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Diamesa sp. on snow. Photo Alyssa M. Anderson.

CHIRONOMUS Journal of Chironomidae Research

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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: *Diamesa* sp. on snow by Alyssa M. Anderson.

Editorial

The Ecology and Taxonomy of Chironomidae (Diptera): A Memorial Issue to Honor Leonard C. Ferrington, Jr.

This memorial issue is published to express our appreciation of Dr. Leonard C. Ferrington, Jr., and his contributions to the field of aquatic entomology, ecology, and especially research on the family Chironomidae. We honor Len and his work in three ways. First, a written tribute portraying a life well lived, including his extensive research record, his many talents and, most importantly, as a man who was loved and respected by family and friends (Bouchard et al. 2021). Second, the extent of Len's influence in chironomid research was exemplified by the 17 international presentations submitted to the 2024 Society for Freshwater Science (SFS) Special Session: Ecology and Taxonomy of Chironomidae (Diptera): A Memorial Session to Honor Leonard C. Ferrington, Jr. (Fig. 1). The meeting was aptly held in Pennsylvania (USA), where Len began his research career. Third, this Memorial Issue includes several of the papers presented at the SFS Special Session and other contributions submitted by researchers who could not attend the 2024 SFS annual meeting yet wished to recognize Len. The authors of these papers include collaborators and colleagues who began work with Len in the 1980s and those who were still working with him through 2021, when he passed away. Len built a community of chironomid researchers during his 41 years in Pennsylvania, Kansas, and Minnesota. The interconnectedness of his research, exemplified by the papers of this Memorial Issue, reflects the exceptional training his students received and the true collaborative nature of Len Ferrington's research.

Figure 1. Some participants from the 2024 SFS Special Session honoring Leonard C. Ferrington, Jr. gather for a photo with Deborah Ferrington, Len Ferrington's wife. Participants and attendees from left to right are Will Bouchard, Hannah Bodmer, Alyssa Anderson, Tracey Anderson, Susan Gresens, Valeria Lencioni, Tessa Durnin, Sabrina Moore, Corrie Nyquist, Kaitlynn Davis, Fabio Laurindo da Silva, Joseph Phillips, and Deborah Ferrington.

Ecology and SFPE

A major theme in Len Ferrington's research was on aquatic ecology and emergence of Chironomidae. Len studied emergence patterns of Chironomidae from mesocosms (Goldhammer et al. 1992), tropical streams (Ferrington et al. 1993), cold springs (Ferrington 1987a), and an estuary (Kranzfelder and Ferrington 2018). Len and others developed an early protocol in the USA for collecting and using surface-floating pupal exuviae (SFPE) in bioassessment and ecology (Ferrington et al. 1991); field and laboratory techniques associated with this protocol were reinforced in a later publication (Kranzfelder et al. 2015). During his tenure at the Kansas Biological Survey of the University of Kansas, Len collaborated with Dr. Tracey Anderson who was then a graduate student. A paper contributed by Anderson et al. (2025) titled "Chironomids shed light on organic matter dynamics in macroinvertebrate communities in prairie pothole lakes in west-central Minnesota, USA" describes the functional contribution of Chironomidae to these unique habitats of the Northern Great Plains, USA. Len's research interest in intermittent streams (see Chou et al. 1999) is connected to the paper contributed by Bouchard (2025) "Long-term emergence patterns of Chironomidae (Diptera) from an intermittent stream." Work by Lencioni et al. (2025) "Diet and functional feeding groups of Chironomidae (Diptera) in Alpine freshwater habitats" honors Len's long-term interest in cold spring ecology.

Life on the Edge

Another theme of Len Ferrington's research was unique and extreme ecosystems such as the hyporheic zone of streams and rock pools (Ferrington 1987b, Egan and Ferrington 2015). His research on cold springs transitioned into the study of winter or hibernal emergence of Chironomidae in the Great Plains and other regions of the Upper Midwest of North America (i.e. Ferrington 2000, Anderson et al. 2011, Nyquist et al. 2020) and elsewhere (e.g. Baranov and Ferrington 2013). Len also began studying cold tolerance of chironomids in Minnesota. His work as well as research by others on these topics is summarized within the contribution of Anderson et al. (2025) "Midges Below Zero: A Review of Hibernal Emergence of Chironomidae in Temperate Regions." Len's enthusiasm for winter research was contagious – he often worked to engage others in winter insect studies, and this is captured in the article "Bugs Below Zero: Communicating Science and Engaging the Public with Winter Active Aquatic Insects and Stream Food Webs" by Anderson et al. (2025). Len's survey work in Tasmania uncovered associations between tidal rock pool chironomids and their symbiotic gut-fungi, Harpellales (Ferrington et al. 2005). Discovering marine chironomids inspired the contribution by Hayford et al. (2025) "*Eretmoptera* from Washington State, USA: maritime or terrestrial midge?"

Taxonomy

Len published taxonomic work throughout his career ranging from descriptions of new species (Coffman et al. 1988, Chen et al. 2017) and genera (Ferrington and Sæther 2006) to major revisionary works (Ferrington and Sæther 2011, da Silva and Ferrington 2018). Some of Len's most important contributions to taxonomy were in co-authoring the keys to Chironomidae of North America (Ferrington et al. 2008, Ferrington and Berg 2019), in which he included provisional taxa. One of these taxa, Orthocladiinae *Genus 5*, has been described as a new species in the paper contributed by Fasbender et al. (2025) "Description of *Parakiefferiella ferringtoni* with discussion of relationships within the *Parakiefferiella* group." *Parachaetocladius lenferringtoni* Bouchard and Namayandeh was described and named in honor of Len (Bouchard and Namayandeh 2024). Molecular analysis supports the species status of *P. lenferringtoni* as shown in the Namayandeh et al. (2025) contribution "DNA analysis confirms the new species *Parachaetocladius lenferringtoni* Bouchard et Namayandeh, 2024 (Chironomidae: Diptera)." DNA analysis was also useful in describing new species and clarifying taxonomy of the Orthocladiinae tribe, Corynoneurini in a paper contributed to this Memorial Issue by Stur et al. (2025) titled "A contribution to the understanding of European Corynoneurini, with the description of three species new to science." Len's interest in hyporheic ecosystems (Ferrington 1987b) and pupal exuviae is shared in Egan's (2025) contribution "*Lopescladius* (Chironomidae) from the Nearctic, including keys and new pupal exuviae descriptions" which includes associated immatures described for the first time as well as range extensions.

Conclusion

We thank all the authors for their contributions to this Memorial Issue. Just as Len inspired many of the contributing authors, we hope the work shared here will encourage and support other chironomid researchers. More information on Len Ferrington's scientific works can be found within Bouchard et al. (2021) and on Google Scholar: https://scholar.google.com/citations?user=ydEaYTEAAAAJ&hl=en&oi=sra.

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A CONTRIBUTION TO THE UNDERSTANDING OF EUROPEAN CORYNONEURINI, WITH THE DESCRIPTION OF THREE SPECIES NEW TO SCIENCE

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https://zoobank.org/7F3C3C7E-ED8A-4373-9816-4298E5CED9A6

Abstract

Northern European *Corynoneura* species with the combination of comparatively short extension of the hind tibia apex and a thick transverse sternapodeme are morphologically similar to species in *Thienemanniella*. Moreover, the generic placement of *Corynoneurella paludosa* Brundin has been debated. We present results from morphological and molecular analyses that clarify the taxonomy of *C. paludosa*. Consequently, we regard *Corynoneurella* as a junior synonym of *Corynoneura,* move *Corynoneurella paludosa* to *Corynoneura* and return *Corynoneurella afra* (Lehmann) to *Thienemanniella*. Our observations also conclude that *Corynoneurella paludosa* sensu Langton is morphologically different from *C. paludosa* (Brundin) and is best placed in *Thienemanniella* as *Thienemanniella langtoni* sp. n. *Corynoneura ferringtoni* sp. n. and *Corynoneura minimagna* sp. n. are described and diagnosed based on adult male morphology and DNA barcodes. We redescribe *Corynoneura minuscula* Brundin, discuss *Corynoneura magna* Brundin, and suggest a solution for the identity of *Corynoneura celeripes* Winnertz. Finally, we provide an identification key to adult males of Holarctic *Corynoneura* species with a short hind tibial extension.

Introduction

Species of the tribe Corynoneurini (Orthocladiinae), also known as the *Corynoneura*-group, are amongst the smallest within the Chironomidae (Diptera), with a typical wing length of 0.9-1.8 mm. The group is species rich and often numerous in all kinds of aquatic and semiaquatic habitats such as streams, creeks, ponds, lakes, bogs and fens, and are recorded from fresh and brackish waters (e.g. Andersen et al. 2013, Moller Pillot 2013). Corynoneurini species are challenging to identify morphologically due to their small size and limited number of diagnostic characters. For adults, it is usually necessary to examine a slide mounted specimen with a cleared abdomen under a compound microscope to see both the external and internal characteristics. The morphology of associated life stages supports species as well as generic boundaries within the tribe (Andersen and Sæther 2005, Wiedenbrug and Trivinho-Strixino 2009). However, immature stages and female adults are unknown for many species and the generic diagnoses might change when more associated life stages are included.

Identification of Chironomidae species using DNA barcoding works very well in most cases given that there is a decent reference library for comparison (Ekrem et al. 2007, Lin et al. 2015, Gadawski et al. 2022). Thus, building a high-quality reference library of Chironomidae makes it possible to include taxonomically challenging taxa like the Corynoneurini in freshwater biomonitoring. Moreover, DNA barcode data has proven valuable in taxonomic studies where species boundaries were uncertain and has contributed to increased knowledge of species diversity for several groups within the Chironomidae as well as association of life stages (Stur and Spies 2011, Stur and Ekrem 2015, Lin et al. 2018, Stur and Ekrem 2020).

Species of the tribe Corynoneurini are characterized by having a wing with an apically fused costa where R1 and R2+3 form a thick clavus (Fig. 1) (Sæther and Kristoffersen 1996). The tribe comprises nine genera, *Corynoneura* Winnertz, 1846; *Corynoneurella* Brundin, 1949; *Ichthyocladius* Fittkau, 1974; *Notocladius* Harrison, 1997; *Onconeura* Andersen et Sæther, 2005; *Physoneura* Ferrington et Sæther, 1995; *Tempisquitoneura* Epler et de la Rosa, 1995; *Thienemanniella* Kieffer, 1911 and *Ubatubaneura* Wiedenbrug et Trivinho-Strixino, 2009 (Wiedenbrug and Trivinho-Strixino 2009, Ashe and O'Connor 2012,). Only species of the genera *Corynoneura*, *Thienemanniella* and *Corynoneurella* are reported from the Holarctic region.

Figure 1. *Corynoneura minimagna* sp. n., male adult. Scale bar = 1 mm.

As more Corynoneurini species are found and described the boundaries between the genera appear increasingly blurred. For instance, morphological traits in adult males such as microtrichia between the ommatidia or oral projections of the transverse sternapodeme, previously regarded as typical of members of the genus *Thienemanniella* (Cranston et al. 1989, Sæther et al. 2000), are now found in several *Corynoneura* species (*C. bodoquena* Wiedenbrug et al., 2012; *C. diogo,* Wiedenbrug et al. 2012; *C. tyrrhena* Moubayed-Breil, 2015*; C. sundukovi* Makarchenko et Makarchenko, 2010) (Makarchenko and Makarchenko 2010, Wiedenbrug et al. 2013, Moubayed-Breil 2015). On the other hand, *Thienemanniella partita* Schlee, 1968, a species which otherwise fits well the definition of *Thienemanniella*, seem to have bare eyes, as does *T. nipponica* Tokunaga, 1936 (Spies and Sæther 2004, Fu et al. 2010a, b, Fu et al. 2020). Currently, the main features to morphologically differentiate *Corynoneura* from *Thienemanniella* seem to be the presence of a keel at the fore trochanter (Schlee 1968, Wiedenbrug et al. 2013) and a relatively short clavus (Spies et al. 2022) in the adult. The presence of a pearl row on the wing-sheath of the pupa (Coffman et al. 1986) (except for *Corynoneura franciscoi* Wiedenbrug, Lamas & Trivinho-Strixino, 2012), and a long four-segmented antennae in the larva (Andersen et al. 2013) define the genus in the immature stages.

Corynoneurella was established by Brundin (1949) who placed the genus between *Corynoneura* and *Thienemanniella*. He considered the non-elongated apex of the hind tibia to be sufficient to separate *Corynoneurella* from *Corynoneura*, even though the other characters fit well with the contemporary diagnosis of the latter genus. Brundin (1949) also stated that *Corynoneurella* might be considered a subgenus of *Corynoneura* in the future since *Corynoneura magna* Brundin, 1949 and *Corynoneura minuscula* Brundin, 1949 also have an atypical hind tibial apex. Schlee (1968) later transferred *Corynoneurella paludosa* to *Coryno-neura* as all diagnostic characters fit his concept of that genus. Langton (1997) re-established the genus *Corynoneurella* based on a pharate male pupa he regarded to be conspecific with *Corynoneurella paludosa* and emended the diagnosis for *Corynoneurella* to include pupal characters. The genus was accepted as valid by Fu et al. (2010a, 2010b) who transferred *Thienemanniella afra* Lehmann, 1981 to *Corynoneurella* mainly based on the pupal characters that fit the diagnosis of *Corynoneurella* sensu Langton (1997). In the world catalogue of Chironomidae, Ashe and O'Connor (2012) listed *Corynoneurella* as a valid genus with the species *Corynoneurella paludosa* and *Corynoneurella afra*.

Here we wish to contribute to the knowledge of the tribe Corynoneurini by clarifying the identity of *Corynoneurella paludosa* and related *Corynoneura* species. We selected *Corynoneura* species where adult males possess a well-developed thick transverse sternapodeme (Fig. 4G) as opposed to a thin transverse apodeme as seen in *Thienemanniella* (Cranston et al. 1989, fig. 9.88E, F) or missing as seen in other *Corynoneura* (Cranston et al. 1989, fig. 9.17G). In addition, these species have a hind tibia with a short apical extension, less than 0.7 times the tibial apex width (Fig. 3B). Through the examination of type material and the association of morphotypes with DNA barcodes, we argue for synonymizing *Corynoneurella* with *Corynoneura*, and describe three species new to science. We also provide a preliminary molecular phylogeny of selected species in *Corynoneura* and *Thienemanniella* to support our decisions.

Methods

Specimen sampling and morphology

Specimens were sampled as part of various projects and field campaigns in Europe with adult midges collected by sweep-nets, Malaise traps or emergence traps near a variety of freshwater habitats. In addition, nominal types and reference material from scientific collections were obtained as loans and compared with freshly collected specimens when possible. Institutional abbreviations: NHRS = Swedish Museum of Natural History, Entomology Collections, Stockholm, Sweden; NTNU-VM = NTNU University Museum, Trondheim, Norway; ZSM = SNSB - Zoologische Staatssammlung München, Munich, Germany (Bavarian State Collection of Zoology); LUOMUS = Finnish Museum of Natural History, Helsinki, Finland; ZFMK = Zoological Research Museum Alexander Koenig, Bonn, Germany; PHL = private collection Peter H. Langton, Northern Ireland.

The terminology used follows Sæther (1980). Tibia of hind leg = ti_3. Measurements of the apex-extension of the hind tibia are according to Schlee (1968: 140, figs 131-133). Setation of apex of hind tibia (Schlee, 1968: 136, figs 111-113), (A = apical seta, S = long spur, s = short spur). Keel at the fore trochanter of the adult according to Schlee (1968 figs 89, 90, 130) and in Figs 4E, 5, 6E, not as interpreted in Fu et al. (2010a, b) and Wiedenbrug et al. (2013).

All photographs except Fig. 1 were taken with a Leica DMC4500 digital camera fitted on a Leica DM6000B compound microscope using differential interference contrast. The software Leica Application Systems X was used to take z-stacked photographs that were assembled using the default settings, with subsequent polishing in Adobe Photoshop. Figure plates were made with Adobe Illustrator. The image in Fig. 1 was taken with a Leica DMC 5400 fitted on a Leica MZ16A stereo microscope using the same software.

Molecular analyses

Three legs from each of 209 individual specimens belonging to 32 species were sampled and shipped to the Centre for Biodiversity Genomics (CBG) at the University of Guelph for molecular analysis as part of the International Barcode of Life initiative. In addition, eight Canadian specimens provided by CBG were included and DNA barcoded. Metadata from all specimens, except the German specimens of *C. ferringtoni* sp. n., is available in the dataset DS-CORYTHIE (DOI: https://dx.doi.org/10.5883/DS-CORYTHIE) in the Barcode of Life Data Systems database (https://boldsystems.org). DNA extractions followed an in-house silica membrane-based protocol at the Canadian Centre for DNA Barcoding. PCR of the 658 bp Cytochrome c oxidase subunit 1 (COI) barcode fragment was performed on all specimens and used a cocktail (C_LepFolF/C_LepFolR) of the established LepF1/LepR1 and Folmer primers (Hernández-Triana et al. 2014). PCR of 18S was performed on a subset of specimens aiming to represent all barcode clusters in the dataset and used the primers 18S_ai and 18S_bi amplifying an approximately 1000bp long fragment of the nuclear small ribosomal unit (Whiting et al. 1997). Both fragments were sequenced bi-directionally using Applied Biosystems Big Dye termination on ABI 3730XL sequencers.

Sequence contigs were aligned using the Muscle algorithm in MEGA 11 (Tamura et al. 2021) with gap opening costs = -200 and gap extension costs = -50. Alignment of COI was trivial as there were no indels. For 18S, the alignment had no indels for the in-group, while three gaps were inserted in the alignment to accommodate the chosen outgroup taxon *Psectrocladius limbatellus*. The software Mesquite 3.70 (Maddison and Maddison 2021) was used to manipulate taxon names and concatenate alignments of COI and 18S sequences. Substitution models for the four potential partitions 1st position COI, 2nd position COI, 3rd position COI and 18S were tested using PartitionFinder2.0 (Lanfear et al. 2017). The resulting two partitions (1) 3rd position COI and (2) 1st position COI, 2nd position COI and 18S received the best BIC scores for the models TRN+I+G (1) and TIM+I+G (2) respectively.

Phylogenetic analyses were conducted with MrBayes 3.2 (Ronquist et al. 2012) implementing the partitions above and the GTR+I+G model (considered to be the closest available model to the TRN and TIM substitution models) as well as the alternative 'model jumping' option in separate runs. All analyses were done with 10 000 000 generations with sampling every 1000 with all parameters and branch lengths unlinked and a burn-in of 10%. Resulting probabilities and other output were examined using Tracer 1.7.2 (Rambaut et al. 2014) to evaluate convergence. Trees were inspected and figures generated using FigTree 1.4.4 (Rambaut 2016).

Results

The phylogenetic consensus trees from the Bayesian analyses all returned a well-supported monophyletic *Thienemanniella* embedded in *Corynoneura* (Fig. 2). However, many of the more basal branches have low posterior probabilities, indicating that the presented relationships between *Thienemanniella* and groups of *Corynoneura* largely are unsupported. Two exceptions are the *scutellata*-group containing *C. arctica*, *C. edwardsi*, *C. scutellata* and three interim species, and the *lobata*-group containing *C. lobata* and six interim species. The *Corynoneura* species that have short hind tibial extensions and thick transverse sternapodemes (*C. ferringtoni* sp. n., *C. minimagna* sp. n., *C. minuscula*, *Corynoneurella paludosa*) do not constitute a monophyletic unit, and there is no molecular evidence that *Corynoneurella paludosa* should be considered a separate taxon at the genus-level.

Corynoneura celeripes Winnertz, 1852

Corynoneura celeripes Winnertz sensu Brundin, 1949:833; Schlee, 1968:18; Fu et al., 2009: 37; Fu et al., 2017: 70; nec sensu Pinder 1978; nec sensu Langton & Pinder, 2007.

Material examined: Two adult males (NHRS-BYWS 000002988 and NHRS-BYWS 000002989, both A288). Sweden, Växjö municipality, lake Innaren, 1947 coll. Brundin NHRS.

One male on microscopy slide (NHRS-BYWS000002199, B25), Sweden, Dalarna, peat bog near Ludvika, leg. L. Brundin, 05.vi.1946.

Diagnostic characters. *Corynoneura celeripes* adult males can be distinguished from other congeners by having a moderately short apical extension on the hind tibia; inferior volsella moderately large, almost triangular, situated at about mid length of gonocoxite; transverse sternapodeme anterior margin straight, thick; phallapodeme short, knife-shaped, joining sternapodeme at caudal curve.

Remarks

During our work with *Corynoneura celeripes* Winnertz, it became obvious that there are two main conceptions of this species in the literature. One following the opinions of Brundin (1949) and Schlee (1968), and one following Pinder (1978) and Langton and Pinder (2007). The latter is probably based on the opinion of Edwards (1924). Unfortunately, Winnertz' type specimens appear to be lost as they likely were part of the insect collection at the Zoological Museum in Bonn that was lost during the Second World War (Hirvenoja and Hirvenoja 1988). According to Horn and Kahle (1935-1937), most of Winnertz' Diptera material was deposited in Bonn.

The original description of *C. celeripes* is based on two females "aus hiesiger Gegend" [from the vicinity] and is almost exclusively based on colour and colour patterns. On the same page, Winnertz (1852) also describes the much darker *C. atra* based on two adult males collected at the same time and place, indicating that this might be the male of *C. celeripes*. Edwards (1924) confirms this synonymy with male and female adult specimens reared from pond water that agree with Winnertz' description but also writes "As this species has no antennal rosette it may not be Winnertz's *C. celeripes, …*". We have adult male specimens that confirm well with Edwards' description as well as with the characters and drawings presented by Pinder (1997) and Langton and Pinder (2007), where females associated with DNA barcodes have strongly pigmented distal half of the costa (BIN BOLD:ACX7382). As this is a character that Winnertz almost certainly would have detected but described the wings of his *C. celeripes* as shimmering whitish, we find it unlikely that Edwards described specimens of the same species as Winnertz. Adult males of *Corynoneura celeripes* sensu Edwards have strongly elongated apices of the hind tibia and the species is therefore not further treated in the present work.

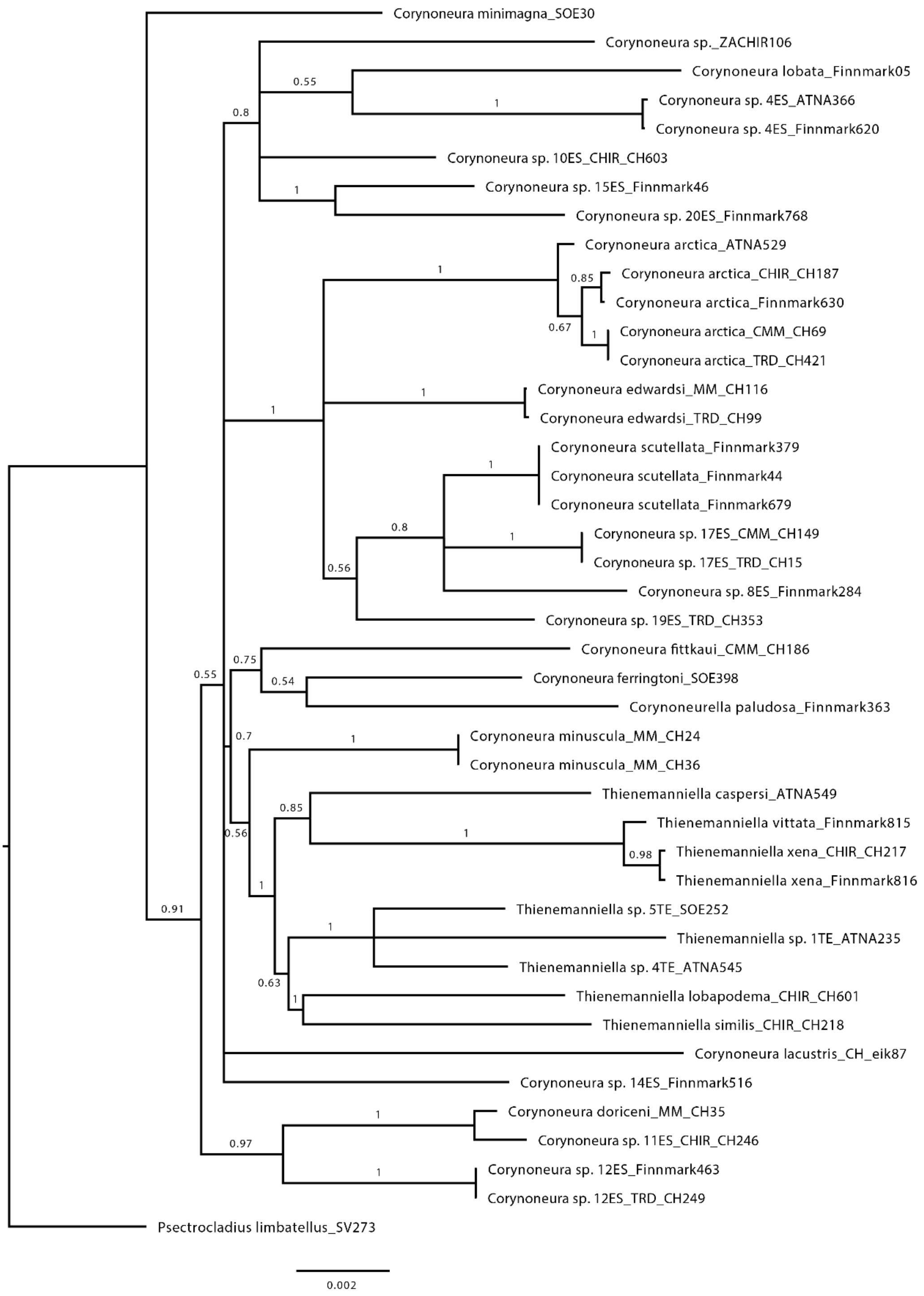

Figure 2. Phylogenetic tree generated using partial COI and 18S sequence data for selected Corynoneurini. Results from Bayesian analysis using 10 million generations and the GTR+I+G substitution model. Posterior probabilities above 50% on branches.

Brundin (1949) recorded *C. celeripes* from several lakes in Sweden, and keyed and figured the hypopygium. He did not describe the coloration in his specimens, but his drawing of the hypopygium resembles those of Edwards in having an obvious inferior volsella curved into a posteriorly pointed apex. The inner margin of the gonostylus, however, is straight in Brundin's drawing (Brundin 1949, fig. 205), while strongly curved in Edwards' drawing (Edwards 1924, fig. 10). We find it likely that Brundin described specimens of *C. celeripes* Winnertz although it is unlikely that he saw type material. Schlee (1968) mentioned *Corynoneura celeripes* as a species in *Corynoneura* with thick transverse sternapodeme and hind tibia with relatively short apex-extension. Schlee (1968) adopted Brundin's concept of the species and examined specimens from Brundin sample no. A288. He described the male adult hind tibia as very little widened and prolonged, with an extension shorter as in *C. edwardsi* Brundin, 1949. Brundin (1949: 833), on the other hand, referred to the hind tibia of *C. celeripes* as moderately strongly prolongated, more prolongated than in *C. minuscula* and *C. magna*. We had the opportunity to examine two adult males from Brundin's sample A288, and although in rather poor condition, the rather short extension of the hind tibia apex is clear (Fig. 3B), as is the posteriorly pointed inferior volsella (Fig. 3A) and the lack of a developed antennal rosette (Fig. 3C). Our examination could also confirm that Schlee's interpretation of the sternapodemes and phallapodemes is correct (Schlee 1968, figs 50-51). Thus, *C. celeripes* sensu Brundin falls within the boundaries of the *Corynoneura* species treated here. Unfortunately, we have not had access to other specimens that belong to *C. celeripes* sensu Brundin and have therefore nothing to add to Schlee's (1968) detailed description of the species. Brundin (1949) reported the distribution of *C. celeripes* to include Sweden, Finland, England, the Netherlands, Belgium, France, Germany, Austria and the USA, but in light of our current knowledge about the diversity in *Corynoneura*, all these records are questionable. The current public records of this species in BOLD are likely misidentified.

***Corynoneura ferringtoni* sp. n.**

https://zoobank.org/C2B073A3-6FD0-4DE1-B603-489E05FF9959

Type material. Holotype: Male adult (NTNU-VM 202004), Norway, Telemark, Drangedal, Sannes-Langen, lakeshore and bog, 59.04388°N, 9.30952°E, 66 m asl, leg. Elisabeth Stur & Patrycja Dominiak, 17.vi.2020, [BOLD ID: MM-CH210]. Paratypes: One male adult (NTNU-VM 145171), Norway, Trøndelag, Røros, Sølendet, kilde C3, 62.691°N, 11.833°E, 780 m asl, leg. T. Ekrem & E. Stur, 11.vi.2006 [BOLD ID: SOE398]. One male adult (NTNU-VM 145170), Norway, Trøndelag, Røros, Sølendet, kildebekk B2 62.689°N, 11.832°E, 785 m, leg. O. Hanssen, 22.VI.2012 [BOLD ID: SOE440]. One male adult (NTNU-VM 124583), Norway, Trøndelag, Røros, Sølendet, Kilde C-1, 62.691°N, 11.833°E, 788 m asl, leg. Aagaard et al., 10.vii.2005 [BOLD ID: SOE35]. Three male adults (ZFMK) Germany, Baden-Württemberg, Nationalpark Schwarzwald, Pfälzer Grube Quelle n° 816 on top, emergence trap, leg. R. Gerecke & S. Wiedenbrug, 13.vi.2016 [BOLD ID: ZFMK-TIS-2.598.342, ZFMK-TIS-2.598.343] and 5.vii.2017 [BOLD ID: ZFMK-TIS-2598404]. Two male adults (ZSM) Germany, Bayern, Allgäu, Oberstdorf, Engelkopf, 1210 m asl, 47.39500°N, 10.21617°E, 13.-29.v.2015, leg. Doczkal & Volgt, [BOLD ID: CCDB24183-E11, CCDB24183-E10].

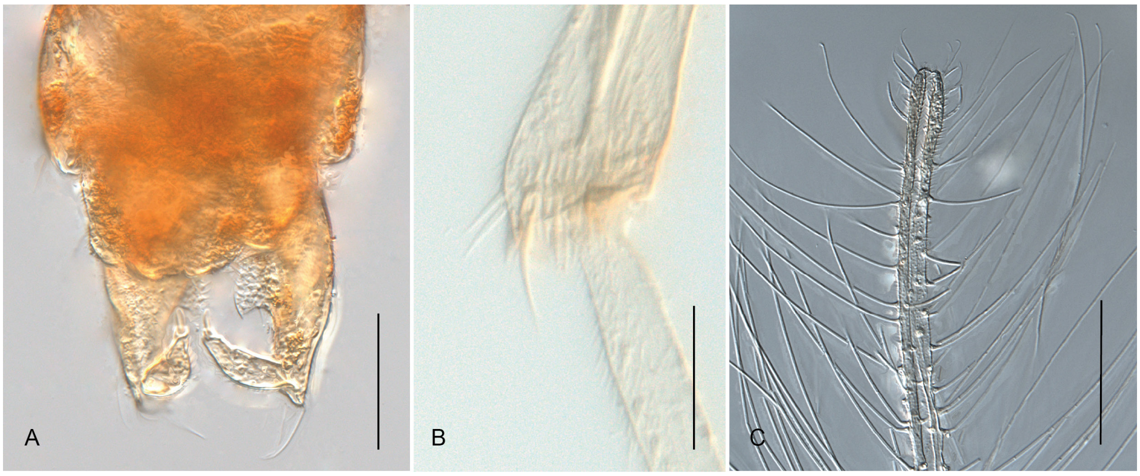

Figure 3. *Corynoneura celeripes* Winnertz sensu Brundin, male adult. A: Hypopygium (NHRS-BYWS000002199), B: Hind tibial apex (NHRS-BYWS000002199), C: Antennal apex (NHRS-BYWS000002989). Scale bars: A, C = 50 µm; B = 30 µm)

Other material examined (Private collection Nicola Reiff): One male adult Austria, Ennstaler Alpen, Tamischbach, Quellhorizont S Ischbauernkopf, rechts, 47.63660°N, 14.70691°E, 955 m asl, 23.vii.2016, leg. H. Haseke & C. Remschak. One male adult Austria, Nationalpark Gesäuse, Einzugsgebiet Mühlbach, Moos- Algenquelle beim Hochsitz 47.63953°N, 14.66209°E, 845 m asl, 01.viii.2014, leg. H. Haseke & C. Remschak.

Etymology. The species is named in honour of our colleague and friend Leonard C. Ferrington, Jr., for his long-standing contributions to Chironomidae research.

Diagnostic characters. *Corynoneura ferringtoni* adult males can be differentiated from other *Corynoneura* species by having a very short apical extension on the hind tibia; superior volsella broadly triangular; inferior volsella small, almost

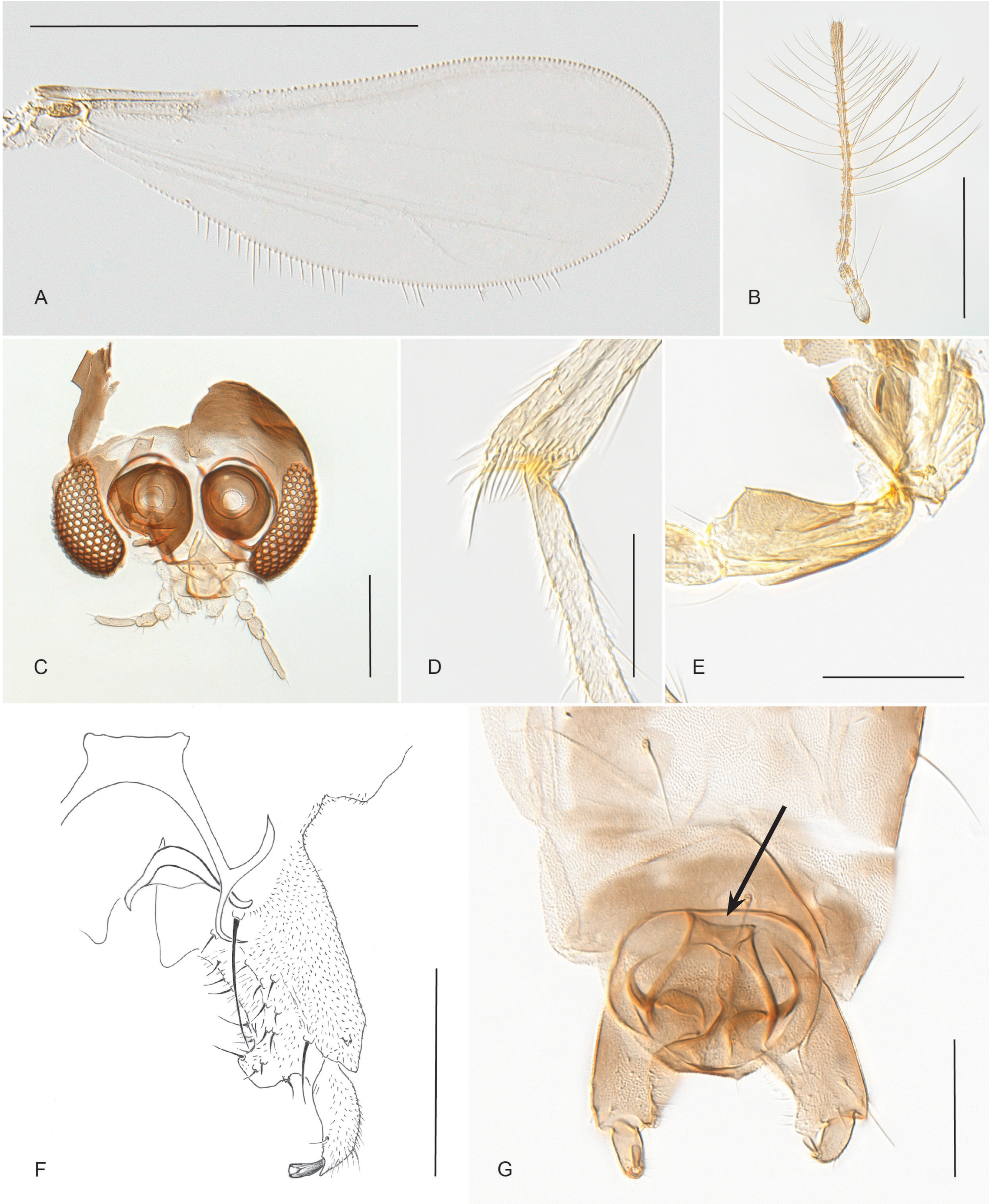

Figure 4. *Corynoneura ferringtoni* sp. n., male adult. A: Wing (NTNU-VM 145171); B: Flagellomeres (NTNU-VM 145171); C: Head (NTNU-VM 145171); D: Apex of hind tibia (NTNU-VM 124583); E: Fore trochanter (NTNU-VM 145171); F: Hypopygium (NTNU-VM 202004), G: Hypopygium (NTNU-VM 145171). Scale bars: A = 500 µm; B = 200 µm; C = 100 µm; D-G = 50 µm. Arrow pointing to the thick transverse sternapodeme.

square, situated distally at inner margin of gonocoxite; transverse sternapodeme anterior margin almost straight, thick, with oral extensions; phallapodeme short with curved, well-sclerotized anterior margin, joining sternapodeme at caudal curve.

Male adult (n = 3)

Colour. Head brown; thorax light brown, scutum, scutellum and postnotum dark brown; halteres white; abdominal tergites light brown, with white rectangular patch at tergite VII; legs light brown.

Head (Fig. 4C). AR = 0.65 - 0.69; antenna (Fig. 4B) with 9 flagellomeres (2); apical flagellomere 170 - 190 µm; flagellomeres with more than one row of setae each; antennal tip slightly enlarged with a median small concavity, with short setae distributed on the apical fourth of the terminal flagellomere.

Thorax. See Table 1.

Wing (Fig. 4A). Wing length 0.77 - 0.79 mm; wing width/wing length 0.38 - 0.40; clavus/wing length 0.24 - 0.25.

Legs (Figs 4D, E). LR_1 0.47 - 0.48 (2); Ti_3 with apical seta slightly curved (Fig. 4D); Ti_3 scale length/apical width 0.42 - 0.47.

Abdominal setation: TII - TV 1, TVI - TVII 3 and TVIII 1.

Hypopygium (Figs 4F, G). Tergite IX with 4-6 setae; laterosternite with 1-2 setae; superior volsella conspicuously broadly triangular; inferior volsella small and almost square, situated distally at gonocoxite inner margin; sternapodeme thick, 25 µm wide with anterior extensions; phallapodeme anterior margin sclerotized, curved, joined with sternapodeme at caudal curve; gonostylus short, crista dorsalis absent (Fig. 4F).

Measurements and other characters in Table 1. Female, pupa and larva not known.

Remarks

The presence of a slightly enlarged antennal tip with a median small concavity, short setae distributed on the apical fourth of the terminal flagellomere, a comparatively short lateral sternapodeme and short phallapodeme are characters that indicate a morphological proximity of *C. ferringtoni* with *C. fittkaui*, *C. celeripes*, *C. paludosa* and *C. makarchenkorum.* The shape and placement of the superior and inferior volsellae of the hypopygium separates *C. ferringtoni* from these species. Distribution: Barcoded records in BIN BOLD:AAI0860 are available from Norway and Germany. In addition, we have seen material from Austria (not DNA barcoded) that fits the diagnosis of *C. ferringtoni*, but these are not considered as part of the type material.

***Corynoneura magna* Brundin, 1949**

Material examined. Holotype: Male adult, remains on microscopy slide (NHRS-BYWS000002205, A63 or A6J) Sweden, Jönköping (Småland), Vetlanda, Lake Skären, leg. L. Brundin, 07.vi.1946.

According to Brundin (1949) the wing length of *C. magna* is 1.8 mm and the species has an AR between 0.8 and 1.0. The apex of the hind tibia resembles *C. minuscula*. The antennal apex, however, is pointed without a rosette, and not concave as in *C. minuscula*. The type material of *C. magna* is one single male with an abdomen separated

Figure 5. *Corynoneura magna* Brundin, 1949, holotype (NHRS-BYWS000002205, A63 or A6J). Fore trochanter. Arrow pointing to the stepwise structural transition of the keel in the distal end. Scale bar = 50 µm.

from the thorax, and a missing hypopygium. The holotype of *C. magna* has a fore trochanter with keel, and bare eyes (Fig. 5). Distribution: Sweden and Finland (Ashe and O'Connor 2012, Tuiskunen and Lindeberg 1986).

Corynoneura minimagna **sp. n.**

https://zoobank.org/1626ADF6-F4A7-4F62-A17D-D6B91A9A3B6E

Holotype: Male adult (NTNU-VM 124528), Norway, Trøndelag, Røros, Sølendet, spring C-1, 62.691°N, 11.833°E, 788 m asl, 04.-10.vii.2005, leg. Aagaard et al. [BOLD ID: SOE30]. Paratypes: Four male adults (NTNU-VM 124550, NTNU-VM 124572, NTNU-VM 124594, NTNU-VM 280870) as holotype [BOLD ID: SOE32, SOE34, SOE36, not in BOLD].

Etymology. The species is named "minimagna" for its superficial resemblance to, but much smaller size than, *C. magna* (almost half the wing length).

Diagnostic characters. *Corynoneura minimagna* can be differentiated from other *Corynoneura* species by having a weak extension of the hind tibia; superior volsella rounded, weak, barely recognizable; inferior volsella a well-developed rounded lobe situated on posterior part of gonocoxite inner margin; transverse sternapodeme thick; phallapodeme with a curved sclerotized anterior margin joined with sternapodeme at caudal curve.

Male adult (n = 4)

Colour. Head brown; thorax light brown; halteres whitish; abdominal tergites light brown, with white rectangular patch at tergite VII; legs light brown.

Head (Figs 6B, C). AR = 0.47 - 0.79; antenna (Fig. 6D) with 10-12 flagellomeres, apical flagellomere 165 - 245µm; flagellomeres with more than one row of setae each; antennal tip tapering, with short setae distributed on the apical fourth of the flagellomere.

Thorax (Fig. 6F). See Table 1.

Wing (Fig. 6A). Wing length 0.95 - 1.03 mm; wing width/wing length 0.32 - 0.35; clavus/wing length 0.31 - 0.33.

Legs. LR_1 0.53-0.57; Ti_3 with apical seta slightly curved (Fig. 6H); Ti_3 scale length/ apical width 0.42 - 0.53.

Abdominal setation (1): TII - TIV 1, TV - TVII 3, TVIII 1; SIII - SVI 2, SVII - SVIII 1.

Hypopygium (Figs 6G, I, J). Tergite IX with 6-10 setae, with concavity on median margin; laterosternite with one seta; superior volsella weakly developed, wide and rounded; inferior volsella a well-developed lobe on posterior part of gonocoxite; sternapodeme thick, 27 µm wide, caudal extensions barely developed; phallapodeme anterior margin curved and sclerotized, joins with sternapodeme on caudal curve; gonostylus curved, crista dorsalis absent.

Measurements and other characters in Table 1. Female, pupa and larva not known.

Remarks

The tapering antennal tip, with short setae distributed on the apical fourth of the flagellomere combined the relative long lateral sternapodeme indicate a morphological proximity of *C. minimagna* to *C. disinflata* and *C. capitanea*. *Corynoneura minimagna* can be separated from these species by the shape and placement of the superior and inferior volsellae of the hypopygium. Distribution: So far only recorded from Norway.

Corynoneura minuscula **Brundin, 1949**

Corynoneura minuscula Brundin, 1949: 698.

Corynoneura aurora Makarchenko et Makarchenko, 2010: 356, syn. n.

Material examined: Syntypes: Three male adults (two on microscopy slide, one in EtOH) (NHRS-BYWS000002202), Sweden, Kronoberg (Småland), Växjö, Lake Växjösjön, leg. L. Brundin, 29.vii.1946. One male adult abdomen on microscopy slide (NHRS-BYWS000002199, B25), Sweden, Dalarna, peat bog near Ludvika, leg. L. Brundin, 05.vi.1946. Other material: Two male adults, Germany, Baden-Württemberg, Bodensee-Gebiet, Buchenseen, leg. F. Reiss, 18.vi.1962. One male adult, Germany, Bavaria, Großer Ostersee bei Lauterbacher Mühle, leg. Burmeister, 30.viii.1981. Two male adults, Germany, Bavaria, am Gröbensee, Osterseen, leg. F. Reiss, 12.ix.1981. One male adult (NTNU-VM 201818), Norway, Telemark, Drangedal, Sannes-Langen, lakeshore and bog, 59.04388°N, 9.30952°E, 66 m asl., leg. Elisabeth Stur & Patrycja Dominiak, 19.viii.2019 [BOLD ID: MM-CH24]. One male adult (NTNU-VM 201830) Norway, Telemark, Drangedal, Haugstjenn ved FV 38, 59.09381°N, 8.95910°E, 90 m asl., Elisabeth Stur & Patrycja Dominiak, leg. 19.viii.2019 [BOLD ID: MM-CH36].

Diagnostic characters

Corynoneura minuscula can be differentiated from other *Corynoneura* species with short apical extension on the hind tibia by the following character combination: Superior volsella small, rounded lobe; inferior volsella well-developed and knob-

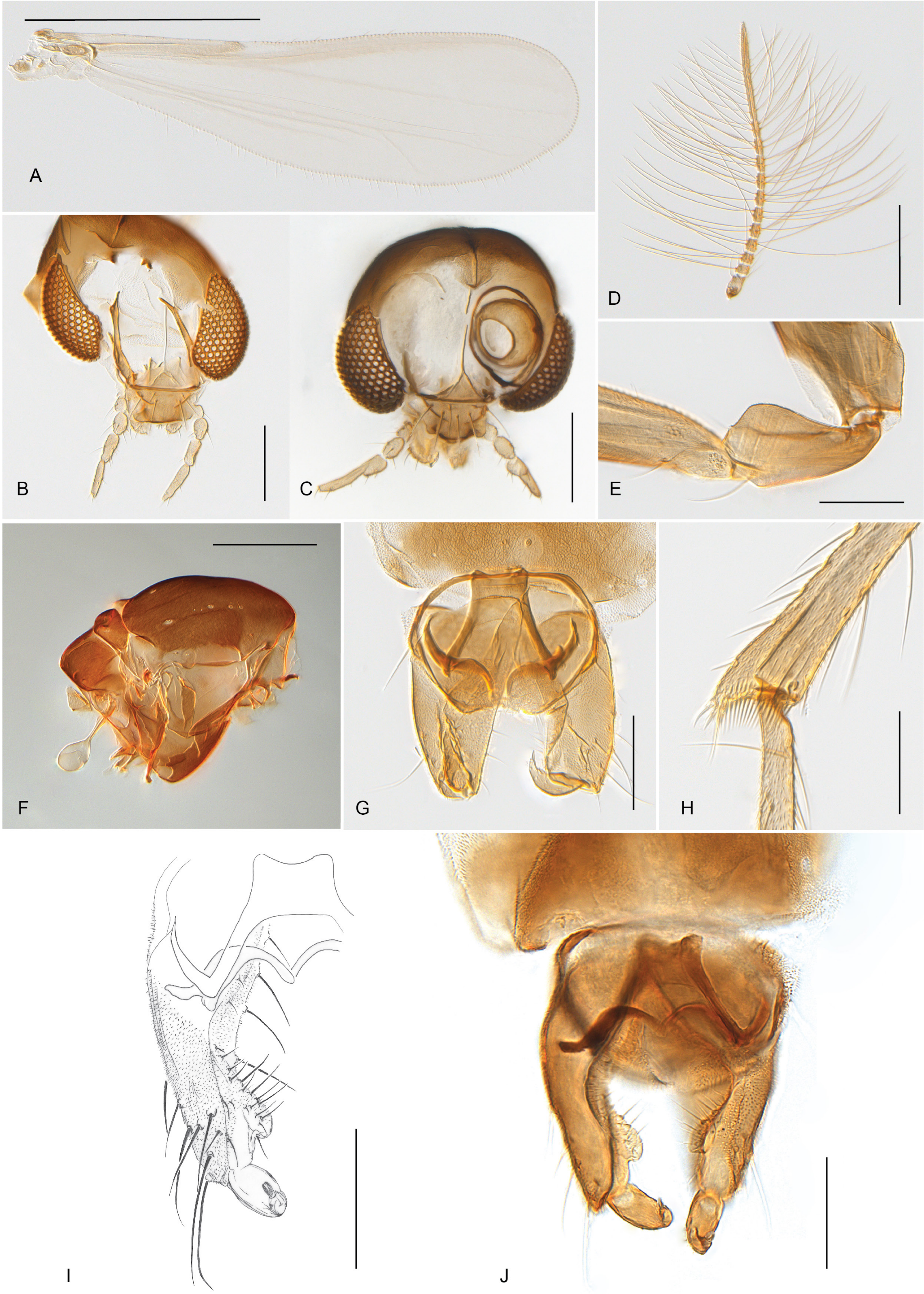

Figure 6. *Corynoneura minimagna* sp. n., male adult. A. Wing (NTNU-VM 124572); B. Head with normal palp (NTNU-VM 124594); C. Head with aberrant palp (NTNU-VM 124550); D. Flagellomeres (NTNU-VM 124572); E. Fore trochanter (NTNU-VM 124528); F. Thorax (NTNU-VM 280870); G. Hypopygium (NTNU-VM 124528); H. Apex of hind tibia (NTNU-VM 124528); I. Hypopygium (NTNU-VM 280870); J. Hypopygium (NTNU-VM 280870). Scale bars: A = 500 µm; D, F = 200 µm; B, C = 100 µm; E, G, H, I, J = 50 µm.

like with hyaline margin, placed at inner margin of gonocoxite at about half length; transverse sternapodeme u-shaped, thick; phallapodeme short with anterior margin sclerotized and rounded.

Male adult (n = 5)

Colour. Head dark brown; thorax brown, scutum, scutellum and postnotum dark brown; halteres white; abdominal tergites dark brown, with light brown rectangular patch at tergite VII; legs light brown.

Head (Figs 7C, F, G). AR = 0.51 - 0.79; antenna (Fig. 7B) with 10 - 12 flagellomeres, apical flagellomere 155 - 190 µm; flagellomeres with more than one row of setae each; antennal tip slightly enlarged and tapering distally, with short setae distributed on the apical fourth of the flagellomere.

Thorax. See Table 1.

Wing. Wing length 0.73 - 0.88 mm (4); wing width/wing length 0.37 - 0.38 (4); clavus/wing length 0.25 - 0.29 (4).

Legs (Figs 7B, E). LR_1 0.54 - 0.60; Ti_3 apical seta slightly curved (Fig. 7D); Ti_3 scale length/apical width 0.46 - 0.54. (Fig. 7B)

Abdominal setation: TII - TV 1, TVI - TVII 3, TVIII 1.

Hypopygium (Figs 7H, I, J). Tergite IX with 4 setae; laterosternite with 2 setae; superior volsella small, rounded lobe; inferior volsella well-developed, rounded knoblike with hyaline margin, situated at half length of the gonocoxite inner margin; transverse sternapodeme thick, 15 – 25 µm wide; phallapodeme short with rounded and sclerotized anterior margin, joined with sternapodeme at its caudal extension (Fig. 7H); gonostylus widened medially with rounded posterior margin, crista dorsalis absent (Figs 7H, I, J).

Measurements and other characters in Table 1.

Remarks

Schlee (1968) defined *C. minuscula* based on material from Germany (Brandenburg, coll. Mothes). He illustrated the apodemes and refers to Brundin's (1949) drawing of the hypopygium. In Schlee (1968), *C. minuscula* keys close to *C. fittkaui* separated by the long inner setae on the superior volsella in *C. fittkaui* and the shape of the gonocoxite inner margin. Fu et al. (2010b) transferred *C. minuscula* to *Thienemanniella* without further explanation. The most likely reason is that they erroneously coded this species with hairy eyes in their taxon-character matrix used in the phylogenetic analysis (Fu et al. 2010: 38). Following Fu et al. (2010b), Ashe and O'Connor (2012) listed *C. minuscula* within the genus *Thienemanniella*.

Makarchenko and Makarchenko (2010) described *C. aurora* based on material they previously identified as *Corynoneura* cf. *minuscula*. Since *Corynoneura minuscula* was transferred to *Thienemanniella* by Fu et al. (2010b), Makarchenko and Makarchenko (2010) described their material as species new to science. They mentioned, however, the necessity of examining Brundin's type material of *Corynoneura minuscula* to confirm whether they are conspecific. Moller Pillot (2013): 53) recognized that *C. minuscula* has bare eyes and considered *C. aurora* Makarchenko *et* Makarchenko, 2010 a possible synonym of *C. minuscula.* We have seen Brundin's syntypes of *Corynoneura minuscula* and can confirm that the eyes are overall bare, except for some small microtrichia at the margin. Moreover, a superior volsella is present, and the fore trochanter has a keel. We thus consider *C. aurora* Makarchenko *et* Makarchenko a synonym of *C. minuscula* Brundin. Distribution: Records on BOLD belonging to BIN BOLD:ADA5504 are collected in Sweden, Finland and Norway. Non-barcoded records additionally from Russia and Germany. According to Ashe and O'Connor (2012) the species is also recorded from Canada, Austria, France and Spain.

Corynoneura paludosa (Brundin, 1949)

Corynoneurella paludosa Brundin, 1949: 830

Corynoneura paludosa (Brundin), Schlee, 1968: 48

Material examined. Syntype: One male adult on microscopy slide (NHRS-BYWS000002197, B34, BRD282), Sweden, Jämtland, Kälarne, Öster-Vontjärn, leg. L. Brundin, 12.vi.1946.

Other material: Two males on microscopy slide (NHRS-BYWS000002196, BRD281), Finland, Kemin Lappi (Lapponia kemensis pars occidentalis), Pohjoinen-Sodankylä, Posolampi (pond Poso), leg. M. Hirvenoja, 01.vii.1961. Six male adults (NTNU-VM 136459, NTNU-VM 200419, NTNU-VM 200420, NTNU-VM 235343, NTNU-VM 235344, NTNU-VM 235345), Norway, Finnmark, Porsanger, Fen near Gaggavann, sweep net, 70.42284°N, 26.73690°E, 106 m asl, leg. T. Ekrem & E. Stur, 17.vi.2010 [BOLD ID: Finnmark363, Finnmark855, Finnmark856]. Two male adults (NTNU-VM 229667, NTNU-VM 229668), Norway, Møre og Romsdal, Trollheimen, Folddalen, Gråhaugfjellet, sweep net, 62.91223°N, 9.11036°E, leg. T. Ekrem, 09.viii.2021 [BOLD ID: NO137, NO138].

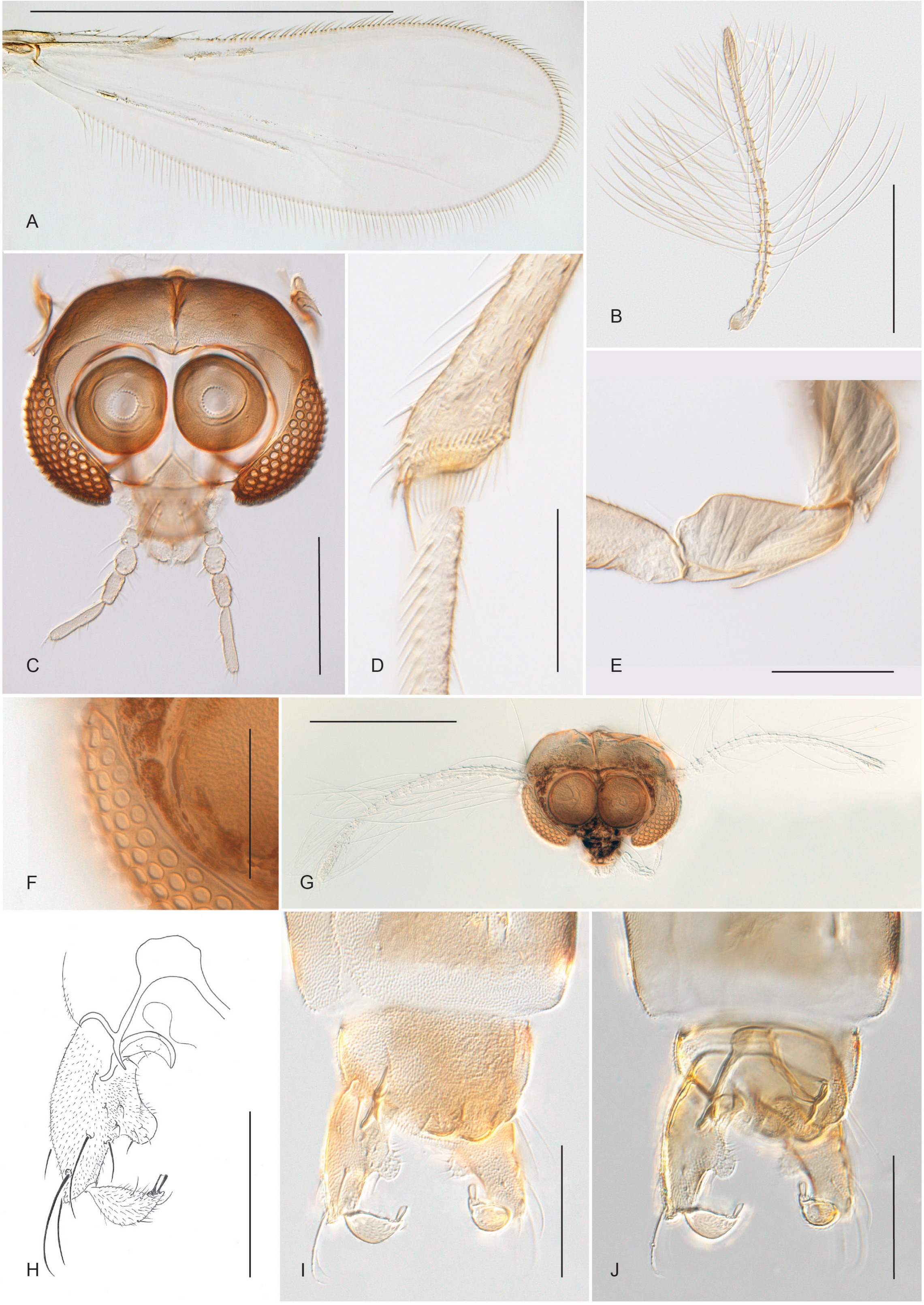

Figure 7. *Corynoneura minuscula,* male adult. A: Wing (NTNU-VM 201830); B: Flagellomeres (NTNU-VM 201830); C: Head (NTNU-VM 201830); D: Apex of hind tibia (NTNU-VM 201830); E: Trochanter (NTNU-VM 201830); F: Eye (syntype), G: Head with antennae (syntype); H-J Hypopygium (NTNU-VM 201830). Scale bars: A = 500 µm; B, G = 200 µm; C = 100 µm; D-F, H-J = 50 µm.

Diagnostic Characters. The male adult of *C. paludosa* can be differentiated from those of other *Corynoneura* species by the following character combination: apex of the hind tibia with very weak elongation; superior volsella well-developed, sclerotized; inferior volsella wide and rounded along the distal half of the gonocoxite; transverse sternapodeme thick; phallapodeme short with posterior margin convex and sclerotized; gonostylus thin, slightly curved.

Male adult (n = 4)

Colour. Head brown; thorax dark brown; halteres white; abdominal tergites brown, with white rectangular patch at tergite VII; legs brown.

Head (Fig. 8C). AR = 0.47 - 0.52. Antenna (Fig. 8B) with 12 flagellomeres, apical flagellomere 150 - 170 µm; flagellomeres with more than one row of setae each; antennal apex enlarged with a median cavity, with short setae distributed on the apical fourth of the flagellomere (Fig. 8B).

Thorax. See Table 1.

Wing (Fig. 8A). Wing length 0.82 - 0.88 mm; wing width/wing length 0.35 - 0.43; clavus/wing length 0.29 - 0.31.

Legs (Figs 8D, E). LR_1 0.49 - 0.5; Ti_3 with apical seta slightly curved (Fig. 8D); Ti_3 scale length/apical width 0.25 - 0.42.

Abdominal setation: TII - TV 1, TVI - TVII 3, TVIII 1 setae.

Hypopygium (Figs 8F, G). Tergite IX with 4-8 setae, two of them longer, situated at the posterior margin; laterosternite with 1-2 setae; superior volsella strongly sclerotized, well-developed, broadly triangular; inferior volsella rounded along posterior half of gonocoxite, with distal concave margin; sternapodeme thick, 18 - 28 µm wide; phallapodeme posterior margin convex and sclerotized, joined with sternapodeme at caudal extension; gonostylus thin, about the same width basally and medially, slightly tapering distally; crista dorsalis absent.

