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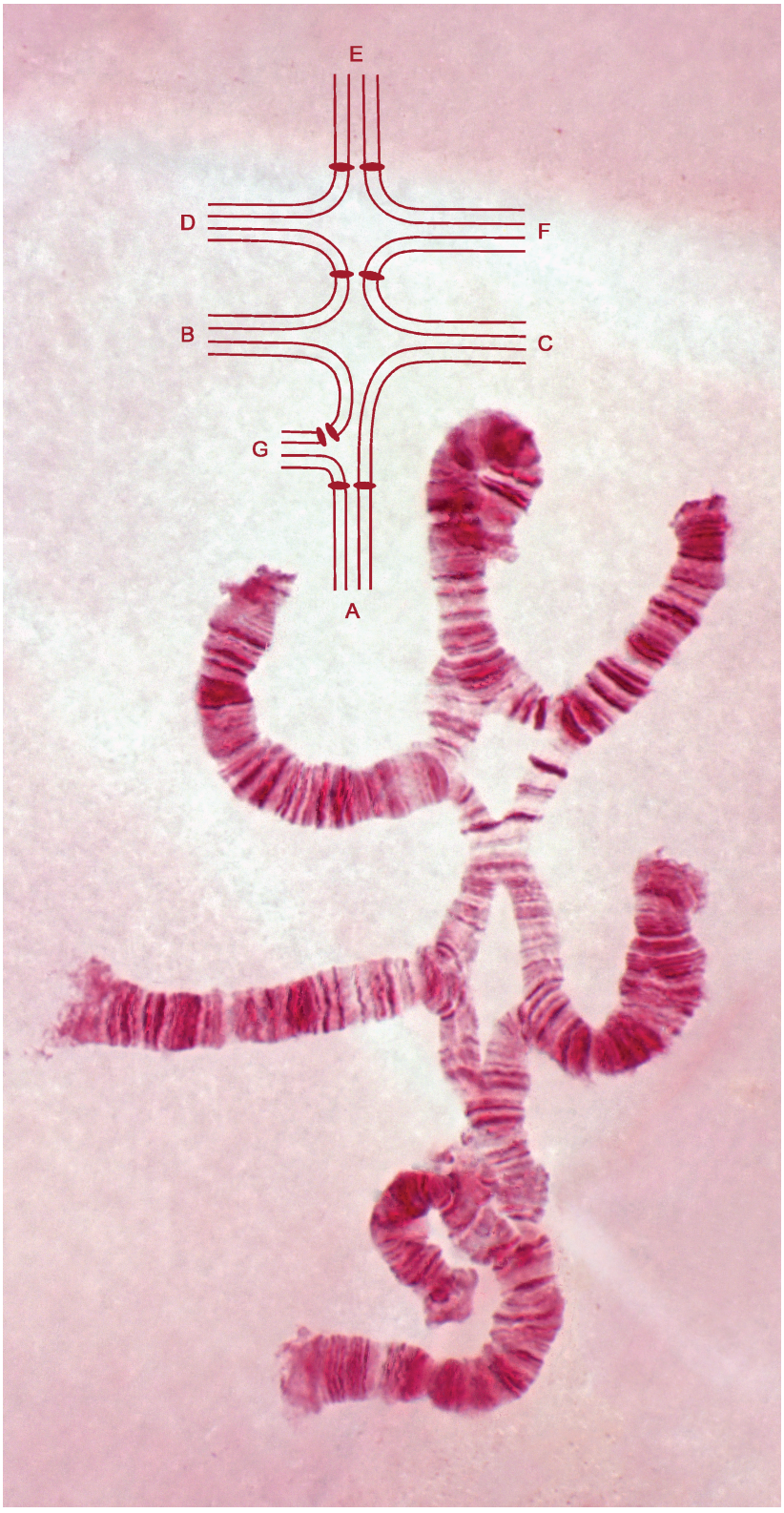

Giant chromosome ring of all eight chromosomes of *Chironomus nuditarsis*, produced by Jürg Fischer (1936-2018) by combined crossing of three midges with different translocations. Photo: Jakob Zbären, Bern.

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*).

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

Front page photo: Giant chromosome ring of all eight chromosomes of *Chironomus nuditarsis*, produced by Jürg Fischer (1936-2018) by combined crossing of three midges with different translocations. Photo: Jakob Zbären, Bern.

Editorial

A passion for Chironomidae

Halfway through my 82nd year I hope that our readers will bear with my reminiscences and observations. My mother reports that my first encounter with invertebrates was when I presented her with a live scorpion on a cushion, saying 'Look!' I cannot recollect what my mother's reaction was, but she had only recently settled in South India and had developed a deep mistrust of legless vertebrates in particular and invertebrates in general. Whatever it was, it didn't stop me looking. By 8 I was chasing butterflies around the compound with a net my mother made me from an old tennis racket. This turned into a scientific enterprise when my father found a book on Indian butterflies; I pored over the descriptions and memorized their scientific names and searched for species I hadn't previously seen as distinct. The butterflies were placed in envelopes, labelled with their binomina and full data. The collection was stored in an old tin suitcase and covered with Gammexane to keep the ants at bay while away at boarding school. Boarding school was in the jungled Palni Hills at 2000 metres. It was a paradise for a budding invertebratologist: the jungles surrounding the school in those days not only harboured elephant and tigers, but teemed with insects – the large beetles were the best fun: we kept the biggest stag beetles we could find in our desks during class time and would fight them during breaks. I would get up early in the morning and sneak out of the school grounds against regulations to shin up the lamp pole in the centre of the Seven Ways Roundabout to find what moths had been attracted overnight (half a century later I visited my old school – the lamp post has gone.). I even contemplated joining the Franciscans, primarily because of their interest in the local plants and animals rather than their ascetic lifestyle; the museum at the monastery is still there today. So it was that when the family moved to England at age 14 I was already a committed entomologist. My first headmaster in England when interviewing me asked what I saw as my future career I said 'An entomologist', to which he replied, 'Well, it's a good hobby'; I replied, 'No Sir, that is to be my life's work'. He was right in a way, but here I am still an entomologist, an amateur, yes, and as such have suffered for lack of funding, but at liberty to follow my interests unrestricted.

The butterflies in England were a disappointment: small in size and few in species. I turned to moths, but by the time I went to university I had switched to beetles; what they lacked in size, they made up for with great diversity. However, that was to change for good after I left university and took up a biology teaching post at a secondary school in the East Anglian Fens, absolutely flat, farmed to the roadside verges and criss-crossed with ditches and drains. Undeterred I took to investigating the beetles of the various waterways, but water beetles in Britain had been extensively researched and I discovered an insect group that was rich in numbers and in species, and little researched. This was the challenge I was looking for.

I started by collecting the adults and used the Royal Entomological Society's Handbook for Chironomidae, by Coe. I soon got into trouble! So I sent Paul Freeman at the British Museum Natural History a couple of specimens I was having particular difficulty with. He sent them back, named. This prompted me to send him a box of about 30 specimens, which I received back by return with a note telling me to visit the Museum where I would be given facilities to name them myself, having access to the collections, the library and Paul Freeman's guidance. After that beetles had only cursory attention: I was hooked! Strange; Lars Brundin told me he too had come to Chironomidae via the Coleoptera. I threw myself into the literature on Chironomidae and discovered that the pupal exuviae were identifiable to species: what a great way of finding what species inhabited a ditch, pond, lake, stream or river by just collecting the floating pupal exuviae! However, many of the specimens I collected were not identifiable using the published keys and descriptions of Thienemann, Lenz, Pagast and others. A more comprehensive key was needed, which I set out to produce, through rearing larvae to adult. The technique I developed used plastic boxes 10x7x4cm with transparent lids, of the sort used to sell cut comb honey. 4mm depth of tap water ensured a large surface to volume, which, together with the large volume of air enclosed, allowed sufficient oxygenation of the water for over a week without removing the lid. The pabulum I used was the mulm (mainly fish excrement) from the bottom of a well-stocked tropical fish tank, which I sterilized with boiling water before adding to the water; only a small squirt from a pipette provides sufficient food for the completion of development. 350 containers, each with 1 larva were stacked on shelves in the utility room and checked morning and night for emerged adults, or dead pharate adults. Any adults were pooted out and the pupal exuviae and dead pharate adults (and when obvious, the larval exuviae) were transferred to vials containing 70% isopropanol until mounting on microscope slides in Euparal, the pupal exuviae on the same slide as the adult. The containers were washed

and restocked with a single larva and replaced on the shelf. Species from high mountain streams to lowland lakes generally completed their development in these containers at about 20^0 C.

This was continued for seven years as part of an external Ph.D. carried out in my spare time as a school teacher. From the Ph.D. 'A key to the Pupal Exuviae of British Chironomidae' was compiled. Not being part of the establishment, publication was rejected, so I borrowed money to publish it myself (1984). I sold the books at cost price and the 200 were all sold, so I got my money back from the venture. At the International Symposium in Bergen (1985), Ole Sæther invited the delegates to a soirée at his home. I was standing, glass in hand, chatting, when approached by Frieder Reiss, who said 'Come'. When Frieder said come, you come! I followed him to the far end of the room where in the corner was seated 'Sepp' Fittkau . Pointing to a chair opposite Fittkau, Frieder said 'Sit', so I sat. 'Dr. Fittkau and I think you should produce a key

like the one you produced for Britain for the West Palaearctic.' I responded by excusing myself: I was a full time school teacher, I hadn't the resources to travel the Continent…. I was stopped 'You must do it', said Frieder. 'You will come to Munich where the collection will be made available to you and space for you to work. There is no funding [What's new?] but you must do it'. So that led to three glorious visits to Munich, where Fittkau vacated his room for me to work in and Frieder supplied me with tray after tray of slides to work on. I was loaned a key to the museum, arrived at 8 and left at 8 each day. I was allowed to borrow specimens so that I could continue work at home. The result was 'A Key to Pupal Exuviae of West Palaearctic Chironomidae' (1991). Once again, I found I had to publish it myself – an extension of my house mortgage was arranged to fund it. In 2001 Henk Visser of the Biodiversity Centre of ETI in Amsterdam contacted me with the offer of turning the 1991 Key into a CD ROM. I agreed, providing we could update it. To this he agreed and the CD ROM was published in 2003. Since then the upgrade of Microsoft windows has rendered it inoperable, but an IT friend has managed to produce a copy that works for both Microsoft and Mac.

In 1995 I had the opportunity to summarize my knowledge of the pupal stage of the Chironomidae in 'The Chironomidae', edited by Armitage, Cranston and Pinder. In 1997 or thereabouts I was asked by the Freshwater Biological Association whether I would update the 'Key to Chironomidae (Adults)' (1978) as it was then out of print and out of date, in collaboration with the author of that work, Clive Pinder. I had, of course, a wide knowledge of the adult males, because the identity of pupal exuviae required identification of the adults reared. This took ten years and was published in 2007. It took that long, because there were frequent additions to the British fauna and nomenclatural changes that required rejigging the keys that had been developed.

Shortly after the publication of the West Palaearctic key I was contacted by Bill Coffman asking if I would turn his massive collection of pupal exuviae into a key for Nearctic species. This resulted in three visits to Pittsburgh and Pymatuning, where we trawled his collections for distinct (and often, not so distinct) morphotypes, with sporadic field excursions to satisfy a basic need of two inveterate collectors! I was invited to Minnesota by Len Ferrington to trawl his collection as well. I set about the Nearctic key with a will, but the momentum dropped when, sadly, Bill died prematurely of pancreatic cancer in 2013. I owe it to Bill and my other Nearctic colleagues to finish this work, if spared, and at present it is making good progress.

I live in Northern Ireland, a watery land with lots of lovely midges. I have just completed a long-term phenology/biodiversity project here, which, I suppose, may never be published. It's completion leaves me free to concentrate on the Nearctic key. I continue to be blessed with a steady hand and reasonable eyesight, enjoy collecting (you can't beat water for enhancing the visual natural environment), looking (yes, still looking!) at these fascinating beasts, informing others through publications and drawing the diagrams to accompany those submissions (I cannot agree more with Alyssa Anderson's views on the value of line drawings (CHIRONOMUS editorial 2013)).

I value the acquaintance of many colleague-friends across the world. COVID-19 and progressive age related physical deterioration willing, I shall see you all in Japan in one and a half year's time. I look forward to that!

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Description of the larvae of *Cricotopus festivellus* (Kieffer 1906) and *Cricotopus diversus* (Boesel 1983) with keys to discrimination of larval, pupal and adult stages (Diptera: Chironomidae)

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Abstract

The larva of two very similar *Cricotopus* species are described for the first time: *Cricotopus diversus* (Nearctic) and *C. festivellus*, new description (West Palaearctic). Confusion can arise depending on the source used for identification of Nearctic *Cricotopus*. The key of LeSage and Harrison (1980) treated adults and exuviae of *C. diversus* as variants of *C. festivellus*. Subsequently Boesel (1983) formally described *C. diversus* and included it in keys to adult *Cricotopus* of the eastern United States. Adults of these species have been distinguished by consistent differences in the pigmentation on the fourth and fifth abdominal tergites; we also confirmed differences in the structure of the male hypopygium. Keys to larvae, pupal exuviae and adult males are presented. Publicly available DNA barcode records document *C. diversus* populations in the Mid-Atlantic US and Ontario, Canada, whereas barcode records of *C. festivellus* were available only for Scandinavia, although this species is widely distributed in Western Europe. These two species are genetically distinct, with 13% mean difference in barcode sequence between species. Both species are reported from rivers and lakes of relatively good water quality.

Introduction

The genus *Cricotopus* van der Wulp 1874 is large, with 218 species distributed widely across most biogeographic regions. It is not surprising given this level of diversity that *Cricotopus* species differ in microhabitat preference and their tolerance to pollution (Haase and Nolte 2008; Moller Pillot 2013, Krosch et al. 2015). *Cricotopus* larvae can be difficult to identify to species, and some are even difficult to distinguish from *Orthocladius* larvae without associated rearing of pupae or adults (Epler 2001, Cuppen and Tempelman 2018). Hirvenoja (1973) revised the genus for the western Palaearctic, but similar treatment of Nearctic *Cricotopus* is lacking and additional species await description (Epler 2001). The status of some closely related pairs of Nearctic and Palaearctic *Cricotopus* species have been subject to debate (Sublette 1964, Oliver 1977, Boesel 1983, Gresens et al. 2012). LeSage and Harrison (1980a) described "*C. festivellus*" from Southern Ontario but noted discrepancies in pigmentation pattern compared with the diagnosis in Hirvenoja (1973). Subsequently, Boesel (1983) considered this variation in his decision to describe *C. diversus* as a distinct Nearctic species, distributed from Michigan and Ohio (including western Lake Erie) to New York and Delaware. The descriptions in LeSage and Harrison (1980a) match those of our adult and pupal *C. diversus*, and we suspect that these were the same species.

Such taxonomic ambiguity complicates bioassessment of water quality, although application of DNA sequence data (i.e., "DNA barcoding") promises to facilitate identification of chironomid larvae (Ekrem et al. 2007, Failla et al. 2016) by referring to a "barcode library" of sequence data from adults and pupae which have been identified based on morphological criteria. Nevertheless, larvae of some species still remain to be associated with their adult and pupal life stages. Here we describe the larval stages of two very similar *Cricotopus* species: Holarctic *C. festivellus* (Kieffer) and the Nearctic endemic *C. diversus* (Boesel) and use DNA barcoding data to compare the genetic distance between these species.

Neither Hirvenoja (1973), LeSage and Harrison (1980a) nor Boesel (1983) described the larval stage. The ability to identify chironomid larvae is needed to connect their tolerance to environmental stressors. In western Europe tolerance values for *C. festivellus* remain unclear because these larvae may have been confused with species of the *cylindraceus* group, as well as with other members of the *festivellus* species group: *C. albiforceps* and *C. flavocinctus* (Moller Pillot 2013). Similarly, there

has been confusion over species' ranges in the Nearctic: LeSage and Harrison (1980a) included *C. festivellus* in their descriptions of *Cricotopus* species from a stream in Ontario, Canada, however their work pre-dated Boesel's (1983) description of *C. diversus* from Lake Erie. Although both authors noted subtle differences in color pattern in the adult form of the Nearctic species compared to Palaearctic *C. festivellus,* both species are listed as occurring in the Nearctic (Ashe and O'Connor 2012).

Materials and Methods

Cricotopus festivellus material was collected in the Netherlands and Norway. In the Netherlands, material originated from a non-natural stream, a moorland pool, a lake and a ditch with seepage. Norwegian specimens were collected from lakes and a pond near Trondheim (details in Table 1), and now reside in the collection of the Norwegian University of Science and Technology (NTNU) University Museum, Trondheim. *Cricotopus diversus* larvae were collected from Baismans Run, a small second-order stream in a forested catchment within Oregon Ridge Park, Baltimore County, Maryland (MD), USA (Fig. 1A, Table 1).

Larvae of *C. diversus* were gently removed from rocks bearing attached algae and water moss, and reared in individual aerated jars at 12:12 (L:D) photoperiod and temperature regime of 18℃: 15℃ (D:N). Larvae were fed with epilithic algae from Baismans Run (Table 1) and water changes were conducted weekly. Jars were inspected daily for emerged adults and associated pupal exuviae. Two legs from each adult were used to obtain COI nucleotide sequence data from the Canadian Centre for DNA Barcoding, following their standard procedures (Ratnasingham and Hebert 2007). These barcode data are available from the Barcode of Life Datasystems (www.boldsystems.org). Specimens were prepared by clearing adults with proteinase K; the adult exoskeleton was dissected and slide-mounted in Euparal with the associated pupal and larval exuviae. Use of morphological terminology follows Sæther (1980).

Molecular genetic data provides an independent line of evidence which complements morphological discrimination of species. Sequence data for the cytochrome oxidase (COI) gene, i.e., "DNA barcodes" were previously obtained for larval *C. diversus* and the Norwegian *C. festivellus* specimens by the Canadian Centre for DNA Barcoding and are publicly available from BOLD, the Barcode of Life Datasystem v4 (www.boldsystems.org; Ratnasingham and Hebert 2007). As part of its goal to expedite the use of barcodes in description and enumeration of species diversity, BOLD maintains a Barcode Index Number (BIN) System, in which an clustering algorithm is used to cluster specimens with similar barcodes (starting

Table 1. Location details of studied material. LA = 4th instar larva; PU = pupa; PEX = pupal exuviae. HC = Hub Cuppen, RW = Rink Wiggers, SG = Susan Gresens, TE = Torbjørn Ekrem

Species	Stage	#	Location	Nearest town	Country	Water type	Lat.	Long.	Coll. date	Leg.
Cricotopus diversus	LA, PU	5,7	Baismans Run	Cockeysville, MD	USA	small forest stream	39.4795	-76.6917	11-Sep-10	SG
Cricotopus diversus	PEX	6	Chimney Branch	Reisterstown, MD	USA	small forest stream	39.4062	-76.8589	1-Jul-02	SG
Cricotopus festivellus	LA, PU	1,1	Grift	Apeldoorn	NL	unnatural stream	52.2117	5.9651	30-Mar-05	HC
Cricotopus festivellus	LA	1	Landweerven	Enschede	NL	moorland pool	52.2367	6.9347	18-May-09	HC
Cricotopus festivellus	LA	1	Veluwerandmeer	Biddinghuizen	NL	lake	52.4154	5.7179	6-Oct-16	RW
Cricotopus festivellus	PEX	1	Den Dulvert	Waspik	NL	ditch with seepage	51.6880	4.9736	4-Aug-92	HC
Cricotopus festivellus	Adult ♂ NO73	1	Lake Målsjøen	Klæbu, Trøndelag	NO	lake	63.2460	10.4374	30-May-11	SG, TE
Cricotopus festivellus	Adult ♂ NO60	1	Lake Målsjøen	Klæbu, Trøndelag	NO	lake	63.2460	10.4374	30-May-11	SG, TE
Cricotopus festivellus	Adult ♀ NO63	1	Bymarka, Blomstertjønna	Trondheim, Trøndelag	NO	upland lake	63.4193	10.2614	31-Jul-11	SG
Cricotopus festivellus	Adult ♂ NO56	1	Ringve botaniske hage	Trondheim, Trøndelag	NO	pond	63.4489	10.4532	24-Jul-11	SG

at about 2% sequence variation) into operational taxonomic units, each identified by a unique code, its BIN. A test of the correspondence of BINs with traditionally defined species in large datasets of well-studied taxa was very high, finding that 89% of BINs corresponded exactly with described species (Ratnasingham and Hebert 2013). In order to compare the genetic diversity within and between species, we accessed the "Public Data Portal BIN Page" on BOLD for BOLD:AAP5924 (*C. diversus*) and BOLD:AAV1707 (*C. festivellus*). A BIN page includes a record list of all specimens in that BIN which are registered on BOLD, with information on their taxonomy, collection location and depository, and links for download of sequence data. The BIN for *C. festivellus* is based on specimens in the collections of the Swedish Museum of Natural History and the NTNU University Museum.

The MEGA7 package (Kumar et al. 2016) was used to compare genetic distances within and between species. FASTA files containing the COI sequences for each BIN were downloaded from BOLD. Alignment of the combined sequences by nucleotide was carried out in MUSCLE; mean genetic distances within species and between *C. diversus* and *C. festivellus* were subsequently calculated.

Taxonomy

***Cricotopus festivellus* Kieffer, 1906:18**

Cricotopus (Cricotopus) festivellus (Kieffer), Hirvenoja, 1973:225

Description of larval *C. festivellus*: based on 4th instar larvae (n = 3). Measurements of bilaterally symmetric structures are reported as mean values per specimen. Head capsule width 334-344 µm (n = 2).

Head capsule yellow, sometimes proximal part light brown. Postoccipital margin dark brown. Bifid S1 setae with branches of similar size; S2 seta simple (Fig. 2A). Antennae 5 segmented, Lauterborn organs well-developed and extending 2/3 – 3/4 the length of antennal segment 3. Total antennal length 85 – 90 µm. Antennal blade extends to last segment, accessory blade half that length. Antennal ratio 1.9-2.1, mean 2.0 (n = 3). Pecten epipharyngis composed of 3 scales. Mandible and 3 inner teeth, brown extending to molar area with wide pale base (Fig. 2A), length 136 µm. Outer margin of mandible smooth. Seta subdentalis yellow, ca. 2 times long as wide with a notched asymmetric tip which reaches the last free mandible tooth. Seta interna with 6 branches. Premandibula simple. Galea of maxilla with two or three rows of pectinate lamellae.

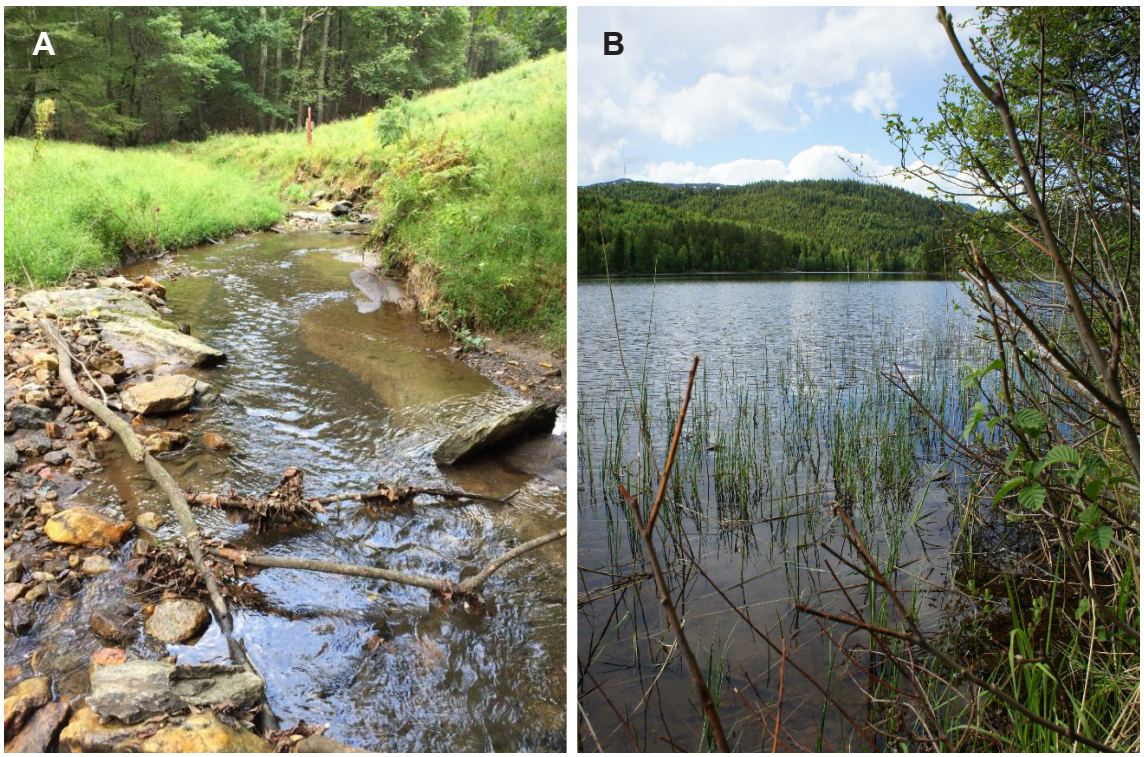

Figure 1. A) Baismans Run (USA) where larvae of *Cricotopus diversus* were collected. B) Lake Målsjøen (Norway) where larvae of *Cricotopus festivellus* were collected.

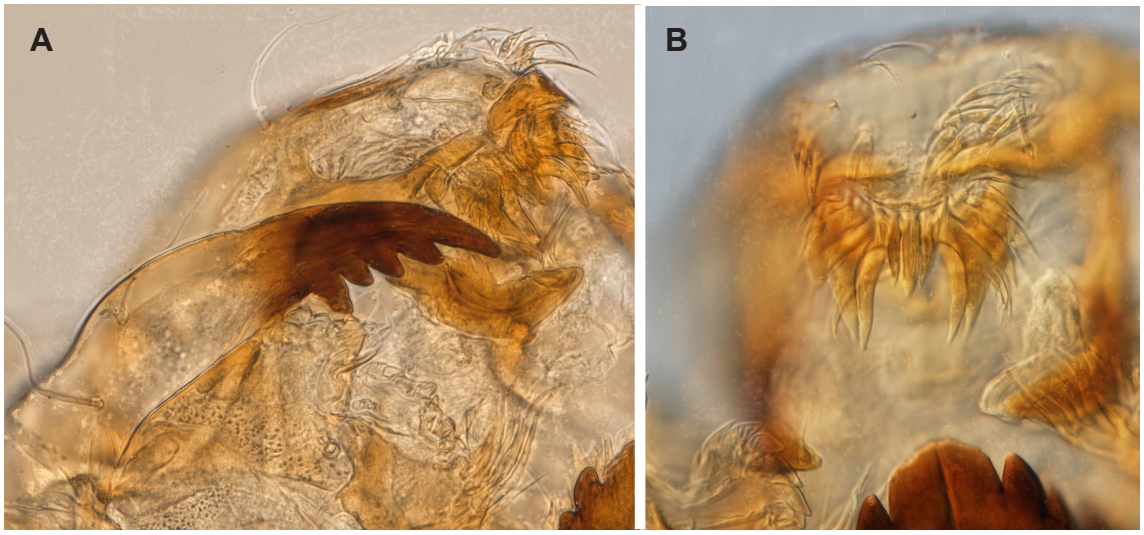

Figure 2. A) *Cricotopus festivellus*; mandible and premandible. Enschede, Landweerven, 18 May 2009. B) *Cricotopus festivellus*; epipharynx and S1. Veluwerandmeer, 5 October 2016.

Mentum with 6 brown lateral teeth; median tooth projecting forward and at most 3 times wider than first lateral tooth; MR (width of median tooth/ width first lateral tooth ranges from 2.7-3.0, mean 2.85 (n = 3). VM-plate reaches1st lateral tooth. Submental setae located at the level of the fourth lateral tooth of the mentum.

Middle-sized claws of the anterior parapods with inner teeth at most half as long as apical tooth (Fig. 6B). Abdominal segments 1 - 6 bear posterolateral setal tufts of 12-24 filaments. Length of tufts from 70 – 240 µm, reaching up to half to ¾ the length of the segment. Setal tufts on segment VII bear 8-10 setae which are shorter than the setae on I - VI. (Fig. 3).

Procercus wider than long, bearing a sclerotized scale and 5-6 apical setae plus 2 fine lateral setae. Supra-anal setae 78 – 83 µm, as long or slightly longer as the anal tubules.

Pupa: The pupa of *Cricotopus festivellus* is described by Hirvenoja (1973) and Langton (1991). Exuviae light brown, characterized by frontal setae located on prefrons, 4 lateral setae on segment VIII with L4 not larger than the other lateral setae. Thoracic horn distally pointed and covered with small spinules. Pedes spurii B obvious on segment II but absent or weak on III. Tergite II usually with an extensive area of small spinules, variable, but at always at least bearing a transverse band of spinules anterior to the hooklet row. Tergites III-VI with median and posterior transverse bands of small points separated, but usually joined laterally, leaving a conspicuous median bare patch in the region of the posterior muscle marks.

