CHIRONOMUS

Journal of Chironomidae Research

No. 39 ISSN 2387-5372 November 2025

CONTENTS

Editorial

Ekrem, T. What is a species? 3

Current Research

Widmann, C., Marle, P., Brodin, Y. A new species of *Parametriocnemus* Goetghebuer, 1931 (Diptera: Chironomidae: Orthocladiinae) from Switzerland and elevation of *P. stylatus adzharicus* Kownacki & Zosidze, 1973 to full species. 4

Donato, A., Zanotto Arpellino, J.P., Siri, A. New distributional records and biogeographic considerations on *Pampacladius gaucho* Donato, Zanotto Arpellino and Siri (Chironomidae: Orthocladiinae) 13

Short Communications

Namayandeh, A., Ghaderi, E., Ram, J.L. Correcting a mistake: *Limnophyes stagnum* Namayandeh, Guerra & Ram, 2024 is not c onspecific with *Limnophyes* sp. 14ES 20

Raunio, J., Brodin, Y. *Cryptotendipes usmaensis* (Pagast, 1931) – a cryptic species of Chironomidae (Insecta, Diptera) 23

Bilalli, A., Musliu, M., Geci, D., Ibrahimi, H., Namayandeh, A. Some new faunistic records of Chironomidae (Diptera) from the Republic of Kosovo 29

Xiang, P., Tang, H. Two peculiar species of Oriental Chironomini (Diptera, Chironomidae) 34

News for the Chironomidae Community

Lin, X-L., Zhang, R-L. The 6th Chinese Symposium on Chironomidology 40

New books 41

Telmatogeton trilobatus at Point Reyes National Seashore, California. Photo: Steve Gaimari June 25th, 2008

CHIRONOMUS Journal of Chironomidae Research

The *CHIRONOMUS Journal of Chironomidae Research* is devoted to all aspects of chironomid research and serves as an up-to-date research journal and news bulletin for the Chironomidae research community. The journal is open access, and can be downloaded free from this website: http://www.ntnu.no/ojs/index.php/chironomus. The publisher is the NTNU Univeristy Museum at the Norwegian University of Science and Technology in Trondheim, Norway.

Research articles for the *CHIRONOMUS Journal* are subject to peer-review. New scientific names are registered in ZooBank (http://zoobank.org).

Contributions to *CHIRONOMUS Journal of Chironomidae Research* should be submitted online through the online journal system: http://www.ntnu.no/ojs/index.php/chironomus following the author guidelines. There are no submission deadline for articles as they will be published continously upon acceptance. News should be submitted by December 1 for inclusion in the yearly issue.

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

NTNU
University Museum

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

Front page photo: *Telmatogeton trilobatus* (Kieffer , 1911) at Point Reyes National Seashore, Marin County, California, USA. Identified by Pete Cranston. Photo: Steve Gaimari June 25th, 2008

Editorial

What is a species?

When I pose this question to our second-year bachelor students, they always offer thoughtful suggestions. The first usually refers to Ernst Mayr's biological species concept, which defines species as groups capable of producing fertile offspring. But, drawing on their field experience with plants and invertebrates from their first year, they quickly bring up the morphological species concept as well. From there, we discuss the strengths and weaknesses of different ways to define species, and how using different concepts and character sets can lead to conflicting conclusions. These discussions are always lively, and I never tire of debating this question with our biology students.

It is fascinating to reflect on the uncertainty surrounding the definition and boundaries of what is arguably the most fundamental unit in biodiversity science. Just imagine trying to conduct conservation work, natural resource management, ecological research or genomics without being able to refer to 'species'. Even food production, trade, and public health rely on our capacity to recognise, differentiate, and name species. The taxonomic baseline we provide is truly essential for discussing and understanding the natural world.

As all readers of *CHIRONOMUS* know, delimiting and distinguishing species in the Chironomidae is far from straightforward. Even species that seem easy to identify for a trained chironomidologist often turn out to be more complex once genetic variation among populations is examined using DNA barcodes. Although genetic data improve our understanding of species boundaries, they do not always give a final answer. Expert interpretation and experience still play an important role. For this reason, an integrative approach that brings together multiple lines of evidence – including morphological, molecular, and life history aspects – is needed to develop the strongest possible species hypotheses (see for instance Raunio and Brodin 2025 in this issue).

Ultimately, this is what taxonomy deals with: species hypotheses that are tested, refined, and evaluated by the scientific community. Sometimes, integrative research leads to new conclusions, as illustrated by the case of *Parametriocnemus adzharicus* in this issue of *CHIRONOMUS* (Widmann et al. 2025). In other cases, new data support existing hypotheses. Either way, the process of getting there—through rigorous, peer-reviewed, and reproducible science—is what drives the field forward.

The *CHIRONOMUS Journal of Chironomidae Research* therefore remains fully committed to publishing high-quality, open-access research that strengthens the taxonomic foundations on which all biodiversity science depends.

Torbjørn Ekrem

Department of Natural History, NTNU University Museum, Norwegian University of Science and Technology, Trondheim, Norway.