Measurements and other characteristics in Table 1. Female, pupa and larva not known.

Remarks

The mounted syntype of *Corynoneurella paludosa* has pubescent eyes, the antennal apex has a median cavity, a dorsal keel on the fore trochanter is present, and the apex of the hind tibia is only slightly extended (see also the drawing of the hypopygium of another syntype in Brundin 1949: 831, fig. 199). Despite the hypopygium of the studied syntype being mounted laterally, the sclerotization of the posterior margin phallapodeme is clearly visible. The sclerotized superior volsella is visible and the gonostylus shows its distinct form as described and illustrated by Brundin (1949). In the literature, the eyes of *Corynoneura* are generally described as bare (e.g. Brundin 1956, Cranston et al. 1989, Goetghebuer 1932) and rarely as pubescent (Fu et al. 2017), and the character "bare eyes" is used in generic keys to differentiate *Corynoneura* from other Corynoneurini (e.g. Cranston et al. 1989, Sæther et al. 2000, Wiedenbrug and Trivinho-Strixino 2009). However, in our experience, specimens of other *Corynoneura* species (e.g. *C. gratias*, *C. arctica*) show sparsely distributed, short microtrichia between the ommatidia. Thus, we suspect that small microtrichia might have been overseen or not interpreted as conspicuous enough to be considered as pubescent in previous studies (Wiedenbrug and Trivinho-Strixino 2011). Moreover, the presence of a pearl row in the pupal wing sheaths of *C. paludosa* further support the placement in *Corynoneura* (Janne Raunio pers. com.)

The well sclerotized phallapodeme posterior margin observed in *Corynoneurella paludosa* (Fig. 8G) is a feature also present in several *Corynoneura* species from the Neotropical region, e.g., *Corynoneura espraiado* Wiedenbrug et al., 2012, and *C. humbertoi* Wiedenbrug et al., 2012. For these species, the immatures are known and fit the diagnosis of *Corynoneura*. In conclusion, we cannot find reliable morphological characters that warrant placement of *C. paludosa* in a genus outside of *Corynoneura*. Distribution: DNA barcoded specimens belonging to BIN BOLD:AAV3049 have been collected in Norway, Finland and Canada. In addition, we have Brundin's type material from Sweden. Previous records from other regions should be compared against the current understanding of the species.

Thienemanniella langtoni **sp. n.**

Corynoneurella paludosa sensu Langton 1997, nec Brundin 1949

https://zoobank.org/13CA9CD7-6AFF-4A0D-AD7E-DAE9A06E7BA8

Etymology. The species is named in honour of Peter H. Langton for his many contributions to Chironomidae taxonomy.

Type material. Holotype: Pharate male (PHL), Spain, Pyrenees, Centre de Recerca d'Alta Muntanya (CRAM) de la Universidad de Barcelona (Vielha), 03.vii.1994, leg. P.H. Langton. Paratypes: 1 pharate male, 3 pupae as holotype.

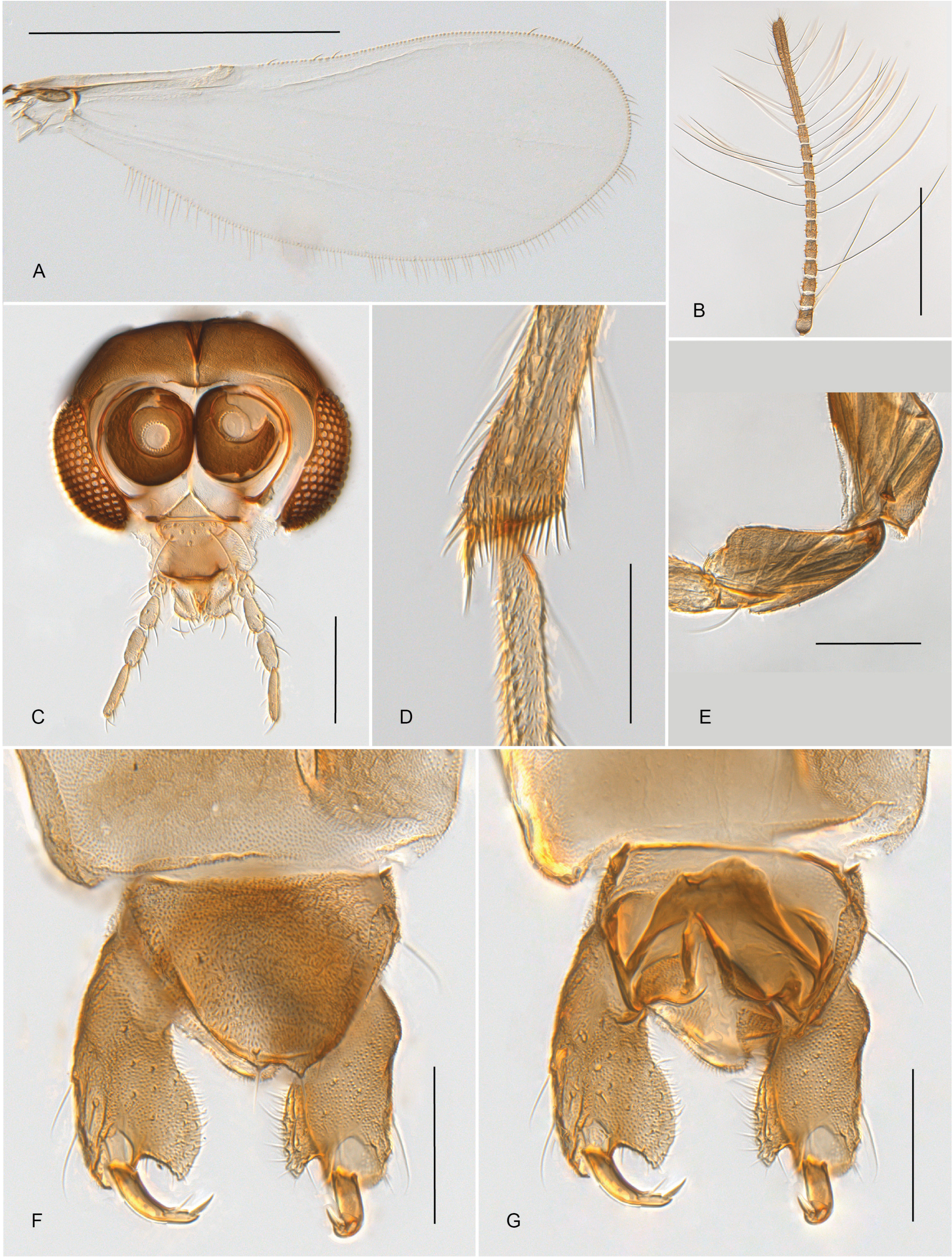

Figure 8. *Corynoneura paludosa* comb. n., male adult (NTNU-VM 136459). A: Wing; B: Flagellomeres; C: Head; D: Apex of hind tibia; E: Trochanter; F, G: Hypopygium. Scale bars: A = 500 µm; B = 200 µm; C = 100 µm; D-G = 50 µm.

Diagnostic characters. *Thienemanniella langtoni* can be separated from other congeners by combining characters of pupae and male adults: the latter having strongly pubescent eyes, fore trochanter with keel not having the stepwise abrupt structural transition in the distal end (as typically seen in *Corynoneura*), straight transverse sternapodeme, superior volsella without microtrichia and setae, inferior volsella well-developed and placed distally on gonocoxite margin. The pupa separates from congeners in having a pearl row on the wing sheaths and long spinules on sternite II.

Table 1. Comparison of metric (in µm) and meristic characters of male adults of *Corynoneura*.

Character/Species	*C. paludosa* (n = 4)	*C. minuscula* (n = 5)	*C. ferringtoni* sp. n. (n = 3)	*C. minimagna* sp. n. (n = 4)	*C. celeripes* (n = 3)
Thorax length	530 (1)	420-465	425-450 (2)	540 (1)	~500
Abdomen length	750–770 (2)	-	680-830	-	~700
Wing length	820–880	730-780 (4)	770-790	950-1030	~800
AR	0.47–0.52	0.51-0.79	0.65-0.69 (2)	0.47-0.79	0.7(1)
Length apical flagellomere	260–280	155-190	170-190 (2)	165-245	210 (1)
flagellomeres	12	10-12	9	10-12	11 (1)
Clypeals	8–11	6 (3)	6-7 (2)	5 (1)	-
Palpomere 3	30-38	20-22	20 (1)	28 (1)	20 (1)
Palpomere 4	33–38	25-30	22 (1)	40 (1)	25 (1)
Palpomere 5	43–55	35-52	45 (1)	50 (1)	52 (1)
Dorsocentrals	5–6	4-5	4-5 (2)	4(1)	-
Prealars	2–3	2	2 (2)	2(1)	-
Scutellars	2	2	2 (2)	2(1)	-
Wing width/wing length	0.35-0.43	0.37-0.38 (4)	0.38-0,40	0.32-0.35	-
Clavus/Wing	0.29-0.31	0.25-0.29 (4)	0.24-0.25	0.31-0.33	-
LR_1	0.49-0.56	0.54-0.60	0.47-0.48 (2)	0.53-0.57	0.53 (1)
Ti_3 apex extension length/ Apex ti3 width	0.25-0.42	0.46-0.54	0.42-0.47 (2)	0.40-0.53	0.5-0.7 (2)
Apical setae on Ti_3	curved	curved	curved	curved	curved
Setae on tergite IX	4-8	4	4-6	6-10	At least 4
Setae on laterosternite TIX	1-2	2	1-2	1	-
Gonocoxite length	85–100	75-77	67-75	102-115	87
Gonostylus length	28–48	20-25	25 (2)	35-45	35 (1)
Crista dorsalis	absent	absent	absent	absent	absent
Transverse stenapodeme	18-28	15-25	25	27	25 (1)

Remarks

Langton (1997) assigned these specimens from the Pyrenees to *Corynoneurella paludosa*, figured the male hypopygium and discussed the taxonomic placement based on adult male and pupal characters. His observations concluded that these specimens have characteristics that place them systematically between *Corynoneura* and *Thienemanniella* and therefore re-erected the genus *Corynoneurella*. However, Langton's specimens do not match the diagnostic characters found in the examined syntype of *Corynoneurella paludosa* Brundin. Even though the inferior volsella and the gonostylus of *Corynoneurella paludosa* sensu Langton resemble the drawing of Brundin´s *C. paludosa*, the superior volsella and the apodemes in these species are clearly different (Langton 1997, fig.

1). To avoid future confusion, we here designate a name for *Corynoneurella paludosa* sensu Langton (1997) and until further molecular and morphological evidence is present, place the species in *Thienemanniella*: The two pharate males have hairy eyes, but the microtrichia are relatively short just barely surpassing the ommatids (Fig. 9A). The trochanter of two examined pharate males is without the same type of keel seen in the *Corynoneura* species in this paper (Fig. 9C), lacking the abrupt stepwise structural transition in the distal end (Fig. 5). The apex of the hind tibia is without extension (Fig. 9D), and the transverse sternapodeme is thin and wide with anterolateral projections (Langton 1997, fig. 1) as is typical for *Thienemanniella*. The ratio of the clavus length/wing length is not yet known since the available specimens were pharate males and pupae. The pupa has cylindrical macrosetae typical for *Thienemanniella,* but as typical for *Corynoneura,* pearl rows are present in the wing sheaths (Fig. 9B). This character has, to our knowledge, not been recorded within *Thienemanniella* previously. Langton (1997) also mentions the presence of long spinules on sternite II in the pupa which is another feature he considers typical for some *Corynoneura* species. We expect the generic diagnoses to be emended as more immature stages of Corynoneurini species are described and therefore choose to tentatively place it in *Thienemanniella* until its placement is otherwise resolved.

As a consequence of the above-described misconception, the recorded distribution of *Corynoneura paludosa* should be reconsidered. For example, Michiels (2004) reported *Corynoneurella paludosa* for the first time for Germany. We were able to study her material and can confirm that these specimens are not *Corynoneura paludosa* sensu Brundin. Moreover, even though they resemble *Thienemanniella langtoni* sp. n., they are clearly different and probably belong to a species new to science that remains to be described.

Thienemanniella afra Lehmann, 1981

Thienemanniella afra was described by Lehmann (1981) based on males with associated pupae. The species was collected from a creek near Kisangani in the Democratic Republic of Congo. Fu and Sæther (2012) placed *Thienemanniella afra*

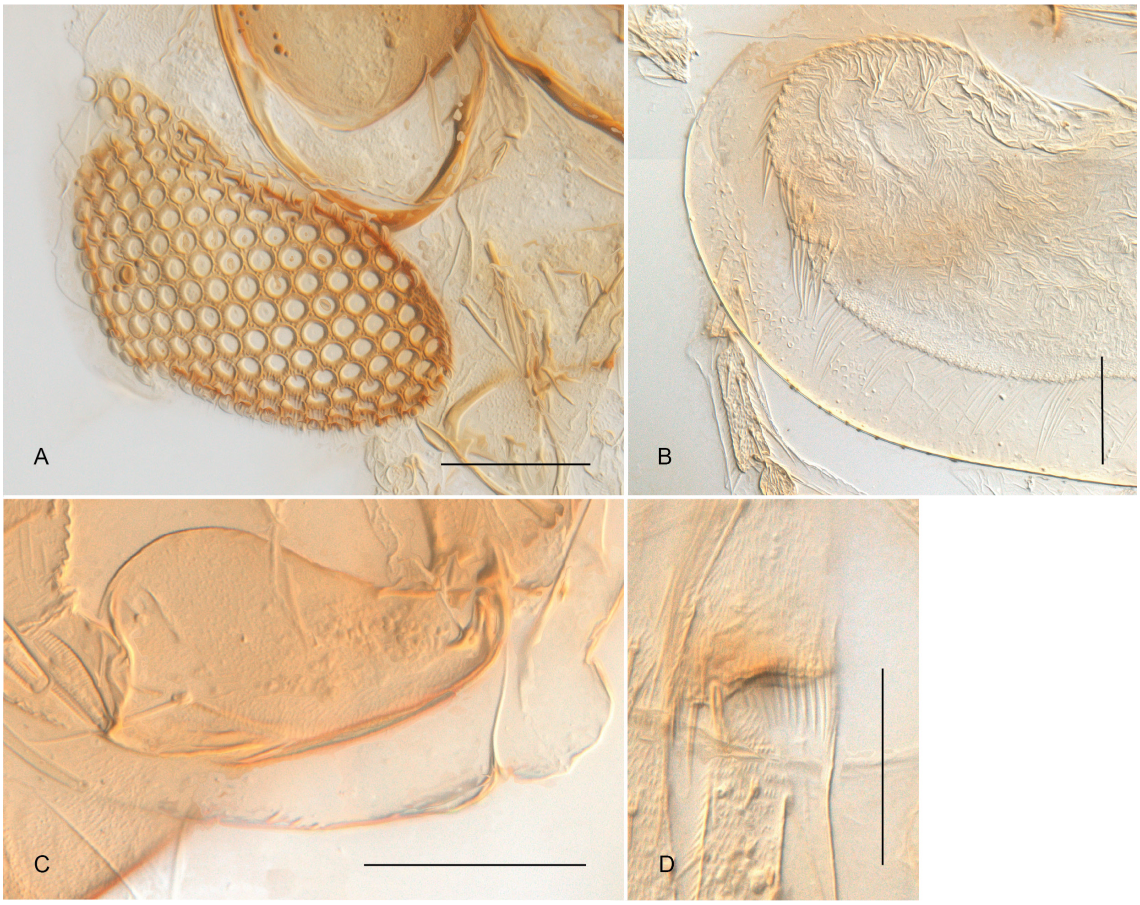

Figure 9. *Thienemanniella langtoni* sp. n., holotype, pharate male. A: Eye; B: wing sheath showing pearl row; C: Fore trochanter; D: Apex of hind tibia. Scale bars = 50 µm.

Lehmann into *Corynoneurella,* suggesting that bare eyes in the adult male and missing pearl row in the pupa places the species in a position between *Thienemanniella* and *Corynoneura*, like *Corynoneurella paludosa* sensu Langton (1997). However, Fu et al. (2010b) mention several *Thienemanniella* species with bare eyes: *T. nipponica, T. flavescens*, *T. flaviscutella*, *T. okigrata*, *T. partita* and *T. togamijika.*

After examining the holo- and paratypes of *Thienemanniella afra* at the ZSM, we noted that the first trochanter does not have a dorsal keel. Based on this and the discussion above, we support Lehmann's original placement of the species and restore it to its original combination *Thienemanniella afra* until its generic assignment is otherwise revised.

Key to *Corynoneura* species with a short elongation of the hind tibial apex.

1. Antennal apex scarcely broadened, with or without small concavity, setae on antennal tip never arranged in form of a rosette; tibial apex of hind leg not elongated, half as long as tibial width or shorter; hind tibia with apical seta (A-seta) slightly curved; hypopygium with transverse sternapodeme present, straight, not pointed; phallapodeme joins posteriorly, or at posterior extension with sternapodeme (see Schlee 1968: 142 figs 151-152); phallapodeme slightly curved (Inner structures of *C. magna* not described by Brundin) 2

- Antennal apex usually without a small apical concavity with or without rosette; tibial apex of hind leg elongated more than half of the tibial width; apical seta (A-seta) curved or s-shaped; hypopygium with transverse sternapodeme straight, slightly curved, or absent - when absent, lateral sternapodeme meeting orally; phallapodeme joins either laterally, or posteriorly, or at posterior extension with sternapodeme (see Schlee 1968: 142 figs 151-153); phallapodeme long or short, and strongly or slightly curved .... not keyed

2. Wing length about 1.8 mm; AR between 0.8 and 1.0; inferior volsella as in Brundin (1949: 831, fig. 202) .... *C. magna* Brundin, 1949

- Wing length shorter than 1.8 mm; other combination of characters 3

3. Phallapodeme strongly sclerotized on convex posterior margin (Fig. 8G); inferior volsella wide, with base along distal half of gonocoxite; gonostylus comparatively thin, slightly curved (Figs 8F, G) .... *C. paludosa* Brundin, 1949

- Phallapodeme strongly sclerotized on anterior margin; inferior volsella considerably smaller with base along less than half of gonocoxite; gonostylus comparatively thin, straight or curved 4

4. Superior volsella short and triangular, bearing several long setae postero-median directed (Schlee 1968: 116, fig. 8) .... *C. fittkaui* Schlee, 1968

- Superior volsella without setae, not as above 5

5. Transverse sternapodeme thin with parallel sided margins; superior volsella long and narrow (Fu et Sæther 2012: 19, fig. 9) .... *C. disinflata* Fu et Sæther, 2012

- Transverse sternapodeme mostly thick, with at least posterior margin concave; superior volsella not as above 6

6. Antennal apex pointed; superior volsella triangular, small, restricted to oral ¼ of gonocoxite margin; inferior volsella small, placed caudally; gonostylus with well-developed megaseta (Fu et Sæther 2012: 12, fig. 5) .... *C. capitanea* Fu et Sæther, 2012

- Character combination not as above 7

7. Inferior volsella situated on distal end of inner margin of gonocoxite (Figs 4F, 6I) 8

- Inferior volsella not situated distally on inner margin of gonocoxite (Fig. 7H) 9

8. Antennal apex blunt with small concavity (Fig. 4B); superior volsella conspicuous and triangular; inferior volsella small, almost square in outline (Fig. 4F) .... *C. ferringtoni* sp. n.

- Antennal apex pointed (Fig. 6D); superior volsella indistinctive, flat, rounded; inferior volsella rounded, posteriorly pointed lobe (Fig. 6I) .... *C. minimagna* sp. n.

9. Inferior volsella small, placed at about ½ length of gonocoxite, rounded, knoblike with hyaline margin (Figs 7H-J), AR 0.5-0.8, wing length less than 0.9 mm .... *C. minuscula* Brundin, 1949

- Inferior volsella broader, placed in distal half of gonocoxite, albeit not far distally 10

10. AR 0.9-1.0, wing length about 1.4 mm, gonostylus curved (Krashenninikov 2012: 85 figs 1-3) .... *C. makarchenkorum* Krasheninnikov, 2012

- AR 0.59-0.70, wing length about 0.9 mm (Schlee 1968), gonostylus with straight inner margin (Fig. 3A, Brundin 1949, fig. 205) .... *C. celeripes* Winnertz, 1852

Discussion

The generation of DNA barcodes from specimens and populations in the *Corynoneura*-group has revealed species-level diversity previously hidden behind similar morphology. The many interim names for species in this group in BOLD is an indication of this, as is the number of Barcode Index Numbers (BINs, Ratnasingham and Hebert (2013)) for this group in the database. Our findings for Europe, thus, is consistent with those of Silva and Wiedenbrug (2014) reported for neotropical taxa. It is perhaps not surprising that minute taxa such *Thienemanniella* and *Corynoneura* hold many undiscovered species as comparable results are found in other nematoceran families (e.g. Hebert et al. 2016, Kjærandsen 2022, Stur and Borkent 2014). However, as we show in this work and elsewhere (e.g. Anderson et al. 2013, Stur and Ekrem 2015), these undiscovered taxa are often not truly cryptic as small and consistent morphological differences usually can be found. Thus, integrative approaches in the analysis of species diversity, including morphological and molecular characters, certainly are useful in the quest to delineate taxonomic units at the species level (Silva and Wiedenbrug 2014).

From our results, it is apparent that as more species in the *Corynoneura*-group are discovered and described, the previous rather clear-cut generic boundaries become less distinct. As an example, Schlee (1968) used the presence of an elongated hind tibial apex in adult males as a synapomorphy for all *Corynoneura*, and shorter elongation to separate *C. paludosa, C. celeripes, C. minuscula* and *C. fittkaui* from the remaining *Corynoneura*. The results from our phylogenetic analysis using COI and 18S data disagrees with this, albeit many of the basal branches have low branch support (Fig. 2). There are, however, groups that appear more robust such as *Thienemanniella*, the *scutellata*-group and the *lobata*-group. Although taxon sampling and geographical representation needs to be improved considerably for a proper comparison, we observe that at least some of these relationships conflict with those presented by Schlee (1968) and Fu et al. (2009). Thus, the task to resolve the phylogeny of the *Corynoneura*-group remains open, and future molecular analyses should include multiple slow-evolving markers (such as 18S and CAD) to increase the robustness of phylogenetic groups above the species-level (Ekrem et al. 2010).

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Lopescladius (Diptera: Chironomidae) from the Nearctic, including keys and new pupal exuviae descriptions

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Abstract

Male adults and pupae of the genus *Lopescladius* from the Nearctic are reviewed. The morphologies of adult male and pupal *Lopescladius* (*Cordiella*) *hyporheicus* Coffman and Roback, 1984, are reexamined and compared to the Neotropical *Cordiella*. The pupa of *Lopescladius* (*Lopescladius*) *inermis* Sæther, 1983, is described, along with comments on the adult male. Three additional pupal morphotypes are described. Keys are included for adult males and pupal exuviae from the Nearctic. Large range expansions are reported for several species.

Introduction

The genus *Lopescladius* Oliveira was first described from the Neotropics, where much of the known diversity exists (Oliveira 1967, Ashe and O'Connor 2012). The original reference to this genus was proposed by Brundin (1966), as "*Cordites*" *nomen nudum*, but without giving a formal description. Sæther (1983) described three additional species, along with six unassociated pupae. Coffman and Roback (1984) described the subgenus *Cordiella*. An emended diagnosis of *Cordiella*, with additional descriptions, was given by Hagenlund et al. (2010).

There are three species described from the Nearctic: *Lopescladius* (*Cordiella*) *hyporheicus* Coffman & Roback, 1984, *L.* (*L.*) *inermis* Sæther, 1983, and *L.* (*L.*) *verruculosus* Sæther, 1983. All three have broad distributions. A described unassociated larva known as *Lopescladius* sp. 1 (Coffman and Roback 1984) is common in the southeastern U.S., but it's unclear if this represents an undescribed species or is a smaller form of *L.* (*L.*) *hyporheicus* (Epler 2001). The pupal exuviae were described for *L.* (*L.*) *verruculosus* by Sæther (1983) and *L.* (*C.*) *hyporheicus* by Coffman and Roback (1984). Across its range, *Lopescladius* larvae occur in sandy erosional and depositional sediments of rivers and streams (Andersen et al. 2013, Hagenlund et al. 2010, Epler 2001).

Materials and Methods

General terminology follows Sæther (1980). Measurements are presented in ranges followed by a mean in parentheses. If only two specimens were measured, the mean was not included.

Pupal exuviae descriptions in this genus usually conflate the term rugulose (fine wrinkling or a striated texture) and granulose (fine granules or a bumpy texture). In most original descriptions, the term rugulose was used for granular structures, although wrinkles may be present in some species at the same locations. For example, in *Lopescladius* the thorax is commonly granular with a rugose prealar area, but rarely is the thorax rugose overall.

In previous descriptions of pupal exuviae in this genus (e.g., Sæther 1983, Coffman and Roback 1984), the term 'spine' is used for large posterior points on abdominal segments. These posterior spines may appear to be on caudal flaps when flipped forward in a slide mount, but *in situ* they do not appear to be on flaps. In general, these features are triangular, and 'thorn' may be a more accurate term, although some of these features, particularly on sternites, are thin and spine-like. Sæther (1980, p. 16) described spines as an "immovable projection of body wall of multicellular origin," differentiated from spinules by size (> 10 µm). Snodgrass (1935) has a similar definition and illustrates both narrow and wide features. For consistency, the word spine will continue to be used.

Slide mounting techniques presumably varied for the specimens examined but were likely similar to those in Wiederholm (1986) and Kranzfelder et al. (2015). Specimens examined are from collections at the University of Minnesota, St. Paul (UMSP, all specimens unless noted), the Academy of Natural Sciences of Drexel University (ANSP), and personal collections (Will Bouchard [RWB], Peter Langton [PHL]).

Diagnoses

For generic and subgeneric diagnoses see Sæther (1983), Coffman and Roback (1984), Coffman et al. (1986), and Hagenlund et al. (2010). Specimens fit prior genus and subgenus diagnoses with the following additions and comments for pupae: the thorax ranges from granulose to rugose to nearly smooth; in many species abdominal reticulation is absent or indistinct; sternite VIII of female often has weak posterior spines laterally; abdominal segment I with 1-2 dorsal and 0-1 ventral taeniate setae; abdominal segments II-VII with taeniate setae at D_2 and D_5, (V_4) and V_5, and the single L seta; abdominal segment VIII with 2 dorsal, 1-2 ventral, and 1 lateral taeniate setae; and in some species the genital sheath has an additional small lobe ventrally.

For adult males: wing length 0.5-1.5 mm; hind tibia comb with 8-14 setae; inferior volsella probably always present but may be transparent and obscured by other features; in subgenus *Cordiella*, inferior volsella with two lobes in all described species; gonostylus does not always have a medial bend.

Lopescladius (*Cordiella*) *hyporheicus* Coffman & Roback, 1984

Material examined. USA: Pennsylvania: Crawford County, Linesville Creek (1 km north of Linesville), 15-VI-1974, leg. Wartinbee, 6 adult males (holotype and paratypes), ANSP. Colorado: Freemont County, Arkansas River at Canyon City, 19-IX-1985, leg. S. J. Herrmann, 1 adult male. New Mexico: Rio Grande River, Otowi Bridge between Santa Fe and Los Alamos, 16-VII-1976, sweep net, 1 adult male. Taos County, Rio Pueblo above the confluence with the Rio Grande, 21-VIII-1991, 1 adult male. Catron County, Negrito Creek at Reserve, 21-IX-1992, leg. D. James, 1 female of unassociated pupal exuviae. Sierra County, Alamosa Creek 14.4 rd. mi. upstream of Monticello, 10-IX-1997, leg. M. Hatch, 1 female of unassociated pupal exuviae. Vacas (Rio de las Vacas?), 31-V-2018, 3 adult males. Washington: Stevens County, Little Pend Orielle River, 21-VII-2021, 1 adult male. Mason County, east fork Satsop River, Shafer State Park, 16-VII-2024, leg. R. W. Bouchard, Jr., 2 females of unassociated pupal exuviae. Montana: Carbon County, Red Lodge Creek above lowest bridge, 3-VIII-2023, 3 adult males. Minnesota: Chisago County, Sunrise River, 23-V-2003 and 2-IX-2023, leg. L. C. Ferrington, Jr., 1 male and 1 female of unassociated pupal exuviae. Rush Creek and Rock Creek, 6-VI-2003, leg. L. C. Ferrington, Jr., 1 male and 1 female of unassociated pupal exuviae.

Adult males and pupal exuviae generally agree with the original descriptions, with the following morphological additions and comments.

Male imago. Thorax and tergites golden to yellowish brown. Unless indicated, n = 1.

Wing. Wing length 1.07-1.12 (1.10) mm (n = 4). Wing length/length of profemur 3.41-3.73 (3.61, n = 3). Venarum ratio (VR) 1.30-1.33 (n = 2). Cu length 644 µm. M length 840 µm. False costal extension faint, 88 µm, ending shortly beyond R_{4+5}. Brachiolum with one seta 30 µm long, other veins bare. Squama bare.

Legs. Fore tibia spur 25-29 µm long (n = 2). Mid tibia spur 28-30 µm long (n = 2). Hind tibia spur 32-35 µm long, and 8-14 setae in comb (n = 2). Width at apex of fore tibia 27 µm; of mid tibia 27 µm; of hind tibia 34 µm.

Abdomen. Tergites II-VII have a single strong medial seta, 68-92 µm long (n = 6 measured on one adult). Sternite II-IV with no setae or insertion points; sternites V-VII with a single strong medial seta, 40-48 µm long (n = 3 measured on one adult).

Hypopygium. Phallapodeme 41-45 µm long (n = 2); transverse sternapodeme 45 µm long. Gonocoxite 66-96 (81) µm long (n = 4). Inferior volsella bifurcate, basomedial branch 10-14 µm long, 6-7 µm wide at base (n = 2), usually tapers to a rounded apex resembling a canine tooth; main branch 34-41 (40) µm long (n = 4), 7-8 µm wide (n = 2, Fig. 1).

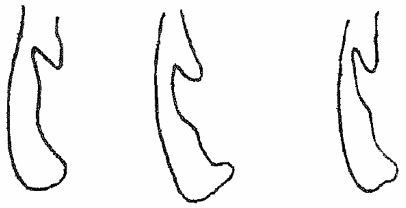

Figure 1. *Lopescladius hyporheicus*, inferior volsella variations.

Pupal exuviae. Total length and abdominal spine counts include ranges from Coffman and Roback (1984). For all other measurements n = 8, unless indicated. See Figs 9-12 in Coffman and Roback (1984).

Total length 2.1-2.5 (2.2) mm.

Cephalothorax: Thorax granulose overall (not rugulose per Coffman and Roback [1984]); prealar weakly granulose; wing sheath often smooth. Leg sheath sclerotization not distinctive.

Abdomen: Largest D_{1-5} setae 70 µm, taeniate. Transverse anterior shagreen 3-5 µm long, arranged in 2-5 irregular rows (Fig. 2 c). Segments VI-VIII may have additional pleurite shagreen. Pleurites with granulation ranging from moderate to weak, sometimes absent. Tergite posterior spine count (n = 10): I 5-17 (10), II 12-20 (15), III 11-22 (15), IV 10-23 (15), V 12-18 (15), VI 11-16 (13), VII 8-16 (11), VIII 6-14 (10). Sternite posterior spine count (n = 10): I 0, II 7-16 (12), III 13-19 (15), IV 11-15 (13), V 11-15 (13), VI 8-14 (12), VII 7-12 (10), VIII 5-13 (9). Sternites II (male and female) and VIII (female) with small spines widely separated. In lateral view, anal lobe digitiform projections conical, 84-129 (103) µm long, 42-56 (50) µm wide at base (L/W_{base} 1.7-2.3), and lacking ventral lobes on the genital sheath (n = 6).

Remarks. The original description and figures of the *L. hyporheicus* male imago (Coffman and Roback 1984) described the inferior volsella as digitiform with a single lobe. Hagenlund et al. (2010) described three Neotropical *Cordiella* with an inferior volsella having a double lobe, including a small anterior branch. A review of specimens from across the Nearctic, including the type material (holotype and five paratype males, Fig. 1) from Pennsylvania, indicate the original description and figures omitted the small, basomedial branch (it can appear to be part of the lateral sternapodeme). Therefore, all *Cordiella* species currently described share a bilobed inferior volsella. The subgenus diagnosis in Hagenlund et al. (2010) otherwise remains correct for *Cordiella*, with only one minor variation: comb of hind leg with 8-14 setae.

Within *Cordiella*, imago features for Nearctic *L. hyporheicus* (Coffman and Roback 1984) are similar to Neotropical *L. vibrissatus* Hagenlund, Andersen *et* Mendes, 2010 and *L. morosus* Hagenlund, Andersen *et* Mendes, 2010. The hypopygium of *L. hyporheicus* is most similar to *L. vibrissatus* in Hagenlund et al. (2010), but the basomedial lobe of the inferior volsella is often shaped like a canine tooth, with a broad base that tapers to a rounded apex (Fig. 1). In addition, the wing length/profemur length in *L. hyporheicus* is larger (3.41-3.73) and the hypopygium ratio (length of gonocoxite/length of gonostylus) is lower (1.56-1.89).

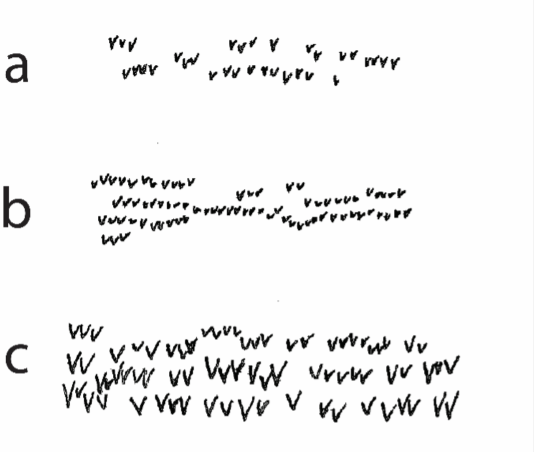

Figure 2. Abdominal shagreen pattern and relative size. a: *Lopescladius* NA3 (2-3 µm); b: *L.* NA2 (1-2 µm); c: *L. hyporheicus* (3-5 µm).

Specimens presented here indicate a broad Nearctic range for *L. hyporheicus*, from Pennsylvania, South Carolina, and Virginia in the east (Ashe and O'Connor 2012), to Minnesota in the Midwest, and Washington, Colorado, Montana, and New Mexico in the west. Larvae have been identified from New Brunswick and Nova Scotia in Canada (Bilyj, pers. comm.).

Lopescladius **(*Lopescladius*)** ***inermis*** **Sæther, 1983**

Material examined. USA: New Mexico: Eddy County, Pecos River at bridge on Hwy 82 between Artesia Loco Hills, sweep net, 1-VIII-1976, leg. J. E. Sublette, 6 adult males. Doña Ana County, Rio Grande River below Leasburg Diversion Dam, UV sample, 29-IX-1992, leg. unknown, 2 adult males. Guadalupe County, Pecos River at Puerto de Luna, Malaise trap, 29-IX-1974 and 12-VII-1976, leg. unknown, 2 adult males. Chaves County, Pecos River at NM Hwy 70, 13-IV-1991, leg. M. Hatch, 3 females and 4 males of unassociated pupal exuviae. Wisconsin: Burnett County, St. Croix River upstream of CCC Bridge, 25-VII-2007, St. Croix County, St. Croix River upstream of Marine-on-St. Croix, 27-VIII-2007, leg. B. E. Schuetz, 3 pharate males with associated pupal exuviae. Minnesota: Pine County, St. Croix River, upstream of Hwy 70, 30-V-2007, leg. B. E. Schuetz, 1 female of unassociated pupal exuviae. Chisago County, Sunrise River and Rush Creek, 6-VI-2003, leg. L. C. Ferrington, Jr., 2 females of unassociated pupal exuviae. Texas: Gregg County, Sabine River, Zone 1 Rte. 149 bridge to outfall 001, Zone 4 outfall 004 to Mason Lake discharge, 7-X-2015 and 9-X-2015, leg. R. W. Bouchard Jr. and R. L. Thomas, 1 male and 1 female of unassociated pupal exuviae, RWB collection. Oklahoma: Beaver County, Cimarron River at Mocane, 16-VII-2019, leg. unknown, 7 pharate males with associated pupal exuviae.

In Sæther (1983) only one adult male was examined. Selected features from thirteen additional adult males are included here to revise the description of *L. (L.) inermis*. Measurements include Sæther's (1983) specimen. Specimens from Sæther (2004) were not included but agree with present measurements. See Sæther (2004, Fig. 1) for a revised illustration of the male hypopygium.

Male imago. Coloration yellow-brown to brown on thorax and legs, pale brown abdomen. Ultimate flagellomere of antenna 100 – 142 (121) µm long, AR 0.35-0.66 (0.51, n = 11). Phallapodeme 69-99

(86) µm long. Gonocoxite 145-180 (165) µm long (n = 10); distance from base of gonocoxite to base of gonostylus 83-116 (94) µm (n = 10); distance from base of gonostylus to apex of gonocoxite 62-83 (71) µm long (n = 9). Gonostylus 41-57 (51) µm long (n = 13) and 9-12 (10) µm wide (n = 12). Hypopigium ratio 2.79-4.02 (3.19, n = 9). Inferior volsella present (see Fig. 1 in Saether 2004), weak and transparent with a pointed apex, no seta; depending on orientation may not be visible; 8-12 (10) um long (n = 10).

Pupal exuviae. Total length 1.27-1.83 (1.67) mm (n = 18), pale yellow to pale brown.

Cephalothorax. Figs. 3 a-b. Granularity strong to weak overall including frontal apotome and antennal sheath; posterior thorax may be rugose instead, wing sheath with reduced granules or smooth. Frontal setae and thoracic horn absent. All thoracic setae weak to very weak, usually difficult to find, no prealars observed; antepronotal, precorneal, and dorsocentral setae similar in size, 18-31 (27) µm long (n = 8). All leg sheaths straight and adjacent, foreleg ends near posterior edge of abdominal segment I, midleg ends near apex of wing sheaths, and hindleg ends near the posterior edge of abdominal segment II (see Figure 3C in Saether [1983]).

Abdomen. Figs. 3 c-d. Lateral setae on segments I-VII weak or absent, narrow taeniate or hairlike, 20-41 (30) µm long (n = 14). No lateral setae observed on segment VIII. Dorsal and ventral setae taeniate, $D_2 + D_5$ and $V_3 + V_5$ lanceolate with wider base and elongate narrow apex; smaller setae on anterior segments, larger setae on posterior segments and in posterior spine rows. Dorsal setae on segments I-II are 28-51 (35) µm long and 2-4 µm wide, segments III-VI are 31-75 (53) µm long and 3-7 µm wide, segments VII-VIII are 34-79 (60) µm long and 3-7 µm wide. Ventral setae on segment I ab-

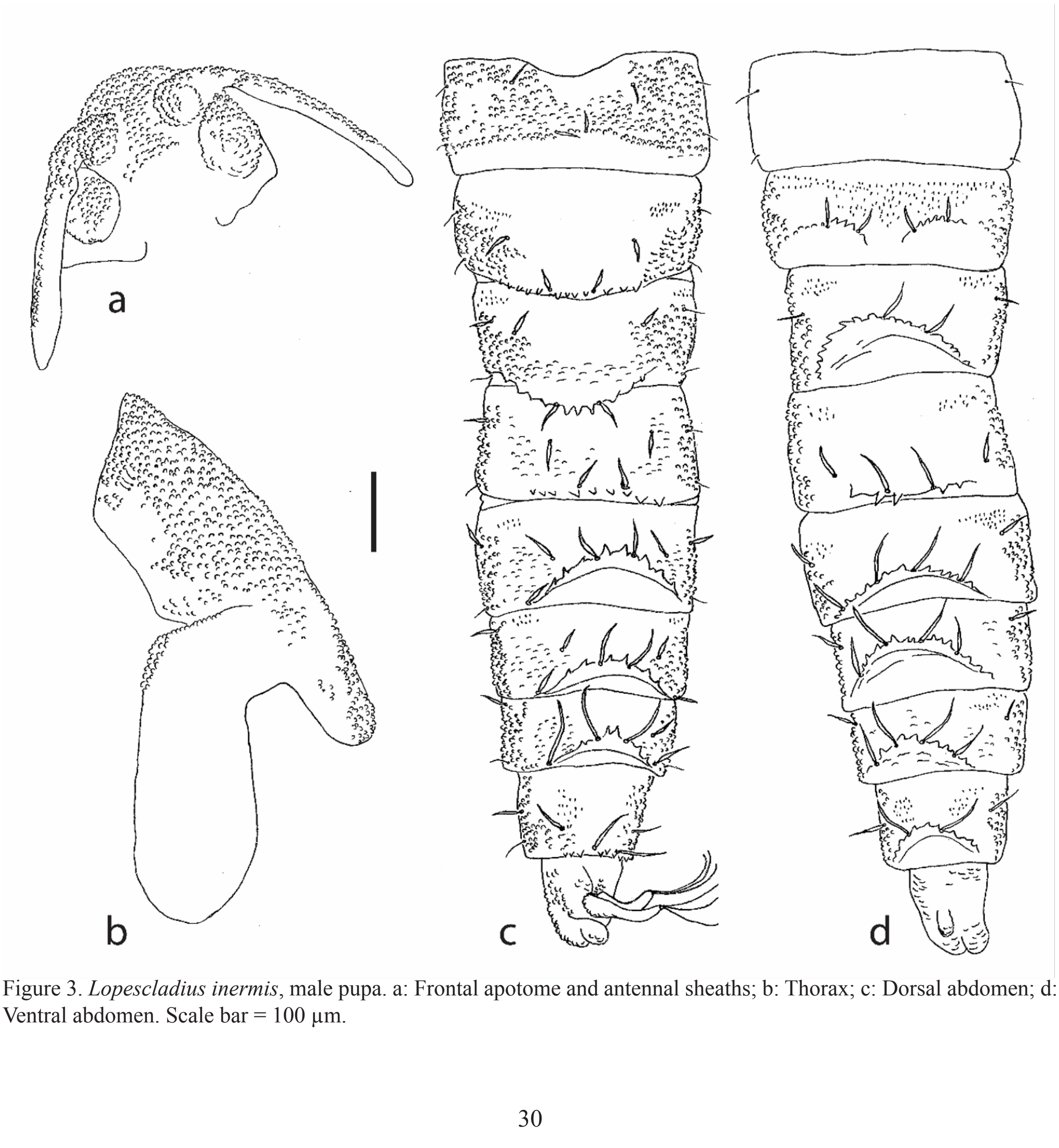

Figure 3. *Lopescladius inermis*, male pupa. a: Frontal apotome and antennal sheaths; b: Thorax; c: Dorsal abdomen; d: Ventral abdomen. Scale bar = 100 µm.

sent, segment II 31-58 (39) µm long and 2-4 µm wide, segments III-VIII 44-82 (62) µm long and 4-8 µm wide. Shagreen variable but never in transverse rows around segments, usually all segments bare; segment II can have sparse anterolateral shagreen on tergite and anterior shagreen on sternite; segment III can be similar to II; segments III-VII may have sparse and light shagreen anterolaterally or above the lateral rugose patches; VIII may have sparse, small tergite or sternite shagreen; anal lobe (IX) bare to wrinkled or sparse shagreen. Lateral edge of abdominal segments usually with rugosity, ranging from strong to weak, occasionally bare; rugosity on anterior segments may extend to tergites, particularly along the spine rows. Caudal spines triangular, usually with pointed apex but may be rounded; on tergite I rugosity often strong along the posterior edge but spines absent. Spines on sternite II have a medial gap between short lateral patches. Spines on sternite VIII absent or present in females, when present there is a wide gap as on sternite II, there are also two posterior medial lobes facing inward.

Tergite posterior spine count (n = 13): I 0, II 5-12 (9), III 7-11 (9), IV 6-11 (9), V 7-11 (9), VI 6-9 (8), VII 6-8 (7), VIII 2-7 (4). Sternite posterior spine count (n = 13): I 0, II 3-8 (7), III 7-13 (10), IV 7-12 (10), V 7-13 (9), VI 5-11 (7), VII 4-7 (6), VIII 2-7 (6) for males, 0-6 for females.In lateral view, anal lobe with cylindrical digitiform projections, 84-112 (95) µm long, 28-34 (29) µm wide at base (L/W_{base} 3.0-3.4), 17-20 (17) µm wide medially; three curving terminal macrosetae 89 (72-103) µm long (n = 6). Genital sheaths wrinkled to lightly rugose, curved dorsally in male, with a small ventral lobe in male and female.

Remarks. New pupal exuviae description. *L. inermis* is easily separated from other Nearctic pupal exuviae by the lack of anterior transverse spinule rows on abdominal segments and abdominal setae that are typically short and lanceolate. Caudal spines appear to be on flaps when the posterior tergite edge is flipped forward, but when not flipped forward there is no flap-like structure.

Distribution and ecology. *L. inermis* was previously known from Kansas and Wisconsin. The range is extended to Minnesota, Texas, Oklahoma, and New Mexico. A specimen in the Sublette collection from 27-VII-1971 was collected in the Wabash River, but the state is not identified on the locality label; it is likely that both Illinois and Indiana are in the range of *L. inermis*.

Lopescladius (*Lopescladius*) *verruculosus* Sæther, 1983

Sæther (1983) described this species from an adult male and paratypes of pupal exuviae in Michoacán, southwest Mexico. A single pupa was also noted from the Mississippi River near Cordova, Illinois. Helping to bridge the geographical gap are two specimens of *L. verruculosus* from the PHL collection from sites in the Rio Grande River at Las Cruces, New Mexico, leg. R. Jacobsen, and Rock Springs Creek at Wakita Springs, Florida, leg. W. P. Coffman (Langton, pers. comm.).

Lopescladius NA1 Langton

Langton (2023 and pers. comm.) describes NA1 as being relatively large at 2.7-3.1 mm long (n = 8); thorax nearly smooth to having shallow granulations anterodorsally; lanceolate abdominal setae; tergite I with small posterior spines; sternite II armament with anterior and posterior bands that are joined medially; distinct, continuous transverse anterior shagreen bands encircling segments; weak lateral abdominal rugosity; anal lobe digitiform projections conical, 88-92 µm long, 40-54 µm wide at base (LxW_{base} is 1.7-2.2), 26-30 µm wide medially, and lacking a ventral lobe on the genital sheath (n = 2).

Remarks and diagnostic characters. *Lopescladius* NA1 is separated from other *Lopescladius* by the weakly rugose anterodorsal thorax and shagreen on sternite II in two transverse rows joined medially. While it is possibly a pale variant of *L. hyporheicus* with reduced granulation, the sternite II shagreen and larger proportions are unique. In contrast to placement in Langton (2023), *L.* NA1 likely belongs in the subgenus *Cordiella* based on caudal tergite I spines, L/W of the anal lobe projection, and lack of a ventral lobe on the genital sheath. *L.* NA1 is known from collections made at Linesville Creek, Pennsylvania (Langton, pers. comm.).

Lopescladius NA2

Material examined. USA, Wisconsin: Burnett County, St. Croix River, CCC Bridge 25-VII-2007 and Riverside Landing 31-V-2007, leg. B. E. Schuetz, 3 males and 1 female of unassociated pupal exuviae.

Pupal exuviae (n = 4). Total length 2.2-2.7 (2.4) mm, golden brown.

Cephalothorax. Figs. 4 a-b. Strongly granulose thorax and frontal apotome, weak posterior granularity, wing sheaths and prealar mostly bare. Frontal setae and thoracic horn absent. All thoracic setae weak, no prealars observed. Two median antepronotals, about 30 µm long (n = 2). Three precorneals in a short line, longest is 41 µm long, the others 20 µm long (n = 2). Dorsocentrals 1-2 together, both about 34 µm long; Dc3-4 together, both about 35 µm long; two groups 180 µm apart (n = 2). All leg sheaths straight; foreleg ends before wing apex, midleg ends at apex, and hindleg ends past wing sheath apex.

Abdomen. Figs. 4 c-d. Lateral seta on segment I weak, 37 µm long (n = 2). Lateral setae on II-VIII 38-55 µm long, narrow taeniate, 2-3 µm wide (n = 4). Dorsal and ventral setae taeniate, 55-86 µm long, 3-7 µm wide (n = 4). Shagreen absent on segment I; anterior transverse rows of very weak 1-2 µm spinules circle segments II-VIII (Fig. 2 b); sternite II with weak medial shagreen, sternite III without additional medial shagreen; segment IX and genital sheath with weak shagreen. Lateral edge with granulose to rugose patch on anterior segments, usually smooth on posterior segments. Posterior medial lobes on segment VIII of female, absent on male. Caudal spines small, 14-20 µm for tallest, triangular with pointed apex or low and rounded. Spines on sternites II with a medial gap, and sternite VIII with (♀) or without (♂) a gap. Tergite I either bare or with caudal rugose knobs, but never spine shaped. Tergite posterior spine count (n = 4): I 0, II 8-10 (9), III 10-13 (11), IV 8-12 (10), V 9-12 (11), VI 9-11 (10), VII 5-8 (7), VIII 4-6 (6). Sternite posterior spine count (n = 4): I 0, II 9-11 (10), III 10-12 (11), IV 8-12 (10), V 8-11 (10), VI 8-9 (8), VII 5-8 (6), VIII 0-8 (4).

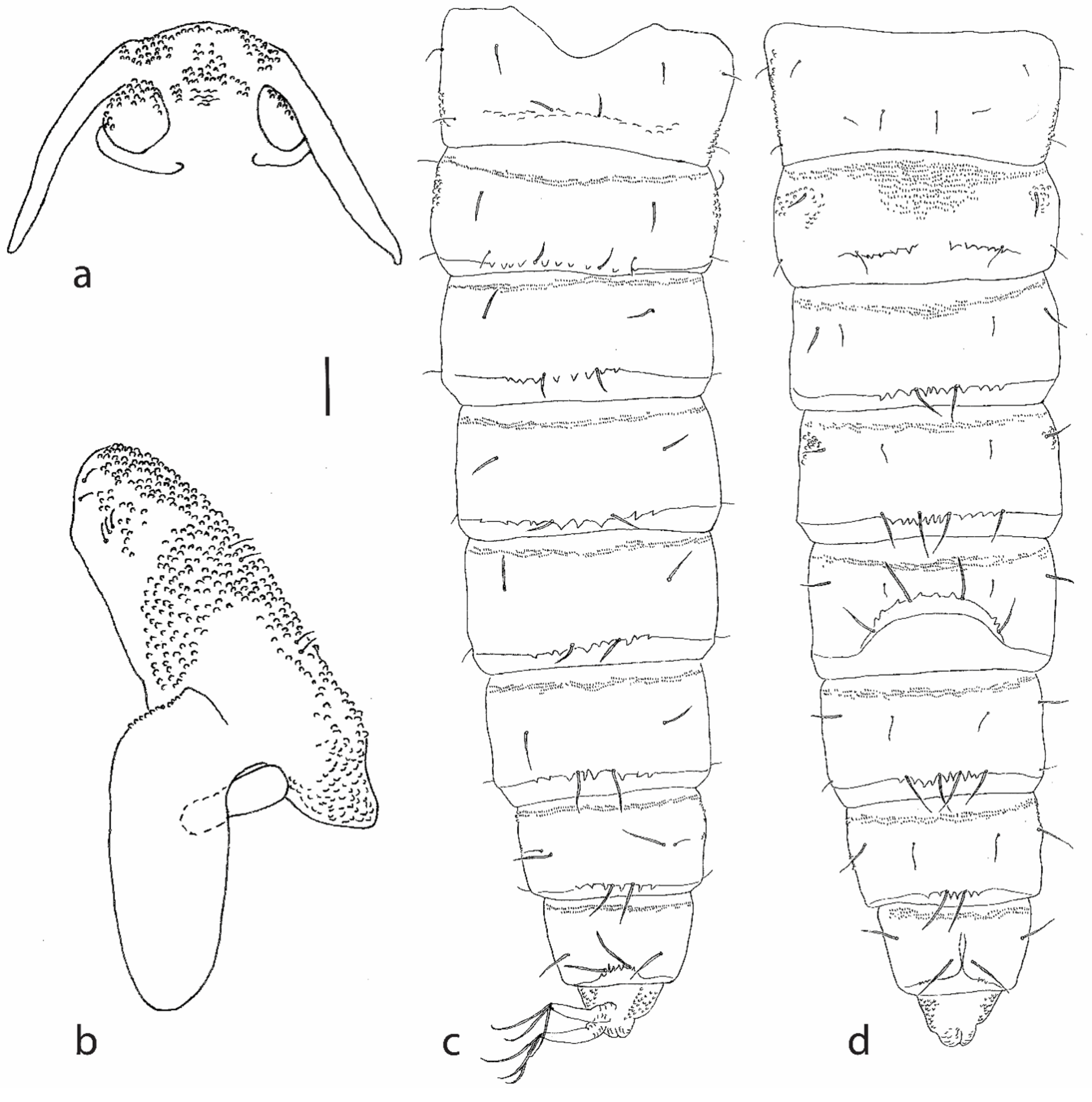

Figure 4. *Lopescladius* NA2, female pupa. a: Frontal apotome and antennal sheaths; b: thorax; c: dorsal abdomen; d: ventral abdomen. Scale bar = 100 µm.

In lateral view, anal lobe projections variable in shape, elongate conical, wrinkled; 118-129 (124) µm long, 40-62 (49) µm wide at base (L/W$_{base}$ 2.0-3.3, n = 6). Small ventral lobes variable, absent to weak. Three curving terminal macrosetae 113-120 µm long (n = 6). Male genital sheaths curve dorsally.

Remarks. To follow nomenclature from Langton (2023), the designation *L.* NA2 is used for this morphotype. Placement of *L.* NA2 in the subgenus *Lopescladius* is tentative because of variable features, including anal lobe projections and secondary ventral lobes. However, the lack of caudal spines on tergite I appears to be an important feature separating subgenera. Overall, *L.* NA2 is similar to *L. hyporheicus* but several features suggest a unique morphology. Anterior transverse shagreen rows are comprised of very small points in compact and elongated rows, compared to larger points in irregular rows for *L. hyporheicus* (Fig. 2). Sternite III is unarmed medially in *L.* NA2. Tergite I spines are absent (or at most a few indicated knobs) in *L.* NA2; low counts and weak spines can occur in *L. hyporheicus* but this appears to be atypical. This morphotype is also similar to *L. verruculosus*, but medial sternite II shagreen (absent in *L. verruculosus*, Langton, pers. comm., n = 12) and larger size of *L.* NA2 will separate these.

Lopescladius NA3

Material examined. USA, Wisconsin: Burnett County, St. Croix River upstream of Riverside boat launch, 31-V-2007, leg. B. E. Schuetz, 1 male of unassociated pupal exuviae. Minnesota: Chisago County, St. Croix River upstream of Sunrise River, 26-VII-2007, leg. B. E. Schuetz, 1 female of unassociated pupal exuviae.

Pupal exuviae (n = 2). Total length 1.82-1.96 mm, pale yellow.

Cephalothorax. Figs 5 a-b. Strongly granulose anterior thorax, weak granulation with bare areas posteriorly; bare to weakly granulose frontal apotome and prefrons; wing sheaths mostly bare. Frontal setae and thoracic horn absent. All thoracic setae weak, no prealars observed. Two median antepronotals, 41 µm long. Three precorneals in a short line, longest is 40 µm long, the others 24 µm long. Dorsocentrals 1-2 paired, both 41 µm long; Dc 3-4 paired, also weak; two groups 120 µm apart. All leg sheaths straight; foreleg ends before wing apex, midleg and hindleg end past wing sheath apex.

Abdomen. Figs. 5 c-d. Lateral seta on segment I weak, 38 µm long (n = 1). Lateral setae on II-VIII 62-70 µm long, 2 µm wide. Dorsal and ventral setae hairlike or narrow taeniate, 34-69 µm long, 2-3 µm wide. Shagreen absent on segment I; anterior transverse rows of weak 2-3 µm spinules circle segments II-VIII; sternite II with widespread weak shagreen, sternite III similar but less widespread; segment IX and genital sheath with weak shagreen. Lateral edge of segment II with light anterior rugosity, all other segments smooth. Posterior medial lobes on segment VIII of female, absent on male. Caudal spines robust, triangular with a pointed apex. Spines on sternites II and VIII have a wide medial gap between short lateral patches. Tergite posterior spine count: I 11-12, II 11-11, III 10-12, IV 7-9, V 8-11, VI 9-10, VII 7-8, VIII 6-7. Sternite posterior spine count: I 0, II 9-11, III 9-11, IV 9, V 7-10, VI 9-10, VII 5-7, VIII 5-8. In lateral view, anal lobe projections conical, wrinkled to lightly granulose; 73-90 (81) µm long, 34-39 (36) µm wide at base (L/W$_{base}$ 2.1-2.3, n = 4). Three curving terminal macrosetae 72-79 µm long (n = 4). Due to mount orientations, uncertain if male genital sheaths curve dorsally. Small ventral lobes on genital sheath absent in both specimens.

Remarks. To match the nomenclature from Langton (2023), the designation *L.* NA3 is used for this morphotype. Many features are similar to *L. hyporheicus*, in particular the strong spines on tergite I, but several features diverge: small size (< 2 mm), pale pigmentation, weaker abdominal setae, very weak circumferential shagreen (2-3 µm), and no lateral rugose abdominal patches. *L.* NA3 likely belongs in the subgenus *Cordiella* based on caudal tergite I spines, L/W of the anal lobe projection, and lack of a ventral lobe on the genital sheath.

The following are Nearctic revisions to keys in Sæther (1983).

Key to known Nearctic male adults of *Lopescladius*

1. Gonocoxite without a caudal extension (Coffman and Roback 1984, Fig. 7); inferior volsella bifurcate with long digitiform lobe and short basomedial branch (Fig. 1) ……………………………... *Lopescladius* (*Cordiella*) *hyporheicus* Coffman and Roback

- Gonocoxite with an elongate caudal extension; inferior volsella simple, small spiniform or triangular (Sæther 2004, Fig. 1) …..........................….... .... *Lopescladius* (*Lopescladius*) 2

2. Antennal ratio (AR) about 0.75; gonocoxite caudal extension tapers to a point, with a conspicuous terminal spine-like seta (Sæther 1983, Fig. 2E) .... *Lopescladius* (*s.str.*) *verruculosus* Sæther

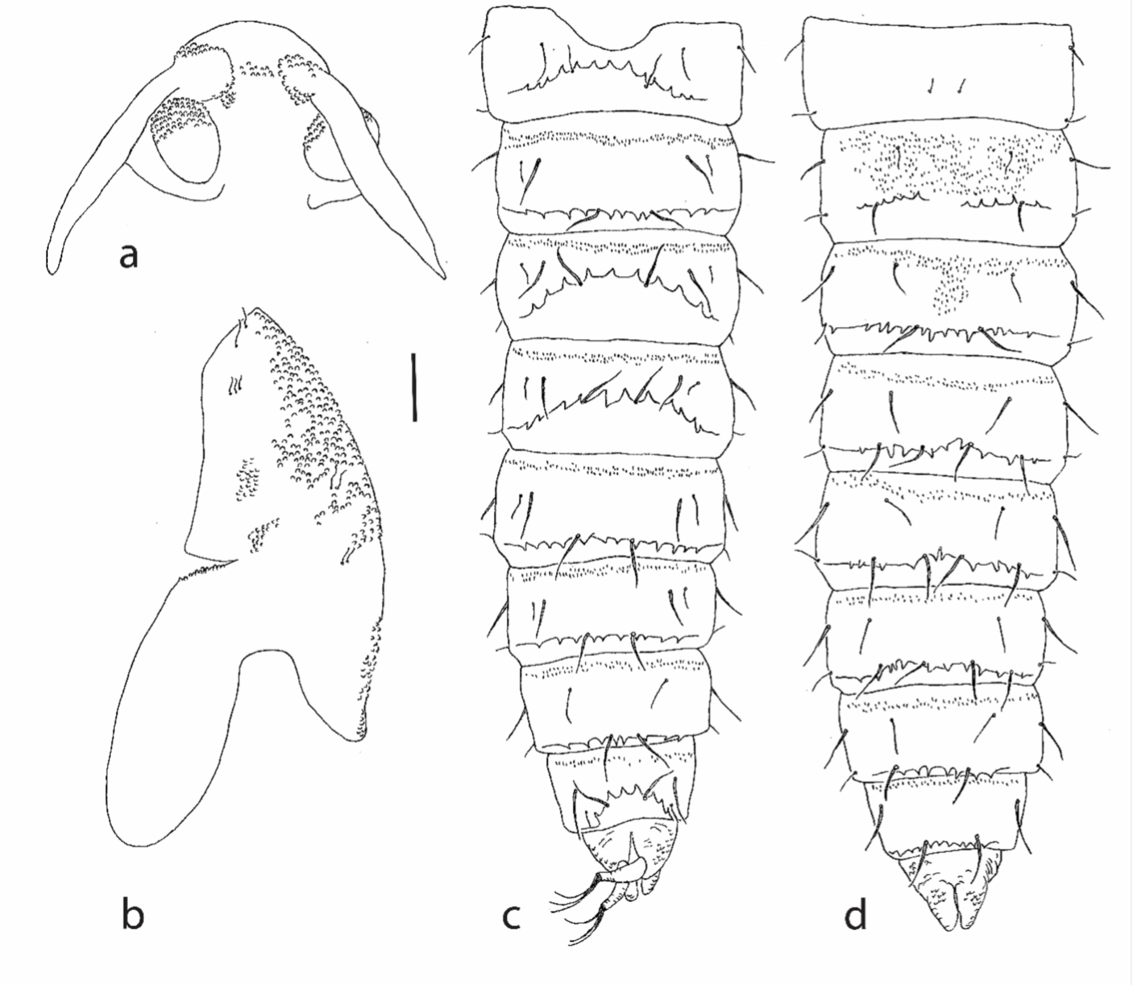

Figure 5. *Lopescladius* NA3, male pupa. a: Frontal apotome and antennal sheaths; b: thorax; c: dorsal abdomen; d: ventral abdomen. Scale bar = 100 µm.