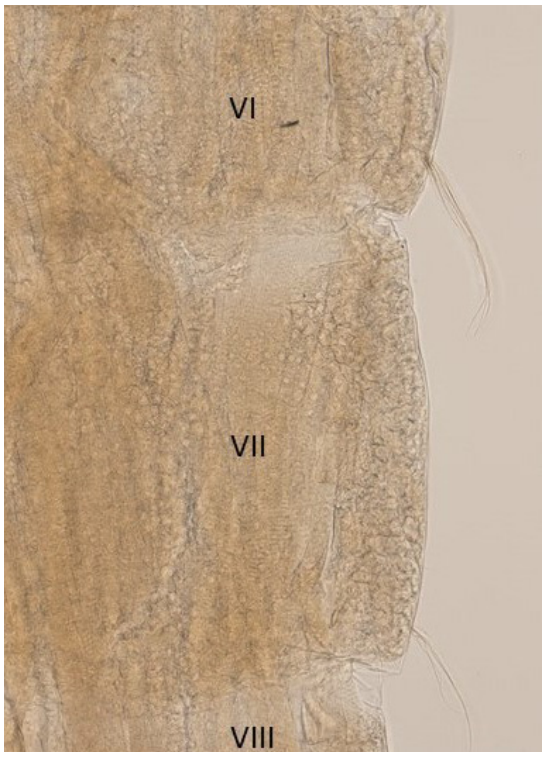

Figure 3. *Cricotopus festivellus*; lateral tufts on segment 6 and 7. Veluwerandmeer, 5 October 2016.

Specimens examined: NTNU University Museum: 200820 (NO56), 200824 (NO60), 200826 (NO63), 200831 (NO73); H. Cuppen personal collection: 3 larvae, one with associated pex, 1 pex (Table 1).

DNA barcodes (BOLD Process IDs): BSCHI522-17, BSCHI708-17, BSCHI731-17, CHRFI512-11, CHRFI729-11, CHRSV514-11, CHRSV515-11, CHRSV517-11 = NO56, CHRSV521-11 = NO60, CHRSV524-11 = NO63, NEACH003-12 = NO73.

***Cricotopus diversus* Boesel, 1983:85**.

nec LeSage and Harrison 1980:94, *C. (s.s.) festivellus*

Description of larval *C. diversus*: based on 4th instar larval exuviae (n = 5) with total length 3.5-4 mm. Measurements of bilaterally symmetric structures are reported as mean values from both sides of specimen. Head capsule light golden, head capsule width = 369 µm (n = 4). Bifid S1 setae with branches of similar size; S2 seta simple. Antennae 5 segmented, Lauterborn organs well-developed and extending 2/3 the length of antennal segment 3. Antennal blade extends to last segment, accessory blade half that length. Antennal ratio (AR = basal segment/distal segments) 1.3-1.6, mean 1.41 (n = 4). Pecten epipharyngis composed of 3 subequal scales. Mandible with 3 inner teeth, color brown extending to molar area with wide pale base. Outer margin of mandible smooth. Seta subdentalis grey, ca. 2 times long as wide with a notched asymmetric tip with one half blunt and the other half produced to a sharp point. Seta interna with 6 branches. Premandible simple. Galea of maxilla with no more than 2 rows of pectinate lamellae along the base of the scale-like marginal lamellae. Mentum with 6 brown lateral teeth; median tooth strongly projecting forward and at least 3 times wider than first lateral tooth MR (width of median tooth/width first lateral tooth ranges from 3.0- 3.8, mean 3.53 (n = 5). The median tooth, first and second lateral teeth are slightly grey compared to the brown lateral teeth. VM-plate reaches only 2nd lateral tooth. Submental setae located at the level of the basal corners of the mentum.

Large claws of the anterior parapods with robust inner teeth at least three quarters as long as apical tooth (Fig. 6A). Abdominal segments 1 - 6 bear posterolateral setal tufts of 10-26 filaments. Length of tufts from 108 – 200 µm, up to half the length of a segment. Setal tufts on segments I and VI bear fewer and shorter setae. Procercus wider than long, bearing a sclerotized scale and 5-6 apical setae plus 2 fine lateral setae.

Pupal exuviae of *C. diversus*: Measurements are mean values (n = 10) unless stated otherwise. Length 3.5-4 mm, general color yellowish, tergites darker, sternites colorless. Frontal warts absent. Frontal setae fine, located on prefrons, 64 µm. Thoracic horn (TH, Fig. 4) length 106 µm, L/W 9.9. Two median antepronotal setae: 153 and 130 µm long. Three precorneal setae: 160, 143, 124 µm, distinctly longer than TH. Notum weakly granular along eclosion line.

Abdomen with Pedes spurii B on segment II, but absent on segment III. Pedes spurii A on sternites 4-6. Hooklet row on tergite 2 with 37-58 hooklets, mean = 47; hooklet row covers 0.42 of width of segment (580 µm). Armament of abdominal tergites (T) as follows: TI bare, TII may bear a very small number of fine points immediately anterior to hooklet row, or points absent. TIII with 2 patches of strong spinules: distinct median and posteromedian patches may merge around a central oval clear area. TIV and TV similar: more extensive median and posteromedian spinule patches are clearly merged, or with small median clear oval (Fig. 8A). TVI spinule patch less extensive; may appear as 2 distinct patches. TVII and TVIII similar: 2 anterolateral patches of fine spinules, less extensive on TVIII. Lateral setae distributed as

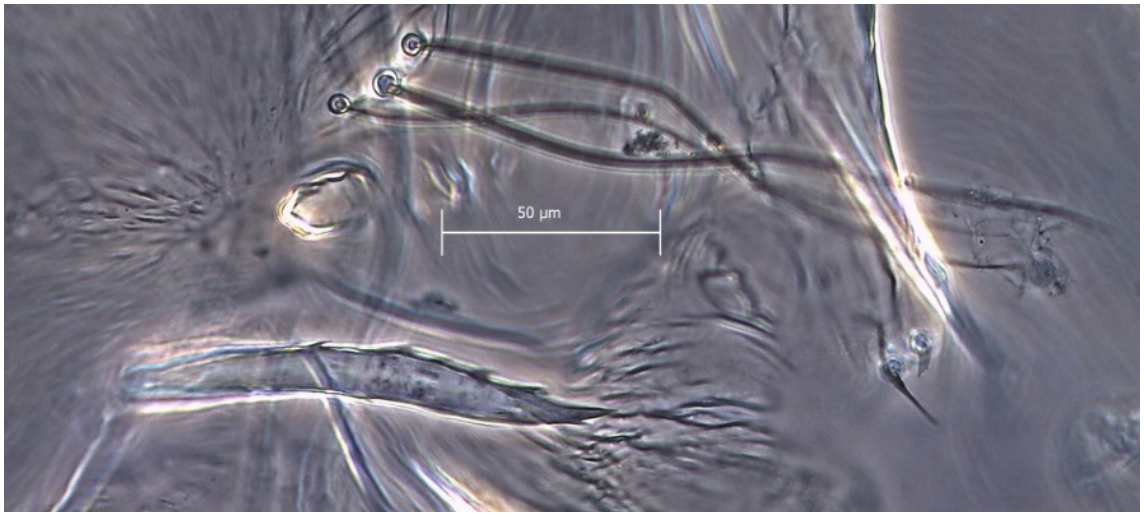

Figure 4. *Cricotopus diversus*; respiratory organ and precorneal setae (400x). Oregon Ridge Park, Baismans Run, 11 September 2010.

follows: one seta on segment 1 and 3 on segments 2-7. Segment 8 bears 4 L setae, with L4 no larger than L3. Sternites SI and SII without fine spinules. SIII and SIV with very fine spinules primarily on lateral areas of sternite. SV and SVI with 2 anterolateral patches of fine spinules and a posteromedian patch. SVII with 2 anterolateral patches of small spinules whereas SVIII bears fine spinules only in lateral areas. Anal lobe slightly wider than long: mean length 199 µm, width 223 (n = 7). The three anal lobe macrosetae of equivalent length, 172 µm (n = 11).

Re-description of adult *C. diversus*, based on holotype, paratypes and MD specimens:

Male: Head and antennae brown, Antennal ratio (AR) 1.4 (Boesel 1983) to 1.49 (MD, n = 3). Thorax brown with lighter humeri; vittae, and notum darker brown to black. Scutellum dark brown with an irregular row of fine bristles. Leg ratio (fore-tarsal segment 1/fore-tibia) = 0.62 (Boesel 1983), LR = 0.61 (MD). Foreleg brown, except for tibia: basal 15% brown, medial white ring and distal 30% brown. Mid and hind legs with brown femur, mid-tibia may have an indistinct light ring, otherwise as hind tibia: light brown darkened distally, tarsi light brown. Wing light brown, halteres yellow. Abdominal TI white/bright yellow. TII highly variable among both the paratypes and MD specimens, ranging from yellow to black, often yellow-white with a brown band covering roughly the middle third of TII. The incisures between TII-TIII and TIII-TIV narrowly light; TIII brown; TIV light on posterior 70-75% length of tergite, anterior brown; TV anterior 30- 40% white with posterior brown. TVI, TVII, TVIII brown with incisures slightly lighter. Hypopygium white. Holotype and most of the slide-mounted paratypes are mounted intact in lateral view, largely obscuring details of the genitalia. Based on three paratypes, the inferior volsella is broad basally, apically bluntly rounded and bending posterior. In two specimens a small triangular spur is present at base of inferior volsella. In MD specimens, the inferior volsella tapers gradually from a broad base to a more conical rounded tip, also bending posterior (Figs 11B, 11C, 12). Gonostylus with strong crista dorsalis.

Female: (based on allotype, paratypes and MD specimens): Antennae with preapical bristle; terminal flagellomere slightly longer than the 3 preceding flagellomeres (i.e., AR = 1.12, n = 4). Thorax brown to dark brown, humeri lighter; vittae, notum darker brown. Scutellum brown with 1 row of fine bristles. Femurs apically brown to black, lighter at base. Fore-tibia brown with median white ring covering half of segment; fore-tarsi brown; LR = 0.58 (Boesel 1983), LR = 0.57 (MD). Mid and hind legs with light tibiae slightly darker distally mid tibia may bear an indistinct light ring; tarsi light brown. Typically, TI, TV and TVI are light yellow/white but may be brownish in dark specimens. Tergite 5 is most consistently light, although it may be brownish in dark specimens (Fig. 10B). Sternites yellow, SIV-SVIII with medial spine patches. Spermathecae oval with ducts posterior; ducts with anterior "S" bend otherwise straight.

Specimens examined: Peabody Museum of Yale University, M. Boesel collection: holotype ♂ + paratype ♂, allotype ♀ and 3 additional ♂ paratypes Put in Bay, Ohio, USA, 21 June 1946; paratype ♀ Oxford, Ohio, USA, 4 June 1978, ♂ paratype - hypopygium mounted separately, Put in Bay, Ohio, USA, 30 June 1924; pinned paratypes 68 ♂♂ and 37 ♀♀ dates span 1925-1976, locations include OH, Michigan, New York, Delaware and Pelee Is. Canada (details in Boesel 1983).

Canadian National Collection of Insects, Ottawa: DRO 32.4-50 1 ♂ + pex Green Creek Ontario 19 June 1967; Towson University Entomology Museum (Barcode Specimen IDs): SEG46 ♀, SEG47♀, SEG48♂, SEG49♀, SEG50♀, SEG51♂, SEG52♂ Baismans Run, Oregon Ridge Park, Baltimore, USA, 11. Sept. 2010; CHIM830 3 pex Chimney Branch, 1 July 2002.

Keys to distinguish *C. diversus* and *C. festivellus* from morphologically similar species

Larvae, numbering adapted from Epler (2001)

16. Mentum with median tooth very wide: 4-6 times as wide as 1st lateral.....*C. flavocinctus*

16'. Mentum with median tooth less wide, 2.8-3.5 times as wide as 1st lateral….....….....16A

16A. Mentum with median tooth projecting strongly forward having a bluntly pointed apex and somewhat angular sides (Fig. 5A) ……..…….. 16B

16A'. Mentum with median tooth not projecting far forward, apex more broadly rounded (dome-shaped) (Fig. 5B) …..…….....……. 17

16B. AR 1.3-1.6; galea of maxilla with no more than 2 rows of pectinate lamellae; medium-size claws on anterior parapods with terminal tooth only slightly longer that the next inner tooth; claw – index* 1.6……...……..…. *C. diversus*

16B' AR 2.0-2.5 galea of maxilla with 3-4 rows of pectinate lamellae; medium-size claws on anterior parapods with terminal tooth much

longer that the next inner tooth; claw – index 3.7……..... *C. cylindraceus*

17. Setal tufts on abdominal segments either absent or reduced, < 50 µm …..... *C. politus*

17'. Setal tufts more developed, about 80- 240 µm in length…….....……18

18. L4 hairs at least half the length of the segment; VM plate reaching first lateral tooth. *C. festivellus*

18'. L4 hairs about 1/4th to 1/3rd of the length of the segment ……..…… 19

19. Galea of maxilla with numerous pectinate lamellae set in 2 or more rows; medium-sized claws of anterior parapods with terminal tooth distinctly longer (ca. twice) than the next inner tooth; basal antennal segment L/W about 3; claw-index 1.9-2.0 …..…. *C. albiforceps*

19'. Galea of maxilla with pectinate lamellae absent or a few set in a single row; medium-size claws on anterior parapods with terminal tooth only slightly longer that the next inner tooth; basal antennal segment L/W about 2; claw-index 1.4….. ……..…. *C. vierriensis*

*Claw-index = ratio length terminal tooth and penultimate tooth of the medium sized claws of the anterior parapods (Fig. 6).

Pupae, numbering adapted from Simpson et al. (1983)

9. Pedes spurii B well developed on abdominal segments II and III …..... *C. cylindraceus* gr.

9'. Pedes spurii B well developed only on abdominal segment II, weakly on III (*C. festivellus* gr.) 13

13. Thoracic horn present ….....…… 14

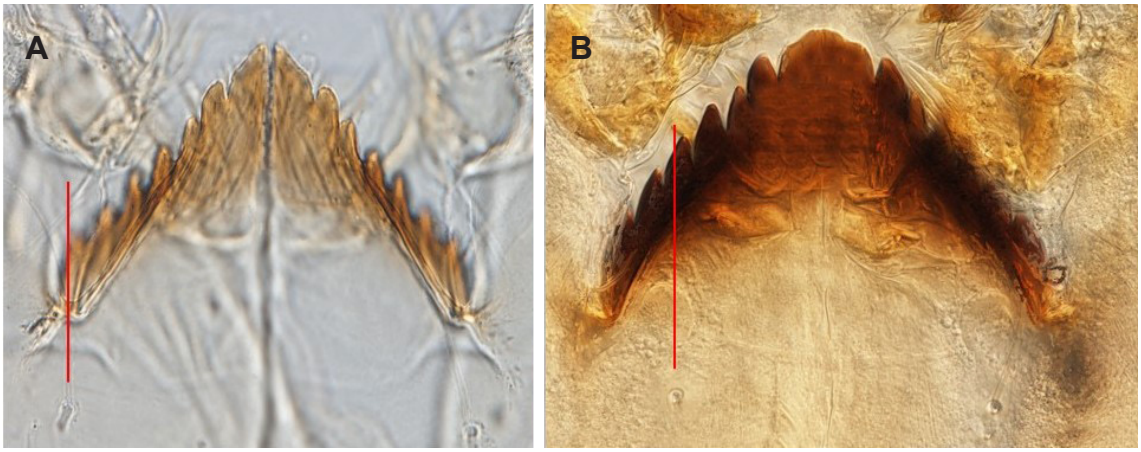

Figure 5. A) *Cricotopus diversus*; mentum with SSm-setae. Oregon Ridge Park, Baismans Run, 11 September 2010. B) *Cricotopus festivellus*; mentum with SSm-setae, position relative to lateral teeth indicated by red vertical line. Veluwerandmeer, 5 October 2016.

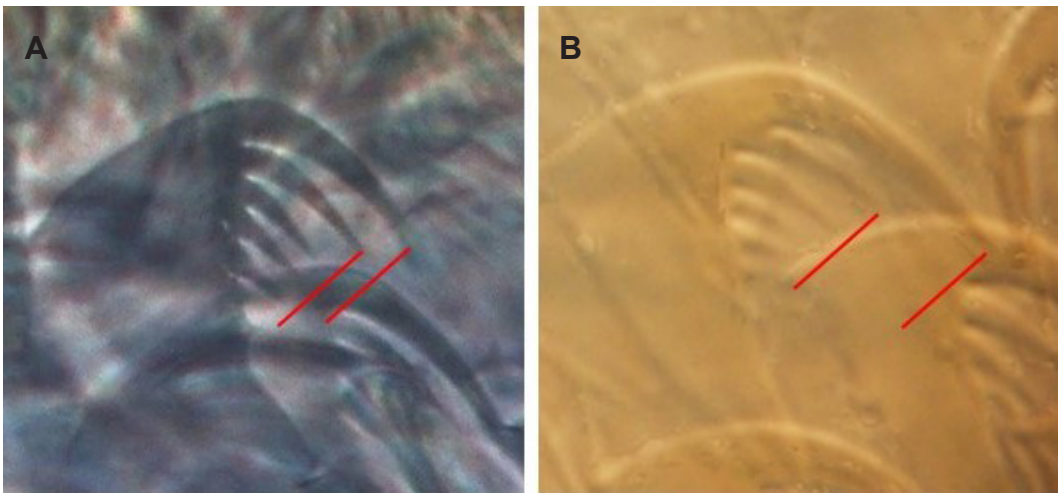

Figure 6. A) Middle sized claws of anterior parapods of *Cricotopus diversus*. Oregon Ridge Park, Baismans Run, 11 September 2010. B) Middle claws of anterior parapods of *Cricotopus festivellus*. Veluwerandmeer, 5 October 2016.

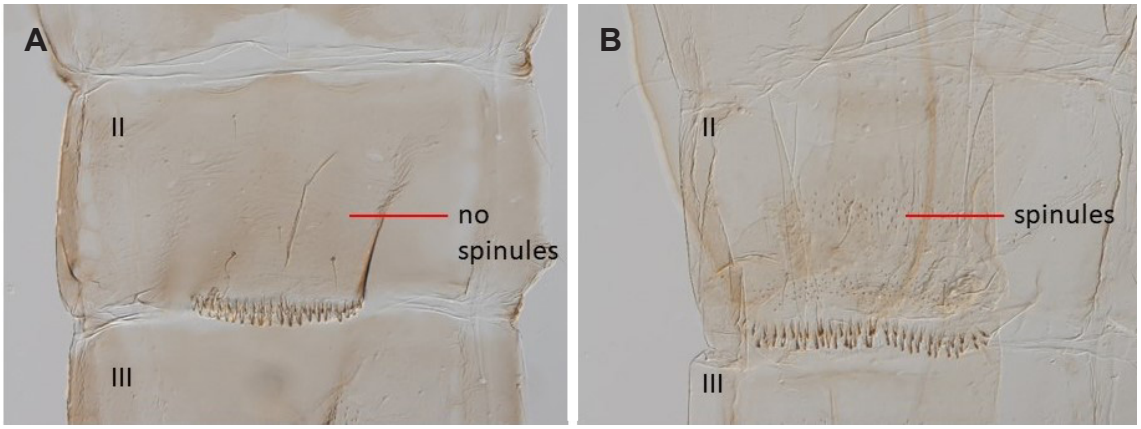

Figure 7. A) *Cricotopus diversus* pupal exuviae segment 2. Oregon Ridge Park, Baismans Run, 11 September 2010. B) *Cricotopus festivellus* exuviae segment 2. Sprangcapelle Waalwijk, 4 August 1992.

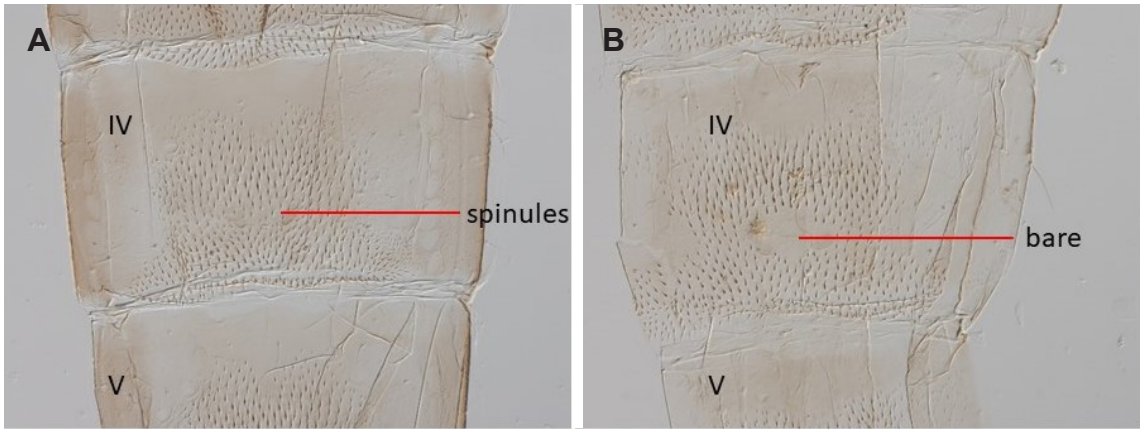

Figure 8. A) *Cricotopus diversus* exuviae segment 4. Oregon Ridge Park, Baismans Run, 11 September 2010. B) *Cricotopus festivellus* exuviae segment 4. Sprangcapelle Waalwijk, 4 August 1992.

13'. Thoracic horn absent ..... *C. flavocinctus*

14. Shagreen on TIV distinctly separated into anterior and posterior fields (i.e., point bands) ...…..... .....……*C. albiforceps*

14'. Shagreen on TIV not separated into separate fields …….…..…….. 15

15. TII with extensive spinule patches or at least a transverse row of spinules (Fig. 7B); TIV median and posterior spinule bands joined laterally, leaving a conspicuous median bare spot in shagreen field (Fig. 8B) ….....…….. *C. festivellus*

15'. TII bare, or with a few isolated spinules anterior to hooklet row (Fig. 7A); TIV median and posterior spinule fields more broadly joined, with or without a small oval median bare spot (Fig. 8A)…. ..... *C. diversus*

Adult males

1. TIV with yellow-white band covering posterior 65-75% of tergite; TV with yellow-white band on anterior 30-40% of tergite (Fig. 9); inferior volsella of gonocoxite broad basally, tapering, bluntly rounded apically and bending posteriomedially… ……..…….*C. diversus*

1'. TIV with yellow-white band covering posterior 30-40% tergite; TV with white or light brown band on anterior 20-30% of tergite (Fig. 10A) inferior volsella of gonocoxite medially broadened (truncated) with apex directed posteriorly *C. festivellus*

Discussion

Genetic evidence supports *C. diversus* as a species separate from *C. festivellus*. Comparison of COI-5P sequence data from 11 *C. festivellus* collected in Norway and Sweden with that of the BIN containing the 7 specimens of *C. diversus* and 7 specimens from Canada showed a mean within-group distance of 0.2% for *C. festivellus* and 1.1% for the Nearctic *C. diversus* group. In contrast, the mean between-species distance was 13%.

Cricotopus diversus larvae were reared and the associated adult males and females have been compared with Boesel's (1983) description and the *C. diversus* type series. Adult male *C. diversus* are distinguished from other *Cricotopus* species

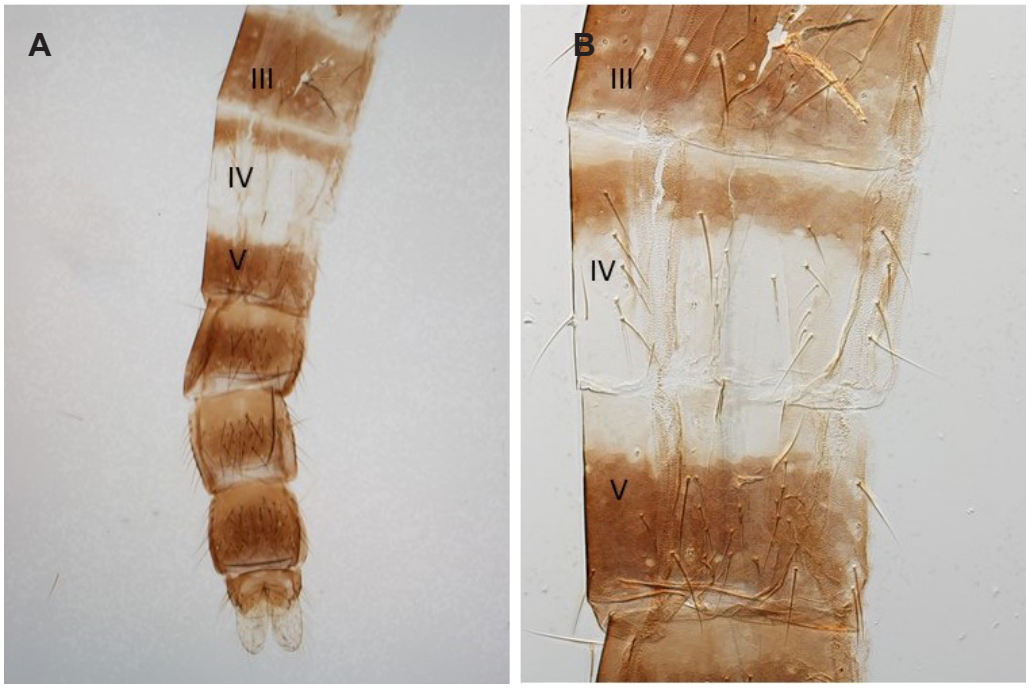

Figure 9. A) *Cricotopus diversus* male abdominal segments 3-8. Oregon Ridge Park, Baismans Run, 11 September 2010. B) *Cricotopus diversus* male, detail segment 4 and 5 (same specimen as in Fig. 9A).

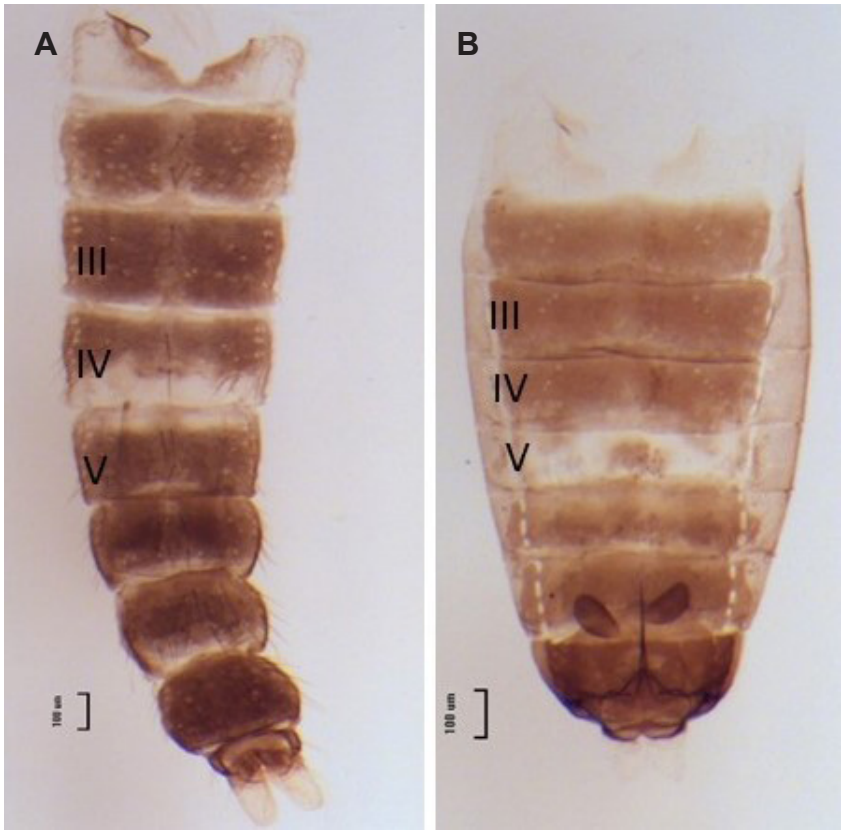

Figure 10. A) *Cricotopus festivellus* male. Målsjøen, Klæbu, 30 May 2011. B) *Cricotopus festivellus* female. Blomstertjønna, Bymarka, Trondheim, 31 July 2011.

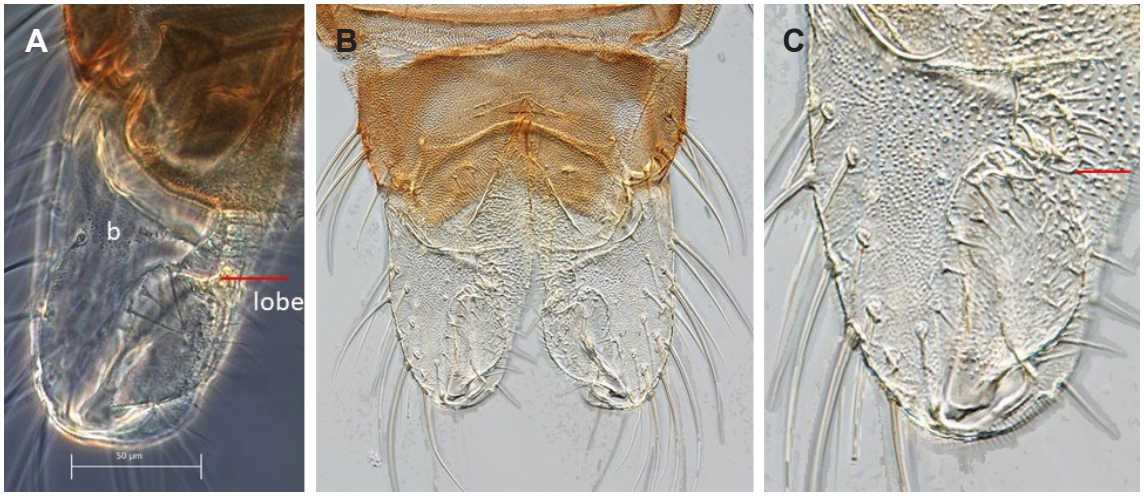

Figure 11. A) *Cricotopus festivellus* hypopygium. "b": gonocoxite; pond, Ringve Botanical Garden, Trondheim, 24 July 2011. B) *Cricotopus diversus* male hypopygium; Oregon Ridge Park, Baismans Run, 11 September 2010. C) *Cricotopus diversus* male hypopygium; arrow: inferior volsella of gonocoxite. Same specimen as in B.

