length (WL) 2.20-2.30 µm. TL/WL = 1.85-1.90. Head: eyes hairy; temporal setae 8-9 including 5-6 inner and 3 outer verticals. Clypeus rectangular with 26-28 setae in 4-5 rows. Palp 5-segmented, length (µm) of segments: 40, 55, 50, 55, 125-130; palpomere 3 (Fig. 17) truncate apically and bearing 3 sensilla coeloconica placed distally. Antenna (Figs 18-21) 6-segmented, 365-385 µm long, segments 1 and 2 (Figs 18-19) fused; segments 3-5 together 150 µm long and sub-equal (about 50 µm each), ultimate flagellomere (Figs 20-21) about 120 µm long, moderately clubbed and bearing a distinct small projection placed medially, distal part with 1 preapical seta and a tuft of curved setae including several sensilla chaetica; antennal groove restricted to last flagellomere. AR 0.46. Thorax: chaetotaxy as in the male; wing: distribution of setae on veins as in the male, squama with 11-13 setae in 1 row. Abdomen. Distribution pattern of setae on median area of tergites II-V as illustrated in Fig. 22: tergite II (2 setae distally), III (3 setae distally), IV (5 setae, 2 anteriorly and 3 distally), V (3 setae distally). Genitalia in dorsal and ventral view as illustrated in Figs 23-26. Notum about 100-110 µm long, rami indistinct. Sternite VIII with 22-24 setae (11-12 on each side of the notum). Gonapophysis VIII (Figs 23-25). Dorsomesal lobe uniformly linear; ventrolateral lobe broad and slightly projecting posteriorly; apodeme lobe undulating three times from base to apex. Seminal capsules 100-105 µm maximum length, 70 µm maximum width, sub-oval with narrowed tip and well sclerotized laterally. Spermathecal ducts with loops and separate openings. Tergite IX (Fig. 26) nearly semi-circular and distinctly divided on its posterior part into two large rounded lobes, with 14 setae including 10 placed laterally (5 on each side) and 4 markedly shorter placed medially. Gonocoxite (Fig. 26) broadly globular, bearing 6-7 short setae. Cercus (Fig. 27) normally developed and narrowed distally.

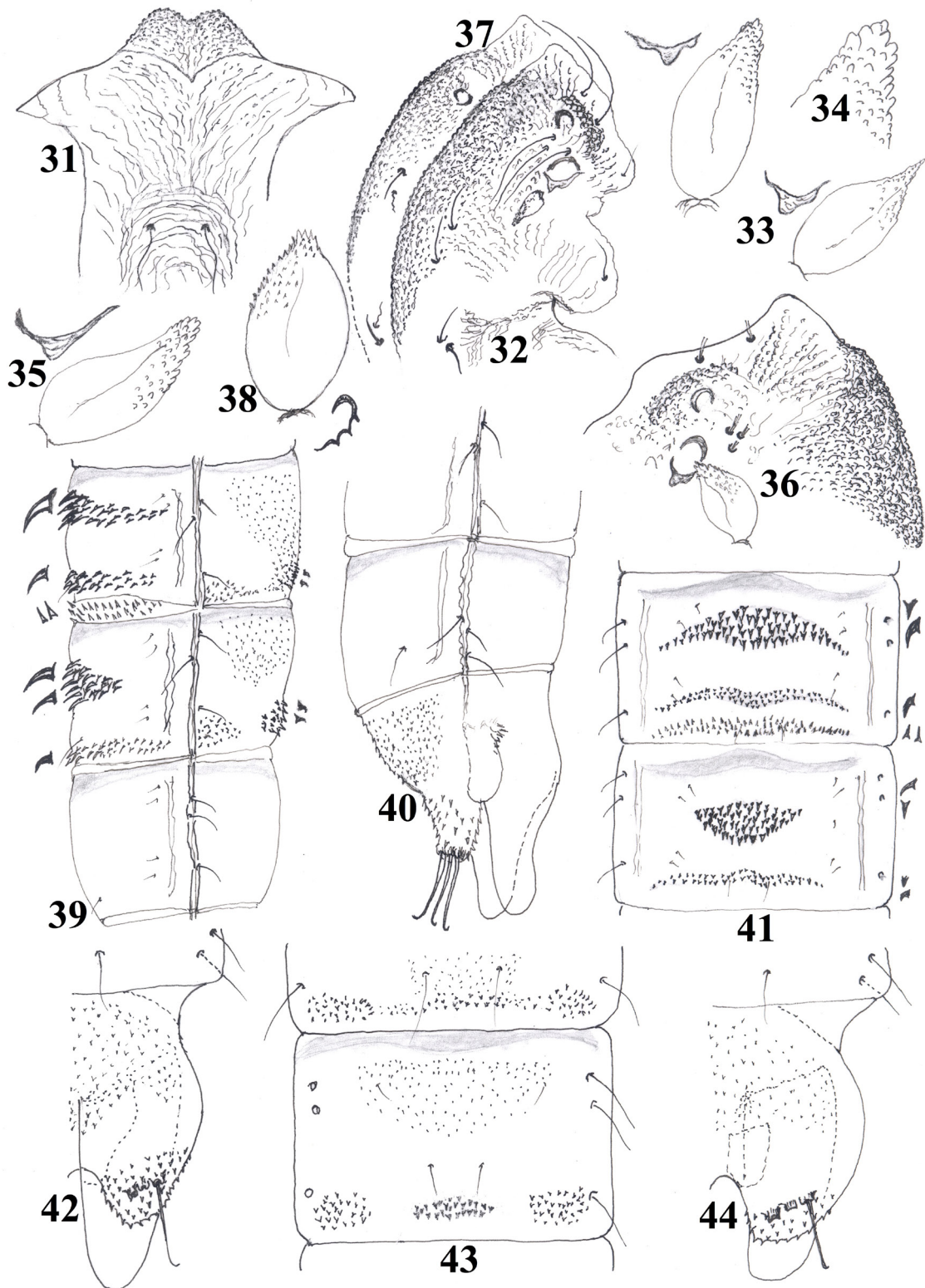
Male pupal exuviae

(n = 15: 7 males, 8 females; Figs 31-36, 39-43)

Colouration contrasting brownish to dark brown with blackish cephalothorax. Frontal apotome with dense wrinkles and granulation; cephalothorax brown to dark brown with blackish anteromedian area, densely wrinkled and granulose,

granulation and wrinkles strongly covering the anteromedian area including the thoracic suture and Dc₁-Dc₂ zone, granulose area along the thoracic suture reaching Dc₃-Dc₄ zone, posteromedian area less granulose, presence of a characteristic transversal posteromedian blackish shading extending between Dc₃-Dc₄ and base of wing sheath. Base of wing sheath covered with blackish bow-like shading. Abdomen including anal segment brown to dark brown; dark brown apophyses distinctly present on segments I/II-VII; muscles marks distinct on segments I-VIII.

Total length 3.60-4.10 mm. Frontal apotome (Fig. 31) distinctly domed with dense granulation, frontal setae bristle-like, inserted on prefrons ventral to antennal sheaths, 45 µm long and separated by only 30 µm. Thorax (Figs 32-36). Anteprenotals 3 including 2 median anteprenotals (130 and 150 µm long) and 1 lateral anteprenotal 60 µm long; 1 prealar 100-110 µm long; precorneals sub-equal (180-190 µm long); dorsocentrals all seta-like, Dc₁ and Dc₂ sub-equal (110-120 µm long), Dc₃ and Dc₄ 40 µm long; distance between Dc₂ to Dc₃ 150 µm, Dc₁ is placed close to Dc₂, Dc₃ and Dc₄ close to one another. Thoracic horn (Figs 33-36) foliate to ellipse shaped with narrowing distal part, toothed apically and distally (mainly on one side), teeth are often distinctly blunt apically. Abdomen. Armament and distribution pattern of patches of spines and points, chaetotaxy and lateral setation of abdominal segments as illustrated in Figs 39-43. Distribution of lateral setae on segments I-VIII: I (2), II-VIII (3). Tergite I bare. Transverse posterior margin of tergite II armed with 1-2 rows of orally projecting hooks, which occupy about 80% of segment width. Anteromedian patches of spines present on tergites III-V (Figs 39, 41), laterally extensive and becoming diamond-like to nearly semi-circular on tergite VI (Figs. 39, 41). Posteromedian transverse patches of spines present on tergites III-VI (Figs 39, 41) not interrupted medially, gradually more extensive laterally and almost reaching muscles marks, size of spines mostly similar. Posteromedian transverse rows of orally projecting spines (Figs 39, 41) restricted to tergites III-V, occupying 75 to 80% of segments width. Pedes spurii B absent. Pedes spurii A present on sternites IV-VI. Armament absent on sternites I-VIII; field of shagreen and points present on anteromedian area of sternites III-VI; posterior transverse rows of spinules present on sternites V-VI, occasionally with a distinct median patch of small spines on sternite VI (Figs 39, 43). Apophyses markedly distinct on tergites I/II-VIII (Figs 28, 80). Anal segment (Figs 40, 42) 200-220 µm long and 300 µm maximum



Figures 31-44. *Cricotopus* spp., male pupal exuviae. *C. latellai* sp. n.: 31, frontal apotome; 32, cephalothorax, chaetotaxy and granulation; 33-35, thoracic horn, three aspects; 34, thoracic horn, details of apical part; 36, cephalothorax, details of anteromedian area. *C. mantetanus*: 37, cephalothorax with dorsocentrals; 38, thoracic horn. *C. latellai* sp. n.: 39, armament and chaetotaxy of abdominal segments V-VII with details of the hook rows on tergites (left) and sternites (right); 40, lateral view of segments VII-VIII and anal segment; 41, armament of tergites V-VI (dorsal view) with median patch of spines and details of the hook rows; 42, anal lobe, right half, dorsal; 43, ventral view of sternites V (distal part) and VI (posteromedian patch of spines); *C. mantetanus*: 44, anal lobe, right half, dorsal.

width, slightly narrowing distally, apical area with small spines extending well above insertion of anal macrosetae. Macrosetae about 100 μm long, stout, pin-shaped and slightly curved apically. Genital sac (Fig. 42) 180-190 μm long, narrowed apically and overreaching apical margin of anal lobe by 65-70 μm .

Final instar larvae

(n = 3, Figs 45-49); 2 of the 3 examined larvae were attached to a pharate pupa.

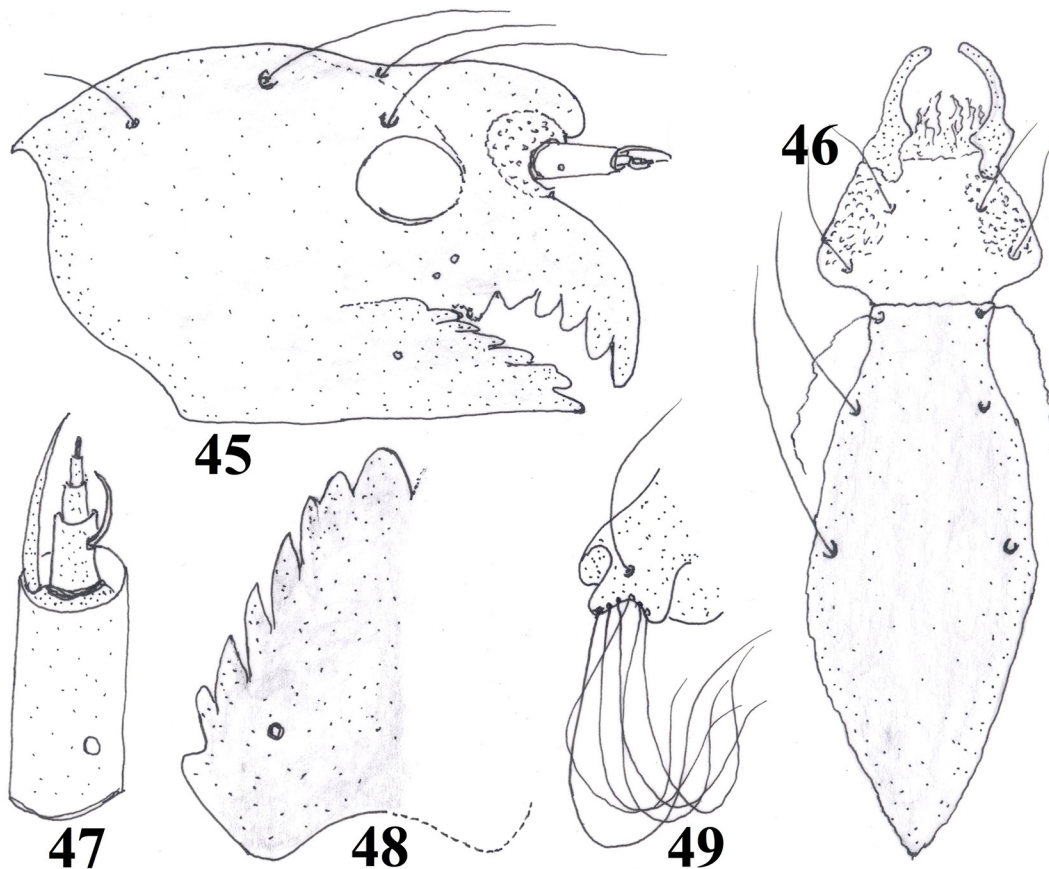
Total length 3.90-4.00 mm, maximum width 0.5-0.6 mm. Colouration contrasting pale greenish to blackish. Head (Fig. 45) blackish with a markedly transparent circular area (clearly visible in lateral view) placed on each side below base of antenna; proximal part of head capsule contrasting brown to dark brown including antennae and epipharyngeal region; mentum totally blackish; anteromedian area of clypeus densely covered with granulation. Thorax, abdomen and anal segment greenish.

Head illustrated in lateral view (Fig. 45). Eye spots broadly circular; clypeus (Fig. 46) nearly trap-

ezoidal, densely granulate on lateral parts; frontal apotome (Fig. 46) 370 μm long, with 3 pairs of lateral setae, median and distal setae are setae-like and about 200 μm long, proximal setae are bristle-like and shorter (about 90 μm long). Antenna (Fig. 47) 5-segmented, 95 μm long; basal segment about 55 μm long and 20 μm maximum width, AR 1.35-1.45; ring organ placed about $\frac{1}{4}$ distance from base of antenna, accessory blade markedly overreaching fifth segment as in *C. storozhenkoi* (Makarchenko & Makarchenko 2016, Fig. 27). Mandible (Fig. 45) with 6-7 teeth, apical tooth nearly as long as combined length of two first lateral teeth. Mentum (Figs 45, 48) composed of 1 median large tooth and 5-6 pairs of lateral teeth, apical pair of teeth smooth and domed, first and second lateral teeth distinctly fused at base. Procercus (Fig. 49) bilobed distally with 6 long setae, dorsal seta about 100 μm long.

Taxonomic position

C. latellai sp. n. can be separated from its morphologically most similar European species *C. mantetanus* by a combination of characters.



Figures 45-49. Larva of *Cricotopus latellai* sp. n. 45, head, right side, lateral view; 46, head capsule, dorsal; 47, antenna; 48, mentum, half part; 49, procercus with dorsal and anal setae, lateral view.

In the male adult: median area of tergites III-IV each with 3 distal setae (Fig. 4), while there is 4 setae (2 anterior and 2 posterior) in *C. mantetanus* (Fig. 6); tergite IX differently shaped in lateral and dorsal view (Figs 2-3) than in *C. mantetanus* (Fig. 5).

In the pupal exuviae: granulation on cephalothorax covering the entire anteromedian area and thoracic suture (Fig. 32), is differently figured in *C. mantetanus* (Fig. 37); dorsocentrals are all setae-like; thoracic horn foliate to ellipsoidal and narrowed distally (Figs 33-34); posteromedian area of sternites VI with a patch of small spines (Fig. 43).

Additional remarks

According to the previously provided key for known male adults and pupal exuviae from the Tyrrhenian Region (Moubayed-Breil 2016), *C. latellai* sp. n. keys near *C. mantetanus* and *C. storozhenkoi* based on the following combination of characters:

outer margin of inferior volsella is rounded for *C. latellai* sp. n. and *C. storozhenkoi*,

shape of the thoracic horn for *C. latellai* sp. n. and *C. mantetanus*, while a nearly similar shape of anal lobe is observed in both *C. latellai* sp. n. and *C. mantetanus*.

Consequently, the main differentiating morphological features found in the male adults and pupal exuviae of the *tremulus*-group can be supplemented based on the combination of characters summarized in the following key of known species from the Tyrrhenian Region.