- Antennal ratio 0.5-0.6; gonocoxite caudal extension tubular with a rounded apex, without spine-like terminal seta (Sæther 1983, Fig. 5D) …..............… *Lopescladius* (*s.str.*) *inermis* Sæther

Key to known Nearctic pupal exuviae of *Lopescladius*

1. Tergite I with a caudal spine row; in lateral view, anal lobe projections conical; small ventral tubercle on genital sheaths always absent …….………………............... subgenus *Cordiella* 2

- Tergite I without a caudal spine row, though may be rugose or with small granulation; in lateral view, anal lobe projections tubular or weakly conical; small ventral tubercle on genital sheaths present or absent ………….………. subgenus *Lopescladius* 4

2. Total length < 2 mm; pale brown; transverse shagreen very weak, 2-3 µm (Fig. 2a); abdomen smooth laterally …..………….. *Lopescladius* NA3

- Total length > 2 mm; pale to golden brown; transverse shagreen stronger (Fig. 2c); abdomen usually granular or rugose laterally, at least on some segments ……...................................………… 3

3. Thoracic granulation strong; sternite II with widespread shagreen but no separate posterior band ……..........................……….. *Lopescladius* (*Cordiella*) *hyporheicus*, Coffman and Roback

- Thoracic granulation weak to nearly smooth; sternite II with a broad anterior and narrow posterior band of shagreen joined medially …..………........……….... *Lopescladius* NA1

4. Abdominal segments bare or with sparse shagreen, never forming transverse rows; abdominal setae lanceolate (Fig. 3 c-d); total length < 2 mm ......……….. *Lopescladius* (*s.str.*) *inermis* Sæther

- Anterior shagreen on abdominal segments II-VIII form transverse rows encircling the segment; abdominal setae taeniate, rarely lanceolate ………. 5

5. Total length > 2 mm; sternite II with shagreen expanded medially (Fig. 4d) ………………………………. *Lopescladius* NA2

- Total length < 2 mm; sternite II shagreen without medial expansion ….. .... *Lopescladius* (*s.str*.) *verruculosus* Sæther

Discussion

Although the first mention of this genus was by Brundin (1966), he did not formally describe it. He did note its unique morphology, including straight leg sheaths of the pupa and cordiform fourth tarsal segments of adults. In the Nearctic, he mentions a record from Vancouver Island. Without description this cannot be ascribed to a species. But the locality and his illustration (Brundin 1966; Fig. 622) suggests this could have been *L. hyporheicus*, which is the most widespread species in the Nearctic. With three associated pupal exuviae and three distinct pupal morphotypes, it appears that only half of the Nearctic diversity are described as adults.

Description of the inferior volsella shape between Nearctic and Neotropical species confirms separation of subgenera, with the subgenus *Cordiella* having a bilobed inferior volsella and the subgenus *Lopescladius* having a spiniform shape (although transparency and small size can make it difficult to observe), which was described as absent in *L. inermis* (Sæther 1983:293). For pupal exuviae, caudal spines on tergite I are probably the most useful feature for separating subgenera, with anal lobe projection shape and ventral tubercle presence on the genital sheaths as valuable secondary traits. Without associated adult males, the three morphotypes have been separated using pupal exuviae.

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MIDGES BELOW ZERO: A REVIEW OF HIBERNAL EMERGENCE OF CHIRONOMIDAE IN TEMPERATE REGIONS

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Abstract

The Chironomidae are one of several groups of aquatic insects with representatives that possess cold hardening strategies that allow pronounced hibernal activity, including species that complete their life and reproduce only during the coldest months of the year. Although these winter-active species are often ignored in aquatic studies, relatively recent research has demonstrated that these insects are not only interesting, but they can also be an important and diverse element of aquatic insect communities. This review synthesizes existing literature on winter-emerging Chironomidae, focusing largely on Holarctic species emerging from temperate streams that are at least partially ice-free throughout the winter season. We found that there are currently at least 215 chironomid taxa present during winter, predominated by Orthocladiinae (n = 127), Chironominae (n = 42) and Diamesinae (n = 35). Our review highlights cold hardening strategies, such as supercooling, that permit winter activity, and we also discuss growth and emergence for species that have been extensively studied, such as *Diamesa mendotae* Muttkowski. Winter-active species tend to be long-lived at cold temperatures, and we discuss how consequences of climate change, including warmer temperatures and reduced snowpack, may negatively impact certain winter-active species. Although there is a growing base of studies featuring winter-active species, our review demonstrates that research is largely restricted to a handful of localities, and autecology studies are limited to only select species. We emphasize the importance of extending field work into the winter season and expanding research on winter Chironomidae to a broader geographic range to better gauge species accounts and enhance our understanding of the importance of winter-emerging Chironomidae.

Introduction

Several aquatic insect groups include species that are active during the winter season in northern temperate regions of the Holarctic when air temperatures can be below 0°C (Lencioni 2004, Soszyńska 2004, Hågvar 2010, Soszyńska-Maj et al. 2016, Bouchard and Gelhaus 2020). These insects exhibit a myriad of physical, behavioral, ecological, and biochemical adaptations to survive freezing and subfreezing temperatures (≤0 °C) (Lee 1989, Block 1990, Oswood et al. 1991, Lencioni 2004, Danks 2008). Most aquatic insects are not active at temperatures below 0°C and have strategies for avoiding (e.g. migration, occupying protected microhabitats) or surviving (e.g. freeze tolerance, depression of supercooling point [SCP]) exposure to freezing temperatures. For example, many aquatic insects in temperate regions occupy or disperse to aquatic habitats that do not freeze in winter (e.g. deep waters and away from habitat margins) or they will burrow into benthic sediments or the hyporheic zone (Danks 2008). Other aquatic insects have the ability to survive freezing and may hibernate in frozen substrates. Additionally, certain aquatic insects can protect themselves from freezing by lowering their SCP. This strategy allows these species to remain active during winter because they do not need to enter hibernation. Among aquatic insects, select Diptera (e.g. Chironomidae, Limoniidae), Plecoptera, Trichoptera, and Ephemeroptera emerge from ice-free aquatic environments as active adults and can be observed walking or mating on the snow or occasionally flying short distances (Lencioni 2004, Bouchard et al. 2009, Hågvar 2010, Soszyńska-Maj et al. 2016, Bouchard and Gelhaus 2020, Anderson et al. 2022). This winter activity is often ignored or treated as a curiosity in aquatic studies, but relatively recent research has demonstrated that these insects can be taxonomically diverse and an important element of aquatic communities.

Although life during winter for ectothermic organisms comes with challenges, including cold and snowy environmental conditions, insects adapted to emerge during this season are afforded some benefits. Winter-emerging species likely encounter reduced risk of predation, as many potential predators are dormant or inactive (e.g. certain insectivorous herpetofauna, mammals, or predatory insects/invertebrates), have migrated (e.g. many bird species), or are active but have reduced metabolic requirements at low temperatures (e.g. fish). Given that relatively few aquatic insect species are adapted to winter development and emergence, competition is also reduced. Although winter is often viewed as an extreme time of year, in some ways it is no more harsh than other seasons. Insects emerging during other seasons often face significant daily air temperature fluctuations; although also true during winter, winter-emerging taxa may find refuge within the snowpack or near the air-water margin of spring-fed streams to buffer them from seasonal fluctuations (e.g. Hågvar 2010, Anderson et al. 2013). Additionally, larvae developing during winter may face reduced probability of flooding or drying events, which can alter habitat and food resources. Lastly, required habitats may be primarily available only during winter for some species. For example, Ferrington and Sæther (1987), describe certain intermittent streams which harbor populations of *Orthocladius (Orthocladius) ferringtoni* Soponis; streams harboring this species flow primarily from fall-spring, therefore, development can only occur over this period when suitable habitat is available. Thus, the cost for aquatic insects to employ successful strategies for surviving subfreezing temperatures can be offset by conditions that may be favorable during the winter months.

The fly family Chironomidae contains many winter-active representatives and is the largest, most diverse group of winter-active invertebrates studied thus far. Studies of winter-emerging Chironomidae are rare as compared to their summer counterparts; however, this is not due to the novelty of the phenomenon. Instead, as suggested 50 years ago by Jonsson and Sandlund (1975), this rarity is because of the dearth of entomological sampling that occurs during the winter season. Although rare in comparison to work during warmer seasons, the emphasis on winter entomological field research and focus on the importance of the findings has increased in recent years. Dr. Leonard Ferrington, Jr., who held long-term positions at both the University of Minnesota and the University of Kansas, championed a significant portion of the winter chironomid research in temperate habitats in recent years, driving or inspiring many research projects in both the Nearctic and Palearctic ecoregions.

This review synthesizes existing literature on winter-emerging Chironomidae, focusing largely on Holarctic species emerging from temperate streams that are at least partially ice-free throughout the winter season. We define winter-emerging as species observed to emerge from streams as adults from December through March. Our discussion is restricted primarily to seasonal, temperate ecosystems that experience freezing or subfreezing temperatures for a significant portion of the year but include discussion outside these areas where appropriate. We cover a suite of topics including the diversity of winter-emerging species, cold hardiness, emergence and growth patterns, longevity, behavior, and the ecological importance of winter emergence.

Diversity

Chironomidae contain over 5,000 species described across all continents including Antarctica and projections suggest up to 20,000 species exist worldwide (Ferrington and Berg 2019). Given the immense species-richness of the group, there is significant diversity when considering ecological adaptations and tolerances. In terms of environmental temperature, certain species are well adapted to extreme highs and occur in habitats such as thermal springs (Hayford et al. 1995) whereas others are cold adapted and can survive near and at subfreezing conditions (Kohshima 1984, Lee 1989, Lencioni 2004, Bouchard et al. 2006a, 2006b, Danks 2007). Chironomidae are thought to have evolved in upper reaches of cool, spring-fed mountain streams in temperate forest regions (Brundin 1966). Danks (1971) further deduced that these ancestral chironomids were cold-adapted given that most major genera include representative species exhibiting cold tolerance. Given this, it is not surprising that many species are known to withstand and even thrive in cold winter temperatures.

Across the 11 chironomid subfamilies and 22 nominal tribes, the Diamesinae, Prodiamesinae, Podonominae, and Orthocladiinae are considered most cold-tolerant (Bouchard 2007, Eggermont and Heiri 2012), although winter-emerging Podonominae are not yet known to the Holarctic. While recognized as somewhat less cold tolerant than Diamesinae and Prodiamesinae (Oliver 1971, Bouchard 2007, Bouchard and Ferrington 2008), Orthocladiinae generally comprise a larger percentage of winter taxa, due in-part to the immense species-richness of the subfamily (e.g. Coffman 1973, Rempel and Harrison 1987, Chou et al. 1999, Anderson et al. 2010, Anderson and Ferrington 2013, Soszyńska-Maj et al. 2016). The subfamilies Tanypodinae and Chironominae are also species rich but are more typical of warmer waters (Coffman and De La Rosa 1998).

However, these patterns do not always hold. Certain genera, such as the Chironominae genus *Micropsectra*, tend to emerge at cooler temperatures and are often present during winter (Table 1), whereas *Corynoneura* and *Parametriocnemus,* while also found in winter, are restricted to warmer temperatures than other winter-emerging orthoclads (e.g. Ferrington 2000, Bouchard and Ferrington 2008, Nyquist et al. 2020). This thermal partitioning may allow species to reduce competition between closely related taxa (Bouchard and Ferrington 2008).

Taxonomic composition and diversity of the winter chironomid community have also been shown to vary both longitudinally within a stream system and over the winter season, following predictable patterns. For example, Nyquist et al. (2020) found that taxonomic composition was variable within a 300m reach in a groundwater-dominated stream due to thermal heterogeneity between sites. Taxa such as *Corynoneura, Parametriocnemus,* and *Eukiefferiella* (Orthocladiinae)*,* were found near a spring source, which provided "winter warm" reaches in the stream. In contrast, *Orthocladius* and *Diamesa,* were found in cooler reaches. Cooler reaches also exhibited greater taxonomic diversity (Nyquist et al. 2020). Studies examining seasonal emergence follow similar patterns, with Diamesinae and Orthocladiinae emerging during the colder period of the winter season and orthoclads predominating throughout winter. Tanytarsini, Chironomini, and Tanypodinae species do not commonly emerge during the winter, but rather begin to emerge at the end of the winter season as temperatures begin to warm; many Diamesinae and Orthocladiinae taxa also follow this pattern (Coffman 1973, Boerger 1981, Chou et al. 1999, Ferrington 2000, Ferrington 2007, Anderson et al. 2010, Anderson and Ferrington 2013, Soszyńska-Maj et al. 2016).

We assessed the literature to document the diversity of winter-emerging Chironomidae found in the Holarctic. Although several cold-adapted taxa are known from glaciers/glacial-fed streams and polar or alpine regions during non-winter months as defined in this paper (e.g. Oliver 1968, Kohshima 1984, Lencioni 2004, Lencioni et al. 2008), our discussion of diversity is restricted to species emerging from December through March. However, certain references (e.g. Soszyńska-Maj et al. 2016) defined winter based on weather or snow cover and/or did not specify specific winter emergence periods. While some taxa from these studies may emerge predominantly in early spring, we include these taxa in our list of winter-active species because they were found to emerge within our defined winter period. We document evidence of emergence either as collections of adult chironomids, or as collections of pupal exuviae. Where species-level designations were not reported or could not be determined from the literature, we record the taxa to the highest defined taxonomic level (e.g. *Diamesa* spp. when multiple undistinguished species were presented or *Diamesa* sp. when one was presented). Each taxon was counted once for each associated region to obtain a conservative estimate of total number of winter-active taxa per region.

Our literature survey yielded 39 studies across the Holarctic that documented the presence of winter chironomid emergence. These records indicate at least 215 taxa present during the winter season (Table 1). Although we recognize that winter may not be the primary emergence period for many of the species recorded, these taxa are documented at low temperatures at some time during the winter period as we define in this paper. We anticipate that certain species that are currently incidental winter emergers may become more common as temperatures become warmer with climate change. The most common winter-active groups from our literature survey are Orthocladiinae (total taxa=127), Diamesinae (total taxa=35), and Tanytarsini (total taxa=28). Chironomini (total taxa=14), Tanypodinae (total taxa=8), and Prodiamesinae (total taxa=3) were also collected from various regions but were less common (Table 1). This pattern holds with what is reported in the literature with orthoclads typically documented as the most abundant (Coffman 1973, Anderson et al. 2010, Anderson and Ferrington 2013, Soszyńska-Maj et al. 2016) and Chironomini and Tanypodinae as the least winter-active subfamilies/tribes in Chironomidae (Coffman and De La Rosa 1998). Table 1 provides a comprehensive overview of these winter-active

Chironomidae, including locations they are known from and literature references.

Figure 1 highlights currently known winter chironomid diversity by region. As for many biodiversity efforts, documentation of winter-active Chironomidae is biased based on human artifacts related to deference for collecting biodiversity data in certain geographic regions (geographic bias), focusing on taxa that are commonly found or studied most often (taxonomic bias), collecting specimens when field work is optimal (temporal bias), or collecting taxa that are easy to see or find based on size or reproductive patterns (bias based on functional traits) (Meyer et al. 2015, Daru and Rodriguez 2023). Some bias may also be driven by public interest (Troudet et al. 2017), and by complex interactions among geographic accessibility and socio-economic factors (Meyer et al. 2015). Thus, the total number of taxa we report for various regions is not only a conservative estimate, but also only represents regions where studies have been conducted and where, most likely, chironomid researchers are located (Fig. 1). Our review also points to research voids, and regions where additional research focus would benefit. For example, Figure 1 shows that Minnesota and Kansas are known to have more than 40 winter-active chironomid taxa, whereas far fewer are documented from neighboring states/provinces. Given the significant amount of work completed in Minnesota and Kansas, led largely by Dr. Ferrington, it is perhaps not surprising that so many species are known in these areas. Additional research focus would undoubtedly yield far more taxa in surrounding areas.

The phenomenon of cryptic species, or biological groups that are morphologically near-identical, incapable of interbreeding, and genetically distinct (Pfenninger and Schwenk 2007), also suggests that the results presented here are conservative. Chironomidae are known to contain many cryptic

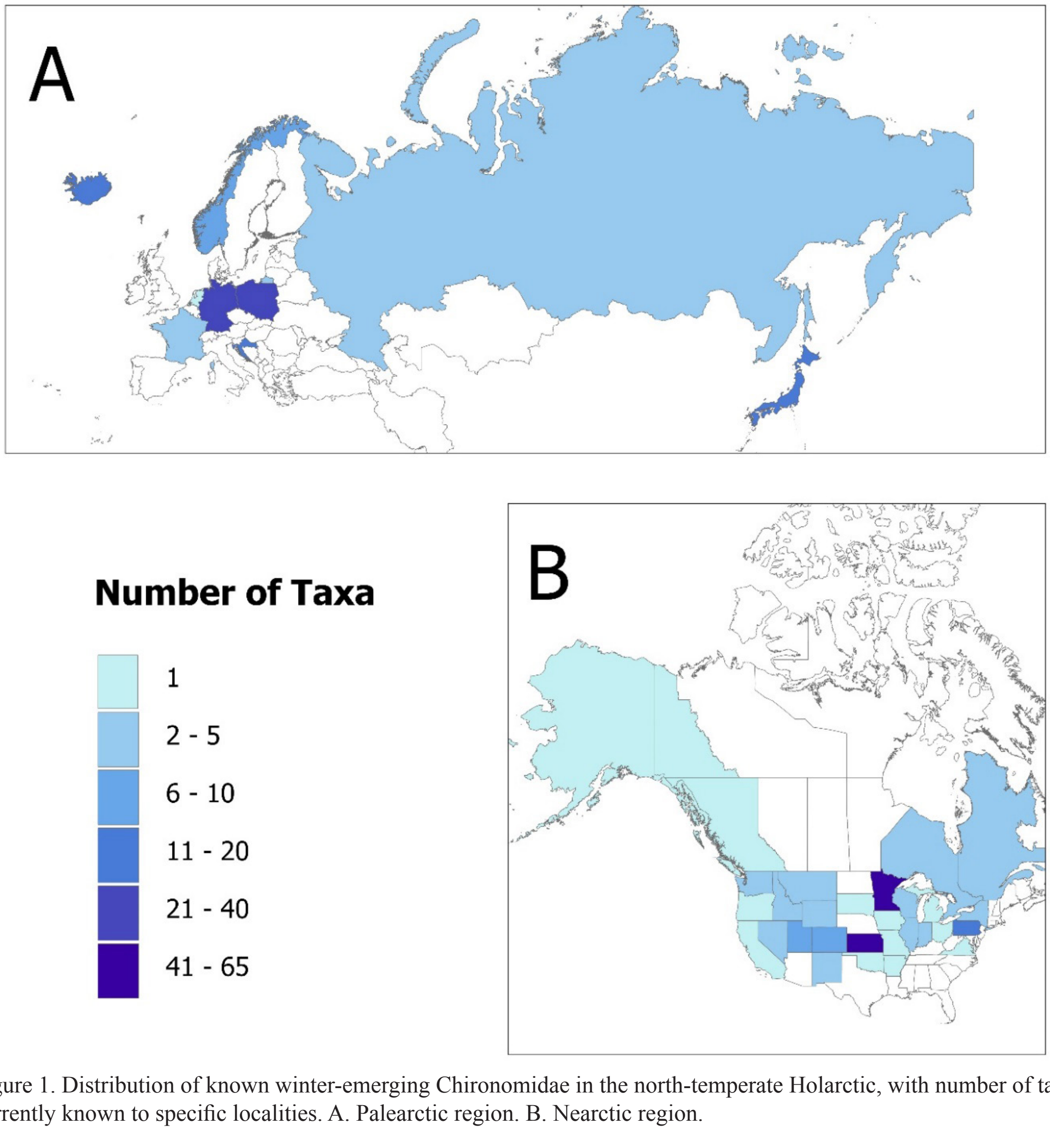

Figure 1. Distribution of known winter-emerging Chironomidae in the north-temperate Holarctic, with number of taxa currently known to specific localities. A. Palearctic region. B. Nearctic region.

Table 1. Taxa with representatives that emerge during winter. Locations of reported emergence and reference sources provided. Where species-level designations were not reported or could not be determined from the literature, we record the taxa to the highest defined taxonomic level (e.g. *Diamesa* spp. when multiple undistinguished species were presented or *Diamesa* sp. when one was presented). Asterisks (*) next to taxonomic names reflect species with junior synonyms; accepted names are used here, while a reference documenting the taxon utilizes a junior synonym.

Taxon	**Location(s) Recorded**	**References**
Orthocladiinae (127 taxa)		
Acricotopus lucens (Zetterstedt, 1850)	Crimea	Baranov and Ferrington 2013
Brillia bifida (Kieffer, 1909)*	Crimea, Croatia, Germany, Poland (lowlands)	Illies 1971, Ringe 1974, Siebert 1980, Baranov and Ferrington 2013, Soszyńska-Maj et al. 2016, Dorić et al. 2024
Brillia flavifrons (Johannsen, 1905)	USA: Minnesota	Bouchard 2007
Brillia retifinis Sæther, 1969	USA: Minnesota	Anderson and Ferrington 2012
Bryophaenocladius akiensis (Sasa, Shimomura & Matsuo, 1991)	Crimea	Baranov and Ferrington 2013
Bryophaenocladius sp.	Crimea	Baranov and Ferrington 2013
Cardiocladius fuscus Kieffer, 1924	Croatia	Dorić et al. 2024
Chaetocladius dentriforceps gr.	USA: Minnesota	Bouchard 2007, Anderson and Ferrington 2012
Chaetocladius dissipatus (Edwards, 1929)	Iceland	Nyquist 2022
Chaetocladius grandilobus Brundin, 1956	Poland (lowlands and mountains)	Soszyńska-Maj et al. 2016
Chaetocladius insolitus Caspers, 1987	Crimea	Baranov and Ferrington 2013
Chaetocladius laminatus Brundin, 1947	Germany, Norway, Poland (lowlands and mountains)	Lehmann 1971, Hågvar and Østbye 1973, Ringe 1974, Soszyńska-Maj et al. 2016
Chaetocladius longivirgatus Stur & Spies, 2011	Germany	Stur and Spies 2011
Chaetocladius nr. *melaleucus/dissipatus*	Iceland	Nyquist 2022
Chaetocladius perennis (Meigen, 1830)	Germany, Poland (lowlands)	Lehmann 1971, Soszyńska-Maj et al. 2016
Chaetocladius piger gr., sp.	Poland (lowlands and mountains), USA: Minnesota	Bouchard 2007, Anderson and Ferrington 2012, Soszyńska-Maj et al. 2016
Chaetocladius spp.	USA: Kansas, Minnesota	Kavanaugh 1984, Chou et al. 1999, Ferrington 2000, Ferrington 2007, Bouchard 2007, Anderson et al. 2010
Corynoneura lacustris Edwards, 1924	Crimea	Baranov and Ferrington 2013
Corynoneura lobata Edwards, 1924	Poland (mountains)	Soszyńska-Maj et al. 2016
Corynoneura spp.	USA: Kansas, Minnesota, Pennsylvania	Coffman 1973, Kavanaugh 1984, Chou et al. 1999, Ferrington 2000, Ferrington 2007, Bouchard 2007, Anderson et al. 2010, Anderson and Ferrington 2012
Cricotopus (*Cricotopus*) *bicinctus* (Meigen, 1818)	Croatia	Dorić et al. 2024
Cricotopus annulator Goetghebuer, 1927	USA: Minnesota	Anderson and Ferrington 2012
Cricotopus spp.	USA: Kansas, Minnesota	Coler 1981, Kavanaugh 1984, Ferrington 2000, Ferrington 2007, Bouchard 2007, Anderson et al. 2010, Anderson and Ferrington 2012
Cricotopus skirwithensis (Edwards, 1929)*	Croatia	Dorić et al. 2024
Cricotopus tremulus (Linnaeus, 1758)	USA: Minnesota	Anderson and Ferrington 2012
Cricotopus triannulatus (Macquart, 1826)	USA: Minnesota	Anderson and Ferrington 2012

Taxon	**Location(s) Recorded**	**References**
Cricotopus trifascia Edwards, 1929	USA: Minnesota, Poland (mountains)	Anderson and Ferrington 2012, Soszyńska-Maj et al. 2016
Diplocladius cultriger Kieffer, 1908	Germany, USA: Kansas	Ringe 1974, Chou et al. 1999, Ferrington 2000, Ferrington 2007, Anderson et al. 2010
Diplocladius sp.	USA: Minnesota, Pennsylvania	Coffman 1973, Bouchard 2007
Eukiefferiella brevicalcar (Kieffer, 1911)	Germany	Ringe 1974
*Eukiefferiella claripennis (*Lundbeck, 1898)	Crimea, Minnesota	Bouchard 2007, Anderson and Ferrington 2012, Baranov and Ferrington 2013
Eukiefferiella devonica (Edwards, 1929)	Germany	Ringe 1974
Eukiefferiella ilkleyensis (Edwards, 1929)	Crimea, USA: Minnesota	Bouchard 2007, Anderson and Ferrington 2012, Baranov and Ferrington 2013
Eukiefferiella minor (Edwards, 1929)	Iceland, Germany	Illies 1971, Ringe 1974, Siebert 1980, Nyquist 2022
Eukiefferiella gracei (Edwards, 1929)*	USA: Minnesota	Anderson and Ferrington 2012
Eukiefferiella spp.	USA: Kansas, Minnesota, Pennsylvania	Coffman 1973, Coler 1981, Kavanaugh 1984, Ferrington 2000, Ferrington 2007, Anderson et al. 2010
Heterotrissocladius marcidus gr.	USA: Minnesota	Anderson and Ferrington 2012
Heterotrissocladius sp.	USA: Kansas	Ferrington 2000, Ferrington 2007, Anderson et al. 2010
Heterotrissocladius subpilosus gr.	USA: Minnesota	Nyquist et al. 2020
Hydrobaenus johannseni (Sublette, 1967)	USA: Kansas, Minnesota, Pennsylvania	Coffman 1973, Coler 1981, Bouchard 2007
Hydrobaenus kisosecundus Sasa & Kondo, 1991	Japan	Kondo and Sasa 1994
Hydrobaenus korneyevi (Baranov, 2011)*	Crimea	Baranov and Ferrington 2013
Hydrobaenus kondoi Sæther, 1989	Japan	Kondo and Sasa 1994
Hydrobaenus nr. *spinnatus*	USA: Kansas	Chou et al. 1999
Hydrobaenus pilipes (Malloch, 1915)	USA: Kansas	Coler 1981, Chou et al. 1999
Hydrobaenus pilipodex Sæther , 1976	USA: Kansas	Chou et al. 1999
Hydrobaenus simferopolus Moubayed-Breil & Baranov, 2018	Crimea	Moubayed-Breil and Baranov 2018
Hydrobaenus spp.	USA: Kansas	Kavanaugh 1984, Ferrington 2000, Ferrington 2007, Anderson et al. 2010
Hydrosmittia kisotriangulata Sasa & Kondo 1993*	Japan	Kondo & Sasa 1994
Limnophyes borealis Goetghebuer, 1933	Canada: British Columbia	Cranston and Oliver 1988
Limnophyes cf. *pumilio* (Holmgren, 1869)	USA: Minnesota	Nyquist et al. 2020
Limnophyes gurgicola (Edwards, 1929)	Croatia	Dorić et al. 2024
Limnophyes minimus (Meigen, 1818)	Crimea	Baranov and Ferrington 2013
Limnophyes natalensis (Kieffer, 1914)	Poland (mountains)	Soszyńska-Maj et al. 2016
Limnophyes pentaplastus (Kieffer, 1921)	Crimea, Poland (mountains)	Baranov and Ferrington 2013, Soszyńska-Maj et al. 2016
Limnophyes prolongatus (Kieffer, 1921)	Germany	Illies 1974, Ringe 1974

Taxon	Location(s) Recorded	References
Limnophyes sp.	USA: Kansas, Minnesota	Kavanaugh 1984, Chou et al. 1999, Ferrington 2000, Ferrington 2007, Bouchard 2007, Anderson et al. 2010
Limnophyes spinigus Sæther, 1991	Crimea	Baranov and Ferrington 2013
Metriocnemus albolineatus (Meigen, 1818)	Poland (lowlands)	Soszyńska-Maj et al. 2016
Metriocnemus eurynotus (Holmgren, 1883)	Crimea, Iceland	Baranov and Ferrington 2013, Nyquist 2022
Metriocnemus fuscipes (Meigen, 1818)	Germany	Ringe 1974
Metriocnemus martinii Thienemann, 1921*	Croatia	Dorić et al. 2024
Metriocnemus picipes (Meigen, 1818)	Poland (mountains)	Soszyńska-Maj et al. 2016
Metriocnemus spp.	Germany, USA: Kansas	Illies 1974, Kavanaugh 1984, Ferrington 2000, Ferrington 2007, Anderson et al. 2010
Nanocladius crassicornis Sæther, 1977	USA: Kansas	Coler 1981
Nanocladius rectinervis (Keiffer, 1911)	USA: Minnesota	Nyquist et al. 2020
Nanocladius sp.	USA: Kansas	Coler 1981, Ferrington 2007, Anderson et al. 2010
Nanocladius spiniplenus Sæther, 1977	USA: Minnesota	Nyquist et al. 2020
Oliveridia hugginsi Ferrington & Sæther, 1987	USA: Kansas	Ferrington 2000, Ferrington 2007, Anderson et al. 2010
Orthocladiinae sp.	Poland (lowlands)	Soszyńska-Maj et al. 2016
Orthocladiinae spp.	Poland (mountains), USA: Kansas, Pennsylvania	Coffman 1973, Ferrington 2007, Anderson et al. 2010, Soszyńska-Maj et al. 2016
Orthocladius (Euorthocladius) abiskoensis Thienemann & Krüger, 1937	USA: Kansas	Kavanaugh 1984, Ferrington 2000, Ferrington 2007, Anderson et al. 2010
Orthocladius (Euorthocladius) rivicola Kieffer, 1911	USA: Kansas, Minnesota	Ferrington 2000, Bouchard 2007, Ferrington 2007, Anderson et al. 2010, Anderson and Ferrington 2012
Orthocladius (Euorthocladius) rivulorum Kieffer, 1909	USA: Kansas, Minnesota	Kavanaugh 1984, Ferrington 2000, Bouchard 2007, Ferrington 2007, Anderson et al. 2010, Anderson and Ferrington 2012
Orthocladius (Euorthocladius) sp.	USA: Kansas, Pennsylvania	Coffman 1973, Coler 1981
Orthocladius (Euorthocladius) thienemanni Kieffer & Thienemann, 1906	Germany, Poland (lowlands), USA: Kansas	Lehmann 1971, Siebert 1980, Chou et al. 1999, Ferrington 2000, Ferrington 2007, Anderson et al. 2010, Soszyńska-Maj et al. 2016
Orthocladius (Orthocladius) appersoni Soponis, 1977	Canada: Yukon Territory	Soponis 1977
Orthocladius (Orthocladius) cf. *lignicola* Kieffer, 1915	Germany, USA: Pennsylvania	Coffman 1973, Ringe 1974
Orthocladius (Orthocladius) ferringtoni Soponis, 1983	USA: Kansas	Ferrington 2000, Ferrington 2007, Anderson et al. 2010
Orthocladius (Orthocladius) frigidus (Zetterstedt, 1838)	Germany, Japan, Poland (mountains), USA: Minnesota	Illies 1971, Lehmann 1971, Ringe 1974, Siebert 1980, Kondo and Sasa 1994, Bouchard 2007, Anderson and Ferrington 2012, Soszyńska-Maj et al. 2016
Orthocladius (Orthocladius) mallochi Kieffer, 1919	USA: Kansas, Minnesota	Ferrington 2000, Ferrington 2007, Anderson et al. 2010, Anderson and Ferrington 2012
Orthocladius (Orthocladius) manitobensis Sæther, 1969	USA: Minnesota	Anderson and Ferrington 2012

Taxon	**Location(s) Recorded**	**References**
Orthocladius (Orthocladius) nigritus Malloch, 1915	USA: Kansas, Minnesota, Pennsylvania, Utah, Virginia	Soponis 1977, Chou et al. 1999, Bouchard 2007, Anderson and Ferrington 2012
Orthocladius (Orthocladius) obumbratus Johannsen, 1905	USA: Illinois, Iowa, Kansas, Minnesota, Pennsylvania, South Dakota	Soponis 1977, Chou et al. 1999, Bouchard 2007, Anderson and Ferrington 2012
Orthocladius (Orthocladius) oliveri Soponis, 1977	USA: Arkensas, Illinois, Indiana, Minnesota, Missouri, New York, Oklahoma, Oregon	Soponis 1977, Bouchard 2007
Orthocladius (Orthocladius) spp.	USA: Kansas, Pennsylvania	Coffman 1973, Coler 1981, Ferrington 2000, Bouchard 2007, Ferrington 2007, Anderson et al. 2010
Orthocladius (Orthocladius) vaillanti Langton & Cranston 1991	USA: Minnesota	Anderson and Ferrington 2012
Orthocladius excavatus Brundin, 1947	Germany	Ringe 1974
Orthocladius glabripennis (Goetghebuer, 1921)	Crimea, Japan	Kondo and Sasa 1994, Baranov and Ferrington 2013
Orthocladius kanii (Tokunaga, 1939)	Japan	Kondo and Sasa 1994
Orthocladius rhyacobius Kieffer, 1911	Poland (mountains)	Soszyńska-Maj et al. 2016
Orthocladius saxicola Kieffer, 1911	Germany	Illies 1971
Orthocladius spp.	Crimea, USA: Minnesota	Anderson and Ferrington 2012, Baranov and Ferrington 2013
Orthocladius tamarutilus Sasa, 1981	Japan	Kondo and Sasa 1994
Orthocladius wetterensis Brundin, 1956	Germany, Poland (lowlands, mountains)	Lehmann 1972, Soszyńska-Maj et al. 2016
Parachaetocladius abnobaeus (Wülker, 1959)	USA: Kansas	Cranston and Oliver 1988
Parachaetocladius lenferringtoni Bouchard, Namayandeh & Hudson 2024	USA: Kansas, Minnesota	Bouchard et al. 2024
Parachaetocladius spp.	USA: Minnesota, Pennsylvania	Coffman 1973, Bouchard 2007
Paracladius conversus (Walker, 1856)	Crimea	Baranov and Ferrington 2013
Paracricotopus niger (Kieffer, 1913)	Poland (mountains)	Soszyńska-Maj et al. 2016
Parakiefferiella spp.	USA: Kansas, Minnesota, Pennsylvania	Coffman 1973, Coler 1981, Ferrington 2000, Bouchard 2007, Ferrington 2007, Anderson et al. 2010, Anderson and Ferrington 2012
Parakiefferiella bathophila (Kieffer, 1912)*	Japan	Kondo and Sasa 1994
Parametriocnemus lundbeckii (Johannsen, 1905)	Kansas	Koler 1981
Parametriocnemus spp.	Croatia, USA: Kansas, Minnesota, Pennsylvania	Coffman 1973, Kavanaugh 1984, Ferrington 2000, Bouchard 2007, Ferrington 2007, Anderson et al. 2010, Anderson and Ferrington 2012, Nyquist et al. 2020, Dorić et al. 2024
Parametriocnemus stylatus (Spärck, 1923)	Crimea, Croatia	Baranov and Ferrington 2013, Dorić et al. 2024
Paraphaenocladius impensus contractus (Walker, 1856)	Crimea	Baranov and Ferrington 2013
Paraphaenocladius sp.	USA: Kansas	Coler 1981, Kavana 1984
Paratrissocladius excerptus (Walker, 1856)	Poland (lowlands)	Soszyńska-Maj et al. 2016

Taxon	Location(s) Recorded	References
Psectrocladius yunoquartus Sasa, 1984	Japan	Kondo and Sasa 1994
Pseudorthocladius sp.	Crimea	Baranov and Ferrington 2013
Pseudosmittia forcipatus (Goetghebuer, 1921)	USA: Kansas	Chou et al. 1999
Rheocricotopus (*Psilocricotopus*) *atripes* (Kieffer, 1913)	Croatia	Dorić et al. 2024
Rheocricotopus fuscipes Kieffer, 1909	Germany, Poland (mountains)	Ringe 1974, Soszyńska-Maj et al. 2016
Rheocricotopus sp.	USA: Kansas	Ferrington 2007, Anderson et al. 2010
Smittia aterrima (Miegen, 1818)*	Japan, Poland (mountains)	Kondo and Sasa 1994, Soszyńska-Maj et al. 2016
Smittia stercoraria Rossaro & Lencioni, 2000	Poland (lowlands)	Soszyńska-Maj et al. 2016
Stilocladius sp.	USA: Minnesota	Bouchard 2007
Thienemannia gracilis Kieffer, 1909	Croatia, Germany, Poland (mountains)	Ringe 1974, Soszyńska-Maj et al. 2016, Dorić et al. 2024
Thienemannia sp.	Poland (mountains)	Soszyńska-Maj et al. 2016
Thienemanniella clavicornis (Kieffer, 1911)	Crimea	Baranov and Ferrington 2013
Thienemanniella obscura Brundin, 1947	Germany	Ringe 1974
Thienemanniella spp.	USA: Kansas, Minnesota, Pennsylvania	Coffman 1973, Kavanaugh 1984, Ferrington 2000, Bouchard 2007, Ferrington 2007, Anderson et al. 2010, Anderson and Ferrington 2012
Thienemanniella vittata (Edwards, 1924)	Crimea, Croatia	Baranov and Ferrington 2013, Dorić et al. 2024
Thienemanniella xena (Roback, 1957)	USA: Minnesota	Bouchard 2007
Trissocladius brevipalpis Kieffer & Thienemann, 1908	Poland (lowlands)	Soszyńska-Maj et al. 2016
*Tvetenia bavarica (*Goetghebuer, 1934)*	Germany	Siebert 1980
Tvetenia spp.	USA: Kansas, Minnesota	Kavanaugh 1984, Ferrington 2000, Bouchard 2007, Ferrington 2007, Anderson et al. 2010, Anderson and Ferrington 2012
Prodiamesinae (3 taxa)		
Odontomesa fulva (Kieffer, 1919)	USA: Minnesota	Bouchard 2007, Anderson and Ferrington 2012
Odontomesa sp.	USA: Kansas	Ferrington 2007, Anderson et al. 2010
Prodiamesa olivacea (Meigen, 1818)	Poland (lowlands), USA: Minnesota	Bouchard 2007, Anderson and Ferrington 2012, Soszyńska-Maj et al. 2016, Nyquist et al. 2020
Diamesinae (35 taxa)		
Diamesa aberrata Lundbeck, 1898	Iceland	Nyquist 2022
Diamesa ancysta Roback, 1959	USA: Colorado, Idaho, Montana, Nevada, Utah, Washington	Hansen and Cook 1976
Diamesa arctica (Boheman, 1865)*	USA: Utah	Young, 1964
Diamesa bertrami Edwards, 1935	Iceland	Nyquist 2022
Diamesa bohemani Goetghebuer, 1932	Iceland	Nyquist 2022
Diamesa cheimatophila Hansen, 1976	USA: New York, Pennsylvania	Hansen and Cook 1976, Ferrington and Masteller 2015
Diamesa chiobates Hansen, 1976	USA: Minnesota, Wisconsin	Hansen and Cook 1976

Taxon	Location(s) Recorded	References
Diemesa cinerella Meigen, 1835	Germany, Poland (mountains)	Ringe 1974, Siebert 1980, Soszyńska-Maj et al. 2016
Diamesa davisi Edwards, 1933	USA: Utah, Wyoming	Hansen and Cook 1976
Diamesa davisi gr.	USA: Colorado	Hermann et al. 1987
Diamesa garretti Sublette & Sublette, 1965	USA: Colorado, Idaho	Hansen and Cook 1976, Hermann et al. 1987
Diamesa hamaticornis Kieffer, 1924	Germany, Poland (mountains)	Illies 1971, Lehmann 1971, Ringe 1974, Soszyńska-Maj et al. 2016
Diamesa heteropus (Coquillett, 1905)	USA: Alaska, Colorado, Idaho, Montana, Nevada, New Mexico, Utah, Washington	Hansen and Cook 1976
Diamesa incallida (Walker, 1856)	Iceland, USA: Wyoming	Hansen and Cook 1976, Nyquist 2022
Diamesa insignipes Kieffer, 1908*	Germany, Poland (mountains), USA: Wyoming	Illies 1971, Lehmann 1971, Ringe 1974, Hansen and Cook 1976, Soszyńska-Maj et al. 2016
Diamesa latitarsis (Goetghebuer, 1921)	Iceland, Norway, Poland (mountains), Pyranees	Serra-Tosio, 1972, Jonsson and Sadlund 1975, Soszyńska-Maj et al. 2016, Nyquist 2022
Diamesa leona Roback, 1957*	Poland (mountains), Russia, USA: Colorado, Idaho, Montana, Nevada, New Mexico, Utah, Washington	Hansen & Cook 1976, Makarchenko 1985, Hermann et al. 1987, Giłka et al. 2013, Soszyńska-Maj et al. 2016, Makarchenko et al. 2022
Diamesa leoniella Hansen, 1976	USA: California, Utah	Hansen and Cook 1976
Diamesa mendotae Muttkowski, 1915	USA: Minnesota, Wisconsin	Hansen and Cook 1976, Mazack 2013, Durnin 2021
Diamesa nivoriunda (Fitch, 1847)	Canada: Ontario, Quebec, USA: Indiana, Minnesota, New York, Wisconsin	Hansen and Cook 1976, Durnin 2021
Diamesa spinacies Sæther, 1969	USA: Idaho, Utah	Hansen and Cook 1976
Diamesa spp.	USA: Kansas, Minnesota, Pennsylvania	Coffman 1973, Kavanaugh 1984 Ferrington 2000, Ferrington 2007, Bouchard 2007, Anderson et al. 2010, Anderson and Ferrington 2012, Nyquist et al. 2020
Diamesa thomasi Serra-Tosio, 1970	Croatia	Baranov et al. 2013
Diamesa tonsa (Haliday, 1856)*	Germany, Norway, Poland (mountains)	Lehmann 1971, Jonsson and Sandlund 1975, Soszyńska-Maj et al. 2016
Diamesa tsutsuii Tokunaga, 1936	Russia (Former USSR regions), Japan	Makarchenko 1985, Makarchenko 2023
Diamesa vockerothi Hansen, 1976	Canada: Ontario, Quebec	Hansen and Cook 1976
Diamesa zernyi Edwards, 1933	Iceland	Nyquist 2022
Pagastia sp.	USA: Minnesota	Mazack 2013
Pagastia partica (Roback, 1957)	USA: Colorado	Hermann et al. 1987
Potthastia sp.	USA: Kansas, Pennsylvania	Coffman 1973, Coler 1981, Ferrington 2000, Ferrington 2007, Anderson et al. 2010
Prodiamesa olivacea (Meigen, 1818)	Germany	Lehmann 1971
Pseudodiamesa branickii (Nowicki, 1873)	Iceland, Norway, Poland (mountains), USA: Colorado	Hågvar and Østbye 1973, Hermann et al. 1987, Soszyńska-Maj et al. 2016, Nyquist 2022

Taxon	**Location(s) Recorded**	**References**
Pseudodiamesa nivosa (Goetghebuer, 1928)	Iceland, Norway	Willassen 2011, Nyquist 2022
Sympotthastia sp.	USA: Kansas	Ferrington 2000, Ferrington 2007, Anderson et al. 2010
Chironomini (14 taxa)		
Biwatendipes motoharui Tokunaga, 1965	Japan	Kondo and Sasa 1994
Chironomus nipponensis Tokunaga, 1940	Japan	Kondo and Sasa 1994
Chironomus riparius Meigen, 1804	USA: Kansas	Coler 1981
Chironomus spp.	USA: Kansas	Coler 1981, Ferrington 2000, Ferrington 2007, Anderson et al. 2010
Dicrotendipes fumidus (Johannsen, 1905)	USA: Kansas, Minnesota	Ferrington 2000, Ferrington 2007, Anderson et al. 2010, Anderson and Ferrington 2012
Dicrotendipes nervosus (Staeger, 1839)	Crimea	Baranov and Ferrington 2013
Dicrotendipes sp.	USA: Kansas	Kavanaugh 1984
Microtendipes sp.	USA: Kansas	Coler 1981
Paracladopelma sp.	USA: Kansas	Coler 1981
Phaenopsectra sp.	USA: Minnesota	Bouchard 2007
Polypedilum trigonum Townes, 1945	USA: Minnesota	Anderson and Ferrington 2012
Polypedilum sp.	USA: Kansas	Anderson et al. 2010, Ferrington 2000, Ferrington 2007
Stictochironomus sticticus (Fabricius, 1781)*	Japan	Kondo and Sasa 1994
Stictochironomus spp.	USA: Kansas, Minnesota	Coler 1981, Kavanaugh 1984, Ferrington 2000, Bouchard 2007, Ferrington 2007, Anderson et al. 2010, Anderson and Ferrington 2012
Tanytarsini (28 taxa)		
Cladotanytarsus atridorsum Kieffer, 1924	Crimea	Baranov and Ferrington 2013
Micropsectra apposita (Walker, 1856)*	Crimea, Croatia, USA: Minnesota	Anderson and Ferrington 2012, Baranov and Ferrington 2013, Dorić et al. 2024
Micropsectra atrofasciata (Kieffer, 1911)	Crimea, France, Germany, Japan	Ringe 1974, Siebert 1980, Stur and Ekrem 2006, Baranov and Ferrington 2013
Micropsectra attenuata Reiss, 1969	USA: Minnesota	Anderson and Ferrington 2012
Micropsectra bavarica Stur & Ekrem, 2006	France, Poland (mountains)	Stur and Ekrem 2006, Soszynska-Maj et al. 2016
Micropsectra bidentata (Goetghebuer, 1921)	Germany	Siebert 1980
Micropsectra dives gr. sp.	USA: Pennsylvania	Coffman 1973
Micropsectra geminata Oliver & Dillon, 1994	USA: Minnesota, Ohio	Oliver and Dillon 1994, Nyquist et al. 2020
Micropsectra junci (Meigen, 1818)	Germany, Poland (lowlands and mountains)	Siebert 1980, Soszynska-Maj et al. 2016
Micropsectra klinki Stur & Ekrem, 2006	Netherlands	Stur and Ekrem 2006
Micropsectra longicrista Stur & Ekrem, 2006	Germany	Stur and Ekrem 2006
Micropsectra nigripila (Johannsen, 1905)	Canada: Ontario, USA: Minnesota	Oliver and Dillon 1994, Bouchard 2007, Anderson and Ferrington 2012
Micropsectra notescens (Walker, 1856)	Poland (mountains)	Soszynska-Maj et al. 2016
Micropsectra pallidula (Meigen, 1830)	Croatia	Dorić et al. 2024
Micropsectra polita (Malloch, 1919)	USA: Minnesota	Bouchard 2007, Anderson and Ferrington 2012

Taxon	Location(s) Recorded	References
Micropsectra spp.	Germany, USA: Kansas, Minnesota, Pennsylvania	Coffman 1973, Ringe 1974, Coler 1981, Kavanaugh 1984, Chau et al. 1999, Ferrington 2000, Bouchard 2007, Ferrington 2007, Anderson et al. 2010, Anderson and Ferrington 2012, Nyquist et al. 2020
Micropsectra subletteorum Anderson, Stur, & Ekrem, 2013	USA: Minnesota	Anderson et al. 2013, Nyquist et al. 2020
Paratanytarsus sp. 1 *laccophilus* gr.	USA: Minnesota	Nyquist et al. 2020
Paratanytarsus sp. 2 *inopertus* gr.	USA: Minnesota	Nyquist et al. 2020
Paratanytarsus spp.	USA: Kansas	Coler 1981, Kavanaugh 1984, Ferrington 2000, Ferrington 2007, Anderson et al. 2010
Rheotanytarsus curtistylus (Goetghebuer, 1921)	Croatia	Dorić et al. 2024
Rheotanytarsus distinctissimus (Brundin, 1947)	USA: Minnesota	Anderson and Ferrington 2012
Rheotanytarsus sp.	USA: Kansas	Kavanaugh 1984
Tanytarsini sp.	USA: Pennsylvania	Coffman 1973
Tanytarsus arduennensis Goetghebuer, 1922	Croatia	Dorić et al. 2024
Tanytarsus nearcticus Butler, 2000	USA: Minnesota	Anderson and Ferrington 2012
Tanytarsus spp.	USA: Kansas	Ferrington 2000, Ferrington 2007, Anderson et al. 2010
Tanytarsus yunosecundus Sasa, 1984	Japan	Kondo and Sasa 1994
Tanypodinae (8 taxa)		
Ablabesmyia (*Ablabesmyia*) *longistyla* Fittkau, 1962	Croatia	Dorić et al. 2024
Ablabesmyia spp.	USA: Kansas	Coler 1981
Conchapelopia rurika Roback, 1957	USA: Kansas	Coler 1981
Conchapelopia sp.	Croatia	Chou et al. 1999, Ferrington 2007, Dorić et al. 2024
Conchapelopia flavifrons (Johannsen, 1905)*	USA: Kansas	Kavanaugh 1984
Psectrotanypus dyari (Coquillett, 1902)	USA: Kansas	Coler 1981
Zavrelimyia sinuosa (Coquillett, 1905)	USA: Kansas, Minnesota	Coler 1981, Kavanaugh 1984, Ferrington 2000, Anderson et al. 2010, Anderson and Ferrington 2012
Zavrelimyia sp.	USA: Kansas	Coler 1981

species and studies suggest that the most accurate identifications include morphological characteristics and molecular techniques used in parallel (Anderson et al. 2013, Montagna et al. 2016, Lin et al. 2018, Makarchenko et al. 2018). For example, Anderson et al. (2013) described three cryptic *Micropsectra* species in spring-fed streams of the Driftless Area of southeastern Minnesota, USA including one known to emerge during winter (*M. subletteorum* Anderson, Stur, & Ekrem) using mitochondrial and nuclear DNA. Additional research in this region by Durnin (2021) suggested that winter-emerging *Diamesa nivoriunda* (Fitch) may contain two or more cryptic species and recommends that testing for reproductive compatibility and further examination of habitat requirements would likely support these results. Stable environmental conditions promote consistent selective pressures whereby organisms become selectively constrained in morphology (Fišer et al. 2018). Spring-fed streams often provide consistent, stable conditions because of the constant input of thermally and chemically stable groundwater (Williams and Vondracek 2010), leading to constant selective pressure. These environments are also often geographically isolated, which may contribute to speciation and the presence of cryptic species (Murphy et al. 2008). As we show here, many winter-emerging chironomids are found in spring-fed streams with recent studies discovering cryptic diversity in these habitats. It is likely that ongoing research will identify additional cryptic species.

Cold Hardiness

Cold hardiness is the ability of an organism to survive at low temperatures. Two different cold-hardiness strategies are observed in aquatic insects, which permit survival at subfreezing temperatures: freeze tolerance and freeze avoidance. Freeze-tolerant insects can raise and control the SCP of haemolymph using ice-nucleating agents (Lencioni 2004), which allows these insects to slow and control the formation of ice crystals within their bodies to avoid damaging tissues. Freeze avoidant species are unable to survive freezing of their haemolymph and have attributes or mechanisms to avoid the freezing of their tissues including lowering their SCP, melanism, increased hairiness, reduced wing size, cocoon construction, and seeking microhabitats that do not freeze (Lencioni 2004). Depending on the habitat, life stage, and life history, different strategies are observed in aquatic insects to survive and in some cases, to remain active when air temperatures are below 0 °C.

Considering Chironomidae are the most widespread and commonly observed winter-active insect family, it is not surprising that some species have been demonstrated to be very cold hardy. Both freeze tolerance and freeze avoidance strategies are documented in the Chironomidae; however, only freeze avoidance is observed in adults, whereas both freeze tolerance and avoidance are observed in larvae. Cold hardiness strategies of the egg and pupal stages are poorly known. Freeze tolerance is most often observed in diapausing Chironomidae larvae in habitats that freeze, such as wetlands, small streams, and the margins of lakes and rivers (Lencioni 2004, Danks 2008), but this strategy has also been documented in larvae from habitats that do not freeze (Bouchard et al. 2006b). Most larvae have not been tested for freeze tolerance, so it is unknown how widespread this ability may be in Chironomidae larvae. Although freeze avoidance is observed in both larvae and adults, specific mechanisms may differ between life stages. Most freeze avoidant larvae seek habitats that do not freeze (e.g. deep lakes, large streams, groundwater fed streams, springs, hyporheic zones; Lencioni 2004). Although freeze-avoidant larvae may diapause in protected habitats, many also remain active during the winter if conditions are suitable. Adult Chironomidae are not known to diapause and many winter-active species may have morphological adaptations (e.g. melanism, increased hairiness) or seek protected microhabitats to avoid freezing. However, an interesting strategy to avoid freezing has been studied in several winter-active midge adults. These species lower their SCP (i.e. the temperature at which they freeze) through the production of antifreezes and thermal hysteresis proteins (Lencioni 2004, Lencioni et al. 2015). The diversity of cold hardiness strategies observed in different life stages and species in Chironomidae demonstrate how well adapted this group is for surviving and thriving in a variety of winter habitats.

Although the ability of many Chironomidae species to diapause as larvae and survive subfreezing temperatures is a fascinating and productive area of research, observations of adult flies in winter habitats are particularly intriguing, given their ability to maintain activity in sub-freezing environments. Many winter-active species are morphologically specialized due to adaptations for surface mating such as reduced antennae, enlarged hypopygium, or wing reduction (e.g. Hansen and Cook 1976, Ferrington and Sæther 1987). In addition, it is unusual for ectothermic species to maintain activity at subzero air temperatures which raises questions regarding how and why these species are active in the winter. These flies are often considered an oddity, but more recent research has indicated that winter activity may be a beneficial strategy in some Chironomidae species. In temperate and high elevation habitats, detailed research has focused on the genus *Diamesa*. Members of this genus are commonly observed in the winter and are often active at temperatures below freezing and are also known to fly at subfreezing temperatures (Young 1969, Hågvar and Østbye 1973). For example, *Diamesa mendotae* Muttkowski is commonly found on snow near groundwater-dominated streams during the winter indicating that winter activity is an integral part of their life cycle, and these winter-active individuals are not "leakage" from the main population (see labile life cycle hypothesis; Ferrington et al. 2010). Associated with observations of adult activity at low temperatures, research on *D. mendotae* has demonstrated that this species can greatly lower its SCPs (-21.6 °C; Bouchard et al. 2006a). The ability to survive low temperatures in a winter-active insect has interesting implications such as increasing the life span of these insects, and with that, the likelihood of locating a mate (Bouchard et al. 2006a). This behavior may be particularly important in habitats where adults are unable to swarm if temperatures are too cool (see section on Longevity and Behavior). A longer life span may be beneficial in surface-mating species because mate searching may require more time than in aerial swarming species and periods of poor weather conditions (e.g. torpor inducing temperatures, high winds) could also lengthen the period between emergence and mating. The ability to survive and remain active at low, subfreezing air temperatures allows species, such as *D. mendotae* to successfully occupy winter habitats and may contribute to their importance in these habitats.

The ability to lower SCPs may be widespread in adult Chironomidae; but has not been extensively studied. Among Chironomidae, Diamesinae are most associated with adult activity at subzero temperatures, but many other subfamilies are also observed in the fall, winter, or spring in temperate habitats where subzero air temperatures are possible (see Diversity section). Although adults of other subfamilies are less likely to be observed at subzero temperatures, there is evidence that they may also have low SCPs. For example, 48 adult midges from several different subfamilies and tribes (Prodiamesinae, Orthocladiinae, Tanytarsini, and Chironomini) collected from a Minnesota stream and a lake in May had average SCPs ranging from -26.9 to -11.0 °C (R.W. Bouchard, Jr., unpublished data). One genus (*Odontomesa*: SCP = -26.1 °C [n = 12]) had an average SCP lower than that of *Diamesa mendotae* (-21.6 °C; Bouchard et al. 2006a) and two genera were only slightly higher than *D. mendotae*: *Stictochironomus*: -20.5 °C (n=4); *Micropsectra*: -20.7 °C (n=7)). These genera are often observed emerging at relatively low temperatures (Bouchard 2007), whereas other genera with higher SCPs (e.g. *Parametriocnemus*, *Chironomus*, *Dicrotendipes*, *Paratendipes*, *Polypedilum*; SCPs -17.9 to -11.7 °C) are less commonly observed emerging at low temperatures. Interestingly, aquatic insects from other families and orders observed from similar Minnesota habitats during winter and early spring have higher SCPs (e.g. *Allocapnia* [Bouchard et al. 2009] and *Phantolabis* [Bouchard and Gelhaus 2020]). Given that Chironomidae are thought to have evolved in cold habitats (Brundin 1966), cold hardiness may be an ancestral trait of the family. Why this ability has apparently been retained in species that are not frequently exposed to subzero temperatures is not clear, although in temperate, polar, and high-altitude regions there may be an evolutionary advantage as low temperatures are possible during the period of adult emergence. The retention of physiological mechanisms associated with cold hardiness may also be adaptive as they can serve other purposes such as resistance to desiccation (Danks 2000). Although only a small fraction of winter-active chironomids has been studied in detail, available research demonstrates that the ability to survive low temperatures is widespread within the family and deserves additional study.

Growth patterns and emergence of winter-active Chironomidae

In temperate regions, we can identify two main habitat types from which winter-active insects emerge: groundwater-fed habitats and surface water-fed habitats. There are gradients between these two types of habitats, but habitats with large emergences of winter-active chironomids are typically not covered with surface ice throughout the winter and water temperatures remain high enough for larval growth and development (Bouchard and Ferrington 2009). In surface water-fed habitats in high latitudes, aquatic habitats are frozen or water temperatures are near 0 °C through much of the winter and most emergence will not occur until the spring thaw, although there may be some limited emergence through cracks or small openings in the ice (Coffman 1973, Hansen and Cook 1976, Herrmann et al. 1987). Large emergences of winter-active midges from surface water-fed habitats occur at lower latitudes where winter air temperatures are high enough to keep the habitat ice free. However, even if ice cover is limited during the winter, water temperatures in these temperate habitats can be low, slowing the growth and developmental rate of chironomid larvae (Nolte and Hoffman 1992, Bouchard and Ferrington 2009, Schütz and Füreder 2019). Regardless, some chironomids are known to successfully use surface water-fed streams in the winter (Ferrington 2000). In contrast, groundwater-fed streams in the winter create a highly suitable habitat for cold stenothermic species as groundwater inputs provide a thermally buffered habitat, yielding ideal water temperatures for growth and development throughout winter. However, even if water temperatures are suitable, the adults of these species, especially those at higher latitudes, may then be challenged by low air temperatures when they emerge.