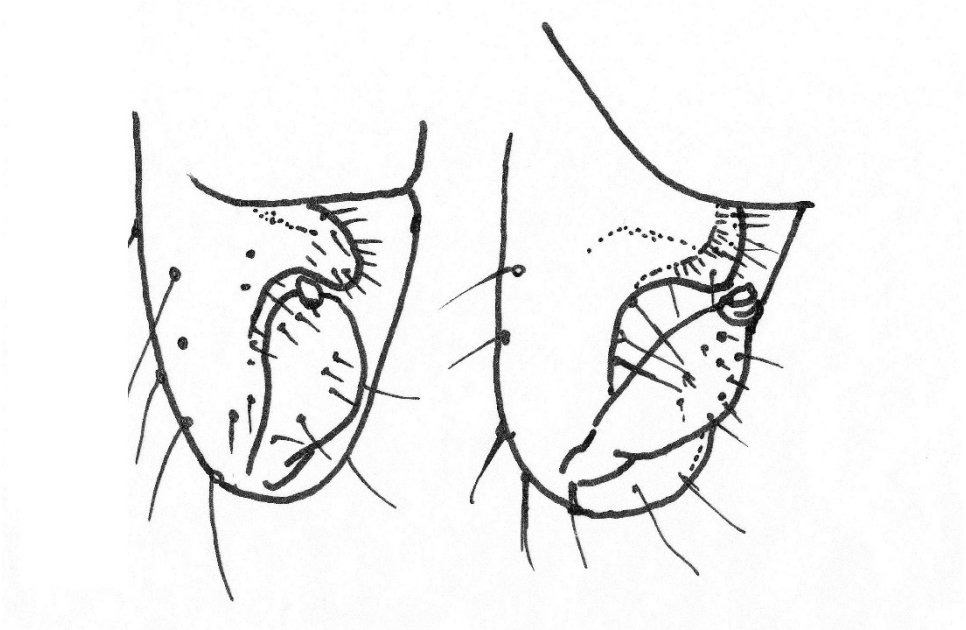

Figure 12. Comparison of inferior volsellae of *C. diversus* (left) and *C. festivellus* (right). Same individuals as Fig. 11.

of eastern North America by the blackish-brown (fuscous) abdomen, except for tergites 1, 4 and 5. Tergite 1 is completely yellow-white, whereas the posterior 2/3 to ¾ of tergite 4 is white, with only the anterior ¼ of tergite 5 white. Tergite 2 is variably darkened, and the incisures between tergites 2 and 3 may bear narrow light bands. Examination of the type series, both slide-mounted and pinned, show this pattern of pigmentation to be very consistent.

The pattern of pigmentation on tergites 4 and 5 of the adult male distinguishes *C. diversus* from *C. festivellus* and other members of the *festivellus* species group (Hirvenoja 1973). In *C. festivellus*, only the posterior ¼ of tergite 4 and the anterior ¼ of tergite 5 are white (Fig. 10); in contrast, tergite 4 of *C. diversus* is largely white with only the anterior ¼ to 1/3 darkened (Fig. 9). Both species have tergite 1 completely white. In addition, the male hypopygium differs in the form of the inferior volsellae, which in *C. festivellus* bend posteriorly, but have a more angular, flattened apex than *C. diversus* (Figs 11-12). In *C. festivellus* the base of the inferior volsellae may bear a slight bifurcation (Hirvenoja 1973) which was observed in two *C. diversus* paratypes.

Identification of adult *Cricotopus* species also relies on the male hypopygium, especially the inferior volsella. Boesel (1983) noted that the angle of view affected the shape of the inferior volsella, and thus his Fig. 8 is ambiguous: the diagram on the left resembles that of *C. festivellus* whereas that on the right matches our *C. diversus*. The holotype and most of the paratypes were slide-mounted intact in lateral view, thus the inferior volsella was either obscured or difficult to examine. However, the hypopygium of one paratype had been dissected and mounted dorsally: this is represented in Fig. 8, diagram on the right (Boesel 1983) and it matches that of our specimens. We conclude that the inferior volsella of *C. diversus* is simple, the broad base tapering to a rounded apex which bends posterio-medially. The base of the inferior volsella is both broad and thick, becoming thinner at the tip, which sometimes is slightly bent out of the plane of focus, thus making the inferior volsella appear slightly angular, but not to be confused with *C. festivellus*.

Female *C. diversus* differ in coloration from males: tergites 1, 5 and 6 may be totally white, although T1 and TVI may be somewhat darkened. The antenna bears an apical bristle. Spermathecae are ovoid with S-curved ducts. Female *C. diversus* appear similar to female *C. festivellus* as figured in Hirvenoja (1973) with tergites 5 and 6 white. We have not found features that consistently distinguish female *C. diversus* from *C. festivellus*.

We found that larval *C. diversus* are most easily distinguished from *C. festivellus*, by the lateral placement of the submental setae: they are placed in line with the outside of the sixth mental tooth in *C. diversus*, whereas they are placed more medially, in line with the fourth or fifth mental tooth in *C. festivellus* (Fig. 5). All other members of the *festivellus* species group have 3 or 4 rows of pectinate lamellae on the galea of the maxilla (Hirvenoja 1973), whereas *C. diversus* has at most two rows of pectinate lamellae.

Cricotopus festivellus can be separated from other (Palearctic) species in the *festivellus* species group, as follows: *C. flavocinctus* has a broader central mental tooth; the median-sized claws of the anterior parapods have a longer subterminal tooth in *C. cylindraceus*. *Cricotopus festivellus* can be distinguished from *C. albiforceps* and *C. vierriensis* by having longer L4 setal tufts (Cuppen and Tempelman 2018).

Did LeSage and Harrison (1980) actually find *C. diversus*? We examined the single pupal-adult male association of *C.* "*festivellus*" (from Green River, Ontario Canada) produced from their study, and confirmed their description of the exuviae. Key features match our description of *C. diversus*: frontal setae on prefrons, ~ 40 µm long; pointed thoracic horn covered with spinules, dimensions184 x 16 µm. Weakly granular along the dorsal eclosion line, pedes spurii B on abdominal segment 2, but weak on segment 3. The distribution of lateral abdominal setae matches that of *C. diversus*: 1 L-seta on segment 1, 3 L-setae on segments 2-7, and 4 L-setae on segment 8. The anterior and posterior spinule patches on tergites III-IV are weakly joined laterally, leaving an oval median bare patch which could be either *C. festivellus* or *C. diversus*. However, tergite II was bare except for a very narrow band of minute spinules just anterior to the hooklet row, which identifies *C. diversus*. Pigmentation of the associated adult male is consistent with *C. diversus*: T1 light, the anterior 40% of TII light, narrow light incisures TII-TIII and TIII-TIV, posterior 70% of TIV and the anterior 35% of TV are light. The inferior volsella was very clearly flattened-conical, tapering and curving postero-medially. Based on this specimen as well as the descriptions in their paper, we conclude that LeSage and Harrison (1980) actually found *C. diversus* in Southern Ontario streams.

Information provided by the BIN system in BOLD shed more light on the degree of relatedness and geographic distribution of these species. Barcode data for *Cricotopus* specimens in BIN AAP5924, which includes *C. diversus* and other specimens collected in Ontario, Canada (Centre for Biodiversity Genomics) show a within-BIN genetic distance of 1.1% suggesting these are the same species. Specimen records in BIN AAV1707, all identified as *C. festivellus* from Norway and Sweden, had a within-BIN genetic distance of only 0.2%, versus a 13% distance with the Nearctic "*diversus*" BIN. Nearctic *C. diversus* have clearly differentiated from west Palaearctic *C. festivellus*.

Ecological notes

Cricotopus festivellus is widely distributed and rather common in Western Europe (Moller Pillot, personal communication; Murray et al. 2018). It inhabits slowly flowing and standing waters with clean water such as lakes (Fig. 1B), large pools in the coastal dune area, ditches in regions with peaty soils and poorly buffered moorland pools.

Cricotopus diversus larvae were found in a small forest stream, in a reach where the forest canopy had been removed for a gas pipeline (Fig. 1A) and the increased light supported visible algal growth (primarily diatoms with some filamentous green

algae) and higher diversity of chironomids than in the adjacent forested reaches of the stream. Although down-cutting of the stream channel and fine sediment deposition was obvious, the water quality of Baismans Run was very good: Gresens and Ferrington (2010) studied chironomid emergence at a site 350 m downstream and measured an average of 8.4 µg/L total phosphorus, 2.1 mg/L nitrate and 144 µS conductivity. A total of 75 chironomid species was observed at this downstream forested reach, based on an 8-month survey of chironomid pupal exuviae (Gresens and Ferrington 2010). Pupal exuviae were also collected from Chimney Branch, another small forested stream (Table 1) with good water quality: 13 µg/L total phosphorus, 1.2 mg/L nitrate and 290 µS/cm conductivity.

LeSage and Harrison (1980b) presented a detailed ecological study of 15 *Cricotopus* species in Salem Creek, Ontario. The stream was enriched by runoff from pasture and row crops, but remained well-oxygenated, with stable cobble-gravel substrate encrusted with marl that supported seasonally abundant algae (diatoms and *Cladophora)*. Here, the preferred microhabitat of *C. "festivellus*" was in a pool with a hard substrate and a thin layer of detritus; the species was absent from nearby rivers impacted by urban and industrial pollution (LeSage and Harrison 1980b). In the Mid Atlantic US, *C. diversus* was found in small woodland streams of good water quality. Canadian BIN records on BOLD point to collections from both lakes and riffle areas of rivers in forested areas and parks. Mating swarms of *C. diversus* were observed in western Lake Erie in first half of the 20th century (Boesel 1983). More recently, Failla et al. (2015) found larvae of *C. bicinctus* (pollution tolerant) and larvae of an unidentified *Cricotopus* sp. in the same region, perhaps reflecting the decline in water quality of Lake Erie. It appears that *C. diversus* prefers lower water velocity and is restricted to situations of moderately good water quality.

Illustrations

Fig. 1, 4, 6A, 10, 11A and 12: S. Gresens; Fig. 2, 3, 5, 6B, 7-9,11B-C: H. Cuppen and D. Tempelman.

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Oropuella, a new genus of Orthocladiinae from the western Nearctic

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

Abstract

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

Introduction

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

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

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

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

Materials and Methods

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

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

Results

Oropuella new genus

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

Type species: *Oropuella eidolon* sp. n.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Head. Dorsomedial eye extension weak. Five temporal setae.

Thorax. Acrostichals ending just before midpoint of scutum.

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

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

Pupa. Fig. 4. Habitus pale.

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

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

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

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

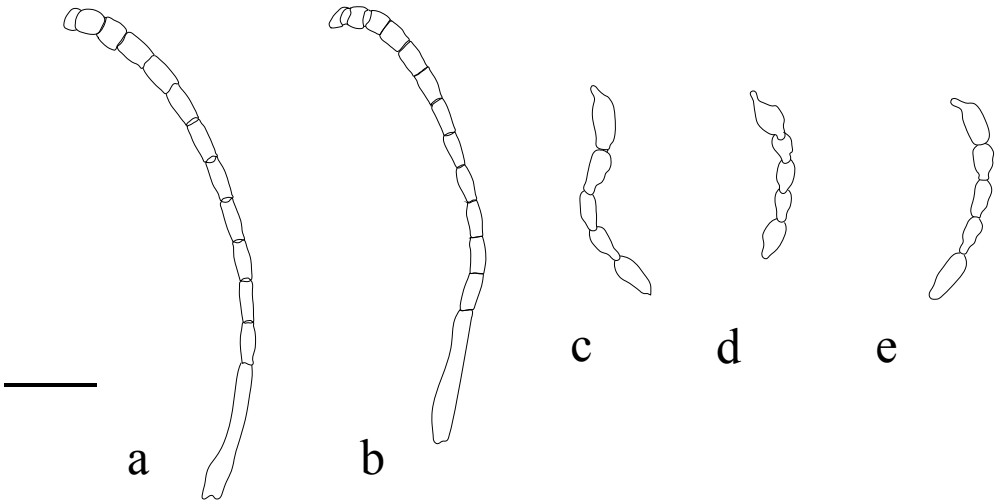

Figure 1. Adult antennae. Male antennae: a) *Oropuella eidolon* n. sp.; b) *Oropuella pallida* n. sp. Female antennae: c) *Oropuella* sp. F1; d) *Oropuella* sp. F2; e) *Oropuella* sp. F3. a), b) and d) exhibit some foreshortening due to the position of the antenna on the specimen; setae omitted; scale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

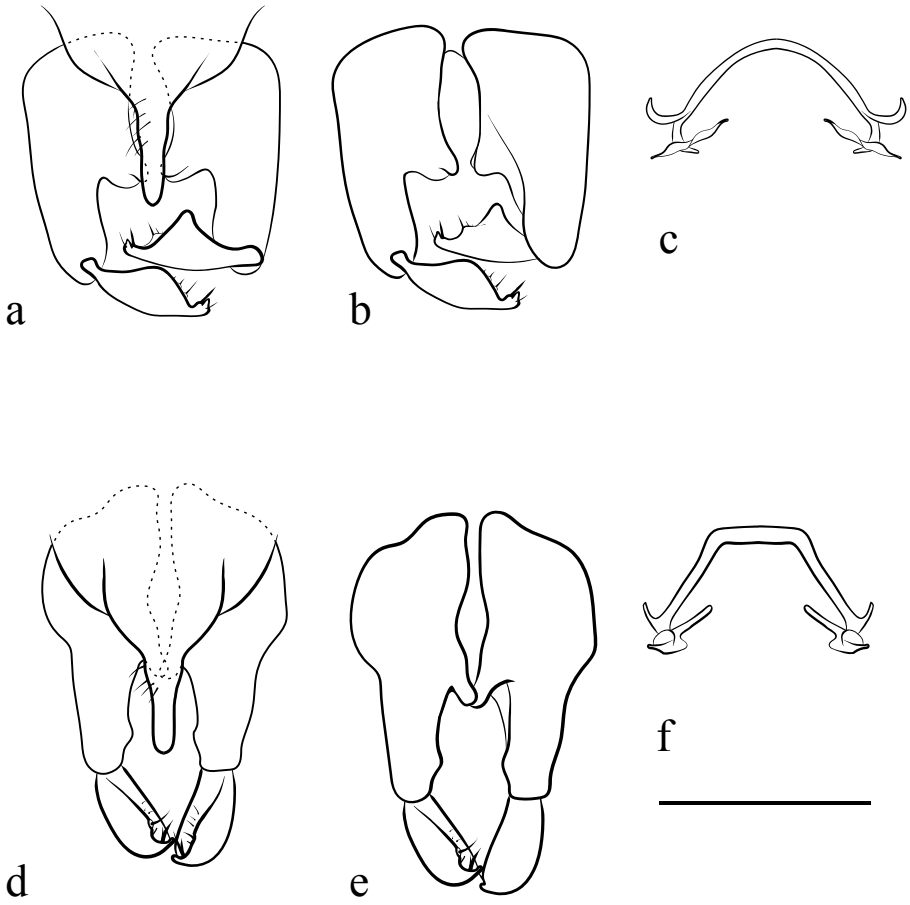

Figure 2. Male genitalia. a–c *Oropuella eidolon* n. sp.: a) anal point and gonopods; b) left gonopod in dorsal view, right gonopod in ventral view; c) sternapodeme and phallapodemes. d–f) *Oropuella pallida* n. sp.: d) anal point and gonopods; e) left gonopod in dorsal view, right gonopod in ventral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

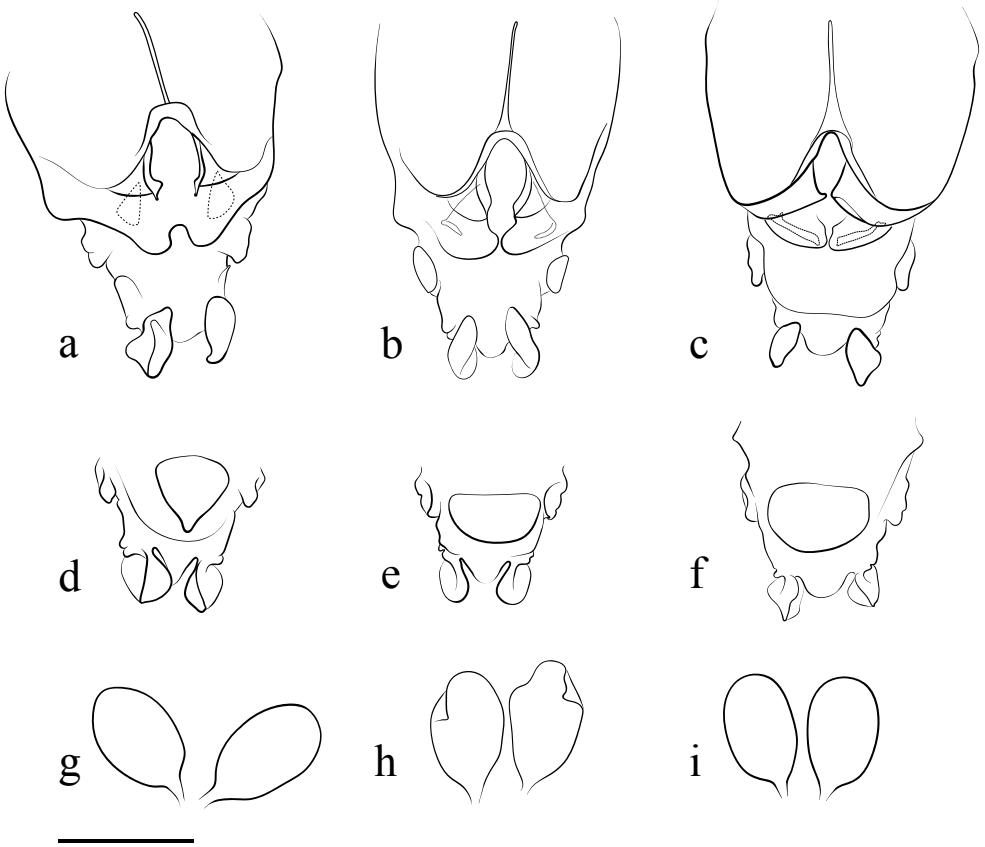

Figure 3. Female genitalia. a–c overall dorsal view: a) *Oropuella* sp. F1; b) *Oropuella* sp. F2; *Oropuella* sp. F3. d–f overall ventral view: d) *Oropuella* sp. F1; e) *Oropuella* sp. F2; f) *Oropuella* sp. F3. g–i spermathecae: g) *Oropuella* sp. F1; h) *Oropuella* sp. F2; i) *Oropuella* sp. F3.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

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

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

Remarks

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

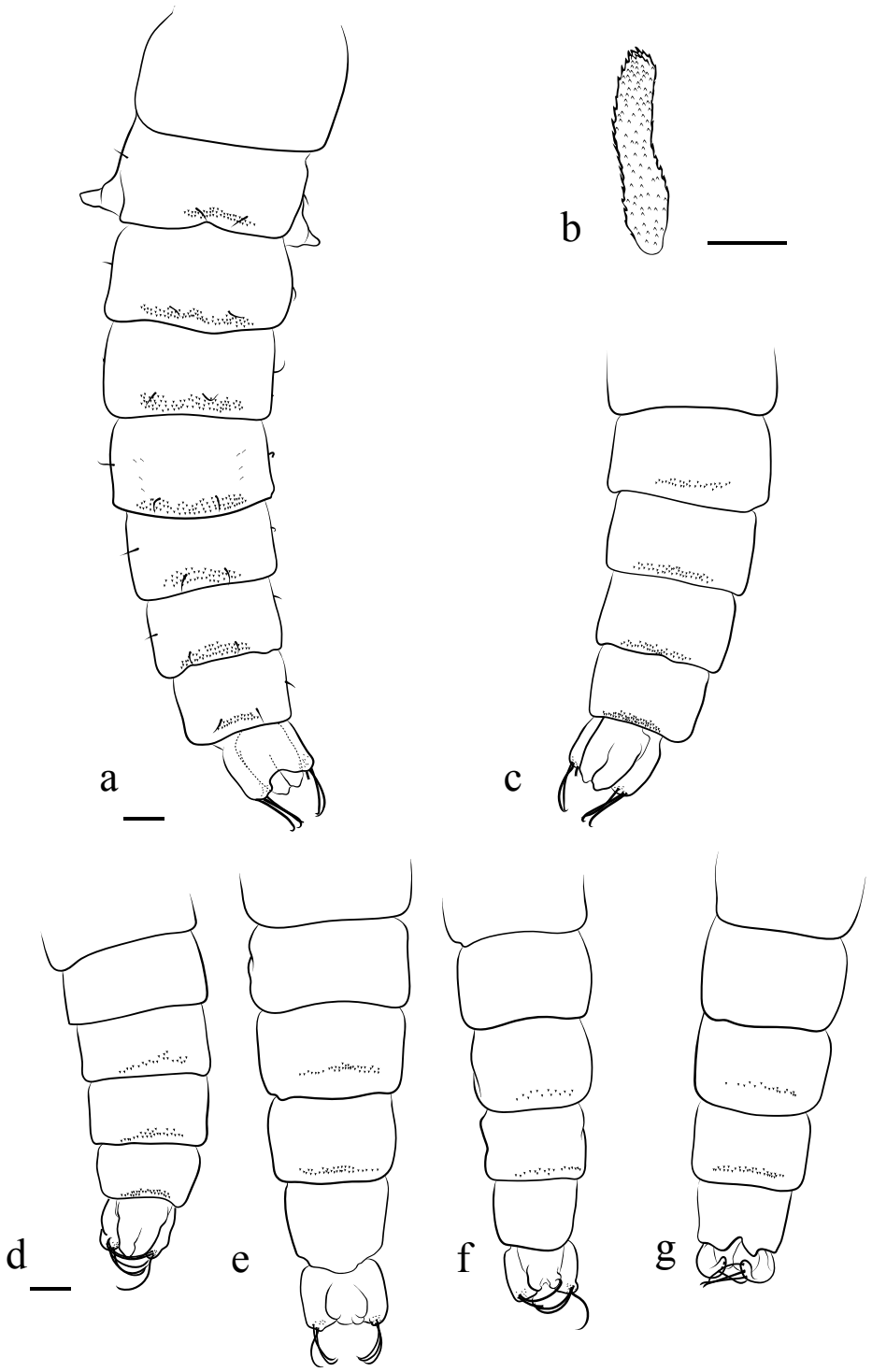

Figure 4. Pupae. a) *Oropuella eidolon* n. sp. complete abdomen, dorsal view; b) *Oropuella* sp. F2 thoracic horn; c) *Oropuella eidolon* n. sp. apical abdominal segments, ventral view; d) *Oropuella pallida* n. sp. apical abdominal segments, ventral view; e) *Oropuella* sp. F1 apical abdominal segments,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

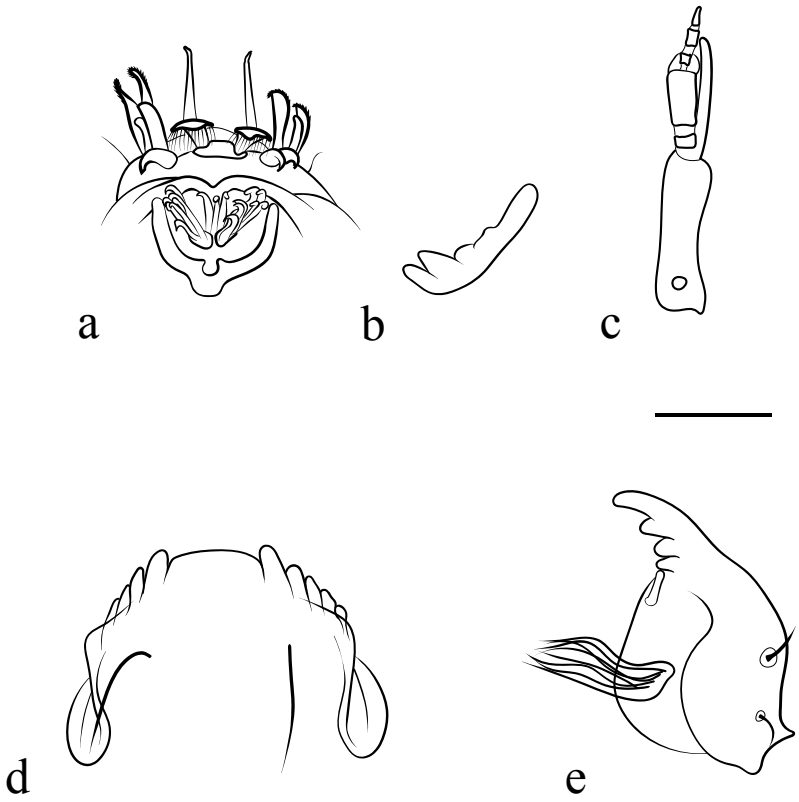

Figure 5. Larval head. A) labral setae; b) premandible; c) antenna; d) mentum and ventromental plates; e) mandible. Scale bar = 25 µm.

Key to the adult males of *Oropuella*

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

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

Key to the adult females of *Oropuella*

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

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

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

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

Key to the pupae of *Oropuella*

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

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

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

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

***Oropuella eidolon* new species**

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

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

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

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

Pupa. Sternites V–VIII with posterior spinule band.

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

with lighter maculations around abdominal setae.

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

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

***Oropuella pallida* new species**

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

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

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

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

Pupa. Sternites VI–VIII with posterior spinule band.

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

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

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

***Oropuella* sp. F1**

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

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

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

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

***Oropuella* sp. F2**

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

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

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

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

***Oropuella* sp. F3**

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

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

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

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

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

COI Barcoding

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

Discussion

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

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

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

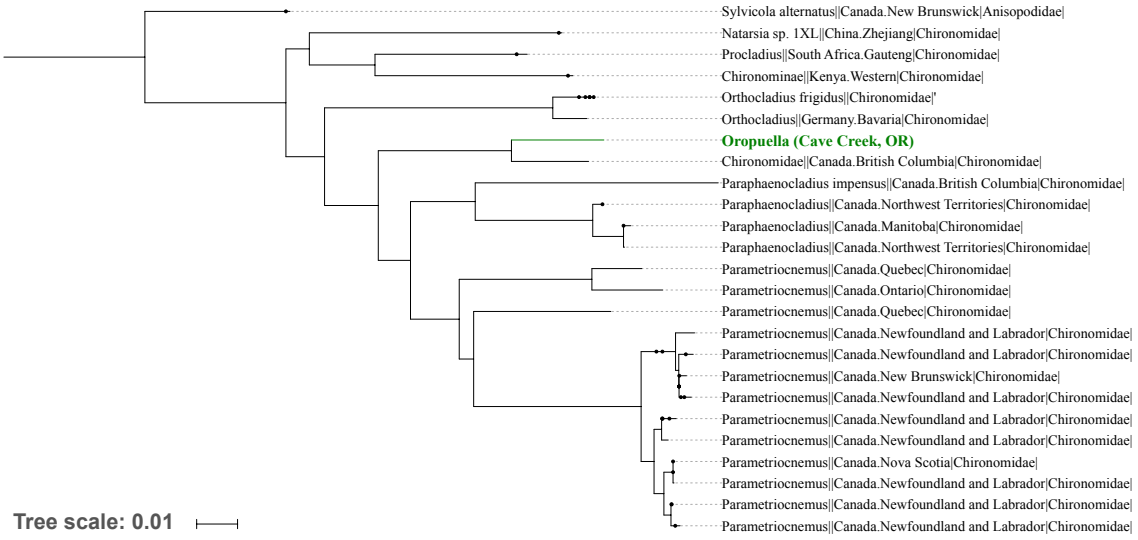

Figure 6. Kimura 2 Parameter neighbor-joining tree of COI barcode sequences for *Oropuella* and related genera. Circles denote nodes with multiple sequences pruned for clarity.

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

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

Antenna without break in second flagellomere ….. 61

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

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

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

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

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

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

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

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

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

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

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

Conclusions

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

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Description of the adult and immature stages of *Clunio ponticus* Michailova, 1980 (Diptera, Chironomidae), from the Black Sea, Varna, Bulgaria

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Abstract

The mature and immature stages of *Clunio ponticus* Michailova, 1980 are diagnosed and described based on associated material recently collected in the marine littoral zone of Varna, St-Konstantin and Helena Resorts, Black Sea (eastern Bulgaria). Male and female adults, pupae and larvae of *C. ponticus* can be easily distinguished from other known European *Clunio* species on the basis of some atypical features found in the male and female adults, pupal exuviae and fourth instar larvae. In addition, the biological cycle (reproduction and emergence) of *C. ponticus* is not synchronized with lunar periodicity (new and full moon) as for some other known *Clunio* species from Europe, but closely related to the typology of the intertidal zone along the coastline of the Black Sea. This indicates that this species is a local biogeographic representative of the 'Pontus Region', which includes the eastern coastline of the Black Sea. Remarks on related known *Clunio* species from Europe with comments on the ecology and geographical distribution of *C. ponticus* are given.