Male adults

1. Inferior volsella pointing downwards at an acute angle, narrow and finger-like (Moubayed-Breil 2016, Fig. 47; Casas & Vilchez-Quero 1992, Figs 1A-B), outer margin not gradually or abruptly bent downwards *C. nevadensis* (Spain, Portugal)

- Inferior volsella not pointing downwards at an acute angle, not narrow and finger-like, outer margin gradually (Figs 7, 9) or abruptly (Fig. 12) bent downwards 2

2. Outer margin of inferior volsella abruptly bent downwards medially at a right angle and with 2 strong dorsal setae on outer edge (Fig. 12; Moubayed-Breil 2016, Figs 6, 8-9) *C. mantetanus* (France)

- Outer margin of inferior volsella rounded and bent gradually downwards, lacking strong dorsal setae on outer edge (Figs 7, 9; Moubayed-Breil 2016, Figs 43, 45) 3

3. Gonostylus with large, broad, apically rounded, strongly projecting crista dorsalis (Figs 10-11); median area of tergites III-IV each with 3 setae placed distally (Fig. 4) *C. latellai* (France, Italy)

- Gonostylus not as described above, with small crista dorsalis (Moubayed-Breil 2016, Figs 11, 43); median area of tergites III-IV each with 5 setae (2 proximal, 3 distal) (Moubayed-Breil 2016, Fig. 5) or tergites III and IV respectively with 4 setae (1 proximal, 3 distal) and 5 setae (2 proximal, 3 distal) (Moubayed-Breil 2016, Fig. 41) 4

4. Gonostylus with rounded posterior margin, crista dorsalis short, tooth-like with rounded apex (Moubayed-Breil 2016, Fig. 11); median area of tergites III-IV with 5 dorsal setae (2 proximal, 3 distal) (Moubayed-Breil 2016, Fig. 5) *C. tremulus* (widespread Holarctic species)

- Gonostylus with sinuous posterior margin, crista dorsalis tooth-like with pointed apex (Moubayed-Breil 2016, Fig. 43); median area of tergite III with 4 setae (1 proximal, 3 distal) and tergite IV with 5 setae (2 proximal, 3 distal) (Moubayed-Breil 2016, Fig. 41)..... *C. royanus* (France, Italy)

Pupal exuviae

1. Thoracic horn over three times as long as broad, elongated to lobe-like, densely to weakly toothed distally on one side or entirely smooth; median patch of spines on tergite VI diamond-like or spinning-top-like; anal lobe broadly rounded apically, densely covered with rows of small spines placed near the posterior margin 2

- Thoracic horn about twice as long as broad, lobe-like or foliate to ellipse-like, entirely smooth or distinctly toothed distally and apically; median patch of spines on tergite VI rounded or nearly semi-circular; anal lobe narrowing distally (Fig. 42), weakly to moderately covered with small spines (Figs 42, 44) 3

2. Granulation on cephalothorax only covering the anterior part of thoracic suture, sparsely covering the anteromedian area; thoracic horn moderately toothed medially, distally and apically on one side; median patch of spines on tergite VI spinning top-like (Moubayed-Breil 2016, Fig. 77) *C. royanus*

- Granulation on cephalothorax sparse and restricted to the anteromedian area close to the thoracic suture; thoracic horn smooth or only toothed apically, occasionally toothed pre-apically on one side; median patch of spines on tergite VI sub-oval to nearly diamond-like *C. nevadensis*

3. Granulation on cephalothorax restricted to the anteromedian area located close to the thoracic suture; thoracic horn balloon-like and entirely smooth (Hirvenoja, 1973, Fig. 113.3); *C. tremulus*

- Granulation on cephalothorax covering at least the anteromedian area and the thoracic suture (Figs 32, 37); thoracic horn foliate to ellipsoidal in shaped and bearing teeth at least apically (Figs 33-35, 38)..... 4

4. Granulation on cephalothorax covering the anteromedian area and thoracic suture but not reaching the Dc_3 - Dc_4 zone (Fig. 37); thoracic horn ellipse shaped (Fig. 38), toothed apically and medially on one side; median patch of spines on tergite VI diamond-like; anal lobe weakly covered with small spines (Fig. 44) *C. mantetanus*

- Granulation on cephalothorax densely covering the antero- and posteromedian area and the thoracic suture, reaching the Dc_3 - Dc_4 zone (Fig. 32); thoracic horn foliate to ellipsoidal (Figs 33-35) with narrowing distal part, apex of teeth are often smooth (Fig. 34); median patch of spines on tergite

VI diamond-like to semi-circular (Fig. 41); anal lobe moderately covered with small spines (Fig. 42)..... *C. latellai*

Ecology and geographical distribution

Localities where the larval, imaginal and pupal material of *C. latellai* sp. n. were collected consist of pristine epirhithral sections located in middle and high sectors of glacial streams (upper basin of the River Po, northwestern Italy, Fig. 50). Environmental data of water are: crystalline to moderately calcareous water, conductivity (about 20-30 μ S/cm for dolomitic areas of the upstream of the Po River; 95-100 μ S/cm for the calcareous upstream of the Casterino stream); temperature 8-12 °C during late spring and summer. Emergence is observed in June and July but apparently extends to late summer. Geographical distribution (Fig. 51) is delimited by the eastern limit of the Tyrrhenian Region, which covers both the Italian and French Maritime Alps. Moreover, *C. latellai* sp. n. belongs to a relict glacial element, which is believed to characterize the Tyrrhenian continental Province where other Tyrrhenian elements have previously been documented by Moubayed-Breil & Ashe (2016) and



Figure 50. Riffles and waterfalls (altitude 1700-1800 m) delimited by the upper basin of the River Po at ‘Pian del Re’, Alpi Marittime, north-western Italy. Photo J. Moubayed-Breil, 09.07.2017.

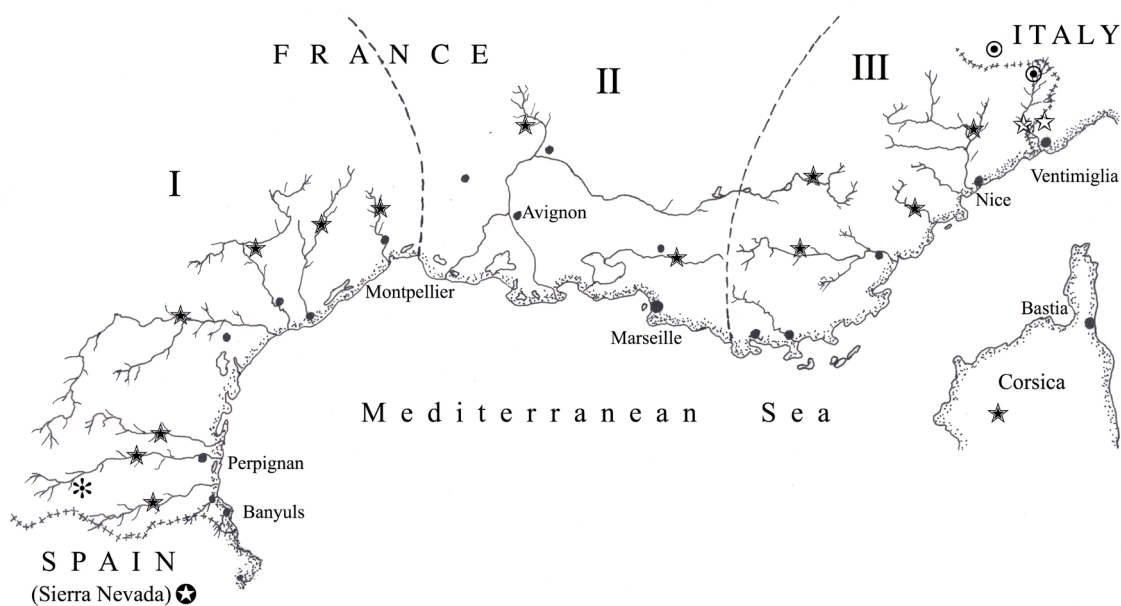


Figure 51. Geographical distribution of the five currently known *Cricotopus* species (*tremulus*-group) in the Tyrrhenian Region delimited by continental France, Italy, Spain and Corsica: *C. latellai* sp. n. ●, *C. mantetanus* *, *C. nevadensis* ⊕, *C. royanus* △, *C. tremulus* ★.

Moubayed-Breil & Orsini (2016). This highlights the importance of some high local glacial mountain ranges in the Mediterranean Region where constructive plans for conservation and preservation of autochthonous glacial relic species must be implemented. Such relic species are considered as biogeographically representative and biological indicators of global warming and climate change.

Associated species in the same habitat to *C. latellai* sp. n. include: *Boreoheptagya legeri* (Goetghebuer, 1933); *Diamesa cinerella* (Meigen, 1835); *D. hamaticornis* Kieffer, 1924; *D. latitarsis* (Goetghebuer, 1921); *D. thomasi* Serra-Tosio, 1970; *D. tonsa* (Haliday, 1856); *D. zernyi* Edwards, 1933; *Pseudodiamesa branickii* (Nowicki, 1873); *P. nivosa* (Goetghebuer, 1928); *Cricotopus* (*Paratrichocladiua*) *spiesi* (Ashe & O'Connor, 2013); *Eukiefferiella ilkleyensis* (Edwards, 1929); *E. fittkaui* Lehmann, 1970; *E. minor* (Edwards, 1929); *Krenosmittia camptophleps* (Edwards, 1929); *Limnophyes gelasinus* Sæther, 1970; *Orthocladus ruffoi* Rossaro & Prato, 1991; *Pseudorthocladus curtistylus* (Goetghebuer, 1921); *Thienemannia corsicana* Moubayed-Breil, 2013; *T. gracilis* Kieffer, 1909; *T. spiesi* Moubayed-Breil & Ashe, 2016 and *Tvetenia bavarica* (Goetghebuer, 1934).

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ed in the discovery and description of *Cricotopus latellai* sp. n. Special thanks are also due to staff of the Maritime Italian Park for providing the authorization to collect material in the protected area of 'Parco del Alpi Marittime'.

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A NEW SPECIES IN THE *RHEOCRICOTOPUS (R.) EFFUSUS* GROUP FROM CANADA WITH A REVIEW OF THE NEARCTIC SPECIES OF *RHEOCRICOTOPUS* AND *PARAMETRIOCNEMUS* (CHIRONOMIDAE: ORTHOCLADIINAE)

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<http://zoobank.org/urn:lsid:zoobank.org:pub:3625C1D8-40F1-44D9-8571-97C481739253>

Abstract

Rheocricotopus (Rheocricotopus) reduncusoides sp. n. is described from Bathurst Island, Nunavut, Canada. Its discovery was made while curating and examining specimens deposited by H. V. Danks in 1969 at the Canadian National Collection in Ottawa, Canada. We also report the first Canadian record of *Parametricnemus hamatus* (Johannsen, 1934) from Québec, based on previously unsorted material deposited at the Canadian National Collection. In this paper we provide distributional records of the Nearctic species of *Rheocricotopus* Thienemann and Harnisch and *Parametricnemus* Goetghebuer, and present a key to the Nearctic adult males of these genera.

Introduction

While curating mounted specimens of Chironomidae at the Canadian National Collection of Insects, Arachnids and Nematodes (CNC), we encountered six specimens that had been placed into the wrong genus. Five of these were adult males from materials previously collected during 1969 Arctic expedition by H.V. Danks and J. R. Byers to Bathurst Island, Nunavut, Canada (Danks and Byers 1972). The Chironomidae from this survey were deposited at CNC for further identification. The original examination of the Bathurst Island material (Danks and Byers 1972) identified 30 species (21 Orthoclaadiinae) across 18 genera. Overall close to 10,000 Chironomidae specimens were collected (Danks and Byers 1972).

Subsequent publications and descriptions were produced from this expedition (Danks 1971, 1980, 1981, Danks and Oliver 1972a, 1972b, Oliver and Danks 1972, personal communication with Dr. H. V. Danks). In spite of this prodigious publishing output that arose from the 1969 Arctic Expedition, specimens remained that required further investigation.

In this paper we describe *Rheocricotopus (Rheocricotopus) reduncusoides* sp. n. a new species in *Rheocricotopus (R.) effusus* group from Nunavut, Canada based on five of these mounted specimens. We also report on a single mounted specimen of *Parametricnemus hamatus* (Johannsen, 1934) collected by D.R. Oliver in Quebec, 1968. Because there are no geographic coordinates associated with this latter specimen (i.e. only province of Quebec is on the label) the exact locality of the collection remains unknown. Nevertheless, this is a new geographic record for Canada. *Parametricnemus hamatus* is only reported in eastern and southeastern USA. In this study we provided additional morphological information for Chironomidae species, a list of species from the collection, and a key to adult male for the Nearctic.

Study area and Methods

In the original field study (Danks and Byers 1972) arthropods were collected within a radius of 4.8 km of a campsite at 75° 43' 00" N, 98° 25' 00" W. The site was located about halfway between the paired inlets of the west and east coast which characterize south-central Bathurst Island. Dry ridges and their upper slopes formed rather typical *Saxifraga* barrens. South of the ridges there was an extensive sedge marsh with numerous shallow ponds and two shallow lakes. The northern part of marsh is adjacent to the Goodsir River which runs southwards before turning eastwards towards Goodsir Inlet (Danks and Byers 1972).

Pan traps were used to collect arthropods both on ridges and in the marsh. Additionally, drift nets were used in several creeks. Further sampling was conducted by searching amongst vegetation, beneath stones, and around carrion by sweeping, especially in the marsh habitat (Danks and Byers 1972). There are no associated reports for the collection of *P. hamatus* in Quebec.

Geographical records are based on Ashe and O'Connor (2012) with additional records mined from the Barcode of Life Data Systems (BOLD Systems), Namayandeh (2016), Namayandeh and Culp (2016) and Namayandeh et al (2012, 2016). We made type specimens of the mounted materials along with new identification labels and returned all the loaned specimens to the CNC. Images were obtained by OMAX A3550U Camera mounted on AMScope compound scope.

In this paper, we use the terminology and abbreviations as defined by Sæther (1980).

***Parametrioctenus* and *Rheocricotopus* species recorded in North America**

Parametrioctenus Goetghebuer consist of 35 named and described species worldwide (Ashe and O'Connor 2012). There are at least seven provisional species that have no formal names, six occurring in the Neotropical region and one in the Oriental region (Ashe and O'Connor 2012). The highest diversity of the genus is in the Palearctic with 21 species with three new records from Palaearctic China including *P. lundbeckii*. Palearctic is a relatively well-studied region and therefore, this high diversity of the species is to be expected. The more northern parts of the Nearctic (NE) are relatively understudied region with only four species listed in the catalogue (Ashe and O'Connor 2012) and one added in this study. Given the cold-stenothermic nature of most *Parametrioctenus* species, finding a new species and/or new geographic records in the northern Nearctic is likely.

Rheocricotopus Thienemann and Harnisch, in comparison, is a diverse and well-studied genus with 72 named species worldwide (Ashe and O'Connor 2012, Moubayed-Breil 2016). So far 44 species have been described or reported from the Palearctic (Ashe and O'Connor 2012, Krasheninnikov and Loskutova 2015, Liu et al. 2014a, b, Moubayed-Breil 2016, Yamamoto and Yamamoto 2017) and 13 from the Nearctic, including the present study. Published records of *Rheocricotopus* species from the Holarctic indicates seven species occur in both the Nearctic and Palearctic regions with more expected overlapping. This is especially true for those species from Far East Russia (Makarchenko and Makarchenko 2005). However, extent of this overlapping needs a detailed investigation. Recent records (BOLD Systems) have revealed that *Rheocricotopus* (*Psilocricotopus*) *chalybeatus* (Edwards 1929), previously only known from the Palearctic also occur in the Nearctic. Furthermore, the occurrence of several species in both the eastern and western Nearctic (BOLD Sys-

tems, Namayandeh 2016, Namayandeh et al. 2016, Namayandeh and Culp 2016) suggest that many species likely have a widespread distribution.

***Parametrioctenus* Goetghebuer**

***Parametrioctenus boreoalpinus* Gowin and Thienemann, 1942**

Adults: Male and female described by Gowin and Thienemann (1942).

Immatures: Pupa and larva Gowin and Thienemann (1942) and Kownacka and Kownacki (1967).

Ecology and Habitat: Larvae inhabit the stony banks of high-altitude streams (Kownacka and Kownacki 1967).

NE: Canada (Alberta, British Columbia, Labrador, Northwest Territories, Nunavut). Widespread in the Palearctic.

***Parametrioctenus eoelivus* Sæther, 1969**

Adults: Male and female described by Sæther (1969).

Immatures: Pupa and larva described by Sæther (1969).

Ecology and Habitat: Larvae inhabit lotic habitats (Sæther 1969).

NE: Canada (Québec); USA (North Carolina, Tennessee). In Palearctic, only recorded in Italy (dubious record).

***Parametrioctenus graminicola* (Lundbeck, 1898)**

Adults: Male described by Sæther (1969) and Sublette (1966). Female is unknown.

Immatures: Unknown.

Ecology and Habitat: Adults collected near lentic habitats (Sæther 1969).

NE: Canada (Alberta, Northwest Territories, Yukon Territory); Greenland. In the Palearctic, recorded only in Far East Russia.

***Parametrioctenus hamatus* (Johannsen, 1934)**

Adults: Male described by Sublette (1967) as *Paraphaenocladus hamatus* (Johannsen 1934). Female is unknown.

Immatures: Unknown. Apparently, the larva of this species was reared by M. J. Bolton. However, no records of larval description are available.

Ecology and Habitat: Adults collected near springs and runs of Cedar Bog, an alkaline fen in Ohio, USA (Bolton 1992).

NE: Canada, first record (Québec); USA (Connecticut, Florida, Maine, New York, North Carolina, Ohio).