Summer poses a different challenge for cold-adapted chironomids in temperate regions. In most habitats, the larvae of these cold stenothermic species presumably aestivate in hyporheic zones during warmer periods (Lencioni 2004). Less commonly, habitats that are sufficiently buffered by groundwater may allow winter-active species to continuously grow throughout a significant portion of the year. For example, multiple species of *Diamesa,* including *D. cheimatophila* Hansen, *D. mendotae,* and *D. nivoriunda* are reported to emerge from September to May (Hansen and Cook 1976). Yet, detailed life histories for most midge species are unknown, and the exact habitat for cold stenothermic species during the summer in temperate regions is not well known in Chironomidae.

The larvae of chironomids with winter-active adults are often cold stenothermic and are limited to habitats with cool water temperatures, including habitats that remain cold year-round or seasonally cold habitats. Based on the typically short duration of the adult stage (hours or days) compared to the larval stage (although see section on Longevity and Behavior), the water temperature at the time of adult emergence is linked to larval thermal requirements. For example, Rossaro (1991) examined optimum water temperature for 127 species of chironomids, finding that larvae of cold stenothermic species are tolerant of only a narrow temperature range, with most exhibiting temperature optima near the minimum tolerance value. Moreover, the study included 12 species of *Diamesa,* a particularly cold-stenothermic genus. Mean water temperature where these larvae were found ranged from 4.2 to 9.8°C, exemplifying the need for low temperatures to facilitate larval development (Rossarro 1991). Therefore, this indicates that for winter-active insects, adults must survive and be active at cold air temperatures to provide suitable habitat for their offspring. As such, the phenology of winter-active chironomids is often linked to the growth and development of the larvae and emergence patterns of adults may be useful for estimating thermal preferences of larval midges. However, this may be complicated by species that diapause, are semivoltine, or those with facultative thermal preferences.

In many temperate habitats, most Chironomidae species likely diapause during winter because aquatic habitats may completely freeze to the benthic substrates (Danks et al. 1994) or water temperatures are too low for growth and development. Many chironomid larvae that are not active in winter can tolerate freezing or avoid freezing by occupying habitats that do not freeze (e.g. forming cocoons, producing ice-nucleation proteins; Danks 1971). In habitats with consistently cold water temperatures (<2 °C), most chironomid larvae typically undergo diapause during winter regardless of whether they are freeze tolerant or freeze avoidant. There are some species, however, that can grow at very low temperatures including species that inhabit streams with maximum temperatures below 1 °C (e.g. *Diamesa*; Burgheer and Ward 2001). *Diamesa incallida* (Walker) (Nolte 1992) and *D. mendotae* (Bouchard and Ferrington 2009) larvae grow continuously during the winter at temperatures below 10 °C and are known to emerge throughout the winter months of temperate regions. This prolonged emergence period may be advantageous, especially for populations that emerge under especially inhospitable winter conditions (Jonsson and Sandlund 1975). However, the ability to produce multiple generations during the winter (e.g. labile life cycle hypothesis) appears to be limited to a small subset of species that are especially well adapted for activity at low temperatures. In general, the life histories of most winter-active species are understudied, especially larval growth and development patterns.

Most winter-active midges appear to be more marginal in terms of their cold hardiness because their activity is often limited to late winter or early spring in northern temperate habitats or regions with mild winters. These species often have a single, distinct emergence period in late winter indicating a univoltine life cycle (e.g. *Parachaetocladius lenferringtoni* Bouchard and Namayandeh [Bouchard et al. 2024], *Chaetocladius* spp. [Bouchard 2007]). Although it is likely that these less cold hardy species with winter-active adults can also grow and develop at low water temperatures, the thermal requirements for the larvae of most species are unknown.

Longevity and Behavior

A general assumption of chironomid biology is that the adult lifespan is short (Armitage 1995). However, winter-active chironomids display surprisingly long lifespans given their small size and the extreme conditions they inhabit (Table 2). Most of our understanding of winter-active chironomid longevity stems from a single focal species: *Diamesa mendotae.* The mean longevity for *D. mendotae* has been determined to be between 12–33 days and maximum longevity between 25-54 days when maintained at 6 °C (Ferrington et al. 2010), which is supported by additional studies on this species (e.g. Ferrington 2019, Anderson et al. 2022, Bodmer et al. 2023). Differences in mean longevity across collection events could reflect site-specific environmental factors, such as higher mean water temperature during larval development, which may influence adult longevity (Bodmer et al. 2023). Sex may also influence longevity, with mean female longevity measured to be 6 days longer than males (Ferrington et al. 2010). However, longevity has also been determined to be significantly influenced by reproductive status,

Table 2. Maximum and mean longevities of *Diamesa* mendotae reported in recent studies of winter-emerging populations in the Midwest USA. Male and female longevities are given separately, unless these values were not individually reported in the below studies.

Study	**Collection Location**	**Collection Date**	**Treatment**	**Sample size (m/f)**	**Max longevity (m/f)**	**Mean longevity (m/f)**
Ferrington et al. 2010	Stream at Pine Needles Preserve; Washington County, MN	13 Feb, 1 Mar, 16 Mar 2001	6°C	470 / 259	48 / 54	18.6
	Stream at Arcola Mills Historic Site; Washington County, MN	16 Mar 2001				
	Valley Creek; Washington County, MN	20 Dec, 26 Dec 2002, 13 Feb 2003				
	Kinnickinnic River; Pierce County, WI (River Falls)	19 Feb, 27 Feb 2003				
	Kinnickinnic River; Pierce County, WI (Downstream)	12 Mar 2003				
	Trout Brook; Dakota County, MN	30 Jan 2003				
Anderson et al. 2013	Kinnickinnic River; St Croix County, WI	16 Feb 2004	Under snow 4 days, then 6°C	10 / 10	48 / 57	41.3 / 42.5
			Under snow 8 days, then 6°C	10 / 10	56 / 57	40.3 / 47
			Under snow 12 days, then 6°C	10 / 10	51 / 58	42.4 / 48.4
			Under snow 16 days, then 6°C	10 / 10	66 / 61	48.3 / 45.8
			Under snow 20 days, then 6°C	10 / 10	62 / 66	47.4 / 43.4
			Under snow 24 days, then 6°C	10 / 10	65 / 61	55 / 50
			Under snow 28 days, then 6°C	10 / 10	60 / 61	44.7 / 50.5
Mazack et al. 2014	Valley Creek; Washington County, MN	3 Mar 2010	–5°C for 7 days, then 6°C	5 / 4	35 / 42	28.4 / 29.5
			–5°C for 14 days, then 6°C	4 / 2	32 / 38	29.2 / 32.5
			–5°C for 21 days, then 6°C	3 / 3	43 / 35	33.3 / 37.3
			–5°C for 28 days, then 6°C	3 / 2	58 / 38	44.7 / 34.5
			–5°C for 34 days, then 6°C	3 / 3	50 / 56	44.3 / 46.3
			–5°C for 42 days, then 6°C	4 / 1	64 / 43	51.5 / 43
			–5°C for 49 days, then 6°C	2 / 0	53 / --	51.5 / --
			–5°C for 56 days, then 6°C	1 / 0	58 / --	58 / --
			–5°C for 63 days, then 6°C	2 / 1	81 / 73	79.5 / 73
			–5°C for 70 days, then 6°C	2 / 1	92 / 76	83 / 76

Study	Collection Location	Collection Date	Treatment	Sample size (m/f)	Max longevity (m/f)	Mean longevity (m/f)
Ferrington 2019	Hay Creek; Goodhue County, MN	17 Feb 2019	6°C	17 / 19	28 / 30	
			18°C	18 / 20	12 / 12	
			24°C	19 / 20	4 / 6	
	Trout Brook; Dakota County, MN	14 Feb 2019	6°C	29 / 13	27 / 28	
			18°C	29 / 13	10 / 8	
			24°C	29 / 13	5 / 2	
	Valley Creek; Washington County, MN	23 Feb 2019	6°C	26 / 12	23 / 19	
			18°C	26 / 12	7 / 5	
			24°C	26 / 12	4 / 4	
Anderson et al. 2022	Tributary to Redwood River; Lyon County, MN	22 Jan, 28 Jan, 6 Feb, 21 Feb, 24 Feb 2020	2°C	92 / 33	60	19.8
			6°C	86 / 48	34	14.9
			Ambient outdoor conditions	58 / 36	37	13.9
Bodmer et al. 2023	Ike's Creek; Hennepin County, MN	5 Jan, 23 Jan 2020	6°C	41 / 6	32 / 20	18.0 / 16.3
			22°C for 24hr, then 6°C	42 / 4	37 / 19	17.0 / 7
	Pickwick Creek; Winona County, MN (Site 1, Upstream)	4 Jan 2021	6°C	39 / 11	26 / 23	15.2 / 14
			22°C for 24hr, then 6°C	37 / 11	30 / 23	14.2 / 13.6
			22°C for 48hr, then 6°C	37 / 12	28 / 30	12.4 / 11.9
	Pickwick Creek; Winona County, MN (Site 2, Downstream)	4 Jan 2021	6°C	36 / 14	26 / 23	16.4 / 15.6
			22°C for 24hr, then 6°C	35 / 13	26 / 20	13.1 / 10
			22°C for 48hr, then 6°C	34 / 13	23 / 18	11.3 / 8.5

NOTES — Ferrington et al. (2010) combines mean and maximum longevity for all streams and mean male and female longevity. Ferrington 2019 does not report mean longevity. Anderson et al. (2022) does not report separate mean and maximum longevities for males and females, and notes that although most specimens were *D. mendotae,* non-*Diamesa* chironomids may be included in estimates.

with males and ovipositing females living significantly longer than non-ovipositing females (Anderson et al. 2022). Further work on chironomid longevity, particularly by investigating additional taxa, is necessary to elucidate the drivers of within and between-population differences in longevity.

Winter-active chironomids often display prolonged adult lifespans under extreme, subfreezing conditions. In one study, all *D. mendotae* survived burial under snow for 4–28 days, with mean longevity increasing in longer treatment durations (Anderson et al. 2013). Similarly, *D. mendotae* maintained at –5 °C for treatments of 7–70 days before returning to 6 °C had higher mean longevities when kept under subfreezing conditions longer, with a maximum longevity of 92 and 76 days for males and females, respectively (Mazack et al. 2014). Chironomid longevity also increased when maintained at constant lower temperatures: chironomids held at constant 2 °C lived significantly longer (19.8 days) than those at constant 6 °C (14.9 days) or exposed to ambient outdoor conditions (13.9 days) (Anderson et al. 2022). Therefore, these cold-adapted insects may be disproportionately impacted as climate change threatens the duration and intensity of cold winter temperatures.

Recent research on chironomid longevity has assessed how cold-adapted taxa are impacted by climate change (e.g. Nyquist et al. 2021, Bodmer et al. 2023). The winter season in north temperate regions is predicted to experience significant warming in coming decades due to climate change (Notaro et al. 2014, Gulev et al. 2021), which intensifies the need for a comprehensive understanding of cold-adapted, winter-active taxa. At constant high temperatures, Icelandic *Diamesa* spp. had mean longevities of 19.9 days when held at 6 °C, compared to 3.8 days at 20 °C (Nyquist et al. 2021). Although sample sizes were small, this pattern also held for other genera in the study, including *Eukiefferiella*, *Micropsectra*, and *Thienemanniella* (Nyquist et al. 2021). Even short-term warm temperature spikes may impact chironomid longevity. *D. mendotae* from southeastern Minnesota had higher longevities when held at constant 6°C than when exposed to 22°C for 24hrs or 48hrs (Bodmer et al. 2023). Short-term high temperature exposure also reduced successful reproduction, with fewer females producing egg masses that hatched into larvae compared to control 6 °C groups (Bodmer et al. 2023). In similar research, short term exposure to 22 °C reduced adult Orthocladiinae longevity (Bodmer and Nyquist 2022). However, the presence of parasitic nematodes unexpectedly increased orthoclad longevity by increasing survivorship in early life (Bodmer and Nyquist 2022). It is therefore critical that future studies address the impacts of winter warming on chironomids and other cold-adapted taxa to better predict the myriad of ways these communities may respond to climate change and warming winters.

Ferrington (2019) proposed a standardized behavioral assay to estimate individual adult activity level. This assay can be used to predict whether or not adult flies could survive under natural conditions to better understand how climate change may impact winter-active Chironomidae fitness. Although winter-active chironomids have relatively long lifespans, only a subset of their total lifespan is spent successfully mating and reproducing (Bodmer et al. 2024). As *D. mendotae* aged, the proportion of time spent performing inactive behaviors, such as lying on their backs in water, increased steadily until death (Bodmer et al. 2024). Flies that displayed active behaviors soon after collection lived significantly longer than inactive flies, which may allow researchers to predict the age of flies collected off snow (Bodmer et al. 2024). These findings illustrate the limitations of using longevity alone to predict changes in population structures as winter warming progresses. Instead, researchers can use behavior to better predict how individual-level responses to warm temperatures may accumulate to produce population and community-level shifts under climate change.

Mating behavior of winter-emerging chironomids is also notable, with some species either mating on the snow surface (e.g. *Chaetocladius laminatus* Brundin*, Diamesa permacer* (Walker)*, D. mendotae* as described by Hågvar and Østbye 1973 and Ferrington et al. 2010) or exhibiting surface-mating behavior, where mating pairs glide across the surface of the water (e.g. *Oliveridia hugginsi* Ferrington and Sæther*,* as described by Ferrington and Sæther 1987). These behaviors contrast with mating swarms more typical of warmer seasons and are better suited for maximizing rapid contact with mates during winter while also limiting the potential of inadvertent wind-blown dispersal (Ferrington and Sæther 1987). Flight may be impeded or impossible due to low temperatures, and smooth, white snow cover can facilitate location of potential mates (Hågvar and Østbye 1973, Anderson et al. 2013). Morphological adaptations follow suit: the winter-active species *Oliveridia hugginsi* and *Orthocladius ferringtoni* Soponis exhibit adaptations, such as broadened and elongated legs, elaboration of the hypopygium, and a reduced antennal plume — traits that favor surface mating as opposed to those needed to recognize and mate with females during flight (Lencioni 2004). Although several winter-emerging species have been observed in flight, especially on warmer winter days (Young 1969, Bouchard and Ferrington 2009, Anderson et al. 2022), brachypterous and apterous conditions are also reported. Wing-variations are reported within *Diamesa leona* Roback (*D. starmachi* Kownacki & Kownacka as a synonym, see Makarchenko et al. 2022). Seasonal wing variation, with brachypterous forms emerging during winter and fully-winged spring to summer-emerging forms are reported from the Palearctic (Giłka et al. 2013 and Makarchenko et al. 2022. Although, Makarchenko et al. (2022) reports that macropterous forms are incapable of flight due to underdeveloped flight muscles. Winter-emerging *D. leona* from Colorado, USA exhibit similar variation; however, both forms occurred together with brachypterous forms reported to dominate populations at higher elevations and macropterous forms more common at lower elevations (Herrmann et al. 1987).

Ecological Importance

Chironomids are important members of stream food webs and are key food sources for fish, birds, and other insects in winter. Research from Minnesota trout streams found that chironomids are an important component of the winter diet of brown trout (*Salmo trutta* Linnaeus) (French et al. 2016) and are actively selected for by brown trout in some streams (Anderson et al. 2016, Cochran-Biederman and Vondracek 2017). Smaller fish tended to exhibit preference for chironomid larvae and exhibited size-selective predation for larger larvae, comprised primarily of late-instar *Diamesa* (Anderson et al. 2016)*.* Additionally, both stomach-content analysis (French et al. 2014, 2016) and stable isotope analysis (French 2014) found chironomids are important prey for brown trout during winter. Research from the Iberian Peninsula has also found that certain overwintering passerine birds, including *Motacilla alba* Linnaeus (White Wagtail), *Saxicola rubicola* Linnaeus (Common Stonechat), and *Erithacus rubecula* Linnaeus (European Robin) feed upon recently emerged chironomids; *E. rubecula* was observed feeding upon chironomids that were visible on the snow surface (Braz et al. 2014).

Some assemblages dominated by *Diamesa* may decline or disappear due to continued climate warming and increasing water temperatures (Dočkalová et al. 2024, Lencioni et al. 2024). Specifically, increases in water temperature have been shown to reduce or halt emergence of *D. mendotae,* with temperatures above 10 °C unsuitable for continued larval growth and survival (Bouchard and Ferrington 2009); maximum growth rates of *D. mendotae* are recorded at water temperatures between 6-8 °C (Mazack 2013). Similarly, a drop in the abundance of *D. steinboecki* Goetghebuer has been reported in kryal streams that exhibited climate-associated temperature increases, along with an increase in overall invertebrate diversity (Lencioni et al. 2022). Considering that the longevity of at least some cold-adapted species decline with higher temperatures, certain chironomid populations may become less abundant (e.g. Nyquist et al. 2021, Bodmer et al. 2023). Communities currently dominated by *Diamesa* may become more diverse as temperatures rise (Dočkalová et al. 2024). The combination of these observations suggests that the winter chironomid community will change; however, other taxa may replace those that are reduced or lost.

What a future cold-adapted chironomid community might include, and whether there may be ecological consequences, is unknown, especially given the current role of *Diamesa* in food webs. A reduction in emergence of some of these winter-active groups or changes in their activity period could lead to mismatches in phenology and asynchrony between species interactions (Kharouba et al. 2018). A study on Diptera in the United Kingdom found that peak flight biomass of active adult flies changed significantly from 1974 to 2014, which could have negative repercussions for birds and other animals that feed on them (Grabener et al. 2020). Additionally, significant differences in the rate of phenological change in trophic levels have been described in marine and freshwater systems (Thackeray et al. 2010). When the rate of phenological change is great enough and the synchrony between species interactions is in different directions, this can decouple relationships resulting in negative fitness consequences for the species involved (Kharouba et al. 2018) and ecosystem disruption (Thackeray et al. 2010). Further work on cold-adapted Chironomidae is necessary to investigate rates of change in key life-stage events like hatching and emergence to be able to draw further conclusions on the effect that climate change may have on food webs reliant on winter emergence.

Winter-emerging taxa appear highly resistant to extreme late summer floods which suggests that certain taxa may exhibit an opportunistic response to flooding disturbance, taking over niches left vacant by less resistant summer taxa (Anderson and Ferrington 2013). Many chironomids use the hyporheos as a “nursery” for eggs, early larvae, or quiescent stages (Chou et al. 1999, Lencioni et al. 2008, Dole-Olivier 2011, Lencioni and Spitale 2015, Mugnai et al. 2019). Among those taxa known to use the hyporheos are several genera within the Orthocladiinae and Diamesinae that include winter-emerging taxa, such as *Diamesa, Chaetocladius, Cricotopus, Parametriocnemus, Orthocladius,* and *Micropsectra* (e.g. Lencioni et al. 2008, Lencioni and Spitale 2015, Mugnai et al. 2019). Use of the hyporheic zone may allow these taxa to avoid displacement and mortality associated with streambed disturbance (Anderson and Ferrington 2013). Additionally, Anderson and Ferrington (2013) suggest the hyporheos is important for predators, such as stream fish recovering from disturbance events that utilize these insects as a food source. The potential consequences of climate change, including the very likely increase in prevalence and intensity of heavy precipitation events that result in flooding (Seneviratne et al. 2021) combined with a likely scenario of reduced prevalence of certain winter-emerging species due to temperature shifts, may result in pronounced differences in stream food webs.

Hågvar (2010) posed the important question of whether insects that emerge from open streams during winter, such as Chironomidae, would adapt to the absence of snow. This is worth consideration as consistent snow cover throughout the winter months is becoming less reliable due to climate change. Recent models for Minnesota, U.S.A., for example, suggest that there will be significantly less snow cover, with 55 fewer days per year with over 2.54 cm of snow (Liess et al. 2022); similarly, temperatures may be 6 °C warmer by the end of the 21st century (Notaro et al. 2014, Liess et al. 2022). This is significant as areas such as the Driftless Region in southeast Minnesota harbor a diverse network of spring-fed streams with at least 60 winter-emerging chironomid taxa. Winter-active chironomids seek refuge within crevices or under the snowpack to avoid and survive adverse winter conditions, and longevity studies suggest some taxa can survive for prolonged periods in these conditions (e.g. Ferrington et al. 2010, Anderson et al. 2013). Mid-winter periods of minimal or non-existent snow cover could be detrimental for insects that would otherwise seek cover from harsh conditions. Species that currently emerge in early-spring are favored under climate change scenarios; however, periodic cold snaps may affect their survival, especially if snow cover is unavailable.

Future Directions and Conclusion

Soszyńska-Maj et al. (2016) stated that winter emergence of Chironomidae should not be viewed as a curiosity, but instead a "strategy that allows these insects to live actively and to disperse widely." The studies we describe here support this claim. None-the-less, research detailing winter-emerging chironomids is limited, and as we demonstrate (Fig 1), research is largely restricted to only a handful of localities and detailed autecology studies of only select species. Expanding research to include a broader geographical range to better gauge species accounts is important, especially to document shifts in species composition under changing climate regimes. Studies documenting cold hardiness, longevity, behavior, and other life-history parameters are largely restricted to only a few taxa, leaving much room for additional research.

Emphasis on extending field work into the winter season is critical to better understand the distribution, diversity, and life history of winter-active insects. As suggested by Figure 1, the presence of winter-active species is simply unknown in many regions because of a lack of winter field surveys. It is also unknown where and in what life stage many winter-emerging species aestivate during warmer months. Furthermore, while we have an understanding of cold hardiness and select life history parameters, such as growth, emergence, and longevity for certain species (e.g. *D. mendotae*), little research documents these parameters in other taxa. In short, there are an endless number of basic biological questions yet untapped when discussing winter-active species.

Although the freeze tolerance and freeze avoidance of some chironomid taxa have been tested (e.g. Lencioni 2004, Bouchard et al. 2009), little work has been conducted on critical thermal maxima (CT_{max}). Additional work in this area would complement studies on thermal stress and reduced longevity by providing a physiological endpoint for testing ambient temperatures and for creating more accurate models of thermal acclimatization. Testing CT_{max} could also provide data on adaptive plasticity since studies on Diptera utilizing acclimatization have found some plasticity in this endpoint measurement and, in combination with the size of the fly, could affect the insect's ability to cope with a warming climate (Weaving et al. 2023). Additionally, obtaining thermal performance curves (TPCs) from laboratory tests and field investigations would also deepen understanding of how temperatures might affect cold-adapted Chironomidae. These studies could be used to identify local adaptation in different ranges and populations of various taxa and for launching further investigations on fitness, plasticity, and acclimatization (Sinclair et al. 2016).

Enhancing the awareness of the winter-emerging insect community is also important. For example, few studies documented in introductory ecology textbooks highlight research conducted during winter and fewer yet focus on invertebrates. Similarly, ecological or environmental-focused community science programs are typically conducted during the summer or growing season. While winter programs can present more logistical challenges considering low temperatures and icy conditions, winter field opportunities can offer profound hands-on learning opportunities for classrooms with various science learning objectives (Swenson and Nyquist 2024). Programs such as *Bugs Below Zero* (Anderson et al. 2025) which is directed at educators and community scientists can help create awareness about winter-active aquatic insects and their value in stream ecosystems and can assist scientists in terms of documenting winter-emerging insects.

As shown here, winter-emerging Chironomidae are not simply a novel occurrence, but an integral and surprisingly diverse component of stream ecosystems worldwide that play important roles in both aquatic and terrestrial food webs. Hibernal species possess behavioral and physiological strategies that allow them to not simply survive but thrive throughout the coldest months of the year, with several species only known to develop, emerge, and reproduce during winter. The study of winter-active Chironomidae and other cold-hardy aquatic insects, while growing, is still in its infancy. Furthermore, what the future holds for many of these species is uncertain, especially considering our warming climate and the fact that research shows that some species are unable to survive in warmer conditions. We hope this review will both enhance the awareness of winter-emerging species and encourage other researchers to take on winter field work, continuing explorations of midges that are active below zero.

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New Records of *Eretmoptera* from Washington State, USA: maritime or terrestrial midge?

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Abstract

Washington State, USA has extensive coastal habitats that extend from marine or estuarine ecosystems upstream to the upper mixing zone where tidal surge and freshwater meet. We document a rare maritime chironomid genus, *Eretmoptera* Kellogg, 1900, from these habitats. The larvae of *Eretmoptera* were identified from 21 samples composed of 17 sites in the Puget Lowlands and Coast Range ecoregions based on a total of 1067 samples examined. Larvae were compared to reference material to confirm identification. We document *Eretmoptera* from low order forested streams in urban and private lands. Many sites sampled were near marine habitat and likely experienced saltwater intrusion while at least six sites were far from saltwater intrusion and were likely fully freshwater. We compare larval habitat for *Eretmoptera* in this study to larval habitat of the sub-Antarctic and Antarctic *E. murphyi* Schaeffer, 1914, the only species in the genus for which larvae have been associated. The georeferenced data provided in this study should spur further research to find and associate all life stages for *Eretmoptera* in Washington State to verify the genus identification and to help solve its taxonomic position within maritime and terrestrial Orthocladiinae.

Introduction

Washington State has extensive seashore, estuarine, and tidal surge habitat with 4870 km (3026 miles) of coastline (NOAA 2025). Our faunistic knowledge of benthic invertebrates inhabiting these systems is at best preliminary, with many taxa never thoroughly surveyed in the region. This is particularly true for insects in nearshore marine environments, estuaries, and tidally influenced rivers and streams. Gaps in this knowledge need to be filled, particularly as biodiversity in aquatic ecosystems is declining at a greater rate than terrestrial habitats due to extensive water use by agriculture and industry combined with increasing threats from invasive species and climate change (Strayer and Dudgeon 2010, Fabricius et al. 2014, Reid et al. 2019). Estuarine and nearshore invertebrate diversity is also declining (Worm et al. 2006), a particular problem for understudied invertebrates of these ecosystems such as maritime Chironomidae. Declines in biodiversity necessitate increased publication of georeferenced biodiversity data (e.g., Costello et al. 2013, Costello et al. 2018, Ball-Damerow et al. 2019) either in biodiversity centered research or as appendices related to ecosystem research. The purpose of this paper is to provide detailed range and ecological information for larvae of a rare maritime chironomid taxon, *Eretmoptera* Kellogg, 1900 found in Washington State.

Eretmoptera browni is the only described species of *Eretmoptera* in North America, the sole other described species being *E. murphyi* Schaeffer, 1914 from Antarctic and sub-Antarctic islands. Adults of *E. browni* are brachypterous (Kellogg 1900, Hashimoto 1976), collected in intertidal habitats in California (Kellogg 1900, Wirth 1949); no larvae or pupae have been associated with the adult of *E. browni* (Andersen et al. 2013). Larval descriptions for the genus are based on larvae of *E. murphyi* with the two species placed in the same genus solely based on adult female taxonomy (Andersen et al. 2013). Most recent publications on the genus center on *E. murphyi* as a terrestrial, parthenogenic Antarctic midge, particularly its role as an invasive species in the subantarctic Signy Island, from which the immature stages have been described (Cranston 1985, Convey 1992, Bartlett et al. 2019).

We have long-term, extensive chironomid data for Washington State generated via collaborations of Rhithron Associates, Inc. (RAI) with state, county, city, and non-profit agencies tasked with bioassessment of the state's aquatic resources. Washington's 4870 km of coastline extend from the upper estuary at the mouth of the Columbia River, around Cape Disappointment and north to Cape Flattery along the outer Pacific Coast, then inland along the

Strait of Juan de Fuca and the Salish Sea. Taxonomists at RAI identified *Eretmoptera* from Washington State. Our goals are to:

- Document new distribution records from Washington State of the genus *Eretmoptera* as currently recognized in Holarctic taxonomic keys,

- Provide a georeferenced database for the new chironomid records,

- Use the results to highlight issues in taxonomy and ecology of this rare and unique taxon in Washington State.

Methods

Chironomid specimens were identified from 1067 sites collected from nearly every part of Washington State, USA. Benthic samples were collected in Washington State from 2001-2024 by various organizations, including city, county, and state agencies. All Chironomidae were processed and identified by RAI taxonomists following state and federal protocols. Taxonomic data for this paper were used with permission from: the Cities of Bellevue, Bellingham, Bainbridge Island, Bothell, Federal Way, Issaquah, Kirkland, and Redmond; King County; Pierce County; Seattle Public Utilities; Snohomish County Public Utilities Division; Vashon Nature Center, LLC; and the Washington State Department of Ecology.

Larvae were identified based on a commonly used and widely respective key (Andersen et al. 2013). *Eretmoptera* larvae were identified based on the following combination of characters (Fig. 1): premandible with five teeth, simple SI and SII setae (Fig. 1a), five-segmented antenna (Fig. 1b), procerci and anal tubules absent from the posterior end of the abdomen (Fig. 1c), mentum with median tooth double and with five lateral teeth (Fig. 1d), mandible with four inner teeth (Fig. 1e). Due to the rare nature of the initial find and based on RAI policy, specimens were verified by an outside taxonomic expert (Peter Cranston). Voucher and reference specimens were retained for some projects at client discretion; these specimens are currently housed at RAI. RAI maintains a Microsoft Access database for all project data. Since RAI as a second party identified samples made by the first parties listed above, only latitudes and longitudes were available, thus we had no direct ecosystem data available for this study. Queries were

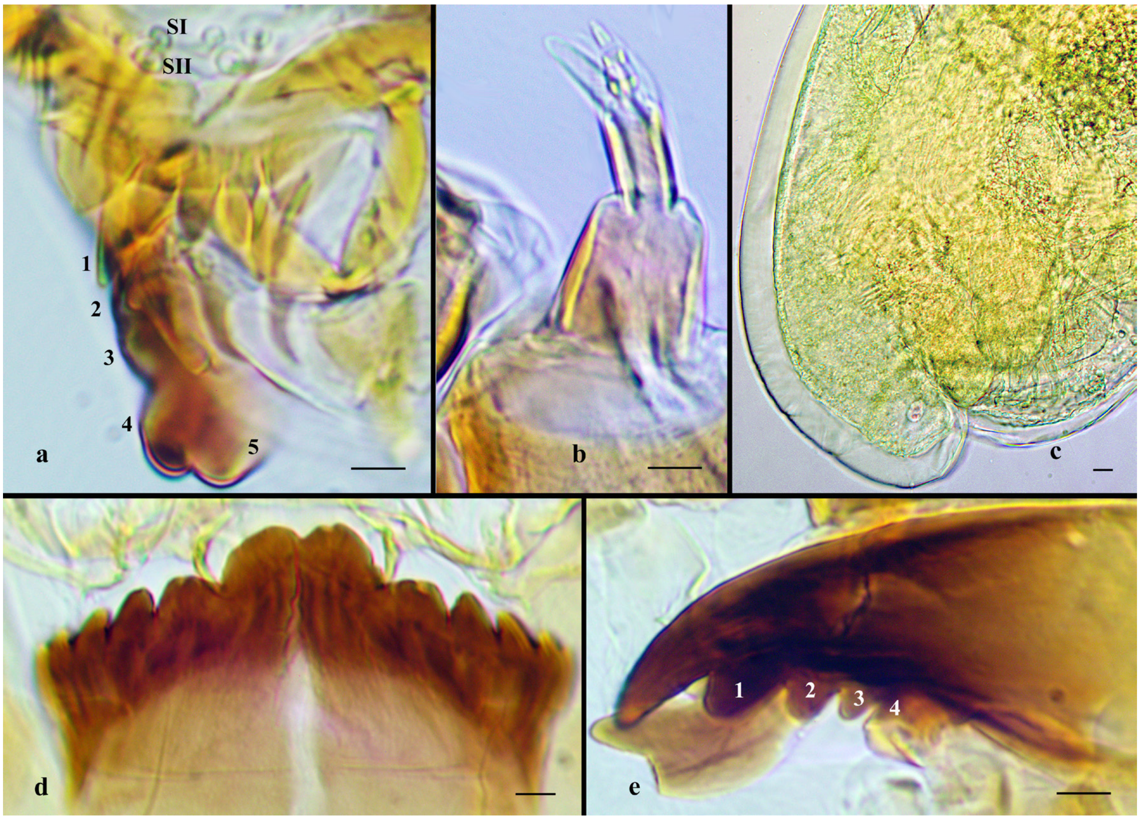

Figure 1. Photomicrograph showing larval head morphology of Eretmopetera from the reference collection at Rhithron Associates, Incorporated: a) premandible with teeth numbered 1-5, and SI and SII setae, b) antenna, c) posterior end of the abdomen, d) mentum, e) mandible with inner teeth numbered 1-4. Scale bar = 100 µm.

conducted to extract location data from records available for publication. Data were exported to Microsoft Excel to create a distribution map generated with SimpleMappr (Shorthouse 2010). We cross-checked latitude and longitude data for all sites retrieved from the RAI database with data for the same sites retrieved from the Washington State Environmental Information Management database (EIM 2021).

To test whether these data represented new records of *Eretmoptera,* literature was retrieved from electronic databases (Web of Science, Google Scholar, PubMed, Bibliography of the Chironomidae) via individual searches or searches through Publish or Perish (Harzing 2007). These databases were reviewed from project inception to the present covering publication dates from 1900 to July 2024 and using keywords such as *Eretmoptera*, *E. browni*, Chironomidae, chironomid, Washington State, Pacific Northwest, Columbia River, and the specific streams, wetlands, and lakes listed below. Distribution of *Eretmoptera* was compared to Nearctic and world catalogs of Chironomidae (Oliver et al. 1990, Oliver and Dillon 1994, Ashe and O'Conner 2012) and then to published literature listing Chironomidae to identify *Eretmoptera* as a new record for the State.

Additional information on larval environments, including estimates of stream width and distance from the marine habitat, was collected. Generalized land use and land cover (i.e. rural or urban, forested or not forested) and estimated size based on estimated stream width were retrieved from Google Earth Pro. Distance of collection sites to marine habitat or freshwater lake was estimated using high resolution aerial imagery from Google Earth Pro (version 7.3.6) and maps of river miles (CBI Data Basin 2025). Estimates of the extent of upstream tidal influence and upstream intrusion of saltwater for tributaries of the Salish Sea (denoted as river kilometer, rkm) were taken from Collins and Sheikh (2005) and Hall et al. (2018). Categories for the Columbia River estuary were taken from Hudson (2014).

Results

We retrieved 195 peer-reviewed research papers from the database searches related to macroinvertebrates in freshwater and estuarine ecosystems in Washington State. Of these, 122 papers contained taxonomic information related to macroinvertebrates, but few papers listed chironomid taxa. Paper publication dates ranged from 1969–2024. Published taxa were found in text or in tabular form within most papers and only one paper included a link to a taxonomic database for their study (Morley et al. 2020). Review of these publications confirmed that *Eretmoptera* represents a new record for Washington State.

Of the 1067 sites in Washington State for which chironomids were identified by RAI taxonomists, *Eretmoptera* larvae were identified from only 17 sites composed of 21 samples. Sites were located in the Puget Lowland and Coast Range level III ecoregions (USEPA 2013) (Fig. 2, Table 1). All Puget Lowland stream sites were small tributaries (~5 m or less in width) of rivers or flowed directly into the Salish Sea (Table 1). These sites flowed through urban, suburban, and exurban locations characterized by riparian forests. Four stream sites were proximal to the Salish Sea with three of these sites located on islands (Table 1). Streams that flowed directly into marine habitat or were tributaries of rivers that flowed into the Salish Sea were located within the estimated historic intrusion of saltwater and within or just above the estimated current intrusion of saltwater in tributaries of the Salish Sea (Table 1). Five of the Puget Lowland sample sites flowed into the freshwater Sammamish Lake (Table 1). Larval habitat was primarily urban forested corridors for all Puget Lowland stream sites (Table 1).

The six Coast Range stream sites were small tributaries (~5 m or less in width) of rivers that flowed into the upper estuary zone of the Columbia River and Willapa Bay (Table 1). These sites flowed through rural areas characterized by sparce to dense forests. One unnamed tributary of the Elochoman River was located above the Columbia River estuary (Table 1). Two stream sites in the Grays River watershed that flow into the Columbia River were at high elevation relative to sea level representing a steep gradient from the Coast Range

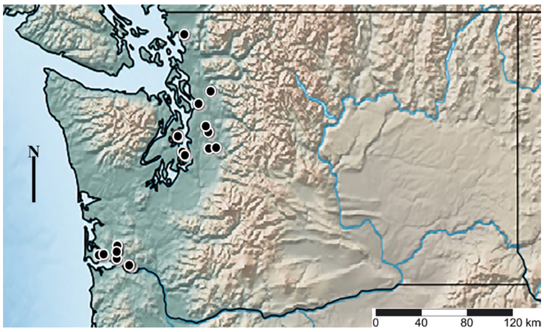

Figure 2. Distribution map of sample site locations where larvae of Eretmoptera were collected in Washington State, USA. Black circles denote sampling locations.

Table 1. Location and watersheds of sites from which *Eretmoptera* were identified and details on site proximity to marine ecosystems and saltwater intrusion.

Number samples/ site	Latitude	Longitude	Distance (rkm)*	Elev (m asl)	Watershed	Receiving aquatic habitat
1	46.4269	-123.5297	37.97	295	Grays R.	Columbia River[1]
1	46.3716	-123.5307	23.92	152	Grays R.	Columbia River[1]
2	46.2902	-123.5541	5.56	32	Harlows Cr.	Columbia River[1]
3	46.2205	-123.3366	8.16	36	Elochoman R.	Columbia River[2]
2	46.3313	-123.8173	27.52	52	Naselle R.	Willapa Bay
1	46.3326	-123.7752	24.58	65	Naselle R.	Willapa Bay
1	47.4763	-122.4815	0.45	10	Vashon Island	Salish Sea
1	47.4537	-122.4442	0.06	3	Vashon Island	Salish Sea
1	47.6653	-122.5679	0.03	10	Bainbridge Island	Salish Sea
1	48.7522	-122.4528	3.34	20	Whatcom Cr.	Salish Sea
1	48.0015	-122.2215	0.37	10	Snohomish R.	Salish Sea
1	48.1445	-122.0254	39.7	60	Stillaguamish R.	Salish Sea
1	47.5195	-122.0386	7.05	58	Issaquah Cr.	L. Sammamish
1	47.6955	-122.0561	11.55	158	Mackey Cr.	L. Sammamish
1	47.7580	-122.1063	15.72	91	Bear Cr.	L. Sammamish
1	47.5259	-121.9405	12.48	179	Issaquah Cr.	L. Sammamish
1	47.6955	-122.0561	8.88	158	Bear Cr.	L. Sammamish

* Indicates distance to receiving aquatic habitat in river kilometers (rkm) based on estimated historic saltwater intrusion upstream for Puget Lowland river (27 rkm, Collins & Sheikh 2005), current estimates (15.9 rkm, Hall et al. 2018). [1]Upper estuary of the Columbia River. [2]Above the upper estuary of the Columbia River. In general, L. = lake, R. = River, Cr. = Creek.

mountains to the Columbia River (Table 1). Larval habitat was primarily forested, located on private forestry lands and other private lands. Tidal influence on the Naselle River reaches nearly to the town of Naselle, 14.5 rkm upstream from Willapa Bay where the river exhibits a drastic decrease in size (Fig. 3). Stream sites in the Naselle River watershed were all low elevation (Table 1). We could not find salinity intrusion studies for the Willapa Bay rivers and so use the upstream estimates for the Salish Sea. Streams sites located in the Naselle River watershed were within the estimated historic intrusion of saltwater and within or just above the estimated current intrusion of saltwater in tributaries of the Salish Sea (Table 1).

Figure 3. Examples of stream sites for this study: a) Naselle River ~15 rkm upstream of the tidal surge plain; b) tidal surge plain for the Naselle River; c) an example of mossy banks typical of streams in Western Washington, USA.

Discussion

Our results highlight the need for publishing biodiversity data. The dearth of published studies that include taxonomic information for Chironomidae from Washington State does not indicate that *Eretmoptera* have not been collected prior to this study, but rather that Chironomidae are not identified or reported at a fine taxonomic resolution (e.g. genus or species). Studies on fish, amphibian, and shorebird diets typically refer to Chironomidae only at the family level (e.g. Wilson 1994, Tyler et al. 1998, Champion et al. 2018). Some research focusing on macroinvertebrates take many taxa to genus or species but leave Chironomidae at subfamily or tribe (e.g. Foster et al. 2020, Claeson et al. 2021). Food web analyses may not include taxa identified to finer resolutions (Wootton 2012). Finally, in some cases taxa were identified to the genus or species level, but only aggregated taxa, metrics, indices and/or functional traits are included in the publication without inclusion of taxa in the text or as supplemental material (Marshalonis & Larson 2018, Larson et al. 2019). In publications that do list chironomid genera and lower-level taxonomic resolution in Washington State (Gaines et al. 1992, Danehy et al. 2021, Morley et al. 2020), *Eretmoptera* was absent.

One of the goals of this study was to characterize larval habitat to examine whether *Eretmoptera* is truly a maritime midge, but our results warrant further investigation. All study sites were connected to marine ecosystems presently or in the past. For example, Lake Sammamish is part of the Lake Washington watershed and Lake Washington had anthropomorphic saltwater intrusions in the 1940s and 1950s (Edmondson 1994), though it seems unlikely that saltwater would have intruded as far upstream as the study sites in the Lake Sammamish watershed (Table 1). Other study streams may represent the upper intrusion of saltwater into freshwater tributaries of marine ecosystems, but the extent of saltwater intrusions used in our research were based on only one river tributary of the Salish Sea (Collins & Sheikh 2005, Hall et al. 2018). It is no coincidence that many study sites were located above the tidal zone since lower reaches or tidal streams were excluded from some of the studies for which chironomids were identified (e.g. Larson et al. 2019). It is possible that more *Eretmoptera* will be found along the tidal reaches of the study streams, if sampled, particularly since the genus was identified for projects that sampled small tidal streams (e.g. Bainbridge and Vashon Islands, Table 1). Salinity concentrations vary greatly in bays and estuaries, so predicting the percent salinity moving upriver proves challenging. Willapa Bay salinity patterns vary based on a suite of physical processes such as wind and wave intensity, currents, and river discharge (Banas et al. 2004, Banas and Hickey 2005). The Columbia River estuaries have even greater variation in salinity than Willapa Bay and the Salish Sea given the profound discharge volume of the lower Columbia River (Hudson 2014). Thus, even if saltwater characterizes some of the sites in this study, salinity varies seasonally based on flows and tides and salinity intrusion has changed significantly over time due to alterations such as diversions, diking, shipping canals, and impoundments (Edmondson 1994, Collins & Sheikh 2005, Hall et al. 2018).

These habitats are far different from the expected larval habitats of *Eretmoptera* based on previous studies of adults associated with California tidal pools (Kellogg 1900, Wirth 1949). Larvae of *E. murphyi* are terrestrial to semi-aquatic in the sub-Antarctic and Arctic regions (Cranston 1985, Convey 1992, Bartlett et al. 2019). Larvae of *E. murphyi* inhabit wet mosses and lichens, peat, soil, and leaf litter; egg-masses seem to be resistant to desiccation (Convey 1992, Bartlett et al. 2019, Bartlett et al. 2020). Possibly, larvae of *Eretmoptera* from Western Washington State inhabit wet moss and peat soils, common habitats along shady and forested streams (Fig. 3). Terrestrial and semi-aquatic larvae are often found in Washington stream samples, especially low order streams. Rather than indicating nearshore or tidal pool larval habitats the results of this study provide ecological evidence supporting phylogenetic analyses placing *Eretmoptera* and some other maritime midge genera in a group of Orthocladiinae "varia" *sensu* Tang *et al.* 2023. Several "maritime" genera group with semi-aquatic/terrestrial orthoclads instead of the marine *Clunio* Haliday and relatives (Cranston *et al.* 2011, Tang et al. 2023). If the *Eretmoptera* documented in this study are *E. browni*, then adults found associated with tidal pools in California may emerge from nearby terrestrial or semiterrestrial substrates such as mosses or lichen like *E. murphyi*, which may explain the lack of success locating larvae in tidal pool habitats.

Since *Eretmoptera* is monotypic for North America, it is tempting to determine the specimens in this study as *E. browni*. However, when it often becomes "known" that a particular genus is monotypic in a region, taxonomists may neglect to assess the species diversity of that group and overlook contradicting information, such as novel habitats or range extensions of preexisting taxa (i.e. *Parochlus kiefferi*, Hayford 2012) or subtle morphological characters. Assumptions of monotypy can change once taxonomists are able to analyze specimens in greater detail and from a range of sites (i.e. *Doncricotopus*, Namayandeh and Beresford 2021), but using presumed monotypy to ascribe a species epithet is particularly common when the specimens being identified lack the characters needed to assess their species level placement. In Chironomidae, most species descriptions are based on adult males, and for many species the immature stages are undescribed. Furthermore, research based on molecular analysis has shown that cryptic species may be found in one and sometimes multiple life stages of Chironomidae (i.e. Anderson et al. 2013). Lack of associated material for *E. browni* has hampered understanding of the taxonomy and systematic placement of the species and genus (Tang et al. 2023). As the immatures have yet to be described for *E. browni* and our material does not have associated adults, we believe it would be inappropriate to assign the Washington specimens to the described species as it is possible the California adults and our Washington larvae are not conspecific. Furthermore, given that the larval description for *Eretmoptera* in widely used Holarctic identification keys (i.e. Andersen et al. 2013) is based on specimens from the far southern reaches of the Southern Hemisphere, a more conclusive identification of specimens as *Eretmoptera* will require DNA analysis and further taxonomic work.

Several taxa in Chironomidae inhabit marine environments (Hashimoto 1976, Tang et al. 2022) and chironomids have been documented from marine and estuarine habitats in Washington State (e.g. Wootton et al 1996). Focusing on these habitats and ecoregions while working to improve our taxonomic understanding of Chironomidae will yield a wealth of biodiversity data for the Pacific Northwest region of North America. Furthermore, sampling that targets specific taxa vastly increases known biodiversity for a region (e.g. Borkent et al., 2018). Although limited, the distribution data in this study (Table 1) is shared to aid in future targeted surveys for adults and pupae of *Eretmoptera* with the goal of finding all three life stages so that the genus' taxonomic placement may be solved. With additional sampling, we hope to further explore whether Washington *Eretmoptera* are marine or terrestrial.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

Author Contributions

BH, paper conceptualization, literature review and analysis, natural history and ecology comments; AF, taxonomic comments; RS, data retrieval, mapping; all authors contributed to writing the manuscript.

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DESCRIPTION OF *PARAKIEFFERIELLA FERRINGTONI* WITH DISCUSSION OF RELATIONSHIPS WITHIN THE *PARAKIEFFERIELLA* GROUP (DIPTERA, CHIRONOMIDAE, ORTHOCLADIINAE)

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https://zoobank.org/ B7C7E3D6-58DF-42B4-ACF4-A03FC63F9FB0

Abstract

Parakiefferiella ferringtoni sp. n., previously recorded as the provisional taxon Orthocladiinae Genus 5 *sensu* Coffman and Ferrington (2008), is described based on larval, pupal, pharate male and female material. This species resides in the Pacific Northwest region of the Nearctic. The morphology of *P. ferringtoni* sp. n. blurs the lines between genera of the *Parakiefferiella* group. We review the state of generic taxonomy of the *Parakiefferiella* group, with an emphasis on providing explicit morphological synapomorphies to delineate each genus, although *Parakiefferiella* Thienemann and *Lappokiefferiella* Tuiskunen have no unambiguous synapomorphies.

Introduction

The Chironomidae fauna of the western Nearctic is poorly known, having only received a cursory survey in the literature (Sæther 1969, Sublette & Sublette 1971, Sublette et al. 1998, Namayandeh & Culp 2016). Despite the paucity of published taxonomic studies, there is a network of organizations conducting aquatic bioassessment in western North America, including federal, state, local and tribal entities (Roper et al. 2010). Immature Chironomidae are an integral part of these programs, typically identified to genus, though a few taxa are taken to species or species group. In addition to established taxa there are also morphotypes which do not conform to current genus concepts (Fasbender 2020).

This study is based on long-term chironomid data generated via collaborations of Rhithron Associates, Inc. (RAI) with state, county, city, and other entities assessing freshwater systems. RAI maintains an internal catalog of provisional taxa designations for morphotypes not conforming to established genera to provide consistent reporting of these taxa. With client permission we also retain unusual specimens from samples, typically larvae and pupae as these are benthic samples. One such taxon found in the Pacific Northwest is Orthocladiinae Genus 5, a provisional taxon previously known only in the pupal stage (Coffman and Ferrington 1996, Ferrington et al. 2008, Ferrington and Berg 2019).

The pupa of Orthocladiinae Genus 5 is distinctive, similar to *Rheosmittia* Brundin, except it has an ovoid thoracic horn and lacks the flattened D5 setae that characterizes that genus. We were able to find associated material of the heretofore unknown larvae, which resemble *Parakiefferiella* Thienemann except for a very narrow median tooth subequal in size and aligned with the first lateral teeth of the mentum. Examination of pupae with pharate adults showed they are of the *Parakiefferiella* group type, but do not key out properly to that genus based on the diagnosis of Cranston et al. (1989). The adults fall into the *Parakiefferiella* group of genera with a curved gonostylus but do not have a medial setal tuft or tubercle on the scutum. Examining a range of material of this unusual taxon and attempting to place it into a genus led us to reexamine the characters used to delimit genera within the *Parakiefferiella* group. Although we did not have access to eclosed adult material, based on the characters of the immatures and the absence of a setal tuft or tubercle along diagnostic male genitalia there is sufficient justification to formally name this taxon (Fasbender 2020) and describe this species as *Parakiefferiella ferringtoni* sp. n.

The decision to place Orthocladiinae Genus 5 within *Parakiefferiella* lead us to examine relationships between *Parakiefferiella* and closely related genera, thereby broadening the scope of this research. The goals of our research are to:

Describe all three life stages of *P. ferringtoni* sp. n.

Suggest amendments to existing keys to include *P. ferringtoni* sp. n.

Update the species keys for Nearctic *Parakiefferiella* to include *P. ferringtoni* sp. n.

Briefly review taxa in the *Parakiefferiella* group (*sensu* Sæther 1983) and the characters that define them.

Methods and materials

The material used in this study originated from 2001-2021 bioassessment programs from the following organizations: the Cities of Bellevue, Bellingham, Bainbridge Island, Bothell, Federal Way, Issaquah, Kirkland, and Redmond; King County; Montana Department of Environmental Quality; Pierce County; Seattle Public Utilities; Snohomish County Public Utilities Division; Vashon Nature Center, LLC; and the Washington State Department of Ecology. Most of the specimens examined were retained by the originating entity, although select individuals have been deposited in the Academy of Natural Sciences of Drexel University [ANSP] or Andrew Fasbender's personal collection [AFPC]. All material examined was mounted on slides unless otherwise noted in the material examined.

Descriptive terminology follows Sæther (1980), except for the use of "ommatrichia" for the microtrichia between ommatidia of the adult eye, epandrium for the structure called "Tergite IX" in Sæther 1980, gonopod for the clasping structure consisting of the gonocoxite and gonostylus, genital fork for the structure called "Gonapophysis IX" in Sæther 1980 and spermathecae (Cumming and Wood 2017). The female description only contains those characters which differ from the male. All measurements are given in µm unless otherwise noted, the anal point was measured from the posterior apex to the anterior setae (as there is not a distinct separation of it from the remainder of the epandrium). Life stage associations were made using the ontogenetic method sensu Hogue and Bedoya Ortiz (1987). Specimens were cleared using Tergazyme© enzymatic detergent, transferred to 99% isopropanol, dissected, and mounted in Canada balsam on microscope slides. A Leica DM1000 compound microscope equipped with an Amscope MU1000 camera was used to examine specimens. The Amscope 4.11 software package was used to capture images and take calibrated measurements. Illustrations were rendered using Inkscape 1. Data were exported to Microsoft Excel to create a distribution map generated with SimpleMappr (Shorthouse 2010). Sites on the distribution map not included in the material examined come from the RAI database.

Our review of taxa and morphological characters in the *Parakiefferiella* group is based on examination by the senior author and characters in Sæther (1983), Coffman et al. (1986), Cranston et al. 1989 and Andersen et al. (2013).

Results

Parakiefferiella ferringtoni sp. n.

https://zoobank.org/BD022F7A-9A53-4862-8E50-71D8E0DE307F

Orthocladiinae Genus 5 (Ferrington et al. 2008, Ferrington and Berg 2019).

Possibly Orthocladiinae gen.? sp.? NA7 and *Parakiefferiella* NA3 (Langton 2023).

Orthocladiinae RAI #0011 (for larva).

Type material. Holotype male pupa (slide), USA, WASHINGTON, *King Co.*, Vashon Island, Shinglemill Creek, 47.4762°N, 122.4815°W, 13.ix.2021, KC21AMB02064 (ANSP-ENT-124842), on slide [ANSP]. Paratypes: 1 male (ANSP-ENT-124843), 2 female pupae (ANSP-ENT-124844, 124846), 1 larva (ANSP-ENT-124847) on slide as holotype [ANSP]; 1 male pupa, USA, MONTANA, *Beaverhead Co.*, Willow Creek at base of Thunderhead Mountain, 45.448°N, 112.8277°W, 15.vii.2019 (ANSP-ENT-124848) on slide [ANSP]; 7 larvae, WASHINGTON, *King Co.*, Cedar River Lower Tributary (0311), 47.4614°N, 122.1255°W, 25.viii.2016 08CED2898_16 KC16AMB2040, in vial [AFPC], 7 larvae as previous, 10.viii.2017 08CED2898_17, KC17AMB4038, in vial [AFPC]; 1 male pupa, High Point Creek, 47.5319°N, 121.9762°W, 1.viii.2018 08ISS4573_18, KC18AMB2018, (ANSP-ENT-124850) on slide [ANSP]; 1 male pupa, Holder Creek, 47.4339°N, 121.9719°W, 9.viii.2017, 08ISS4730_17, KC17AMB4035 (ANSP-ENT-124851, on slide [ANSP]); 3 larvae, Holder Creek, 47.5319°N, 121.9762°W, 1.vii.2018, 08ISS4573_18, in vial [AFPC]; 7 larvae, Vashon Island, Christenson Creek, 47.4027°N, 122.5169°W, 20.ix.2017 KC-17VAS001, in vial [AFPC]; 1 larva, *Kitsap Co.* Chico Creek, 24.viii.2011, in vial [AFPC]; 1 female pupa, Garst Creek, 29.viii.2011, in vial [AFPC]; 2 female pupae, 5 larvae, Little Anderson Creek 29.viii.2011, in vial [AFPC]; 1 male (ANSP-ENT-124852), 1 female pupa (ANSP-ENT-124854) *Pacific Co.*, Basin 7315, 46.3241°N, 123.7668°W, 26.iv.2016, WADOE16MM019, slide [ANSP]; 5 female pupae (2 with associated larval exuviae) as previous, in vial [AFPC]).

Etymology. This species is named in honor of Leonard "Len" Ferrington Jr., in recognition of his contributions to Nearctic chironomid studies and mentorship of the last author.

Diagnostic characters. *Adult male (pharate).* As per *Parakiefferiella* except scutum without medial tubercle or setal tuft, inferior volsella subquadrate, with anteromedial corner rounded, glabrous and expanded beyond medial margin. *Pupa.* Thoracic horn ovoid with scale-like spinules, tergites with band of dark triangular spinules posteriorly, anal lobes coming to an acute point, without setal fringe, macrosetae absent or if present minute (< 0.1 x length of anal lobe). *Larva.* SI plumose, mentum with single median tooth subequal in width and height to first laterals giving the appearance of three median teeth, ventromental plates not extending beyond lateral teeth, antenna with hair-like 6th segment.

Description. *Adult male (pharate).* Figs 1, 3a. Measurements (n = 3): Flagellomeres 17–31 (18), 15–20 (18), 16–20 (19), 28–29 (29), 34–45 (42), 37–42 (39), 39–42 (40), 38–42 (41), 34–44 (38), 35–40 (39), 34–39 (35), 32–38 (37), 128–203 (175); AR 0.33–0.50 (0.44); anal point 42–46 (44); virga 61–75 (63); gonocoxite 113–121(115); gonostylus 40–45 (43). Habitus pale.

Head. Verticals absent, 1 postorbital. Antenna with 13 flagellomeres, plume present. Eyes reniform. Clypeus ovoid but not inflated, tapering ventrally, with five setae on dorsal margin. Tentorium not visible on specimens examined. Maxillary palpus with five segments.

Thorax. Antepronotum not narrowed medially, setae absent. Acrostichals absent, 1 dorsocentral above scutellum, 3 uniserial prealars, single scutellar. Postnotum bare.

Wing. Veins without setae, venation otherwise not apparent.

Legs. Sensilla chaetica absent. Tibial spur formula 1-1-2. Pseudospurs absent. Pulvilli lanceolate, 2/3 length of tarsal claws.

Abdomen. Tergites II–V with three pairs of setae in arched row, middle pair set distinctly anterior to lateral pairs; tergites VII and VIII similar, but with first lateral pair of setae moved medially directly posterior to medial pair of setae; tergite VI variable, resembling either anterior or posterior tergites. Sternites I–IV without setae, V–VIII with irregularly spaced setae.

Hypopygium. 2–3 setae on each side of anal point. Lateral margins of gonocoxite straight; superior volsella low and rounded, inferior volsella protruding and squared off apically: anteromedial corner glabrous, rounded and slightly expanding beyond straight medial margin, posteromedial corner with brush of fine setae. Gonostylus curve starts at 2/3rd of the length, megaseta forked, with small tooth directly ventrally. Very small lentiform pars ventralis present. Sternapodeme trapezoidal, lateral apices of oral projections hooked, anterior apices

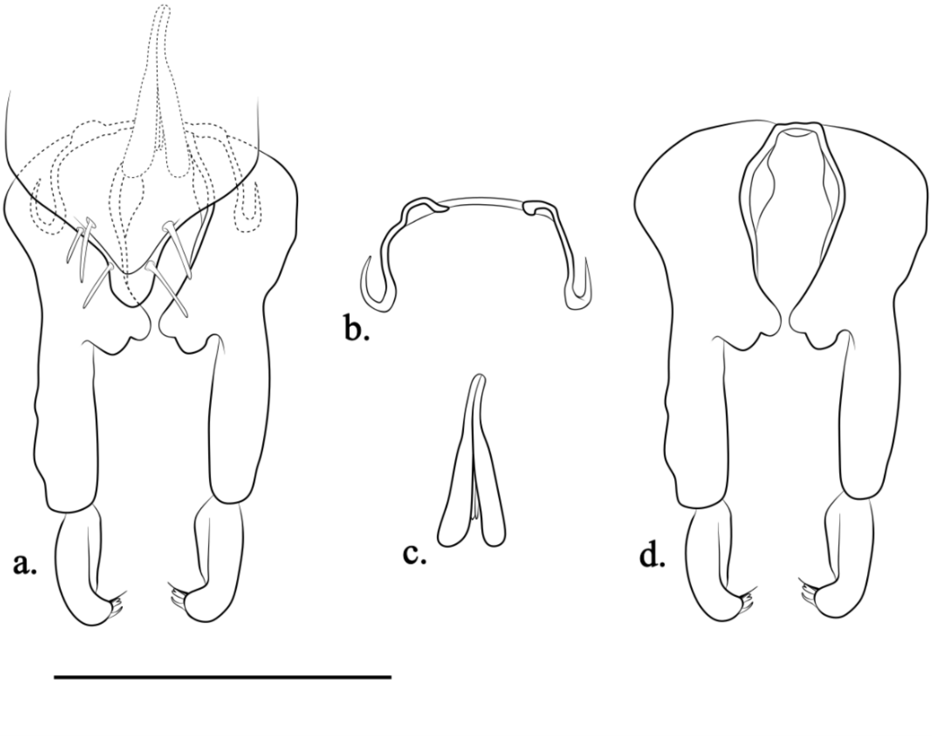

Figure 1. *Parakiefferiella ferringtoni* sp. n. male genitalia: a. dorsal view (overall), b. transverse sternapodeme, c. virga, d. gonopods, unobscured. Scale bar 100µm.

low and quadrate; phallapodemes inconspicuous. Virga with two flattened dorsal scales expanding posteriorly, curving in a C shape to an acute ventral terminus.