References

Raunio, J. and Brodin, Y. 2025. *Cryptotendipes usmaensis* (Pagast, 1931) – a cryptic species of Chironomidae (Insecta, Diptera). *CHIRONOMUS Journal of Chironomidae Research*. 39: 23-28. DOI: https://doi.org/10.5324/cjcr.v0i39.6375

Widmann, C., Marle, P. and Brodin, Y. 2025. A new species of *Parametriocnemus* Goetghebuer, 1931 (Diptera: Chironomidae: Orthocladiinae) from Switzerland and elevation of *P. stylatus adzharicus* Kownacki & Zosidze, 1973 to full species. - *CHIRONOMUS Journal of Chironomidae Research*. 39 (Jun. 2025), 4–12. DOI: https://doi.org/10.5324/cjcr.v0i39.6282

A new species of *Parametriocnemus* Goetghebuer, 1931 (Diptera: Chironomidae: Orthocladiinae) from Switzerland and elevation of *P. stylatus adzharicus* Kownacki & Zosidze, 1973 to full species

Christian Widmann[1], Pierre Marle[1,2] & Yngve Brodin[3]

[1]*Naturéum - State Museum of Natural Sciences, Department of Zoology, Lausanne, Switzerland.*
E-mail: christian.widmann@unil.ch. Corresponding author.
[2]*Laboratory of Aquatic Ecology and Biology, Department F.-A. Forel for Environmental and Aquatic Sciences, Institute for Environmental Sciences, Faculty of Sciences, Earth and Environment Sciences, University of Geneva, Geneva, Switzerland.*
E-mail: pierre.marle@unige.ch
[3]*Swedish Museum of Natural History. Department of Zoology, Stockholm, Sweden.*
E-mail: tav77ygg@gmail.com

https://zoobank.org/6DC4249C-6BA0-4FFB-AB11-68F4F664E0F0

Abstract

Parametriocnemus lausannensis Widmann, Marle & Brodin sp. n. is described and figured based on five barcoded specimens collected in the city of Lausanne, Switzerland. This species is morphologically similar to *P. stylatus* (Spärck, 1923), *P. lundbeckii* (Johannsen, 1905), *P. scotti* (Freeman, 1953) and *P. adzharicus* Kownacki & Zosidze, 1973 which is raised here from subspecies to species level. Males of *P. lausannensis* can be separated from related species by the squarish shape of the inferior volsella and by its DNA barcodes.

Introduction

The genus *Parametriocnemus* lies within the subfamily Orthocladiinae. The genus was initially described as a subgenus of *Metriocnemus* van der Wulp by Goetghebuer (Goetghebuer 1932), based on *M. stylatus* Kieffer, 1924. It was raised to the genus level by Brundin (1956). The morphologically closest relatives to *Parametriocnemus* are *Paraphaenocladius, Metriocnemus* and *Heterotrissocladius* (Cranston et al. 1989, Sæther et al. 2000).

Adult males of *Parametriocnemus* can be distinguished from other Orthocladiinae genera by a combination of characters described in Cranston et al. (1989). Wings have macrotrichia on the membrane, vein R_{4+5} ends opposite or slightly proximal or distal of vein M_{3+4} (much proximal and closer to end of Cu_1 in *Paraphaenocladius*), costa is distinctly extended (unlike in *Heterotrissocladius*), mid and hind leg tarsomeres are without pseudospurs (which are present in *Metriocnemus*), and in many species there is a markedly long parallel-sided dorsal eye extension (not long in the other genera).

As most genera of Chironomidae present in Europe, *Parametriocnemus* have recognized species in all continents except Antarctica, including some unidentified species in South America (Ashe and O'Connor 2012).

The larvae of *Parametriocnemus* are known from springs or running water from lowland to alpine regions. In the closely related genus *Paraphaenocladius*, the larvae are mostly terrestrial or semi-aquatic, but some might be fully aquatic in running water. In *Heterotrissocladius,* the larvae are mostly found in stagnant water, preferably lakes, but some also occur in running water and springs. Most species of *Heterotrissocladius* inhabit cold oligotrophic freshwater lakes (Sæther et al. 2000).

Ashe & O'Connor (2012) listed 35 accepted species of *Parametriocnemus* worldwide. Two species from China have been added since then (Li et al. 2013).

The cytochrome c oxidase subunit 1 (hereafter referred to COI) sequences of *Parametriocnemus* available in the Barcode of Life Data Systems (BOLD) (Ratnasingham et al. 2024) are grouped into 51 genetic clusters (Barcode Index Numbers, BINs). However, most *Parametriocnemus* COI genetic clusters have not been assigned a species level identification.

As part of an inventory species found at the first author's home, five chironomids were collected, four males and a one female that turned out to correspond to a species new to science. The new species, *Parametriocnemus lausanniensis* Widmann,

Marle & Brodin sp. n., is described and figured below.

Material and methods

Four males and one female of *Parametriocnemus lausannensis* were found dead inside a house at the type locality. Non-destructive DNA extraction and sequencing of the COI locus was performed in accordance with the methods described in Widmann et al. (2023), and in Widmann and Bächli (2022). The specimens were later mounted in Euparal on microscopy slides.