***Parametricnemus lundbeckii* (Johannsen, 1905)**

Adults: A detailed description of adults are given by Sæther (1969) and Sublette (1967).

Immatures: Pupa and larva described by Sæther (1969), and Namayandeh and Culp (2016). Larva described by Namayandeh et al. (2012), and Simpson and Bode (1980).

Ecology and Habitat: Larvae inhabit clean piedmont and mountain streams (McShaffrey and Olive 1985, Simpson and Bode 1980).

NE: Canada (Alberta, Northwest Territories, Ontario, Québec, Saskatchewan); USA (Alabama, Arizona, California, Florida, Georgia, Michigan, New Mexico, New York, North Carolina, Ohio, South Carolina, Texas). In the Palearctic, records were made by E. Stur and T. Ekrem (2010) in Norway as part of Barcode of Life, Centre for Biodiversity Genomics (Ratnasingham and Hebert 2007). Also, recently this species was reported from China by Li et al. (2013).

***Parametricnemus vespertinus* Sæther, 1969**

Adults: Male described by Sæther (1969). Female unknown.

Immatures: Pupa described by Sæther (1969). Larva unknown.

Ecology and Habitat: Larvae likely inhabit lotic habitats (Sæther 1969).

NE: Canada (Alberta).

Rheocricotopus Thienemann and Harnisch

***atripes* group:**

***Rheocricotopus (Psilocricotopus) glabricollis* (Meigen, 1830)**

Adults: Male described by Lehman (1969), Sæther (1985), Makarchenko and Makarchenko (2005), hypopygium in key by Pinder (1978). Female described by Sæther (1985).

Immatures: Pupa described by Sæther (1985), figs. in Lehman (1969) under *R. gouini*, and in key by Langton (1991). Larva described in Namayandeh (2016), and in key by Epler (2001).

Ecology and Habitat: Larvae inhabit lotic habitats (Hudson et al. 1990).

NE: Canada (Manitoba, New Brunswick); USA (Georgia, North Carolina, Ohio, Pennsylvania,

South Carolina, Tennessee). Widespread in the Palearctic.

***chalybeatus* group:**

***Rheocricotopus (Psilocricotopus) chalybeatus* (Edwards, 1929)**

Adults: Male described by Lehman (1969), Sæther (1985), hypopygium in key by Pinder (1978). Female described by Sæther (1985).

Immatures: Pupa described by Sæther (1985), in key by Langton (1991), figures in Lehman (1969). Larva by Cranston (1982).

Ecology and Habitat: Larvae inhabit springs and streams (Cranston 1982, Lehman 1971).

NE: Canada (Nunavut). Widespread in the Palearctic.

***Rheocricotopus (Psilocricotopus) chapmani* (Edwards, 1935)**

Adults: Male and female described by Sæther (1985).

Immatures: Unknown.

Ecology and Habitat: Adults collected near lentic and lotic habitats (Sæther 1985).

NE: Canada (Northwest Territories, Nunavut, Yukon Territory); Greenland. In the Palearctic, recorded in Finland and Norway.

***Rheocricotopus (Psilocricotopus) robacki* (Beck and Beck, 1964)**

Adults: Male described by (Beck and Beck 1964) as *Trichocladus robacki* and by Sæther (1969) as *R. kenorensis*. Female described by Sæther (1985).

Immatures: Pupa and larva described by Sæther (1985). Larva in key by Epler (2001).

Ecology and Habitat: Fast flowing streams (Sæther 1969).

NE: Canada (Alberta, British Columbia, Ontario, Saskatchewan, Yukon Territory); USA (Alabama, Arizona, California, Florida, Georgia, Mississippi, Montana, New York, North Carolina, Ohio, Pennsylvania, South Carolina, South Dakota, Tennessee). Recently reported from the Palaeartic China (Xinjiang Uyghur Auto. Region) by Liu et al. (2014a).

***effusus* group:**

***Rheocricotopus (Rheocricotopus) effusoides* Sæther, 1985**

Adults: Male and female described by Sæther (1985).

Immatures: Pupa and larva described by Sæther (1985).

Ecology and Habitat: Larvae inhabit lotic habitats (Sæther 1985).

NE: USA (Ohio, South Dakota).

Rheocricotopus (Rheocricotopus) effusus (Walker, 1856)

Adults: Male described by Albu (1968), Lehman (1969), Sæther (1985), Makarchenko and Makarchenko (2005), hypopygium in key by Pinder (1978). Female described by Sæther (1985).

Immatures: Pupa described by Sæther (1985), in key by Langton (1991). Larva described by Cranston (1982), Sæther (1985), and in key by Epler (2001).

Ecology and Habitat: Larvae mainly inhabit springs (Thienemann 1954).

NE: Canada (Alberta, Northwest Territories); USA (Illinois, North Carolina, South Carolina, South Dakota). Widespread in the Palearctic.

Rheocricotopus (Rheocricotopus) pauciseta Sæther, 1969

Adults: Male described by Sæther (1969), and Makarchenko and Makarchenko (2005). Female is unknown.

Immatures: Pupa and larva described by Sæther (1969) and in key by Epler (2001).

Ecology and Habitat: Lotic habitats (Hudson et al. 1990).

NE: Canada (Alberta, British Columbia, Labrador); USA (North Carolina). In the Palearctic Far East Russia, recently from China (Sichuan Province), Tibet by Liu et al. (2014b).

Rheocricotopus (Rheocricotopus) reduncusoides sp. n.

Adults: Male described in this study. Female is unknown.

Immatures: Unknown.

Ecology and Habitat: Specimens were collected probably near creeks close to the marshland in Barthurst Island (Danks and Byers 1972).

NE: Canada (Nunavut).

Rheocricotopus (Rheocricotopus) unidentatus Sæther and Schnell, 1988

Adults: Male and female described by Sæther and Schnell (1988).

Immatures: Pupae and larva described by Sæther

and Schnell (1988). Larva in key by Epler (2001) and described by Namayandeh et al. (2012).

Ecology and Habitat: Larvae inhabit streams and springs (Sæther and Schnell 1988). Namayandeh et al. (2012) collected the larvae from leaf litter in headwater streams located on the Precambrian Shield.

NE: Canada (Ontario); USA (North Carolina, Ohio).

fuscipes group:

Rheocricotopus (Rheocricotopus) amplicristatus Sæther, 1985

Adults: Male and female described by Sæther (1985).

Immatures: Unknown.

Ecology and Habitat: Adults collected near creeks (Sæther 1985).

NE: USA (Georgia, South Carolina).

Rheocricotopus (Rheocricotopus) eminellobus Sæther, 1969

Adults: Male described by Sæther (1969), and Makarchenko and Makarchenko (2005). Female described by Sæther (1969).

Immatures: Pupa described by Sæther (1969). Larva described by Sæther (1985), in key by Epler (2001), and Namayandeh et al. (2012, 2016).

Ecology and Habitat: Larvae are shredders and inhabit leaf litter in running waters (Namayandeh et al. 2012).

NE: Canada (Alberta, Labrador, Ontario); USA (North Carolina, Ohio, South Carolina, Tennessee). In the Palearctic, found only in the Far East Russia.

godavarius group:

Rheocricotopus (Psilocricotopus) conflusirus Sæther, 1985

Adults: Male described by Sæther (1985). Female is unknown.

Immatures: Unknown.

Ecology and Habitat: Adults collected near reservoirs (Sæther 1985).

NE: USA (South Carolina).

tuberculatus group:

Rheocricotopus (Rheocricotopus) tuberculatus Caldwell, 1984

Adults: Male and female described by Caldwell (1984) and Sæther (1985).

Immatures: Pupae described by Caldwell (1984) and Sæther (1985). Larva by Caldwell (1984), Namayandeh et al. (2012), and in key by Epler (2001).

Ecology and Habitat: In Georgia and North Carolina larvae occurred in second and third order piedmont streams feeding on detritus and diatoms (Caldwell, 1984). Namayandeh et al. (2012) collected the larvae from leaf litter in headwater streams located on the Precambrian Shield.

NE: Canada (Ontario); USA (Florida, Georgia, North Carolina, South Carolina, Tennessee).

Descriptions

***Parametricnemus hamatus* (Johannsen, 1934)**

(Figs 1a–e)

Material examined. *Parametricnemus* (1 ♂), Old Man Joe Mt. (No such location was found in Québec), Québec, 26 April 1968, Collected by D. R. Oliver, No. CH 767

Diagnostic characters. Virga with 2–3 long narrow branches. AnP with inflated basal 1/2 to 2/3 with 4 stout setae on each side. IVo narrow and apically hooked. Gc with large projecting CD.

Male (n=1). Total length = 3.2 mm

Coloration of slide-mounted specimen: Male head and thorax light brown, abdomen golden-brown, halter hyaline to slightly golden, and wing golden-brown.

Head. Antenna (Fig. 1a), with 13 flagellomeres, ultimate flagellomere (L = 606 µm) with apical setae (L = 35 µm); AR = 1.3. Eyes bare, with parallel-sided dorsomedial extension (Fig. 1b). Tentorium L = 147 µm (Fig. 1b). Four coronal setae, six orbitals in single row on each side, Clypeus squared (L = 64 µm, W = 68 µm), bearing nine setae. Palpomeres p₁₋₅, lengths (µm): 36, 42, 141, 143, 207; p₃ with 2 sensilla chaetica.

Thorax. As in Fig. 1c. Antropronotals 11, uniserial; prealars 6, uniserial. Scutum with six setae in single row.

Wing. Wing with fine punctation and hairs covering most of membrane (Fig. 1d). L = 2.1 mm, W =

0.6 mm. R with 24 setae, R₁ with 15, R₄₊₅ with 60 setae. Costa extends pass R₄₊₅. R₄₊₅ distal to M₃₊₄. Cu curved.

Legs. Fore legs with two spurs (Ls = 42 µm, 30 µm), mid tibia with two spurs (Ls = 34 µm, 24 µm) and hind tibia with 2 spurs (Ls = 59 µm, 19 µm) and comb with 12–13 stout setae. Pseudospurs are absent. Mid and hind femurs with keel. Pulvilli reduced. Lengths and proportions of legs in Table 1.

Hypopygium. As in Fig. 1e. Segment IX bilobed. Virga present with 2–3 long narrow branches. Anal point L = 61 µm. Superior volsella tapered. Inferior volsella narrow and apically hooked-shaped. Gonocoxite longer than wide (L = 203 µm, W = 85 µm) with cluster of long setae medially just below the inferior volsella. Gonostyle more or less rectangular; crista dorsalis large triangular-shaped and strongly projecting above dorsal margin; megaseta L = 13 µm. HR = 3.8, HV = 2.4.

Remarks. The species was first described by Johannsen, 1934 as *Metriocnemus hamatus* and indicated its close relationship with *Meteriocnemus lundbecki* Johannsen differing from the latter only in coloration and hypopygium. Sublette (1967) re-described the species as *Paraphaenocladus hamatus* (Joh.) with illustration of the male hypopygium.

Key to the known Nearctic adult male *Parametricnemus* Goetghebuer

Abbreviations: AnP = Anal Point; CD = Crista Dorsalis; Gc = Gonocoxite; Gs = Gonostyle; IVo = Inferior Volsella; SVo = Superior Volsella; T = Tergite.

1a. TIX with AnP short not reaching the IVo (Sæther 1969, fig.62)...***P. grammicola* (Lundbeck)**

1b. TIX with AnP longer, reaching the IVo.....**2**

2a. IVo narrow, finger-like with hooked apex (Fig. 1e; Sublette 1967, fig. 35).....***P. hamatus* (Johannsen)**

2b. IVo with broad base and rounded apex.....**3**

3a. AnP very long reaching beyond IVo; CD en-

Table 1. Male leg lengths (µm) and proportions of *Parametricnemus hamatus*.

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
P₁	851	898	733	406	281	191	99	0.82	2.5	2.4	2.4
P₂	826	756	446	218	155	102	88	0.59	3.6	3.6	2.7
P₃	835	819	522	252	170	113	94	0.64	3.5	3.2	4.4

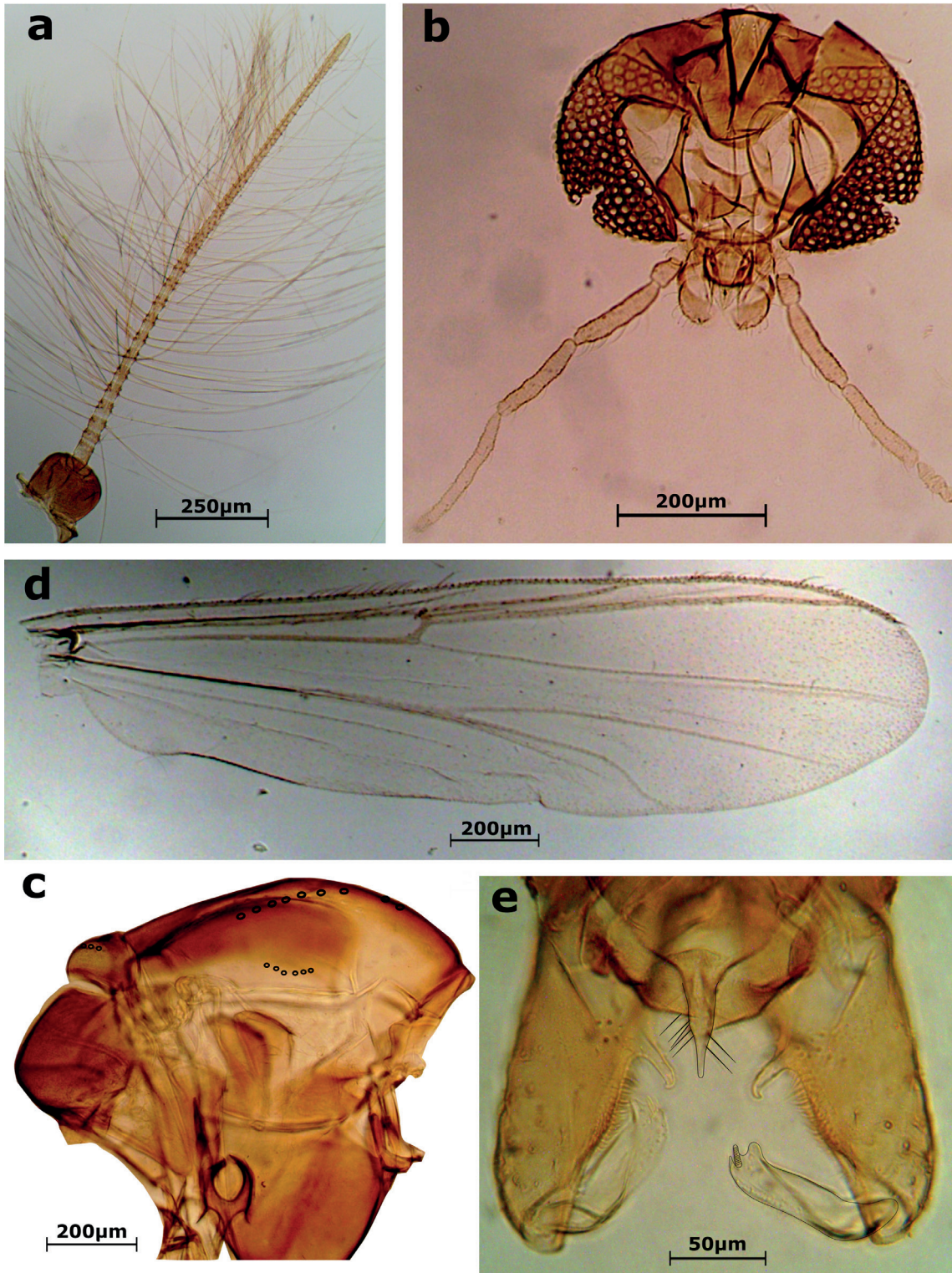


Figure 1. *Parametrioctenemus hamatus* (Johannsen) male. a) antenna, b) head, c) thorax lateral view, d) wing, e) hypopygium.

larged, broadly projecting above the inner margin of Gs (Sæther 1969, fig. 65).....

.....*P. vespertinus* Sæther

3b. AnP short, reaching only as far as IVo; CD either inconspicuous or present as small preapical to oth.....**4**

4a. IVo with a broad basal attachment about half the length of Gc. AnP with weak lateral setae (Sæther 1969, fig. 63; Sublette 1967, fig. 33).....
.....*P. lundbeckii* (Johannsen)*

4b. IVo with narrow basal attachment about quarter length of Gc. AnP with bristle-like setae.....**5**

5a. Gs angular distally with dorsal ridge ending in small sharply pointed CD (Sæther 1969, fig. 58)...
.....*P. eoelivus* Sæther

5b. Gs somewhat rounded distally with weak dorsal margin merging with poorly defined CD (Gowin and Thienemann 1942, fig. 2).....
.....*P. boreoalpinus* Gowin and Thienemann

*Will also key to *Parametriocnemus stylatus* (Spärck, 1923), a Palearctic species. *Parametriocnemus lundbeckii* and *P. stylatus* closely resemble each other. The two species can partially be separated based on the characters of gonostyle. The gonostyle of *P. stylatus* is much narrower and tube-shape. It also lacks the prominent crista dorsalis present in most variations of *P. lundbeckii*. Sæther (1969) suggested that the synonymy of both species cannot be excluded. However, the two species can be separated based on partial COI DNA sequences (DNA barcodes).