Introduction

Data on the taxonomy and geographical distribution of known marine species belonging to the genus *Clunio* Haliday, 1855 (Strenzke 1960; Olander & Palmén 1968; Neumann 1976; Sæther 1977; Heimbach 1978; Michailova 1980a, 1980b; Coffman et al. 1986; Cranston et al. 1989; Langton 1991; Neumann et al. 1997; Langton & Pinder 2007; Tasdemir 2010; Ashe & O'Connor 2012; Kaiser & Heckel 2012; Sæther & Spies 2013; Moubayed-Breil & Ashe 2012; Andersen et al. 2013; Moubayed-Breil et al. 2013; Moubayed-Breil & Ashe 2016; Abdelsalam, 2017) show that there are currently five known valid species from the sea coasts of Europe and neighbouring areas: *C. balticus* Heimbach, 1978; *C. boudouresquei* Moubayed-Breil, 2019; *C. marinus* Haliday, 1855; *C. mediterraneus* Neumann, 1966 and *C. ponticus* Michailova, 1980. For *C. ponticus* the larva was described based on the species-specific cytogenetic characteristics and SEM analysis of some male characters (Michailova 1980a, 1980b).

In this paper, *C. ponticus* is diagnosed and described as male and female adults, pupal exuviae and larvae based on recent investigations conducted by P. Michailova in the Black Sea at Varna seashore (eastern Bulgaria). Based on some atypical characters found in the male adult (frontal area of head, palpomeres, ridge of tergites VIII, apodemes of hypopygium, gonostylus), female adult (palp, apodemes of gonapophysis VIII), pupal exuviae (absence of hook rows on sternites) and larva (median tooth of mentum widely domed), this species appears to belong to a local marine biogeographic representative of the 'Pontus Region', which includes the eastern coastline of the Black Sea.

Larval stages of *C. ponticus* are marine dwellers of the intertidal zone along the seacoast of Varna, where various types of perturbation and degradation of the marine habitats have been observed over the last four decades. The type locality where larvae and pupae were collected consists of rocky shores with a dense population of *Cladophora* algae.

The *C. ponticus* community at the Varna locality is perceived to be a potential environmental indicator of the Varna seashore, where changes in ecological conditions of the intertidal zone presumably are the results of human activities and global warming.

Material and methods

Material composed of adults, pupae and larvae of *C. ponticus* was collected using standard methods: troubleau net (mesh 500 µm) for the benthos (lar-

vae and pupae) and individuals floating on the surface of the water; sweep net for flying adults. The examined material of male and female adults was preserved in 96% ethanol, then cleared of musculature in 90% lactic acid (head, thorax, abdomen and anal segment) for 60 to 80 minutes but this can be left overnight at room temperature without any detrimental effect or damage. The specimens were checked under a binocular microscope after 20 minutes in lactic acid to determine how the clearing was progressing. When clearing was complete the specimens were washed in two changes of 50 to 60% ethanol to ensure that all traces of lactic acid were removed. The specimens were then mounted in polyvinyl lactophenol. Before the final slide mounting, the hypopygium including tergites VIII- IX and anal point, the gonocoxite and the gonostylus, were viewed ventrally and laterally to examine and draw from both sides all the necessary details. The ventral view of hypopygium was illustrated with the anal point and tergite IX omitted. The abdominal segments I-VII of the male adult was preserved in 85% ethanol for an eventual DNA analysis.

Morphological terminology and measurements follow those of Sæther (1980), Langton (1991) and Langton & Pinder (2007) for the imagines, pupal exuviae and larvae. Taxonomic remarks on some related known species from Europe with comments on the ecology of *C. ponticus* are given.

Description

***Clunio ponticus* Michailova, 1980**

Holotype and paratypes in Institute of Biodiversity and Ecosystem research, Bulgarian Academy of Sciences, Sofia, Bulgaria (Michailova 1980b).

Material examined

Topotypes: 30 male adults, 2 female adults, 2 pharate females, 1 male pupal exuviae, 7 larva, Varna, Bulgaria. rocky seashores at Varna beach, St. Konstantin and Helena Resorts (43°13'45" N and 28° 0'30'' E) (locus typicus), 26.VI.2019, leg. P. Michailova. Water temperature: low 10-12 °C; high 22-25 °C.

Material is deposited in the authors' collections as follows: 5 male adults (JM); 23 male adults including 7 mounted on slides and 14 preserved in ethanol 95%, 2 female adults, 7 larvae (PM); 1 male adult, 2 pharate females, 1 male pupal exuviae (PHL). 1 male adult mounted on 1 slide is deposited in the Zoologische Staatssammlung München (ZSM), Germany.

Diagnostic characters

Although *C. ponticus* is keyed near *C. mediterraneus* and *C. boudouresquei*, some atypical morphological characters found in male and female adults and pupal exuviae enable us to separate this species from its congeners. The species is also distinguished by its ecology and the stability of its biological cycle, which is not related to the lunar periodicity as for most populations of *C. marinus, C. mediterraneus* and *C. boudouresquei*. The following combination of morphological characters will separate *C. ponticus* from other related species:

Male adult: frontal margin of head atypically straight and not projecting. Antenna 10-segmented; pedicels conical, closely inserted at their base on midline of head; segment 1 globular with 1 long seta; segment 2 linearly elongated and swollen proximally and distally, bearing 2 long setae on distal half and 5-6 shorter setae apically; last flagellomere as long as the 3 preceding segments, slightly bent apically; AR 0.40-0.43. Palp 2-segmented, palpomere 2 sub-circular with 2 long setae (1 dorsal and 1 ventral), inner apical margin pointed. Ridge of tergite VIII long, drop-shaped with 8 median setae (4 on each side of the midline). Hypopygium. Inferior volsella wider at base, narrowing distally and ending with parallel-sided margins; basal apodeme umbrella-shaped, caudal apodeme with 3 long, thin, spine-like extensions, anterior one distinctly curved downwards. Gonostylus with basal tooth crochet-shaped and conspicuous, posterior area with 4 pointed and characteristic teeth; crista dorsalis a wide lobe, long extended and occupying about 90% of the anterior side; megaseta absent.

Female adult: antenna 7-segmented. Segment 3 as long as the 2 preceding segments, last flagellomere slightly longer than the 3 preceding segments, AR about 0.45; apical segment of palp sub-circular, bearing 2 long setae (1 dorsal, 1 ventral) and several shorter setae located close to the margin; tergite VIII with a large drop-like posterior margin; gonocoxite elongated vertically and weakly prominent; tergite IX ellipsoidal to egg-shaped; gonapophysis VIII, dorsomesal lobe convex medially, apodeme lobe swollen medially and thicker distally, seminal sac ovoid; cercus bean-shaped with 2 long dorsal setae located on 1 side.

Pupal exuviae: anterior transverse rows of points present on tergite II-VII, not broken medially on any tergite; posterior transverse rows of hooks present on sternites III-VII; posterior transverse rows of hooks absent on sternites.

Fourth instar larvae: clypeus trapezoidal; setae 1 and 2 plumose, occasionally with branches; setae 3 and 4 are simple. Antenna 5-segmented; ring organ located close to pedestal; segment 2 with a lancet-shaped style reaching apex of segment 4; segments 3 and 4 sub-equal. Median tooth of mentum widely domed, and smooth. Mandible with 5 teeth. Pecten epipharyngis with lanceolate setae on both sides.

Male adult

(n = 6; Figs 1-5)

Clunio sp. 1, Moubayed-Breil & Dominici (2019)

Total length 2.70-2.90 mm. Wing length 1.35-1.40 mm, TL/WL = 2-2.10. General colouration contrasting brown to dark brown. Head and antennae dark brown; thorax contrasting light brown to brown with dark brown mesonotal stripes; wing pale translucent; legs brown to dark brown; tergites I-VII brown, tergite VIII and hypopygium distinctly contrasting light brown to dark brown.

Head (Figs 1A, 2A-E). Eyes sub-circular without dorsomedian extension, densely hairy with long and short pin-like microtrichiae; microtrichiae absent from inner lateral eye margin, outer posterior margin lacking setae. Frontal area of head (Figs 1A, 2A, 2D-E) atypically with a straight anterior margin, vertex not projecting; temporals 4 consist of 2 inner and 2 outer verticals, postorbitals absent. Antenna (Figs 1B-C, 2A-C) 10-segmented, about 575-585 µm long, bearing a few short setae located on all segments; pedicels (Fig. 2A) conical (funnel-like) with sclerotized margins, closely-connected at their base and inserted on the midline of head; segments 1-2 (Figs 1B, 2A-B), respectively 70 and 165-175 µm long, segment 1 globular with 1 long seta, segment 2 linearly elongated, swollen in its proximal and distal parts, thinner medially, bearing 2 long setae on distal half and 5-6 shorter setae apically; segments 7-9 globular, nearly sub-equal (20-25 µm long), bearing sensilla chaetica; ultimate flagellomere (Figs 1C, 2B-C) 140-150 µm long, about 35 µm maximum width, as long as the 3 preceding segments, thumb-like, bearing 6-7 long setae; antennal groove reaching segment 2; AR 0.40-0.43. Palp (Figs 1D, 2D-E) 2-segmented, lacking sensilla clavata; palpomere 2 similarly shaped, sub-circular, about 30 µm long, with 2 long setae (1 dorsal and 1 ventral), inner apical margin projecting and pointed apically. Clypeus semi-circular and bare.

Thorax. Antepronotum well-developed, distinctly domed with joined lobes. Antepronotals absent; acrostichals 2-3 starting close to antepronotum; dorsocentrals 3 in 1 row; prealars absent; scutellum with 6 stout setae located 3 on each side of the midline. Wing. Brachiolum with 1 stout seta; number of setae on veins: R, 4-5; R_1 4-5 located distally; remaining veins and squama bare. Legs (Figs 3A-D). Femur of PI distinctly broad basally (about 180-85 µm maximum width), femur of PII and PIII are narrower (about 50-55 µm). Tibial spurs of third leg curved apically (Fig. 3C); length (in µm) of tibial spurs: PI, 30; PII, 45; PIII, 50 µm long. Tarsomeres ta_3 and ta_4 of PI and PII (35 and 30 µm long) shorter than tarsomere ta_5 (45 and 60), while only tarsomere ta_4 of PIII (45) is shorter than tarsomere ta_5 (70). Tarsomere ta_3 of third leg is long and thin (Fig. 3C). Value of SV (Table 1) of PII and PIII (8.64 and 10.64) is much higher than in PI (4.67). Sensilla chaetica present on tibia and tarsomere ta_1 of PI-PIII, those on tibiae are located apically. Length (µm) and proportions of legs as in Table 1.

Abdomen and anal segment (Figs 1 E-L, 4A-E). Ridge of tergite VIII (Fig. 1E) 70-75 µm long, broad drop-shaped with a slightly narrowing pointed apex; 8 setae are located medially (4 on each side of the caudal part of ridge). Hypopygium in dorsal and ventral view as in Figs 1F-L and Figs 4B-C; dorsal view (Figs 1F, 4 B), ventral view with tergite IX omitted (Figs 1H-I, 4C). Tergite IX 265-275 µm long, 200 µm maximum width at base and 100-120 µm at apex, anal point absent; dorsal side (Figs 1F, 4B) densely covered with macrotrichia-like setae reclinate (orally directed) setae about 35-40 short setae; ventral side (Figs 1H-I) with a semi-circular posterior lamella covered with macrotrichia. Apodemes consist of 4 distinct parts (basal, axial, lateral and caudal): basal apodeme (ba, Figs 1G, 4B-C) about 200 µm maximum width, umbrella-shaped with anterior side convex; axial apodeme (aa, Figs 1 G-H, 4B-C) about 235-245 µm long, ending with a bi-lobed semi-circular apical expansion; lateral apodeme (la, Figs 1H, 4C) 270-280 µm long, inwardly bent distally to connect with caudal apodeme; caudal apodeme (ca, Figs 1H-I, 4C) on each lateral side with 3 long, pointed, spine-like extensions, basal one claw-like with a downwardly curved apex, the two others are upwardly projecting apically. Gonocoxite (Figs 1F, 4C, 4E) about 420-450 µm long, 160-170 µm maximum width, distal inner area with a dense group of proclinate short setae. Inferior volsella (Figs 1F, 4C) about 70 µm long in its median part and about 35 µm in its distal half, conical basally and nearly parallel-sided in its distal half, densely covered with short upwardly directed setae. Gonostylus (Figs 1J-L, 4C-E, 5A-D) 240-250 µm long, inverted triangular, much thinner in median and distal parts, arched with

rounded posterior angle, antero-lateral end projecting upwards into a pointed anterior apex; basal part with a well-sclerotized tooth, crochet-like and conspicuous, rounded at base and pointed apically; posterior part (Figs 1K-L, 4D, 5C) with 4 pointed teeth (clearly visible when viewed laterally, as in Figs 1K-L), posterior one is much longer than the 3 others. Crista dorsalis (Figs 2J, 4D, 5C) well-developed, a single wide lobe occupying about 90% of the anterior side; megaseta absent.

Table 1. Male adult of *Clunio ponticus*. Length (μm) and proportions of fore- (PI), mid- (PII) and hind (PIII) legs.

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
PI	485	595	125	38	35	30	45	0.21	8.14	8.64	0.70
PII	610	560	110	50	35	35	60	0.20	7.11	10.64	2.50
PIII	530	520	225	190	122	45	70	0.43	2.99	4.67	1.40

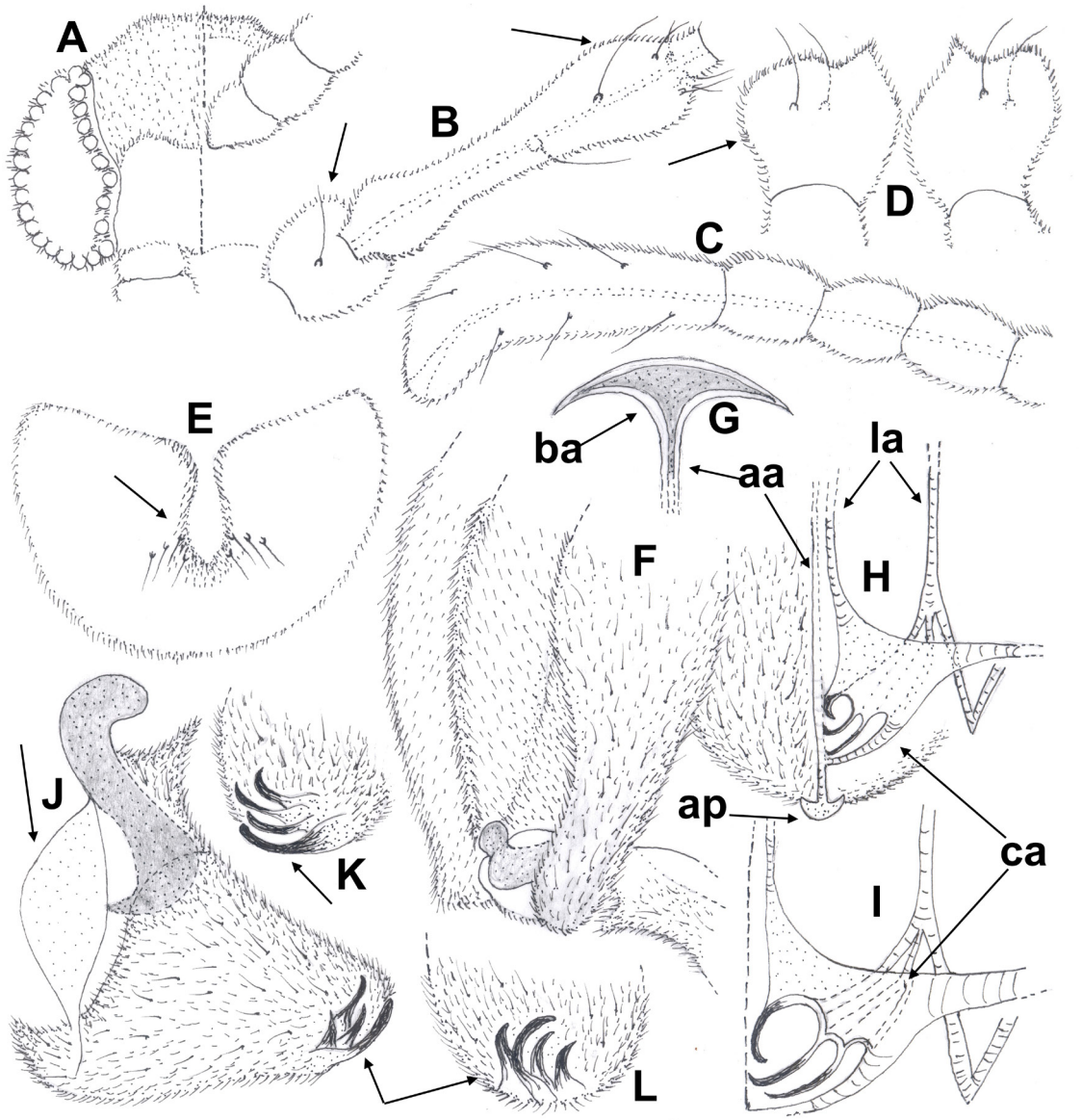

Figure 1. Male adult of *Clunio ponticus*. A) head in dorsal and ventral view; B) antenna, segments 1-2; C) antenna, segments 7-9 and last flagellomere; D) palp, palpomeres 2 (left and right); E) ridge of tergite VIII; F) hypopygium: gonocoxite, inferior volsella, tergite IX, axial apodeme and apical projection, dorsal view; G) basal and axial sternapodeme, dorsal view; H-I) lateral and caudal apodemes in ventral view; J) gonostylus, dorsal view; K-L) apex of gonostylus, different views. ba = basal apodeme; aa = axial apodeme; la = lateral apodeme; ca = caudal apodeme. The arrows indicate some distinguishing characters.

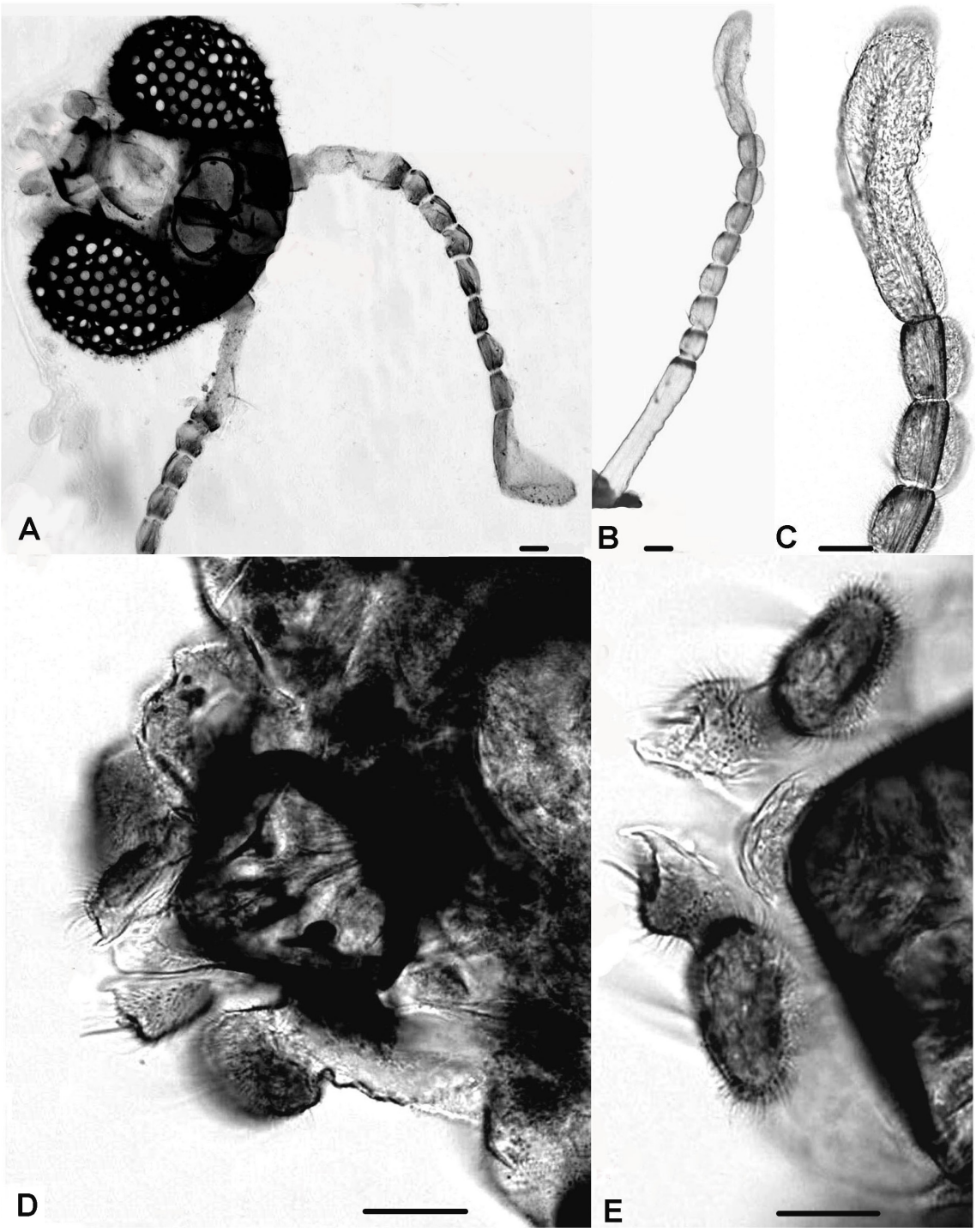

Figure 2. Male adult of *Clunio ponticus*. A) head (frontal view) and antenna; B) antenna, segments 2-10; C) antenna, segments 7-9 and last flagellomere; D-E) palp, palpomere left and right. Scale bar = 10 µm.

Figure 3. Male adult of *Clunio ponticus*. Legs, tibial spurs and tarsomeres of fore leg (A), mid leg (B) and hind leg (C-D). Scale bar = 10 μm.

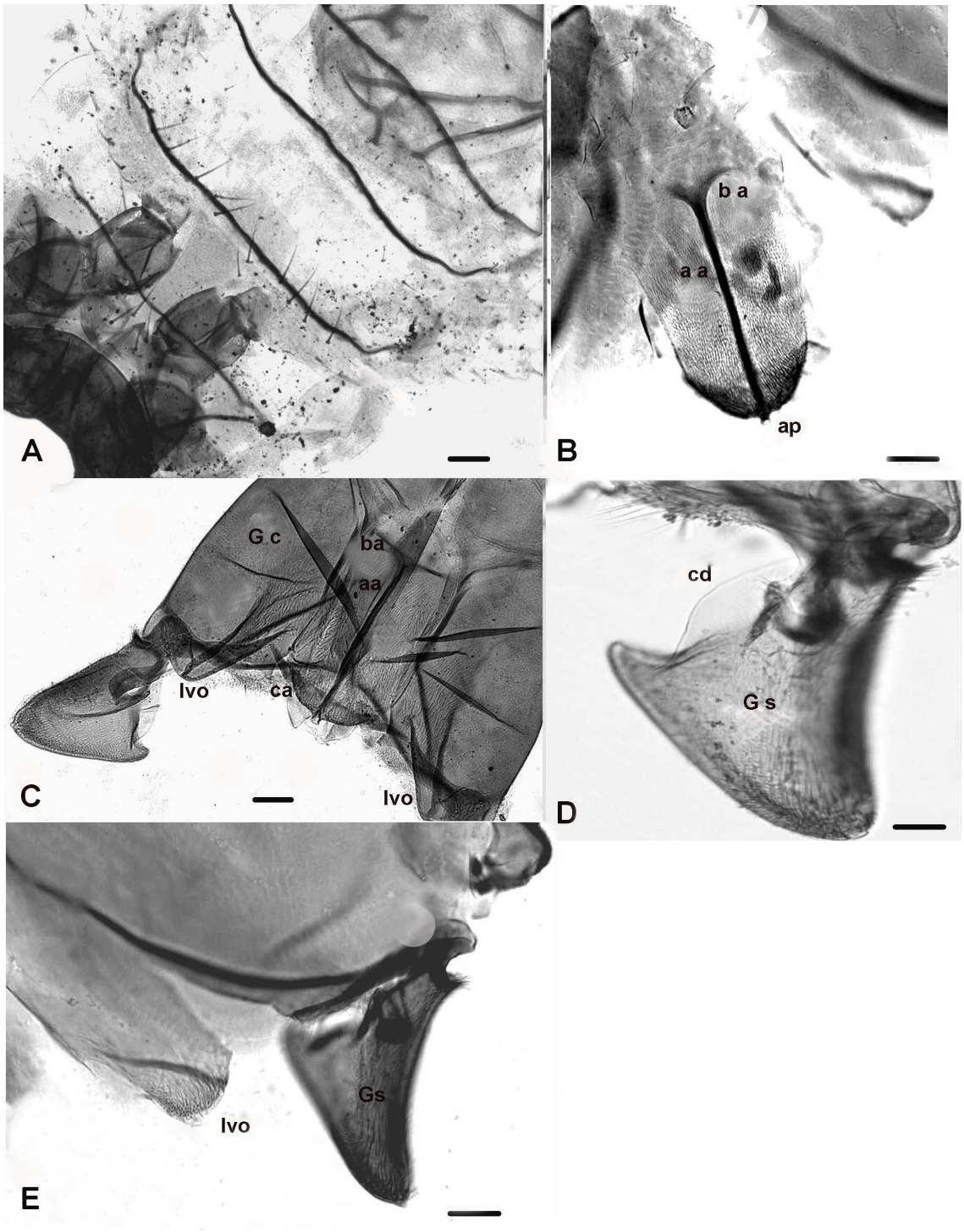

Figure 4. Male adult of *Clunio ponticus*. A) chaetotaxy of first abdominal segments; B) tergite IX with basal (ba) and axial (aa) apodemes and apical projection (ap); C) hypopygium with gonocoxite (Gc), inferior volsella (Ivo), apodemes and gonostylus (Gs); D) gonostylus with crista dorsalis (cd); E) Inferior volsella and gonostylus. Scale bar = 10 μm.

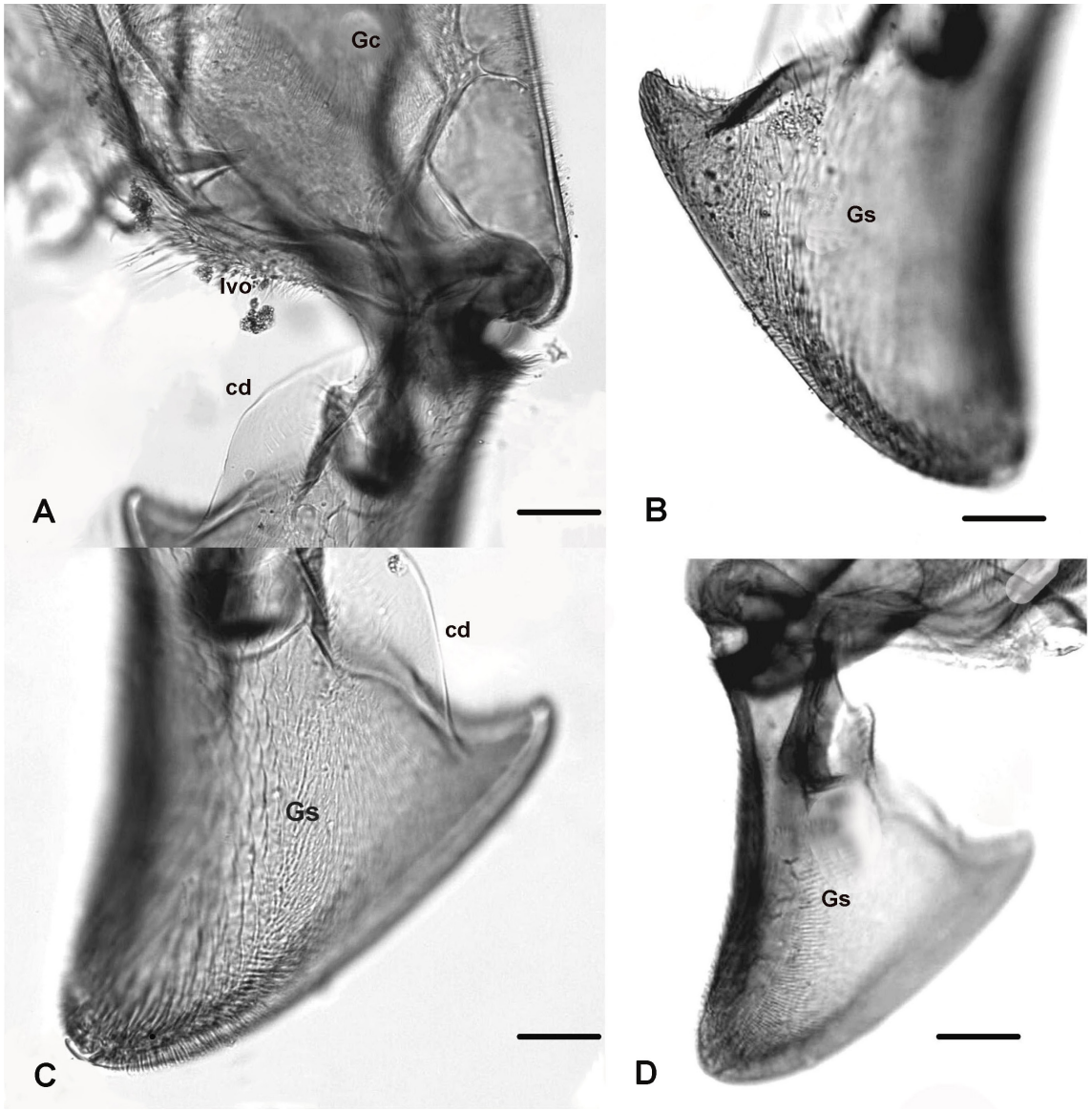

Figure 5. Male adult of *Clunio ponticus*. A) gonocoxite (Gc), inferior volsella (Ivo) and crista dorsalis (cd); B-D) different aspects of gonostylus. Scale bar = 10 µm.