Adult female (*pharate*). Figs 2, 3b. Measurements (n = 3): Flagellomeres 21–44 (39), 33–42 (35), 31–42 (40), 33–41 (38), 76–138 (97); AR (n = 1) 0.6; cercus 58–78 (73) genital fork 125–150 (151), notum 83–97 (92), ramus 42– 54 (48); spermathecae (n = 2) 45–57 x 36–39, 46–58 x 32–43. Habitus pale.

Head. 1-2 postorbitals. Antenna with 5 flagellomeres, plume absent.

Genitalia. Tergite IX semicircular, with 6-10 setae, without projections or lobes. Gonapophysis VIII dorsomedial lobe rounded triangular, medial margins parallel and lined with setae; ventrolateral lobe rounded with brush of long setae on ventral surface. Apodeme lobes indistinct.

Pupa. Figs 4, 5b-d. Measurements: overall length (n = 5) 1.8–2.2 (2.0) mm; thoracic horn length (n

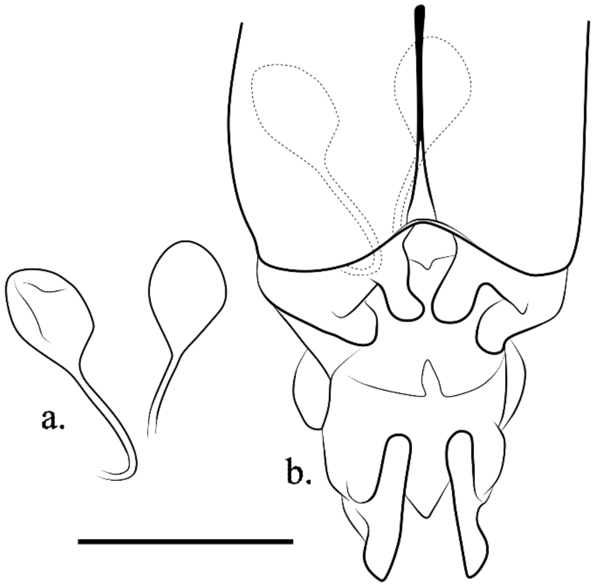

Figure 2. *Parakiefferiella ferringtoni* sp. n. female genitalia: a. spermathecae, b. ventral view (overall). Scale bar 100μm.

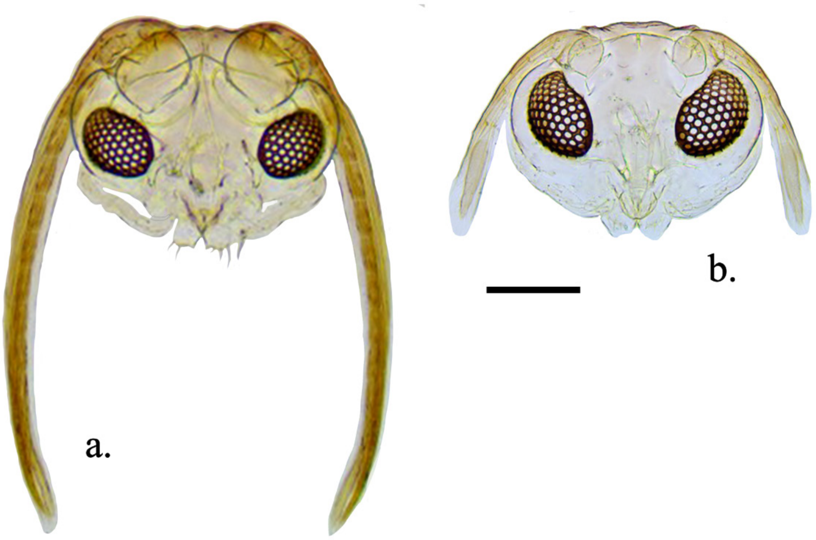

Figure 3. *Parakiefferiella ferringtoni* sp. n. adult heads: a. male, b. female. Scale bar 100μm.

= 3) 64–72 (66), anal lobe length (n = 3) 167–181 (171); female genital sac (n = 3) 76–114 (109); male genital sac (n = 3) 174–199 (183). Habitus pale.

Cephalothorax. Cephalic tubercles and frontal warts absent. Frontal setae absent. Antennal sheath smooth. Postorbitals absent. Antepronotum with one median and one lateral seta, both simple. Thoracic horn ovoid, 1.5x longer than broad, 70–80 μm; surface with scale-like texturing. 2–3 precorneals present, other thoracic setae absent. Anterodorsal surface of thorax rugose.

Abdomen. Tergites I and VII-VIII without shagreen, tergites II-VI with minute widely spaced triangular shagreen (Fig. 5d). Posterior margins of tergites II-VII with dark triangular spinules, conjunctives III and IV with medial patch of hyaline spines. Pedes spuria A and B absent. Sternites without shagreen or spinules. Abdominal setation: tergite I 5 D, 0 L, 4V; tergites II-VII 5D, 3L, 3V; VIII 3 D, 3L, 1V. No taeniae. Anal lobe without rounded lateral section narrowing to acute point curved dorsally, with irregular serrations scattered towards apex. No fringe present, macrosetae often absent. When present 0–3 minute macrosetae (< 0.1 x length of anal lobe) at 3/4th of the length of anal lobe, placed dorsolaterally. Male genital sac subequal in length to anal lobes, with apical papilla developed only as a slight tapering at apex; female genital sac trapezoidal, extending 1/3 anal lobe length.

Larva. Figs 5a, 6. Measurements (n = 11): overall length 2.7–3.2 (2.9) mm. Overall habitus pale, cranium light yellow.

Antenna. 6 flagellomeres, 1.3x length of mandible, antennal ratio 0.8. Ring organ not apparent on specimens examined. Blade reaching the apex of flagellomere 4. 2nd flagellomere weakly expanding

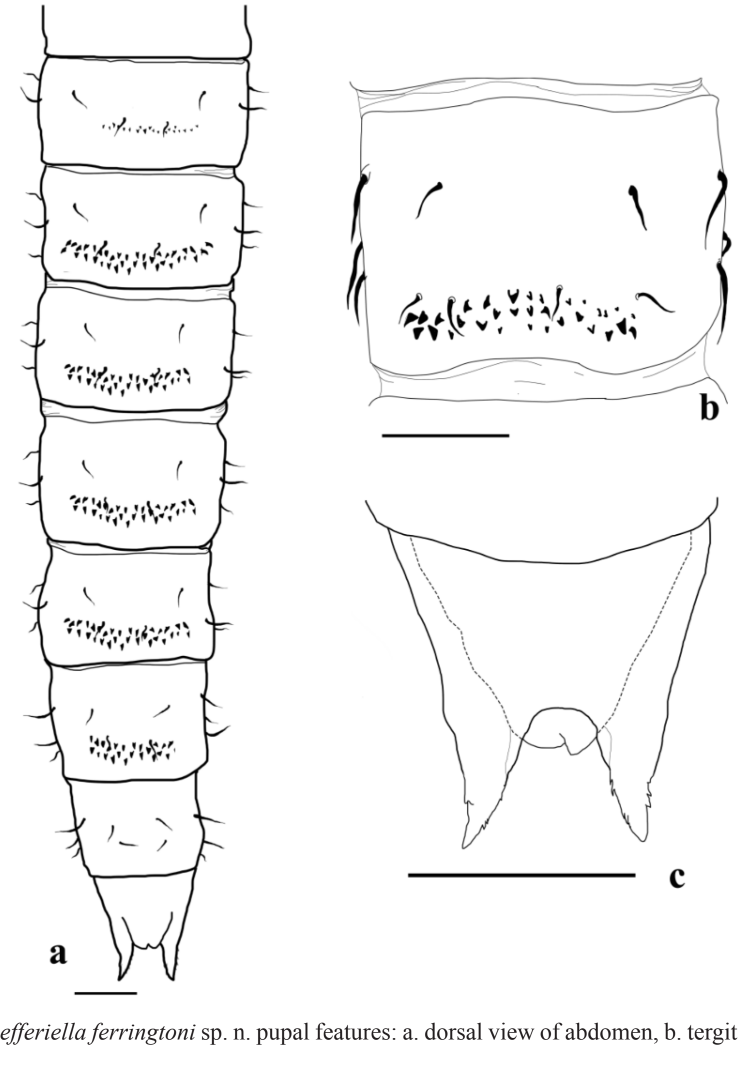

Figure 4. *Parakiefferiella ferringtoni* sp. n. pupal features: a. dorsal view of abdomen, b. tergite VII, c. anal lobes. Scale bars 100µm.

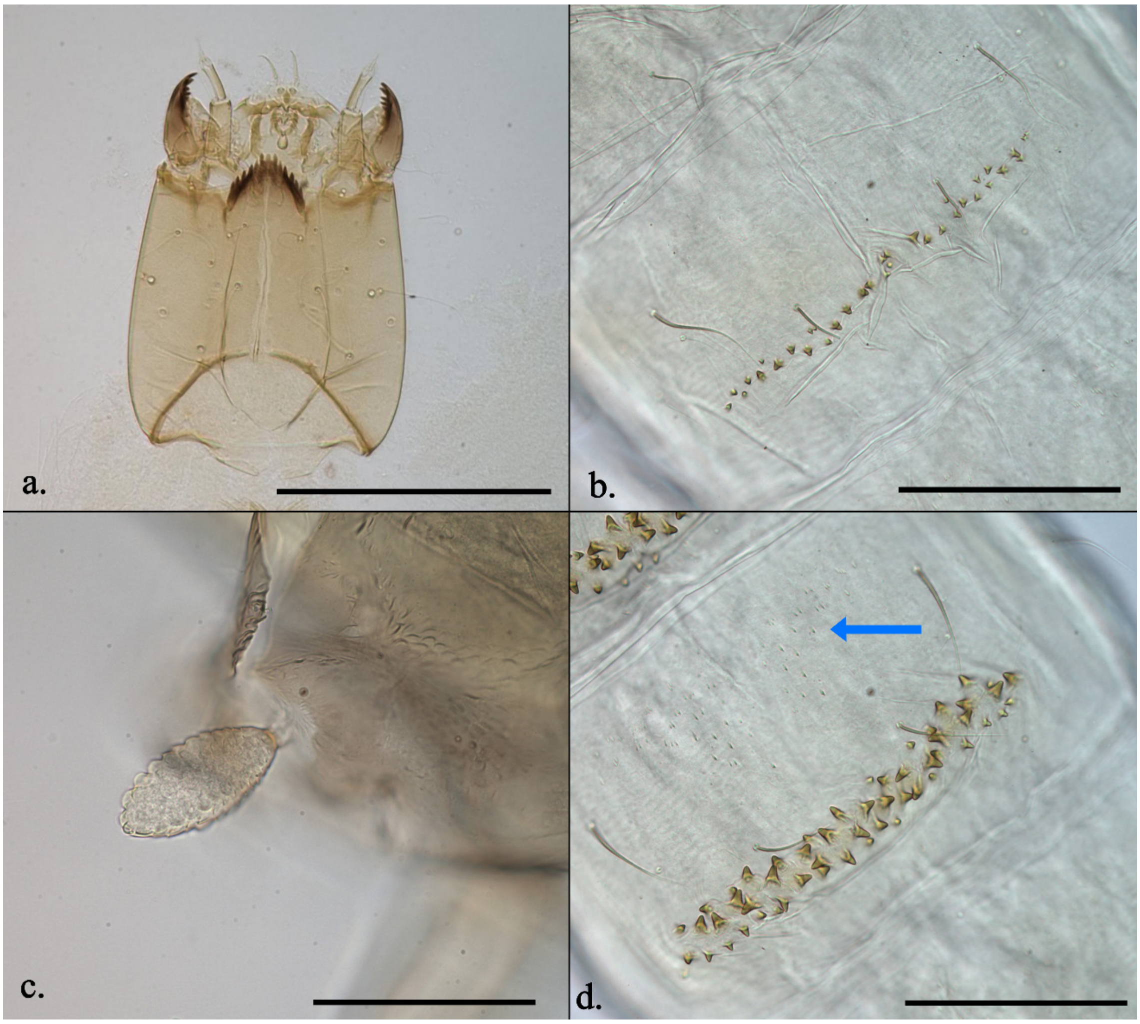

Figure 5. *Parakiefferiella ferringtoni* sp. n. photomicrographs: a. larval head capsule, b. pupal tergite II, c. pupal thoracic horn, d. pupal tergite IV, blue arrow indicates shagreen spinules. Scale bars 100µm.

towards apex. Lauterborn organs distinct, extending to apex of 3rd flagellomere. Flagellomeres 4 and 5 ~2x length of flagellomere 3, flagellomere 6 hairlike.

Labro-epipharyngeal region. SI broad, plumose. SII large, bladelike, other S setae reduced. Chaeta media reduced. Labral lamellae absent. Pecten epipharyngis with three, minute spines. Chaetulae laterales simple. Premandible with 2 apical teeth, 1 broad inner tooth dorsally; brush absent.

Mandible. With 3 teeth inner teeth, apical tooth ~0.3 length of inner teeth. Seta subdentalis with hooked apex, extending to first inner tooth; seta interna divided to base with numerous simple branches, difficult to discern, not extending beyond the margin of the mola.

Mentum. Triangular, with median tooth subequal in width and height to first laterals, creating a trifid appearance, projecting above five other pairs of lateral teeth. Ventromental plates narrow, paralleling lateral teeth, do not extend beyond them. Setae submenti placed further than halfway up the mentum, well anterior to ventromental plate apex. Beard absent. Hypopharyngeal scales absent.

Body. Anterior parapods separate, claws serrate. Posterior parapods 2.75–3x longer than wide, claws simple. Procerci length subequal to width, with 4–5 anal setae longer than posterior parapods. Supraanal setae shorter than posterior parapods. Anal tubules shorter than posterior parapods, apex pointed. Abdominal segments without prominent setae, cuticle appearing waxy.

Distribution. Pacific Northwest and Northern Rocky Mountains (Fig. 7).

Remarks. The pupa of *P. ferringtoni* was keyed as “Genus 5” in Coffman and Ferrington (1996), Ferrington et al. (2008) and Ferrington and Berg (2019). This species also keys to “Orthocladiinae

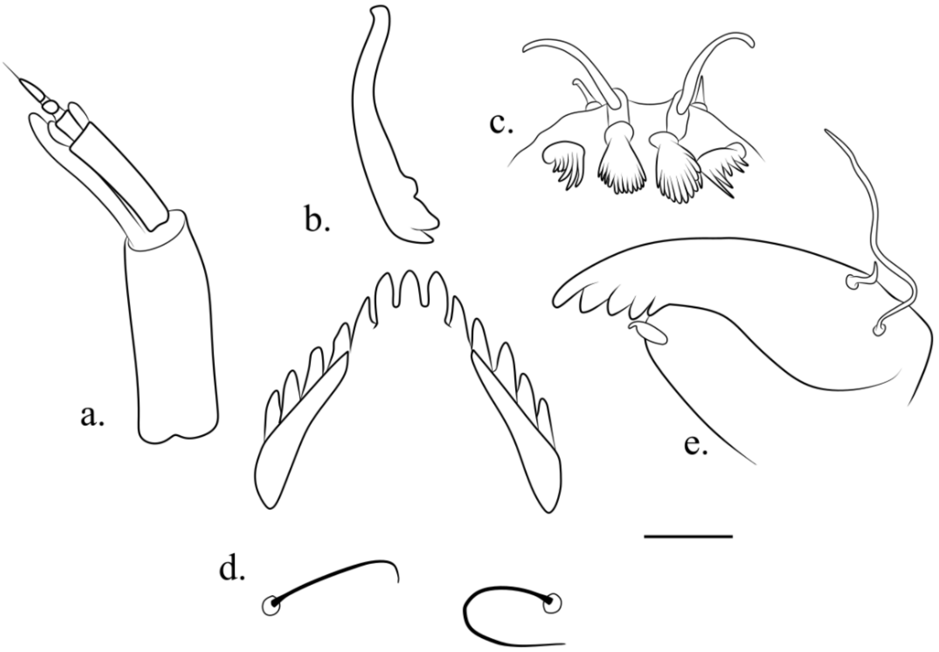

Figure 6. *Parakiefferiella ferringtoni* sp. n. larva: a. antenna, b. premandible, c. labral setae, d. mentum, e. mandible. Scale bar 100µm.

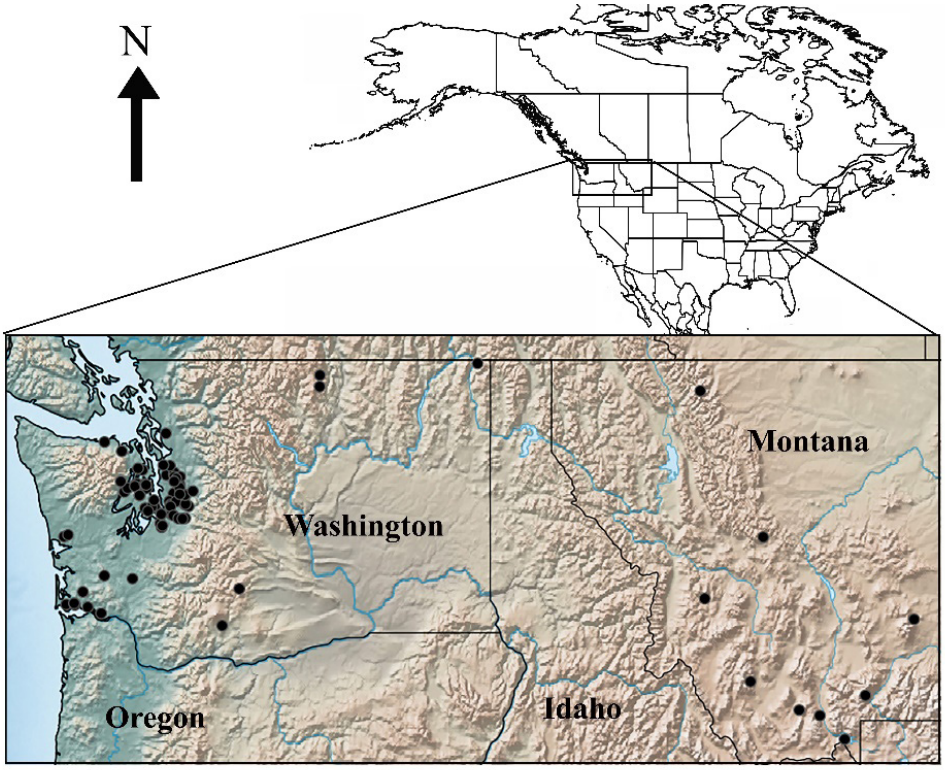

Figure 7. Distribution map of *Parakiefferiella ferringtoni* sp. n. examined for this study. Black circles indicate sampling localities for material examined.

gen.? sp.? NA7" in Langton (2023). However, the thoracic horn is not as tapering as illustrated for that provisional taxon. *Parakiefferiella ferringtoni* also has some similarities to *Parakiefferiella* NA3 in the same key, although the configuration of L setae appears slightly different and *P. ferringtoni* lacks a hook row on segment II. RAI has found associated larval specimens and pharate adults on which this description is based. *Parakiefferiella ferringtoni* is similar in the larval stage to other *Parakiefferiella* in having a hairlike 6th antennal flagellomere and to *Rheosmittia* (Cranston and Sæther 1986) in overall habitus, especially the conical head capsule, though it has significant differences from both genera. It differs primarily from other *Parakiefferiella* in the structure of the median tooth of the mentum. The median and first pair of lateral teeth are subequal in width and are of the same height in *P. ferringtoni* versus typical *Parakiefferiella* where the median tooth is wider than the first laterals and projects anterior to them. Additionally, the apical tooth of the mandible is subequal to or longer than the inner teeth in most *Parakiefferiella,* whereas it is subequal in size to the distal inner tooth in *P. ferringtoni. Rheosmittia* differs in the labral SI setae, which are simple in *Rheosmittia,* and plumose in *P. ferringtoni*. The hypopharyngeal scales of *Rheosmittia* are absent in *P. ferringtoni* and setae submenti reduced in *Rheosmittia* but normally developed in *P. ferringtoni*. *Rheosmittia* has a five segmented antenna with the second flagellomere divided by a desclerotized break, whereas the antenna is six segmented in *P. ferringtoni*.

While the three medial teeth of *P. ferringtoni* could be interpreted as a trifid median tooth, the presence of five other pairs of lateral teeth indicated the outer medial teeth are actually the first pair of laterals, as other species of *Parakiefferiella* have six pairs of lateral teeth. Thus the larva of *P. ferringtoni* correctly keys out to *Parakiefferiella* in couplet 72 of Andersen et al. (2013), provided the three subequal medial teeth of the mentum are correctly interpreted as a single median tooth and the first pair of lateral teeth. These three subequal teeth of uniform height are unique among *Parakiefferiella* larvae and readily allow the diagnosis of *P. ferringtoni* from the other species for which larvae are known. While there are some species in other genera with a similar configuration of three subequal teeth medially on the mentum (i.e. *Parorthocladius*, some *Cricotopus*), genus level characters readily distinguish them from *P. ferringtoni*.

The number of anal macrosetae is quite variable between pupal specimens, with many having no macrosetae while other individuals from the same locality have 1–3 minute macrosetae on the dorsolateral surface about 3/4 down the length of the anal lobe. We have even examined one specimen with two macrosetae on the left anal lobe and only one on the right. Setal sockets were not observed in pupal specimens lacking anal macrosetae, indicating this variability is not merely loss due to damage or wear.

The adult of *P. ferringtoni* keys out to the genus *Krenosmittia* based on Cranston et al. (1989) couplet 103. As published their key does not correctly handle *Rheosmittia* without a scutal tubercle. The key could be modified to incorporate *P. ferringtoni* by adding the following couplet between couplets 103 and 104:

Gonostylus straight, or at most weakly curved along its length .... *Krenosmittia*

- Gonostylus curved apically .... *Parakiefferiella ferringtoni* and *Rheosmittia* (in part)

Parakiefferiella ferringtoni cannot reliably be distinguished at the genus level from *Rheosmittia* species without a scutal tubercle except through details of the male genitalia. The genus *Hanocladius* also keys out in the same couplet (Wang and Sæther 2002), but can be distinguished from the *Parakiefferiella* group by the presence of scapellate acrostichals and acute anal point (see Discussion).

Natural history. Immatures of *P. ferringtoni* were collected from low-order Puget Sound urban streams, and the taxon is typically collected in cool, low-order streams often at low elevation, but it was also collected from two higher-elevation streams along the east side of the Cascade Mountains and from the Duckabush River on the Olympic Peninsula. In Montana *P. ferringtoni* has been recorded from mountain streams, including areas in the early stages of regrowth following fire. Most records of mature pupae are from late July or August, though this may be an artifact of collecting procedures for biomonitoring as those times are favored for sampling by agencies in western North America. There is at least one record from southwest Washington of mature pupae in April, suggesting a possible bivoltine phenology.

Key to Nearctic *Parakiefferiella* pupae

This key was adapted from Langton 1991, Langton and Visser 2003 and Langton 2023 with reference to Sæther 1969, Sublette 1970 and Cranston and Oliver 1988 for *Parakiefferiella subaterrima* Malloch and Makarchenko and Makarchenko

2010 for *Parakiefferiella rara*. We suggest cross referencing any specimens with Langton 2023, as that publication keys thirteen additional provisional taxa. The pupa of *Parakiefferiella finnmarkica* Tuiskunen, reported by Hebert et al. 2025 from Canada, is currently unknown.

1. Anal lobes without macrosetae, or if macrosetae present minute and less than 0.1x length of anal lobe, margins of anal lobes irregularly serrate .... *Parakiefferiella ferringtoni*

- Anal lobes with prominent macrosetae 0.2–0.35x anal lobe length. Margins of anal lobes not serrate 2

2. Anal lobe points >1.5x length anal lobe base, anal lobe tapering evenly from base to apex 3

- Anal lobe points <1.5x length anal lobe base, apical points of anal lobe distinctly constricted from base 4

3. Abdominal segments with lateral setae small and inconspicuous, <0.2x length of the abdominal segment .... *Parakiefferiella gracillima* Kieffer

- Abdominal segments with strong lateral setae, >0.2x length of the abdominal segment .... *Parakiefferiella rara* Makarchenko and Makarchenko

4. Thoracic horn absent .... *Parakiefferiella subaterrima* Malloch

- Thoracic horn present 5

5. Pedes spurii B absent .... *Parakiefferiella nigra* Brundin

- Pedes spurii B present 6

6. Thoracic horn smooth (without points/spinules), tergite VII with point patch of spinules .... *Parakiefferiella coronata* Edwards

- Thoracic horn with points/spinules, tergite VII without distinct point patch, though shagreen may be present 7

7. Anterior shagreen bands of tergites widely broken medially, width of gap subequal to median shagreen band... *Parakiefferiella smolandica* Brundin

- Anterior shagreen bands of tergites complete or with a narrow gap medially, if gap present much narrower than median shagreen band 8

8. Anterior shagreen band on tergite III with points smaller than those of median shagreen band .... *Parakiefferiella bathophila* Kieffer

- Anterior shagreen band on tergite III with points larger than those of median shagreen band .... *Parakiefferiella scandica* Brundin

Key to Nearctic *Parakiefferiella* adult males

This key was adapted from Cranston et al. 1989 with reference to Caldwell 1996.

1. Terminal segment of maxillary palpus length > 10x its width .... *Epoicocladius* Šulc & Zavrel

- Terminal segment of maxillary palpus < 10x its width 2

2. Gonostylus straight, or at most slightly curved evenly along its length 3

- Gonostylus distinctly curved apically 5

3. Costa extended beyond R_{4+5} .... *Krenosmittia* Thienemann & Kruger

- Costa not extended beyond R_{4+5} 4

4. Length of terminal maxillary palpomere < 2x its width, scutal tuft absent, virga present .... *Lapposmittia* Thienemann

- Length of terminal maxillary palpomere > 2x its width, scutal tuft present, virga absent .... *Acamptocladius* Brundin

5. Scutum without medial tubercle or tuft of setae .. .. *Parakiefferiella ferringtoni*

- Scutum with medial tubercle and/or tuft of setae 6

6. Scutum with medial tubercle bare of setae .... *Rheosmittia arcuata* Caldwell

- Scutum with medial tuft of setae, with or without tubercle .... other *Parakiefferiella* Thienemann

Discussion

Placement of Parakiefferiella ferringtoni

Parakiefferiella ferringtoni is aberrant and requires a revision of the diagnoses for the pupal and adult stage of its genus. In some ways it seems like an intermediate between *Parakiefferiella* and *Rheosmittia*, having more elongate anal lobe apices than most *Parakiefferiella* pupae (though *Parakiefferiella gracillima* Kieffer is longer), reduced or absent anal macrosetae, and the adult lacking a scutal tubercle or tuft. However, it has none of the distinctive synapomorphies that demarcate *Rheosmittia* in the larval and pupal stages (see below), and the only features that would preclude it from being placed in *Parakiefferiella* is the absence of a scutal tuft in the adult and anal macrosetae in the pupa.

As the presence of a scutal tubercle is variable between species of *Rheosmittia*, it is not unreasonable to suspect the homologous setal tuft may vary within *Parakiefferiella.* Similarly, variable number of minute anal macrosetae found in some specimens of *P. ferringtoni* suggest an autapomorphic reduction from the three well developed macrosetae found in "normal" *Parakiefferiella*. As the other features of *P. ferringtoni* fall within the variation already established in *Parakiefferiella* we decided to place it in that genus and emend the generic description.

Generic concepts in the Parakiefferiella group.

During the early parts of our study we believed *P. ferringtoni* may have merited placement in its own genus, but as we examined other members within the genus group it became clear that the delineations between some of the genera were based on plesiomorphies and autapomorphies. We decided to reexamine the limits of the *Parakiefferiella* group and the genera within by searching for synapomorphies to define each genus.

This was complicated by uncertainty about the nearest relatives of the *Parakiefferiella* group within Orthocladiinae. While there have been both molecular (Cranston et al. 2011) and morphological (such as Mendes et al. 2004a, 2004b, Mendes and Andersen 2008, Pinho et al. 2009, Andersen et al. 2024) phylogenies produced, each only contains a fraction of the genera of Orthocladiinae and there are substantial differences in relationships between the various phylogenies. The morphological phylogenies have consistently recovered *Parakiefferiella* and *Krenosmittia* as sister taxa when both have been included, but most of the other genera historically included in the *Parakiefferiella* group have never been included in computational morphological or molecular analyses. It is unclear what genus or group of genera represents the sister group of the *Parakiefferiella* group (or even what genera should be included within the group) without falling back on older intuitive assertions of relationships between genera.

Sæther (1983) provided a definition of the *Parakiefferiella* group containing *Epoicocladius* Šulc & Zavrel, *Gynnidocladius* Sublette and Wirth*, Krenosmittia* Thienemann and Krüger*, Lopescladius* Oliveira*, Parakiefferiella, Sætheriella* Halvorsen and *Stilocladius* Rossaro. The sole synapomorphy for grouping these species provided by Sæther (1983) was "Anal lobe of pupa with an apical more or less well-developed elongation." This was despite the fact that the immatures of *Gynnidocladius* and *Sætheriella* are unknown and the configuration of the anal lobes of *Lopescladius* and *Stilocladius* (cylindrical or rounded with apical macrosetae) are different than the anal lobes of other members of the group (having acutely pointed apices and subapical macrosetae). There are also other genera with extended anal lobes, including ones with pointed apices such as *Acamptocladius* Brundin and *Parachaetocladius* Wülker. While *Epoicocladius*, *Krenosmitta* and *Parakiefferiella* share several characters in the adult stage, the adults of the other species included in Sæther (1983) are quite heterogenous. Sæther and Andersen (1995) placed their new genus *Ionthosmittia* Sæther and Andersen in the *Parakiefferiella* group based on "wing features," including the evidently bare squama and extended costa (the immatures of *Ionthosmittia* are unknown). There have been other genera, such as *Hanocladius* Sæther and Wang (2002) and *Trondia* Ferrington and Sæther (2006), which have been noted to be similar to *Parakiefferiella* group taxa but were not explicitly included in the genus group.

A broad characterization of the *Parakiefferiella* group could be those taxa with the anal lobes terminating in acute points in the pupa and the adult male without a prominent subapical seta of the antenna, acrostichal setae absent (except for a posteriorly located medial setal tuft in some taxa), no setae on the squama and anal point broad and extending beyond the posterior margin of the epandrium. This characterization would include *Acamptocladius*, *Epoicocladius, Krenosmitta, Lapposmittia* Brundin*, Parakiefferiella, Rheosmittia* and probably *Lappokiefferiella* Tuiskunen and *Qiniella* Wang and Sæther (for which pupae are unknown). This excludes *Lopescladius* by the blunt apex of the anal lobe and absence of the anal point (Sæther 1983, Caldwell 1996), *Sætheriella* and *Ionthosmittia* by the presence of acrostichals (Halvorsen 1982, Sæther and Andersen 1995) and *Stilocladius* by the presence of acrostichals, rounded apex of the anal lobe and elongate, acute anal point (Sæther 1982, Coffman et al. 1986). Cranston et al. 2011 recovered *Lopescladius* as sister to *Stictocladius* and related to the *Brillia* group of genera rather than the *Parakiefferiella* group in their molecular study. *Gynnidocladius* is harder to ascertain based on the original description (Sublette and Wirth 1980), the curved apex of the gonostylus is found in some *Parakiefferiella* group taxa and it appears that the acrostichals are absent in their illustration of the thorax (though this feature is not mentioned in the text of the description). However, the anal point does not extend beyond the posterior margin of the epandrium (Sublette and Wirth 1980) which would exclude it from our concept of the group. For the purpose of this discussion we will not treat *Gynnidocladius*, though it, *Ionthosmittia* and *Sætheriella* merit reexamination when additional material becomes available – especially the immature stages.

Delimitation of genera in the Parakiefferiella group and morphological characters of phylogenetic significance

As described above, the *Parakiefferiella* group is herein broadly characterized as those taxa with the anal lobes terminating in acute points in the pupa, and the adult male without a prominent subapical seta of the antenna, few or no acrostichal setae, no setae on the squama and anal point broad and extending beyond the posterior margin of tergite IX. This characterization would include *Acamptocladius*, *Epoicocladius, Krenosmitta, Lapposmittia, Parakiefferiella, Rheosmittia* and probably *Lappokiefferiella* and *Qiniella* (for which pupae are unknown). Putative synapomorphies to support each genus follow:

Acamptocladius: Larva with dorsomentum toothed, separated medially by hyaline ventromentum. Pupa with L setae on posterior segments branched apically (also found in *Parametriocnemus* Goetghebuer). Adult with virga absent (shared with numerous other genera outside the *Parakiefferiella* group).

Epoicocladius: Larva with long dark body setae, large procerci, mentum with 4-8 median teeth. Pupa with dense fringe of long lateral setae on abdomen. Adult with long terminal maxillary palpomere (>10x width).

Krenosmittia: Larva with elongate maxillary palp, at least one anal seta longer than ¼ body length (shared with *Parachaetocladius* and *Pseudorthocladius*). Pupa with prominent rows of spines on posterior margins of abdominal tergites and sternites (shared with numerous other genera outside the *Parakiefferiella* group). No adult synapomorphies.

Lappokiefferiella: Larva and pupa unknown. No adult synapomorphies.

Lapposmittia: Larva and pupa without unambiguous synapomorphies. Adult with terminal maxillary palpomere length >2x width (also found in some species of *Ionthosmittia*, Sasa and Okazawa 1994, Yamamoto et al. 2015).

Parakiefferiella: Larva with six antennal segments (shared with other genera, ambiguous depending on interpretation of *Rheosmittia* antenna).

Qiniella: Larva and pupa unknown. Adult with trifid gonostylus.

Rheosmittia: Larva with elongate antenna with partially desclerotized break in second article, premandible with numerous comb-like teeth, hyaline mentum, and flattened, toothed hypopharyngeal scales. Pupa with flattened D5 setae on abdominal tergites. No adult synapomorphies.

Our review thus found six of the genera had at least one putative synapomorphy, but *Parakiefferiella* appears to be defined by symplesiomorphies shared with some of the other genera. *Lappokiefferiella* is dubiously distinct, defined by a lack of features (lack of extended costa and presence of a virga) that would allow placement in one of the established genera as the immature stages are unknown. Understanding the placement of *P. ferringtoni* within the genus and placement of *Parakiefferiella* within *Parakiefferiella* group requires systematic revision of the group, ideally using integrative taxonomic methods. Establishing reliable relationships within the *Parakiefferiella* group will require more comprehensive genus sampling than is available in previous morphological and molecular phylogenies of the subfamily. Such a project is outside the scope of the current study, but in our investigation we found many morphological characters representing synapomorphies to define genera or of potential utility in resolving relationships both within and between genera. Without an unambiguous sister taxon to *Parakiefferiella* it is impossible to establish polarity for many of these morphological characters, but we include discussion of these characters even if their relevance to the phylogeny remains unclear.

Larvae

Larval antenna with terminal flagellomere minute and hair-like. *Parakiefferiella* and *Rheosmittia* have a hair-like terminal flagellomere (Andersen et al. 2013), the 6th antennal article in *Parakiefferiella* and the fifth in *Rheosmittia*, suggesting the close relationship of these taxa. There are more distantly related orthoclad genera (*Parametriocnemus*, *Oropuella* and *Stilocladius* among others) that also have hair-like terminal flagellomeres (Andersen et al. 2013, Fasbender 2020).

Number of larval antennal flagellomeres. Larvae of the *Parakiefferiella* group have from four to six flagellomeres, the number of which seem to be consistent within genera. *Epoicocladius* and *Krenosmittia* have four flagellomeres, *Acamptocladius, Lapposmittia* and *Rheosmittia* five, and *Parakiefferiella* six (Andersen et al. 2013). Having six flagellomeres is the only putative synapomorphy we could find for *Parakiefferiella*, though the unusual structure of *Rheosmittia*'s second flagellomere (elongate, Lauterborn organs absent; Cranston and Sæther 1986, Caldwell 1996) may represent a fusion of flagellomeres 2 and 3. If so, the *Rheosmittia* antenna form could derived from the *Parakiefferiella* type.

Antennal length <25% head capsule length; Antennal length >25% head capsule length. The elongate antenna of *Rheosmittia* (Andersen et al. 2013), along with the unusual structure of the second article as described above, are synapomorphies defining that genus.

Premandible with 1–2 apical teeth; with 3–4 apical teeth; with numerous comb-like teeth. Epoicocladius, Krenosmittia and *Parakiefferiella* have 1–2 apical teeth on the premandible (Andersen et al. 2013), which is probably the ancestral condition of the *Parakiefferiella* group. *Acamptocladius* and *Lapposmittia* have 3–4 apical teeth (Andersen et al. 2013), a potential synapomorphy uniting those genera. *Rheosmittia* has numerous fine teeth on the premandible (Andersen et al. 2013), which could potentially indicate a relationship with *Acamptocladius* + *Lapposmittia*, but also could be an independently derived condition related to its psammophilic habits.

Larval maxillary palp length < 2.5x width; > 2.5x width. The sole unambiguous synapomorphy we could find for *Krenosmittia* is the elongate maxillary palp of the larva (Sæther 1983), at least 2.5x longer than wide. The maxillary palp is as long or longer than the length from the mola of the mandible to the tip of its apical tooth. In all other genera within the *Parakiefferiella* group the length of the larval maxillary palp is less than 2.5x its basal width.

Hypopharynx without flattened scales; hypopharynx with pair of flattened, toothed scales. The development of a pair of large hypopharyngeal scales (Andersen et al. 2013) is an autapomorphy unique to *Rheosmittia* probably related to its psammophilic lifestyle.

Larvae with median and lateral portions of mentum contiguous; toothed lateral portions of dorsomentum distinctly separated by hyaline ventromentum medially; mentum completely hyaline. The genus *Acamptocladius* is unique among the *Parakiefferiella* group in having the dorsomentum divided, divided by a hyaline area of ventromentum (Andersen et al. 2013). In Orthocladiinae this is also found in *Trichochilus* (Andersen et al. 2013), which does not appear to be closely related, and Orthocladiinae sp. C Sæther (1982). The undescribed pupa of Orthocladiinae sp. C is very similar to *Acamptocladius* and *Parakiefferiella* (Epler 2001), suggesting it is a member of the *Parakiefferiella* group – possibly an undescribed species of *Acamptocladius.*

Only *Rheosmittia* has a completely hyaline mentum, but its structure (dorso- and ventromentum not separate, at most fifteen teeth, no untoothed gap medially (Andersen et al. 2013) suggest it was derived independently of the condition found in *Acamptocladius*.

Larvae with 15 or fewer teeth on the mentum; more than 15 teeth on the mentum. Most taxa in the *Parakiefferiella* group have fifteen or fewer teeth on the mentum, while *Acamptocladius, Epoicocladius* and *Lapposmittia* have sixteen or more (Andersen et al. 2013), a potential synapomorphy linking those taxa. Additionally, *Epoicocladius* has 4–6 median teeth of the mentum set in a linear row, which is a clear synapomorphy defining that genus.

Larvae without strong setae on abdomen; with strong setae on abdomen. Epoicocladius is the only member of the *Parakiefferiella* group with strong body setae as larvae (Andersen et al. 2013), another synapomorphy supporting its distinctness.

Procerci cylindrical, <25% the length of the anal tubules and not heavily sclerotized; procerci conical, >50% the length of the anal tubules and heavily sclerotized. The large, conical and heavily sclerotized procerci are a synapomorphy defining *Epoicocladius*.

At least one anal seta 25% of larval body length. Only *Krenosmittia* larvae have elongate anal setae within the *Parakiefferiella* group (Andersen et al. 2013). Mendes and Andersen 2008 recovered the *Parakiefferiella* group as sister to the *Pseudorthocladius* group (*Doithrix, Georthocladius, Parachaetocladius* and *Pseudorthocladius*; Sæther and Sublette 1983) in a reweighted parsimony analysis of their morphological dataset – though this relationship was not found in the original unweighted analysis. *Parachaetocladius* and *Pseudorthocladius* are the other two genera with elongate anal setae as larvae, and if the *Parakiefferiella* and *Pseudorthocladius* groups are sister taxa the presence of elongate anal setae could be a symplesiomorphy and provide support to *Krenosmittia* being sister to the remainder of the *Parakiefferiella* group.

Pupae

Thoracic horn absent; elongate; ovoid. The presence/absence of thoracic horns is highly variable between and within genera of Orthocladiinae, and as such is a poor indicator of relationships. When present, the thoracic horn of most orthoclads is distinctly elongate, rather than ovoid as found in *Epoicocladius*, most *Parakiefferiella* and some *Acamptocladius*. However, ovoid thoracic horns are also found in *Parorthocladius* and some *Euki-*

efferiella (Coffman et al. 1986) – suggesting this character is potentially susceptible to homoplasy.

Thoracic horn with scale-like texture. Many Orthocladiinae have pointed spicules covering at least part of their thoracic horn including most of the *Parakiefferiella* group (Sæther 1983). In many species of *Parakiefferiella* this texture has become distinctly rounded, as can be found in *P. ferringtoni,* which may indicate relationships within *Parakiefferiella*.

Posterior margins of abdominal tergites and sternites with spines or strong spinules. Krenosmittia is the only member of the *Parakiefferiella* group with rows of erect triangular spines on the posterior margins of its abdominal tergites and sternites, though this character is found in several other genera (Coffman et al. 1986). Most notable is the *Pseudorthocladius* group, recovered as sister to the *Parakiefferiella* group by Mendes and Andersen (2008).

Some species of *Parakiefferiella*, including *P. ferringtoni*, have darkened subacute spinules along the posterior margins of the abdominal tergites, which may indicate relationships between them.

Posterior margin of Tergite II with medial spine patch. Hooklets on the posterior margin of Tergite II are found in the pupae of Chironominae, suggesting that the presence of hooks or spines in the same area is plesiomorphic for Orthocladiinae – though the presence and type of hooks/spines are quite variable throughout the subfamily (Coffman et al. 1986). *Lapposmittia, Rheosmittia* and some *Parakiefferiella* (including *P. ferringtoni*) lack spines at the posterior margin of Tergite II. The polarity of this character is unclear.

Abdominal segments with three or fewer strong L *setae; with four or more strong* L *setae; with complete setal fringe. Acamptocladius* and *Lapposmittia* have four strong L setae on their posterior abdominal segments (Coffman et al. 1986), a potential synapomorphy uniting the two taxa. The L setae on segment VIII of *Acamptocladius* are additionally bifid (Coffman et al. 1986), a synapomorphy defining that genus. *Epoicocladius* has a uniform setal fringe on the lateral margins of its abdominal segments (Coffman et al. 1986), again serving as a diagnostic synapomorphy. The development of more extensive chaetotaxy on the abdomen could be a synapomorphy grouping these three taxa.

D_5 *seta trichoid;* D_5 *seta flattened and leaflike. Rheosmittia* is the only orthoclad with flattened, leaflike dorsal setae on the abdominal tergites (Coffman et al. 1986), a synapomorphy of the genus.

Anal lobes with serrations or spinules apically. Rheosmittia and some species of *Parakiefferiella* (including *P. ferringtoni*) have serrations/spines on the apical portion of the anal lobe (Langton 2023). This could be an indication of the close relationship between these taxa.

Margins of anal lobe straight; rounded basally. Krenosmittia, Rheosmittia and some species of *Parakiefferiella* such as *P. ferringtoni* and *P. gracillima* have the lateral margin of the anal lobe largely straight or evenly tapering to the apex (Coffman et al. 1986). The other taxa of the *Parakiefferiella* group have the lateral margin at the base of the anal lobe rounded, swelling somewhat laterally (Coffman et al. 1986). Based on the distribution of other characters within the *Parakiefferiella* group, the straight lateral margin was probably not homologous between *Krenosmittia* and the other taxa with that character state, though it may be a synapomorphy between *Rheosmittia* and those aberrant *Parakiefferiella.*

Anal lobe macrosetae present; reduced or absent. All members of the *Parakiefferiella* group have three macrosetae except for *Rheosmittia* (Coffman et al. 1986). There other informal taxa keyed by Langton (2023), either unplaced to genus (Orthocladiinae gen? sp? NA7 and NA8) or placed as *Parakiefferiella* (*P.* NA2 and NA3) that have a reduced number of anal setae or lack them entirely. *Parakiefferiella ferringtoni* has a variable number of reduced anal setae, suggesting there may be an evolutionary series leading to the reduction and finally absence of macrosetae.

Adults

Eye without ommatrichia; eye with ommatrichia. Rheosmittia is the only genus of the *Parakiefferiella* group with ommatrichia, though *Parakiefferiella scandica* Brundin also has them (Cranston and Sæther 1986), another instance of the blurred delineation between *Parakiefferiella* and *Rheosmittia*.

Adult male terminal maxillary palpomere length 2-10x width;<2x width; >10x width. Most taxa within the *Parakiefferiella* group have a "normal" terminal article of the maxillary palp 3–6x longer than wide (Cranston et al. 1989). *Lapposmittia* has a shortened apical palpomere <2x length/width (Cranston et al. 1989), which is probably an adaptation to its Arctic habitat, much as the male antenna has a reduced number of flagellomeres and lacks a plume. The elongate maxillary terminal palpomere of *Epoicocladius* (>10x length/width, Cranston et al. 1989) is clearly synapomorphic for that genus.

Scutum with tubercle or tuft. Acamptocladius, Epoicocladius, Lappokiefferiella and most *Parakiefferiella* have a tuft of setae located medially on the scutum (Cranston et al. 1989, Tuiskunen 1986), while some species of *Rheosmittia* have a tubercle in the same location (Caldwell 1996, Cranston and Sæther 1986, Cranston et al. 1989). This character is probably a synapomorphy for these genera, and its absence in *P. ferringtoni* and some *Rheosmittia* are probably reversals – suggesting that its absence in *Lapposmittia* may not be an indicator to exclude that genus from close relationship with *Acamptocladius*. Whether the structure presents as a tuft or tubercle may have some phylogenetic utility, as most of these groups have a setal tuft while *Rheosmittia* has a tubercle with no tuft. Interestingly, *Parakiefferiella coronata* Edwards has a combined tubercle and tuft, suggesting that the structure may have started as a setal tuft and then transitioned into a tubercle within *Parakiefferiella*.

Costa extending beyond R_{4+5}*. Acamptocladius, Lappokiefferiella* and *Lapposmittia* have the costa ending at R_{4+5}, while *Epoicocladius, Krenosmittia, Parakiefferiella, Qiniella* and *Rheosmittia* have C extending beyond R_{4+5} (Cranston et al. 1989). It is unclear which state represents the plesiomorphic condition within the group, and this character is variable between species in many genera outside the *Parakiefferiella* group (Cranston et al. 1989). The state of this character is unknown for *P. ferringtoni*, as we only had pharate specimens for examination.

Gonostylus curved apically. Having the apex of the gonostylus curved apically is a putative synapomorphy of *Parakiefferiella* + *Rheosmittia*. However, this character is found in unrelated orthoclads (ex. *Heleniella curtistyla* Sæther) and is subject to interpretation, as the apparent degree of curvature of the gonostylus can differ between specimens of the same species.

Gonostylus undivided; Gonostylus trifid. Qiniella is known solely from adults, defined by the male having a trifid gonostylus. Wang and Sæther (1998) noted in the initial description of the genus that they resembled *Krenosmittia* except for said distinctive gonostylus. As *Krenosmittia* adults are diagnosed within the *Parakiefferiella* group solely by plesiomorphies, this suggests *Qiniella* is outside the *Acamptocladius, Epoicocladius, Lappokiefferiella, Parakiefferiella, Rheosmittia* lineage. As the genus is based only on a single adult autapomorphy and the immatures are unknown *Qiniella* deserves further investigation as to whether it truly represents a distinct genus or just an unusual species group within one of the other genera.

Virga present; absent. All taxa within the *Parakiefferiella* group have a virga except *Acamptocladius* (Cranston et al. 1989), its absence is thus a synapomorphy of that genus.

The monophyly of Parakiefferiella and status of Lappokiefferiella and Rheosmittia.

Parakiefferiella been noted as in need of revision in North America (Epler 2001, Namayandeh pers. comm.) with many undescribed species and possible conspecificity of European names with Nearctic taxa (Epler 2001). Epler keyed seven larval taxa from the southeast United States, while Langton (2023) keyed seventeen pupal taxa, thirteen unnamed. With the description of *P. ferringtoni* there are only six named species of *Parakiefferiella* recorded from North America.

Understanding the diversity of *Parakiefferiella* is complicated by the ambiguous characters used to diagnose the genus. During our study we were able to find only one potential unambiguous synapomorphy for the genus, the presence of six larval flagellomeres. This is a very weak character, especially as the structure of *Rheosmittia*'s antenna could be derived from the *Parakiefferiella* configuration by the fusion of flagellomeres 2 and 3 as described above. The other characters used to diagnose *Parakiefferiella* within the genus group are: scutal tuft present, costa extended beyond R_{4+5}, and the gonostylus curved apically. The scutal tuft is shared with several other genera, and the evidently homologous tubercle seen in *Rheosmittia* can also be found in *P. coronata*. The extended costa is shared with *Epoicocladius, Krenosmittia* and *Rheosmittia*; if *Krenosmittia* is sister the remainder of the *Parakiefferiella* group this may represent a plesiomorphy. The curved gonostylus appears to be a synapomorphy within the *Parakiefferiella* group, but is shared with *Lappokiefferiella* and *Rheosmittia*. We are thus led to ask, is the current concept of *Parakiefferiella* paraphyletic if *Rheosmittia* and possibly *Lappokiefferiella* are treated as separate genera?

Rheosmittia is clearly a monophyletic group, with several synapomorphies related to its specialization in sand substrates during the immature stages as noted above. The curved gonostylus has been used to suggest a close relationship between *Rheosmittia* and *Parakiefferiella*, but as noted above the characters that separate *Parakiefferiella* are symplesiomorphies. There is one character of the larval mouthparts that could suggest a relationship between *Rheosmittia* and *Acamptocladius* + *Lappokiefferiella* (more than two apical teeth on the premandible), but this evidence seems weak since *Rheosmittia* has far more teeth in a comb-like arrangement that could have been independently derived from its psammophilic lifestyle. In our opinion the thread like terminal antennal flagellomere in the larva, curved gonostylus, and the reduction or absence of anal lobe macrosetae in the pupae of some *Parakiefferiella* indicate a closer relationship between that taxon and *Rheosmittia.* The reduction of anal lobe macrosetae, narrowing of the basal part of the anal lobe and serrations on the apical part of the anal lobe as found in *P. ferringtoni, P. gracillima* and some undescribed *Parakiefferiella* suggest an evolutionary series leading to *Rheosmittia*. Thus, we present the hypothesis that *Rheosmittia* is probably a morphologically divergent specialist lineage within *Parakiefferiella* as the latter is currently defined.

Lappokiefferiella is another enigmatic lineage which confounds our understanding of *Parakiefferiella*. Being known solely in the adult stage has hindered placement of *Lappokiefferiella platytarsus,* described in a monotypic genus because the species could not be conclusively placed between *Acamptocladius, Lapposmittia* or *Parakiefferiella* (Tuiskunen and Lindeberg, 1986). The species was excluded from *Acamptocladius* by the presence of a virga, and *Parakiefferiella* by the absence of a costal extension. Both characters have been found to be variable within genera outside the *Parakiefferiella* group (Sæther and Wang 1995, Cranston et al. 1989). It lacks the reduced antennal plume and small terminal segment of the maxillary palp found in *Lapposmittia*, though those are autapomorphies probably related to adaptations to the arctic environment as seen in numerous other groups. The gonostylus appears somewhat curved apically, which could indicate this species is just another aberrant *Parakiefferiella*, as the extent of costal extension is variable in genera outside the *Parakiefferiella* group. Being defined solely by a lack synapomorphies and lacking autapomorphies of its own, *L. platytarsus* is possibly an aberrant species of one of the three aforementioned genera. Finding the immature stages should settle the placement of this species.

The current generic classification of Orthocladiinae needs substantial revision, as many genera are based on non-cladistic morphological character formulae instead of synapomorphies. This limits the utility of genera, since many are artificial and potentially unstable assemblages of species. Numerous genera have been described based on autapomorphic characters without consideration of whether they may represent a derived group within another genus, while others have been established for species that do not fit an existing generic diagnosis or “don’t key out properly.”

While we encountered non-cladistic genera in the *Parakiefferiella* group, the problem is by no means limited to this group. Most groupings of orthoclad genera the first author examined in detail contain probable paraphyletic taxa and genera defined by non-synapomorphies to more or less of an extent. Resolving these issues is complicated by the fact that there has yet to be a definitive phylogeny covering the majority of genera in the subfamily, which is needed to provide a backbone to understand character polarity and discover proper synapomorphies. This has created something of a “chicken and egg” conundrum: to create an accurate phylogeny to understand generic synapomorphies requires revision at the species level, yet to properly place species in genera requires a cladistic framework.

Conclusion

Parakiefferiella ferringtoni is an unusual species of *Parakiefferiella* found in the Western U.S. Amended keys for all three life stages of Nearctic *Parakiefferiella* including *P. ferringtoni* should aid in taxonomic and ecological research. Documenting and describing this species uncovered problems with how genera are delineated in the *Parakiefferiella* group. While six of the eight genera we included within the group appear monophyletic, *Parakiefferiella* is probably paraphyletic with *Rheosmittia* as a specialized psammophilic lineage arising within it. *Lappokiefferiella* is dubiously distinct, and discovery of the immature stages should help solidify the placement of that monotypic genus.

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LONG-TERM EMERGENCE PATTERNS OF CHIRONOMIDAE (DIPTERA) FROM AN INTERMITTENT STREAM

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Abstract

In non-perennial aquatic habitats, dry periods influence the aquatic community by filtering organisms based on their ability to survive periods of drying or to recolonize the habitat quickly. Although intermittent streams often support interesting, specialized species, they often support a less species-rich community compared to perennial habitats. I collected Chironomidae from an intermittent, 3rd order stream biweekly for 6 years using surface floating pupal exuviae. The stream regularly dried in the summer every year, but the duration of the dry period varied among years (15-146 days). In total, 158 non-biting midge taxa were identified, but many species (65) were rare and only occurred in 1-2 samples. Most common taxa had regular seasonal emergence patterns, but some taxa had irregular emergence patterns that differed year to year. Following dry periods, Chironomidae resumed emergence within 2-3 weeks after the stream began flowing again and, in some years, sample richness was higher after flow resumed than before. The length of the dry period in the stream did not affect annual taxa richness, but lower annual taxa richness was observed in years with higher accumulated discharge and more frequent summer spates. Comparison of midge communities from this intermittent site to a downstream perennial site found no difference in taxonomic richness. However, the emergence period was shorter and the number of taxa present in individual samples was lower at the intermittent site. The equivalent richness was achieved in the intermittent site through higher temporal β diversity. This pattern may have been the result of higher disturbance in the intermittent site due to spates and drying which could contribute to greater habitat heterogeneity and promote colonization of new taxa. Most taxa at the perennial site were also found in the intermittent site indicating that many midge taxa in these habitats are facultative. Intermittent streams are usually expected to only support a depauperate community of facultative species, but these habitats can support interesting and diverse communities that should be conserved.

Introduction

Intermittent and ephemeral streams comprise more than half of stream miles in the United States with the proportion of non-perennial aquatic habitats increasing in arid regions (Levick et al. 2008, Fesenmyer et al. 2021). However, society often assigns lower value to intermittent and ephemeral streams, wetlands, and lakes compared to perennial habitats. As a result, non-perennial habitats are afforded less stringent protections from human activities including prevention of water quality degradation or destruction through dredging and filling (Fesenmyer et al. 2021). The importance of these habitats is often minimized because they are thought to provide fewer benefits to humans and wildlife. Non-perennial habitats are often considered to support lower biodiversity and have limited human uses such as fishing and boating. However, they provide many important benefits such as flood mitigation, processing and sequestration of pollutants, habitat for aquatic and terrestrial life, and cultural or aesthetic benefits (Koundouri et al. 2017). Given the large proportion of stream miles that can be classified as non-perennial and the important benefits these waters provide, there is value in protecting these habitats.

Undervaluation of intermittent streams is associated with the most obvious characteristic of these waters – they are dry for part of the year. These dry periods suggest that for part of the year, the habitat does not provide the benefits that would typically be supported by an environment with surface water (e.g., fishing, boating, aesthetics). Drying streams may also be viewed negatively as they may become pooled and a source for organisms that can be harmful to humans (e.g., mosquitoes; Williams 1987). Furthermore, dry periods in intermittent habitats appear to make the habitat unsuitable for aquatic organisms which reduces biodiversity. This assumption is not unreasonable because the drying of an aquatic habitat is a type of disturbance that can have negative impacts on aquatic organisms. Stream discharge can also be more variable in non-perennial streams, and these habitats may be exposed to both dry periods and disruptive spates. In addition to hydrology, chemical and physical parameters are also more variable in intermittent streams which create additional challenges for organisms in these waters (Williams 1987). Temperature and dissolved oxygen can vary more seasonally and daily than in perennial streams due to smaller water volumes and periods of low flow. The challenges of surviving in intermittent streams creates a filter that eliminates many aquatic organisms not adapted to such conditions (Wiggins et al. 1980). As a result, ephemeral and intermittent streams have been demonstrated to support lower species richness and diversity compared to similar perennial habitats (Williams 1996, Williams 2006, Meyer and Meyer 2000, Rüegg and Robinson 2004). However, species inhabiting non-perennial habitats have a variety of physiological and behavioral attributes that allow survival during dry periods (e.g., drying resistant life stages [eggs, cocoons], movement to hyporheic zones, rapid colonization). As a result, non-perennial habitats can still support a diverse and unique assemblage or aquatic organisms.

The family Chironomidae includes species that are fully aquatic, semiaquatic, and terrestrial. Although most species are considered fully aquatic, there are aquatic species that are facultative and can survive dry periods. Drought-resistance strategies observed in Chironomidae include drought resistant larvae, formation of cocoons, movement to wetted habitats (e.g., hyporheic zone or pools), and rapid recolonization (Blanchart et al. 1987, Frouz et al. 2003, Frouz and Matěna 2015). However, many detailed studies of drought resistance in chironomids have focused on unusual ephemeral habitats (e.g., Hinton 1960, Kitching 1972, McLachlan and Ladle 2001). As a result, the specific mechanisms used by most chironomid species in ephemeral and intermittent streams are not as well studied or known (Frouz et al. 2003). Generally, chironomids in intermittent streams likely use a variety of strategies observed in other aquatic invertebrates and in chironomids in other habitats. Studies documenting the chironomid diversity from temporary streams have reported both lower (Boulton and Suter 1986, Rüegg and Robinson 2004) or equivalent (Feminella 1996, Casas and Langton 2008) taxa richness in temporary streams compared to perennial streams. In some cases, the richness of chironomids exceeded 100 taxa in intermittent streams (e.g., Casas and Langton (2008)). Given the high diversity in this family and the range of strategies for surviving dry conditions observed in the Chironomidae, this family is an important component of intermittent habitats (e.g., Williams 1996, Rüegg and Robinson 2004).

The objective of this study was to document the diversity and emergence patterns of chironomids from an intermittent stream over multiple years. Long-term studies of aquatic insects are limited due to the effort required, but they may provide additional insight into how environmental factors, particularly antecedent conditions, influence the diversity of aquatic communities and the phenology of species. I studied the emergence of chironomids over six years from an intermittent stream site to document the diversity of midges in an intermittent habitat and to determine if there are species that are dependent on these habitats. In addition, by sampling this stream for six years, the annual variability in hydrology can be documented and linked to attributes of the chironomid community (e.g., α and β diversity, phenology). In this study, I also compared Chironomidae emergence from the intermittent stream reach to a perennial reach on the same stream. Overall, the objective is to characterize chironomid communities in intermittent streams and to describe how changes in hydrology affect this biological assemblage. This information is important to develop strategies to conserve these habitats as they face risks from anthropogenic alteration, water pollution, and climate change.