***Rheocricotopus (Rheocricotopus) reduncusoides* sp. n.**

<http://zoobank.org/urn:lsid:zoobank.org:act:BD1AEE59-F9A2-4EBB-9BB6-791096FFB959>

(Figs 2a–c & 3a–c)

Material examined. Holotype: 1 ♂ slide-mounted in Canada Balsam, Bathurst Island, Nunavut, 75° 43' N, 98° 25' W, 10 July 1969, Coll. H.V. Danks, CH 1214 (CNC). Paratypes: 4 ♂♂ slide-mounted in Canada Balsam, Bathurst Island, Nunavut, 75° 43' N, 98° 25' W, 10 July 1969, Coll. H.V. Danks,

CH 1214 (CNC). *Rheocricotopus (Rheocricotopus) reduncus* Sæther and Schnell, 1988: 3 Paratypes, ♂♂, Jostedøla River at Inlet to small lake, Luster, Sogn and Fjordane, Norway, 23/7' 86, A. Fjellheim and A. Schnell (ZMB NO. 116).

Etymology: From Latin, *reduncus* meaning curved or hooked backward which refers to the shape of caudomedian projection of superior volsella, and the epithet of the most closely related species, and New Latin *oides*, referring to the likeness of form.

Diagnostic characters. HP small to indistinct. SVo with long caudomedian projections evenly curved, medially projected and opposing each other, apex slightly pointed. Sternapodme broad horizontal band. IVo simple, small, blunt, slightly dilated at the apex. AR = 0.75. BR₁₋₃ = 1.4, 1.2, 1.4. AnP 67 µm long with 13–19 setae. Gs with slight bent distally, CD developed, long and low. HR = 1.6.

Male (n = 5). Total length = 3.1mm

Coloration of slide-mounted specimen: Head and thorax dark brown, abdomen golden-brown, halter light brown with dorsolaterals darker, and wing yellowish brown.

Head. As in Fig. 2a. Antenna (Fig. 2b) with 13 flagellomeres, ultimate flagellomere 332 µm long, AR = 0.72–0.79 (0.75). Tentorium L = 152 µm. 4 Coronal setae. Temporal setae consisting of: 3 postorbitals, 1 inner verticals, 2 outer verticals present. Clypeus wider than long (L = 119 µm, W = 132 µm), bearing six setae. Palpomere p₁₋₅ lengths (µm): 51, 55, 79, 88, 144; sensilla clavata difficult to see on 3rd palpomere.

Thorax. As in Fig. 2c. 7–8 anteprenotals. 4–5 (4) acrostichals close to anteprenotum (L = 11 µm). Dorsocentrals 7–9, uniserial (Ls = 57–69 µm). Prelars 2–3.

Wing. As in Fig. 3a. Wing with fine punctation. L = 2 mm, W = 0.5 mm. Squama with 4–5 setae, brachiolum with 1 seta, R with 5–6 setae, R₁ bare, R₄₊₅ with 2 setae. Costa slightly extends past R₄₊₅ (L = 30 µm).

Legs. Fore legs with tibial spur 41 µm long, mid tibial spurs 25 and 16 µ long, hind tibial spurs 44 and 23 µ long, comb with 15–17 setae. Pseudospurs

Table 2. Male leg lengths (µm) and proportions of *Rheocricotopus (R.) reduncusoides* sp. n.

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
P₁	671	738	459	304	227	128	108	0.6	2.4	3.1	1.4
P₂	702	666	320	189	145	82	94	0.5	3.3	4.3	1.2
P₃	690	773	393	221	182	92	92	0.5	3.2	3.7	1.4

are absent on t_1 and t_2 of mid and hind legs. Mid and hind femur with keel. Pulvilli well-developed, almost half as long as the claws. Lengths and proportions of legs in Table 2.

Hypopygium. As in Fig. 3b. Laterosternite IX with 6–7 setae ($L = 25 \mu\text{m}$). Phallapodme (59–66) $64 \mu\text{m}$ long; sternapodeme (90–105) $99 \mu\text{m}$ long. Anal point mostly hyaline, triangular with 13–19 setae, (56–89) $67 \mu\text{m}$ long. Superior volsella (Fig. 3b–c) with caudomedian projections long, finger-

like, curved evenly before meeting medially, $L = 84 \mu\text{m}$. Inferior volsella simple triangular, lobe with blunt tip and slightly dilated at apex, $L = 19 \mu\text{m}$. Gonocoxite $278 \mu\text{m}$ long. Gonostyle curved with slight bent distally (Fig. 3b), $L = 171 \mu\text{m}$. Crista dorsalis long and low, megaseta $L = 17 \mu\text{m}$. $HR = 1.6$, $HV = 1.8$.

Remarks. This species is closely related to the *Rheocricotopus* (*Rheocricotopus*) *reduncus*. Combination of lower AR, lower BR, higher number of

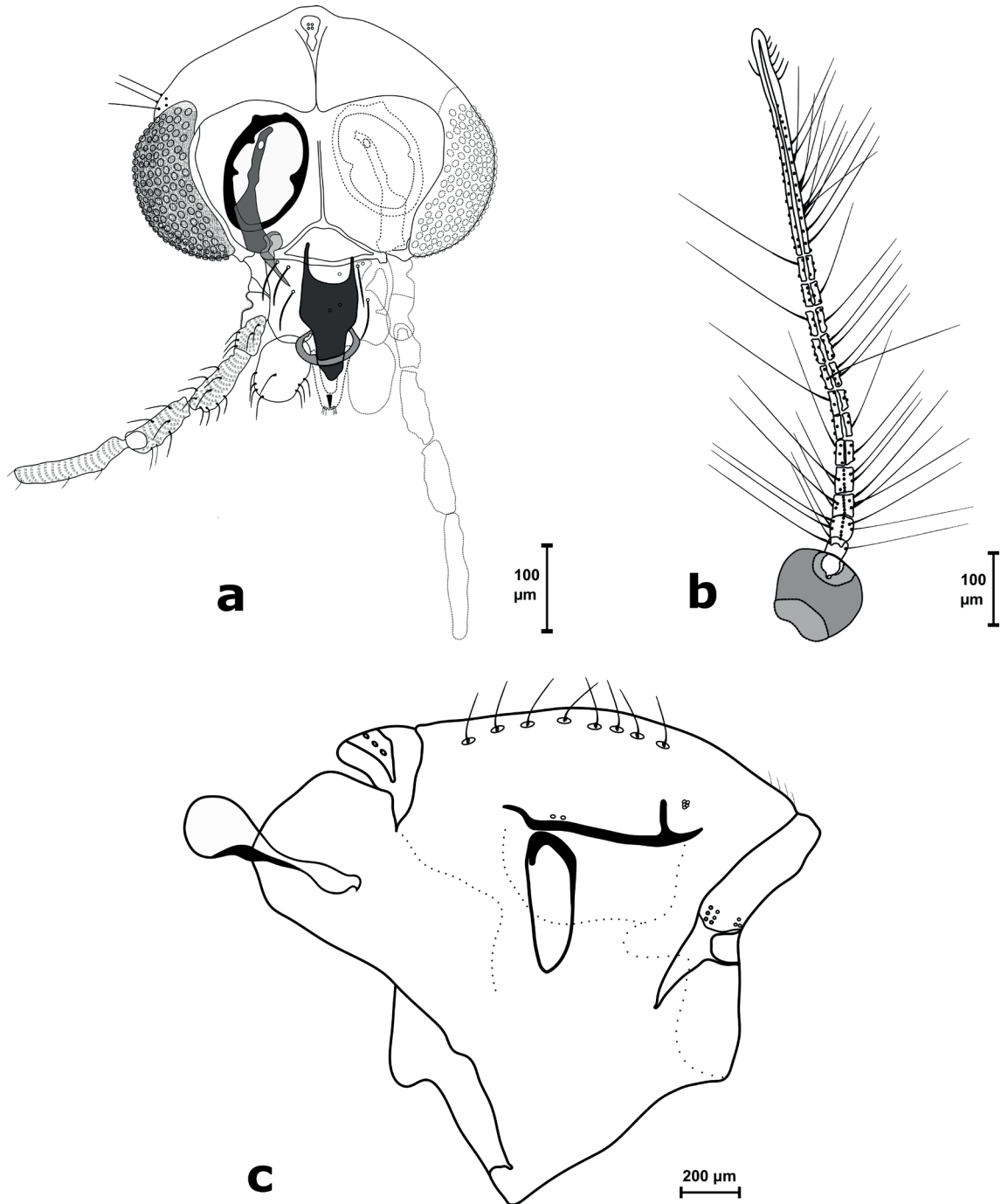


Figure 2. *Rheocricotopus* (*R.*) *reduncusoides* sp. n. male. a) head, b) antenna, c) thorax lateral view.

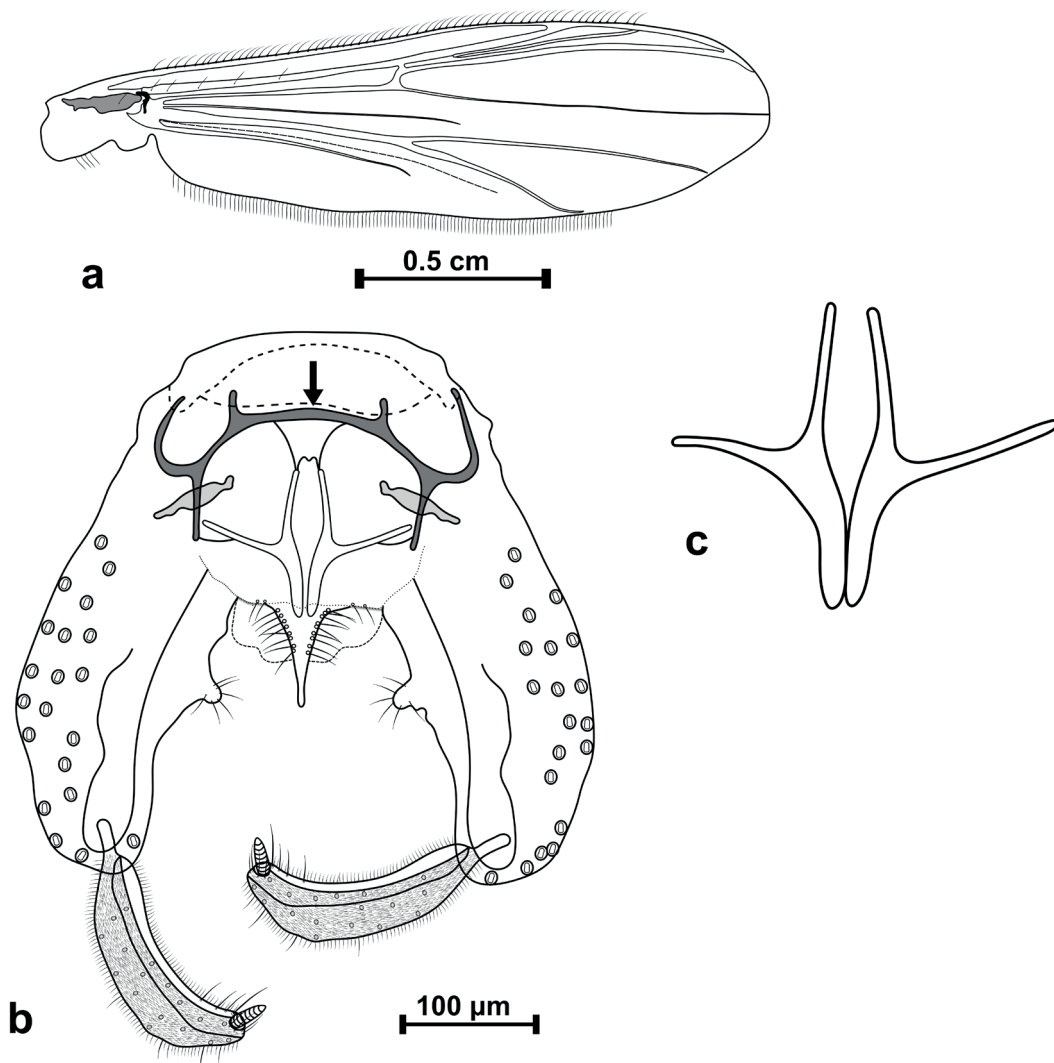


Figure 3. *Rheocricotopus (R.) reduncusoides* sp. n. male. a) wing, b) hypopygium, arrow indicates the sternapodeme, c) superior volsella caudomedian projection.

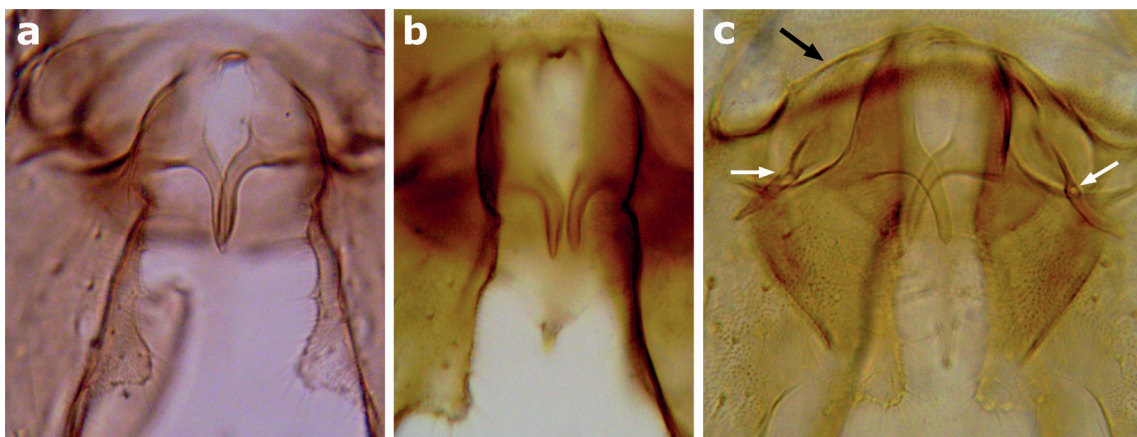


Figure 4. *Rheocricotopus (R.) reduncus* Sæther and Schnell, male paratypes from Norway. a) caudomedian projection and inferior volsella, b) variation of the caudomedian projection and inferior volsella, c) sternapodeme (black arrow) and phallapodeme (white arrows).

Table 3. Comparison of some members of *Rheocricotopus (R.) effusus* group's adult male. Modified from Sæther and Schnell (1988). Ac = Acrostichals, AnP = Anal Point; CD = Crista Dorsalis, Gs = Gonostyle, HP = Humeral Pit; IVo = Inferior Volsella; SVo = Superior Volsella, L = Lengths in μm .

Species	AR	No. Dc	No. Ac	HP	LR ₁	AnP L
<i>R. unidentatus</i>	1.10–1.36	18–22	30–36	Large ellipsoid	0.71	41–60
<i>R. effusus</i>	0.99–1.33	9–16	18–26	Large ellipsoid	0.70–0.74	<75
<i>R. effusoides</i>	1.39–1.69	12–16	14–21	Large ellipsoid	0.64–0.67	75–98
<i>R. pauciseta</i>	0.6–0.83	5–7	12–17	small	0.62–0.74	36–40
<i>R. reduncus</i>	0.83–1.17	7–12	3–9	small	0.62–0.67	38
<i>R. reduncusoides</i>	0.72–0.79	7–9	4–5	small	0.60–0.66	56–89

	AnP setae	HR	Stern-apodeme	SVo	IVo	Gs
<i>R. unidentatus</i>	8–12	2.42–2.50	Arched upward	Tooth-like caudomedian projection	Large, simple with pointed apex	Curved evenly
<i>R. effusus</i>	5–13	—	Arched upward	Tooth-like caudomedian projection	Large, simple with pointed apex	Curved evenly with low and long CD
<i>R. effusoides</i>	15–12	1.92–2.07	Arched upward	Tooth-like caudomedian projection	Large, simple with slightly hooked apex	Curved evenly with low and long CD
<i>R. pauciseta</i>	6–8	2.61–2.69	Arched upward	Triangular	Divided into 2 small lobes with dorsal one naked	Plump, broad at apex, without CD
<i>R. reduncus</i>	6–11	1.82–2.00	Narrow and arched upward	Mainly bent, short digitiform caudomedian projection, caudally appearing closely parallel to one another or overlapping each other	Small, simple with blunt tip. Evenly tapered at the apex	Long, curved evenly with very low CD that may appear absent
<i>R. reduncusoides</i>	12–19	1.50–1.80	Broad horizontal band	Evenly curved, long digitiform caudomedian projections medially projected and opposing each other	Small, simple, slightly dilated at the apex	Long, slightly bent distally, CD developed, long and low

setae on AnP, longer AnP, a more evenly curved caudomedian projection of SVo (Figs 3b–c; compared to mainly bent in *R. reduncus* Fig. 4a–b), apically dilated IVo, broad horizontal sternapodeme (Fig. 3b; compared to narrow arched in *R. reduncus*, Fig. 4c), lower HR and HV separates the two species.