Construction of a neighbor joining (NJ) identity tree was done using sequences available from BOLD. Sequences from each BIN attributed to *Parametriocnemus* were selected for the alignment as well as sequences that were not labelled at the genus or species level but that were nevertheless close to other *Parametriocnemus* COI sequences. For each BIN, one sequence of 658 bp per country was randomly selected. If no 658 bp long sequences were available for a given country, the nearest longest sequences were selected. The selected sequences have been placed in a BOLD data set called DS-PARAMETR (DOI: dx.doi.org/10.5883/DS-PARAMETR). Alignment was performed using the online Clustal Omega tool (Goujon et al. 2010, Sievers et al. 2011) using the default parameters. The percent identity (PID) NJ tree was then constructed from this alignment using the Jalview program (v. 2.11.4.1) (Waterhouse et al. 2009). In Jalview, the method for the generation of the PID tree using the NJ algorithm relies on raw pairwise distances derived from sequence comparisons. The generated tree was saved in Newick format and further formatted using the FigTree (v1.4.4) software (Institute of Evolutionary Biology, University of Edinburgh; available at: http://tree.bio.ed.ac.uk/software/figtree). The illustrations were created using the software Inkscape (v1.1; Inkscape Project 2020) and the Olympus software Preciv Core Pro (v1.2).

Morphological species-level identifications of available BOLD records were done by Viktor Q. Baranov (*P. scotti*), Godtfred A. Halvorsen (*P. lundbeckii*), Valerie Levesque-Beaudin (*P. boreoalpinus*), Xiaolong Lin (*P. scotti*), C. S. Logan (*P. boreoalpinus*), Renee Miskie (*P. boreoalpinus*), Mikko Pentinsaari (*P. stylatus*), Kate Perez (*P. adzharicus*), Mateusz Plociennik (*P. stylatus*), Trey Simmons (*P. boreoalpinus*), Elisabeth Stur (*P. adzharicus*, *P. boreoalpinus*, *P. lundbeckii*, *P. stylatus*), Angela Telfer (*P. boreoalpinus*), Christian Widmann (*P. adzharicus*), Sofia Wiedenbrug (*P. stylatus*), Monica Young (*P. boreoalpinus*).

Results

The NJ tree in Fig. 1 shows that *P. lausannensis*, *P lundbeckii* (BOLD:AAP6586) and *P. scotti* (BOLD:ADY1862) (Baranov et al. 2024) form distinct and well separated clusters. *P. stylatus adzharicus* forms two close clusters containing four BINs (BOLD:AAI2687, BOLD:ACT9205, BOLD:ADA7271, BOLD:AAW0334). Under the heading *P. adzharicus* below, the rationale to raise *P. stylatus adzharicus* to the species level is explained.

P. stylatus, not including *P. stylatus adzharicus*, forms three clearly separated clusters containing 4 BINs (Fig. 1). As explained below, we were able to select one of these BINs as belonging to the nominal *P. stylatus*. The other BINs are likely to correspond to species that have yet to be formally described.

Comparison of pairwise genetic distances among barcodes showed that *P. lausannensis* has a within-species (intraspecific) variance of about 0.9% and an interspecific distance of about 12.5% to its nearest neighbor *P. scotti* (BOLD:ADY1682) from Namibia (Fig. 1). The NJ tree shows that the interspecific distance is greater when considering the other morphologically similar species *P. stylatus, P. adzharicus* and *P. lundbeckii,* all present in Europe. There is a shorter genetic distance between *P. stylatus*, *P. lundbeckii* and *P. adzharicus* compared to *P. lausannensis* (Fig. 1). *P. scotti*, *P. stylatus*, *P. lundbeckii*, *P. adzharicus* and *P. lausannensis* have superficially similar hypopygia but this similarity is not reflected in the NJ tree.

Parametriocnemus lausannensis sp. n.

https://zoobank.org/0F74C45D-D843-4229-A9F7-C31C48BC3A51

Material examined

Holotype: 1 male adult, Switzerland, Lausanne, N 46.534 E 6.656, 652 m above sea level, at a home window, 22.viii.2023, leg. C. Widmann, museum ID: GBIFCH00618687, BOLD ID: VALM135-24. Paratypes: 3 male adults and 1 female adult (museum IDs GBIFCH00618677, GBIFCH00618694, GBIFCH00618695 and GBIFCH00618689; BOLD ID: VALM134-24, VALM137-24, VALM138-24, VALM136-24; respectively).

The holotype and paratypes are housed in the collection of the State Museum of Natural Sciences, Department of Zoology, Lausanne, Switzerland (leg. Christian Widmann; det. Christian Widmann). Important identification features are shown in Figs 2-4. Additional images can be found on the