Materials and Methods

Site descriptions

The main study site was located on the Credit River, a 3rd order stream with a 60 km^2 drainage area (Figure 1a; Table 1). The watershed is dominated by forest, row crop, and open field, but the stream at the sample site is located within or borders a regional park and is protected. The riparian zone at the sample site and approximately 2 km upstream from the sample site is forested. As a result, the site was largely shaded, and extensive leaf matter was present in the stream after leaf abscission. Although the stream was well shaded, periphyton, filamentous algae, and mosses were present and sometimes in high abundance. When flow was present, the stream width was on average 6.1 m wide. The proportion of different substrate types was visually estimated during sampling and was comprised of 16% boulder, 30% cobble, 24% gravel, 20% sand, and 11% silt. During periods when the stream was flowing, the sample reach consisted of 55% run, 35% riffle, and 10% pool. During three sampling visits, no flow was detected and only pool habitats were present.

Intermittent streams are defined as channels that do not flow during certain times of the year, but during the rest of the year have sustained flows

Figure 1. Credit River sampling sites on May 19, 2004: a) intermittent site and b) perennial site.

Table 1. Description of study sites.

Stream name	Hydrology	County	Coordinates (WGS 84)	Elevation (m)	SampleYear(s)
Credit River	Intermittent	Scott	44.7086°, -93.3506	280	2003-2008
Credit River	Perennial	Scott	44.7608°, -93.3424°	240	2004

(USEPA and USACE 2020). This contrasts with ephemeral streams which may only flow in response to precipitation events. The intermittent stream studied here meets this definition as it did not only flow in response to rainfall but rather it flowed during much of the year and regularly dried in the late summer or fall (Figure S1). The length of the period the stream was dry varied from year to year and ranged from 15-146 d. This stream is surface-water fed and receives most of its water from surface runoff and shallow subsurface flow and not thermally, buffered groundwater. As a result, the stream was completely frozen during the winter although during warm periods some winters, the stream was open or there were small openings in the ice cover. For example, the winter of 2005/2006 was warmer than other years and the stream was only frozen for the first part of the winter (December-January). Typically, this reach of the Credit River was frozen during the entire winter. It thawed in March and flowed continuously until it dried in July or August. The stream remained dry until it rewetted in September (often following fall leaf abscission) except for fall 2003 when it did not flow again until the following March. Although there was normally only one dry period, in 2008 the summer and fall was drier than usual and there were multiple drying events in the fall. After the stream began flowing again in the fall, it became partially or wholly ice covered in December.

A perennial site on the Credit River was also sampled for comparison with the intermittent site (Figure 1b; Table 1). The perennial site was approximately 14 km downstream of the intermittent site and was also a 3rd order stream although the drainage area was twice as large as the upstream site (120 km^2). The watershed was dominated by developed land, open field, forest, and row crop. Although surrounded by a developed watershed, the stream near the sample site had a largely forested riparian zone. The stream was on average 4.8 m wide. Substrates were visually estimated during sampling and were comprised of 4% boulder, 26% cobble, 28% gravel, 23% sand, and 19% silt. The sample reach consisted of 30% run, 27% riffle, and 44% pool. The downstream site on the Credit River was similar to the upstream site in many regards, but differed in one important attribute for this study, the downstream site was perennial. Discharge differed between the two stream sites in 2004, although the stream widths were similar. Discharge during sample events averaged 0.18 (0.00-1.23) m^3/s at the intermittent site and 0.42 (0.06-3.27) m^3/s at the perennial site. There were some other differences between these stream sites that could have affected aquatic life. This downstream reach likely receives more groundwater and is warmer in the winter than the upstream reach. As a result, parts of the perennial stream reach remained open most of the winter and some emergence of midges were observed throughout the winter when air temperatures were suitable. The composition of substrates also differed slightly, and water quality may also differ but both sites are affected by anthropogenic impacts including increased nutrient loading and elevated chloride (MPCA 2018). Although these differences were present between the two sites, it was assumed that hydrological differences would be the most important difference affecting the Chironomidae communities between sites.

Stream discharge measurement and modelling

Stream discharge for the intermittent site on the Credit River was measured or modeled using several methods. Discharge was directly measured during 13 sampling visits using a Marsh-McBirney Flo-Mate 2000. To determine stream discharge, water velocity was measured 4/10 from the bottom of the stream at a minimum of eight locations along a cross-section transect in a stream run. Discharge was calculated by multiplying the water velocity for each interval by the interval area (i.e., depth multiplied by the interval width) and summing these values. For 67 visits, the stream cross-section area was measured, and stage height was measured during 21 visits. Flow was not measured when ice cover or extensive edge ice was present or when the stream was pooled or dry. In addition, on four visits no stream discharge information was collected. To estimate stream discharge for all sample events when discharge was not directly measured at the intermittent site on the Credit River, several models were developed including: 1) cross section area to stream discharge, 2) stage to cross section area, and 3) Credit River stream discharge from a nearby, continuously-gaged stream site (Vermillion River). A linear model was initially considered for the cross-section area to stream discharge model, but it was apparent that despite a high R^2, the relationship was nonlinear. The best fit was determined to be a combination of models (Figure 2a). At cross-section areas below 1 m^2, a second-order, polynomial model was used (Discharge = 0.2138 • Cross Section Area^2 - 0.0152 • Cross Section Area – 0.0001, $R^2 = 0.97$) and above a cross-section are of 1 m^2, a logarithmic model was used (Discharge = 1.0642 • ln(Cross Section Area) + 0.1961; $R^2 = 0.99$). This approach approximated the results of a generalized additive model but permitted extrapolation for visits when the cross-section area was >2 m^2. A least-squares regression was fitted to estimate stream cross-section area from stage height (Cross Section Area = 6.0268 • Stage + 0.6183; $R^2 = 0.99$; Figure 2b). To estimate daily discharge at the Credit River site, stream discharge data was obtained from a nearby stream site with continuous discharge measurements during the sampling period (2003-2008). A United States Geological Survey (USGS) station on the Vermillion River (near Empire, MN; station number: 05345000) was selected as this was the closest station (24 km) with continuous data and on a similarly sized stream. A segmented regression model (Figure 3) was used to estimate stream discharge at the Credit River site using the Vermillion River gage data (< 4.836 °C: Credit Discharge = 0.0142 • Vermillion Discharge - 0.0237; > 4.836 °C: Credit Discharge = 0.1121 • Vermillion Discharge - 0.4974; $R^2 = 0.82$). Using the Vermillion River model, stream discharge at the intermittent Credit River site could be estimated for days when the site was not visited or not measured. Stream discharge was not estimated for days the stream was frozen. Polynomial and logarithmic models were fitted using the "lm" function (R Core Team 2025). Segmented regression was performed using the "segmented" function in the segmented package (Muggeo 2008).

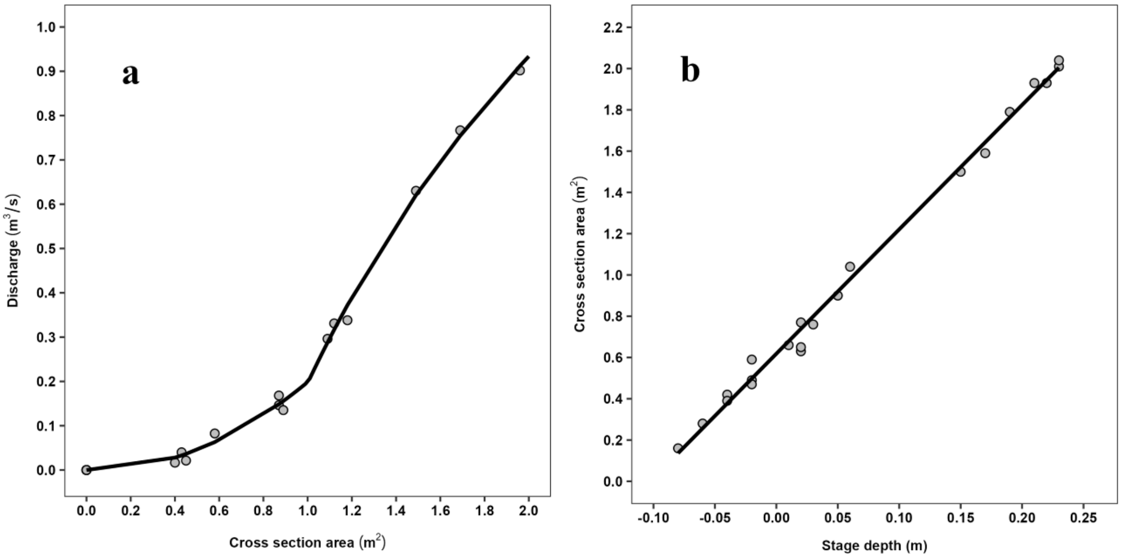

Figure 2. Models used to estimate discharge at the intermittent sites on the Credit River using a) cross section to model discharge ($R^2 = 0.97$-0.99, n = 54) and b) stage to model cross section area ($R^2 = 0.99$, n = 21).

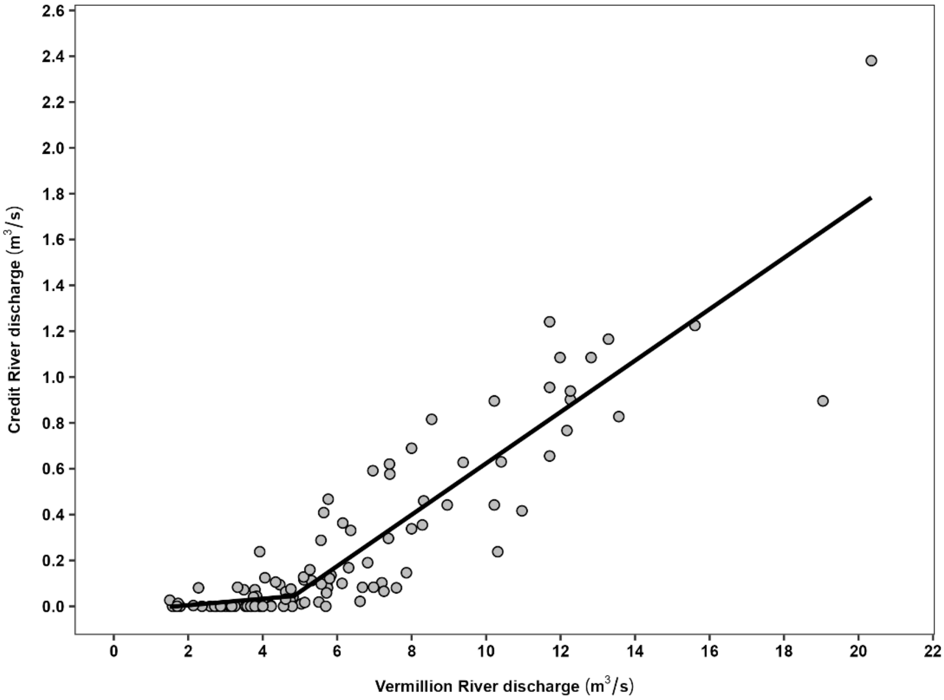

Figure 3. Model used to estimate flow at the intermittent site on the Credit River using stream gage data from a site on the Vermilion River ($R^2 = 0.82$, n = 109).

Water temperature measurement and modelling

Onset HOBO TidbiT temperature loggers were deployed in both Credit River sites. In the intermittent Credit River site, a temperature logger was deployed from March 27, 2003 through September 8, 2004. A temperature logger was deployed in the perennial site from January 9, 2024 through September 8, 2004. Water temperature was measured every 15 minutes during deployment. Using the 15-minute interval readings mean daily temperatures were determined by averaging temperature readings during each 24-hour period. In the intermittent site, there were periods when the temperature logger recorded air temperature, and these measurements were removed from the dataset.

To estimate water temperatures in both stream sites when water temperature was not directly measured, models were developed to estimate water temperature using air temperature data from a station at the Minneapolis Flying Cloud Airport (Midwestern Regional Climate Center 2024). This station was 11 km (perennial site) and 15 km (intermittent site) from the sampling stations. A least squares regression was used to fit the relationship between the three-day average of air temperature and water temperature (Water Temperature = 1.0455 • 3-Day Average Air Temperature - 0.54869; $R^2 = 0.92$; Figure 4a). When this model predicted negative mean water temperatures, these values were replaced with 0 °C as it was assumed that water temperatures would not be much below 0 °C. A simple linear regression was used for the intermittent site because there were few recordings at low air temperatures due to the lack of flow during the winter of 2003. However, this was not the case for the perennial site and a segmented regression was used to fit the broken-stick relationship. Linear models were fitted using the "lm" function (R Core Team 2025). Segmented regression was performed using the "segmented" function in the segmented package (Muggeo 2008). The breakpoint was determined to be at a three-day average of air temperature of -2.189 °C and different linear regressions were fitted to the data on either side of this breakpoint (< -2.189 °C: Water Temperature = 0.0063 • 3-Day Average Air Temperature + 0.4007; > -2.189 °C: Water Temperature = 0.8198 • 3-Day Average Air Temperature + 2.1819; $R^2 = 0.95$; Figure 4b).

Collection and processing of surface-floating pupal exuviae samples

Chironomids are often the most taxonomically rich group in many aquatic habitats (Coffman 1973) and this high richness, along with challenges with identifying chironomid larvae, make the study of whole midge communities in detail difficult. Sampling of surface floating pupal exuviae (SFPE) is more effective relative to common methods of collecting larval chironomids from benthic sediments because it is easier to collect taxa from a

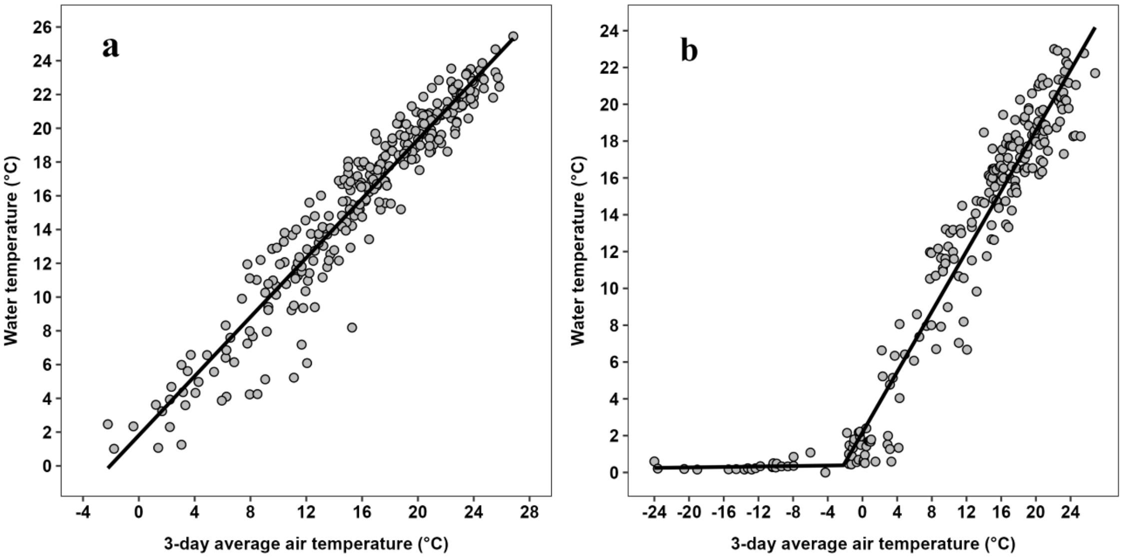

Figure 4. Regression of 3-day mean daily air temperature measures from Minneapolis Flying Cloud Airport and mean daily water temperatures from a) intermittent and b) perennial Credit River sites.

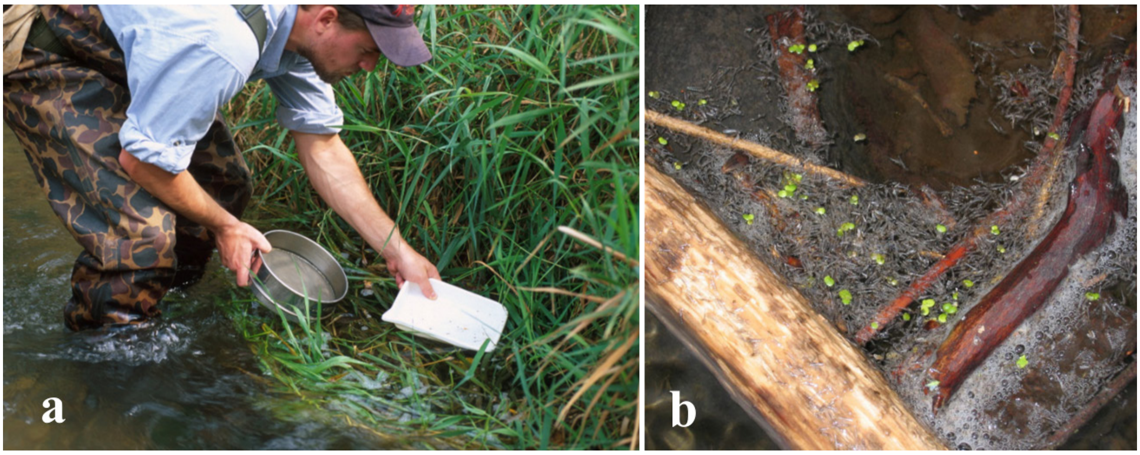

Figure 5. a) Sampling surface floating pupal exvuiae from vegetation and b) accumulation of pupal exuviae behind woody debris.

wide range of habitats, including those difficult to sample with a kick net (e.g., wood, hyporheos, deep water; Wilson 1994, Raunio et al., 2007, Ruse 2010, Kranzfelder et al., 2015). This method permits determination of timing of emergence and can largely produce species-level data. Chironomidae SFPE samples were collected bi-weekly following the methods of Ferrington et al. (1991) and Kranzfelder et al. (2015). The intermittent site was visited 154 times and the perennial site 26 times. If the stream was dry or completely frozen, no sample was collected. Samples consisted of a timed 10-minute sampling period within an approximately 100 m sample reach. Working upstream, SFPE were sampled by scooping exuviae into a pan from areas where they collect (e.g., snags, in vegetation, eddies) and pouring this material through a 125-µm sieve (i.e., standard testing sieve no. 120) (Figure 5). Samples were transferred to 118-ml jars and preserved with 75% ethanol.

In the laboratory, samples were sieved and rinsed in a 125-µm sieve to remove the preservative. A small portion of the sample was placed in a picking tray, and under a dissecting microscope, SFPE were picked from the sample into 3.7-ml vials with 75% ethanol. After a complete pass of the sample tray, the sample was swirled and scanned again. This was repeated until two successive passes did not recover any additional SFPE. Picking of specimens was halted when the entire sample was picked or the total number reached 300 specimens (Bouchard and Ferrington 2011). Specimens that were broken, covered in extensive fungal hyphae, or dried and compressed were not picked or counted to avoid identification problems or uncertainty regarding the age of the SFPE. Whole pupae or SFPE with adults still attached were also not counted; however, this material was picked from the sample to aid with identification of SFPE. Before slide mounting, specimens were divided into morphotaxa under a dissecting microscope. Exuviae were dehydrated in 95% ethanol, dissected, and slide mounted in Euparal as described by Ferrington and Berg (2019). In cases where a particular morphotype was abundant (>40 specimens), only 25% of these specimens were slide mounted and identified. The identity of the remaining unmounted specimens was estimated based on the proportions of each taxon in the slide mounted material. Identifications were made under a compound microscope at 100-400X. Genus-level and some species group identifications were made using Ferrington and Berg (2019) and Wiederholm (1986). Species-level identifications and species-group identifications were based on numerous additional sources. Morphospecies that did not match known pupae were assigned morphospecies codes. Morphospecies with "RWB" are part of the author's taxon numbering system for North American pupal exuviae and morphospecies with "NA" are from Langton (2023).

Analyses

All statistical analyses and plots were generated using R 4.4.2 (R Core Team, 2024). Species richness estimates for the cumulative dataset and for each sampling year were calculated using the nonparametric Chao1 estimator from the "wiqid" package (Meredith 2022). The relationship between annual accumulated discharge and temperature (water and air) on annual taxa richness was assessed using generalized additive models. Accumulated degree days for air and water temperature was calculated by summing the mean daily temperature above a reference temperature of 10 °C. Accumulated annual discharge was determined by estimating the total discharge (cubic hectometers) per year. Generalized additive models were fitted using the "gam" function in the "mgcv" package (Wood 2017).

Taxa richness and taxon specific patterns were visually assessed against temperature, stream discharge, and seasonal timing to identify emergence patterns that may be related to exogenous cues (e.g., temperature and discharge). Emergence patterns were categorized based on the timing of emergence peaks. Emergence pattern categories included: winter, winter/spring, spring, spring/summer, spring/fall, summer, summer/fall, fall, continuous, and irregular. Continuous emerging taxa were present through much of the spring, summer, and fall. Taxa with irregular emergence patterns emerged during different seasons year to year. Some of these taxa with irregular emergence patterns may have had relatively synchronous emergence peaks, but the timing varied among years. Some taxa had low total abundance and there is lower confidence in the assigned emergence patterns for these taxa.

To analyze how the community changed as a function of time between samples (i.e., taxonomic turnover), dissimilarity between sample pairs was calculated using Bray-Curtis dissimilarity coefficients for 2004 samples for both the intermittent and perennial sites. Calculation of dissimilarity was limited to sample pairs during the period of observed emergence from the intermittent site (April 7 through November 17). The winter, early spring, and late fall samples were eliminated to compare sample dissimilarities for the same time period from both sites. Time between samples was used as a variable so it was important that the time frame was consistent between the two sites. Bray-Curtis dissimilarity coefficients were calculated using "vegdist" function in the "vegan" package (Oksanen et al., 2020). The average dissimilarity values were graphed as a function of the number of weeks between each sample pair.

Results

Discharge and temperature patterns

Discharge and water temperature followed largely predictable patterns across the 6 years of sampling from the intermittent site (Figure 6). The thermal regime in the intermittent site followed a regular seasonal pattern with mean daily water temperatures in the winter near 0 °C and summer maximum temperatures usually >20 °C (Figure 6). Discharge was not as predictable as water temperature, but in general discharge was higher in the spring and lower in late summer and fall (Figure 6). Every year a series of spates were observed, but their timing varied among years. There were always a series of spates during the spring and summer, however, in some years, e.g., 2004 and 2005, there were also late summer/fall spates. The number of days the stream flowed each year varied from 219 to 350 days (Table 2). The length of individual dry periods also varied between years and lasted from 2 weeks (2005) to 7 months (2003/2004); however, in most years, the dry period lasted 4 to 6 weeks (2004, 2006, 2007, 2008). The intermittent site regularly dried in July or August and in most years rewetted in August or September. In two years (2003, 2008), the late summer or fall

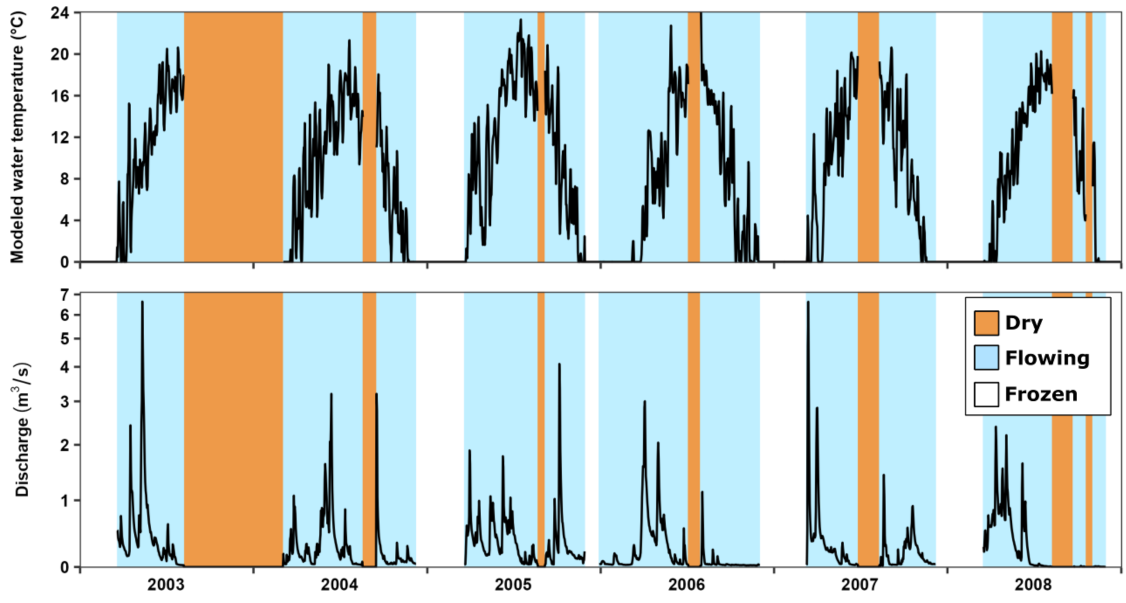

Figure 6. Long-term (2003-2008) estimated temperature (top) and discharge (bottom) for the intermittent Credit River site.

Table 2. Annual taxa richness patterns from the intermittent site on the Credit River.

Year	Days with flow	Total taxa	% Cumulative site richness	New taxa	Cumulative taxa	Unique taxa
2003	219	67	42%	67	67	5
2004	273	90	57%	38	105	5
2005	350	61	39%	6	111	3
2006	340	103	65%	22	133	14
2007	320	91	58%	19	152	17
2008	307	87	55%	7	158	7

was drier than normal. In 2003, the stream did not rewet until the next spring and in 2008, there were multiple dry periods with discharge remaining low when flow resumed.

Intermittent stream emergence patterns

The intermittent site on the Credit River was visited 154 times and a SFPE sample was collected on 99 of these visits. Samples were not collected during 55 visits because the stream was dry (27 visits) or frozen (28 visits). In 14 of the SFPE samples collected, no exuviae were present. The remaining 85 samples produced at least one specimen. Over 6 years of sampling, a total of 14,560 specimens were picked and identified, which included 158 species and morphotaxa. The number of taxa collected each year ranged from 61 to 103 taxa (Table 2). On average, 83 (15 std. dev.) taxa or approximately half of the cumulative 6-year total, was collected each year. New taxa were collected each sample year indicating that not all taxa at this site were detected over the 6 years of sampling (Table 2; Figure 7). The Chao1 richness estimator, projected that 199 taxa (175-256) were present at the intermittent site during the 6-year sample period and therefore (Table 3), 41 taxa were missed by this sampling effort. The plurality of taxa (32%) were only collected in a single year, but 19% of taxa were collected in all six years (Table S1).

Taxa richness per sample followed a regular seasonal pattern with higher taxa richness in samples from late spring to early fall and lower richness in the early spring and late fall (Figure 8). Although richness was low in the spring, emergence was detected immediately or shortly after the stream thawed or began flowing again in the spring. The timing of the highest richness observed differed between years, and occurred in late spring, summer, or early fall (May, June, July, August, or September; Figure 8). For example, peak emergence richness was detected in May in 2005, but in 2006 the greatest richness was in August. In most years, the peak in richness of emerging chironomids occurred before the stream dried in July or August (Figure 8). However, in some years higher (2006) or equal (2007) richness was observed in the late summer or fall after the stream began to flow again

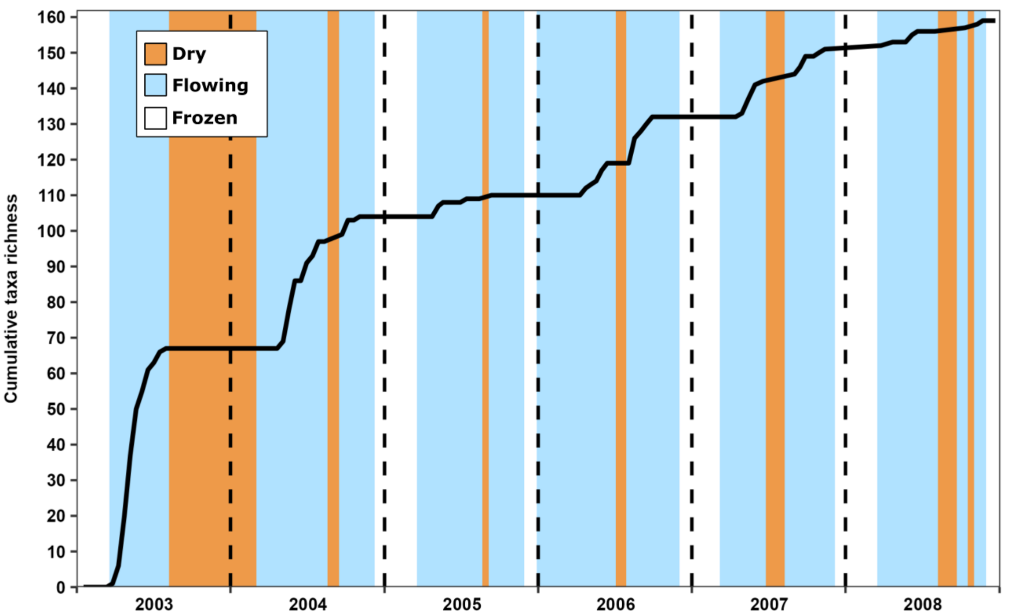

Figure 7. Taxa accumulation curve as a function of time for samples collected from the intermittent site on the Credit River.

Table 3. Total number of taxa collected each year with species richness estimates and 95% confidence intervals.

Year	Number of taxa	Chao estimate	Lower estimate	Upper estimate
2003	67	92	74	160
2004	90	119	101	169
2005	61	75	65	108
2006	103	175	131	287
2007	91	121	103	172
2008	87	126	101	195
All	158	199	175	256

following the dry period. After the stream resumed flowing in August or September, Chironomidae emergence quickly resumed and in most years, emergence was detected in the first sample collected after flow resumed (Figure 8). Emergence halted at or shortly before the stream became ice covered in December.

Total annual taxa richness was assessed against accumulated annual discharge, water temperature, and air temperature. Although 6 years of biweekly monitoring data is a relatively large dataset, when aggregated to annual totals, it results in only 6 data points and limited conclusions can be drawn from these results. However, there are possibly some patterns that can be observed with these data. The year with the lowest accumulated stream discharge (2006) had the highest taxa richness and the year with the highest accumulated stream discharge (2005) had the lowest taxa richness. Taxa richness declined with increasing annual discharge (adjusted $R^2 = 0.60$) although this pattern was largely driven by 2 years (2003, 2005; Figure 9a). Examination of discharge patterns over time in relation to sample taxa richness indicated a possible effect of spates on taxa richness. Sample taxa richness often declined following a spate (> $2m^3/s$) although this pattern was not observed following every spate (Figure 10). There was a unimodal taxa richness pattern along a gradient of increasing annual water temperature (adjusted $R^2 = 0.84$), although much of this pattern was due to a single year (2005). Finally, there was a weak, negative relationship between annual taxa richness and accumulated air temperature (adjusted $R^2 = 0.06$). Both accumulated temperature and stream discharge were highest in 2005, the year with the fewest taxa collected.

Different emergence patterns could be documented in the more abundant species which broadly included seasonal, continuous, and irregular emergence patterns (Table S1). Most taxa had distinct seasonal peaks that were generally consistent in terms of timing from year to year as would be expected in a temperate stream. The most common taxon in the study, *Hydrobaenus* RWB pe1, had a very large

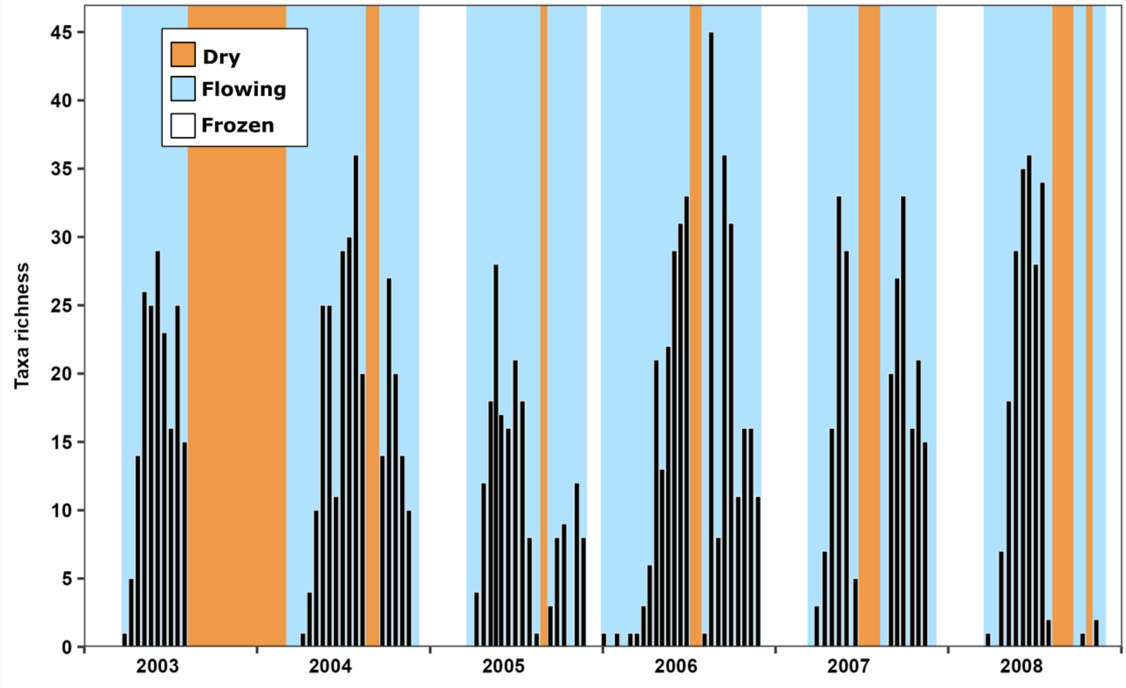

Figure 8. Taxa richness for individual samples from the intermittent site on the Credit River.

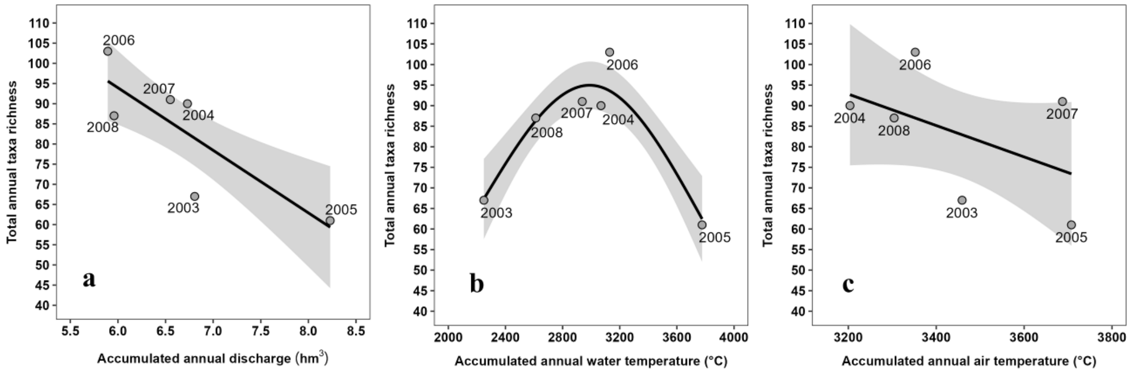

Figure 9. Total annual taxa richness as a function of accumulated a) annual discharge (modeled), b) water temperature (modeled), and c) air temperature (Minneapolis Flying Cloud Airport station).

and distinct emergence in the spring (Figure 11a). Other species that had a distinct spring emergence period included *Chaetocladius* RWB pe5, *Orthocladius* (*O.*) *clarkei*, and *Polypedilum* RWB pe8 (Table S1). Some species had two distinct emergence peaks during the spring and fall (Figure 11b; e.g., *Orthocladius* (*O.*) *obumbratus*, *Orthocladius* (*O.*) *mallochi*). Several species were restricted to summer emergence (Figure 11c; *Polypedilum* (*U.*) *obtusum*, *Microtendipes* RWB pe1, *Tanytarsus* RWB pe10, *Orthocladius* (*O.*) *carlatus*, and *Corynoneura* RWB pe3). Interestingly, several of these summer emerging taxa were absent or had low abundance in 2005 (*P.* (*U.*) *obtusum* (absent), *Microtendipes* RWB pe1 (absent), *Tanytarsus* RWB pe10 (low abundance)). Many of the most common taxa were categorized as having continuous emergence patterns as they were present during much of the open water season (Figure 11d; e.g., *Thienemanniella* RWB pe1, *Orthocladius* (*O.*) *oliveri*, *Hydrobaenus pillipes*, *Corynoneura* RWB pe1, *Parametriocnemus* RWB pe1, *Cricotopus* (*C.*) *bicinctus*, *Eukiefferiella* RWB pe2, *Tvetenia* RWB pe1, *Micropsectra nigripila*, *Paratanytarsus inopertus* gr. RWB pe1, *Parametriocnemus* RWB pe4). Many taxa had inconsistent emergence timing year to year (i.e., irregular) which was only detectable due to the long-term sampling effort (Figure 11e; e.g., *Tanytarsus lobiger*, *Orthocladius* (*Euorthocladius*) *abiskoensis*). Some taxa did appear to have patterns that suggested extirpation or colonization patterns. For example, *Rheotanytarsus exiguus* and *Polypedilum* (*Tripodura*) RWB. pe2 were present in high number in 2003, absent or largely absent from 2004-2006, and reappeared in 2007 and 2008. *Paratanytarsus inopertus* gr.

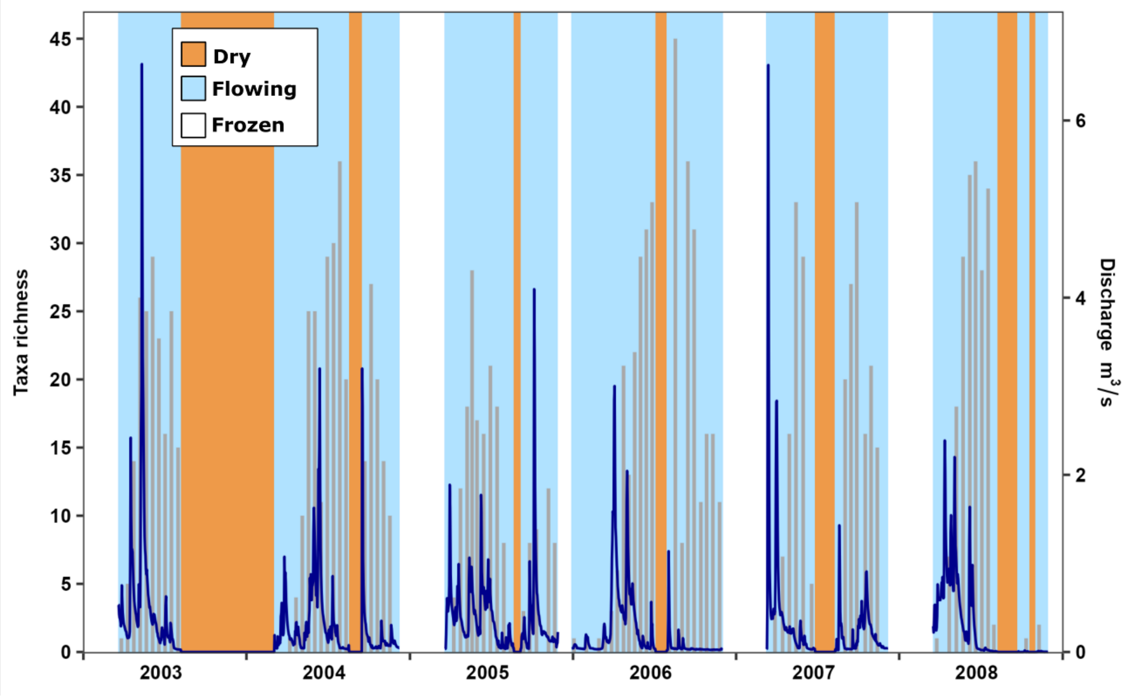

Figure 10. Sample taxa richness and modeled discharge (dark blue line) from the intermittent site on the Credit River.

RWB. pe3 was absent in 2003 but had a steady increase in abundance from 2004 to 2008. The specific drivers for these patterns in presence and abundance on an annual basis were not obvious.

Comparison with a perennial stream site

Seasonal trends in water temperature were similar between the intermittent and perennial sites on the Credit River, but water temperatures were more variable in the perennial site (Figure 12). The number of sample events from which SFPE were collected differed between the two sites (Figure 12). Fifteen samples from the intermittent site contained SFPE in 2004 compared to 23 samples with SFPE in the perennial site. The smaller number of sample events which produced SFPE in the intermittent site was due to greater ice cover during winter and the drying of the stream in late summer/early fall. The number of taxa present in samples differed between the two stream sites. At the perennial site, taxa richness of individual samples equaled or exceeded 25 taxa in 12 samples but at the intermittent site only 6 samples had at least 25 taxa (Figure 12). The average number of taxa per sample was 21 in the perennial site and 18 in the intermittent site. Despite these differences, total annual richness was identical at these sites in 2004 (90 taxa). The composition and pattern of Chironomidae subfamilies were similar between the two sites with Orthocladiinae taxa richness dominating most samples followed by Chironominae. Orthocladiinae was a large component of most samples throughout the year whereas Chironominae were only a high proportion of taxa richness during the spring, summer, and early fall. Tanypodinae, Diamesinae, and Prodiamesinae were a low proportion of the taxa with Tanypodinae occurring in spring and summer, Diamesinae largely limited to winter, early spring, and late fall, and Prodiamesinae collected sporadically throughout the year (Figure 12).

A total of 124 taxa were collected from both sites on the Credit River in 2004. Fifty-six (45.2%) of the taxa collected were shared between the two sites. A total of 68 taxa were only found at one site or the other with 34 (27.4%) taxa were unique to each site. When all 6 years of sampling from the intermittent site is included, 21 additional taxa that were collected from the perennial site were recorded from the intermittent site (13 taxa still not observed). Several of the taxa which were unique to the intermittent site are taxa associated with intermittent habitats including *Hydrobaenus* RWB pe1, *O.* (*Eu.*) *abiskoensis*, *Paraphaenocladius nasthecus*, and *Allocladius* sp. *Hydrobaenus* RWB pe1 was the most common species collected at the intermittent site, but it was completely absent from the perennial site. The most abundant taxon at the perennial site was *Parakiefferiella* RWB pe3 but this taxon was also present in moderate numbers in the intermittent site.

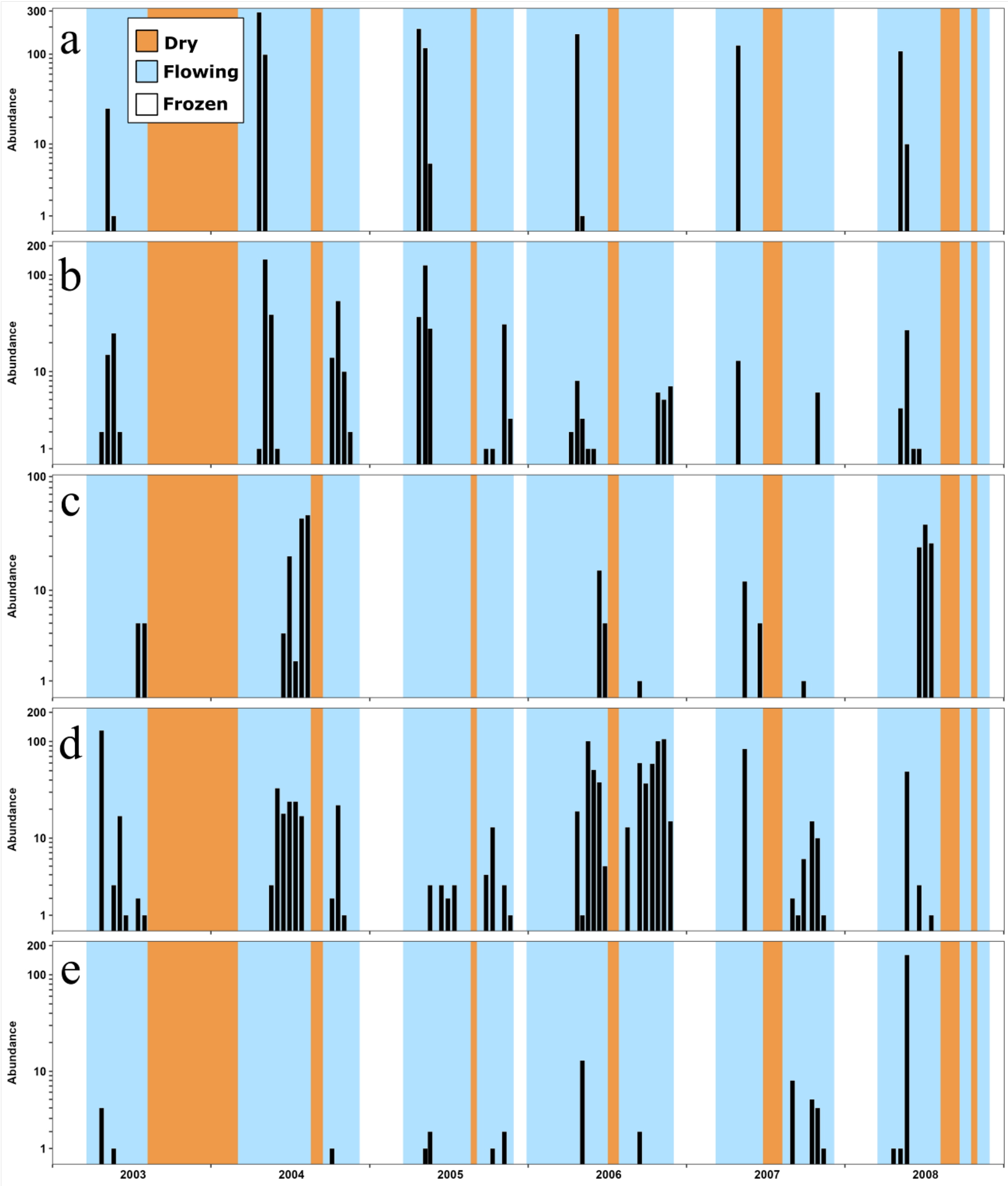

Figure 11. Relative abundance per sample during the six-year sampling period for example species: a. *Hydrobaenus* RWB pe1 (spring), d. *Orthocladius (O.) obumbratus* (spring/fall), c. *Microtendipes* RWB pe1 (summer), d. *Thienemanniella* RWB pe1 (continuous), and e. *Orthocladius (Euorthocladius) abiskoensis* (irregular).

Dissimilarity in taxa composition between sample dates was higher overall at the intermittent site (Figure 13). At the intermittent site, dissimilarity between samples was on average very high (0.88) and exceeded 0.90 when paired samples were separated by 8-18 weeks. Average dissimilarity for the perennial site was also high (0.72) with a largely consistent increase in dissimilarity as the time interval between samples increased. Both the intermittent and perennial sites had a drop in dissimilarity when paired samples were collected between 20 and 28 weeks apart. This decline was smaller and occurred at a higher temporal separation of samples in the perennial site. This decline in dissimilarity corresponded to a comparison of spring and fall samples indicating that these samples were more similar to each other than less temporally separated summer samples.

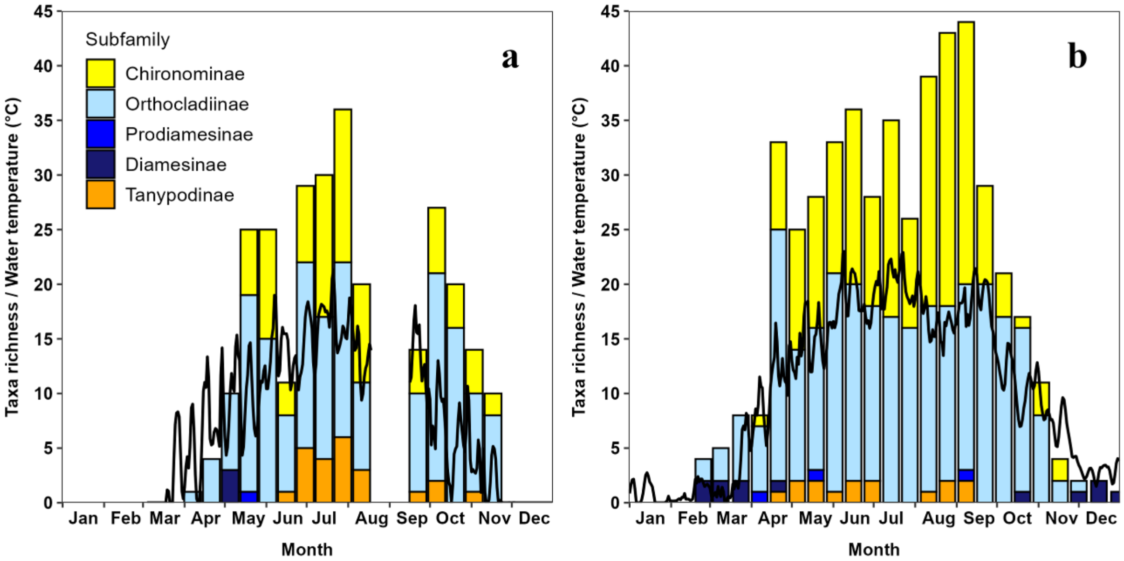

Figure 12. Subfamily taxa richness for each surface-floating pupal exuviae sample collected from a) intermittent and b) perennial sites on the Credit River. The black lines are daily mean water temperatures.

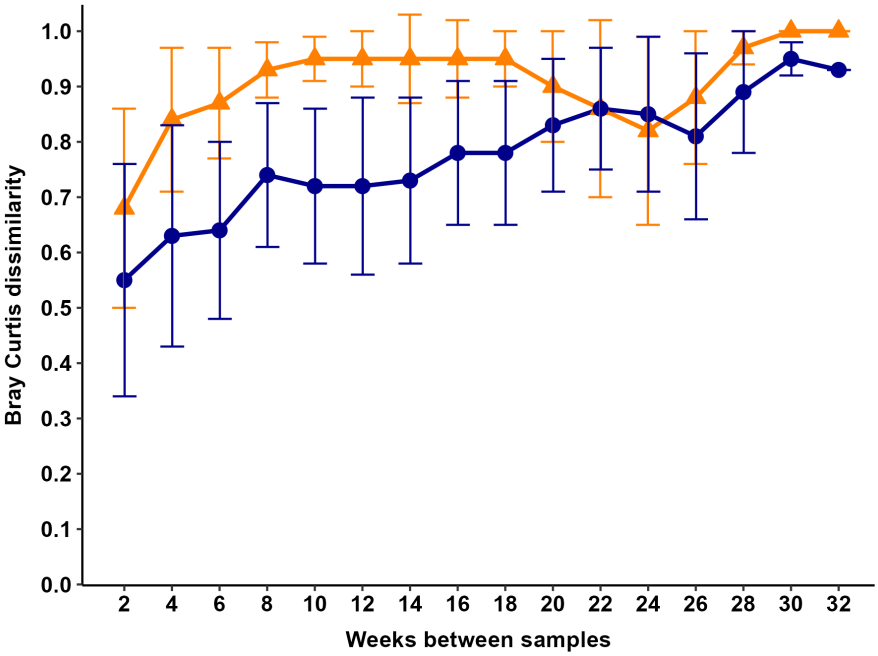

Figure 13. Average Bray Curtis dissimilarity as a function of time between samples for the intermittent (orange triangles) and perennial (blue circles) sites on the Credit River (bars bracketing the points are standard deviation).

Discussion

Comparison with a perennial stream site

Both intermittent and perennial sites were sampled on the Credit River in 2004 to provide a comparison of Chironomidae diversity patterns between intermittent and perennial stream reaches. The most distinct and presumably most important difference between these sites is stream discharge. There were other differences in the characteristics of these sites including thermal regime, substrates, and water quality that could affect the midge community. However, these factors only differed minimally between sites and were assumed to affect chironomids less than discharge. Furthermore, these smaller differences in stream attributes should not obscure important changes in the midges resulting from hydrological differences. Contrary to expectations, total annual taxa richness was identical at the intermittent and perennial sites during 2004 (90 taxa). The intermittent site matched the taxa richness at the perennial site even though there were 8 fewer samples containing SFPE from the intermittent site (15 sample intermittent, 23 samples perennial). In addition, there were on average fewer taxa in individual samples from the intermittent site compared to the perennial site. The equal total annual taxa richness was achieved in the intermittent site through higher temporal β diversity between samples. This attribute was also apparent in the higher dissimilarity between paired samples, especially between paired samples separated by short temporal gaps (2-18 weeks). The more variable discharge (e.g., more disruptive spates and dry periods) may have contributed to the high dissimilarity between summer samples at the intermittent site. Higher species turnover and diversity in intermittent streams may be the result of more colonization of new species due to spates that alter and disrupt substrates and dry periods (Boulton and Suter 1986, Chou et al. 1999, Casas and Langton 2008).

Aquatic insect species may be divided into different categories based on their tolerance of flow permanence including: 1) taxa found only in perennial habitats, 2) facultative taxa that are found both in perennial and temporary habitats, and 3) specialist taxa in temporary habitats (Williams and Hynes 1977, Feminella 1996). Examination of the taxa present in the intermittent and perennial sites on the Credit River permits categorization of taxa into these types of tolerance to flow permanence. Forty-five percent of taxa were collected from both sites and therefore these stream reaches shared a high proportion of their chironomid communities. However, the majority of taxa were only collected at one of the sites indicating differences between the intermittent and perennial sites. Large differences between the aquatic insect and chironomid communities in perennial and intermittent streams have been documented (Dance and Hynes 1979, Wiggins et al. 1980, Williams 1987, Williams 1996). However, some of the differences between the two sites in my study could be a failure to detect some rare species that are actually present at both sites. When including all 6 years of sampling at the intermittent site, only 13 taxa recorded at the perennial site did not occur in the intermittent site samples. This does not consider additional taxa that would have been likely collected from the perennial site if 5 more years of sampling was performed, but it suggests that given a sufficient sampling effort, most taxa likely occur at both sites. If the taxa collected at the intermittent site are compared to a larger study of 12 perennial streams in the region (Bouchard 2007), only 10% (28 taxa) of the taxa collected in the intermittent site were not collected from the perennial streams. Many of the species inhabiting perennial streams appear to be tolerant to dry periods or at least capable of recolonization following dry periods. My study supports the patterns observed by others demonstrating that most species are facultative and occur in both perennial and intermittent streams (e.g., Boulton and Suter 1986, Feminella 1996, Casas and Langton 2008).

Richness and diversity

Documentation of species diversity supports species conservation work and the use of these species in ecological and biological assessment studies. Habitats with high diversity or unique taxa will be given greater priority for protection. Conversely, habitats with low diversity, whether actual or perceived, will be assigned lesser value and be at greater risk of degradation or destruction. Such is the case for non-perennial habitats. This long-term study of Chironomidae emergence from an intermittent stream demonstrated that non-perennial streams can support a high diversity of Chironomidae (158 taxa). Several studies have documented more than 100 species of chironomids from single stream sites in temperate regions (e.g., Coffman (1973): 141 taxa; Siebert (1980) 134 taxa; Boerger (1981): 112 taxa; Becker and Wagner (2004):110 taxa; Bouchard (2007): five streams – 107–131 taxa; Casas and Langton (2008): 159 taxa). However, most of these studies are from perennial streams and only include a single year of study. The high estimate in the current study of an intermittent stream was influenced by the higher-than-typical effort which spanned 6 years. On an annual basis, 61-103 taxa were recorded and only one year exceeded 100 taxa. Most studies of Chironomidae in streams only include a single year or season of sampling, but there are several that spanned multiple years (e.g., Siebert 1980, Feminella 1996), including several that studied non-perennial streams (Chou et al. 1999, Langton and Casas 1999, Casas and Langton 2008). All three of the studies on intermittent streams also used a similar methodology (i.e., biweekly collections of SFPE) which allows a good comparison between studies. Chou et al. (1999) collected SFPE exuviae from an intermittent stream in Kansas over three years, but only 55 taxa were recorded which was assumed to be due to the small stream size (1st order). Casas and Langton (2008) is more comparable to the current study because it compared intermittent and perennial stream sites over multiple years (5 years). No difference in total taxa richness was identified between the two habitats (159 taxa) (Casas and Langton 2008) and their total taxa richness was very similar to the intermittent stream in Minnesota (158 taxa). The current study and Casas and Langton (2008) both studied intermittent streams that remained flowing for a relatively high proportion of the year for at least some years. The taxa richness observed in these detailed studies of chironomid emergence from temporary streams indicates that high taxa richness, comparable to that in perennial streams, is likely maintained until the length of the dry period is sufficiently long (e.g., >2 months) to filter out facultative species. Although relatively short dry periods in streams do not appear to eliminate facultative species, this short dry period does appear to create a suitable habitat for species adapted to temporary habitats. Additional research is needed to determine how aquatic insect communities in lotic habitats shift along gradients of intermittency (e.g., Feminella 1996).

Virtually any sampling design will fail to detect species that are actually present in the study area. Estimation of true taxa richness can be useful to identify sampling effectiveness or for conservation purposes. In the intermittent stream, the taxa accumulation curve and species richness estimator indicated that despite a high sampling effort, chironomid taxa were missed in this study (Figure 7). One hundred and ninety-nine taxa (95% confidence interval: 175-256 taxa) were estimated to be present in the intermittent site during the 6-year study, although there is uncertainty with the results of species richness estimators. The presence of rare taxa creates difficulties for determining species richness. Many of the taxa collected from the intermittent site can be considered rare because they were only collected during one (27%) or two (14%) sample events. The high proportion of rare species increases uncertainty in species richness estimators (Gotelli and Chao 2013). Sample size is also an important factor for a study's ability to detect species. This study relied on a 300-count subsample to maintain a reasonable processing effort as SFPE samples can often contain 1000s of specimens. Bouchard and Ferrington (2011) determined that a 300-count subsample will be sufficient to capture most taxa. However, compared to a 1000-count sample, a 300-count subsample will miss on average 21% of the taxa richness in a sample from a surface-water fed stream. Therefore, it can be concluded that taxa were missed because of the subsample size used in this study. Regardless of the true number of taxa in this habitat during the 6-year sample period, individual years only documented 39-65% of the cumulative 6-year taxa richness indicating that a single year is not sufficient to fully characterize the total richness at a site over a longer period.

The high taxa richness recorded from this intermittent stream raises questions regarding how the diversity from this site (α diversity) compares to regional diversity (γ diversity). Casas and Langton (2008) determined that 36% of the known Iberian fauna was represented in a 5-year study of the chironomids from an intermittent stream. A similar comparison cannot be made for the intermittent site on the Credit River because a comprehensive catalogue of Chironomidae in the Great Lakes Region of North America has not been produced. However, a narrower comparison of the intermittent site on the Credit River can be made by comparing the taxa collected from the intermittent stream to the cumulative richness from 12 perennial streams in the region (Bouchard 2007). Based on this comparison, 54% of the total pool (290 taxa) were present in the intermittent site. However, this is an overestimate of the proportion of regional γ diversity at the intermittent site as the taxa list from the 12 streams does not include chironomids that occur in other habitats such as lakes, wetlands, large rivers, phytotelmata, and terrestrial habitats. In general, the diversity of Chironomidae based on pupal exuviae is less well documented in the Nearctic compared to the Palearctic. Although considerable work toward this effort by researchers such as Ferrington and Berg (2019) and Langton (2023) has improved our knowledge of pupal taxonomy in the Nearctic, considerable work is still required. In particular, fewer species names can be assigned to pupal morphotypes which create difficulty when harmonizing nomenclature between studies. This demonstrates a need to better understand regional diversity patterns, even in regions such as the Great Lakes Region of North America where many studies on the Chironomidae have been carried out. To conserve species, a reasonable determination of regional γ diversity is valuable, but this is an area of research that is lacking especially for taxonomically challenging groups like the Chironomidae.