Female adult

(n = 3; Figs 6-8)

Small as in all female *Clunio* species. Total length 1.55-1.65 mm. General colouration less contrasting than in the male adult; head dark brown with light brown antennae; thorax brownish; legs brown with blackish claws; tergites I-VII brownish, tergite VIII and anal segment distinctly contrasting light brown to dark brown.

Head (Figs 6A-B, 7A-B). Eyes densely hairy, subcircular without dorso-median extension, microtrichiae absent from inner and lateral eye margin, outer posterior margin lacking setae. Frontal area straight (Figs 7A-B); temporals 3, including 1 inner and 2 outer verticals. Antenna 7-segmented (Figs 6A; 7C), about 190-200 µm long; last flagellomere 55-60 µm long, swollen proximally and nearly parallel-sided in its distal half; antennal groove reaching segment 2; AR 0.45. Palp (Figs 6B, 7A-B) 2-segmented; segment 1, indistinct; palpomere 2 about 20 µm long, sub-circular to globular, bearing 2 long fine setae.

Thorax. Chaetotaxy indistinct and difficult to observe. Legs (Figs 7D-G). Tibia of PII and PIII nearly equal (115 and 118 µm long); tarsomeres ta_2-ta_4 of PI and PIII equal in size. Femur of PI is much wider (85 µm) than in PII-PIII (60 and 55); tibia of PIII is wider (50 µm) than in PI and PII (40 µm each); tibial spur (Fig. 7F) strongly curved apically. LR value of PIII (0.54) much higher than in PI and PII (0.28 and 0.25); SV value of PII (9.02) much higher than in PIII (4.32). Few sensilla chaetica present on tibia and tarsomere ta_1 of PI, PII and PIII. Length (in µm) and proportions of legs as in Table 2.

Table 2. Female adult of *Clunio ponticus*. Length (µm) and proportions of fore- (PI), mid- (PII) and hind (PIII) legs.

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
PI	123	124	33	16	15	16	33	0.28	3.53	7.37	0.72
PII	140	115	29	17	14	16	34	0.25	3.55	9.02	0.75
PIII	155	118	63	32	44	18	37	0.54	2.59	4.32	1.58

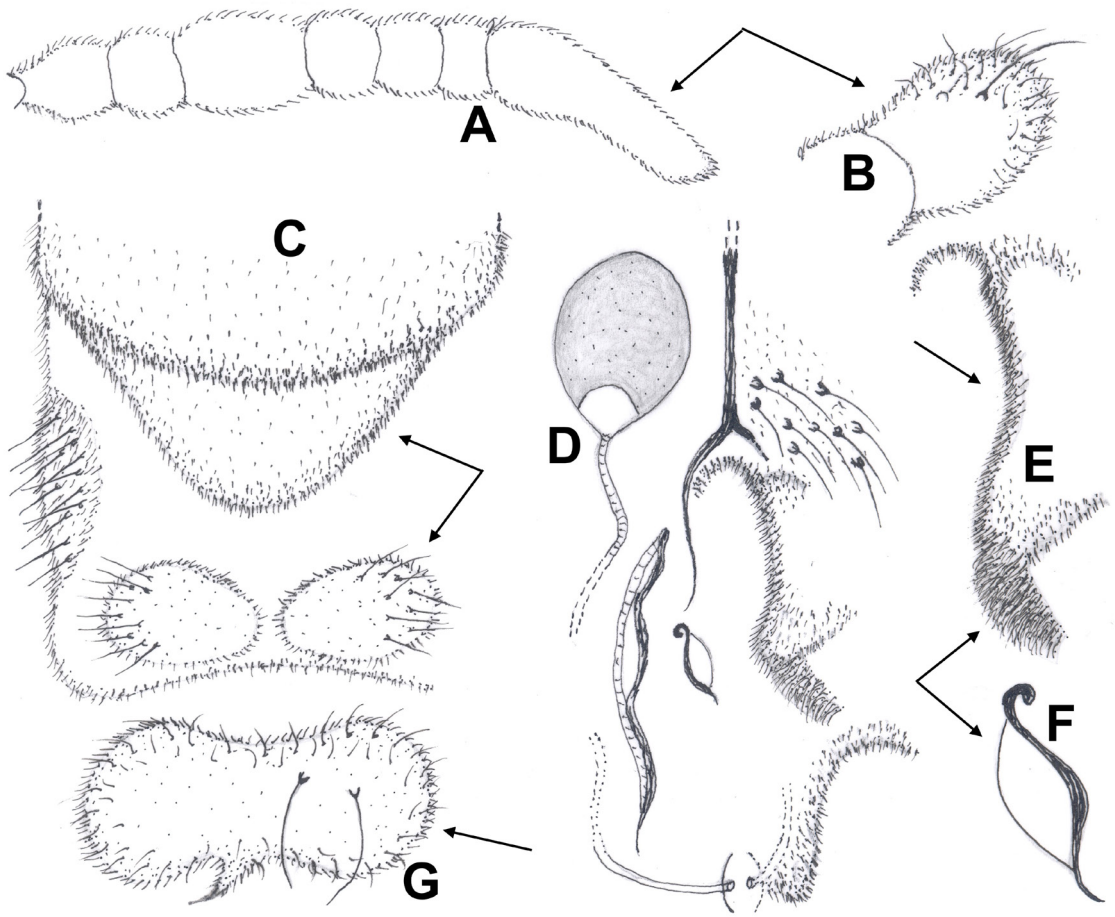

Figure 6. Female adult of *Clunio ponticus*. A) antenna; B) palp; C) tergite VIII, gonocoxite and tergite IX; D) gonapophysis VIII with notum and seminal capsule; E) dorsomesal lobe; F) apodeme lobe; G) cercus. The arrows indicate some distinguishing characters.

Abdomen. Anal segment (Fig. 6C, dorsal; Figs 6D and 8, ventral) about 230 µm long, 210 µm maximum width at base, 110 µm wide at caudal part. Genitalia (Figs 6E-F; 8). Notum about 140 µm long with separate long and sinuous rami, which are almost connected to the sternal axial apodeme. Sternite VIII with 20-22 setae (10-11 on each side of the notum). Gonapophysis VIII (Figs 6D-F, 8). Dorsomesal lobe (Figs 6D-E, 8) concave medially and projecting in both proximal and apical parts; ventrolateral lobe projecting outwards, broader basally and slightly narrowing distally; apodeme lobe (Fig. 6D, right; Fig. 6F, left) distinctly swollen in its median part and thicker distally. Seminal capsules about 60 µm long, 30 µm maximum width, sub-oval, well-sclerotized except for its basal part. Spermathecal ducts with loops and separate openings. Tergite IX (Figs 6C, 8) egg-shaped, distinctly divided, with 18-20 setae (9-10 on each side). Gonocoxite (Figs 6C, 8) bearing 11-12 setae, weakly prominent, well-developed and widely extended vertically along the lateral margin. Cercus (Figs 6G, 8) bean-shaped with sub-equal parts, bearing 2 long dorsal setae located on 1 side.

Pupal exuviae

(n = 3: 1 male exuviae and 2 female pharate adults)

(Male pupal exuviae, Figs 9 A-E)

Exuvial length 2 mm. Colourless. Frontal apotome

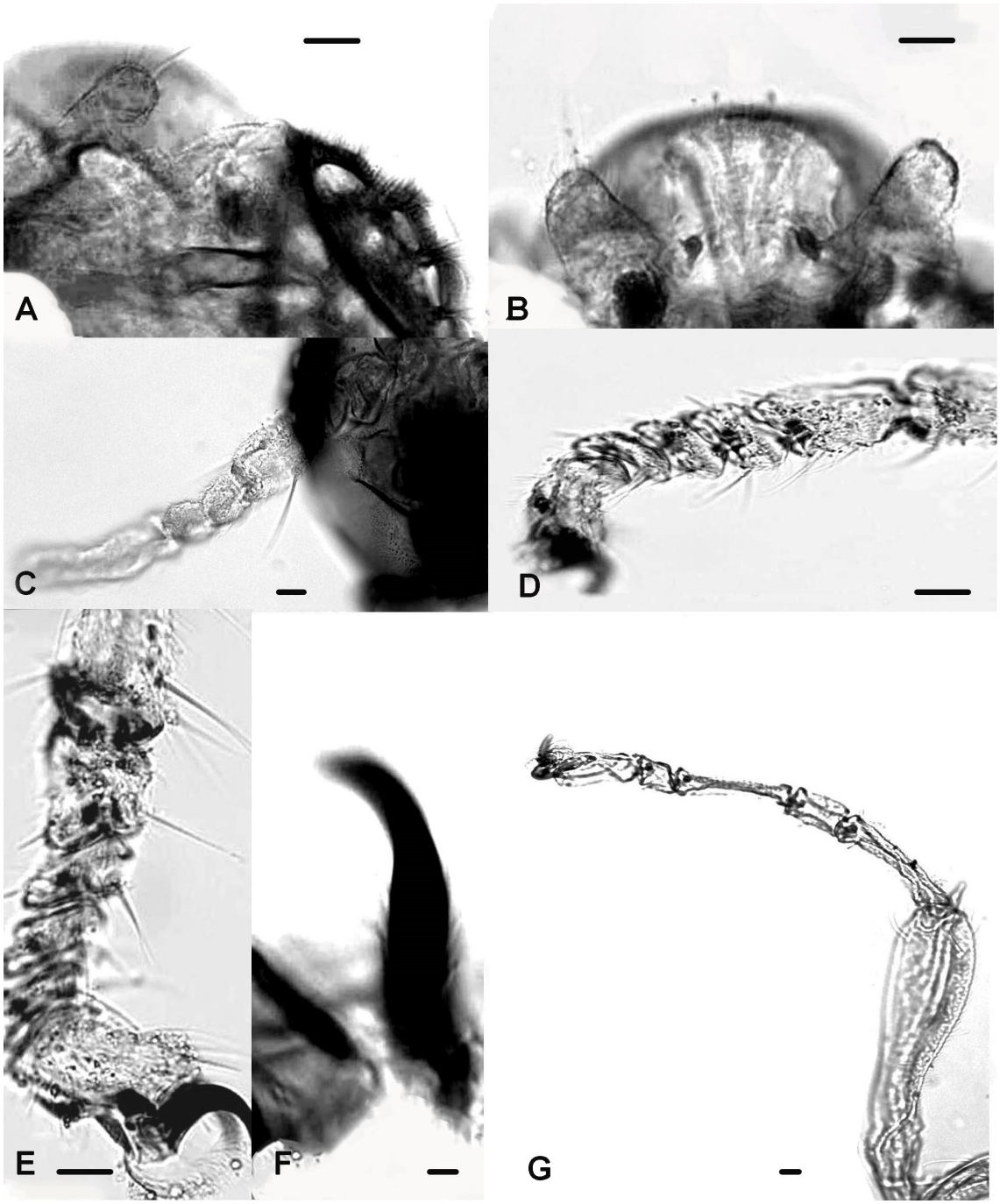

Figure 7. Female adult of *Clunio ponticus*. A-B) head, frontal area and palp; C) antenna, segments 4-6 and last flagellomere; D) tarsomeres of fore leg; E) tarsomeres of mid leg; F) tibial spur of hind leg; G) tarsomeres of hind leg. Scale bar = 10 µm.

rectangular, the free apex truncate. Thorax smooth apart from a small patch of tubercles by suture a little behind middle (Fig. 9C). 4 dorsocentral setae about 50 µm long; setae 1, 2 separated by 25 µm, 2, 3 by 35 µm and 3, 4 by 50 µm (in general, setae on *Clunio* exuviae are very small; other than the dorsocentrals, neither frontal setae nor abdominal setae have been detected in these specimens). Wing sheath 200 µm long, without nose or pearl row.

Abdominal tergites I-VII (Figs 9A-B) with a continuous anterior transverse band of colourless points; III-VII with a posterior transverse row of small hooks extending the width of the tergite. Sternites unarmed except VIII which has a posterior transverse band of minute points, restricted to the middle half in the male (Fig. 9A), but extending to the lateral margins in the female (Fig. 9D). Anal tergite without lobes, truncate apically and bearing at the postero-lateral corners two strong spines

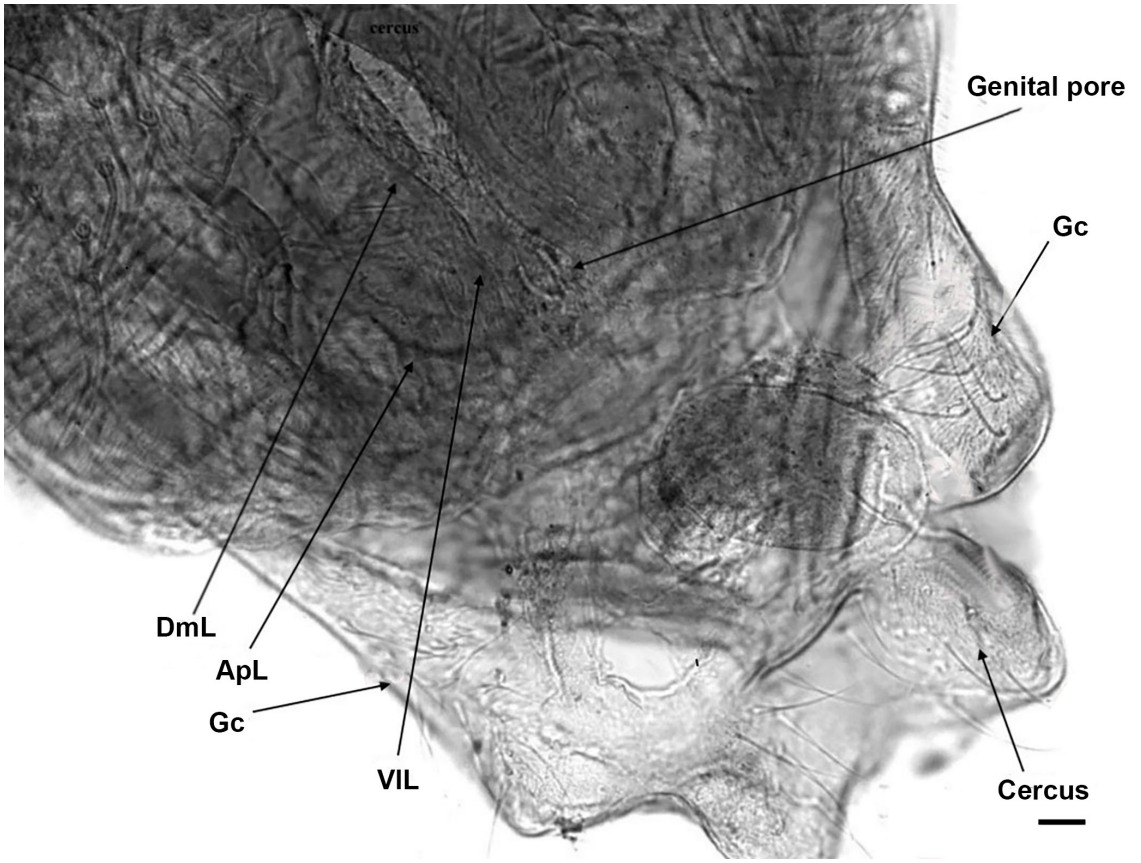

Figure 8. Female adult of *Clunio ponticus*. Genitalia in ventral view including gonapophysis VIII: sternite VIII, dorsomesal lobe (DmL), apodeme lobe (ApL), ventrolateral lobe (VlL), genital pore, gonocoxite (Gc) and cercus. Scale bar = 10 µm.

(Figs 9D-E). Female genital sheaths are restricted to a circular patch beneath the anal segment (Fig. 9D), whereas those of the male are massive, far exceeding the anal segment both laterally and posteriorly, parallel-sided for the anterior half, thence narrowed inwards to a papillate point (Fig. 9E).

Fourth instar larva

(n = 7; Figures 10-12)

Small sized species. TL about 6 mm long. General colouration yellowish green; head dark brown; mentum with dark brown teeth; frontal apotome dark brown, mandible with contrasting brown to pale teeth.

Head. Clypeus, frontal apotome with setae I-IV and labrum as in figures 10 and 12A. Clypeus trapezoidal with wide posterior margin, dense granulation present on both sides; setae 1 and 2 plumose, occasionally with branches; setae 3 and 4 simple; setae S1 located close to the anterior margin, setae S2 inserted medio-laterally. Frontal apotome (Figs 10, 12A) ellipsoidal except in its basal part; setae S3-S5 located near the lateral margin: S3 close to the anterior margin, S4 antero-medially, S5 on median area. Mandible (Figs 10, 11A) with 5 teeth; apical one 100-110 µm long and 34-46 µm maximum width. Mandible subdentalis almost triangular and thin, with uneven sides, reaching the last (posterior) tooth; seta interna consists of 5 strong and conspicuous branches. Mentum (Fig. 11B); median tooth, smooth, widely domed and rounded, the four remaining lateral teeth are rounded apically and progressively decreasing in size from 1 to 4; in some individuals the number of lateral teeth is different from both sides. Ventromental plates poorly developed; seta submenti 42 µm long, well-developed and not branched; distance between setae submenti is recorded in Table 3. Antenna (Fig. 11C) 5-segmented, length of segments (in µm) as in Table 3; segment 1 with ring organ located at mid length of segment; segment 1 with a blade long, reaching apex of 4th segment and divided in two parts; segment 2 with thin style reaching end of segment 4; segments 3 and 4 sub-equal; Lauterborn organ weak. Premandible (Fig. 11D) bare, with a blunt tooth and inner blunt tooth, toothed proximally and blunt medially. Pecten epipharyngis (Fig. 12A) consists of two fused structures at base and bearing lanceolate setae on both sides.

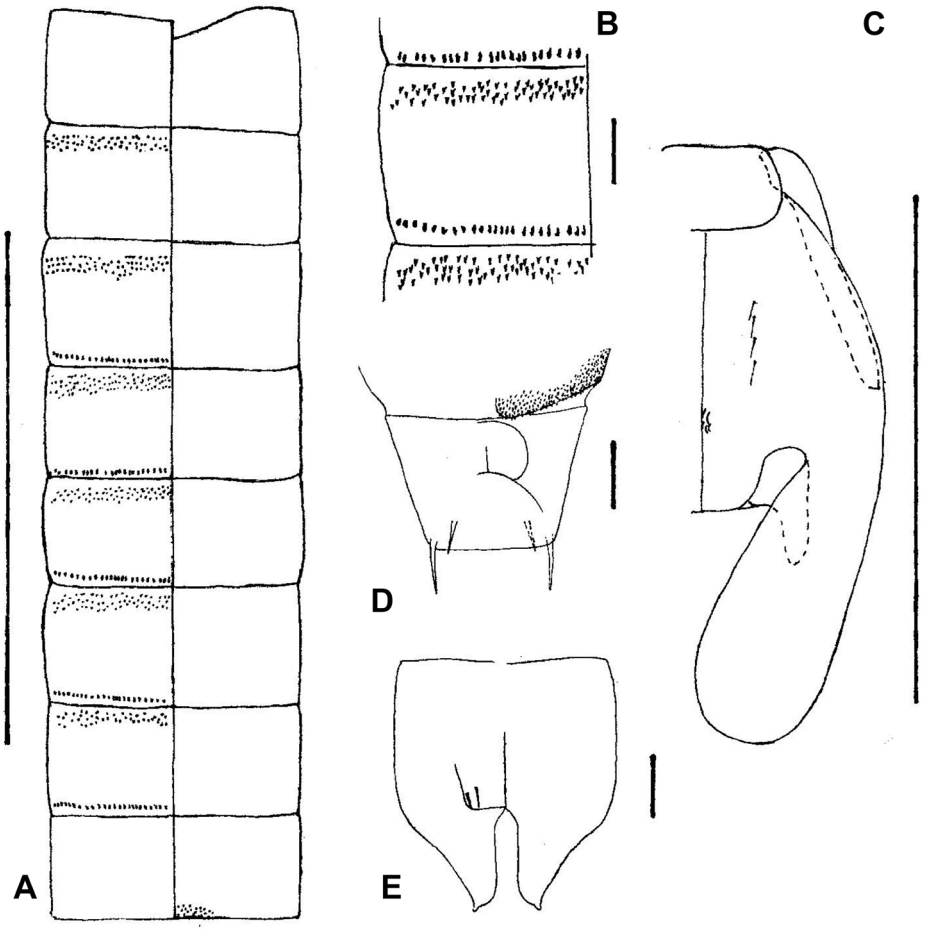

Figure 9. Pupal exuviae of *Clunio ponticus*. A) Abdominal segments I-VIII of male exuviae (dorsal left, ventral right); B) tergites IV (posterior) to VI (anterior); C) female segment VIII (posterior) and anal segment (left dorsal, right ventral); D) male anal segment (dorsal left, ventral right); E) frontal apotome and thorax. Scale bars: A = 1 mm; B-D = 100 µm; E = 500 µm.

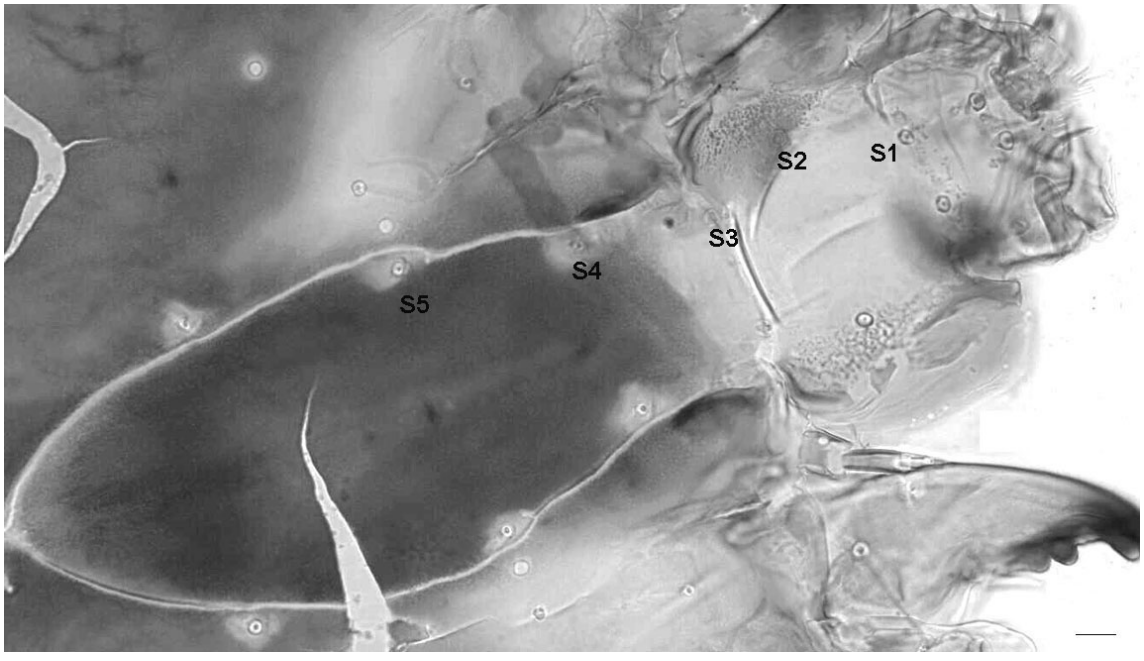

Figure 10. Larva of *Clunio ponticus*. Clypeus and frontal apotome with setae S1 to S5. Scale bar = 10 µm.

Maxilla (Fig. 12B) with well-developed maxillary palp, bearing 1 single seta, located laterally; lacinia with several simple maxillary chaetae. Posterior parapods (Fig. 12C) about 90 µm long, anal setae (as) about 65 µm long, supra-anal setae, (sa) 130-135 µm long; ventral tubules absent.

Table 3. Measurements in the larva of *Clunio ponticus* (in µm). Antennal segments 1-2; width of median tooth of mentum; distance between mental setae; distance from antennal base to ring organ of antennal segment 1.

Larva	Ant. segment 1	Ant. segment 2	Mentum tooth	Mental setae	Base to ring organ
1	9	16	20	59.45	5
2	9	16	24.6	49.2	5.3
3	9	16	20	47.8	5
4	9	14.7	24.6	44.9	5

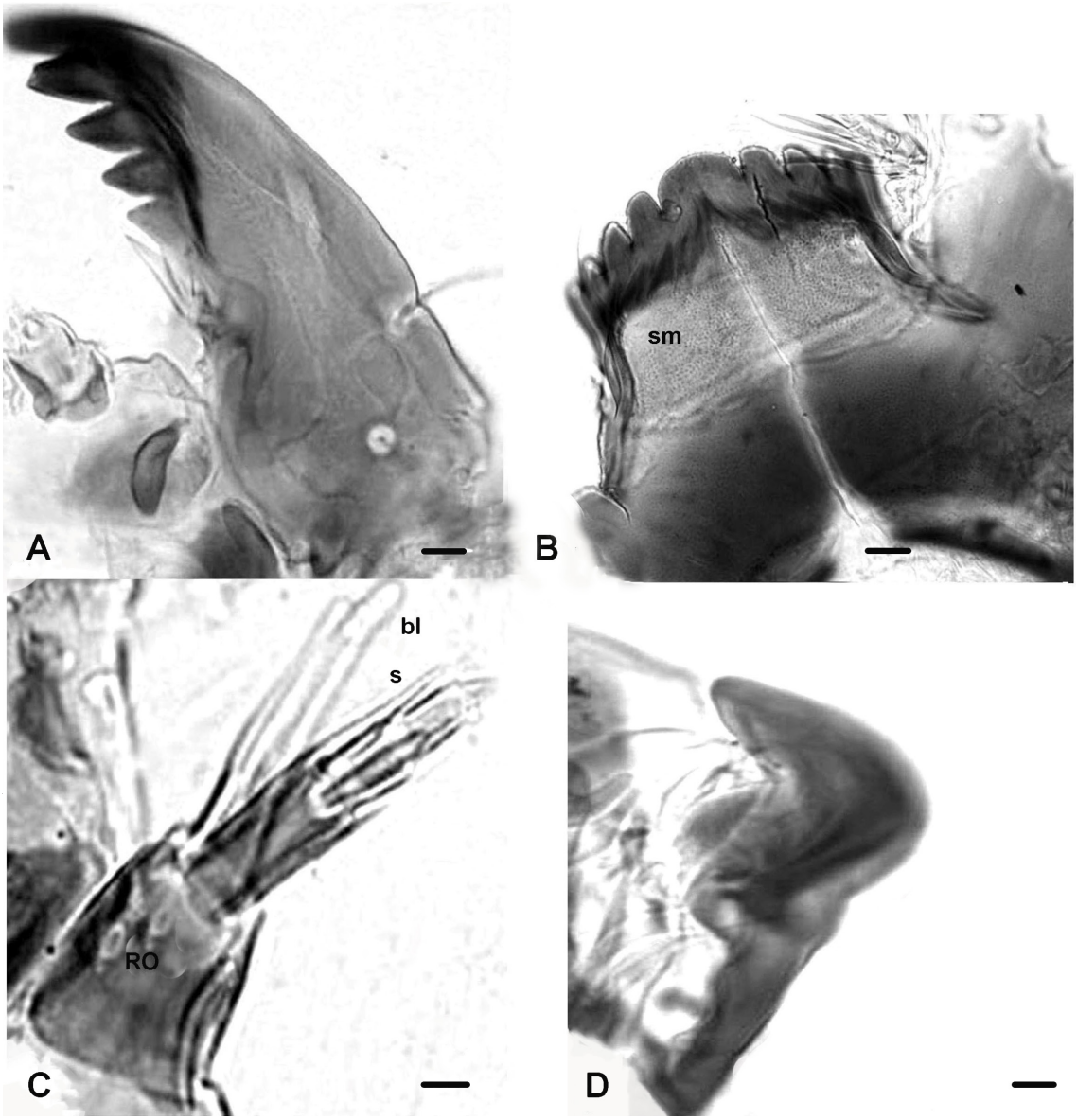

Figure 11. Larva of *Clunio ponticus*. A) mandible; B) mentum with median and lateral teeth; C) antenna, blade (bl), style (s); D) premandible. Scale bar = 10 µm.