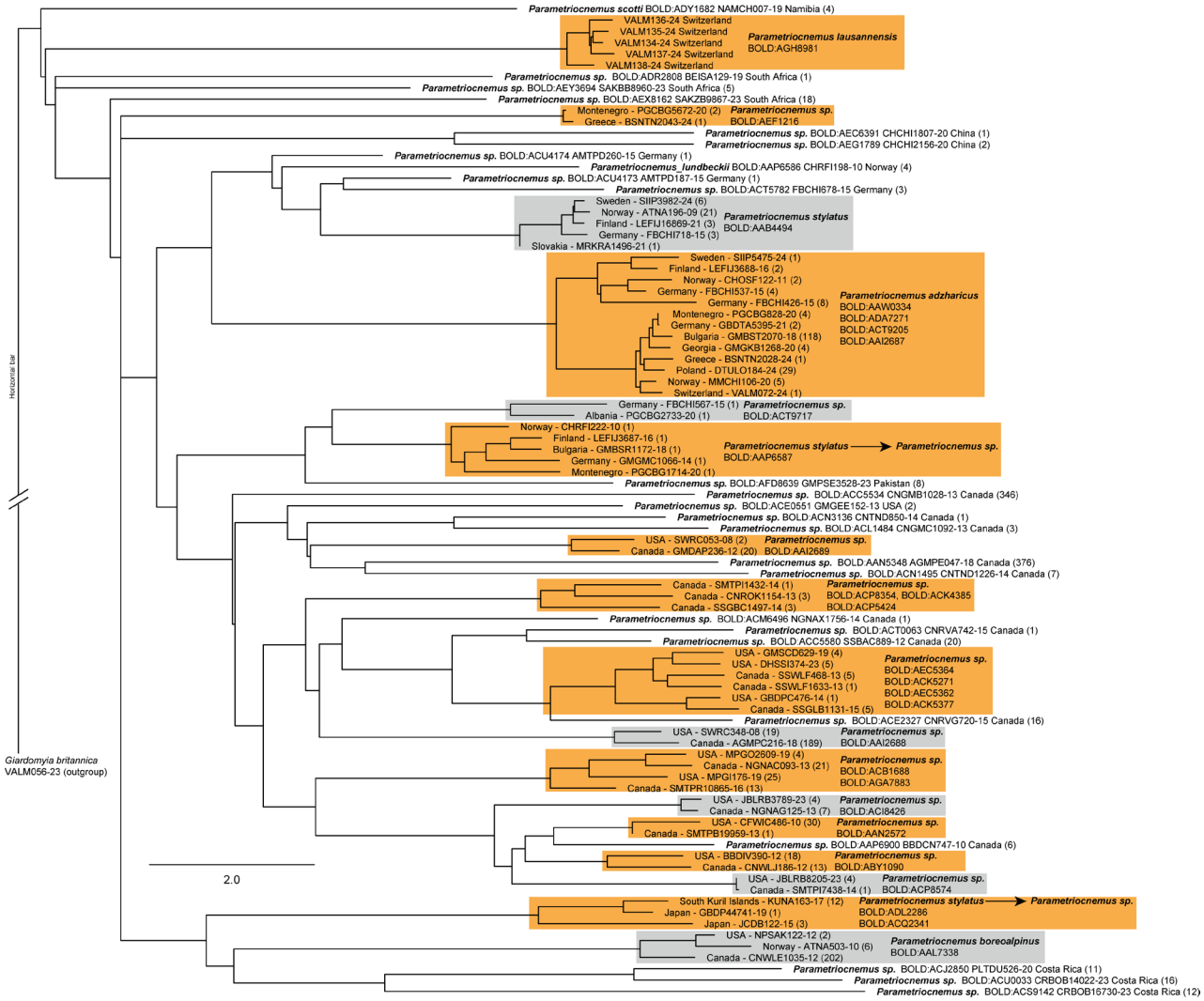


Figure 1. Parametriocnemus NJ tree based on COI sequences available in BOLD. One sequence per BIN cluster and per country was used, BOLD process ID is indicated in each terminal. For P. lausannensis, all available sequences were included. The number of available COI sequences for a given country in each BIN cluster is indicated in parenthesis. Suggested species clusters are highlighted in orange or grey. In some instances, we have grouped several BIN clusters into what could be considered one species when the differences between the clusters were <3.5%.

iNaturalist website (https://www.inaturalist.org/home; observation IDs: 245260497, 245263339, 245264553, 245279203, 245282230; for example, to see specimen 5, use this address: https://www.inaturalist.org/observations/245282230).

Specimen numbers are mentioned in the figures. The holotype corresponds to specimen 2, the female to specimen 3 and the other three male paratypes to specimen 1, 4, and 5 (museum ID GBIFCH00618677, GBIFCH00618694, GBIFCH00618695, respectively).

Type locality: a house located in a residential area; two small gardens on each side of the house, one with a small pond without fish; the house is 50 m away from the Vuachère River, a tributary of Lake Geneva located just over 3 km from the capture site.

Etymology

The species is named *lausannensis* after the town in which it was collected.

Diagnosis

Males of *Parametriocnemus lausannensis* can be separated from all other known *Parametriocnemus* species worldwide by the following combination of characters: inferior volsella rectangular with apical angle towards gonocoxite strongly acute, virga absent, anal point parallel-sided to slightly expanded apically with its top reaching or exceeding inferior volsella end, apical lobes on tergite IX well defined at each side of anal point, AR 1.1-1.4, antenna with 6 very long inwards curved sensilla chaetica subapically.

Description

Adult male (Figs 2a, b, 3a, c, e, 4a, c)

Body length (n = 4), from terminalia to front of scutum = 2.2-2.5 mm; total length (n = 4) (clypeus to gonostylus) = 2.3-2.7 mm.

Head and antenna: eyes dorsally strongly extended, the eye ratio (distance between dorsal ends of eyes/width of scape) = 0.9 (n = 2); palpomere 5, the apical one longer than the others (Fig. 3e), the basal one very small and barely discernable; the distal end of the antenna wedge-shaped (Fig. 3e; blue arrowhead) with 6 very long inwards curved sensilla chaetica (Fig. 4a), antenna ratio (AR) = 1.1-1.4 (n = 7). Sensilla chaetica are hyaline and uncolored, originating from shallow holes on the antennae, whereas the long setae of the antenna (cut off in Figs. 4a-b) are generally colored and originate from a setal base that remains on the antenna if the seta is lost.