The high chironomid diversity in the intermittent Credit River is surprising given the challenges aquatic insects face in non-perennial habitats. This indicates that many of these species can either survive dry periods through physiological or behavioral mechanisms or they can rapidly recolonize the habitat. This stream flowed for much of the summer providing a long window for colonization of the habitat with sufficient time to complete at least one generation. However, many taxa appear to have the ability to survive the mid-summer dry periods. For example, after the stream began flowing again in late summer or fall, emergence usually resumed within 1-3 weeks following the dry period. This suggests that many taxa were present in the stream during the dry period and were able to rapidly resume and complete development once water was present in the stream again. Many chironomid species can survive periods of drying through physiological or behavioral mechanisms (e.g., anhydrobiosis, cocoon building, migration to the hyporheos; Frouz et al. 2003), which may also explain the diversity of this family in the stream. The intermittent site of the Credit River was dominated by areas of coarse substrates which suggest that Chironomidae larvae at this site can retreat to the hyporheos during dry periods. There were several taxa that were specifically related to non-permanent habitats (e.g., *Hydrobaenus* RWB pe1, *O.* (*Eu.*) *abiskoensis*, *P. nasthecus*, and *Allocladius* sp.). These species were absent from the perennial site and their known natural history also suggests they have adaptations to semiaquatic or intermittent habitats (e.g., drying resistant life stages, ability to rapidly colonize habitats; Cranston et al. 2007, Casas and Langton 2008, Andersen et al. 2013). One of these taxa, *Hydrobaenus* RWB pe1, was the most abundant taxon collected in the intermittent stream due to large, synchronized pulses of emergence in the early spring. Langton and Casas (1999) identified a species (*Orthocladius calvus*) which was a dominant taxon in an intermittent stream but absent from a nearby perennial stream; *O. calvus* is documented as an early colonizer of rewetted habitats. *Hydrobaenus* RWB pe1 was present in all years and is likely a permanent resident of the intermittent stream with adaptations for surviving dry periods. Some species of *Hydrobaenus* are well known to colonize intermittent habitats (e.g., Grodhaus 1980, Kondo 1996, Cranston et al. 2007). *Orthocladius (Eu.) abiskoensis*, a species largely collected at high latitudes in the Holarctic region (Soponis 1990), was relatively abundant at this intermittent site. This species has also been collected from an intermittent site in Kansas (Chou et al. 1999). This suggests that at lower latitudes it may be largely limited to non-perennial habitats. Surprisingly, there were 4 species of *Diamesa* present at the intermittent site (Table S1). Although this genus is known from non-perennial habitats (e.g., Langton and Casas 1999, Williams 2006), this cold-adapted genus is more commonly encountered in Minnesota from groundwater fed streams. Because its emergence is limited to winter and early spring, it is likely that larvae aestivate in the stream in the hyporheic zone. Although there is evidence for the drought resistance traits in this midge community, the specific mechanisms used by many of the midges to successfully occupy a non-perennial habitat cannot be determined here.

Discharge and water temperature effects on Chironomidae diversity

Temperature is often considered to be the most important factor influencing the timing of growth and development of aquatic insects in temperate streams (Ward and Stanford 1982). In streams that experience strong seasonal gradients in temperature (e.g., with daily mean temperatures 0-25 °C), water temperatures will be outside of the thermal optima for a given species for some part of the year. As a result, species inhabiting these streams will have periods of diapause or quiescence when growth or development is halted. These regular periods of inactivity serve to synchronize the phenology of species and often species will emerge at predictable times based on their thermal optima. The seasonal emergence patterns in the intermittent stream were typical for a temperate, surface-water fed stream. Taxa richness was low in the winter, early spring, and late fall and was dominated by Orthocladiinae and Diamesinae. Orthocladiinae also dominated much of the rest of the year with Chironominae and Tanypodinae reaching their highest diversity in the summer. This seasonal pattern of richness among the subfamilies is commonly observed in perennial temperate streams (e.g., Armitage 1995, Coffman and de la Rosa 1998, Bouchard 2007). This pattern differs from that of tropical streams which have weaker seasonal variability temperature (Coffman and de la Rosa 1998, Bouchard and Ferrington 2023). However, there are other factors in streams that influence the timing of development including stream discharge and input of allochthonous matter.

The hydrology of a stream is an important factor that influences the composition and life history of aquatic insects. Discharge can have acute disruptive impacts on aquatic invertebrates during high flows (e.g., spates), low flows, and dry periods. Stream intermittency has obvious and direct effects on Chironomidae and other aquatic insects. When a stream dries, this halts emergence of aquatic species and could disrupt the life cycle if refugia are not available or if the species is not adapted to periods of drying. Longer periods of drying are expected to have a greater impact on aquatic species so less taxonomic diversity would be predicted to be supported in habitats or during years with less flow. For example, an intermittent stream in Kansas studied for 3 years had a much longer dry period one year (134 days of streamflow) and only 6 chironomid species were collected compared to 29 and 49 taxa in two other wetter years. There is a decrease in the richness of species emerging from these habitats even when water is retained in pools, but flow ceases (Chou et al. 1999, Casas and Langton 2008). In the present study, a similar pattern was observed with only 0-8 taxa present during sampling events when the stream was pooled. The decline in species richness during periods of no or low flow and an absence of emergence when the stream is dry suggests that the years of this study with longer dry periods should support lower taxa richness. However, this was not the case and there was no clear relationship between the number of days the stream was dry each year and the number of taxa collected. In fact, the opposite pattern was observed when accumulated annual flow was compared against annual taxa richness (Figure 9). Higher flows or spates are disruptive to chironomids which may explain this pattern. The lowest taxa richness was recorded in 2005, a year with numerous spates throughout spring and summer (Figure 6). Taxa richness in 2003 was also low and although this year did not have a series of summer spates, the discharge was high (> 6 m^3/s) in May. In 2007, taxa richness was high (91 taxa) and there were also some spates with very high flows, but these occurred in March and April. This suggests that the frequency and timing of spates is important in determining taxa richness in intermittent streams. If there are spates associated with late winter and early spring snow melt or rainfall, this may be less disruptive than spates during periods when temperatures are warmer and more species are active (e.g., late spring and summer). A negative relationship between spates and chironomid richness was also noted by Chou et al. (1999) who suggested that lower richness during wetter years may be due to scouring flows. This has also been hypothesized to be a determinant of abundance in tropical streams (Ramírez and Pringle 1998, Silva et al. 2009). However, the magnitude and timing of spates may result in different effects on chironomid communities. Scouring spates, especially during periods when chironomids are not dormant, may reduce diversity for days or weeks following the spate. Chironomids and other invertebrates in intermittent streams may be resistant to long-term effects of spates and recover within weeks (Miller and Golladay 1996), but repeated spates during the growing season could have an accumulative, negative effect.

Spates may have a positive effect on macroinvertebrate richness under some conditions. Casas and Langton (2008) hypothesized that spates in an intermittent stream increased temporal β diversity and increased α diversity. In the Credit River, the increased β diversity may be due to spates, but the effects of intermittency could also be important. The combined periodic disturbance caused by spates and drying may allow for the colonization of new species and therefore increase α diversity in the long-term. How this might impact intermittent systems as climate change alters their hydrology is not clear; however, this study does indicate negative relationships between increased flows and temperatures and chironomid taxa richness. Increases in peak flows and decreases in base flows are predicted to occur in the midwestern United States (Demaria et al. 2016) which will impact aquatic insect communities. However, forecasting specific long-term trends in species diversity is more difficult because there are effects that can be observed at different time scales and the relationships between hydrology, temperature, and species diversity can differ depending on the scale.

Conclusions

Disturbance caused by high variability in discharge, including spates and periods of drying, has an important effect on Chironomidae communities. High flows disrupt substrates and can cause displacement or a need for aquatic insects to move to protected habitats within the stream (e.g., hyporheic zone). Although aquatic insects, particularly Chironomidae, have demonstrated resilience to such disturbances (Miller and Golladay 1996, Anderson and Ferrington 2013), they have short-term impacts on communities (Casas and Langton 2008). Low flows and drying can also be a form of disturbance which require migration to the hyporheic zone or resistance to drying. Regardless of the mechanism for survival, dry periods will be disruptive through energetic costs (e.g., movement, cocoon construction) and delays in growth and development. For some chironomid species, dry periods will cause extirpation or at least a decline in the population and the species will need to recolonize the habitat. For species that cannot survive dry periods and require a year or more to develop, they will be limited to perennial sites. Thus, species attributes determine the species that occupy the habitat and their phenologies. However, many Chironomidae species are apparently facultative and capable of inhabiting both perennial and intermittent streams. There is also a smaller subset of taxa that are more abundant or are only found in intermittent streams. Together these taxa demonstrate that intermittent streams can support diverse aquatic insect communities, including unique taxa that do not occur in other habitats.

The long-term study of an intermittent stream in Minnesota (USA) has demonstrated that non-perennial streams contain unique taxa and these habitats can support a high diversity of aquatic insects comparable to that of perennial systems. However, non-perennial habitats are less often studied which highlights the need for additional research on non-perennial habitats. Long-term studies of aquatic habitats are also important to understand the effects of antecedent conditions. Due to the constraints of time and funding, it is not common for aquatic studies to extend beyond a year or two, but long-term patterns in flow and other variables affect aquatic insect communities. This is especially true in intermittent habitats which are more dynamic and have more variability in the aquatic insect community over time. A failure to account for such variability in sampling design and analysis can result in an underestimation of diversity and could affect the identification of other important factors such as pollution. This study also adds to the research demonstrating the importance of non-perennial, freshwater habitats (e.g., high species diversity, unique species) and the need for their conservation (Williams 1996, Casas and Langton 2008). However, this requires a shift in opinions as many consider non-perennial habitats to be less valuable. Conservation becomes more important for non-perennial habitats in this region as climate change is expected to increase temperatures and extreme weather events including droughts and heavy rain events. These threats, coupled with a low priority for conservation, has the potential to cause widespread disruption and extirpation to the diverse communities and unique taxa supported by non-perennial habitats.

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CHIRONOMIDS SHED LIGHT ON ORGANIC MATTER DYNAMICS IN MACROINVERTEBRATE COMMUNITIES IN PRAIRIE POTHOLE LAKES IN WEST-CENTRAL MINNESOTA, USA

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Abstract

The Prairie Pothole Region (PPR) in North America is characterized by a high density of wetlands that can range from temporary habitats to small lakes. Much of the PPR has been converted to agriculture resulting in outright habitat loss, eutrophication, sedimentation, introduction of agrochemicals, and the introduction of invasive plants and animals. We compared two prairie pothole lakes, Cottonwood Lake and Page Lake, to assess the functional roles and trophic structure of the macroinvertebrate communities in this highly altered landscape. We collected macroinvertebrate samples from littoral and profundal regions of each lake with an Ekman grab sampler in winters of 2007-2008. In fall 2012, 15 samples were collected from the near shore aquatic vegetation of Cottonwood Lake using D-frame nets. Macroinvertebrates, including Chironomidae, were identified to genus or morphospecies and assigned to functional feeding groups (FF-groups). Taxon richness of littoral and profundal samples were comparable in Cottonwood Lake but was significantly lower in the profundal zone of Page Lake. Macroinvertebrate abundance was significantly lower in Page Lake. Chironomidae made up 55% and 38% of the abundance in Cottonwood and Page Lakes, respectively and the similarity in chironomid composition was only 53%. Significantly more shredders, including the chironomid *Glyptotendipes*, occurred in Cottonwood Lake, including in the profundal zone. Macrophytes in Cottonwood Lake support additional taxa and shed light on the importance of near-shore sources of organic matter as a food resource. The FF-group distribution in Cottonwood Lake suggests that macroinvertebrates are taking advantage of a continuum of available organic matter resources that is dominated by coarse particulate organic matter (CPOM) near shore and by fine particulate organic matter (FPOM) in habitats farther from shore. In contrast, Page Lake had limited sources of organic matter; very few shredders were collected from littoral samples in Page Lake. In profundal samples most taxa were collector gatherers (*Chironomus*) or predators (*Procladius* and other Tanypodinae). Macroinvertebrates in Page Lake appear to be resource limited suggesting that the extensive algal blooms that characterize this eutrophic lake do not provide a comparable food source to that found in Cottonwood Lake. Analysis of the macroinvertebrate communities allowed us to discern the functional role of macroinvertebrates, including the Chironomidae, in processing organic matter in these highly impacted systems.

Introduction

Macroinvertebrates are diverse and abundant in freshwater habitats where they play critical roles in aquatic food webs and contribute to ecosystem function such as processing of organic matter. Because they exhibit a wide range of preferred environmental conditions and tolerances to anthropogenic impacts including habitat alteration and a variety of pollutants, macroinvertebrates have long been used as bioindicators of the relative health of the habitats in which they occur. This practice is well established in lotic ecosystems where Ephemeroptera, Plecoptera, and Trichoptera, which contain many sensitive species, are common indicators of ecosystem health (e.g. Zweig and Rabeni 2001). While perhaps not as widely used in lakes, macroinvertebrates have shed light on environmental conditions, particularly the trophic state of lentic systems (Sæther 1979, Poikane et al. 2016).

In contrast, evaluating the health of wetland habitats based on macroinvertebrate community composition, particularly in the Prairie Pothole Region (PPR) of North America has proven more challenging (e.g. Tangen et al. 2003, Gleason and Rooney 2017). Part of this challenge stems from the diversity of habitats that fall under the umbrella of "wetland," which can range from temporary habitats to small lakes. The PPR is an area spanning several states and Canadian provinces in central North America that is characterized by a particularly high density of depressional wetlands. Much of the PPR has been converted to agricultural land use resulting in loss of 50-90% of wetland habitat since colonization by Europeans (Euliss et al. 2006, Dahl 2014). As smaller wetlands were drained there has been an increase in larger wetlands and lakes (McLean et al. 2016a, McLean et al. 2020, Vanderhoof and Alexander 2016). Besides habitat loss, anthropogenic stressors include eutrophication, sedimentation, introduction of agrochemicals, and the introduction of invasive plants and animals. Even before widespread human impact, this region was naturally subject to extremes in temperature and variable precipitation, so macroinvertebrate communities in the PPR are comprised of resilient species that have physiological, life history, and behavioral adaptations to survive in harsh environments (Euliss et al. 2006).

Another possible hinderance to understanding macroinvertebrates in prairie potholes is that the taxonomic resolution applied to the macroinvertebrate community is inconsistent, particularly when it comes to the Chironomidae. Some studies identify chironomids to genus (e.g. Campbell et al. 2009, Olson et al. 1995), others may treat the predaceous Tanypodinae separately from the rest of the family (e.g. Rennie and Jackson 2005), still others identify chironomid midges only to the family level (e.g. Hentges and Stewart 2010, McLean et al. 2016b). This is unfortunate because chironomids consistently make up a significant portion of aquatic macroinvertebrate abundance and diversity (e.g. Euliss et al. 2006, Oertli 1993, Ferrington et al. 2008, Lemes-Silva et al. 2014). Multiple studies have revealed that the Chironomidae are not a monolithic ecological entity, but rather a lineage that contains species that exhibit a range of environmental tolerances, habitat preferences and trophic strategies (e.g. Belle and Goedkoop 2021) and that occupy different trophic levels in aquatic food chains (Berg 1995). Some authors provide sound arguments that chironomids should not be considered one group in food web studies (Reuss et al. 2013). While the resilient macroinvertebrates that inhabit prairie potholes may not reliably serve as indicators of environmental impact, they may still provide insight into ecosystem function in habitats that are threatened by human activities and even global climate change.

We studied the macroinvertebrate community in two prairie pothole lakes in west-central Minnesota, USA. Though Cottonwood Lake and Page Lake both occur in a landscape dominated by agriculture, adjacent land cover near Cottonwood Lake has more tree-cover. We sought to compare benthic macroinvertebrate communities from littoral and profundal zones of these two lakes. To further evaluate the influence of allochthonous and autochthonous organic matter on the macroinvertebrate community, we sampled macroinvertebrates from near-shore macrophytes and algae.

Materials and Methods

Study Area

Study lakes were in the Northern Glaciated Plains Ecoregion in west-central Minnesota, USA. The landscape was historically dominated by prairie but has largely been converted to row-crop and animal production agriculture. This ecoregion is in the Prairie Pothole Region (PPR), which is characterized by an abundance of depressional wetlands and lakes, i.e. potholes, that formed as the glaciers retreated ca. 14,000 years ago. Most prairie pothole wetlands are naturally fishless and provide valuable habitat for waterfowl and other nongame wildlife.

We studied two prairie pothole lakes, Cottonwood Lake (Grant County) and Page Lake (Stevens County) in west-central Minnesota. Unlike most prairie potholes, these lakes are managed to support piscivorous gamefish, particularly walleye (Percidae: *Sander vitreus*), though several other species, both native and introduced, occur in both lakes (Table 1). Percent tree cover within 50 m of shore was 32% higher at Cottonwood Lake than Page Lake (Table 1). Submerged and emergent macrophytes were common at Cottonwood Lake but were rare in Page Lake (Table 1). Minnesota Department of Natural Resources monitoring suggests that both lakes are eutrophic, though Cottonwood Lake has lower phosphorus levels and higher water clarity than Page Lake (MN DNR LakeFinder; Table 1.)

Data Collection and Analysis

In the winters of 2007 and 2008 benthic macroinvertebrate samples were collected from Page Lake and Cottonwood Lake. Winter sampling ensured a consistent community when no emergence or oviposition was occurring and allowed for undergraduate students to engage in research during the academic year. Samples were collected by lowering an Ekman grab through a hole in the ice and retrieving 0.02 m^2 of substrate from the bottom of the lake. The volume of grab samples was not

Table 1. Characteristics of Cottonwood Lake and Page Lake, the two prairie pothole lakes located in the Northern Glaciated Plains Ecoregion in west-central Minnesota, USA. Data from Minnesota, Department of Natural Resources LakeFinder, unless noted.

Parameter	Cottonwood Lake	Page Lake
Lake Area (Ha)	103	152
Maximum depth (m)	4.9	3.7
% Tree cover in 50 m buffer*	78	46
10-year-average water clarity (m)	3	2
10-year-average phosphorus (ppb)	161	176
Walleye Stocking	Yes (fingerings)	Yes (fry)
Common Carp	No	Yes
Submerged/emergent macrophytes**	Common	Rare

*ArcGIS10, MN DNR Data Deli

** Field observations

quantified, though seemed comparable between lakes after washing through a 500-micron sieve. From each lake a series of samples from the littoral zone were collected < 50 m from shore. An additional series of samples were collected from the profundal zone > 100 m from shore. Fifteen replicate samples were collected from each habitat in Cottonwood Lake and the profundal zone of Page Lake. Due to weather and ice conditions, only ten samples were collected from the littoral zone in Page Lake.

To assess the association of macroinvertebrates with coarse particulate organic matter (CPOM) in fall 2012, 15 macroinvertebrate samples were systematically collected from the near shore aquatic vegetation of Cottonwood Lake using D-frame nets. Samples were collected from emergent vegetation (primarily *Scirpus*) (N = 6), submerged vegetation, (primarily *Potamogeton* spp.) (N = 6) and filamentous algae (N = 3). Each substrate type was disturbed for 30 seconds and dislodged material, including macroinvertebrates were collected. Near shore aquatic vegetation was extremely rare in Page Lake and was not sampled. Samples from different types of near shore organic matter were not evaluated separately for this analysis.

Both winter benthic samples and samples from near shore vegetation were washed through a 500-micron sieve and preserved in 95% ethanol. Specimens were sorted from sample debris and identified to the lowest possible taxonomic level, typically genus. Functional feeding groups (FF groups) were assigned based on Merritt et al. (2008) and Thorp and Covich (2001).

For winter samples, average macroinvertebrate taxon richness and abundance (log x+1) between lakes and habitat zones were compared using a fully crossed 2-way analysis of variance (ANOVA), followed by the Tukey's multiple comparison of means to evaluate significance of differences among pairs of variables (version 4.4.0, R Core Team 2024). Abundance of shredders, collector gatherers and predators within each habitat zone was compared using 1-way ANOVA followed by the Bonferroni post hoc test. Jaccard's Similarity Index was used to compare the chironomid communities in the two lakes. Organic matter samples, which were collected for a subsequent undergraduate research project, were used to provide a more complete view of the macroinvertebrate community and to help inform hypotheses about organic matter processing in Cottonwood Lake.

Results

Winter Samples

Thirty-five taxa occurred in winter samples, 24 from each of Cottonwood and Page Lakes (Table 2). Seventeen genera (36%) belonged to Chironomidae. Oligochaete worms, and the dipteran lineages Ceratopogonidae, *Chironomus*, and *Procladius*, were the only groups to occur in littoral and profundal samples in both lakes. Both lakes contained 8 unique macroinvertebrate taxa.

Mean taxon richness differed significantly among habitats (2-way ANOVA, $p << 0.01$) but not between lakes, though this difference was driven by lower taxon richness in the Page Lake profundal zone (Fig. 1). At Cottonwood Lake, littoral and profundal samples had similar taxon richness (means of 10 and 8, respectively), while at Page Lake littoral and profundal samples had twice the richness of profundal samples (mean 12 and 6, respectively). Overall, the profundal zone in Page Lake had significantly lower taxon richness than all other habitats ($p < 0.01$; Fig. 1).

Table 2. Macroinvertebrate taxa collected from Cottonwood Lake (CW) and Page Lake (PG). Taxa were assigned to functional feeding-groups (FF-Group) based on Merritt et al. (2008) and Thorp and Covich (2001). (Collector-Gatherers = Coll-Gather, Collector Filterers = Coll-Filter). Number of occurrences in near-shore organic samples (CW only) and winter samples (littoral/profundal) from both lakes are reported.

Taxon			FF-Group	CW Organic (N = 15)	CW Winter (N = 15 / 15)	PG Winter (N = 10 / 15)
Annelida						
		Oligochaeta	Coll-Gather	1	12 / 12	10 / 14
		Hirudinea	Predator	8	0 / 0	2 / 0
Mollusca						
		Gastropod 1	Scraper	10	3 / 0	0 / 0
		Gastropod 2	Scraper	6	1 / 0	0 / 0
		Gastropod 3	Scraper	5	0 / 0	0 / 0
		Bivalve	Coll-Filter	0	0 / 0	1 / 0
Arthropoda						
	Arachnida					
		Hydrachnida	Predator	4	4 / 14	0 / 13
Crustacea						
		Orconectes	Shredder	6	0 / 2	0 / 0
		Gammarus	Shredder	4	0 / 0	0 / 0
		Hyalella	Shredder	15	14 / 13	3 / 0
Insecta						
	Ephemeroptera					
		Callibaetis	Coll-Gather	8	2 / 1	4 / 0
		Caenis	Coll-Gather	7	7 / 0	7 / 0
		Hexagenia	Coll-Gather	0	0 / 0	6 / 0
	Odonata					
		Enallagma	Predator	14	8 / 0	0 / 0
		Anax	Predator	3	0 / 0	0 / 0
	Hemiptera					
		Belostoma	Predator	2	0 / 0	0 / 0
		Corixidae		14	1 / 0	5 / 4
		Ranatra	Predator	2	0 / 0	0 / 0
		Notonectidae	Predator	6	0 / 0	0 / 0
		Neoplea	Predator	1	0 / 0	0 / 0
	Coleoptera					
		Dytiscidae	Predator	4	0 / 0	0 / 0
		Dubiraphia	Scraper	0	0 / 0	1 / 0
		Peltodytes	Piercer	2	0 / 0	0 / 0
	Trichoptera					
		Oecetis	Predator	2	9 / 1	2 / 0
		*Phrygane*a	Shredder	1	0 / 0	0 / 0
		Polycentropodidae	Predator	0	0 / 0	1 / 0
	Diptera					
		Chaoborus	Predator	0	1 / 9	0 / 0

Taxon	FF-Group	CW Organic (N = 15)	CW Winter (N = 15 / 15)	PG Winter (N = 10 / 15)
Ceratopogonidae	Predator	0	14 / 8	9 / 15
Chironomus	Coll-Gather	3	9 / 14	4 / 14
Cryptochironomus	Predator	3	12 / 11	4 / 0
Dicrotendipes	Coll-Gather	0	9 / 0	4 / 1
Endochironomus	Coll-Gather	3	0 / 0	0 / 0
Glyptotendipes	Shredder	6	15 / 11	0 / 0
Polypedilum	Coll-Gather	3	3 / 0	10 / 0
Pseudochironomus	Coll-Gather	0	0 / 0	5 / 0
Cladotanytarsus	Coll-Gather	3	9 / 0	4 / 1
Tanytarsus	Coll-Gather	3	1 / 0	1 / 0
Corynoneura	Coll-Gather	1	0 / 0	0 / 0
Cricotopus	Coll-Gather	11	3 / 0	2 / 0
Eukiefferiella	Shredder	0	1 / 0	0 / 0
Tvetenia	Coll-Gather	4	0 / 0	0 / 0
Ablabesmyia	Predator	0	5 / 4	0 / 0
Coelotanypus	Predator	0	0 / 0	1 / 0
Procladius	Predator	0	14 / 15	8 / 14
Tanypus	Predator	0	1 / 0	3 / 1
Empididae	Predator	1	0 / 0	0 / 0
Psychodidae	Coll-Gather	0	0 / 0	1 / 0
Total No. Taxa 47		33	25 (24 / 13)	25 (24 / 9)

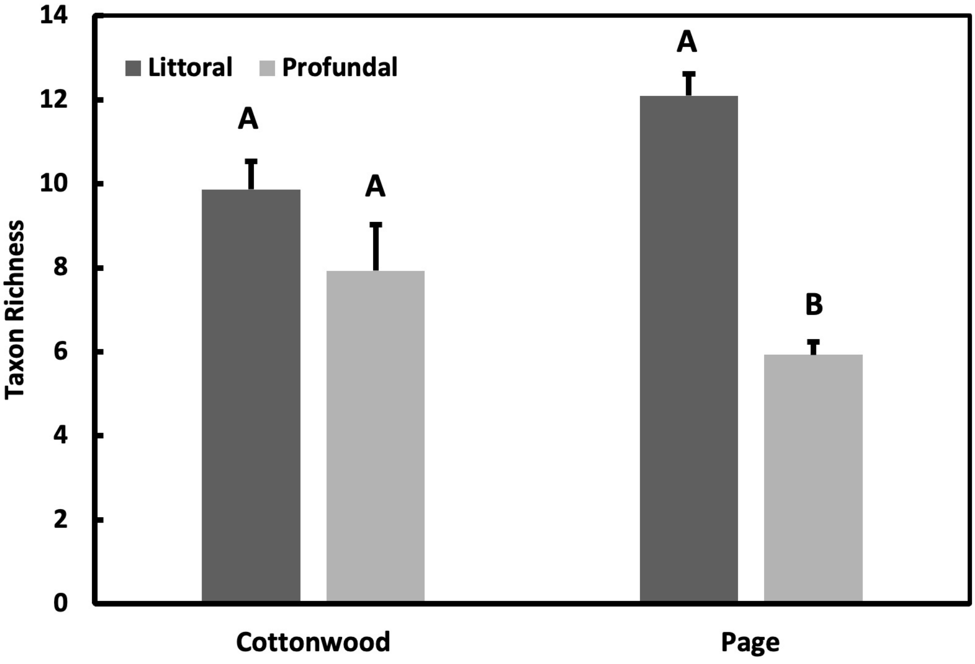

Figure 1. Mean taxon richness (+SE) for littoral and profundal samples from Cottonwood and Page Lake. Taxon richness was compared among lakes and habitats (2-way ANOVA and Tukey's multiple comparison of means test). Different upper-case letters indicate significant differences among habitats.

Macroinvertebrate abundance was significantly greater in Cottonwood Lake (mean 238) than Page Lake (mean 98; $p << 0.01$; Fig. 2). The profundal zone in Page Lake, on average, supported significantly fewer macroinvertebrates than either habitat in Cottonwood Lake, but did not differ in abundance from the Page Lake littoral zone (Fig. 2). The interaction between lake and habitat was marginally statistically significant ($p = 0.07$), driven by lower abundances of macroinvertebrates in the Page Lake profundal zone (mean 73) compared to the Cottonwood Lake profundal (mean 227; $p < 0.01$) and littoral zones (mean 250; $p < 0.01$). The results suggest that the profundal zone in Page Lake, on average, supported significantly fewer macroinvertebrates than either habitat in Cottonwood Lake. However, the difference between mean abundance in Page Lake littoral and profundal zones was not statistically significant ($p = 0.13$; Fig. 2). Chironomidae represented 55% of abundance in Cottonwood Lake and 38% of the abundance in Cottonwood and Page Lakes. Although chironomid richness was comparable between lakes, chironomid communities in the two lakes were not the same (Jaccard Coefficient of Similarity = 0.53).

Shredders were the most abundant functional feeding group in the Cottonwood Lake littoral zone, whereas collector gatherers and predators numerically dominated in the profundal zone (Fig. 3, Fig. 5). In contrast, few shredders were collected from the littoral zone in Page Lake; no shredders were collected from the profundal zone (Fig. 3). Mean abundance of collector gatherers and predators was similar across all habitats. The trophically diverse Chironomidae contributed to observed patterns in functional feeding group distribution within each lake (Fig. 4). Chironomid shredders were more abundant in the littoral zone of Cottonwood Lake compared to the profundal zone. Chironomid collector gatherers were more abundant farther from shore in profundal samples in Cottonwood Lake. Most chironomids in Page Lake were predators and no chironomid shredders were collected.

Fall Near-Shore Organic Matter Samples

Near-shore organic matter samples collected from Cottonwood Lake in the fall contained 33 taxa, 14 of which were not collected from littoral or profundal zones in the winter. Most of the unique taxa in organic matter were larger shredders including *Gammarus* (Amphipoda: Gammaridae) and *Phryganea* (Trichoptera: Phyrganeidae) or predators that actively swim or crawl among the more complex habitat provided by macrophytes and leaf litter such as *Anax* (Odonata: Aeshnidae) and adult Hemiptera and Coleoptera (Table 2). Chironomids found only in the near-shore organic matter samples (*Corynoneura*, *Tvetenia*, and *Endochirono-*

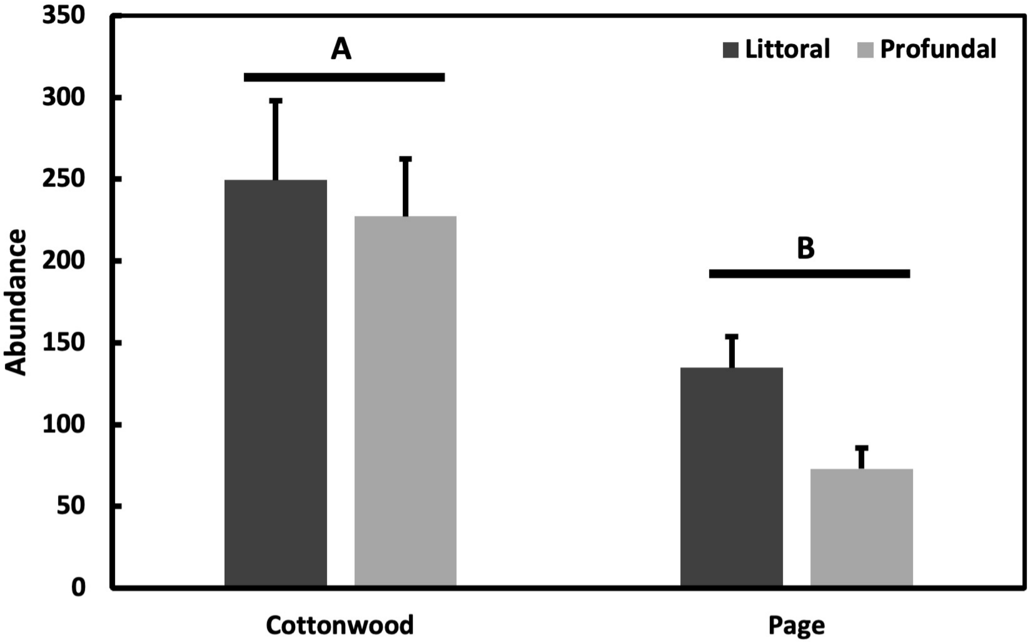

Figure 2. Mean abundance (+SE) of aquatic macroinvertebrates in Ekman grab samples from littoral and profundal samples collected from Cottonwood Lake and Page Lake. Abundance was compared among lakes and habitats (2-way ANOVA and Tukey's multiple comparison of means test). Different upper-case letters indicate significant differences among lakes.

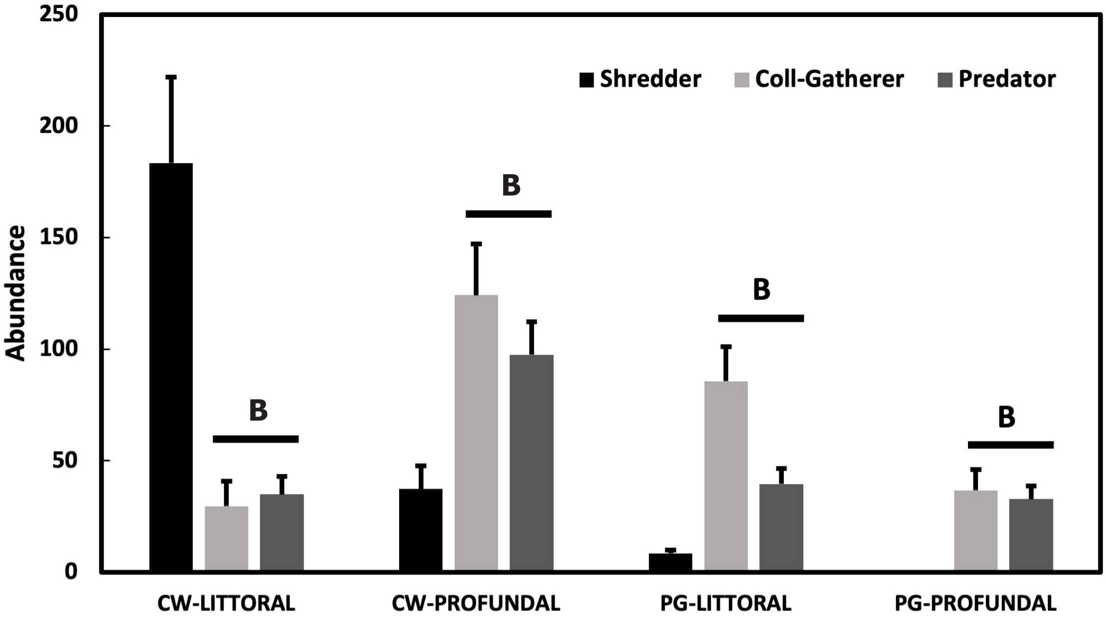

Figure 3. Mean (+SE) abundance of macroinvertebrate shredders, collector gatherers, and predators in Ekman grab samples from littoral and profundal zones from Cottonwood Lake (CW) and Page Lake (PG). Abundances of each FF-group were compared for each habitat (1-way ANOVA and the Bonferroni post-hoc test). Different upper-case letters indicate significant differences in FF-groups within each habitat.

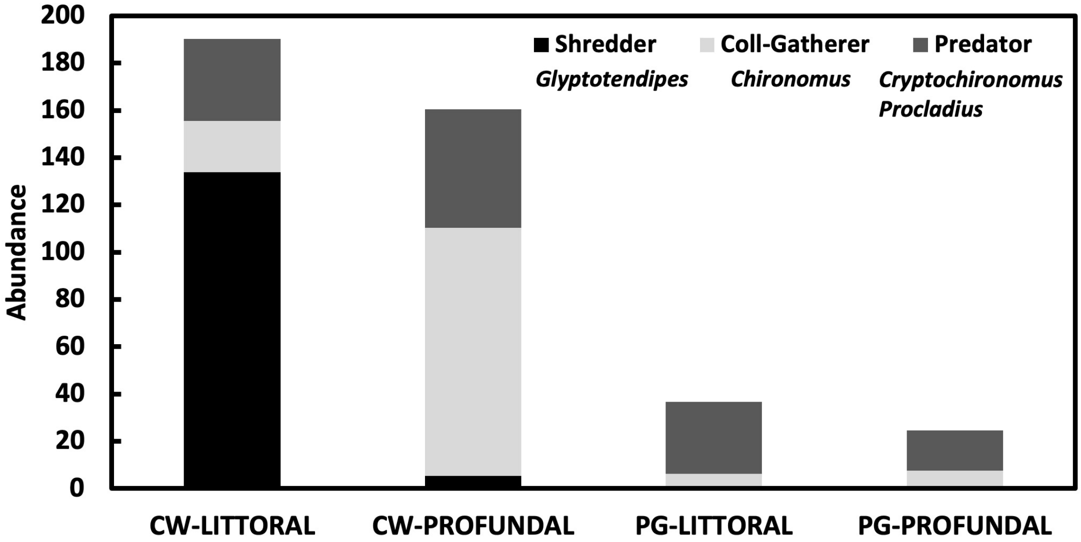

Figure 4. Total abundance of Chironomidae collected in littoral and profundal Ekman grab samples from Cottonwood Lake (CW) and Page Lake (PG). Abundances of chironomid shredders, collector-gatherers (Coll-Gatherer) and predators are indicated. The most common chironomid groups for each functional feeding group are listed.

mus), were all collector gatherers and were infrequently collected. As in littoral samples, shredders were the most abundant functional feeding group (Fig. 5a). Unlike any of the winter samples, scrapers, primarily gastropods, were common in nearshore organic matter (Fig. 5).

Discussion

While both study lakes occur in landscapes dominated by agriculture and are managed to support recreational angling, they exhibit different habitat conditions. Cottonwood Lake has more tree cover within a 50 m buffer, supports abundant submerged and emergent macrophytes, and does not have invasive carp, suggesting it may provide an example

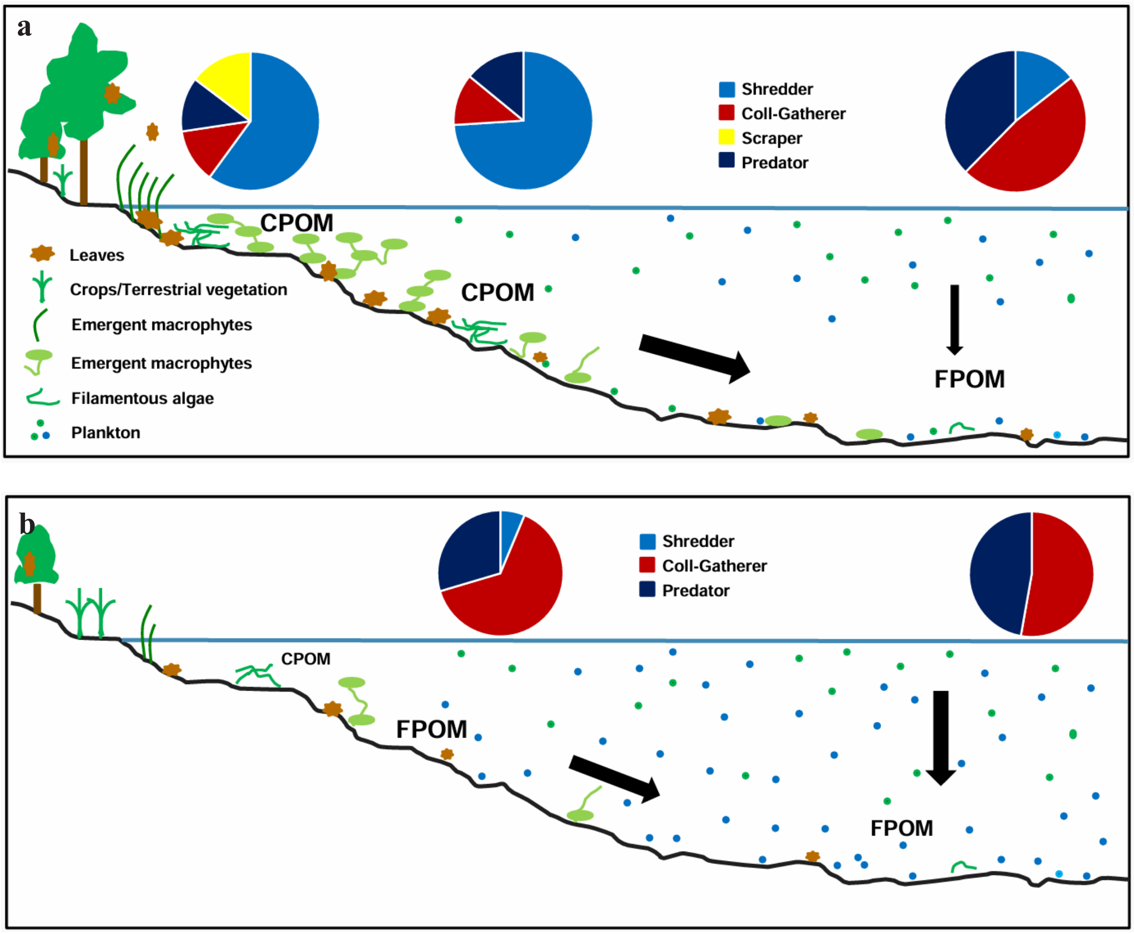

Figure 5. Schematic view of the distribution of particulate organic matter source in a) Cottonwood Lake and b) Page Lake based on relative abundance of macroinvertebrate functional-feeding groups in winter samples from littoral and profundal regions of both lakes and from near-shore organic matter (Cottonwood Lake only). Size of font of indicates relative amounts of coarse particulate organic matter (CPOM) and fine particulate organic matter (FPOM) hypothesized in each region of the lake.

of relatively high-quality conditions in this highly altered landscape. In contrast, Page Lake has invasive carp, few macrophytes and lower water clarity, thus exhibiting many of the environmental challenges that plague lakes in the area. Analysis of the macroinvertebrate communities in each lake also reveal differences between the two lakes.

Macroinvertebrate communities from Cottonwood Lake and Page Lake are consistent with what has been reported from other prairie pothole lakes and wetlands — Chironomidae were the most abundant and diverse macroinvertebrates in littoral and profundal samples from both lakes and relatively few mayflies and caddisflies were collected (Euliss et al. 2006, Oertli 1993, Ferrington et al. 2008, Lemes-Silva et al. 2014). Taxa in both lakes are well adapted to live in the harsh conditions that typify the PPR, particularly benthic anoxia (sensu Euliss et al. 2006). For example, oligochaete worms and midges, especially *Chironomus* and *Procladius*, which are often found in low oxygen conditions (Hershey and Lamberti 2001), were frequently collected in littoral and profundal samples in both lakes (Table 2). Additionally, many adult Coleoptera and Hemiptera, which use atmospheric oxygen and can disperse among habitats, occurred in organic matter samples from Cottonwood Lake.

Taxon richness in winter samples did not differ significantly between the two lakes. While there was insufficient macrophyte cover to collect meaningful samples in these habitats at Page Lake, the widespread occurrence of submerged and emergent macrophytes in Cottonwood Lake yielded 14 additional taxa in fall sampling, suggesting that Cottonwood Lake has the potential to support higher abundance and greater richness of macroinvertebrates than Page Lake.

Winter macroinvertebrate abundances further suggest that Cottonwood Lake provides better habitat for macroinvertebrate communities. Mean macroinvertebrate abundance in Cottonwood Lake was comparable in littoral and profundal environments and was significantly greater than mean abundance in Page Lake. The greater abundance of macroinvertebrates throughout Cottonwood Lake indicates that this lake provides relatively favorable environmental conditions for freshwater organisms even in a highly altered landscape.

By identifying chironomids to the level of genus, we were able to discern meaningful differences in functional feeding groups between the two lakes (Fig. 3). In Cottonwood Lake shredders were significantly more abundant than collector gatherers and predators in the littoral zone but were the least abundant FF-group in the profundal zone, which was dominated by collector-gatherers and predators. Most chironomids in the littoral region of Cottonwood Lake were shredders, which feed in/on coarse particulate organic matter. Shredders including *Glyptotendipes* (and the amphipod, *Hyalella* sp.) also occurred in profundal samples, though in lower numbers. Conversely, collector-gatherers, such as *Chironomus*, comprised a small part of the midge community in the littoral region, but were very abundant in profundal samples.

In Page Lake there were very few shredders and the relative distribution of FF-groups was similar to that of the Cottonwood Lake profundal zone. The relative abundance of predators and collector gatherers were similar the littoral and profundal zones of Page Lake, indicating limited differentiation in community structure and function in these two "habitats." In contrast, Cottonwood Lake had greater abundance of collector-gatherers in the profundal zone than in the littoral zone, suggesting clear differentiation in FF-groups by habitat. The observed patterns in distribution of FF-groups were discernable because chironomids were identified to genus, which allowed us to recognize different feeding strategies found within this diverse family (Ferrington, et al. 2008; Fig. 4).

The FF-group distribution in Cottonwood Lake suggests that macroinvertebrates are taking advantage of a continuum of available organic matter resources that is dominated by coarse particulate organic matter (CPOM) in the littoral region of the lake and by fine particulate organic matter (FPOM) deeper habitats that are farther from shore. As reported for other ecosystems, particularly streams, it is likely that littoral macroinvertebrates facilitate the breakdown of CPOM into FPOM and that macroinvertebrates are distributed in a way to take advantage of the resources they are best adapted to exploit (e.g. Vannote et al. 1980, Dieterich et al. 1997). The low number of shredders in the Page Lake littoral zone and their complete absence in profundal samples suggest there is less CPOM available near shore and consequently less FPOM available in the profundal zone. This is consistent with the observed paucity of macrophytes and relatively low tree-cover within 50 m of the shoreline, compared to Cottonwood Lake (Table 1).

Macroinvertebrate samples from near-shore sources of organic matter in Cottonwood Lake provide a more complete picture of trophic dynamics in the macroinvertebrate community. As expected, organic matter samples were dominated by shredders, including larger organisms such as crayfish and phryganeid caddisflies. Scrapers, collector-gatherers, and predators occurred in approximately equal numbers (Fig 5a). CPOM, whether it originates from the adjacent landscape (i.e. leaf litter) or in-lake primary productivity (i.e. submerged and emergent macrophytes), is a valuable resource for many macroinvertebrates and seems to ultimately support benthic production throughout the lake (e.g. Batzer 1998). Whether due to feeding and production of feces by shredders, breakdown by microbes, or other physical means, CPOM contributes to the pool of FPOM, which serves as a food resource for collector-gatherers. Page Lake had fewer potential sources of CPOM (i.e. few macrophytes, less tree cover) (Fig. 5b) and supported fewer shredders that would facilitate processing of organic matter required by collector-gatherers. The relatively small inputs of CPOM and low abundance of benthic macroinvertebrates suggest that higher trophic levels may be food limited in Page Lake.

It seems counter-intuitive that organisms in a eutrophic lake could be food limited, but much of the productivity in Page Lake is in the form of dense annual blooms of phytoplankton, dominated by cyanobacteria. The combination of low water clarity due to phytoplankton and benthic disturbance by common carp paired with possible effects of allelopathic chemicals produced by cyanobacteria (Mohammed 2017) create an environment that is unfavorable for macrophyte growth. As in other systems, habitat simplification and lack of physical structure provided by submerged and emergent macrophytes influences the diversity and abundance of macroinvertebrates that the system can support (e.g. Olson et al. 1995). Cyanobacteria and other phytoplankton, which senesce and enter the benthic detrital pool in late summer/early fall do not provide a sufficient food resource to support robust macroinvertebrate populations. In contrast, systems with food originating from either the adjacent landscape or emergent and submergent macrophytes provide a variety of food resources to sustain macroinvertebrate communities following the senescence of planktonic primary producers. While some studies have shown that cyanobacteria may be consumed by zooplankton and benthic macroinvertebrates (Yu et al. 2013), invertebrates commonly avoid or reject cyanobacteria as a food source (Vanderploeg et al. 2001) and cyanotoxins are toxic to many macroinvertebrates (Fadel et al. 2023, Stephanian et al. 2020). Determining if and how benthic macroinvertebrates use detrital cyanobacteria and whether this food resource is sufficient to support macroinvertebrates throughout the winter should be further investigated.

Prairie pothole lakes are consistently subjected to human impacts due to agricultural practices that alter the natural vegetation and hydrology of the landscape, augment nutrient availability, deposit other agrochemicals, and aid the establishment of invasive plants and animals (Euliss et al. 2006), all of which may be exacerbated by changing temperature and precipitation patterns associated with climate change (McLean et al. 2020). Despite this plethora of stressors, hardy macroinvertebrate communities of prairie pothole lakes and wetlands continue to play critical functional roles in organic matter processing and the food webs in these highly impacted systems

Our analysis suggests that organic matter deriving from either autochthonous or allochthonous sources near shore provide a critical resource for benthic macroinvertebrates throughout the lake. This conclusion is consistent with theoretical models for processing organic matter in streams (Vannote et al. 1980). Recognizing different feeding strategies of the Chironomidae, which are frequently all lumped together as collector-gatherers made this interpretation of organic matter dynamics in prairie pothole lakes possible. More detailed analysis of detrital food resources, gut content analysis of benthic macroinvertebrates, and the consumption of macroinvertebrates by fish and other vertebrate predators will provide more insight into the trophic dynamics of these environments.

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DNA ANALYSIS CONFIRMS THE SPECIES *PARACHAETOCLADIUS LENFERRINGTONI* BOUCHARD *ET* NAMAYANDEH, 2024 (CHIRONOMIDAE: DIPTERA)

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Abstract

In this study, we confirm the status of *Parachaetocladius lenferringtoni* Bouchard *et* Namayandeh, 2024, using molecular evidence. Using distance-based ABGD, ASAP, and K2P techniques, we determined that the minimum interspecific distance between sequences of *P. lenferringtoni* and *Parachaetocladius abnobaeus* (Wülker, 1959) was 14.8%, sufficient to maintain the species level. Based on the results obtained, we suggest that the threshold of 3% is sufficient to set the species limit for the *Parachaetocladius* species. Therefore, our molecular analyses of *P. lenferringtoni* specimens further support its status as a new Nearctic species distinguished from *Parachaetocladius abnobaeus* (Wülker, 1959).

Introduction

The genus *Parachaetocladius* is characterized by univoltine, cold-stenothermic species inhabiting spring and spring-fed lotic freshwaters (Ferrington 1987, Wülker, 1959, Bouchard et al., 2024). In the Nearctic, the genus is so far represented by five described species (Sæther and Sublette 1983, Namayandeh et al. 2020, Bouchard et al. 2024) and two morphospecies, described as pupae (Langton 2023). Bouchard et al. (2024) previously demonstrated morphological differences between the Nearctic *Parachaetocladius abnobaeus* (Wülker, 1959) (= *Parachaetocladius hudsoni* Sæther & Sublette, 1983) and those from the Palearctic, suggesting the restoration of *P. hudsoni* as a species. Bouchard et al. (2024) further described and erected a new species, *Parachaetocladius lenferringtoni* Bouchard & Namayandeh, based on adult males from Kansas and Manitoba previously identified as *P. abnobaeus* (= *P. hudsoni*), as well as additional adults and immatures from Minnesota, Nebraska, and Wisconsin. The characteristics of the adult male's head and hypopygium and the pupal tergal shagreen, spines, and anal lobe supported the delineation of a new species. At the time of their study, Bouchard et al. (2024) were unable to generate barcode sequences from DNA extracted from the type material. In this study, we successfully extracted sufficient DNA from the tissues of voucher specimens to amplify and provide molecular evidence for the presence of this new species.

Combining morphological and molecular methods has been beneficial to tease out new Chironomidae species, particularly those of cryptic species. For instance, by utilizing mitochondrial and nuclear DNA, Anderson et al. (2013) separated *Micropsectra subletteorum* Anderson, Stur & Ekrem, 2013 from other morphologically similar species in the *M. notescens* group and also separated *Micropsectra neoappendica* Anderson, Stur & Ekrem, 2013 from the morphologically similar *M. appendica* Stur and Ekrem, 2006. Additionally, by utilizing the mitochondrial cytochrome oxidase (COI) gene, Gilka et al. (2018) were able to erect *Tanytarsus latens* Giłka, Paasivirta, Gadawski & Grabowski, 2018 as a new species, which morphologically resembled *Tanytarsus occultus* Brundin, 1949 and *Tanytarsus desertor* Giłka & Paasivirta, 2007. Since separating some *Parachaetocladius* species is difficult, it may be necessary to utilize molecular methods combined with examination of the morphology of the adult and immature stages.

Materials and methods

Molecular analysis

Specimens were collected using the methods outlined in Bouchard et al. (2024). We extracted the genomic DNA of two *P. lenferringtoni* sequences (PCHAT-01, PCHAT-02) from the entire tissues of the adults using the Qiagen DNA Blood and Tissue Kit (Qiagen, Inc., Germantown, MD). A 658 base pair fragment of the cytochrome oxidase subunit 1 (COI) was amplified using the universal primers LCO1490 and HCO2198 (Folmer et al. 1994). DNA amplification was carried out in

24.5 µl reactions of 0.5 µl of each primer, 10 µl of PCR water, 12.5 µl of SSO Advanced SYBR Supermix, and 1.5 µl of template DNA. The amplification cycles were performed using an initial denaturation step of 95°C for four minutes, followed by 40 cycles of 95°C for 30s, 50°C for 30s, 72°C for 1.5 minutes, and a final extension at 72°C for ten minutes. This was followed by DNA denaturation steps that went from 65°C to 95°C with 0.5°C intervals. The above reactions were conducted in the laboratory of Dr. Jeffrey Ram, Department of Physiology, Wayne State University, Michigan, USA. The amplicons were shipped to GENEWIZ, a subsidiary of Azenta Life Sciences, for bi-directional Sanger Sequencing.

A total of 56 sequences were used in the phylogenetic analyses including: 33 *Pseudorthocladius* (*Pseudorthocladius*) *curtistylus* (Goetghebuer, 1921) and *Pseudorthocladius* (*Pseudorthocladius*) *pilosipennis* Brundin, 1956, 16 *Georthocladius*, 6 *P. abnobaeus* and *Parachaetocladius akanoctavus* Sasa *et* Kamimura, 1987*,* and 1 *Orthocladius carlatus* (Roback, 1957) as an outgroup. We submitted new sequences to the BOLD database (http://dx.doi.org/10.5883/DS-PCHAT). The list of all sequences studied, and BOLD species IDs are provided in Supplementary Material Table S1.

The phylogenetic trees were obtained based on two methods: Neighbour-Joining (NJ) and Maximum Likelihood (ML). DNA sequences were aligned using Clustal X version 2.1 software to construct the BI tree (Larkin et al. 2007). We determined the best model for nucleotide substitution using ModelTest-NG v0.1.7 by the Exelixis Lab (Darriba et al. 2020) and chose the best model using the Akaike Information Criterion (AIC). We constructed the ML trees using raxmlGUI 2.0 software (Edler et al. 2021) and with 10,000 Bootstrap repeats. The Neighbour-Joining tree with 10,000 bootstrap replications and K2P distances were produced in MEGA X (Kumar et al. 2018). Trees constructed in the NJ and ML models were visualized in FigTree v. 1.4.2 (Rambaut 2014).

To determine the limits of "molecular species", three species delimitation methods were used based on distance: Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012), Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al. 2021), and K2P distances. ABGD was run with P min = 0.001, P max = 0.1, and a gap width of 1.5, all for 10 steps to calculate the barcode gap in the distribution of pairwise differences. ASAP was run with Kimura (K80) ts/tv = 2.0 setting. For the K2P distance, we set the threshold to 3%. Because the number of *Parachaetocladius* sequences available in BOLD and NCBI was limited, we used sequences from its closest genera, *Pseudorthocladius* and *Georthocladius,* to determine the intraspecific and interspecific K2P distances between the three species in *Parachaetocladius*.

Results and Discussion

The length of the sequences was found to be 685 base pairs. The nucleotide composition was as follows: Cytosine (C) constituted 18%, Guanine (G) constituted 17%, Adenine (A) constituted 26%, and Thymine (T) constituted 39%. No additions or deletions were observed in the sequence. Furthermore, the GC content was calculated and determined to be 35%.

The phylogenetic trees generated in our study indicate the presence of six species. The sequences belonging to the three species of *Parachaetocladius, P. lenferringtoni*, *P. abnobaeus*, and *P. akanoctavus*, cluster within their own groups. As we expected from the previous morphological assessment, the molecular results confirm the presence of a new species, *P. lenferringtoni* (Fig. 1).

Based on the intraspecific K2P distances obtained for the analyzed sequences in this study, the lowest distances belonged to *P. lenferringtoni* (0.0%) and *P. akanoctavus* (0.0%), and the highest to *P. pilosipennis* (1.0%). The intraspecific K2P distance for the remaining species is as follows: *P. curtistylus*, 0.6%; *P. abnobaeus,* 0.84%; and *Georthocladius* sp., 0.95%. The average interspecific distance between all analyzed sequences was 11.0%. The minimum interspecific distance between sequences of *Parachaetocladius* was 14.8%, which belonged to *P. lenferringtoni* and *P. abnobaeus*, the closest species in this genus. This value is sufficient to maintain the species level (Ekrem et al. 2007, 2010, Montagna et al. 2016).

Using ABGD, there is a gap between the highest intraspecific K2P distance (0.03 or 3%) and the lowest interspecific K2P distance (0.13 or 13%). This gap (i.e., for sequences used) suggests that if the distance between two sequences is less than 3%, the sequences belong to the same species. If it is more than 13%, the sequences belong to two different species (Fig. 2). The ASPA result indicates the same results as those of ABGD (Supplementary file 1-Figure S1). Based on the results obtained from these tests, all five species analyzed in this study are placed in the correct classification and represent separate species. While it is premature to establish a criterion for *Parachaetocladius* species in the absence of a more complete DNA library for this genus, this study's 10% gap, determined

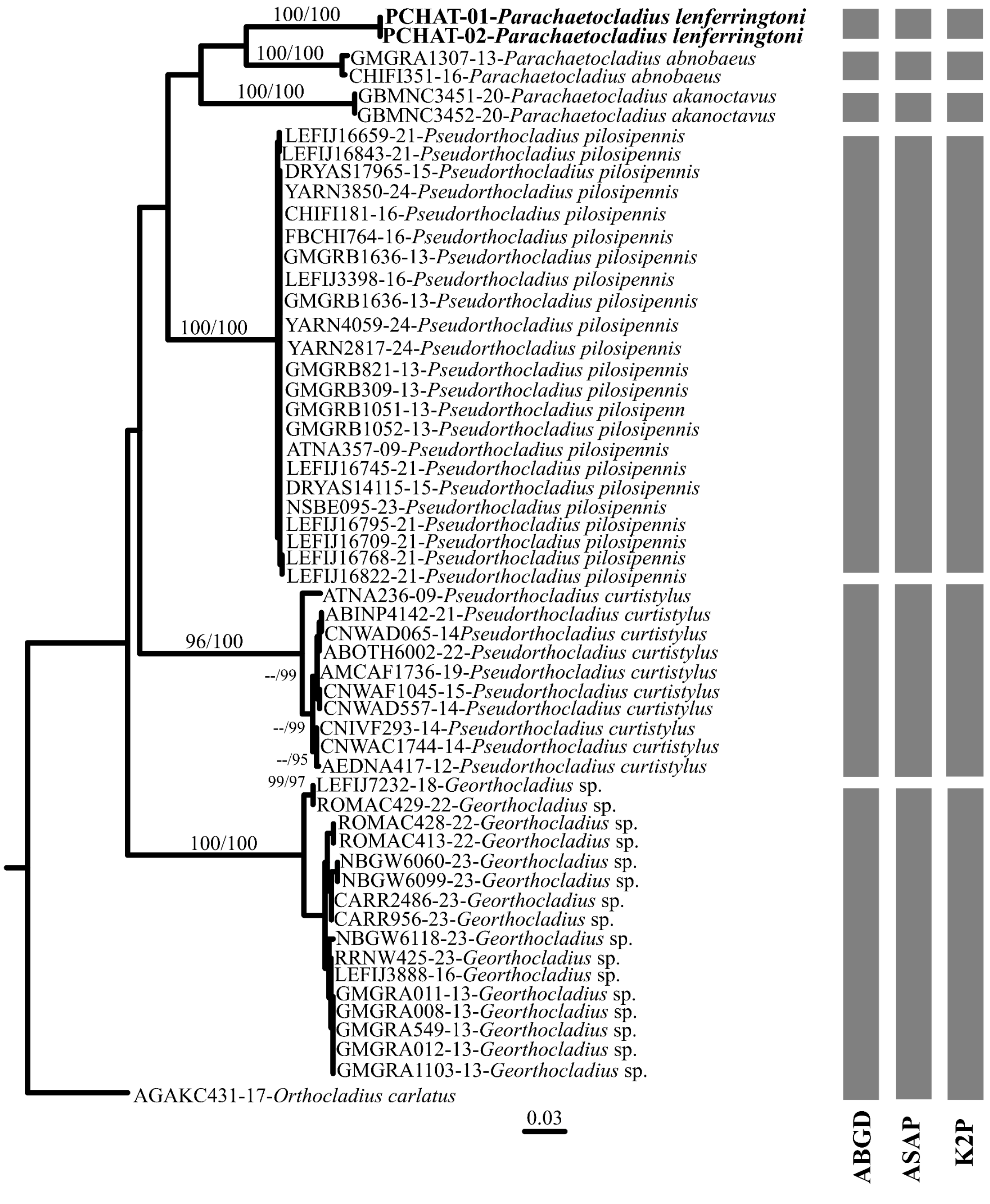

Figure 1. Neighbor-Joining (NJ), Maximum Likelihood (ML) trees of the genera *Georthocladius* Strenzke, 1941, *Parachaetocladius* Wülker 1959, *Pseudorthocladius* Goetghebuer, 1932, and one outgroup *Orthocladius carlatus* (Roback, 1957) inferred from the COI nucleotide sequence data (658 bp). Numbers on branches represent the bootstrap value for Neighbor-Joining (NJ) and Maximum Likelihood (ML) (10000 replicates; values < 95 were omitted). The gray bars on the right are clusters estimated using three molecular species delimitation methods: ABGD, ASAP, and K2P (species delimitation based on the K2P distance, new species threshold value of 3% for sequences used).