Based on the form of SVo this species belongs to the *effusus* group. Given the similarity of *R. reduncus*, *R. reduncuoides* with other species within the *effusus* group a re-evaluation of the distinguishing characters is required to separate the species. In the *effusus* group the anal point length and thoracic chaetotaxy characters may not be sufficient to separate the species in this group. Sæther and Schnell (1988) provided a somewhat better alternative for separating species in this group by listing the main comparative characters in a table. Following their example, we modified and corrected some of these characters and added few more (Table 3).

A notable change to the characters given by Sæther and Schnell (1988) is the AR range of *R. reduncus*. The Norwegian specimens of *R. reduncus* described by Sæther and Schnell (1988) have AR 0.83–0.90 while Makarchenko and Makarchenko (2005) described the species from Far Eastern Russia with AR range of 1.16–1.17. This somewhat changes the state of characters given by Sæther (1985) and discussed by Sæther and Schnell (1988). If we are to consider Makarchenko and Makarchenko (2005) description of *R. reduncus* then the trend 8 of Sæther’s (1985) classification of the genus, describing a male AR of 0.6–0.8, does not longer hold for this species. Consequently, the characteristic of AR should no longer be considered apomorphic for *R. reduncus*. For 1st of trend 7 in Sæther’s (1985) both *R. reduncus* and *R. reduncusoides* are synapomorphous (digitiform caudomedian projection) and symplesiomorphous for second (with small humeral pit). The broad horizontal sternapodeme of *R. reduncusoides* is autapomorphic for this species.

Key to the known Nearctic adult male *Rheocricotopus* Thienemann and Harnisch (Modified from Sæther, 1985)

Abbreviations: AnP = Anal Point; CD = Crista Dorsalis; Gc = Gonocoxite; Gs = Gonostyle; HP = Humeral Pit; IVo = Inferior Volsella; SVo = Superior Volsella.

1a. Gs either with prominent preapical triangular CD or it’s bent distally, and CD is more apical. SVo broadly rounded, never with projection. Subgenus *Psilocricotopus*.....2

1b. Gs either without apparent CD or its long, low and rounded distally. SVo with or without caudomedian projection. Subgenus *Rheocricotopus*.....6

2a. HP of thorax small and indistinct. (Sæther 1985, fig. 2B). AR < 0.7.....*R. (P.) confusirus* Sæther

2b. HP of thorax large and distinct. AR > 0.7.....3

3a. HP very large, rectangular (Sæther 1985, fig. 11B). Gs is not bent upwards distally (Sæther 1985, fig. 11D).....*R. (P.) glabricollis* (Meigen)

3b. HP moderately large, ovoid or circular, if very large and rectangular then Gs is bent upwards distally.....4

4a. Gs with CD tooth-like located apically next to the megaseta (Lehmann 1969, fig. 1; Pinder 1978, fig. 38c).....*R. (P.) chalybeatus* (Edwards)

4b. Gs with triangular preapical CD distinctly separated from megaseta.....5

5a. Gs strongly bent distally (Sæther 1969, fig. 44) Costa not produced.....*R. (P.) robacki* (Beck and Beck)

5b. Gs not strongly bent (Sæther 1985, fig. 4D). Costa moderately produced (Sæther, 1985, fig. 4C)*R. (P.) chapmani* (Edwards)

6a. SVo with or without caudomedian projection.....7

6b. SVo broadly rounded without caudomedian projection.....9

7a. SVo triangular without distinct caudomedian projection (Sæther 1971, fig. 8D). IVo distally divided into two lobes. (Sæther 1969, fig.47).....*R. (R.) pauciseta* Sæther

7b. SVo with distinct caudomedian projection. IVo simple.....8

8a. HP small (Fig. 2c). SVo with long finger-like caudomedian projections that meet medially (Fig. 3b–c).....*R. (R.) reduncusoides* sp. n.*

8b. HP large. SVo conical with short tapered caudomedian projections (Sæther 1985, figs. 18b, d & e; Sæther and Schnell 1988, figs. 3b & d)*R. (R.) effusoides* (Walker), *R. (R.) effusus* Sæther, *R. (R.) unidentatus* Sæther and Schnell†

9a. CD weak to absent. AR = 0.66–0.97. Costa with definite extension $\geq 15 \mu\text{m}$10

9b. CD present as long distally rounded ridge (Sæther 1985, fig.16C). AR ≥ 1.0 . Costa extension barely indicated < 15 μm (Sæther 1985, fig.16B).....*R. (R.) amplicristatus* Sæther

10a. Gc with inner proximal margin bulges slightly before it meets the IVo that strongly projects medially (Sæther 1969, fig. 43; Makarchenko and Makarchenko 2005, fig. 16). Costal extension ~ 30 µm.....**R. (R.) eminellobus Sæther***

10b. Gc with inner proximal margin continuing straight where it meets the IVo which ends in a small posteriorly directed lobe. (Sæther 1985, fig.15C; Caldwell 1984, fig. 2). Costal extension 15–30 µm.....**R. (R.) tuberculatus Caldwell†**

*Will also key to *R. reduncus* Sæther and Schnell, a Palearctic species. See diagnosis and remarks under *R. reduncusoides*, and Table 3 to separate the two species.

†Theses three species in the *effusus* group are difficult to separate. See Table 3 for combination of characters, distinguishing the species in the *effusus* group.

‡Adult male of *R. eminellobus* and *R. tuberculatus* are very similar. Key above can partially separate the two species. Female, pupa and larva of two species are quite distinguishable.

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DESCRIPTIONS OF FOUR NEARCTIC *PROCLADIUS* SKUSE PUPAL EXUVIAE

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Abstract

Associated *Procladius* Skuse (*Holotanypus* Roback) material from the Sublette collection was found to have specimens with undescribed immature stages. Pupal exuviae of two species are described for the first time: *P. barbatulus* Sublette and *P. clavus* Roback. Adult and larval features are included for *P. barbatulus*. In addition, specimens of Nearctic *P. denticulatus* Sublette are compared to Palearctic *P. signatus* (Zetterstedt) to determine if exuviae features can separate these species or suggest that *P. signatus* is a senior synonym. Finally, associated pupal and larval traits of *P. prolongatus* Roback are compared to the original description, corroborating the theory that features are unique enough to warrant subgeneric status.

Introduction

Within *Procladius* Skuse, *Holotanypus* Roback is a diverse subgenus in the Nearctic region with 18 described species (Ashe and O'Connor 2009), of which six have not previously been described in the pupal stage. While comparing recently collected material to the Sublette collection at the University of Minnesota, a number of associated specimens were noted within *Procladius*, including two species (*P. barbatulus* Sublette and *P. clavus* Roback) with undescribed pupal exuviae. Along with these, we also compare an associated specimen of the Nearctic *P. denticulatus* Sublette to the morphologically similar Palearctic *P. signatus* (Zetterstedt) to determine if the pupal stage can be used to better distinguish these species. Finally, we used eight associated rearings from Alaska to reevaluate if *P. prolongatus* Roback is unique enough to have a separate subgenus designation (Roback 1980, 1982).

Materials and Methods

All specimens in the University of Minnesota, St. Paul (UMSP) collection were originally identified by J. E. Sublette and adult features were reconfirmed for this study using Roback (1971). *P.*

prolongatus reared associations were provided by Malcom Butler and will be added to the UMSP collection. Measurements and terminology follow Sæther (1980). Additional specimens of *P. denticulatus* were identified by W. P. Coffman and D. Oliver, while additional specimens of *P. signatus* were identified by P. H. Langton.

Results

Procladius (Holotanypus) barbatulus Sublette

Material examined. USA: California, 1.7 miles east of Benton Hot Springs, 21-VIII-67, col. Jon Martin.

Adult male (n = 1). Total length approximately 3.7 mm.

Head. AR 1.80. Temporal setae 23-24. Clypeus with 15 setae. Palpomere lengths (µm): 50, 70, 105, 148, 238.

Thorax. Anteprenotals 8-9. Dorsocentrals 19. Prealarials 16-17. Acrostichals 40. Prescutellars 7. Supraalar 1. Scutellars 22.

Wing. VR 1.46. Brachiolum with 3 setae; R with 14-17 setae, R₁ with 13 setae, R₄₊₅ with 19-23 setae. Squama with 32-36 setae. Extended part of costa 100 µm long. Wing length 2.1 mm. Total length/wing length 1.76. Dark spot over r-m extending to part of m-cu.

Legs. Spur of front tibia 55 µm. Spurs of middle tibia 38-40 µm and 48-50 µm. Spurs of hind tibia 38 µm and 73 µm. Width at apex of front tibia 58-60 µm; middle tibia 53-58 µm; hind tibia 70-73 µm. Comb with 11 setae; shortest seta 30 µm long, longest seta 58-63 µm long. One pseudospur present on ta₁ of all legs and one pseudospur on ta₂ of mid and hind legs. Pseudospurs 30-43 µm long. Additional measurements and proportions are in Table 1.

Hypopygium. Tergite IX with 21 setae. Transverse sternapodeme 195 µm. Phallapodeme 75 µm. Strut ratio 2.84. Gonocoxite 213 µm long, gonostylus

Table 1. *Procladius barbatulus*, male. Lengths (in μm) and proportions of legs.

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅
p ₁	770, 780	940, 970	690	340	250	170	120
p ₂	850	910, 940	580, 590	300	210, 230	140, 150	100
p ₃	770, 780	1050	700, 740	350, 370	250, 260	160, 170	120, 130
	LR	BV	SV	BR			
p ₁	0.61	2.73	2.42	2.92			
p ₂	0.70, 0.71	3.08, 3.12	2.92, 2.95	2.69			
p ₃	0.65, 0.67	2.86, 2.88	2.51, 2.56	2.71, 2.85			

100 μm long, gonostylus ratio 10. HR 2.13; HV 3.70.

Pupa (n = 1). Total length 4.80 mm.

Cephalothorax. Faint brown, mostly translucent. Thoracic horn (Fig. 1) brown; 380 μm long; with triangular teeth, sometimes rounded apically; no reticulation; plastron plate length 75 μm ; horn width, plastron plate width and apical constriction of horn chamber not measurable.

Abdomen. Color pattern 1, entirely translucent. Shagreen (Fig. 2) of T IV medially with small points (1-2 μm) often arranged in short rows and similar in size to medial points on T VIII. LS taeni-

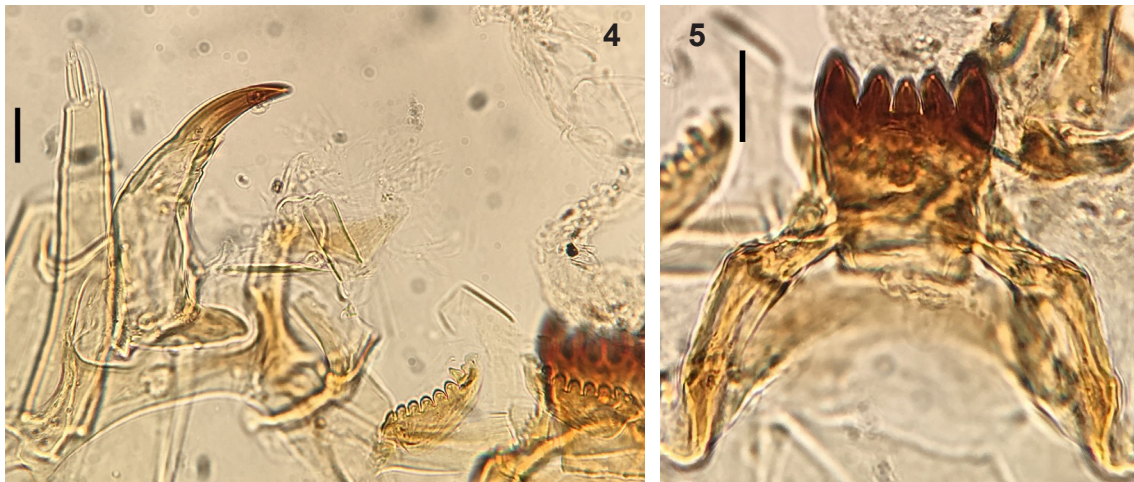
ae of VII at 0.45, 0.53, 0.80, 0.93 segment length. LS taeniae of VIII at 0.30, 0.48, 0.63, 0.80, 0.93 segment length. Anal lobe length 500 μm ; 35-36 spines on outer margin, often bifid or trifid; largest spines 25-27 μm long with spine size similar for much of the margin; smaller spines apically with inner 2-3 spines on a protuberance (Fig. 3).

Larva (n = 1)

Head. Head capsule length about 650 μm . Antenna in Fig. 4. Antennal segment 1-4 lengths (μm): 155-157, 20, 5-6, 2. AR 3.95-4.05. Basal antennal segment 30-33 μm wide; 4.8-5.2 times as long as wide; ring organ 0.73 from base; blade 27.5 μm long. Mandible 135-140 μm long. Maxilla features



Figures 1-3. 1, *Procladius barbatulus* thoracic horn (Scale = 0.1 mm); 2, tergite IV medial shagreen (Scale = 0.025 mm); 3, tergite VIII and anal lobe (Scale = 0.1 mm).



Figures 4-5. 4, *Procladius barbatulus* antenna, mandible, and dorsosomental teeth; 5, ligula (Scale = 0.025 mm).

not distinguishable. Dorsosomental teeth in Fig. 4 and ligula in Fig. 5.

Abdomen. Features not distinguishable.

Remarks. As only the second specimen reported, after the holotype, adult and larval traits were included for comparison to Sublette (1964) and Roback (1971). In Roback (1980) this specimen keys to couplet 18 for *P. freemani*, and in Sæther (2010) to couplet 9 and *P. freemani*. With only a single specimen, it is not certain that any feature can be used to distinguish *P. barbatulus* from *P. freemani* in the pupal stage. Features span all three of Roback's variants (with var. 2 the best fit) and are also similar to specimens described by Sæther. The larva keys to couplet 14 in Roback and appears indistinguishable from other species in this couplet.

Habitat may be the same as most *P. freemani*, which is typically lakes, and the type specimen is from Hume Lake, a reservoir in Sequoia National Forest. Based on slide information, the Benton Hot Springs specimen presented here may be from spring-fed desert streams or related pools.

Known range for *P. barbatulus* is California, USA, in Fresno and Mono Counties.

***Procladius (Holotanypus) clavus* Roback**

Material examined. Canada: British Columbia, Box 4 Lk., Riske Cr. B.C., 1-VI-1970, col. R. Cannings.

Adult males (n = 3).

Hypopygium. Tergite IX with 44-62, 53 setae (n = 3). Transverse sternapodeme 280-307, 296 μ m long (n = 3). Phallapodeme 100-113, 106 μ m long (n = 6). Strut ratio 2.10-2.50, 2.26 (n = 6). Gonocoxite 220-250, 239 μ m long (n = 6). Gonostylus 118-138, 130 μ m long (n = 6). No lobes present

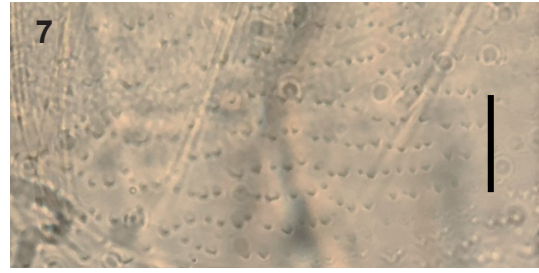
for measuring the gonostylus ratio. HR 1.72-1.92, 1.84 (n = 6).

Pupa (n = 3). Total length 6.37 mm, range 6.1-6.5 mm

Cephalothorax. Faint brown, mostly translucent. Thoracic horn (Fig. 6) 450-520, 483 μ m long (n = 4); 140-150, 145 μ m wide (n = 2); with teeth; no reticulation; plastron plate 70-98, 84 μ m long (n = 4); 106-120, 113 μ m wide (n = 2); apical constriction of horn chamber 70-85, 78 μ m wide (n = 2); length/width of horn 3.27-3.85, 3.56 (n = 2); width of plastron plate/width of horn 0.79-0.80, 0.80 (n = 2); width of plastron plate/width of constriction 1.41-1.51, 1.46 (n = 2).

Abdomen. Color pattern 1, entirely translucent. Shagreen (Fig. 7) of T IV medially with small points (2 μ m) arranged in loosely connected, elongate rows and smaller than median points of T VIII (3-5 μ m). LS taeniae of VII at 0.50, 0.69, 0.86, 0.96 (n = 3) segment length. LS taeniae of VIII at 0.30, 0.45, 0.61, 0.77, 0.93 (n = 3) segment length. Anal lobe length 700-820, 787 μ m; 43-54, 49 (n = 5) spines on outer margin; largest spines (22-30 μ m) posterolateral, usually with gaps narrower than spine base; basal spines broadly triangular; apical spines 10-15 μ m, directed posteriorly, and usually on a small protuberance or clustered together with a shared base (Fig. 8).