Thorax (Fig. 4c): 13-14 acrostichals (n = 4), 15-22 dorsocentrals on each side (n = 6), 8-12 scutellars (n = 4).

Wings (Fig. 3c): length (from base of squama) = 1.9 mm (n = 4), wing ratio (length/width) = 4.0-4.4 (n = 4), macrotricha on wing membrane from almost the wing base to the apical end, fork of Cu opposite or slightly distal of RM, 5-6 seta on squama (n = 7), end of R_{4+5} opposite end of M_{3+4}, anal lobe not produced.

Legs: leg ratio (LR = metatarsus length/tibia length) for leg 1, 2, and 3 equal to 0.70-0.75 (n = 8), 0.52-0.58 (n = 7), 0.60-0.64 (n = 7), respect-

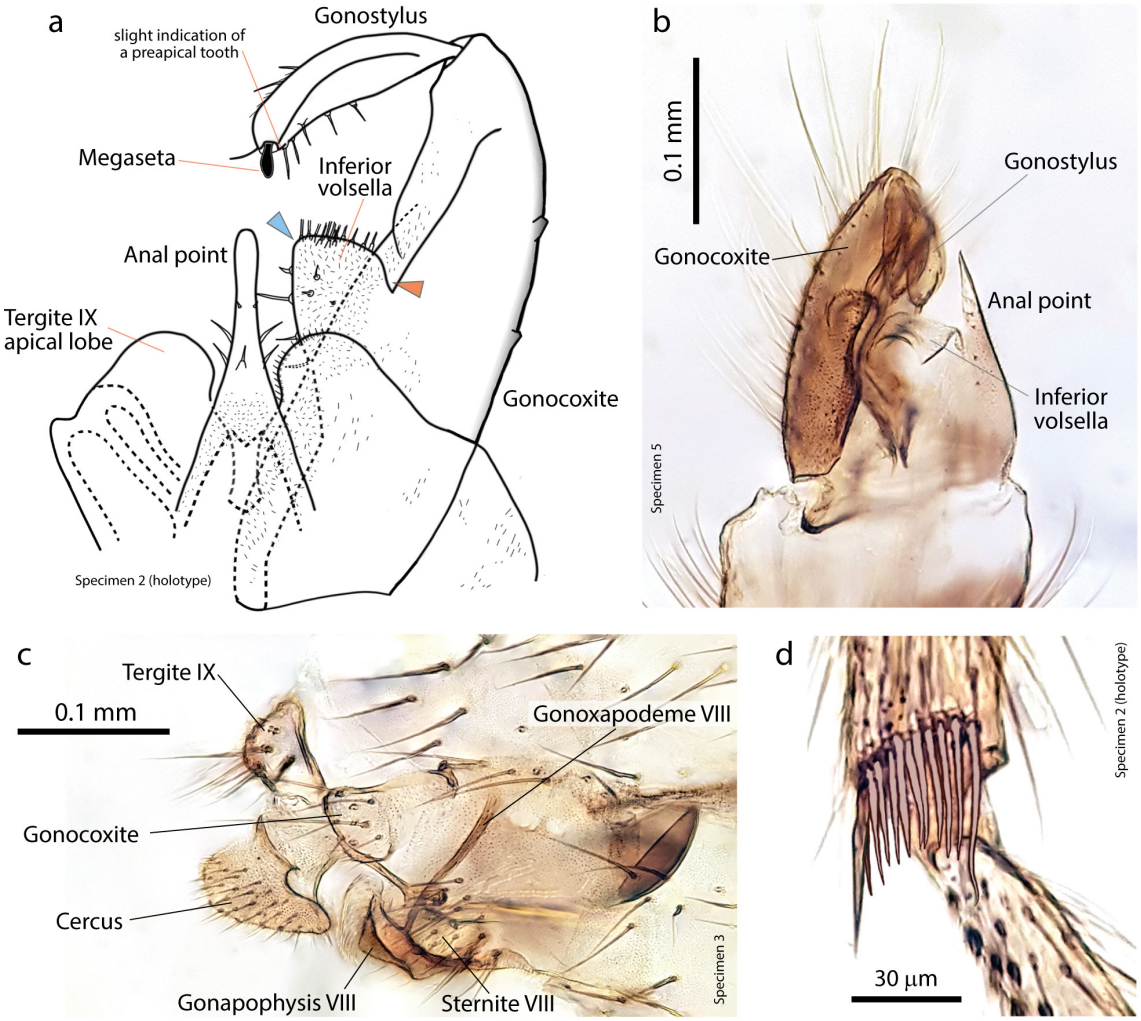


Figure 2. Genitalia and tibial comb of *Parametriocnemus lausannensis*. a, hypopygium dorsal view; b, hypopygium lateral view; c, female genitalia; d, male hind tibial comb. Orange arrowhead: distal angle (indentation between the inferior volsella and the gonocoxite), blue arrowhead: apical angle of the inferior volsella. Terminology of the female genitalia according to Sæther (1977).