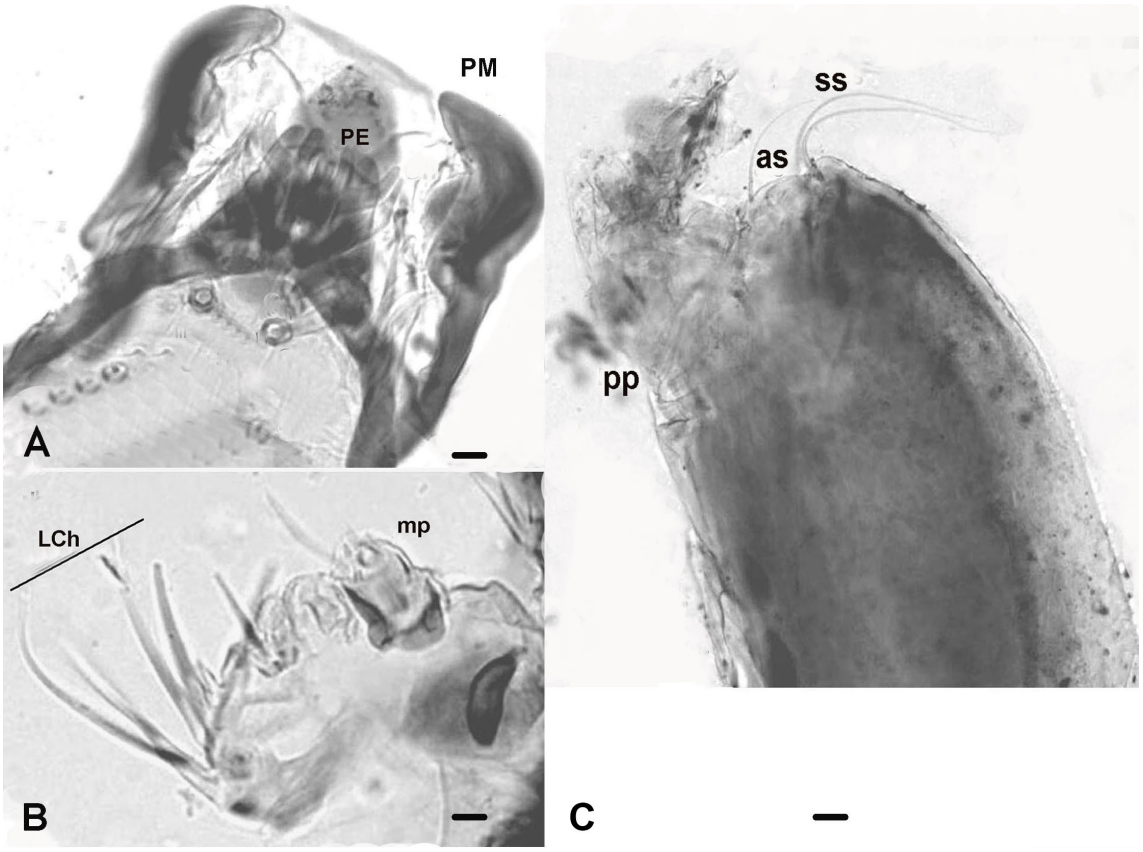

Figure 12. Larva of *Clunio ponticus*: A) premandible (PM), clypeus and frontal apotome, pecten epipharyngis (PE); B) maxilla with maxillary palp (mp) and lacinial chaetae (LCh); C) caudal part with posterior parapods (pp) and anal setae (as, ss). Scale bar = 10 µm.

Differential diagnosis

C. ponticus is easily separable from other known European *Clunio* species by the following morphological characters:

Male adult:

Frontal area of head with a straight anterior margin (Figs 1A, 2A), is projecting in both *C. marinus* (Moubayed-Breil 2019, figs 1a-b) and *C. boudouresquei* (Moubayed-Breil & Dominici 2019, figs 1c-d).

Segment 2 of antenna (Figs 1B, 2A-B) globular and swollen proximally and distally, are differently shaped in *C. boudouresquei* (Moubayed-Breil & Dominici 2019, fig. 1e).

Palpomere 2 sub-circular and similarly shaped (Figs 1D; 2D-E), differently shaped in *C. boudouresquei* (Moubayed-Breil & Dominici 2019, Figs 1g-h) and *C. marinus* (Strenzke 1960, fig. 1; Moubayed-Breil 2019, fig. 1n).

Ridge of tergite VIII (Fig. 1E) broad drop-shaped, narrower in *C. boudouresquei* (Moubayed-Breil & Dominici 2019) and cylindrical to inverted conical in *C. marinus* (Strenzke 1960, figs 12-13).

Caudal apodeme composed of 3 long claws (Figs 1H-I), bearing brush and differently shaped claws in both *C. boudouresquei* and *C. mediterraneus* (Moubayed-Breil & Dominici 2019, figs 2b, 2e).

Megaseta absent on gonostylus (Figs 1J, 4C-D, 5A-D), present in *C. boudouresquei* (Moubayed-Breil & Dominici 2019, fig. 2c).

Apex of gonostylus with several unequal tubercles (Figs 1K-L), consisting of only a single finger-like tubercle in both *C. boudouresquei* and *C. mediterraneus* (Moubayed-Breil & Dominici 2019, figs 2b-c, 2f).

Female adult:

Frontal area straight (Figs 7A-B), orally projecting in *C. marinus*; palpomere 2 sub-circular (Figs 7A-B), sub-rectangular in *C. marinus* (Moubayed-Breil & Dominici 2019, fig. 2n).

Segment 3 of antenna nearly globular (Figs 6A, 7C), elongated and parallel-sided in *C. marinus* (Strenzke 1960, fig. 15).

Proximal and posterior parts of dorsomesal lobe not projecting (Figs 6D-E), prominent in *C. boudouresquei* (Moubayed-Breil & Dominici 2019,

figs b, d); cercus bi-lobed medially (Fig. 6G), differently figured in *C. marinus* and *C. boudouresquei* (Strenzke 1960, fig. 19; Moubayed-Breil & Dominici 2019, fig. 4b).

Pupal exuviae:

Anterior transverse band of points on tergites II-VII complete on all tergites (Figs 9A-B), broken medially on tergite II in *C. boudouresquei* (Moubayed-Breil & Dominici 2019, fig. 5c).

Posterior transverse band of points absent on tergites, present on tergites II-VII or III-VII in *C. boudouresquei, C. mediterraneus* and *C. marinus.*

Transverse row of hooks absent on sternites (Fig. 9A), present on sternites IV-VII in *C. marinus* (Coffman et al 1986, fig. 9. 11c), V-VII in *C. boudouresquei* (Moubayed-Breil & Dominici 2019, Fig. 5a) and V-VI *C. mediterraneus* (Moubayed-Breil & Dominici 2019, fig. 5b).

Larva:

Blade of antenna reaching apex of segment 4 (Fig. 11C), only reaching proximal half in *C. marinus* (Strenzke 1960, fig. 25) and *C.* sp. 1 (Abdelsalam 2017, fig. 5), reaching segment 5 in *C. mediterraneus* (Tasdemir, 2010, fig. 3).

Median tooth of mentum broadly domed and smooth (Fig. 11B), triangularly rounded apically in *C. marinus* (Strenzke 1960, fig. 31), semi-circular in *C. mediterraneus* (Tasdemir, 2010, fig. 4) and sub-trapezoidal in *C.* sp. 1 (Abdelsalam 2017, fig. 5).

DNA sequencing of species-specific genetic markers will be performed later to see how genetically divergent *C. ponticus* is compared to related species of the genus *Clunio.*

Ecology and geographical distribution

Larval and pupal stages of *C. ponticus* are exclusively confined to the intertidal rocky shores of the Black Sea at Varna, St. Konstantin and the Helena Resort coastline (Eastern Bulgaria, Figs 13-14), where a dense population of marine algae (*Cladophora* spp.) occurs.

While the biological cycle (reproduction and emergence) is synchronized with the lunar cycle (new and full moon) for *C. boudouresquei, C. marinus* and *C. mediterraneus* (Neumann 1976, Neumann et al. 1997, Kaiser & Heckel 2012, Moubayed-Breil & Dominici 2019), that of *C. ponticus* is independent of the lunar periodicity but closely related to the typology of the marine intertidal zone, which consists of mostly submerged habitat along the coastline of the Black Sea. Emergence of *C. ponticus* is observed early in the morning in slight tide and has no semi-lunar periodicity.

Figure 13. Type locality at Varna seashores, St. Konstantin and Helena Resort (Black Sea, eastern Bulgaria), where the topotypes of *C. ponticus* were collected. Photo P. Michailova, 26.VI.2019.

Figure 14. Varna coast, St. Konstantin and Helena Resort (Black Sea, eastern Bulgaria): modified marine habitats by recent constructions at the neareby seashores. Photo P. Michailova, 26.VI.2019.

Larvae live in general among the filiform marine algae but sometimes inhabit tubes built with sandy to muddy sediment among stones on the seabed. Copulation takes place mainly on the water surface during low tide: the female dragged along by the male in end-to-end position. Male adults emerge from May to early July slightly earlier than the females: they occur early in the morning between 5 and 7 am.

The littoral and mid-littoral marine zones of the type locality where new material of *C. ponticus* was recently collected have been much degraded over the last four decades by various perturbations related to human activities and tourism (Fig. 14).

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Evidence of parthenogenetic populations from the *Paratanytarsus laccophilus* species group (Diptera: Chironomidae) in the Alaskan Arctic

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Abstract

Parthenogenesis, reproduction without fertilization, is not common in the Chironomidae (Diptera), a family of insects with more than 6,000 described species. Nonetheless, parthenogenetic species and strains have been documented in at least three subfamilies (the Chironominae, Orthocladiinae, and Telmatogoninae), spanning 17 genera and ~30 species. One such species, *Paratanytarsus laccophilus* Edwards 1929, is known to be parthenogenetic in a small portion of its range in Finland, with most other European populations of this species showing evidence of sexual reproduction. We present evidence of parthenogenetic populations from the *Paratanytarsus laccophilus* species group in the Nearctic, specifically a High Arctic site near Utqiaġvik (formerly Barrow), Alaska. During May-July of 2015 and 2016, we sampled emerging adult chironomids and pupal exuviae daily to document insect emergence phenologies. Across 15 local populations, all 623 pupal exuviae collected from the *P. laccophilus* species group were female. Larvae reared from two populations under controlled temperature treatments emerged as female adults (N=37). When isolated, these reared female adults oviposited, and eggs hatched successfully. These progeny were reared for another 12-13 days, reaching second instar larvae when they were preserved at the end of our field season. Taken together, this evidence strongly indicates parthenogenesis from the *P. laccophilus* species group at this location. This species was not previously documented at Utqiaġvik. Although parthenogenetic, their emergence at this location was highly synchronized. In the harsh environment of arctic Alaska, the fitness rewards of parthenogenesis are likely great. Indeed, chironomid parthenogenesis in the northern hemisphere is most commonly documented from far-northern extremes and in extreme habitats.

Introduction

While sexual reproduction is widespread in the animal kingdom, parthenogenesis is a widespread form of asexual reproduction in which there is no fertilization of the egg (Suomalainen 1962; Lynch 1984). Parthenogenesis in animals evolves most commonly as an artifact of hybridization (Carew et al. 2013) and is most often thelytokous, *i.e.* where all asexually produced offspring are female. Because offspring are genetically monotonous with mutation as the only source of variation (Lynch 1984), parthenogenesis is often considered an evolutionary dead end (Darlington 1946; Mayr 1970; Smith 1978). Nonetheless, patterns exist in the distribution of parthenogenetic forms that suggest this reproductive strategy could be evolutionarily advantageous in certain cases.

"Geographic parthenogenesis" describes the observation that parthenogenesis tends to evolve in habitats that are different from where their closely related, sexually-reproducing relatives reside (Vandel 1928). Indeed, parthenogenetic species are most commonly documented from high latitudes, especially in harsh habitat conditions (Lynch 1984). Proposals articulated to account for this phenomenon include the "biotic-uncertainty" hypothesis (Ghiselin 1974), and the "tangled bank" hypothesis (Bell 1982). The biotic-uncertainty hypothesis postulates that parthenogens tend to persist in harsh environments that are relatively barren biologically, and an environment with minimal biotic interaction favors the persistence of genetically monotonous lineages. The tangled bank hypothesis argues that harsh environments are less niche-specialized because they are frequently disturbed. This selects against genetically diverse progeny pre-adapted to fill a diverse array of niches, providing opportunities for clonal populations. These rather intuitive hypotheses include many assumptions, remain untested, and are unsatisfactory in explaining geographic parthenogenesis (Lynch 1984).

Although parthenogenesis is rather widespread across some insect groups such as the Hymenoptera (Suomalainen 1962), it is relatively uncommon in the Chironomidae (Lindeberg 1951; Armitage et al. 1995; Gokhman and Kuznetsova 2017). These non-biting midge flies are often the most common and species-rich macroinvertebrate taxon in freshwater habitats, with estimates of the total richness of this family surpassing 10,000 species (Armitage et al. 1995), with the tropics still poorly studied (Ferrington 2007).

Of the at least 6,434 described chironomid species to date (ISC 2017; Stur and Ekrem 2020), less than 0.5% (~30 species) are known as parthenogens (Table 1). These represent 3 of the 11 extant chironomid subfamilies (Cranston et al. 2012): the Chironominae, Orthocladiinae, and Telmatogoninae. In the Chironominae, there are 17 parthenogenetic species found among 7 genera (Grimm 1870; Thienemann 1954; Lindeberg 1958; Lindeberg 1971; Grodhaus 1971; Oliver and Danks 1972; Armitage et al. 1995; Langton 1998; Donato and Paggi 2008; Porter and Martin 2011); in the

Table 1. Chironomid taxa with evidence of parthenogenesis as of 2020.

Subfamily	Taxon	Reference
Chironominae	*Tanytarsus norvegicus* (Kieffer, 1924)	Lindeberg 1971
	Tanytarsus gregarius (Kieffer, 1909)	Lindeberg 1971
	Tanytarsus sp. *(sylvaticus)*	Lindeberg 1971
	Tanytarsus sp. *(lestagei)*	Lindeberg 1971
	Tanytarsus heliomesonyctios (Langton, 1999)	Langton 1998
	Paratanytarsus grimmii (Schneider, 1885)	Grimm 1870
	Paratanytarsus laccophilus (Edwards, 1929)	Lindeberg 1958
	Paratanytarsus sp. (*boiemicus*)	Lindeberg 1971
	Micropsectra silvesterae (Langton, 1999)	Langton 1998
	Micropsectra sp. (*nigripila*)	Armitage et al. 1995
	Micropsectra sedna (Oliver, 1976)	Porter et al. 2011
	Chironomus atrella (Townes, 1945)	Grodhaus 1971
	Chironomus attenuatus (Walker, 1848)	Grodhaus 1971
	Chironomus stigmaterus (Say, 1823)	Grodhaus 1971
	Lauterborniella sp.	Oliver et al. 1972
	Polypedilum parthenogeneticum (Donato and Paggi, 2008)	Donato et al. 2008
	Zavreliella marmorata (Wulp, 1858)	Thienemann 1954
Orthocladiinae	*Corynoneura celeripes* (Winnertz, 1852)	Edwards 1919
	Corynoneura donovani (Forsyth, 1971)	Forsyth 1971
	Corynoneura scutellata (Winnertz, 1846)	Edward et al. 1968
	Limnophyes minimus (Meigen, 1818)	Armitage et al. 1995
	Limnophyes vestitus (Skuse, 1889)	Forsyth 1971
	Abiskomyia virgo (Edwards, 1937)	Armitage et al. 1995
	Bryophaenocladius furcatus (Kieffer, 1916)	Armitage et al. 1995
	Eretmoptera murphyi (Schaeffer, 1914)	Armitage et al. 1995
	Metriocnemus abdominoflavatus (Picado, 1913)	Thienemann 1954
	Phytotelmatocladius delarosai (Epler, 2010)	Siri et al. 2014
	Pseudosmittia baueri (Strenzke, 1960)	Armitage et al. 1995
	Troglocladius hajdi (Andersen, Baranov et Hagenlund, 2016)	Andersen et al. 2016
Telmatogetoninae	*Telmatogeton amphibious* (Eaton, 1875)	Crafford 1971
	Telmatogeton sp.	Delettre et al. 2003

Orthocladiinae there are 12 species from 9 genera (Edwards 1919; Thienemann 1954; Edward and Colless 1968; Forsyth 1971; Armitage et al. 1995; Siri and Donato 2014; Andersen et al. 2016; Bartlett et al. 2018); and in the Telmatogetoninae there are two species from the type genus *Telmatogeton* (Crafford 1971; Delettre et al. 2003).

Of all these parthenogenetic species (Table 1), the best represented genera are *Tanytarsus*, *Paratanytarsus*, *Micropsectra*, *Corynoneura*, and *Limnophyes*, while at the other extreme some genera show very limited evidence of parthenogenesis (e.g. *Chironomus*) (Oliver and Danks 1972; Armitage et al. 1995). Although most parthenogenetic chironomids are found in arctic and subarctic habitats (Armitage et al. 1995), others are reported from sub-Antarctic islands (Crafford 1971; Delettre et al. 2003), phytotelmata in Argentina (Siri et al. 2014), and a cave in Croatia (Andersen et al. 2016). Furthermore, since many of these parthenogenetic chironomids are triploid and of hybrid origin, they are permanent genetic heterozygotes that may be more apt to find certain habitats suitable (Carew et al. 2013). Considering the wide range of habitats and the taxonomic difficulty of identifying female midges, parthenogenesis in the Chironomidae may be more common than previously believed (Ekrem et al. 2010; Stur and Ekrem 2020).

Not only do parthenogenetic chironomids vary in habitat, but they also vary in the extent of their parthenogenesis. Some of the taxa in Table 1 are strictly parthenogenetic, like *Paratanytarsus grimmii*, the globally-distributed, notorious pest of water-supply systems (Carew et al. 2013). Other taxa (e.g. *Tanytarsus heliomesonyctios*) exhibit population-specific parthenogenesis, with parthenogenesis evident for populations that occur in relatively harsh environmental conditions (Orel and Semenchenko 2019; Stur and Ekrem 2020). This is also the case for *Paratanytarsus laccophilus*. Lindeberg (1958, 1971) documented parthenogenetic populations of *P. laccophilus* in rock pools on the isles of the Gulf of Finland, yet found bisexual reproduction in all other locations where he studied this species. Here we present evidence of additional parthenogenetic populations from the *P. laccophilus* species group, found in the North American High Arctic near Utqiaġvik, Alaska.

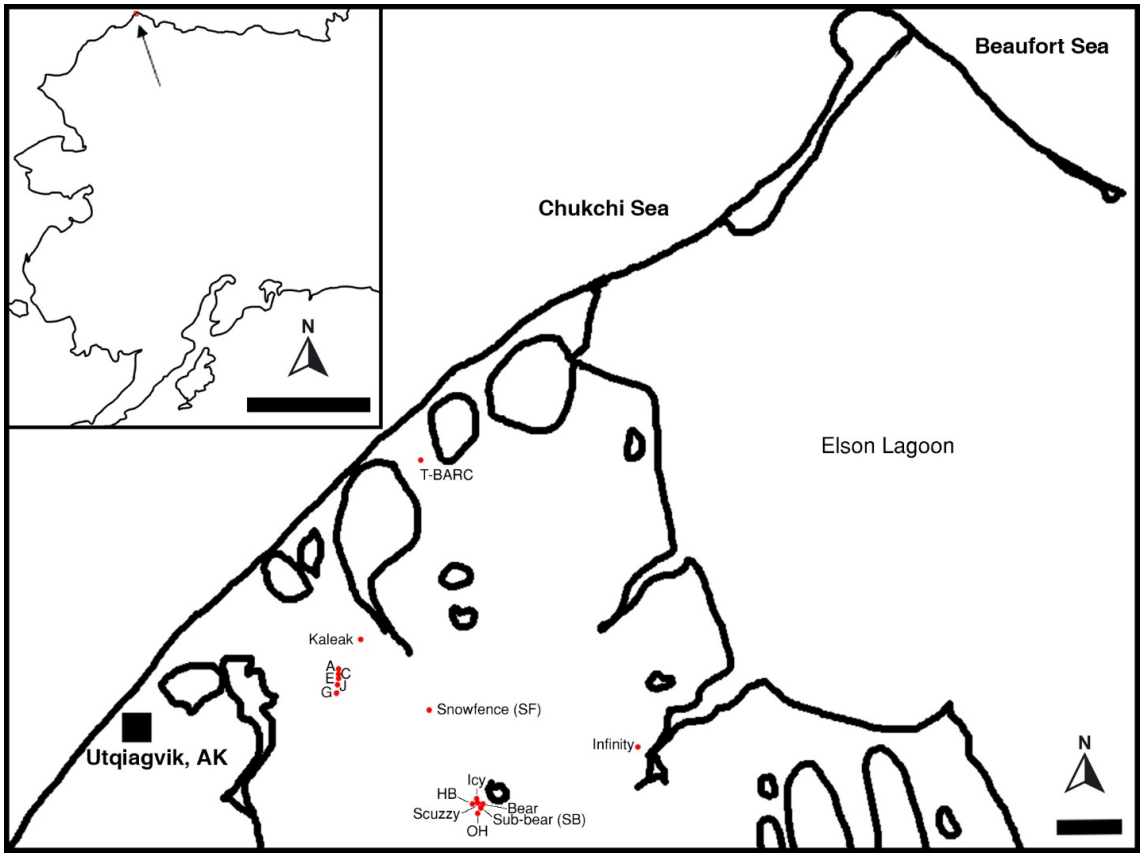

Figure 1. Map of the field site at Utqiaġvik, Alaska. Individuals of the *Paratanytarsus laccophilus* species group were taken from the fifteen tundra ponds labeled (red dots). Scale bars = 500 km (inset), and 1 km.

Materials and Methods

The study site is located at Utqiaġvik (formerly Barrow), Alaska (71°17'27.5"N 156°47'18.5"W). We sampled a total of fourteen tundra ponds (Fig. 1) within an 8 km radius of the village for chironomid pupal exuviae (PEs) across the 2015 and 2016 emergence seasons (*i.e.* from thaw in mid-late May to late July, when chironomid emergence ended). We conducted this phenological sampling daily in both years, with Ponds A, C, E, J, G, and Kaleak sampled on even days, while Ponds Bear, OH, HB, Icy, and Sub-Bear sampled on odd days. Ponds Snowfence and Scuzzy were part of the odd day sampling in 2015 only, and Pond Infinity was sampled only twice in 2015.

Ponds A, C, E, J, G, OH, Bear, and Infinity are low-centered polygon ponds (Liljedahl et al. 2012) with a maximum depth of 0.5 m, surface areas largely consisting of open water, and ranging in size from ~175 m^2 to 900 m^2 total area. Ponds HB and Icy are degrading ice-wedge ponds (Liljedahl et al. 2012) with a maximum depth of 2 m, largely open water, and size ranging from ~120 m^2 to 180 m^2 total area. Ponds Kaleak, Snowfence, Sub-Bear, and Scuzzy are the smallest ponds (areas ~18 m^2 to 750 $m^{2)}$ and shallowest (maximum depth ~30 cm) and are thoroughly vegetated with *Carex* and *Arctophila.* Sub-Bear, and Scuzzy are so shallow they lost most standing water by late June).

Samples consisted of five 1 m dip net sweeps taken on the downwind side of the pond to collect surface-floating PEs and emerging or failed adults. Pooled net contents were stored in 35% ethanol in the field, and subsequently transferred to 70% ethanol. Sampled PEs were sorted, sexed, and tallied in the lab under a dissecting microscope, and identified to species group according to Wiederholm (1986).

While processing 2015 field samples, it became evident that individuals of the *P. laccophilus* species group might be parthenogenetic due to a lack of males. In 2016 we took larval samples for rearing under controlled temperature conditions in the lab. These larvae came from Kaleak Pond on 19 and 28 June, and from Pond T-BARC (Fig. 1) on 30 June. Small tanytarsine larvae hypothesized to include individuals of the *P. laccophilus* species group were batch-placed into rearing cups (replicated thrice) with detritus and pond water and incubated at eight temperature treatments ranging from 5-25 °C (as this rearing setup was part of another study testing chironomid developmental responses to temperature). These lab incubations consisted of eight oxygenated aquaria controlled for water temperature (±1°C) with EcoPlus™ aquarium chillers and monitored with Onset® Hoboware temperature loggers. We monitored larvae and pupae daily for development and emergence of adult flies. For each emerged specimen, we recorded temperature treatment, source pond, species, sex (confirmed by examination of PE genital structures under a dissecting microscope), and date of eclosion. We immediately preserved emerged adults and their pupal exuviae (PEs) in 70% EtOH, except when an emerged singleton from the *P. laccophilus* species group was confirmed (see below). Adults were photographed laterally under a Wild dissecting microscope (Fig. 2) in a thin film of 70% EtOH - just enough to submerse the specimen such that individuals would lie laterally. Photomicroscopy was done with a Canon EOS Rebel T3i camera attached to a trinocular mount.

We isolated reared singletons of the *P. laccophilus* species group that were confirmed as virgin (i.e. no other species or conspecifics had emerged in that daily cohort) by first locating the adult midge beneath the mesh of the rearing cup by shining a light through the base of the cup (Fig. 3a). When a potential specimen was located, we carefully coaxed her to the mesh of the lid (were she not already there). As adults of this species are fast and agile fliers, we took care to prevent escapees. Once the female was on the mesh, (Fig. 3a), we carefully unscrewed the lid and quickly placed it on the table, trapping the fly. We then located and identified the lone PE within the opened rearing cup to confirm it was of the *P. laccophilus* species group (Fig. 3b). If confirmed, we prepared a new rearing cup containing only pond water. The lid with the trapped female clinging to the mesh was then quickly transferred to the new rearing cup. We monitored these isolated individuals for behavior, and ultimately, oviposition. If oviposition took place, we monitored and photographed the egg mass daily under a dissecting microscope, watching for egg development and hatching. Once hatched and larvulae had consumed the gelatinous mass surrounding the eggs, we added filtered (50 µm) pond detritus (free of exotic chironomid larvae) to each microcosm.

We used JMP 14 Pro Statistical Discovery™ for statistical analysis and graphical output. Degree hours (DH) for each emerged specimen were calculated as mean temperature experienced*total hours for development.

Results

Over the 2015 and 2016 field seasons, we collected a total of 623 PEs of the *P. laccophilus* species

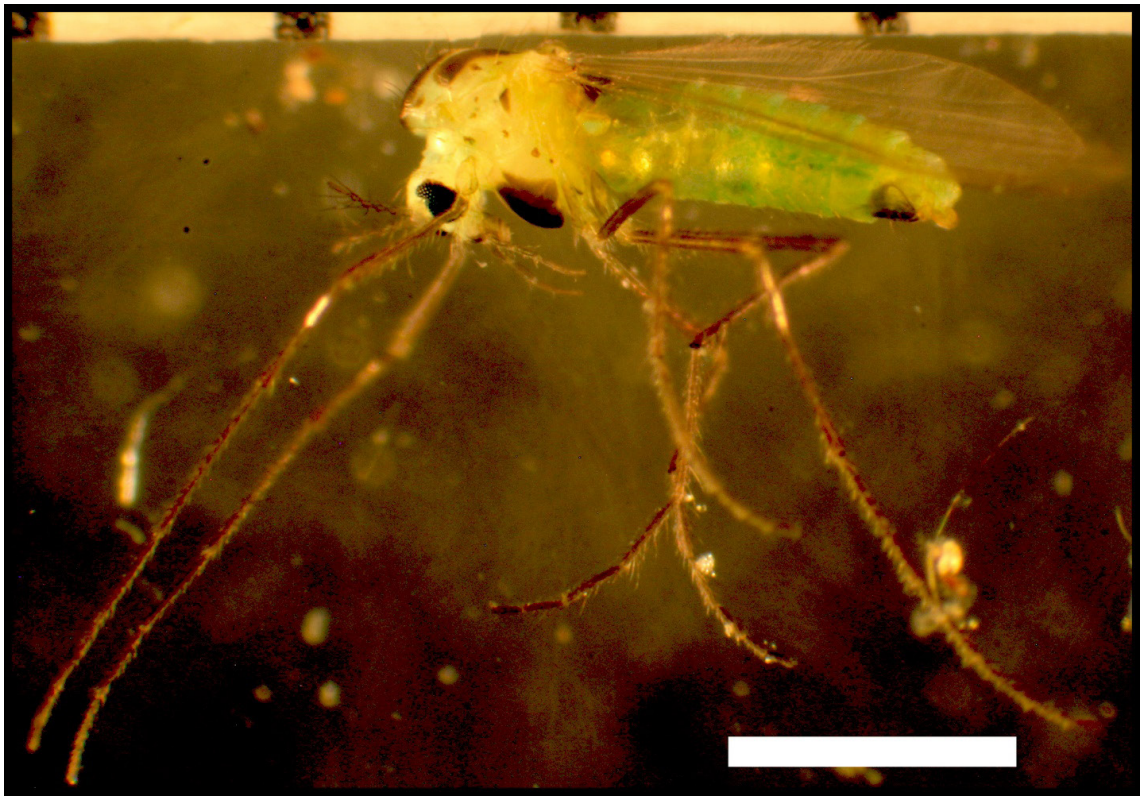

Figure 2. Lateral view of a recently emerged individual (female) of the *Paratanytarsus laccophilus* species group in Utqiaġvik, Alaska. The specimen was submerged in a thin film of 70% EtOH immediately after it emerged from a rearing cup in the lab, and her natural coloration was captured via photomicroscopy. Scale bar = 1 mm.