Remarks. All specimens were paratype, making adult features difficult to observe so only hypopygium measurements were included. In Roback (1980) these specimens key to couplet 13, in Sæther (2010) to couplet 6, and are similar to *P. culiciformis*. Sæther's measurements of *P. culiciformis* were generally similar to Sublette's Riske Creek specimens, but it is possible that *P. clavus* can be distinguished by the wider apical constric-



Figures 6-8. 6, *Procladius clavus* thoracic horn (Scale = 0.1 mm); 7, tergite IV median shagreen (Scale = 0.025 mm); 8, segment VIII and anal lobe (Scale = 0.1 mm).

tion of the horn chamber, which also leads to a smaller ratio for width of plastron plate/width of constriction. Sæther describes *P. culiciformis* anal lobe inner margins as having small spines, which are not present in *clavus* specimens. Finally, figures of the thoracic horn in both Sæther and Roback show the *P. culiciformis* apical constriction as having a rather long neck, while in *clavus* the neck is short enough that it was hard to determine the width, and therefore the horn may resemble *P. paragretis* or *P. ruris*.

Known range is British Columbia and Nunavut, Canada.

***Procladius (Holotanypus) prolongatus* Roback**

Material examined. USA: Alaska, Loon Pond, a tundra pond on the west edge of Prudhoe Bay, 3-VII-1980, col. Malcom Butler.

Pupa (n = 8), 4 males, 4 females. Total length 7.65-8.47, 8.02 mm (n = 8)

Cephalothorax. Light brown. Thoracic horn (Fig. 9) 630-760, 682 µm long (n = 16); 240-300, 265 µm wide (n = 13); covered evenly with short teeth; no reticulation; plastron plate 140-200, 170 µm long (n = 16); 252-330, 279 µm wide (n = 13); apical constriction of horn chamber 110-148, 130 µm wide (n = 5) with a short neck often obscured

by the large, dark plastron plate; an oval aperture between the neck and atrium is often elongate and distinct; length/width of horn 2.3-2.78, 2.57; width of plastron plate/width of constriction 1.89-2.35, 2.06 (n = 5). Respiratory atrium, neck and plastron plate dark brown. Plastron plate width usually equal to or wider than horn width.

Abdomen. Color pattern 3, but usually a solid light brown with no pale patches. Shagreen (Fig. 10) of T IV medially with elongate (4-7 µm) single sharp points. Shagreen on T VIII similar in size, shape, and pattern, although less robust than on other tergites. LS taeniae of VII at 0.38, 0.62, 0.81, 0.96 (n = 6) segment length. LS taeniae of VIII at 0.16, 0.35, 0.57, 0.78, 0.95 (n = 6) segment length. Anal lobe (Fig. 11) length 920-1000, 964 µm; 30-48, 40 (n = 16) spines on outer margin; largest spines 40-70 µm posterolateral, with distance between spines equal or wider than width of spine bases; smallest apical and lateral spines 10-20 µm long; posterior edge indented, apical spines clustered on a projection (except one specimen with no projection and no indentation along posterior edge).

Remarks. Roback (1980) described features that suggested subgeneric status for *P. prolongatus*, and he formally implemented this distinction among *Holotanypus* groups in Roback (1982). From associated Prudhoe Bay material, we can

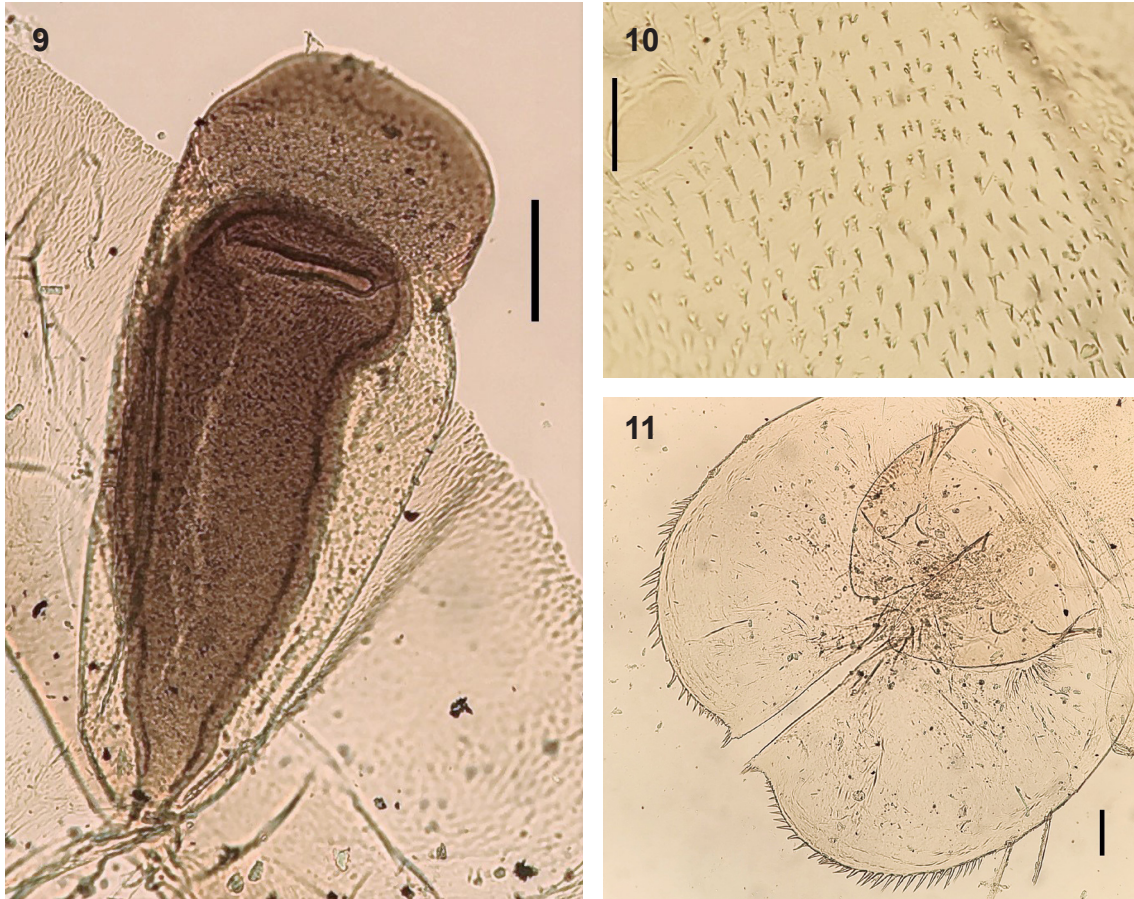


Figure 9-11. 9, *Procladius prolongatus* thoracic horn (Scale = 0.1 mm); 10, tergite IV medial shagreen (Scale = 0.025 mm); 11, anal lobe (Scale = 0.1 mm).

provide insight into the immature traits used to support the subgenus.

The anal lobes from Roback's Barrow, Alaska, specimens were described as "unusually shaped" with a "projecting outer curve". The Prudhoe Bay specimens usually do have a rounded lateral shape, but none are as expansive as in Roback's description and some also have lateral edges that are straight. Based on descriptions in Roback (1980) and material from Isle Royale, Michigan, *P. dentus* also has a large anal lobe that can be rounded laterally. *P. dentus* and *prolongatus* can both have a fold near the respiratory organ neck, but in both species this feature is not always observed. Therefore, these two features are not diagnostic and without a series of specimens may create confusion when using the key in Roback (1980). These species may be reliably separated by tergite IV and VIII shagreen, which is elongate (4-7 μm) in *P. prolongatus* and short (1-4 μm) in *P. dentus*. In addition, the number of spines on the anal lobe will distinguish these species, with 30-48 in *P. prolongatus* and 56-63 (from Isle Royale) and 68 (from the single specimen reported by Roback) in *P. dentus*.

Larvae have one proleg that is apparently diagnostic in *Procladius*, with a wide, triangular base and a tight apical hook (see figure 244, Roback 1980). Prudhoe Bay tundra pond specimens have this feature. Larvae of *P. dentus*, though undescribed, should presumably have simple proleg claws, similar to all other known larvae in *Holotanypus* (Roback 1982).

Known range is Nunavut, Canada, and Alaska, USA.

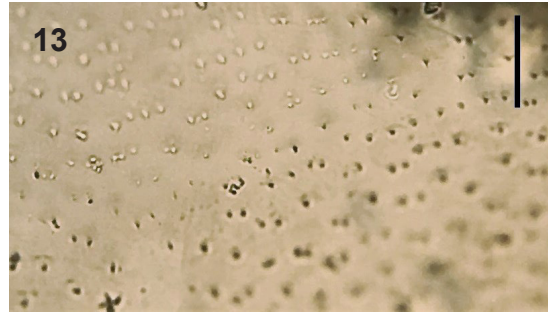
Procladius (Holotanypus) denticulatus Sublette

Material examined. USA: New Hampshire, Mirror Lake, 14-24-VI-74, reared male, col. Rhoda Walter.

Pupa (n = 1). Total length 5.45 mm.

Cephalothorax. Thoracic horn 420-430 μm long; 100-110 μm wide; with teeth; atrial wall with weak reticulation; plastron plate length 50 μm , width 90 μm ; apical constriction of horn chamber 62 μm wide; length/width of horn 3.9-4.2; width of plastron plate/width of horn 0.82; width of plastron plate/width of constriction 1.45 (Fig. 12).

Abdomen. Tergites with color pattern 3, minimal



Figures 12-14. *Procladius denticulatus*. 12, thoracic horn (Scale = 0.1 mm); 13, tergite IV medial shagreen (Scale = 0.025 mm); 14, segment VIII and anal lobe (Scale = 0.1 mm).

contrast. Shagreen of T IV medially with small points (1-2 μm , Fig. 13) often arranged in short rows and smaller than median points on T VIII (3-4 μm). LS taeniae of VII at 0.62, 0.81, 0.93, 0.98 segment length. LS taeniae of VIII at 0.30, 0.50, 0.67, 0.83, 0.93 segment length. Anal lobe length 530 μm ; 29 spines on outer margin; largest spines (33-37 μm) posterolateral with gaps wider than the base of the spines; anterior and apical spines smaller (as small as 5 μm), more closely set, with one apical spine on a small protuberance apically (Fig. 14).

Remarks. Separating this species from *P. signatus*, which has been suggested as a possible senior synonym (Roback 1971), is difficult since the two species are exceedingly alike. Including an additional three specimens (collected by W. P. Coffman and D. Oliver) to the one described here, we observe that on the whole *P. denticulatus* is smaller (ThL 400-430 μm), whereas *P. signatus* is larger (ThL 480-624 μm , mean = 546, n = 13). Roback (1980) gives the range for *P. denticulatus* ThL as 370-540 μm (mean = 468) and Sæther (2010) a ThL range of 393-577 μm (mean = 469), so there is overlap. However, the means are well separated so ThL should be a useful feature unless a specimen falls into the intermediate range and geographic provenance is unknown. Size may be useful for total exuviae length as well, with a range of 5.7-8.5 mm (mean = 6.6) for *P. signatus* (Langton 1991).

In addition, points on tergite IV are smaller on *P. denticulatus* (1-2 μm), which are smaller than on tergite VIII (3-4 μm), whereas points on the two tergites for *P. signatus* are of similar size (4 μm , Langton 1991). This feature is independent of exuviae size. A final structural difference that may hold for *P. signatus* is that the strong points of the thoracic horn wall continue over the 'shoulder', where the horn constricts apically, and onto the 'hood', the apical region containing the plastron plate (Fig. 15). In contrast, *P. denticulatus* points on the hood are reduced in size and density.

Procladius denticulatus is widespread in the northern Nearctic.

Discussion

For the two newly described pupal exuviae, *P. barbatulus* does not appear to be distinguishable morphologically from *P. freemani*, but *P. clavus* does have a distinct set of features and appears to be most similar to *P. culiciformis*. In *P. prolongatus* there are distinct features in immature life stages that will separate it from similar species.

Whether *P. denticulatus* and *P. signatus* are at two ends of a cline from the Eastern Palearctic to the Nearctic, or if they are two distinct species that are only slightly diverged from a common ancestor, cannot be fully answered here. But our results do suggest that most specimens can be separated using the thoracic horn length and tergite IV sha-

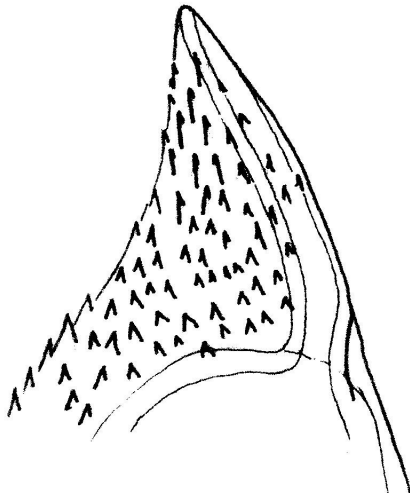


Figure 15. *Procladius signatus*, apex of thoracic horn.

green. As Roback (1980) points out, some intermediate specimens will complicate identification, particularly when a comparing a “larger series from a broad geographical range.”

Two exuvial features may be worth consideration for future *Procladius* revisions. First, variability in the pattern of tergite shagreen shows this is not necessarily a stable feature in some *Procladius* species, yet spinule size and comparison between T IV and T VIII appear useful. Finally, variation in size and distribution of points on the ‘shoulder’ and ‘hood’ of the thoracic horn should be investigated in this genus.

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Oviposition behavior and host records for the parasitic midge *Trichochilus lacteipennis* (Johannsen) (Chironomidae: Orthocladiinae)

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Abstract

Adult female *Trichochilus lacteipennis* were observed to extrude long strings of eggs (up to 15 cm) in flight over a lake in Maine, USA. Once extruded females dropped to the water surface and released the strings. Larvae of *T. lacteipennis* are parasitic on *Elliptio complanata* at this site and probably additional unionid mussel species elsewhere. Based on our dissections of parasitized mussels and previous reports, it appears *T. lacteipennis* spend all or nearly all of their larval life within their mussel host, with first instars initially free-living inside the mantle. At some point larvae enter the marsupium and complete larval development on a diet of mussel eggs and/or glochidia before pupating within the marsupium.

Results

On still mornings between 0700 and 0900 from 17 to 21 June, 2015, we observed an unusual oviposition behavior by a (then) undetermined species of Orthocladiinae over Lake Umbagog, along the New Hampshire/Maine border (USA; 44.8038°N, 71.0072°W). Females were seen flying from the nearby riparian forest canopy each slowly extruding a long string of eggs (Figs 1-2; also, cover of Chironomus Journal of Chironomidae Research, Vol. 29 [2016]). This extrusion lasted up to several minutes as the midges hovered from 2-10 m over the water surface, from near the shore to about 50 m offshore. Strings up to 15 cm in length were extruded, after which females dropped to the water surface, released the strings, then flew off.



Figure 1. Two adult female *Trichochilus lacteipennis* hovering over Lake Umbagog, in the process of extruding strings of eggs.



Figure 2. A female *Trichochilus lacteipennis* with egg string fully extruded, as it descended toward the water surface just prior to releasing the eggs.

Backlit by the low morning sun and observed against the dark, shady, mostly coniferous riparian forest, individual midges with their egg strings could be seen easily from distances up to 170 m. Three ovipositing females were collected and preserved in ethanol. All three were subsequently sequenced for COI (two by BOLD and one at the Stroud Water Research Center; see Appendix). Two individuals had identical haplotypes and the third differed by about 5%, suggesting the possibility of more than one species. No close matches to either haplotype were found in existing databases.

On June 19-25, 2016, the collection site was revisited. Once again, many ovipositing females were observed on calm mornings between 0700 and 0900. One of us (Roberts) recorded video and another (Funk) recorded still images of the oviposition behavior. These were later combined into a video that was posted on YouTube (<https://www.youtube.com/watch?v=z2ugoE1y15o>). Egg strings from two individuals were collected into separate 30 ml sample bottles filled with filtered (0.45 μ), sterilized stream water from White Clay Creek, Pennsylvania, USA (Fig. 3). These were stored in a cooler (temperature ranging from 10-15°C) for 5 days, then transferred to a 20° water bath and a 15:9 (light:dark) photoperiod for 6 weeks, during which time no development was observed. At the end of this period the eggs appeared to be dead, although there was no obvious microbial activity. Nevertheless, both vessels were then stepped down to 4° (at constant darkness) for 5 months to simulate a “winter”, then stepped back up to 20° (15:9). By this time the eggs were clearly dead.

In January, 2017 four female specimens (including the three 2015 specimens with known COI sequences plus a fourth unsequenced specimen from 2016) were sent to Martin Spies in Munich, Germany, who was able to identify them as *Trichochilus lacteipennis* (Johannsen). These specimens now reside in the Bavarian State Collection of Zoology (ZSM = Zoologische Staatssammlung München).

The genus *Trichochilus* was erected by Sæther (1985) for *Trichochilus lacteipennis* (Johannsen, 1908), at that time known only from the adult female holotype. In the 1990s Matthew Gray, then an undergraduate student at the University of Pittsburgh, sent pupal exuviae, pupae, males, and females to William Coffman for identification. These had been reared from larvae found in gills (and presumably feeding on glochidia) of the unionid mussel *Elliptio complanata* in Ansonia, Pennsylvania, USA. Dr. Peter H. Langton (who was visiting Coffman at the time) identified the females as *Trichochilus lacteipennis*. Coffman gave the material to Rick Jacobsen for description, and a manuscript is now in preparation. Epler (2001) and Andersen et al. (2013) provided figures and descriptions of larvae based on this material.