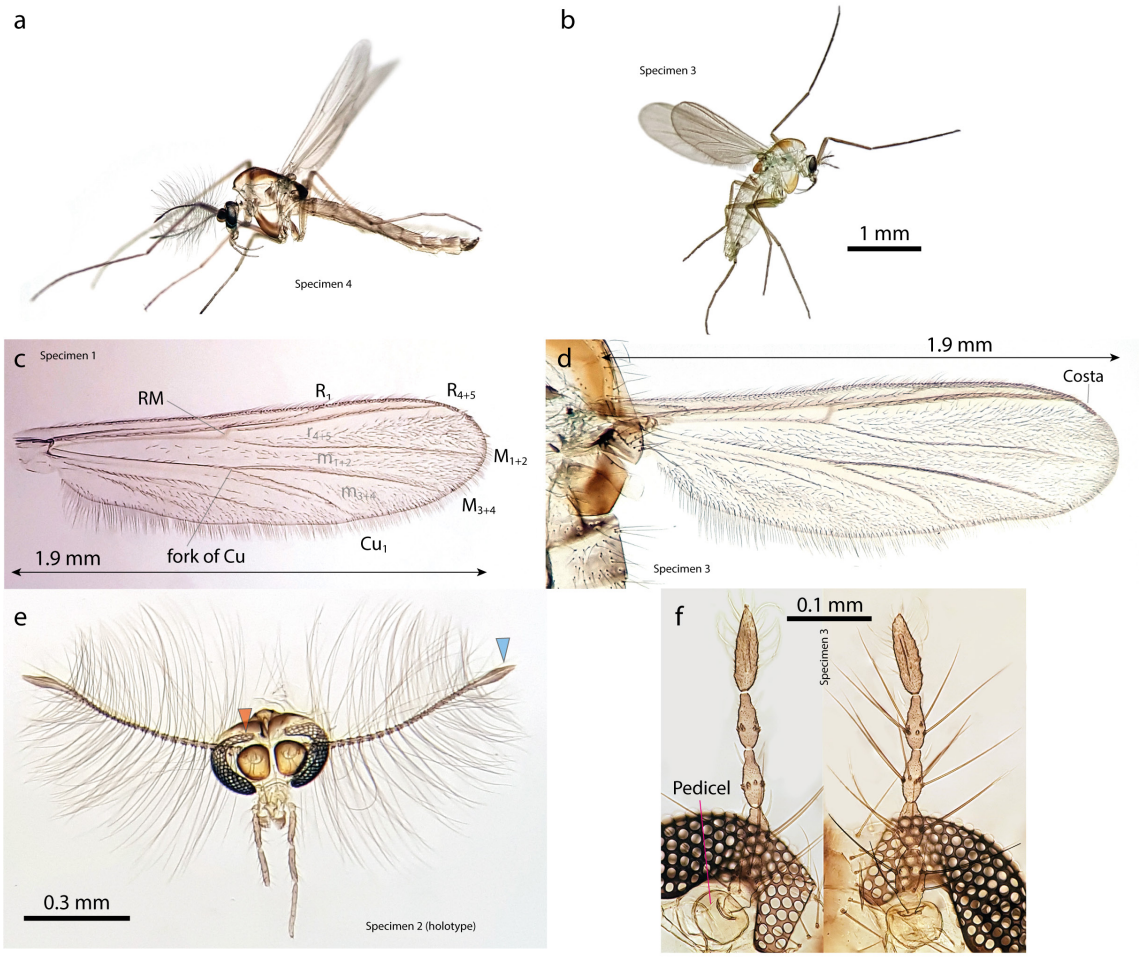


Figure 3. *Parametriocnemus lausannensis*. a, male habitus; b, female habitus; c, male wing; d, female wing; e, male head with antennae; f, female head with antennae. Orange arrowhead points to dorsal extension of the male eye. Blue arrowhead points to wedge-shaped end of male antenna.

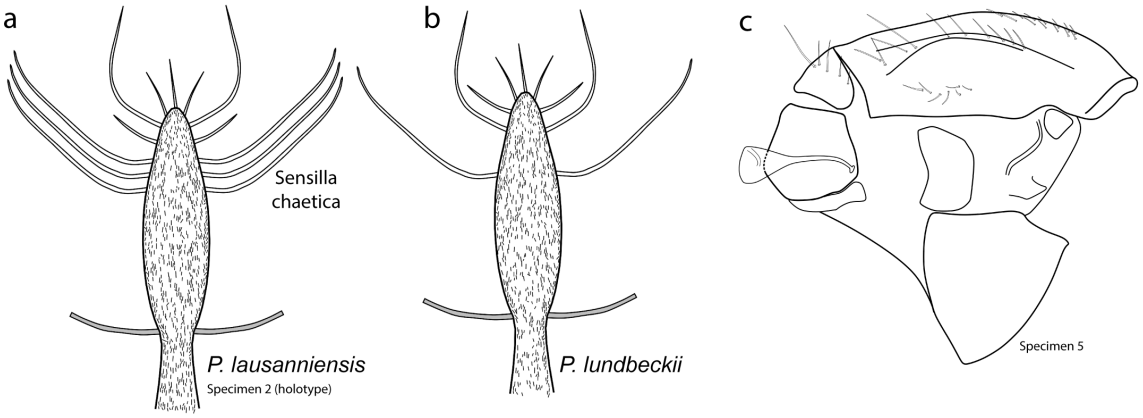


Figure 4. Male antenna apex and thorax. a, *P. lausannensis* with multiple longish sensilla chaetica; b, *P. lundbeckii* (specimen from Sweden stored in the Swedish Museum of Natural History) with fewer sensilla chaetica; c, thorax of *P. lausannensis* (male, lateral view).

ively. The hind tibia comb (Fig. 2d) is made of 8-10 spines bordered on one side by a spur. The spine on the outer side is longer than the others and slightly twisted distally.