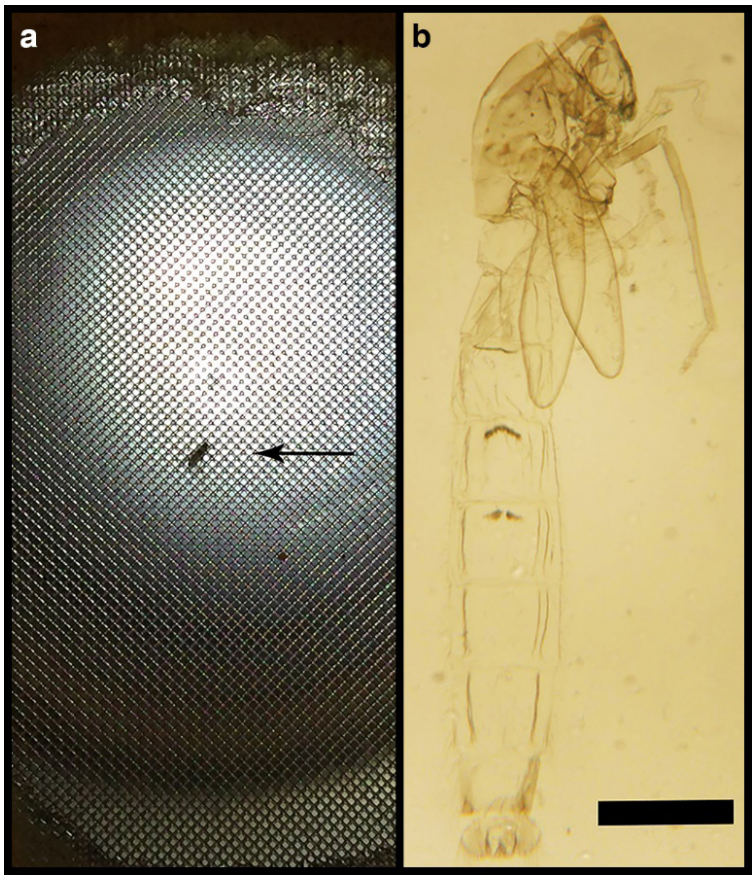

Figure 3. a) A freshly emerged, virgin individual of the *Paratanytarsus laccophilus* species group (arrow) clinging to the mesh lid of the new rearing cup to which it was transferred. The isolated female was then monitored daily for signs of oviposition. b) The pupal exuviae (PE) collected from the larval rearing cup, used to confirm species-group identification. Note the diagnostic patterning on abdominal tergites III and IV (Wiederholm 1986). Scale bar in (b) = 0.5 mm.

group, identified according to Wiederholm (1986). All specimens were female, and came from fourteen tundra ponds (Fig. 1) representing a range of habitat types (see methods). We also reared 37 individuals of the *P. laccophilus* species group (all female), from larvae, collected from two pond sources, to emergence under lab conditions (Table 2).

Kaleak Pond produced the most specimens of the *P. laccophilus* species group of all habitats across both years, and the PE sweep data from this pond offers insight into the species' emergence phenology (Fig. 4). This species showed highly synchronized emergence, even compared to other, sexually-reproducing species at Utqiaġvik (Butler 1980a). In 2015, the peak emergence date (the date on which the greatest number of this species emerged for a given year) for individuals of the *P. laccophilus* species group (Julian date 170) was also the first day PEs of this species were collected from this pond, and these comprised 76% (139/183) of that season's total. Our 2015 sampling in Kaleak began 20 days earlier (on Julian date 150: 30 May), and no PEs of this species were collected on any of the 13 day-specific samples prior to peak emergence on Julian date 170 (19 June). In 2016, 72% (242/338) of all collected individuals of this species emerged in three consecutive samples from 4-8 July (Julian dates 186, 188, and 190), with the peak for this year being 8 July (Fig. 4a), again documenting a high degree of emergence synchrony. In 2015 and 2016 the median date of emergence was also the date of peak emergence in both years (Julian dates 170 and 190 respectively).

Despite this synchrony, this species' emergence timing varied across years. Thaw dates were nearly identical in Kaleak Pond between 2015 and 2016, with only a two-day difference - 2015 having the later start (Fig. 4b). Yet peak emergence of this species in 2015 was ~20 days earlier than in 2016 (Fig. 4a). This difference in phenological emergence pulse corresponds to a difference of more than 3,800 DH between the two years. In 2015, the median degree hour experience from thaw to emergence was 4,102 (mean: 5,339 ± 378 [95% CI]), while in 2016 it was 7,986 (mean: 8,026 ± 132 [95% CI]). Comparing median values, the DH experienced from thaw to emergence in 2015 was just 51% of that experienced in 2016.

Of the 37 individual larvae reared adults (all were female) in the lab, three emerged as singletons. These were transferred into their own rearing cups and tracked for behavior and oviposition. These adult females were most often seen clinging to the mesh lid (Fig. 3a), but were sometimes observed skittering across, or resting on the water's surface in the rearing cup. We found one adult "belly-up" on the surface of the water three days after she eclosed and was moved to her isolated cup (Table 3). The adult was confirmed dead, and no signs of progeny were discovered in the rearing cup (*e.g.* no egg mass or larvae).

Table 2. Collection information for individuals of the *Paratanytarsus laccophilus* species group in the present study.

N (2015, 2016)	N (total)	% ♀	Pond	Coordinates	Sample type
(183, 338)	521	100	Kaleak	71°17'54.36"N, 156°41'46.32"W	PE sweep
(36, NA)	36	100	Snowfence	71°17'23.52"N, 156°39'45.09"W	PE sweep
(1, 12)	13	100	Icy	71°16'39.46"N, 156°38'31.29"W	PE sweep
(4, 3)	7	100	J	71°17'36.75"N, 156°42'5.43"W	PE sweep
(2, 5)	7	100	G	71°17'32.84"N, 156°42'3.55"W	PE sweep
(2, 4)	6	100	Sub-bear	71°16'36.36"N, 156°38'24.19"W	PE sweep
(3, 2)	5	100	A	71°17'41.33"N, 156°42'9.39"W	PE sweep
(1, 4)	5	100	HB	71°16'38.42"N, 156°38'32.46"W	PE sweep
(5, NA)	5	100	Scuzzy	71°16'38.72"N, 156°38'31.49"W	PE sweep
(0, 5)	5	100	Bear	71°16'37.11"N, 156°38'22.99"W	PE sweep
(4, NA)	4	100	Infinity	71°17'4.26"N, 156°34'22.65"W	PE sweep
(1, 2)	3	100	C	71°17'40.21"N, 156°42'8.06"W	PE sweep
(1, 2)	3	100	E	71°17'38.85"N, 156°42'6.81"W	PE sweep
(1, 2)	3	100	OH	71°16'35.25"N, 156°38'27.91"W	PE sweep
(NA, 34)	34	100	Kaleak	71°17'54.36"N, 156°41'46.32"W	Lab rearing
(NA, 3)	3	100	T-BARC	71°19'25.18"N, 156°40'7.30"W	Lab rearing
N(All)	660	100	15	NA	

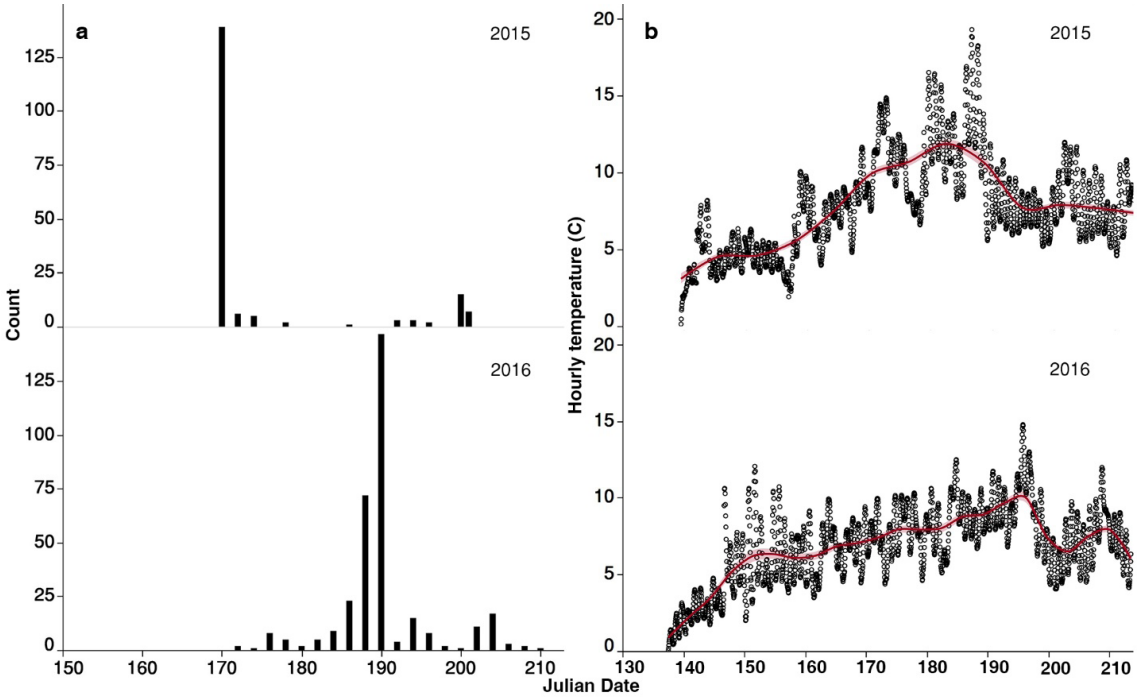

Figure 4. a) Emergence synchrony of individuals of the *Paratanytarsus laccophilus* species group as evidenced by pupal exuviae count vs. sample date (Julian) in 2015 and 2016 from Kaleak Pond. In both years sampling began on Julian day 150 (30 May and 29 May, respectively). In 2015 sampling concluded on Julian date 201 (20 July), and in 2016 on day 213 (31 July). b) Hourly temperature (°C) vs. Julian date from a Hoboware data logger in Kaleak Pond. Thaw was defined according to McEwen and Butler (2018) using the pond-specific logger. In 2015 pond thaw occurred on day 139 (19 May), and in 2016 on day 137 (16 May). The red curve through the data points represents the cubic spline with a bootstrap confidence region at lambda = 0.05.

The other adults died 2-3 days after eclosion. Upon inspection, a single egg mass was immediately noticeable in the bottom of each cup (Fig. 5a-b). These egg masses consisted of a string of eggs in a spiral, within an outer, oval-shaped, gelatinous mass. After 2-5 days (depending on treatment) the eggs hatched (Fig. 5c-g). Larvulae remained within the spiral of the egg rope (e.g. Fig. 5g) in the hours immediately after hatch. By the next day they were moving within the outer gelatinous matrix of the egg mass or freely moving about the rearing cup. The egg mass morphology, time between eclosion and deposition of the egg mass, egg number, time to hatch, and larvulae behavior are all concordant with Lindeberg's (1958) pioneering work describing parthenogenetic populations of *P. laccophilus* in southern Finland.

After we added pond detritus to the rearing cups, the newly hatched larvulae grew and developed in their respective temperature treatments for the remaining duration of the field season. As this species emerged rather late in the season (Fig. 4a) compared to known chironomid phenologies at Utqiaġvik (Butler 1980a; Butler 1982; Braegelman 2016; Lackmann and Butler 2018; Butler and Braegelman 2018), there was little time for additional larval development as our field season ended on 31 July. We preserved the thriving larvae on 28 July and found numerous larval exuviae (LEs) in each rearing cup, indicating that these parthenogenetically-produced larvae had molted to the second larval instar.

Table 3. Individual adults from the *Paratanytarsus laccophilus* species group reared in isolation and their subsequent life history events. All three individuals came from Kaleak Pond. Individual (I); Temperature treatment (Trt); Larval exuviae (LEs)

I	Trt. (°C)	Eclosion	Oviposit	Adult perished	# of eggs	Hatch	LEs present
1	18.3	2 July	NA	5 July	NA	NA	NA
2	15.3	8 July	11 July	11 July	109	16 July	28 July
3	21.0	11 July	13 July	13 July	87	15 July	28 July

Discussion

In cases like *Paratanytarsus laccophilus*, where parthenogenesis appears population-specific, it remains unclear if populations are facultatively parthenogenetic in certain environments, or if obligatory bisexual and asexual strains have evolved independently and now coexist globally (Lindeberg 1971; Oliver and Danks 1972; Armitage et al. 1995). No matter how it evolved, parthenogenesis in a chironomid at Utqiaġvik, AK should not be surprising. The study site is located in the High Arctic where temperatures are low, winds are high, and the overwintering period encompasses most of the year (Butler 1980a). The fitness benefits of being parthenogenetic in such a habitat are likely great because every individual in the population is female and is theoretically capable of producing offspring herself. The risk involved in finding a mate is avoided, and only one individual is hypothetically necessary to establish a population in a new habitat (Lynch 1984). This allows for rapid population growth and productivity. Sexually reproducing species tend to require more individuals for population establishment to be successful, because future generations may be unlikely to find mates if founding numbers are low (Suomalainen 1962; Lynch 1984; Bartlett et al. 2018; 2020).

It is unknown when individuals of the *Paratanytarsus laccophilus* species group colonized Utqiaġvik. Similar to what has been completed for *Paratanytarsus grimmii* (Carew et al. 2013), genetic analyses on populations of *P. laccophilus* at a global scale (e.g. from Finland and Alaska) would be valuable in understanding this history, and in determining the exact taxonomic position of these Alaskan populations. Such genetic information could provide insight on the dispersal of *P. laccophilus* over evolutionary time and provide a basis for interpreting the species' present trajectory. It is interesting to note that this species group was not documented in the Utqiaġvik chironomid fauna during the 1970s (Lougheed et al. 2011), and that the habitats where this species was most abundant in the present study (thoroughly-vegetated, shallow tundra ponds) have also increased substantially over this timeframe (Liljedahl et al. 2016). The species may simply have gone undetected during the 1970s because their habitat type was not as frequently sampled as it was in the 2010s. It is also possible that this parthenogenetic species is a recent invader of this polar extreme, similar to the parthenogenetic chironomid *Eretmoptera murphyi* that recently invaded Antarctica (Bartlett et al. 2018; 2020), but this remains unknown.

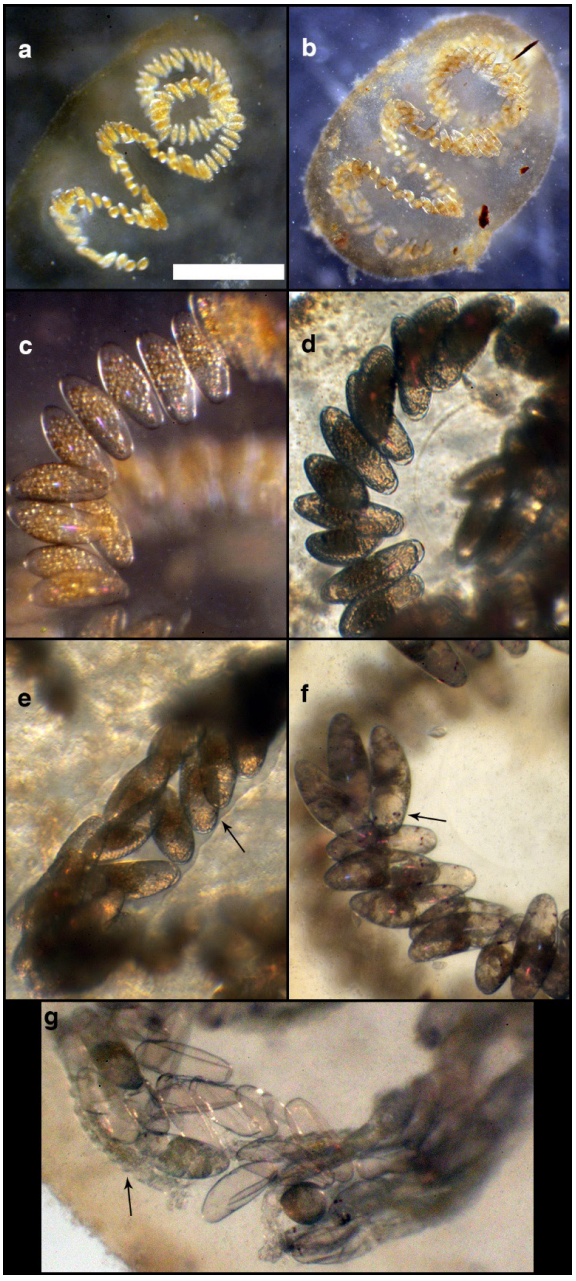

Figure 5. Development of parthenogenetic *Paratanytarsus laccophilus* species-group progeny in the 15.3 °C treatment in 2016. a) An egg mass freshly laid on 11 July. b) The same egg mass on 15 July, just before hatch. c-g) Daily progression of development. **c**) Eggs on 12 July 2016 at one day old. d) At two days old. e) At three days old, eye spots (arrow) and head capsules were evident inside the egg. f) At four days old, eye spots and head capsules (arrow) were well-defined within the egg. g) Larvulae (arrow) hatched on 16 July, five days after oviposition. Several vacated eggs are clearly notable. Scale bar = 1 mm for a and b, and 250 µm for c-g.

The highly synchronous emergence of this Alaskan population is intriguing considering males are lacking and females reproduce parthenogenetically. Thus, selection for synchronized emergence for the purpose of mate-finding, as proposed by Butler (1980a), would seem unnecessary. Perhaps their synchronized emergence is a heritage trait from sexually reproducing ancestors. Still, other factors (*e.g.* optimal temperatures and polarized light) might maintain such eclosion synchrony, even as there is continuous light during the arctic growing season, but this remains to be studied. In contrast, the parthenogenetic and brachypterous *E. murhpyi* that has invaded Antarctica ecloses asynchronously across a broad phenology (2-3 months) (Bartlett et al. 2018; 2020).

Even though individuals of the *P. laccophilus* species group emerged highly synchronously (i.e. >70% of total emergence occurred within a 4-day interval in both years), their seasonal timing and total heat sum from spring thaw to eclosion differed substantially (Fig. 4). This may suggest that larvae overwinter at different stages of development on a year-to-year basis depending on how much they can develop prior to the fall freeze, or that other environmental variables, that have so far gone unstudied, are significant drivers of their emergence timing. In the current climate change era, arctic warming is amplified (IPCC 2014). If individuals of the *P. laccophilus* species group colonized the Alaskan Arctic with the past 50 years, perhaps they are pre-adapted for this rapidly evolving tundra landscape. Migratory shorebirds are also well-documented to feed on chironomids at this location (Braegelman 2016). If these populations thrive under such a warming climate and evolving landscape, it might benefit some avian insectivores.

Conclusion

We present evidence of parthenogenesis in an arctic Alaskan chironomid of the *Paratanytarsus laccophilus* species group. This evidence suggests a reevaluation of geographic parthenogenesis in *P. laccophilus* is necessary. It may turn out that Lindeberg's original hypothesis of geographic parthenogenesis in this species is correct (1958), even though he later discarded it (1971). Although parthenogenesis in the Chironomidae is generally considered uncommon (Armitage et al. 1995), population-specific incidences such as *P. laccophilus* suggest other sexually reproducing species may have parthenogenetic strains that are not yet known.

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Chironomus calligraphus Goeldi, 1905 and *C. hawaiiensis* Grimshaw, 1901 are sibling species

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Abstract

Although Spies et al.'s (2002) thorough analysis of a small sample of the then available specimens of *Chironomus calligraphus* Goeldi,1905 suggested two distinctly different haplotypes in the mitochondrial *COII* gene, the corresponding morphological and cytological evidence kept the authors from concluding that there were two species involved. Further obstacles were unusual aspects of the molecular data and the occurrence of both these haplotypes in samples from the Brazilian type locality by Fittkau (1965) from which he had fixed a neotype. This neotype is slide mounted and can no longer yield molecular data. The present author's analysis of additional material, including the available BARCODE sequences, has shown the existence of two forms, different from those found in the flawed *COII* analysis, and with largely overlapping geographic distributions. One of these forms occurs in Hawaii where it is morphologically indistinguishable from *C. hawaiiensis* Grimshaw, 1901. It is recommended to apply this name, which takes nomenclatural precedence to the form found in Hawaii, and the name *C. calligraphus* to the form found to be more common in Fittkau's type-locality samples.

History

Chironomus calligraphus was originally described by Goeldi (1905) from 'numerous males and females caught as well as reared' from several water tanks in the botanical garden at Belém, Para, Brazil but according to Fittkau (1965: 209) all those syntypes have been lost (lodgement in the Museum of Berne would seem to have been the most likely event). Fittkau re-collected "several times in the course of various years" (*loc. cit.*) and designated a male that had been mass-reared from an egg mass as the neotype (slide mounted and in Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil). Many specimens of all life stages from Fittkau's samples are in the Zoologische Staatssammlung München (ZSM), and several of them were used in the analysis by Spies et al. (2002).

A 'nuisance' species common in southern California was identified as *C. calligraphus* and the species redescribed by Spies et al. (2002) from a comparison of the available material as well as drawing together the data from previous publications (e.g. Roback 1962, Spies 2000). Spies et al. (2002) discussed that their morphological and cytological data were consistent with a single variable species, or two geographically separated ones, but that both these possibilities looked incompatible with the molecular sequences they had obtained for a fragment of the mitochondrial cytochrome c oxidase subunit II (*COII*) (e.g. GeneBank accessions AJ310770; AJ311529). These sequences showed 2 widely separated haplotype groups with a mean p-distance of 21.7% between them, but each group contained specimens from both Brazil and California, i.e. material collected about 9000 km and 30 years apart. Moreover, while the genetic variation was ordinary within one haplotype group (pairwise p-distances up to 2.4%) it was 0.0% in the other group. Under these circumstances and with no consistent morphological and no apparent cytological differences corresponding to the genetic group differences, Spies et al. (2002) took the conservative decision not to propose a taxonomic split within *C. calligraphus* Goeldi. In addition, the question as to which subdivision would keep the name *C. calligraphus* was impossible to answer, as the genetic make-up of the slide-mounted male fixed as the neotype by Fittkau is unlikely to ever be determinable.

Present situation

Subsequently I have received more specimens of the *C. calligraphus* phenotype from other localities in California, Florida and Kansas, as well as from Hawaii. I also had larvae from the original Huntington Beach and adults from Coyote Creek localities studied by Spies et al. (2002).

In addition to morphological and cytological analyses, some of these specimens have been barcoded for the conventional mitochondrial cytochrome c oxidase subunit I (*COI*) fragment using the Folmer et al. (1994) primers: LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198

(5′-TAAACTTCAGGGTGACCAAAAAATCA-3′), with PCR products sent to Macrogen Inc. Seoul, Republic of Korea for sequencing. These sequences are in GenBank (MW378322-MW378327). As well, many sequences are also available in the BOLD Database or in GenBank (e.g. KF278357 from Proulx et al. (2013); KX051951(Tahiti)).

These sequences again indicate two groups, placed in separate Barcode Index Numbers (BINs) in the BOLD Database (Ratnasingham and Hebert 2013). These groups cannot be compared to those from the previous *COII* analyses, because a search of the sequence of the invariant group in GenBank showed no high similarity to the few available *Chironomus* sequences, but instead to the syrphid *Cheilosia canicularis* (GenBank accession AY055210, 92%). The following best matches are all syrphids, which strongly suggests that the two *COII* sequence identified as *Chironomus calligraphus* (GenBank accessions AJ311529, AJ310775) are based on samples contaminated by a species of Syrphidae. The arguments below are therefore based only on the *COI* results. The *COI* groups have been called Type 1 and Type 2 by Martin (2020).

These two *COI* types are in nearest neighbour BINs, separated by a pairwise difference of only about 1.6% and with several different sequences within each BIN. The observed distance between the two barcode BINs is sufficient to conclude that these represent sibling species, since examples from other sibling species in *Chironomus* show that they may differ by much smaller distances, down to ten bases or less (Martin 2011) and there are also cases where sibling, or even morphologically distinct species differ by so few bases that they are included in the same barcode BIN, e.g. *C. staegeri* Lundbeck, 1898, *C. frommeri* Sublette & Sublette, 1972, and *C. 'tigris'* (or species r of Butler et al. 1995) are all in BIN BOLD:AAP3004.

The barcode results (above) indicated that both groups co-occur in some localities (California and Central America) while only one form occurs in other areas – e.g. type 1 in Florida and Kansas, type 2 in Hawaii and Tahiti.

Further morphological and cytological analyses show little consistent difference between the two types. It is possible that there is a difference in the proportions of the fore legs of the males, with the LR1 of type 1 about 1.7-1.9, and that of type 2 about 1.59-1.65, but this needs confirmation from a larger number of specimens (and see below). Most banding patterns of the polytene chromosomes are also identical between the two groups, with only two rare polymorphic sequences in Type 1 that have not been found in type 2. The figure in Spies et al. (2002) is from Type 2 at Huntington Beach, California, with the rare sequences from type 1 and others from specimens of unknown type in South America, shown by brackets above the inverted area. It should be noted that no specialised staining techniques have been attempted to check whether there are differences in the distribution of heterochromatin or repetitive elements between the two forms, as has been demonstrated for some other sibling species, e.g. the *C. plumosus* group (Michailova 1987).

The presence of type 2 of *C. calligraphus* in Hawaii raised another consideration regarding identity of the *C. calligraphus* forms. Some of these specimens were collected by the author with assistance of entomologists from the Bishop Museum as *Chironomus hawaiiensis* Grimshaw, 1901 beginning in 1967, with some provided by other workers.

Samples were available from: Waikiki (19.33°N, 157.72°W) 3-ix-1967, adult male, JM; Honolulu, vicinity of Bishop Museum (19.33°N, 154.88°W) 8-ii-1969 larvae collected and reared to adults by E. Drake.; Ka'elepulu Pond, Kailaua (21.42°N, 157.72°W) 31-viii-1970 larvae, JM & E. Drake; Kahana Iki stream 1.6 Km south of Kailaua (21.38°N, 157.77°W) 31-viii-1970, E. Drake & JM– all Oahu; Kealia Pond, Kihei (20.75°N,156.45°W) Maui 8-xi-1999, M. Nishimoto.

Some larvae have been examined cytologically, and three from three different localities on two of the Hawaiian Islands have been able to be sequenced for the barcode sequence of *COI,* as outlined above, with at least one of them also initially identified as *C. calligraphus* on the basis of the chromosomal banding pattern. The *COI* barcode sequences are *C. calligraphus* Type 2. There are two possibilities here that need to be considered: that *C. hawaiiensis* is conspecific with *C. calligraphus* Type 2, or that *C. calligraphus* has invaded Hawaii and replaced *C. hawaiiensis* on at least Oahu and Maui. Given that Williams (1944) quotes R.C.L Perkins as stating that 'the status of *C. hawaiiensis* as a native insect was very doubtful' the question should probably be whether the introduction of *C. calligraphus* occurred before or after the description of *C. hawaiiensis.*

The possibility of its introduction after the description of *C. hawaiiensis* only needs to be considered if there is reason to consider *C. hawaiiensis* morphologically or cytologically distinct from *C. calligraphus.* In the

former case, the name *C. hawaiiensis* would have precedence as it was published before Goeldi's description of *C. calligraphus*. All published descriptions of *C. hawaiiensis* lack detail and the larva and pupa are represented only in illustrations by Williams (1944). Hardy (1960) provided more details on the adult male but not enough to allow distinction from *C. calligraphus* type 2, and his description of the coloration would be consistent with *C. calligraphus*. As well, his illustration of the male hypopygium is very similar to that of Hawaiian "*C. calligraphus*" males in the present authors collection (Fig. 1): gonostylus moderately swollen and reducing over the posterior third/half; inferior volsella reaching about 2/3 thirds of the length of the anal point (right-hand figure below) or 1/3 of the gonostylus; the shape of the anal point is obviously affected by the mounting (see below) but that on the right below is relatively long and narrow as in Hardy's figure. Hardy also depicts about 10 setae on TIX in multiple pale patches as in the figure and data below.

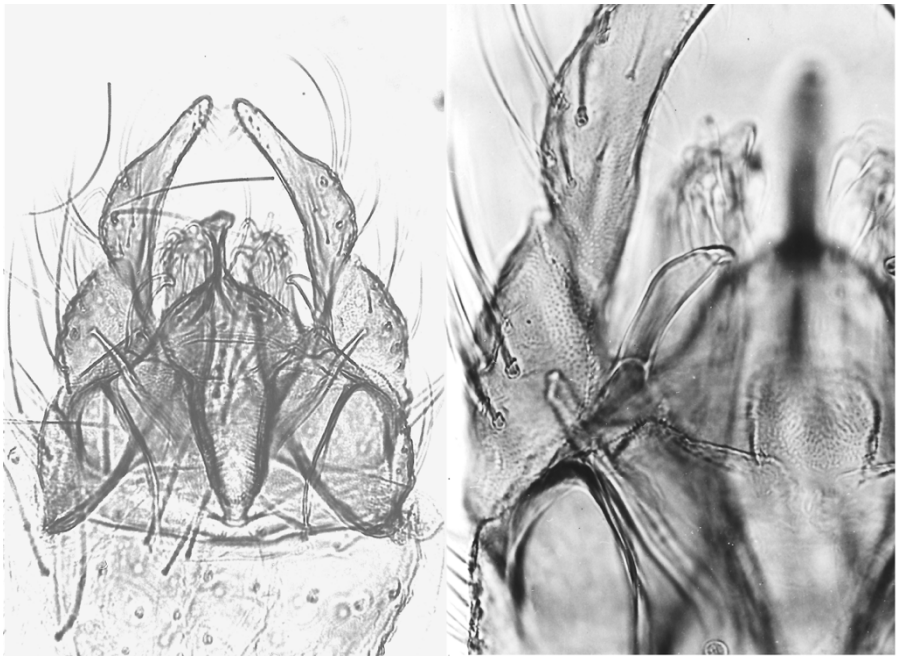

Figure 1. Male hypopygium (left) and superior volsella (right) of *C. hawaiiensis* from Oahu, Hawaii.

On my behalf, Martin Spies has kindly taken a loan of four of the six original syntypes of *C. hawaiiensis* (3 adults of either sex) from the Natural History Museum (NHM) in London, and provided the following details on two of the males:

First syntype: Wing length 2.61 mm; AR 3.0; LR1 1.70; fore tibia length 1000 µm.

Second syntype: Wing length 2.44 mm, antenna and fore tarsi missing; fore tibia length 960 µm.

Abdominal marking between that of Figs. 1a and 1c of Spies et al. (2002).

Superior volsellae also similar to those seen in specimens of *C. calligraphus* (*op. cit.:* figs 2-3).