Figure 3. A string of *Trichochilus lacteipennis* eggs suspended in water near the bottom of a glass rearing vessel.

We visited the Lake Umbagog site again from 18-24 June, 2017, with the intent to collect more *Trichochilus* eggs and adults, and to look for their immatures in *Elliptio complanata*, which we knew to be abundant in the lake. Weather during all 5 mornings of our 2017 stay was windy and/or rainy during the 0700-0900 window within which we had observed oviposition flights in previous years. Probably for this reason, only a few ovipositing *Trichochilus* were seen, and only one captured. The egg strings extruded by these females were much shorter than those observed in previous years. Long strings such as those in Figs 1-2 must surely present a great deal of wind resistance, thus requiring relatively still conditions for controlled flight. The single string collected in 2017 was placed in a 30 ml vessel similar to the ones used in 2016, but this time containing unfiltered water from Lake Umbagog. This vessel was stored in a cooler until 27 June when it was transferred to a 20° (15:9 photoperiod) water bath in the lab. By 30 June most eggs had hatched.

We also searched for pupal exuviae by skimming the water surface near the lake margin with a small aquarium net on several occasions during the 2017 visit. Several species of Chironominae and Tanypodinae were abundant in these collections. Only 7 orthoclads were found and all turned out to be *Trichochilus*.

In addition, 118 *Elliptio complanata* and one *Pyganodon* sp. were collected from the vicinity of the oviposition site. Adductor muscles were severed with a scalpel and the clams opened. For each individual, tissues were first flushed with a jet of lake water into a small pan and carefully examined for the presence of chironomids. Then all the larger bodies of soft tissue were incised and examined for the presence of chironomids within. Nine *Elliptio* contained *Trichochilus* (total: 19 larvae and 25 pupae, most with associated larval exuviae). All chironomid specimens were preserved in 95% ethanol. Shells from mussels that contained *Trichochilus* were collected and dried, and small samples of foot tissue were preserved in 95% ethanol. All *Trichochilus* larvae were mature, some showing pupal structures within. All pupae appeared to

have transformed only recently (none were pharate). *Trichochilus* larvae and pupae were found only within the outer, marsupial gill lamellae and were revealed by incision of this structure (Fig. 4). When more than one individual was present (up to 6 were found in a single lamella) these were conspicuously clustered in the dorsal portion of that lamella. Larval structures of these specimens match the figures and descriptions by Epler (2001) and Andersen et al. (2013).

Gordon et al. (1978) reported parasitic relationships between another orthoclad, *Baeoetenus bicolor* Sæther, 1976, and the unionids *Pyganodon cataracta* and *Anadonta implicata* from a reservoir in New Brunswick, Canada. *Baeoetenus* larvae appeared to invade mussels in late winter as 3rd instars and actively feed on gill tissue. They reached 4th instar around ice out and pupated in late May or early June, with emergence completed by late June or early July. Both larvae and pupae inhabited tubes constructed of particulate organic material, and were normally attached to anterodorsal surfaces of the gills near the labial palps of *P. cataracta*. As many as 3 larvae were found on the same gill and feeding damage was evident, with as much as 50% of gill tissue absent.

In addition to *Baeoetenus bicolor*, Gordon et al. (1978) found a total of 860 larvae of another, unknown orthoclad (“near *Phycoidella*”) in *Pyganodon cataracta* (but not in three other unionids examined, including *Elliptio complanata*). These larvae were collected by severing the mussels’ adductor muscles and washing the tissues with a jet of water over a dish. Only first instars were found and these had a similar seasonal pattern of occurrence as *Baeoetenus*, “with a substantial decrease in June, followed by a sharp rise throughout July and early August.” The apparent absence of any later instars or evidence of feeding damage led the authors to postulate that the association was not parasitic and that the larvae left the mussels to pass the remaining instars elsewhere. Roback (1979) provided figures and a description of these first instar larvae (as “Genus near *Phycoidella*” sensu Sæther) based on the collections by Gordon et al. (1978) as well as on material from five genera of unionids collected in Louisiana. Epler (2001) considered these to be *Trichochilus* despite some differences between Roback’s (1979) description and the fourth instar *Trichochilus* larvae from Pennsylvania, e.g. the presence of a “comb-like row of preapical setae” on the mandible in Roback’s first instars, which Epler suggested might disappear in later instars. Our *Trichochilus* hatchlings are similar



Figure 4. Four *Trichochilus lacteipennis* pupae revealed by incision of the outer lamella (marsupium) of the right gill of an *Elliptio complanata*.

to the brief description given by Roback (1979) (including the presence of the “comb-like row of preapical setae”), except that each anterior proleg on our specimens has one very conspicuous comb-like claw in addition to three large simple ones (plus some smaller structures basally). Thus, our observations confirm Epler’s (2001) suggestion that the mandibular setae disappear in later instars. Similar to the final instar, the ventromentum in our specimens is toothless and the dorsomentum has 5 teeth on each side. Not mentioned by Roback but evident in our specimens, the S I setae are simple (as in the final instar) and the premandible has at least 4 teeth.

Given all the above evidence, it seems likely that *Trichochilus* spend all or nearly all of their larval life within their mussel host, starting with first instars free-living inside the mantle and later entering the marsupium to complete larval development (rather than beginning life in some other habitat). If this is true, one is left to wonder why Gordon et al. (1978) did not find later instars or pupae. A clue may be found in their Methods section: larvae were collected by severing the mussels’ adductor muscles and washing the tissues with a jet of water over a dish; no indication is given that they examined the contents of the marsupium. The 4th instars and pupae we found in *Elliptio complanata* were only revealed by incision of the marsupium. It therefore seems possible that later stages were present inside the marsupium of *Pyganodon cataracta* in June but were overlooked by Gordon et al. (1978). Another, much less plausible, explanation is that first instars leave *Pyganodon cataracta* and enter *Elliptio complanata* to complete their larval lives. Gordon et al. (1978) examined 67 *Elliptio complanata* during the infestation period (using the same washing technique as in *Pyganodon*) and reported the absence of midge larvae or pupae, but again, presence of the latter within the *Elliptio* marsupium might have been missed.

We did not find any first instar *Trichochilus* in the *Elliptio complanata* mussels examined in 2017. As these dissections were performed in the field, without a microscope, it is possible that first instars were overlooked. However, considering what we know of their seasonality it seems likely that only fourth instars and pupae would be present inside mussels during that time period (18-24 June).

Although the diet of first instars is unknown, at some point in their development *Trichochilus* larvae clearly become parasitic. Guts of fourth instars were full of mussel eggs and/or glochidia. Nevertheless, we thought it worth a try to start some of our hatchlings out on a diatom/biofilm diet. We placed approximately 50 each in two vessels with some filtered (200 μ) periphyton slurry and an air stone at 20° (15:9 photoperiod). None survived for more than a couple of days.

Although the production of gelatinous strings of eggs is widespread in the Chironomidae (Nolte 1993), these are generally produced at or near the water’s surface, and attached to stones or other objects. To our knowledge the extrusion of long strings of eggs in flight is reported here for the first time (although something similar was recently observed in *Hydrobaenus biwaquartus* (Sasa & Kawai, 1987); Hongqu Tang, personal communication). *Trichochilus* egg strings sank slowly in our test vessels, appearing to be only slightly denser than the water. They did not stick to the glass, but remained in suspension near the bottom of the vessel. If they behave similarly in the lake it is likely that they drift around with the currents near the lake bottom and might eventually enter the siphon of a mussel. Given the failure of eggs collected in 2016 to hatch, we had hypothesized that egg strings might need to enter and become lodged in the gills of a mussel in order to stimulate hatching. However, the fact that eggs collected in 2017 hatched quickly in a glass vessel of lake water leads us to reject that hypothesis. Thus, the significance (if any) of the unusual method of oviposition observed in *Trichochilus* remains unclear.

Pending the availability of resources, we plan to sequence mitochondrial COI for the 44 immature specimens collected from *Elliptio* in 2017. This data might prove helpful in the interpretation of the considerable morphological variation evident in available specimens (R. Jacobsen and M. Spies, personal communication), as well as provide a measure of genetic diversity and possibly the demographics of infection.

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First record of the genus *Heterotrissocladius* (Chironomidae: Orthoclaadiinae) from the Neotropical region

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Abstract

Here we report the occurrence of *Heterotrissocladius* (Diptera: Chironomidae: Orthoclaadiinae) for the first time in Central America, as well as in the Neotropical region. The report is based on sub-fossil chironomid head capsules from surface sediments of a high elevation lake, Lago Magdalena, Guatemala.

Introduction

Central America is an area particularly interesting for ecological and biogeographical studies, since it represents a bridge between two main biogeographical realms, the Nearctic and the Neotropical regions. Recent years have seen increased activity regarding chironomid systematics in the Neotropical region (e.g. Oliveira et al. 2013, Trivinho-Strixino et al. 2013, 2015, Andersen and Pinho 2014, Silva et al. 2014a,b, Andersen et al. 2015, Silva and Wiedenbrug 2015, Siri et al. 2015, Parise and Pinho 2016, Silva and Oliveira 2016, Silva and Ferrington 2018). At the same time, paleolimnological research, i.e. study of lake sediments as environmental archives, in Central America and the Caribbean region remains fragmentary. Indeed, lake sediments can be a valuable source of information not only of past environmental changes, but also recent distribution of species, since the sediment accumulating in the deepest part of a lake represents the mixture of the biological community from different parts of the lake as well as accumulation of communities from different time periods. Sediment examination thus saves time usually spent sampling in various locations and dates (Frey 1976). While some organisms are not well-preserved in sediments, chironomid larvae remain in good condition due to their chitinized head capsules.

A recent investigation of sub-fossil Chironomidae from surface sediments of lakes in Guatemala yielded an orthoclad species that was not readily identifiable, suggesting that it was a species unrecorded in Central America. Further detailed examination of this material revealed that the species belongs to the genus *Heterotrissocladius* Spärck, more specifically to *Heterotrissocladius marcidus*-type (Brooks et al. 2007). Here we report the occurrence of *Heterotrissocladius* (Diptera: Chironomidae: Orthoclaadiinae) for the first time in Central America, as well as in the Neotropical region.

Material and Methods

Surface sediment samples were collected from Lake Magdalena, located at 15.5426 °N, 91.3956 °W, in Huehuetenango Province, Guatemala, on September 18, 2013. Magdalena is a small, shallow and high altitude lake (approximately 2,800 m a.s.l.), mostly surrounded by coniferous cloud forest composed almost exclusively of Guatemala fir with a few scattered pines and a thick ground cover of moss. Prior to sampling, the following physical and chemical parameters of the surface water were recorded: max. depth 2.8 m, Secchi depth 2.8 m, surface water temperature 12.5 °C, pH 8.8, conductivity 331 µS m⁻¹ and O₂ content 6.2 mg L⁻¹ (for more details see Wojewódka et al. 2016).

Ekman grab sediment samples were taken from the deepest part and the littoral zone of the lake. Sediment samples were placed in plastic bottles (100 mL) immediately after collection, and stored under refrigeration. Extraction of head capsules from sediment samples followed the methodology described by Walker and Paterson (1985). Samples were deflocculated in 10% KOH overnight without heating and subsequently passed through a 90 µm mesh sieve. Chironomid head capsules were removed under a stereomicroscope and slide-mounted in Berlese mounting media. Identification followed Brooks et al. (2007) and Andersen et al. (2013).

Results and Discussion

The littoral surface sediment sample yielded 97 chironomid remains, four of which were tentatively identified as *Heterotrissocladius marcidus*-type (sensu Brooks et al. 2007) (Fig. 1). The genus belongs to the subfamily Orthocladiinae and was erected by Spärck (1923) based on descriptions of adults of *Metriocnemus cubitalis* Kieffer, a junior synonym of *Chironomus marcidus* Walker, which has been carried as *Heterotrissocladius marcidus* (Walker). The genus contains more than 20 species (Ashe and O'Connor 2012) and is subdivided into three species groups: *subpilosus*, *maeaeri* and *marcidus*. Stur and Wiedenbrug (2005) reported additional, undescribed, *Heterotrissocladius* species in the Zoologische Staatssammlung München, which may indicate an additional species group in this genus.

Heterotrissocladius is closely related to *Paratrissocladius*, having similar larval antenna. However, *Heterotrissocladius* possesses 5 pairs of lateral teeth on mentum, wide ventromental plates, bifid premandible, plumose SI and weak, serrate spines on pecten epipharyngis (Andersen et al. 2013), which guarantee its distinction.

The species within *Heterotrissocladius* are predominantly Holarctic in distribution and are often found in lakes (littoral to profundal), ponds, puddles, and in all sizes of flowing waters (Andersen et al. 2013). Larvae of the most widespread species of the genus, *H. marcidus*, occur throughout Europe (Spies and Sæther 2013) and North America (Epler 2001), and are also among the most common insects in alpine and subalpine lakes (Sæther 1975, Krno et al. 2006, Boggero et al. 2006, Bitušik et al. 2006, Füreder et al. 2006, Lods-Crozet et al. 2012). The four described paleo-morphotypes have different temperature optima, with *H. marcidus*-type being the least restricted to cold waters (Brooks et al. 2007).

The chironomid fauna of Central America includes multiple genera with worldwide distributions, as well as genera that are primarily Holarctic or Neotropical (Epler 2017). The Neotropical genera are typically an extension of the warm adapted fauna of lowland South America (Watson and Heyn 1992). Within this group, genera such as *Diplosmittia*, *Goeldichironomus*, *Labrundinia*, *Lipurometriocnemus*, *Pentaneura* and *Polypedilum* (Asheum) possess a Pan-American distribution, having secondarily dispersed into the

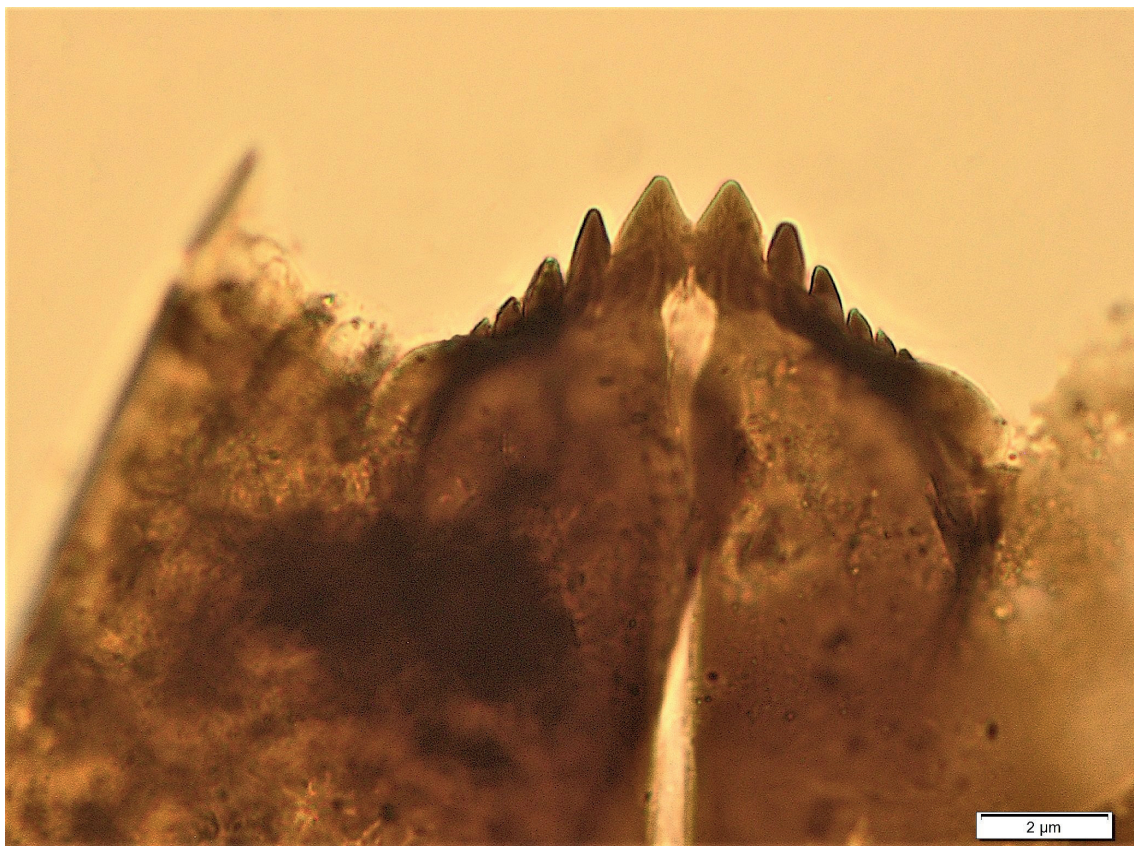


Figure 1. Head capsule of the subfossil *Heterotrissocladius* Spärck found in the surface sediment of the Lake Magdalena, Guatemala, autumn 2003.

southern Nearctic via Central America or the Caribbean (Reiss and Sublette 1985). Other genera such as *Heterotrissocladius* and *Orthocladius* are most widespread in the Holarctic region (Silva et al. 2015). This first-report of a widespread genus in the Neotropical region highlights the need of additional inventories in order to better comprehend the diversity patterns of the chironomid communities in Central America and in the Greater Antilles archipelago.