Abdomen and hypopygium (Figs 2a, b): tergites brownish, tergite IX with a distinct rounded lobe at each side of the anal point, no virga; gonostylus slender in side view (length/width = 2.8-3.2, n = 5) (Fig. 2a) but broader in oblique dorsal view (length/width 3.7-4.4), without crista dorsalis and only with a slight indication of a preapical tooth; when bent inwards, the gonostyli reach well beyond the tip of the inferior volsella (Fig. 2b); anal point long, apically expanded, with a blunt end when viewed from above (Fig. 2a) or with a very pointed end in side view (Fig. 2b), anal point reaches the distal end of the inferior volsella, microtrichia found up to half to three quarter of the anal point; inferior volsella squarish, its apical angle close to 90° (72-87°, n = 4) (Fig. 2a, blue arrowhead), and distal angle at junction to gonocoxite acute (50-60°, n = 4) (Fig. 2a, red arrowhead).

Adult female (Figs 2c, 3b, d, f)

Body length = 1.9 mm; same wing length as males (1.9 mm) but wings broader (wing ratio = 3.5); eye ratio larger (1.9); antenna 7-segmented, including scape and pedicel; the flagellomeres bottle-shaped except the oval apical one that is also the longest one (Fig. 3f). Same number of acrostichals as in the males but striking doubling of other seta: 34-36 dorsocentrals on each side, 18 scutellars and 8 setae on squama. Female genitalia sideview shown in Fig. 2c.

Remarks

The male hypopygium of *P. lausannensis* (Fig. 2) is morphologically close to that of *P. stylatus* (Spärck, 1923), *P. lundbeckii* (Johannsen, 1905), *P. stylatus adzharicus* Kownacki & Zosidze, 1973 and *P scotti* (Freeman, 1953). These five taxa all have eyes with a long dorsal extension (Fig. 3e, orange arrowhead), antenna apically with very long curved sensilla chaetica (Fig. 4a), wing membrane with macrotrichia from inner third to apex (Figure 3c,d), division of wing veins M_{3+4} and Cu_1 opposite or slightly distal of vein RM (Fig. 3c,d), tergite IX with a distinct rounded lobe at each side of the anal point, a long, slender anal point almost or as long as the gonostylus, and the inferior volsella well-developed (Fig. 2a,b).

The form of the inferior volsella is the major diagnostic feature to distinguish *P. lausannensis* from these similar species. In *P. lausannensis*, the distal angle of the inferior volsella at the junction to the gonocoxite is 50-60° (Fig. 2a, red arrowhead). This angle is less acute in *P. scotti* (70-80°) (Baranov et al. 2024, Freeman 1954, Lehmann 1979), and rectangular to slightly obtuse in *P. adzharicus* (100-110°) (Kownacki and Zosidze 1973), *P. lundbeckii* (90-110°) (Cranston et al. 1989, Sæther 1969, Sublette 1967) and *P. stylatus* (90-110°) (Brundin 1956, Langton and Pinder 2007a, Langton and Pinder 2007b). In addition, the inferior volsella of *P. lausannensis* is squarish with a length/width of 0.8-0.9, whereas it is triangular, conical or semicircular with a length/width of 0.5-0.7 in the other species. This morphological trait represents the primary diagnostic feature for distinguishing *P. lausannensis* from its congeners.

Another possibly important diagnostic feature concerns the number of curved sensilla chaetica at the apex of the male antenna. In *P. lausannensis*, there are 6 of them (Fig. 4a). The closely related species, *P. lundbeckii* (Fig. 4b), *P. adzharicus*, *P. stylatus* have 2-4 curved sensilla chaetica and there are around 10 in *P. scotti*.

P. arciger (Kieffer, 1925) could also be confused with *P. lausannensis*. However, *P. arciger* should be considered as a *nomen dubium* or even more likely a synonym of *P. stylatus* as stated already by Edwards (1929) and suggested as possible by Gouin (1956). The description of *P. arciger* is insufficient in providing diagnostic features allowing the separation of the two species (Goetghebuer and Lenz 1940, Kieffer 1925). We have tried, without success, to locate the types of *P. arciger* in museum collections. They might therefore have been lost. In the key provided by Goetghebuer and Lenz (1940), the male antenna of *P. arciger* is described to have 3-4 apical sensilla chaetica, whereas *P. lausannensis* has 6. The only available illustration of *P. arciger* depicts antenna of the female (Goetghebuer and Lenz 1940, text fig. 4). In this illustration, the last segment of the antenna is oval and shorter than the preceding segment, in contrast to what is seen in the female antenna of *P. lausannensis* where the last segment is more elongated and distinctly longer than the preceding one (Fig. 3f). Hence, the female of *P. lausannensis* is clearly different from *P. arciger*.

***Parametriocnemus adzharicus* Kownacki & Zosidze, 1973, stat. nov.**

Parametriocnemus stylatus adzharicus was described in 1973 as a subspecies of *P. stylatus* (Kownacki and Zosidze 1973). At the time, the only clear differences between the nominal subspecies and *adzharicus* were found in the pupae. This was the reason why Kownacki and Zosidze placed the collected specimens in a subspecies of *P. stylatus*.