Thus, these syntypes are consistent with being conspecific with *C. calligraphus*. Martin Spies also queried with the NHM the possibility of attempting to obtain DNA sequence from at least one of the types, but has not received any constructive response.

Additional morphological details are available from two males, identified as *C. hawaiiensis,* from localities on Oahu. One of these is the male whose hypopygium is shown in Fig. 1, the other is reared from the sample of larvae with the *COI* sequence of *C. calligraphus* Type 2.

Wing length 2.28 and 3.52 mm; Antennal plume with a wide dark band, AR 2.93 and 3.52. Clypeus with about 28 setae; palps (micron) 60: 45:191: 200: 308; P5/P4 1.41 and 1.67.

Thoracic setae: acrostichal at least 11 and 17; dorsolateral 18 and 21; prealar 5; supraalar 1, scutellar 18 and 22 in two rows.

Fore LR 1.65 and 1.67; Fem/Ti 1.16 and 1.17; Mid LR 0.59 and 0.63; Hind LR 0.59 and 0.63. These LR1 are in the range suggested for *C. calligraphus* Type 2 (above) but taken along with the measurement from the syntype cast doubt on how reliable this character might be.

Abdomen with dark, posteriorly pointed, triangular area on midline with generally fairly narrow transverse band at or near anterior margin; 5-7 setae (11 in Fig. 1) in multiple pale patches on tergite IX. Hypopygium as in Fig. 1.

These data suggest no significant differences between these later specimens and the available descriptions of *C. hawaiiensis,* or the extra data from the two syntypes. Since there is no evidence that *C. calligraphus* Type 2 differs from *C. hawaiiensis,* there seems to be no reason to postulate a subsequent introduction of the former species.

While it would be nice to have molecular sequence to confirm this result, the logical conclusion from the above discussion is that *C. hawaiiensisis* is an earlier description of the species currently designated as Type 2 of *C. calligraphus.* This decision is not affected by which form of *C. calligraphus* is the true type form. Therefore, I would propose that *C. calligraphus* Type 2 is conspecific with *C. hawaiiensis* Grimshaw, 1901. This means that *C. hawaiiensis,* previously only known from Hawaii has a much wider distribution including Tahiti, western North America and Central and South America. This would be compatible with the view *C. hawaiiensis* was a man-caused introduction to Hawaii, as it would be quite likely that it originated in the Americas and was transported to Hawaii, and presumably also Tahiti (see BIN BOLD:AAP1715).

Concerning the name *C. calligraphus*, the most practical decision is to continue to use it for *C. calligraphus* Type 1 since this type is not conspecific with *C. hawaiiensis* and therefore not a synonym.

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I am grateful to Martin Spies for many helpful discussions on this problem and for providing examination results on ZSM specimens from California, as well as on syntypes of *C. hawaiiensis*, and for providing specimens from his California collections. I am also indebted to Peter S. Cranston and Michelle Sanford for specimens from California; Eugene Drake and Mike Nishimoto for specimens from Hawaii, and to Barbara Coler for specimens from Kansas. Peter Cranston and Torbjørn Ekrem also provided useful comments on drafts of this manuscript.

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Description of the pupa of *Tanytarsus alatus* Paggi (1992) (Chironominae: Tanytarsini)

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Abstract

The pupa of *Tanytarsus alatus* Paggi (1992) is described and figured for the first time. Notes for comparison with other species of the genus are provided, as well as brief notes on its geographic distribution.

Introduction

The species *Tanytarsus alatus* was described by Paggi (1992) based on the male adult. Later, Sanseverino and Fittkau (2007) transferred this species to the genus *Caladomyia* after a reinterpretation of the characters of the male genitalia. The genus *Caladomyia* was erected by Säwedal (1981) based on the posteriorly directed bars on the hypopygial anal point as a synapomorphy. In later works (Reiss 1972, Paggi 1992, Sublette and Sasa 1994, Trivinho Strixino and Strixino 2000, Reiff 2000), more species were described or transfered from the genus *Tanytarsus* van der Wulp, and after the revision of Trivinho Strixino (2012) the genus comprised 31 species. Recently, Lin et al (2018) analyzed *Tanytarsus* sensu lato based on the combined analysis of five nuclear markers and concluded that *Tanytarsus* is paraphyletic with *Caladomyia* Säwedal placed among South American *Tanytarsus*, *Virgatanytarsus* Pinder as part of a Gondwanan clade, and *Corynocera* Zetterstedt within the *Tanytarsus norvegicus* (Kieffer) species group. Lin et al (2018) formally synonymized *Caladomyia* and *Virgatanytarsus* with *Tanytarsus*.

In the present study, the pupa of *Tanytarsus alatus* is described, its taxonomic relationships are discussed in the group of *Tanytarsus* species with the posteriorly directed bars on the hypopygial anal point, and its geographic distribution is updated.

Material and methods

Collection of a pharate adult, along with several pupal exuviae from the same stream, allowed for life-stage association and description of the pupa of *Tanytarsus alatus*. A microscope slide for the pharate adult was prepared by clearing the specimen with 10% KOH; neutralization with glacial acetic acid; dehydration in 80%, 96% and 100% ethanol and mounting in Canada Balsam. Pupal exuviae were mounted with the cephalothorax separated from the abdomen and split into two halves along the mid-dorsal ecdysial opening with the outer surface facing upwards. Morphological terminology and measurement standards follow Sæther (1980) and Langton (2004); the values are rounded off to the nearest 5 µm unless otherwise stated and measurements are given as ranges. The material is deposited in the collection of the Instituto de Limnología "Dr. Raul A. Ringuelet", Argentina (ILPLA).

Results

***Tanytarsus alatus* Paggi (1992)**

(Figs 1A–I)

Tanytarsus alatus Paggi 1992: 302.
Caladomyia alata Sanseverino and Fittkau 2007: 266 (new combination).
Tanytarsus alatus, Lin et al. 2018: 667 (new combination).

Material examined. ARGENTINA: 1 pharate pupa, Buenos Aires, Carnaval stream, 34.870588° S, 58.089776° W, 07-ix-2017, D-net, J.P. Zanotto Arpellino (ILPLA); 5 pupal exuviae, Buñirigo stream, 35.143919° S, 57.570168° W, 07-viii-2018, D-net, J.P. Zanotto Arpellino (ILPLA).

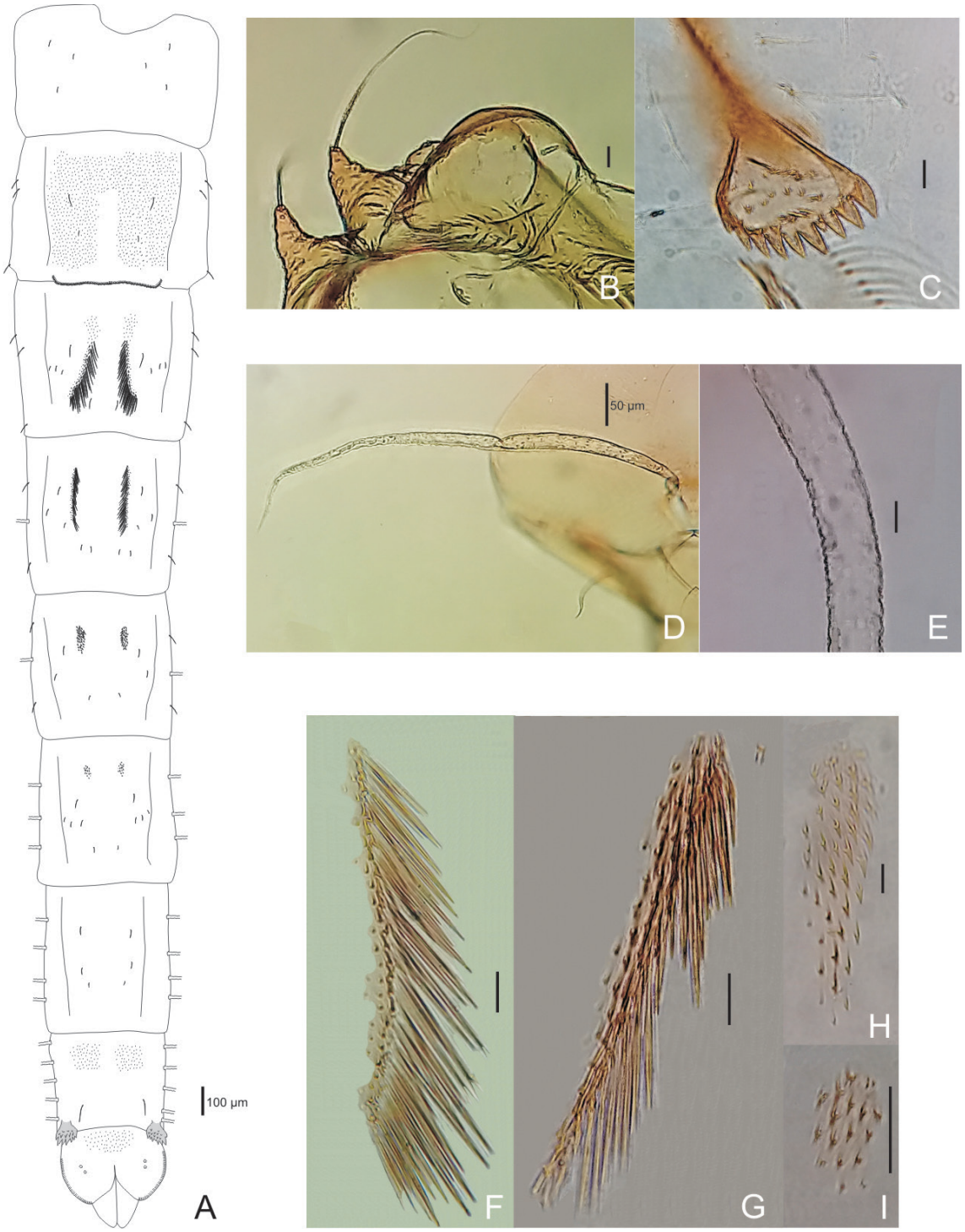

Figure 1. *Tanytarsus alatus* Paggi (1992) pupa. A) abdomen, dorsal view; B) frontal tubercles; C) posterolateral comb of abdominal segment VIII; D) thoracic horn; E) detail of the pores in the thoracic horn; F) patch on tergite III; G) patch on tergite IV; H) spinules on tergite V; I) spinules on tergite VI. Scale bar = 10 µm, except otherwise stated.

Pupal exuviae (n = 6)

Color: slightly brownish. Total length 3.83–4.7 (5) mm; cephalothorax length 0.84–1.2 mm (5); abdomen length 2.82–3.5 mm (5).

Cephalothorax. Frontal setae elongate, slender, 118–144 (5) µm long; cephalic tubercles present (Fig. 1B). Thoracic horn slender with small pores (Figs 1D-E). Thorax smooth; wing sheath with short nose. Thoracic setation: 3 precorneals: 144–176, 116–170, 126–142 (5) µm long, and 2 lateral antepronotals. Dc1–4 present and situated in two groups widely separated; distance between Dc1–Dc3 217–284 µm.

Abdomen (Fig. 1A). Tergite I bare; T II with central field of fine shagreen; T III with longitudinal paired bands of long spines (Fig. 1F); T IV with a paired long bands of hair-like fine spines in slightly curved line (Fig. 1G); T V–VI with anterior pair of semi-oval patches of short single spinules (Figs 1H–I); T VII bare. T VIII with anterior paired patches of fine shagreen. Hook row continuous, with approximately 200–250 hooklets. Segment VIII with posterolateral combs consisting of 5–10 large marginal teeth and 14–31 overlapping ventral teeth (Fig. 1C). Anal lobe with an oval field of fine shagreen; 37–48 taeniae and 2 dorsal setae taeniae. Segment II with 3 L setae; segments III–IV with 3 setae, middle setae teniate; segment V–VI with 3 teniate setae; segment VII with 4 taeniae; segment VIII with 5 taeniae.

Discussion

The presence of paired longitudinal bands of long spines on TIII and IV, patches of spinules in the anterior part of TIII–V separated, segments V and VI with 3 taeniate setae, lateral antepronotal setae without spinules, spine bands on TIV nearly parallel or slightly divergent posteriorly fits the description of *T. bruneola* (Trivinho Strixino) and *T. kapilei* (Trivinho Strixino) in the key to pupa by Trivinho Strixino (2012). However, those species differ from *T. alatus* by the possession of a wrinkled frontal apotome and lacking cephalic tubercles, by having patches on T V–VI with short multiple spines, and no anterior paired patches of fine shagreen on T VIII, nor an oval field of fine shagreen on the anal lobe. Sanseverino and Fittkau (2007) described the occurrence of short spines on T IV–VI sitting close to each other or placed on the same base and giving the appearance of multiple spines, a useful character to recognize the previously *Caladomyia* pupae. The character was not found to be consistent within the genus as several species possess single spinules on T IV–VI. This is also the case for *Tanytarsus alatus*.

The type locality of *Tanytarsus alatus* is Embalse Arroyito and the paratypes were collected in Marimenuco, both localities belonging to Neuquen province of Argentina. These localities are in the Monte province of the biogeographic scheme of Cabrera and Willink (1973). This province covers sandy plains, plateaus and low mountain slopes, with a dry and warm climate in its northern portion and dry and cool in the south. Precipitation is scarce with 80 mm per year in the north and 250 mm in the south, and the temperature between 13° and 17°C on annual average respectively. The predominant vegetation type is the xerophilous scrubland or sammophilic or halophilic shrub steppe. The new records presented in this study together with male adults collected in piedmont areas of Tandilia and Ventania mountain hills (both in Buenos Aires province, Argentina) extends the geographic distribution of this species northwards. These localities occur in the Pampean province (Cabrera and Willink 1973) that occupies the plains of eastern Argentina between 31° and 39° south latitude. It extends over horizontal or slightly undulated plains, with some low-rise mountain ranges (up to 1200 m in Ventania Mountain Hills) that emerge as islands. There are slow rivers and numerous lagoons of fresh or brackish water. The climate is warm temperate, with rains throughout the year that decrease from north to south and from east to west (1100 to about 600 mm per year). The average annual temperature ranges between 13° and 17°C. The dominant vegetation is the grass steppe, there are also grasslands, sammophilic steppes, halophilic steppes, marginal forests and various types of hydrophilic vegetation.

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First record of *Nubensia nubens* (Edwards 1929) (Diptera: Chironomidae) for Bulgaria

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Abstract

Nubensia nubens (Edwards 1929) is reported for the first time in Bulgaria from two lowland rivers, tributaries of the Danube (eastern Europe). Larvae of the species were found incidentally in samples collected for an ichthyological study, in the gut contents of *Neogobius melanostomus* (Pallas 1814) (Actinopterygii: Gobiidae), and in a benthic sample. The habitat conditions at the corresponding sites differed from those of previous records. Our finding underlines the potential of non-standard supporting methods, such as analyzing fish gut contents for discovering rare species that are hard to record by standard hydrobiological methods.

Introduction

Recently the larvae and adult female of *Nubensia nubens* (Edwards 1929) were described and placement of the species in a separate genus was suggested (Spies and Dettinger-Klemm 2015). Older records referred to the species under different names, for example, "*Polypedilum* (*Pentapedilum*) *nubens* Edwards, 1929" (e.g. Sæther and Spies 2013), *"Polypedilum* sp. A" for subfossil larvae (Brooks *et al.* 2007: 102); for a complete list of synonyms, see Spies and Dettinger-Klemm (2015: 110). The species has been reported from many countries in Europe (Czech Republic, France (including Corsica), Great Britain (including Northern Ireland), Ireland, Italy, North Macedonia, Norway, Portugal, Spain, Switzerland; see Sæther and Spies 2013) and from more eastern parts of the Palaearctic region (Armenia, Azerbaijan, Georgia, Iran, Iraq, Israel, Jordan, Lebanon, Russia, Syria, Turkey; see Oyewo and Sæther 2008). In addition, Bitušík and Trnková (2019) gave a new record from Albania, based on a single pupal exuviae. Moreover, *N. nubens* has been recorded very recently in Croatia (from Vrana Lake; Dorić et al., unpublished) and in Malta (A. Móra, pers. comm.).

The present study focuses on two Bulgarian tributaries flowing into the Bulgarian-Romanian sector of the Danube River (Eastern Europe). A detailed list of Chironomidae (231 taxa in total) from this region was compiled about 25 years ago (Russev et al. 1994) with no subsequent major contributions or revisions. In it, there are no records of the older synonyms of *N. nubens*. More recently, hydrobiological studies have produced taxonomic lists of the Chironomidae from standing water bodies in the same region (Trichkova et al. 2013); however, no synonyms of *N. nubens* are mentioned. Thus, this paper gives the first record of *Nubensia nubens* (Edwards 1929) for the Bulgarian fauna.

Material and methods

The study area includes the Iskar and Vit rivers, which flow in northern Bulgaria (Table 1). Both rivers are lowland tributaries of the Danube (R7 river type according to the Water Framework Directive (WFD) river typology (Directive 60/2000/EC; Cheshmedjiev et al. 2010)). The Iskar River is the longest inland river in Bulgaria with a length of 368 km and a discharge of 54.5 m^3s^{-1} (Hristova 2012). At the sampling point bottom sediments included mainly gravel and pebbles with silt and sand accumulated near the riverbank. The Vit River is 188 km long with a discharge of 14.3 m^3s^{-1} (Hristova 2012). The bottom substrate is composed mostly of shale bedrock, with zones of gravel and pebbles. Submerged vegetation was present in both rivers, mostly *Myriophyllum* spp., *Najas minor* All. and *Stuckenia pectinata* (L.) Börmer.

Sampling on the Vit and Iskar rivers was conducted on 1st and 5th October 2017, respectively. At each sampling site, physical and chemical parameters, such as water temperature, pH, oxygen concentration and conductivity, were measured using portable devices (Hanna Inc.). Water velocity was measured with a portable water flow probe (model FP101, Global Water Instrumentation, Inc., USA). Fish and macroinvertebrate samples were collected for an ichthyological study using electrofishing (SAMUS, 200/350 V, 3/12 A, 45-50 Hz) and a Hess sampler (ISO 8265:1988; frame size 0.3 by 0.3 m; mesh size 0.5 mm).

Table 1. Basic characteristics of sampling sites where *N. nubens* was recorded.

	Unit	Vit River	Iskar River
Coordinates		N 43.4078694° E 24.5217917°	N 43.5186500° E 24.2250194°
Altitude	m a.s.l.	63	51
Distance form Danube	km	53	41
Temperature	°C	15.9	14.0
Oxygen concentration	mg L^{-1}	13.4	9.0
Oxygen saturation	%	133	100
pH		9.9	9.3
Conductivity	µS cm^{-1}	483	446
Flow velocity	m s^{-1}	0.4	0.3

From the obtained benthic samples and fish gut contents, Chironomidae larvae and pupae were sorted and identified. Head capsules of the larvae were mounted on permanent slides with Swann's solution. Individuals of *Nubensia nubens* were identified using the larva description by Spies and Dettinger-Klemm (2015). The morphology of our larvae agrees in all described details. The identified material was deposited in the Dipterological collection of the Department of General and Applied Hydrobiology, Sofia University, Bulgaria.

Results and discussion

Larvae of *Nubensia nubens* were observed both in fish gut contents and macrozoobenthic samples. A single individual was found intact in the gut of a *Neogobius melanostomus* from the Iskar River (Fig. 1), where other invertebrates (mostly Chironomidae) contributed to the overall gut content. Most Chironomidae larvae and pupae in the analysed guts were only slightly damaged due to digestion and taxonomically distinguishable (a total of 26 genera were observed in 350 fish: unpublished data). A single larva of *N. nubens* was found in a benthic sample from the Vit River taken from gravel and cobble substrate and submerged vegetation.

Most of the previous records of *N. nubens* are from standing water bodies and fine substrates (Moller Pillot 2013; Murray et al. 2015; Spies & Dettinger-Klemm 2015) with only few records from fast flowing rivers (Michiels 2004 cited in Moller Pillot 2013). The present report is from a lowland river with coarse bottom substrate. Several small lakes and ponds are situated across the Vit River's left bank, near the sampling point: it cannot be excluded that the observed individual originated from these standing water bodies. This could explain the low abundance of *N. nubens* in the sample, while Spies and Dettinger-Klemm (2015) reported >1000 individuals/m² in summer, however this might be also due to the season of sampling and/or differences in habitat type. There are no such standing water bodies near the sampling site of the Iskar River. Here the examined material was part of fish gut contents so it might have originated from a variety of sections in the river – pools or slow flowing bankside mesohabitat. *Nubensia nubens* is thought to live mainly in waters with low conductivity (< 200 µS cm^{-1}) (Ruse 2002 cited in Moller Pillot 2013), while in the present study the species was recorded from water with higher conductivity (>400 µS cm^{-1}, Table 1).

The material sampled by Spies and Dettinger-Klemm (2015) is from eutrophic waters. In contrast, the rivers studied by us are water bodies with "good" ecological status (Dashinov personal observations following Directive 60/2000/EC). The records of *N. nubens* from Albania are also from oligotrophic waters (Schneider et al. 2014; Bitušík and Trnková 2019). In these cases, however, the species was observed in very low abundances. Thus, larvae of *N. nubens* appear to be thriving more in eutrophic waters, as Spies and Dettinger-Klemm (2015) observed. While the present record of *Nubensia nubens* is the first one for Bulgaria, there are probably more suitable habitats in the Bulgarian Danube basin that the species might occupy, but this needs to be evaluated by further studies.

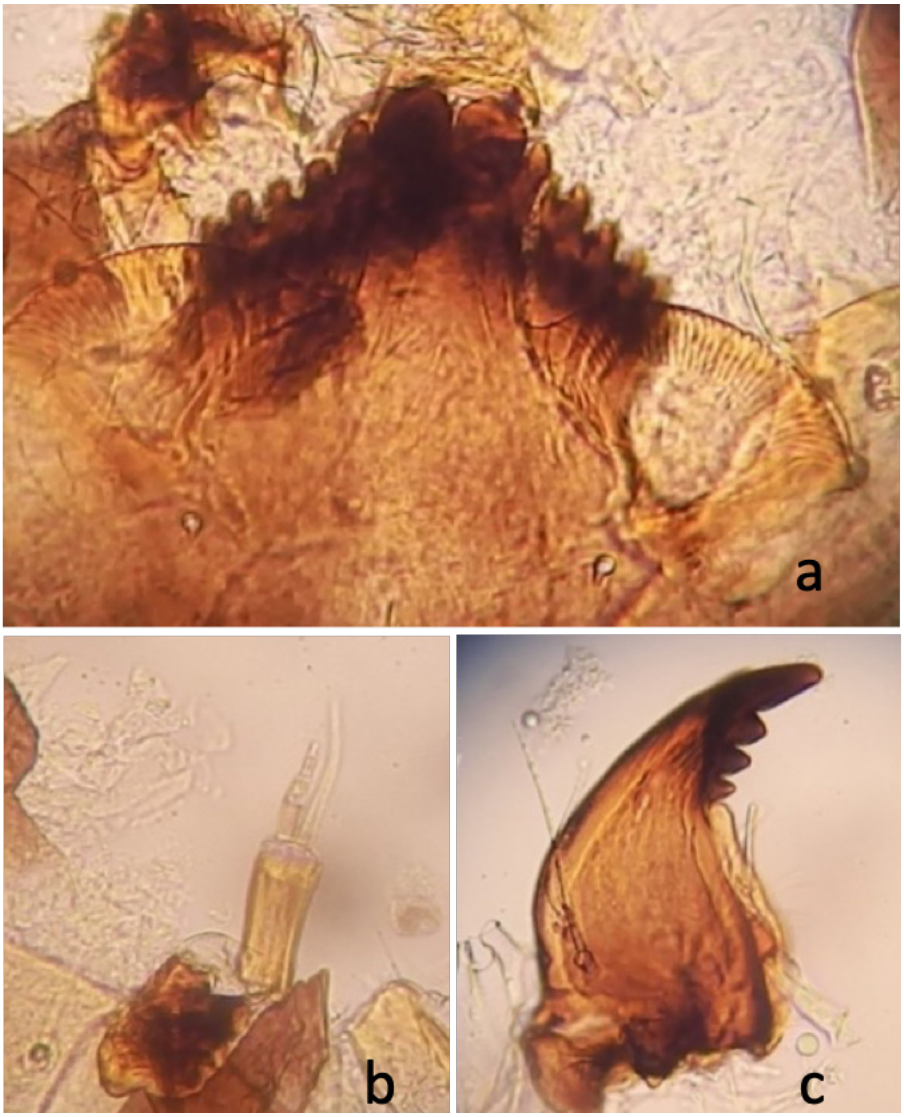

Figure 1. Photos of *Nubensia nubens* from the gut of *Neogobius melanostomus* a. mentum; b. antenna; c. mandible. Photo D. Dashinov

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The 21st International Symposium on Chironomidae

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The 21st International Symposium on Chironomidae was originally scheduled for Tsukuba, Japan in July 2021. However, the COVID-19 pandemic is far from ending and Tokyo Summer Olympic Games were postponed to 2021. Many international conferences including the International Congress of Entomology or the Congress of the International Society of Limnology were also reported to next year. The organizing committee wishes to welcome the whole community of chironomid researchers to Tsukuba under safer and the best possible conditions. For this reason, the organizing committee decided to postpone the symposium to summer 2022. As many colleagues agreed, a physical meeting will yield more fruitful exchanges in our field rather than an online symposium. This is also one point that motivated our decision to postpone the 21st International Symposium on Chironomidae to 2022. As soon as the new dates for the Symposium are fixed, we will make a new announcements in the Chironomus Journal, on the Chironomid Home Page and in the Chironomidae list server (chironomidae@vm.ntnu.no).

We wish you all good health and look forward to welcome all of you in Tsukuba!

Jürg Fischer 1936-2018 - in memoriam

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After a full and varied life, PD Dr. Jürg Fischer died in 2018, secluded in Ascona, southern Switzerland. Many elder researchers had known him from the International Symposia on Chironomidae, by actively corresponding with him or through his publications.

Jürg Fischer in Ascona, 2015. Photo: Barbara Streit-Fischer

Jürg Fischer was born in Bern and received his education as a primary school teacher and then as a secondary school teacher. He taught at various levels for several years in different schools in the canton of Bern before studying zoology, botany and chemistry at the Faculty of Natural Sciences (University of Bern). Already during his diploma thesis as well as his dissertation, was he dealing with chironomids in the department of Prof. Siegfried Rosin (Fischer 1978) at the Zoological Institute. After having finished his dissertation, "On the Reproductive Biology of *Chironomus nuditarsis* Str.", he worked as a senior assistant in the aforementioned department and made essential contributions to its development. Jürg Fischer did not only have an enormously broad knowledge, but he was also very talented in practical/technical matters, which proved its worth in the development of a carefully maintained and well thought-out chironomid breeding program and the construction of sophisticated experimental set-ups. Influenced by Rosin's mathematical and biostatistical knowledge, his experiments were always far-sightedly planned with regard to their verification. After his habilitation, Fischer became heavily involved in the teaching of zoology, especially ecological genetics. Collaborators of the department regarded Jürg Fischer as a reliable colleague and friend. Thanks to his pedagogical-didactic skill, his teaching at the university was very popular among biology students as well as medical or pharmaceutical students. While many truly appreciated his open and direct expression of opinion, others were less pleased with this characteristic. His insect courses and excursions were particularly well attended. Jürg Fischer was also interested in other areas of zoology. He collaborated, for example, several times in outdoor studies on the rock ptarmigan (*Lagopus muta*) in the Swiss Alps.

His list of publications manifests two main areas of chironomid research:
(1) Effects of chromosomal structures or mutations on reproduction and selection. For the time being, this work was mostly done in cooperation with Siegfried Rosin. After a six-month stay at the University of British Columbia in Vancouver, investigations were carried out in collaboration with researchers from Bern, with Dr. Herbert Tichy in Tübingen and especially with Prof. Paraskeva Michailova in Sofia as well as with Russian researchers. Particular emphasis should be laid on his original work on translocations, which was made possible thanks to his ability (acquired with patience and perseverance) to mate midges individually. Unique were, among other things, his translocation 'chromosome rings' of up to all eight chromosomes of *Chironomus nuditarsis*, produced from several translocation breedings; they thus provided fundamentals for population and species vitality and for species evolution.

(2) Thanks to Fischer's basic research on the development and physiology of *Chironomus plumosus* and *Ch. nuditarsis* in particular, several studies on dormancy and eclosion activity could be carried out.

Due to the early death of Prof. Rosin and the subsequent failure to reoccupy the professorship, Jürg Fischer's internal working conditions were unfortunately to become very difficult for years, above all due to resentment and even bullying. He found consolation in his good relations with the international family of chironomid researchers. However, his early withdrawal from chironomid research was something he could not really cope with for the rest of his life.

Nevertheless, his dry sense of humour continued part of his personality. Furthermore, Jürg Fischer's ability to remember and memorize was impressive: He could quote longer literary texts or poems at will and by heart on any appropriate occasions; who else but φ (his usual signature abbreviation) could, just for fun, correctly recite 100 decimal places of the mathematical constant π. In addition to his manifold biological commitment, he played various musical instruments and thus counterbalanced his professional work. For years, Jürg Fischer was known in the Bernese jazz scene as an excellent bass player.

He always stayed loyal to his friends despite his gradual withdrawal in his later years, a behaviour probably also caused by health reasons. Until his death, Jürg Fischer received unwavering support from his family, consisting of his partner, his children and grandchildren. His work and his enthusiasm are an incentive for us to continue working in his spirit. In many ways, φ remains a role model for us all.

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