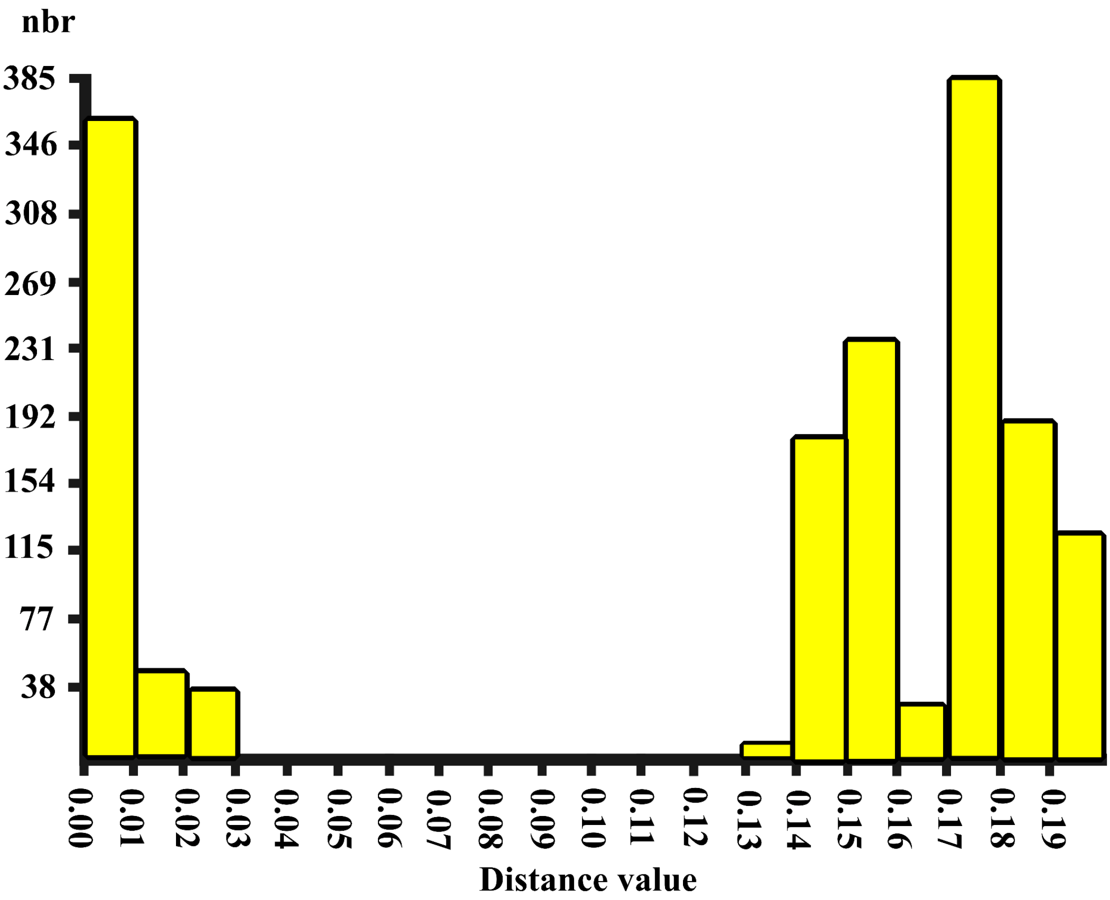

Figure 2. Histograms of genetic distance estimates from ABGD (Automatic Barcode Gap Discovery) for a partition analysis of 56 cytochrome oxidase subunit1 sequences of the *Georthocladius*, *Parachaetocladius,* and *Pseudorthocladius* spp. nbr = number of runs.

using distance-based techniques (such as ABGD and ASAP), is large enough (i.e., much larger than previous studies) to support delimitation of *P. lenferringtoni* from that of *P. abnobaeus* and *P. akanoctavus*. The tree-based and distance-based genetic approaches have been successfully used in recent studies to illustrate the delimitation of Chironomidae species. However, the species threshold (maximum intraspecific distance) of the distance-based methods between chironomid genera varies considerably. For example, the threshold for the *Tanytarsus* species was set at 4–5% by Lin et al. (2015), and within the same genus, the threshold was set at 1–6% for the *Tanytarsus mendax* group by Giłka et al. (2018). For other genera, the selected thresholds have also varied, including 5–8% for *Polypedilum* species (Song et al. 2018) and 4-6% for *Stenochironomus* species (Song et al. 2022). Based on the results obtained in this study, we suggest that the threshold of 3% is sufficient to set the species limit for the *Parachaetocladius* species. The results of the molecular analyses in this study further support our morphological findings in confirming the validity of *P. lenferringtoni.*

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DIET AND FUNCTIONAL FEEDING GROUPS OF CHIRONOMIDAE (DIPTERA) LARVAE IN ALPINE FRESHWATER HABITATS

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Abstract

A gut content analysis (GCA) was performed to quantify the relative use of food resources by larvae of chironomids (Diptera: Chironomidae) inhabiting different Alpine freshwater habitats fed by meltwater (kryal), groundwater (krenal) and mixed waters (glacio-rhithral, proglacial pond) in the Italian Alps (Trentino). GCA was performed on the 13 most frequent and abundant taxa in these habitats: *Diamesa bertrami, Diamesa latitarsis, Diamesa steinboecki, Diamesa zernyi, Pseudokiefferiella parva, Eukiefferiella minor, Metriocnemus eurynotus* gr., *Parametriocnemus stylatus, Thienemanniella clavicornis, Tvetenia calvescens, Macropelopia* sp., *Zavrelimyia* sp., *Micropsectra atrofasciata* gr. Guts were removed, mounted in Canada Balsam, and examined under a microscope (1000x). The gut of each individual was assumed to be 100% full, and proportions of the different food items were estimated using a 10x10 grid designed with the NIS-BR software. Food items were divided into 10 categories: Mineral Material, Animal Tissue, Algae (except diatoms), Diatoms, Plant tissue, Amorphous detritus, Lichens, Bryophytes, Fungi, and Pollen. The gut content of different taxa contained significantly different ($p < 0.05$) food categories based on a non-parametric statistical test. Differences in the diet were observed between species living in the same habitat type and classified into the same trophic category (detritivores, grazers, or predators) and individuals belonging to the same species living in different habitat types. A certain trophic flexibility and omnivory was found, which may facilitate the adaptation of chironomids to changes in available resources due to glacier retreat.

Introduction

Chironomids (Diptera: Chironomidae), commonly known as non-biting midges, are a highly diverse and ubiquitous family of dipteran insects. They inhabit a vast array of aquatic and semi-aquatic environments worldwide ranging from oligotrophic mountain streams to eutrophic lowland lakes, temporary ponds, peat bogs, wet soil, and marine waters (Armitage et al. 1995) and are the sole insect taxon in specific habitats such as glacier-fed streams and springs (e.g., Lindegaard 1995, Płóciennik et al. 2016, Lencioni 2018).

The life cycle of most species involves an aquatic larval stage, which can last from weeks to years, followed by a brief pupal stage and an emergent adult. As primary consumers, larval chironomids play a major role in aquatic food webs, facilitating the transfer of energy from lower trophic levels (e.g., algae, detritus) to higher consumers such as fish, birds, and terrestrial invertebrates (Tokeshi 1995, Hågvar et al. 2016). Given their significant contribution to ecosystem functioning and their widespread distribution, understanding the dietary habits of chironomid larvae is crucial for elucidating nutrient cycling, energy flow, and the overall health of aquatic ecosystems, especially where they dominate the invertebrate community, such as alpine streams and springs (Lencioni et al. 2012, Rossaro et al. 2016). Resource partitioning among phylogenetically close species has been a fundamental concept in the development of modern ecology, serving as a primary structuring force for communities (Pulliam 2000, Butakka et al. 2016). Analysing the larval diet at the species level provides crucial information for assessing a species' ecological role in any given ecosystem. This may help us to understand the interactions between a species and its environment and between different species. Resulting information may lead to enhanced understanding of trophic community structures, ultimately providing better understanding of how these insects colonize specific habitats (Ingvason et al. 2004). Despite numerous studies carried out on Chironomidae, detailed investigations into the specific dietary components and feeding behaviours of alpine chironomid species remain limited.

Previous studies have ascribed chironomid genera and species to different trophic groups (e.g. detritivores, predators, algivores, phytophages, xylophages, parasites, and commensals) and different feeding strategies (gatherers, suckers, filterers, shredders, scrapers) (e.g. Coffman and Ferrington, 1996, Tavares-Cromar and Williams, 1997, da Silva et al., 2008, Sanseverino and Nessimian, 2008, Galizzi et al., 2012, and Merritt et al., 2019). Moog and Hartmann (2017) merged trophic groups with trophic strategies, recognizing 10 main feeding types (recognized by the authors also as “functional feeding groups”) with associated specific food sources for chironomid larvae (Table 1): shredders, grazers, scrapers, raspers, active filter-feeders, eddy filterers, passive filter feeders, detritus feeders (gathering collectors), leaf borers, miners, piercers, xylophagous, predators, parasites, other feeding types/omnivorous. Armitage et al. (1995) recognized that most genera and species exhibit multiple feeding strategies and belong *de facto* to different trophic groups.

Additionally, what larvae eat may vary with the developmental stage, i.e. early instar larvae (I–II instars) could feed differently from mature larvae (III–IV instars) (Baker and McLachlan 1979, Olafsson 1992, Banegas and Rocha 2023). Seasonality and food availability may also drive larval feeding strategies (algae, detritus and associated microorganisms, plant fragments, decomposing wood, fungi, pollen, and invertebrates) (Berg 1995, Henriques-Oliveira et al. 2003, Sanseverino and Nessimian 2008); furthermore, these factors are both influenced by environmental conditions and substrate type (Butakka et al., 2016). This makes the trophic categorization of chironomid species a challenging topic.

This study investigates the trophic ecology of chironomid larvae from various aquatic habitats in glacial catchments of the Alps. New insights are given on the ecological role of chironomids in streams and ponds with different glacial influence as well as on the degree of dietary specialization, and their capacity of using different food sources. The information gathered is expected to be highly valuable to foresee how glacial taxa will adjust their feeding habits in response to the habitat shifts and glacial recession currently underway due to global warming (Wilkes et al. 2023).

Materials and Methods

Study area and sampling sites

We analysed larvae collected in late summer 2022 from seven sites in four catchments (Fig. 1, Table 2), in three different mountainous groups in the Italian Alps (Trentino Province): Conca/Carè Alto

Table 1. Functional feeding groups (FFGs) according to Moog and Hartmann (2017). CPOM = Coarse Particulate Organic Matter -particles between 50 µm and 1 mm); FPOM = Fine Particulate Organic Matter – particles < 50 µm.

FFGs	Abbreviation	Sources of food
Shredders	SHR	Fallen leaves, plant tissue, CPOM
Grazers	GRA	Epilithic algal tissues, biofilm, partially POM
Scrapers, raspers		Endo and epilithic algal tissues, partially tissues of living plants
Active filter-feeders	AFIL	Food in water current is actively filtered
Eddy filterers		Suspended FPOM, micro prey is whirled
Passive filter feeders	PFIL	Food brought by flowing water current
Detritus feeders (gathering collectors)	DET	Sedimented FPOM
Leaf borers, miners	MIN	Leaves of aquatic plants
Piercers		Algae and cells of aquatic plants
Xylophagous	XYL	Woody debris
Predators	PRE	Prey
Parasites	PAR	Host
Other feeding types	OTH	Cannot be classified into this scheme
Omnivorous animals		Diverse

Figure 1. Sampling sites (Northern Italy, Rhaetian Alps, 46°N).

Table 2. Sampling sites features. Glacier surface data from Smiraglia and Diolaiuti (2015). Chl *a* = chlorophyll *a*. GI = Glacial Index.

Sampling site	Stream	Type	Altitude (m a.s.l.)	Distance from the source in 2015 (km)	Feeding glacier surface in 2015 (km^2)	GI	Pfankuch index	Chl *a* ($\mu g/cm^2$)
AG1	Agola	kryal	2623	0	0.53	1	39	0.001
AGpond	Agola	proglacial pond	2596	0.02	0.53	0.95	15	0.067
MA1	Mandrone	kryal	2569	0.05	10.14	0.98	47	0.245
CR0	Careser	kryal	2858	0.75	1.39	0.61	58	0.008
CR2	Careser	glacio-rhithral	2642	2.29	1.39	0.34	44	0.134
CR1bis	Careser tributary	krenal	2645	1.36	-	0	15	2.228
C7	Conca tributary	krenal	2170	1.55	-	0	16	1.221

(site C7) and Mandrone (site MA1) in the Adamello-Presanella Mountain Group, Careser (sites CR0, CR1bis and CR2) in the Ortles-CevedaleMountain group, and Agola (AG1 and AGpond) in the Brenta Dolomites. All sites are located above the treeline (> 2000 m a.s.l.). Three sites are fed by meltwater (kryal type: AG1, MA1 and CR1), two by groundwater (krenal type: C7 and CR1bis), and two with mixed origin (one glacio-rhithral type: CR2 and a proglacial pond: AGpond). Based on the origin, these sites have different glacial influence, quantifiable as Glacial Index (GI), and calculated by combining the glacier area with distance from the glacier terminus according to Jacobsen and Dangles (2012). The index varies from 0 (no glacial influence) to 1 (maximum glacial influence). For each site, substrate stability was evaluated using the Pfankuch Index (Pfankuch 1975), which is a visual and subjective index that varies from 15 (maximum stability) to 60 (minimum stability). Primary production was measured as chlorophyll *a* from biofilm (Biggs and Kilroy 2000). Biofilm was collected by scrubbing submerged pebbles of known surface area (3x3 cm^2) with a toothbrush.

Samples were filtered in the field using a vacuum pump with glass microfiber filters (Whatman GF/CTM, 1.2 µm). Filters were stored frozen until analysis. In the lab, chlorophyll *a* was extracted with 90% acetone and the concentration was read at 665 nm and 750 nm, according to Steinman et al. (2017). Overall, the sites with the highest glacial influence and the lowest substrate stability were CR0 and MA1, followed by CR2 and AG1 (Table 2). These sites also exhibited the lowest primary production, with the exception of MA1, which had significant presence of large thalli of the crysophyte *Hydrurus foetidus* Villars. The highest primary production was measured in the two krenal sites (CR1bis and C7), where bryophytes were also present.

Sampling of larvae and gut content analysis

Larvae were collected in the morning using a pond net (100 µm mesh size) using the kick sampling technique in five different microhabitats/site for two minutes each. Additional samples were collected using a drift net (100 µm mesh size) for 1 hour in each site. Larvae were preserved in 75% ethanol immediately after sampling. Sorting, species identification, and larval instar confirmation were performed under a stereomicroscope (Leica S Apo 10X-80X coupled with Flexcam C5) after preparing permanent slides in Canada Balsam following separating the gut (see details below) under a compound microscope (Nikon Eclipse Ci, up to 1000x magnification) according to: McCauley (1974), Schmid (1993), Epler (2001), Janecek (2007), Andersen et al. (2013), Vallenduuk and Pillot (2014), Ferrington and Berg (2019).

Gut content analysis (GCA) was performed on a selection of species, including 13 taxa that represent the most frequent and abundant taxon in these habitats according to Lencioni et al. (2021): *Diamesa bertrami, Diamesa latitarsis, Diamesa steinboecki, Diamesa zernyi, Pseudokiefferiella parva, Eukiefferiella minor, Metriocnemus eurynotus* gr., *Parametriocnemus stylatus, Thienemanniella clavicornis, Tvetenia calvescens, Macropelopia* sp., *Zavrelimyia* sp., *Micropsectra atrofasciata* gr. Mature larvae (III-IV instar) were available for all species, while early instars (LI and LII) were only available for a few species and only a few specimens were collected. For this reason, we report in this work only the results of the mature larvae, as we are aware of the fact that assigning a trophic group or feeding strategy based on a single larval stage is not sufficient to define the diet of a species. In any case, in our opinion, the data is still of interest for beginning to explore the diet of cryophilic species that have not yet been studied in depth. In Table 3 we report the functional feeding groups (FFG) assigned *a priori* to the 13 taxa according to Moog and Hartmann (2017) (Table 1); however, for *Metriocnemus eurynotus* gr. we based FFG on Pillot (2013).

Guts of 3-4 individuals per taxon were removed, for a total of 72 individuals analysed. We verified that the intestine did not empty when pressed under the cover slip. Proportions of the different

Table 3. Feeding groups assigned to the 13 chironomid taxa according to Moog and Hartmann (2017); the scores assigned to *Metriocnemus eurynotus* gr. were derived from its feeding habits, described by Pillot (2013). The symbol * indicates the major feeding preference while the symbol + indicates a secondary preference. Numbers indicate the score attributed to the feeding group (from 1 to 10).

Taxon	GRA	AFIL	DET	MIN	PRE
Diamesa bertrami Edwards, 1935	*	-	+	-	+
Diamesa latitarsis (Goetghebuer, 1921)	*	-	+	-	+
Diamesa steinboecki Goetghebuer, 1933	*	-	+	-	+
Diamesa zernyi Edwards, 1933	*	-	+	-	+
Pseudokiefferiella parva (Edwards, 1932)	*	-	+	-	+
Eukiefferiella minor (Edwards, 1929)	8	-	2	-	-
Metriocnemus eurynotus gr.	*	-	*	-	-
Parametriocnemus stylatus (Kieffer, 1924)	*	-	*	-	-
Thienemanniella clavicornis (Kieffer, 1911)	6	-	4	-	-
Tvetenia calvescens (Edwards, 1929)	7	-	3	-	-
Macropelopia sp.	-	-	2	-	8
Zavrelimyia sp.	-	-	-	-	10
Micropsectra atrofasciata gr.	1	1	8	-	-

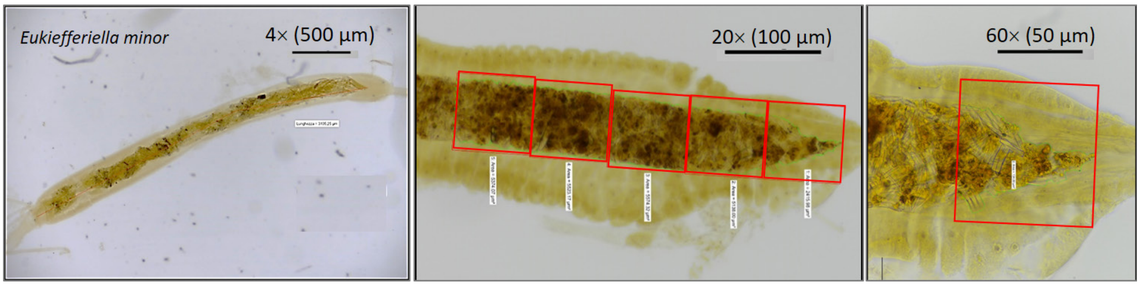

Figure 2. Gut of one IV-instar larvae of *Eukiefferiella minor*. Red frame represents the grid designed with the NIS-BR software. The length of the gut was measured at 4x magnification (on the left), details in each quadrant at 20-60x magnification. The numbers on the white background are the measured lengths and areas, automatically displayed on the image in minute font.

food items were estimated using a 10x10 grid designed with the NIS-BR software (camera DS-Ri2 on Nikon Eclipse) (Fig. 2). Specifically, the total length of the intestine was measured at 4x magnification and then it was virtually divided into ten subsections of equal size (red squares/rectangles in Fig. 2). A photograph was taken of each of these ten squares. The areas occupied by different food categories within each square were identified and measured using magnification of 40x or 60x. The percentage of area occupied by each food category was calculated by first determining the area relative to the sum of all recorded food categories within each gut subsection, and then averaging these percentages across the entire gut following the methodology of Cheshire et al. (2005).

Food items were divided into 10 categories, according to Cheshire et al. (2005): MM = Mineral Material, AT = Animal Tissue, ALG = Algae (except diatoms), DIA = Diatoms, PT = Plant tissue, AD = Amorphous detritus (FPOM), LIC = Lichens, BRYO = Bryophytes, FUN = Fungi (including fungal spores and hyphae), and POL = Pollen. We modified these categories according to Moog & Hartmann (2017) with plant tissue fragments < 1 mm in PT and AD as only FPOM. Food items were associated with FFGs (Table 4). Diatoms were identified according to Barber and Haworth (1981) and Hofmann et al. (2011). Beyond diatoms, efforts were made to identify other resistant microalgal structures, utilizing specialized literature on Alpine stream phytobenthos, such as Entwisle et al. (1997), Dillard (1999), Komárek and Anagnostidis (2007, 2008), Komárek (2013), and Cantonati et al. (2015).

Inter-individual, inter-species, and inter-site significant differences ($p< 0.05$) for percentage of food categories were tested with the Mann-Whitney non-parametric U test and one way ANOVA, performed using STATISTICA version 12.0 (®Statsoft).

Results and Discussion

Catalogue of gut contents

The first catalogue of the gut contents of alpine chironomids (e.g., Figs 3-6) includes a long list of algae, most of which were diatoms (Table 5) which was expected as diatoms are generally dominant in headwaters (Bert et al. 2024). The majority of algae with soft structures were unidentifiable due to the rapid digestion of carbohydrates. However, resistant structures like siliceous diatom cell walls persisted. Cells belonging to this group were often found emptied of their contents but with intact skeletons even after expulsion. Similarly, thick-walled cells, including cysts, other resistance structures, or certain green algae (e.g., *Cosmarium*) and a cyanobacteria (e.g., *Chamaesiphon*, very common in glacial habitats), remained undigested despite enzymatic activity. In Alpine streams, significant cyst-forming algae components of the phytobenthos are chrysophytes, specifically *Hydrurus foetidus* and *Phaeodermatium rivulare* (Rott et al. loc. cit.). Therefore, unidentified algal cysts found in this work are likely attributable to one of these two species.

Table 4. Food items categories and associated dietary groups.

Food Categories	Associated FFG
MM – Mineral Material	DET - Detritivores
AT – Animal Tissue	PRE - Predators
ALG – Algae (except diatoms)	GRA – Grazers
DIA - Diatoms	GRA - Grazers
PT – Plant tissue	SHR – Shredders
AD – Amorphous detritus	DET - Detritivores
LIC - Lichens	GRA – Grazers
BRYO – Bryophytes	SHR – Shredders
FUN - Fungi	DET – Detritivores
POL - Pollen	DET – Detritivores

To illustrate the variety of habitat types and food categories from the most abundant species found in different sites, we selected four representative species as examples: *T. calvescens* (from site CR-1bis) (Fig. 3), *P. parva* (from site C7) (Fig. 4), *M. atrofasciata* gr. (from site C7) (Fig. 5), *D. steinboecki* (from AG1 and MA1), and *D. zernyi* (from CR0 and MA1) (Fig. 6). The gut contents of these four taxa can be seen in Figures 3-5 and show vegetal groups, which are mostly represented in high altitude rhithral and krenal streams (i.e., mosses, thallous chrysophytes), and some of the most common diatom taxa composing the epiphytic communities growing on mosses on circumneutral to slightly acidic waters (i.e., elongated *Fragilaria* spp., *Eunotia* sp., *Odonthdium aff mesodon,* and *Tabellaria flocculosa*) (Rott et al. 2006, Cantonati et al., 2012, Rotta et al. 2018). Pollen grains, fungal spores, and chrysophyte spores were also represented. In contrast, the gut contents in Figure 5 were mainly composed of mineral material and very scarce vegetal cells (chrysophte cysts of *H. foetidus* and coccal cyanobacteria), thus reflecting the very sparse vegetation and periphytic assemblages characterising kryal streams

Mineral material in some individuals of *Diamesa* spp. comprised 99% of gut contents (Fig. 6). Among the MM, only a few cysts of crysophytes such as *Hydrurus foetidus* and a few colonies of *Chamaesiphon* where observed. We can argue that MM is ingested, not digested by the animal, and then expelled. In environments with significant glacial influence, some MM ingestion could also be involuntary due to high turbidity. We associated the presence of MM in the gut with a detritivorous feeding habit, where organisms likely consume the organic coating on mineral particles. Additionally, larvae might ingest MM for the microorganisms (bacteria, fungi, viruses) associated with it, as also argued by Sharp et al. (1999), Logue et al. (2004), Hodson et al. (2008), and Clitherow et al. (2013). Therefore, for these animals living in habitats with low productivity, bacteria may be an important food source.

Inter-individual variability within the same population

Inter-individual variability was observed within individuals of the same population for all species (Fig. 7). For example, in Figure 7A the comparison between three individuals of *P. parva* collected from the same site (C7) is shown, highlighting that this species preferentially consumes bryophytes, although they were absent in the gut of one specimen (individual 1) where bryophytes were replaced by diatoms (68%). Similarly, *D. zernyi* from C7 (Fig. 7B) consumed diatoms if present, however in one individual, amorphous detritus

Table 5. List of diatoms identified in larval gut of chironomids collected in four study sites belonging to three habitat types.

	C7 (krenal)	CR1bis (krenal)	CR2 (glacio-rhithral)	AGpond (proglacial pond)
Achnantes sp.	x		x	x
Achnantidium minutissimum	x	x		
Aulacoseira sp.	x			
Brachysira sp.		x		
Caloneis cf. sp.		x		
Cymbella sp.	x	x	x	
Cymbopleura sp.		x		
Diatoma cf *mesodon*	x	x		
Diatoma sp.	x	*x*		
Eunotia sp.	x	x	x	
Fragilaria arcus		x		
Fragilaria sp.	x		x	
Fragilaria ulna		x		
Gomphonema sp.	x	x	x	
Nitzschia sp.	x	x		
Pinnularia sp.		x		
Tabellaria sp.	x	x		

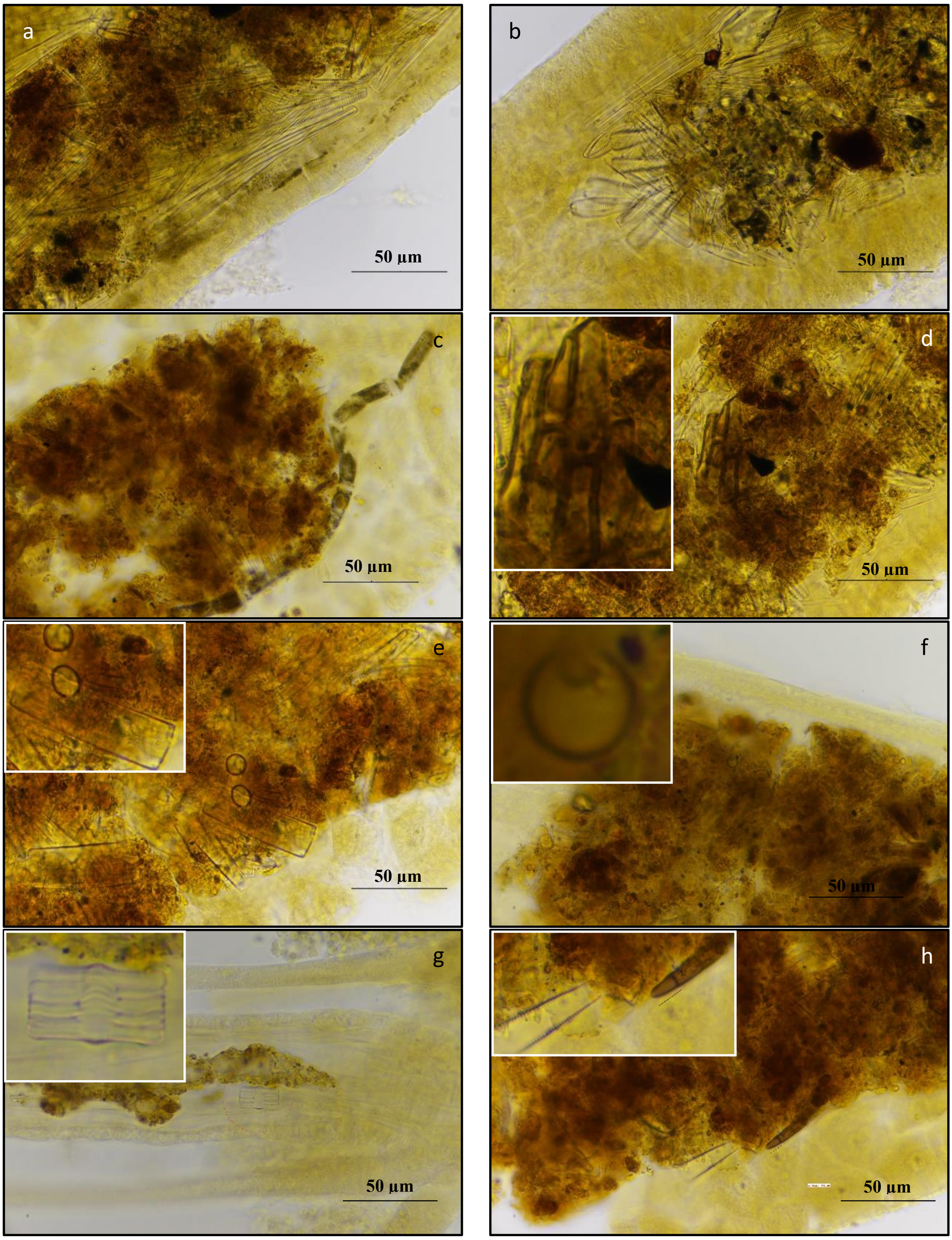

Figure 3. *Tvetenia calvescens* (site CR1bis). a. *Fragilaria aff. ulna* (DIA); b. *Gomphonema* sp. and *Fragilaria* sp. (DIA); c. cf *Klebsormidium rivulare* (ALG); d. Plant tissue (PT); e. Chrysophyte cysts (ALG) and *Eunotia* sp. (DIA); f. Chrysophyte cyst (ALG); g. *Tabellaria flocculosa* (DIA); h. Fungal spores (FUN) and *Gomphonema* sp. (DIA).

was the dominant food type. In *T. calvescens* (Fig. 7C) amourphous detritus was the dominant food type consumed by two specimens but a third specimen consumed more diatoms. Intra-specific differences in gut content of chironomid larvae were observed also by other authors (e.g. Lemes-Silva et al. 2014). The frequently observed significant inter-individual dietary variation was addressed by some authors as being shaped by resource availability at the microhabitat level (e.g. Lemes-Silva et al. 2014) and by intra-specific competition for food by others (Wiley 1981).

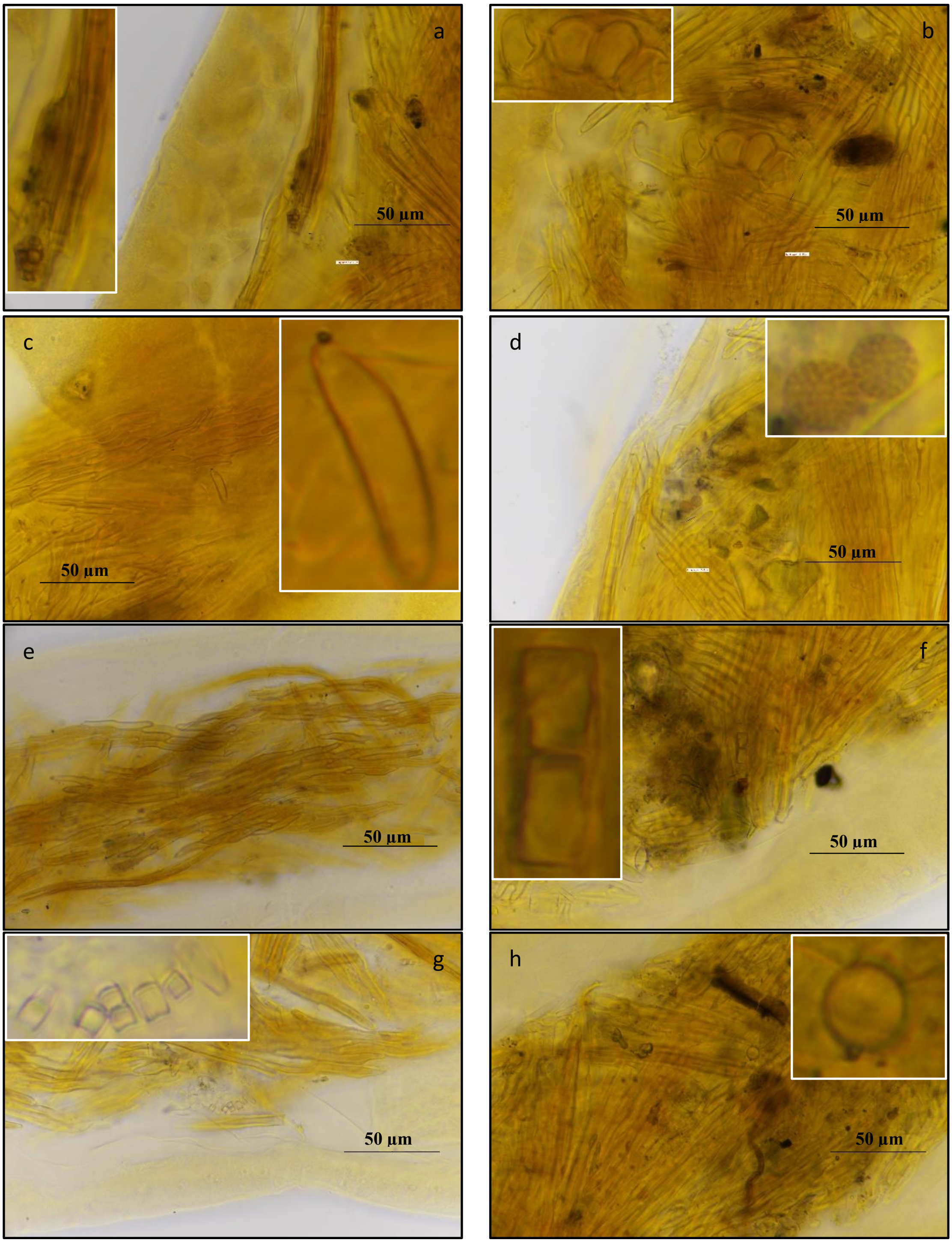

Figure 4. *Pseudokiefferiella parva* (site C7). a. Filamentous cyanobacterium (ALG); b. Plant tissue with empty cells of unidentified green algae (PT, ALG); c. *Eunotia* sp. (DIA); d. Pollen grains (POL); e. Moss (MOS); f, g. *Odonthidium* aff. *mesodon* (DIA); h. Chrysophyte cyst (ALG).

Inter-species variability from the same habitat type

Significant differences were observed between species living in the same habitat type and classified *a priori* (i.e., based on Moog and Hartmann, 2017) into the same feeding group (e.g., detritivorous, grazer, or predator). In Figure 8 we provide an example of five grazer-detritivorous (GRA-DET) species collected in the krenal site CR1bis. According to our analysis, *D. zernyi* and *P. stylatus* can be classified as DET-GRA, *E. minor* as GRA-DET, *P. parva* as GRA-DET-SHR (SHR = shredder; with bryophytes representing 53% of ingested

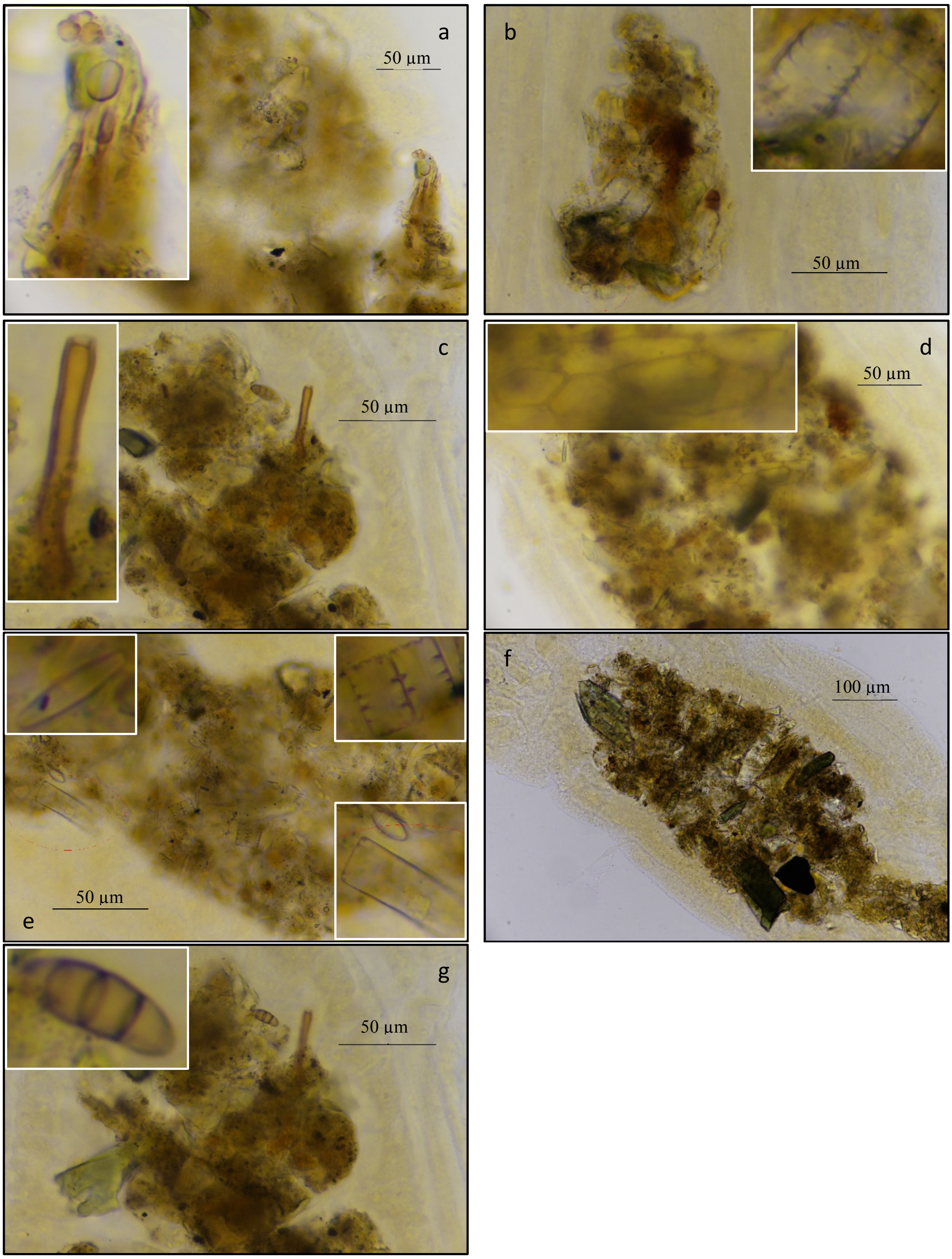

Figure 5. *Micropsectra atrofasciata* gr. (site C7). a. Filamentous cyanophyte (ALG); b. *Diatoma* sp. (DIA); c. Fungal hypha (FUN); d. Plant tissue (PT); e. On the top left *Achnantidium minutissimum*, on the top right *Diatoma* sp. and on the bottom right *Eunotia* sp. (DIA); f. Mineral material (MM) and amorphous debris (AD); g. Fungal spores (FUN).

food), and *T. calvescens* as DET-GRA-SHR (also eating plants, fungi pollen and lichens, altogether 5% of the gut content). Amorphous detritus was much more prevalent in *D. zernyi* and *P. stylatus* than in the other species, whereas *P. parva* seems to prefer bryophytes and can also behave as a shredder which differs from its *a priori* categorisation (Table 3). These results suggest that, at least for IV instar larvae, species sharing the same habitat type or site and generally classified within the same trophic category may exhibit different diets (with high inter-individual variability, e.g., Fig. 7).

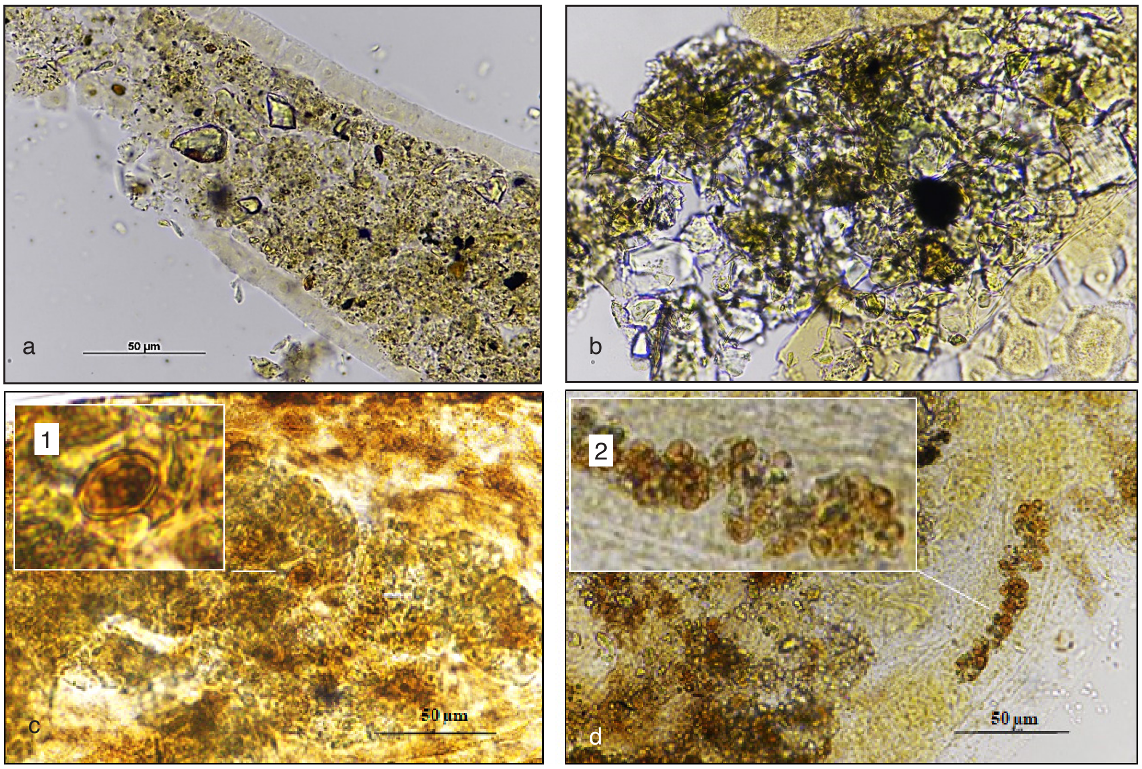

Figure 6. a. *Diamesa zernyi.* Mineral material (MM) and amorphous debris (AD) from site CR0; b. *Diamesa zernyi*. Mineral material (MM) and amorphous debris (AD) from site MA1; c. *Diamesa steinboecki*. 1) *Hydrurus foetidus* cyst (ALG) from site MA1 and d. 2) *Chamaesiphon* sp. (Cyanobacteria) (ALG) from site AG1.

Inter-habitat type variability within the same species

Larvae of the same species living in different types of habitats have gut contents with significantly different percentages of food categories. This was observed for most species. For example, larvae of *D. zernyi* (Fig. 9) living in kryal habitats have very similar stomach contents, dominated by MM followed by AD. Although not significant, the highest percentage of MM was observed in larvae from the CRO site, which was where the lowest primary production levels were measured (Table 2). However, in the gut of larvae collected in C7, diatoms significantly prevailed, suggesting that where diatoms are available, *D. zernyi* will feed preferentially on them. These differences between individuals of the same species from different habitats emphasize opportunistic behavior and suggest some flexibility in the feeding habitats in these species.

Conclusion

We assessed food item preferences according to taxonomy and habitat type with different primary production and we assume different algal abundance and composition. It is interesting to note that individuals belonging to the same species can be opportunistic and exhibit different diets in different habitat types. Opportunism is expected to be more frequent in glacier-fed streams than in groundwater-fed and/or downstream reaches with a broader array of resources available, which can better support specialist feeding behaviours (Sertić Perić et al., 2021). Generally, if diatoms are present, all chironomid species eat them, probably due to their high nutritional quality. In fact, based on the composition and concentration of fatty acids, diatoms are characterized as potentially excellent food resources, whereas other groups, such as chrysophytes and green algae are characterized as intermediate and cyanobacteria as low-quality food resources for herbivores in freshwater habitats (Taipale et al. 2013). Algal nutritional quality is generally of high importance for the fitness of consumers and for sustaining stream food webs (Guo et al. 2016, Niedrist et al. 2018).

Overall, the diet (based on what was present in the gut of late instar larvae) and feeding group of the different species were reviewed. A certain trophic flexibility and omnivory was found, which may facilitate the adaptation of chironomids to changes in available resources due to glacier retreat.

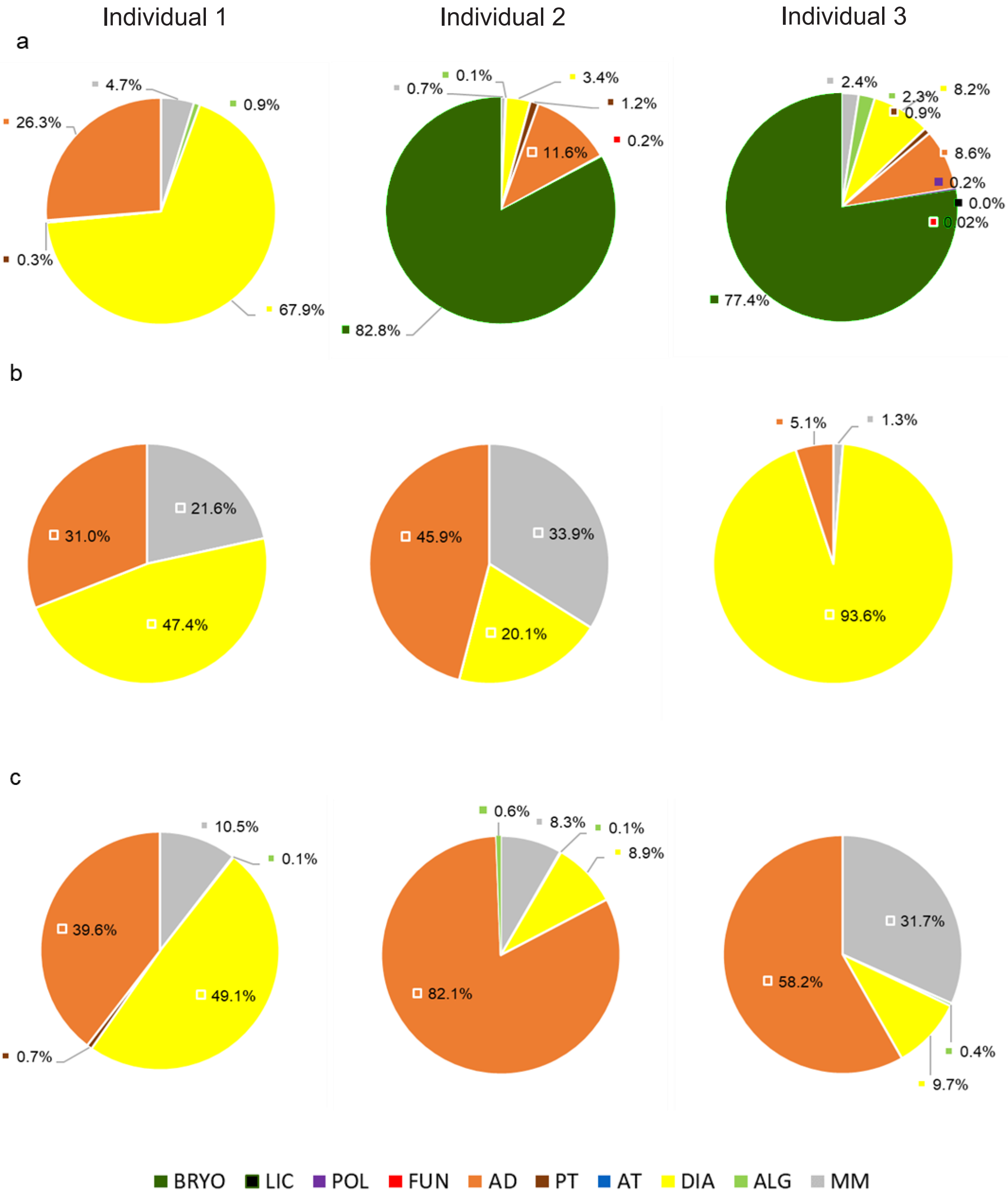

Figure 7. Inter-individual variability observed in three individuals of the same species from the same site (C7). Numbers in the legend refer to the 10 food items reported in Table 3. a. *P. parva* larvae from site C7; b. *D. zernyi* from C7; c. *T. calvescens* from CR1bis. MM = Mineral Material, AT = Animal Tissue, ALG = Algae (except diatoms), DIA = Diatoms, PT = Plant tissue, AD = Amorphous detritus, LIC = Lichens, BRYO = Bryophytes, FUN = Fungi (including fungal spores and hyphae) and POL = Pollen.

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The first author dedicates this article to her friend and mentor, Len C. Ferrington Jr.

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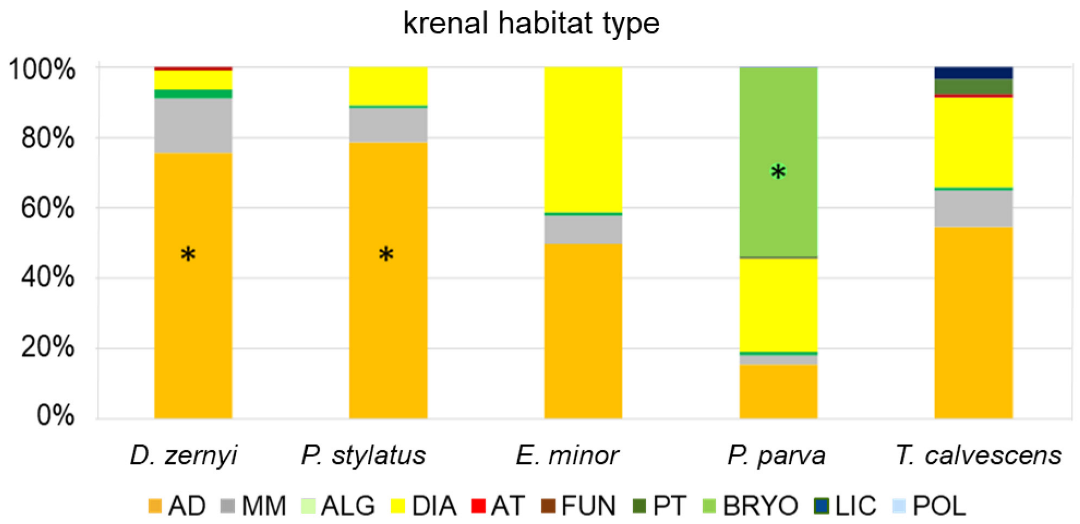

Figure 8. Relative composition of gut contents in five species from a krenal habitat type (site CR1bis). * = significant different food items ($p < 0.05$). AD = Amorphous detritus, MM = Mineral Material, ALG = Algae (except diatoms), DIA = Diatoms, AT = Animal Tissue, FUN = Fungi, PT = Plant tissue, BRYO = Bryophytes, LIC = Lichens, POL = Pollen. For each species, n=3 specimens were examined.

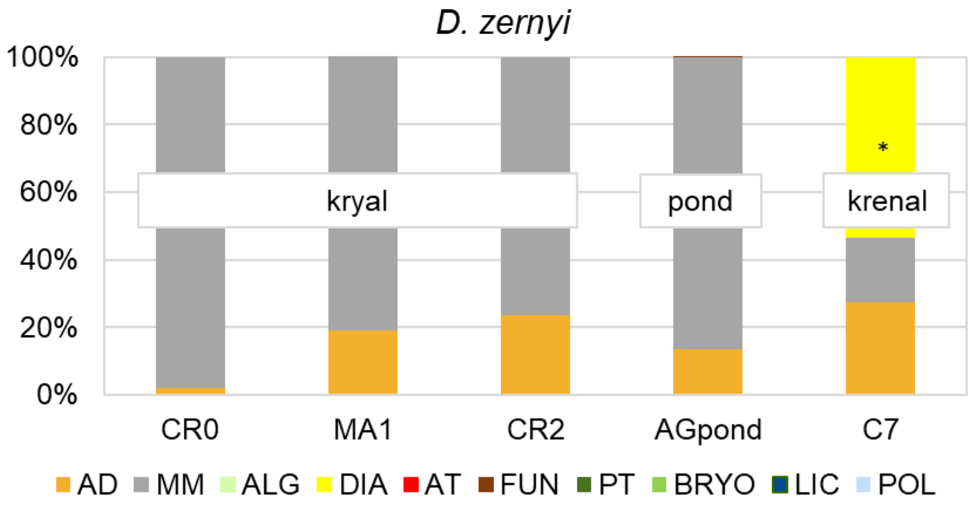

Figure 9. Diet of *Diamesa zernyi* from different sites and habitat types. CRO, MA1 and CR2 = kryal; AGpond = proglacial pond; C7 = krenal. * = significant different food item ($p < 0.05$). AD = Amorphous detritus, MM = Mineral Material, ALG = Algae (except diatoms), DIA = Diatoms, AT = Animal Tissue, FUN = Fungi, PT = Plant tissue, BRYO = Bryophytes, LIC = Lichens, POL = Pollen. For each site, n = 3 specimens were examined.

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Bugs Below Zero: Communicating Science and Engaging the Public with Winter Active Aquatic Insects and Stream Food Webs

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Abstract

Winter is typically viewed as a time when insects are thought to be inactive; however, some aquatic insects (e.g., chironomid flies, stoneflies, and mayflies) have species which complete multiple life stages and emerge as active, terrestrial adults during winter. These insects have adaptations that permit survival at low temperatures and are known to occur in seasonally cold environments worldwide. However, awareness and education around these specialized insects are lacking partially due to the limited research and education centered around winter aquatic ecology. The *Bugs Below Zero* project, started in 2019, aims to enhance awareness and increase appreciation for winter-adapted aquatic insects, providing opportunities for the public to engage in community science efforts collecting data on these insect groups. The program has received positive reception in classroom and outreach settings and has successfully provided multimedia educational materials to hundreds of educators and data collection opportunities to numerous volunteer groups and classrooms. With the help of volunteers, the *Bugs Below Zero* team aims to add to the body of scientific knowledge about winter active insects and to continue educating students and community members about these organisms, their role in food webs, and their conservation needs.

Introduction

Aquatic insects that have recently completed their life cycle and emerged from water bodies are everywhere during the summer. However, relatively few people consider what happens to these insects during winter. Several groups of aquatic insects, including select Plecoptera, Ephemeroptera, Trichoptera, and Diptera (e.g., chironomids, craneflies) are adapted to emerge during winter (Lencioni 2004, Hågvar 2010, Soszyńska-Maj et al. 2016, Bouchard and Gelhaus 2020, Anderson et al. 2025/this issue). In particular, the temperature-modifying influence of groundwater springs keep groundwater-dominated stream sections “winter warm” and ice-free, permitting certain aquatic insects to complete their life cycle and emerge as reproductively active adults (Bouchard and Ferrington 2009, Anderson and Ferrington 2013, Baranov and Ferrington 2013, Nyquist et al. 2020). These winter-active species are well adapted to low temperatures, and exhibit behavioral, physiological, and biochemical adaptations that allow them to thrive during the cold winter season (e.g. Anderson et al. 2025/this issue).

The Chironomidae are among the most common winter-emerging aquatic insects, with over 220 taxa worldwide currently recorded as emerging during winter (Anderson et al. 2025/this issue). Chironomids are often seen walking or mating on snowbanks flanking ice-free groundwater-fed streams or even flying short distances. Research shows that these winter-emerging species are important components of winter food webs as a prey resource for certain fish (French et al. 2014, 2016, Anderson et al. 2016, Cochran-Biederman and Vondracek 2017) and birds (Braz et al. 2014).

Despite the long winter season in northern temperate regions, there is still limited awareness and understanding of cold-adapted insects and the role they play in groundwater-dominated streams. The *Bugs Below Zero* project (BBZ; www.bugsbelowzero.com), conceptualized by Dr. Len Ferrington, Jr. and a team of interdisciplinary, Minnesota-based researchers in 2019, aims to raise awareness and enhance the understanding of chironomids and other winter-active aquatic insects. BBZ strives to achieve this via a participatory science program that can guide research, along with offering classroom education and community outreach opportunities. Specific overarching goals of BBZ include: 1) raising awareness about the winter life of aquatic insects; 2) helping others to understand the value of chironomids and other aquatic insects and to appreciate their importance within stream food webs; and 3) gaining assistance from community scientists looking for and documenting winter-emerging insects.

BBZ includes resources on winter-active insect species and basic identification, kindergarten (K) to grade 16 classroom resources and activities, and information and directions for a community participatory science program where participants walk short sections of stream bank to collect and submit data about chironomids and other aquatic insects that are active on the snow (Fig 1). Volunteers are asked to count and identify insects they observe (chironomids, caddisflies, stoneflies, mayflies), differentiate sex when feasible (e.g., in chironomids), document behavior, and take pictures to help researchers verify identification. Participants are also encouraged to make observations related to weather, stream conditions, and habitat. Data are submitted via the *Anecdata* community science app at: https://www.anecdata.org/projects/view/949. Findings submitted via *Anecdata* provide researchers, agencies, classrooms, and community volunteers with open access data on winter insect activity and winter stream conditions over time, with an additional underlying goal of allowing participants to directly engage with and appreciate winter insect activity.

While winters often feel long, the field season is short; enlisting help from community scientists allows researchers to better understand the winter insect community. The participatory science project has the potential to help scientists understand how variation in winter insect abundance and behavior differs among streams, sexes, taxa, under different air or water temperatures, and at different distances from the stream. This information may also inform natural resource managers as scientists are yet unsure how ecosystems, and the species within, will be impacted under scenarios of climate change.

Outreach and Reception

The BBZ project has garnered attention through its website, social media channels, blog posts, mailings, videos, podcasts, webinars, partner newsletters, media relations, and in-person outreach events. In 2023 and 2024 alone, the *Bugs Below Zero* team had more than 1,000 people attend in-person and live events and more than 2,000 unique visitors to the website.

The BBZ team has developed partnerships with natural history museums, nature centers, and nonprofit organizations like the Bell Museum, Belwin Conservancy, and Minnesota Valley National Wildlife Refuge to host interactive and educational programs for families, community members, and K–grade 12 classrooms. These programs offer an opportunity to handle insects, practice identifying them, and to participate in educational games that build knowledge of winter stream ecology and stream food webs (Figs 2 & 3). The team

Figure 1. Dr. Len Ferrington (left) and students searching for and collecting winter-active Chironomidae from a spring-fed stream in Minnesota, USA.

has also created supporting educational resources for K–grade 12 teachers, higher education classrooms, informal educators, and families, which are available on the website. For example, the team created an ethogram activity focused on documenting insect behavior in the field, instructions on how to use training datasets, videos that introduce stream environments and insects, and guides to support participants interested in collecting field data. These resources have been shared with over 500 educators, and BBZ activities have also been featured in multiple media interviews. Research conducted by the BBZ team shows there is growing interest and motivation to participate in winter community science, and educators are interested in involving classrooms in data collection (Swenson and Nyquist 2024). Specifically, educators view BBZ as an opportunity to break routine and provide students with relevant, hands-on experience in the field that creates enthusiasm for environmental issues, enhances connections and community, and contributes to meaningful science. Yet, barriers to participation exist, such as time constraints, access to field sites particularly during winter which presents additional field-safety concerns, and equity across the classroom, including mobility at stream sites and access to appropriate outerwear (Swenson and Nyquist 2024).

Conclusion

So far, the BBZ program has had positive reception from participants of its various activities with some sharing that they have learned about winter-active insects for the first time and have gained more confidence in identifying them. While BBZ is based in Minnesota, the team welcomes participants from northern-temperate states and countries around the world with the goal of increasing community participation in collecting data on winter-active insects and furthering knowledge and conservation of these uniquely adapted insect groups.

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Figure 2. Live aquatic insects used in demonstrations and workshops.

Figure 3. Middle school students learning about winter stream ecology and the importance of aquatic insects.

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