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Memories of D. (Don) H.D. Edward 1933-2018.

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My friend and colleague Donald Herbert Deshon Edward died in June 2018 at the age of 85 following a decline in health in the previous months. Thus ended a long career entirely based at the University of Western Australia on the banks of the Swan River in Perth. Don Edward was a versatile aquatic biologist who had a particular affection for the stranger life histories amongst the many chironomids that surrounded him in the south-west of Western Australia. Although quiet on the publication front in his later years (Fig. 2E), he continued to sort samples from projects, including many in Western Australia and from Papua New Guinea for Andrew Storey's monitoring studies. However, Don justifiably should be remembered for his early recognition of 'Gondwanan' elements in Australia and great expansion of Brundin's (1966) revelations of austral vicariant patterns amongst the Chironomidae. Don firmly placed Western Australia on the map of Gondwana.

A career-refocus to midge systematics and biogeography started when Don sent some adult chironomids to Don Colless, my predecessor as curator of Diptera at the Australian National Insect Collection in CSIRO, Canberra. Don Colless and Canadian Dipterist Anthony Downes published in *Nature* (1967) the observation that the female of this species was mandibulate - but the publication lacked identification of the midge and made no mention of Don Edward's role in their collection. Subsequently this species was identified as belonging to *Archaeochlus*, a southern African genus of Podonominae described by Brundin (1966), who recognised the long proboscis but not the well-developed mandibles and laciniae enclosed therein.

Don commenced his summary of the Australian Chironomidae across the continent (Edward 1986) and simultaneously set out to investigate the biogeographic conundrum of these gondwanan taxa. With his experience of studying 'granite midges' in western Australia (e.g. Fig. 1A) (Withers and Edward 1997) Don understood that 'dark stains' caused by bacteria, cyanobacteria and algae including epilithic diatoms' showed the course in which seasonal water flowed and where midge larvae would develop (Fig. 1B, 2B). So Don set off for Africa where visited the eastern montane South African locations (Fig. 1C) from which Brundin (1966) had described two *Archaeochlus* species. Don successfully collected and reared both species to provide the basis for descriptions of the previously unknown immature stages. Later on his circum-global trip he visited Patagonia, also very much in Brundin's footsteps and obtained fresh material of Podonominae and Aphroteniinae, and then dropped by the Natural History Museum in London, where I was then working. By chance I had recognised mandibulate female podonomines from a major collection made in Namibia (and Angola) by entomologists of that Museum. Thus Don and I started our long-term collaboration, resulting in substantially increased understanding of *Archaeochlus* (Cranston, Edward & Colless 1987). We found these flies to be more widely present in southern Africa and western Australia, and not solely limited to ephemeral waters on granite outcrop seepages such as Ameib in Namibia (Fig. 1D) - but also in sporadically flowing rivers of arid desert after heavy seasonal rains.

Seeking new material to understand better austral ('Gondwanan') Chironomidae, Don and I awaited the onset of the next wet season in Namibia, fortunately coincidental with the Willi Hennig Society meeting (#XV) in Cape Town which we linked to a subsequent meeting of 'Southern Connections' in Valdivia, Chile. Field work associated with both these meetings, and further visits to austral biomes led to a series of publications. These including the expansion of the austral subfamily Aphroteniinae, addition of a new and speciose genus *Botryocladius* to the 'gondwanan' Orthocladiinae and an early use of molecular data (Cranston, Edward & Cook 2002) to establish the monophyly and putative dating of the Australian species (now placed in *Austrochilus*) as the sister clade to the southern African *Archaeochlus*. Material collected included many rearings that continue to contribute to ongoing projects including notably *Riethia* and a most recently published *Paraskusella* (Cranston 2018) in a paper dedicated to Don and including a new species named in his honour.

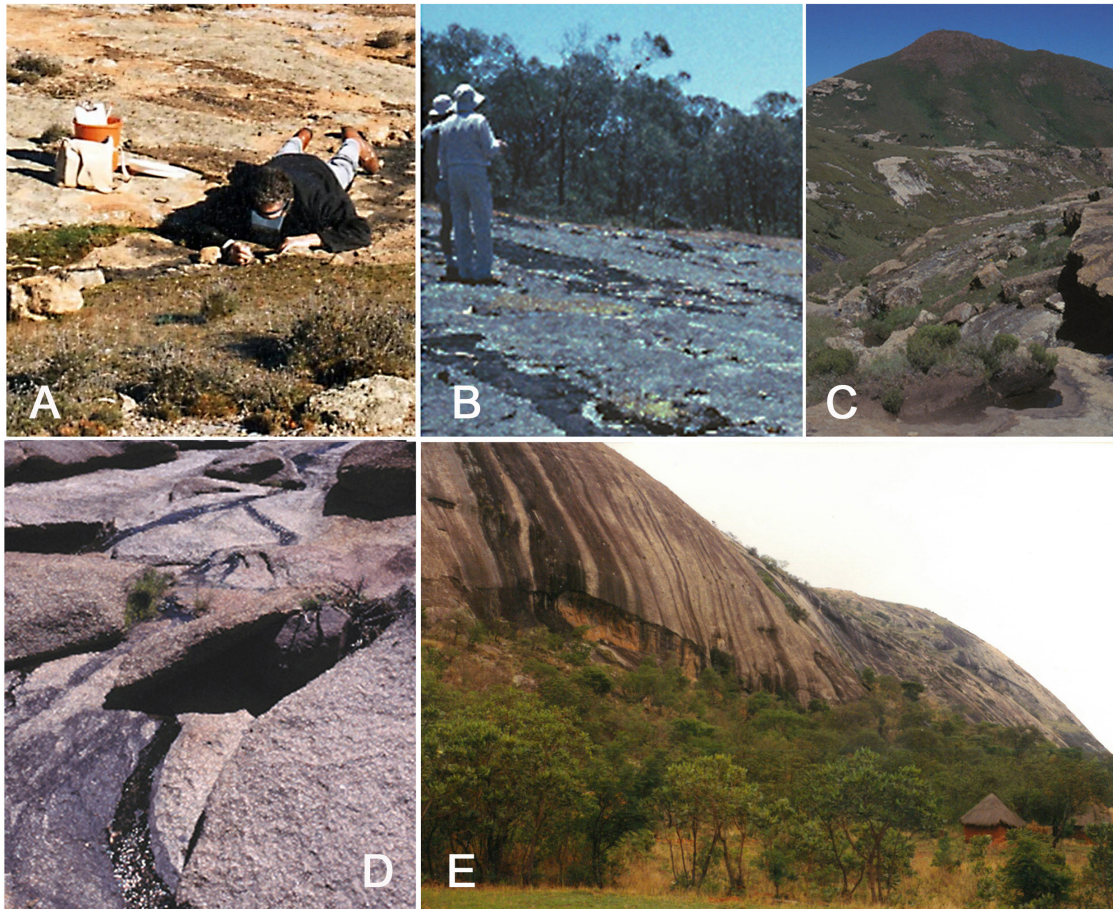


Figure 1. A. Don Edward prone, with ‘head gear’, on a Western Australian granite; B. Don and the author surveying summer-dry Yorkrakine Rock, Western Australia; C. Type locality of *Archaeochlus drakensbergensis*, Lesotho/South Africa; D. Localities for *Archaeochlus* sp., Ameib Farm, Namibia; E. Localities of *Afrochilus*, Ngoma Kurira, Zimbabwe. Photographs by Gullan and Cranston, except A. unknown.

This summary of field work and resulting publications conceals what a kindly and stalwart companion and generous and ethical colleague that Don always was. Although with ‘crook knees’ and an age disadvantage over me of a decade and a half, Don scrambled around exfoliating rocks without complaint when seeking the habitats of his favourite midges. Writing to his daughter Julie when informed of Don’s death, I recalled the ‘quick hit’ trip to seek *Afrochilus* in Zimbabwe that epitomised the man he was. This genus was known only from adults collected by Arthur Harrison and described by Paul Freeman (Cranston 2010b) as the first African podonomine midge. Arthur, another gentleman amongst chironomidologists (Cranston 2010a), had provided excellent details of ‘Ngoma Kurira’, an extensive area of granite domes an hour or so drive north of Harare (Fig. 1E, 2A). With a clapped-out rental car, potholed roads, police checkpoints and no Google maps in those days (late 1996) we headed out only with Arthur’s hand-sketched instructions. This worked well, but when we sought local permission to climb the largest dome, we were told it was too dangerous to do so alone, and we must have an armed guard to accompany us. We were not told the nature of the threat, be-it terrorism, kidnap, lions or buffalo or all of the above - but we accepted and all scrambled up the granite until a beautiful stream came into view in exactly the habitat expected for the podonomine midges that Don was so familiar with in Western Australia (Fig. 2B). Heading upstream we found *Afrochilus* in all stages, with adults emerging in front of our eyes. We were rather blasé about these events, but truthfully the conjunction of appropriate but unpredictable rains, access to the site and politics of Zimbabwe had to conjoin. Whatever, we collected all stages with Don recruiting our guard to assist (Fig. 2A). then descended with care, and paid the guard who insisted we share a beer - with the entire male community of his village. Soon we all moved from commercial beer to ‘scud’ the local sorghum beer and the scene got noisy and we had difficulty slipping away at dusk. Penny Gullan the “designated” driver was the only one capable and sober. So we had achieved our entomological objective by the end of day one (of three), but what to do next?



Figure 2. Don (part obscured) showing *Afrochlus* to the local guide, at type locality, Ngoma Kurira, Zimbabwe; B. Yorkrakine Rock, Western Australia showing dark staining of past (and future) water flow where *Austrochlus* develops; C. Bagdad Lake, Rottne Island, Western Australia; D. Don and Penny Gullan, at breakfast, Lake Street, Northbridge, Perth, 1998; E. Don Edward at ease, 2016. Photographs by Gullan and Cranston, except C, Don Bradshaw; E., unknown.

Fortunately, England had a one-day game of cricket against Zimbabwe, so Don and I learnt much about Zimbabwe cricket and politics and English cricket frailty as we joined spectators tending their braais (barbecues) in the stands, of course with more beer. Encounters with heavily armed police and army and long queues for food, and black market currency exchanges were offered: Don remained unflappably avuncular throughout.

Don was a meticulous taxonomist, truly enjoying the complete process from field to individual rearing, to slide making and even in making the required morphological measurements. His eye for a species based on morphology of any life stage was excellent, and molecular data substantially supports his concepts. He believed, and provided good evidence, that larvae usually could be identified without dissection. This is invaluable in routine biomonitoring with chironomids taken to species level, as he advocated (Edward, Storey & Smith 2000). He could do so because he first learnt the gross larval features that could be used and only then validated against selected slide mounts. Many characters held good for higher level too and I never knew him to be wrong, and never for the substantial south-west Western Australian biota. For this fauna, Don developed a coded system that became widely adopted by state agencies and students of aquatic biodiversity. With encouragement and assistance of Anna Leung and Adrian Pinder, Don contributed his expertise to photographic keys (Leung, Pinder and Edward 2011, Edward, Leung & Pinder 2013) including tabulation of associations between many coded taxa and their formal taxonomic names if available. Although codes continue to be incorporated into revisionary studies, genus-by-genus, they remain invaluable, especially in the Chironominae that are not yet covered by keys.

Don's love for the southern part of his state included a personal protectiveness over his beloved Rottne Island (Fig. 2C) where he ran regular and well-received field classes. It was his idea that the 'field trip' following the 12th International Chironomidae meeting held in Canberra should be in the west, thereby joining the Australian Society for Limnology congress on 'Rotto'. Don also loved the heathland creeks of the extreme south coast for their distinctive 'temperate Gondwanan' chironomids such as *Riethia*, *Botryocladus* and *Stictocladus*. Furthermore, Don's proprietorial interest in streams of the jarrah forests of the State encouraged many students to undertake research projects on the scientific natural history of the area.

Don was a well-regarded teacher of undergraduates across biological sciences and natural history, and trained a succession of applied aquatic biologists at graduate level. When the federal government commenced a national action plan for biological monitoring of the continent's waterways, Don and his cadre were ready to go, and to insist that they would monitor at species level, despite what 'the feds' proposed.

Another proactive side of Don was his membership and participation in the professional societies, and his encouragement of UWA students to follow his example - in attending meetings, presenting their research and in due course to assist in the running of the societies. For three decades, Don was a regular attendee at national and international meetings of the Australian Society of Limnology, the International Society of Limnology (SIL) and the International Symposium on Chironomidae, as were his collaborators, students and colleagues. Don usually could be found in a comfortable armchair (“sparing the knees, you know”) in the company of other patriarchs of the field (I am thinking of Ian Bayly, Patrick de Deckker, Sam Lake, Brian Timms and Bill Williams amongst many others), all equally at home with the applied and the theoretical. As with many researchers of Chironomidae, Don rarely considered himself (narrowly) to be an entomologist but an aquatic biologist - as did Frieder Reiss, Sepp Fittkau, Bill Coffman, Sam Roback and contemporaries - interested in the total life history and autecology of species.

It would be remiss of me not to acknowledge two other sides of Don’s life - his interest in antiques and in wines including viticulture. All his life he collected and traded antiques, and to stay in his lovely bijou cottage in Lake Street, in Northbridge in the heart of downtown Perth was to sleep in an antique store with treasures everywhere including beneath the floorboards. Don was an excellent host, even growing his own avocados (long before hipsters latched onto them), serving them at breakfast on his plant-filled yard (Fig. 2D).

Early in his career Don had a vineyard in the Swan Valley and became skilled in all aspects of the wine business. It is said that he ‘lost’ this vineyard in a divorce although I cannot vouch for this. Andrew Storey recalls a conjunction of chironomids and wine in July 1988 during the International Conference on Chironomidae in Debrecen, Hungary. This meeting allowed Don to fulfil an ambition to visit the home of Tokaj wines beloved of Don’s father, who had never managed to visit the region. Embarked on the post-conference tour that included encounters with dark-eyed Romany ladies, antiques shops, and an amazing circus act in a local night club involving flame-eating and beds of nails, after two days of travel sorely testing Don’s knees they finally arrived in Tokaj. The wine was stored in the catacombs of the local cathedral with mould ‘dripping’ off the walls. Don unexpectedly starred by assisting a struggling Hungarian translator to interpret for the local vintner the more technical aspects of making Tokaj wines. From the essential ingredient of late-ripened grapes, left on the vines into winter, to the importance of the *Botrytis cinerea* mould that concentrates sugars and flavours, to the addition of bags of raisins to enhance baumé – Don knew it all and saved the day. Conference participants were spellbound and well informed! Don liked to recount this tale with great pleasure – made all the sweeter through his impromptu role.

As a State wine show judge Don taught me much. In South Africa in the mid-90s both of us were under-impressed with many wines sampled in tasting rooms and restaurants. Don turned this into a personalised month-long wine appreciation course in asking why did I (and he) not like certain wines, thereby giving me the critical language to identify which elements fell short. With quality wines this education likely would not have transpired. With hindsight the Cabernet Sauvignon, Shiraz, Pinot Noir, Chardonnay and Sauvignon Blanc that we drank all lingered in apartheid-constrained isolation. We completely missed progressive wineries that were improving through international exchange of winemakers including graduate Australians. And we were slow to discover South African Pinotage (an endemic hybrid of Pinot Noir and Cinsault) was a wonderful full-bodied red to challenge European classics. Worse still, we overlooked Chenin Blanc, usually a cask wine but producing an excellent aromatic dry white only if pruned early of 80% of the fruit. My month of immersion in critical wine appreciation from Don was invaluable subsequently in Patagonia where we ‘discovered’ Malbec in Argentina and in Chile, Carménère, a Bordeaux classic ‘long lost’ to phylloxera in France. We had no reservations about these wines, both little known to Australians but predictably destined for international greatness.

The congruence of good wines and cool-temperate streams was obvious and our shared fondness for both nearly led to purchase of prospective grape-producing land in Manjimup, south of Perth, then known only for pome fruit, but with a climate well-matched to Bordeaux. However others would pay a premium, and my presence in Australia, albeit 3500 km to the east, was ending as an offer from the University of California arrived. Surely it was for the best - Penny Gullan and I relocated for a decade to a property in California with Italian varietal grapes already well established, and Don’s ability to prune thousands of grape vines was diminishing due to his ageing wrists.

Don Edward was a one-off, a true gentleman and scholar, with profound good humour and manners that survived into an age when these traits are scarcer. This is perhaps true in ever-more competitive academia where Don's style and breadth of interests are poorly regarded by our franchised universities. Don's relatively unheralded contributions to systematics and gondwanan biogeography are significant and important to a wider field than chironomids.

Vale Don: friend and mentor, on midges, wine and how to live a life to the full.

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