Specimens attributed to *P. stylatus adzharicus* aggregate into a well-separated COI cluster (Fig. 1) composed of four BINs (BOLD:AAW0334, BOLD:AAI2687, BOLD:ACT9205 and BOLD:ADA7271). Specimens from this cluster have been collected mostly from Eastern and Northern European countries (Fig. 1). We suggest that *P. adzharicus* be considered as a valid species. We base this suggestion on the following arguments: Clustering of DNA barcodes shows that the BINs mentioned above form a genetic cluster with about 3.1% intraspecific variance (Fig. 1), clearly divergent from other clusters of *P. stylatus* (Fig. 1). The observed intraspecific genetic distance is within the range for many well-defined species of Chironomidae (Carew and Hoffmann 2015, Ekrem et al. 2018, Lin et al. 2015). Males of *P. adzharicus* can be separated from *P. stylatus* by a lower antenna AR (0.6-0.7 versus 0.8-1.2) and the apex structure of the anal point (narrowing versus parallel-sided or slightly expanded). The pupae can be separated from *P. stylatus* by characters given by Kownacki and Zosidze (1973). Dr. Elisabeth Stur identified specimens of *P. stylatus adzharicus* within the BINs BOLD:AAI2687, BOLD:ADA7271 and BOLD:AAW0334. Based on information available to her, she supports raising *P. adzharicus* to species level (pers. comm. to the first author, 2021). In addition, all morphologically similar species, *P. stylatus, P. lundbeckii, P. scotti* and *P. lausannensis* have barcodes in BOLD with BINs well separated from the four BINs that we now attribute to *P. adzharicus*.

Discussion

More than 50 BINs have been attributed to *Parametriocnemus* in BOLD. Previous studies have indicated that a 4.5-5.0% divergence can be expected within species of Chironomidae (Ekrem et al. 2018). Based on this, we can estimate that the tree generated from the COI sequences corresponding to these BIN clusters contains about 40 species (Fig. 1). As it is likely that not all valid species have had their COI locus sequenced, the total number of *Parametriocnemus* species may be greater than 40, although we must keep in mind that a *bone fide Parametriocnemus* species can be dispersed into several COI clusters and BINs. There are several published examples where apparently well-defined insect species segregate into multiple BINs. In a few cases involving Chironomidae, the genetic difference at the COI locus between specimens of a given species could be as great as 10% (Lin et al. 2017, Song et al. 2016), although one can not exclude the possibility that such as large genetic difference is due to the presence of cryptic species. Additionally, some of the COI clusters in Fig. 1 may not belong to *Parametriocnemus* but to closely related genera such as *Paraphaenocladius* and *Metriocnemus* as COI is known to be too divergent to be useful in generic assignments (Ekrem et al. 2007). The use of more slowly evolving genetic markers than COI would certainly be useful for a more precise species allocation within the genus *Parametriocnemus*.

Another issue associated with barcoding and species naming efforts of *Parametriocnemus* specimens is that 8 BINs in BOLD have been attributed to *P. stylatus*, including the 4 clusters that we now assign to *P. adzharicus* (Fig. 1). The *adzharicus* clusters have an intraspecific divergence of less than 3.1%, which, based on previous studies (Ekrem et al. 2018, Brodin 2025), is consistent with the notion that these clusters correspond to one species.

The remaining 4 BINs containing specimens identified as *P. stylatus* (BOLD:AAB4494, BOLD:AAP6587, BOLD:ADL2286 and BOLD:ACQ2341) form 3 distinct clusters in our NJ tree. These clusters are 8.5%-11.7% different from each other at the COI sequence level (Fig. 1). The BIN corresponding to the BOLD:AAB4494 cluster contains eight photos of the male hypopygium that agree well with illustrations of the hypopygium of *P. stylatus* in Brundin (1956), Langton and Pinder (2007b) and Sasa and Kikuchi (1995), including the sharp triangular tooth (crista dorsalis) close to the megaseta and the bilobed inferior volsella. We consider this BIN to represent *P. stylatus*, further strengthened by the fact that Dr. E. Stur identified twenty specimens of the BIN from Norway and Germany to this species.

Concerning the BOLD:AAP6587 cluster, the only photo of the male hypopygium of this BIN in BOLD is similar to *P. stylatus* in many aspects but seems to have a gonostylus with a low longish lobe before the megaseta, which does not agree with the published illustrations of *P. stylatus*. We suggest that this BIN should not be assigned to *P. stylatus*. Further studies are needed to reveal the species name corresponding to this BIN and whether the neighboring BIN (BOLD:ACT9717) belongs to this species or not.

The BOLD:ACQ2341 and BOLD:ADL2286 COI sequences are only 2.3% apart. They form a distinct cluster in our tree (Fig. 1). BOLD:ACQ2341 contains 3 specimens all identified as *P. stylatus*, one by Dr. R. Ueno, the others without the identifier's names. BOLD:ADL2286 contains 8 males identified as *Parametriocnemus* sp. by Dr.

E. Stur. Only one specimen is identified as *P. stylatus* but without information of the identifier's name. As Dr. Stur did not identify any males of this cluster to a particular species, we suggest that the cluster formed by the BOLD:ACQ2341 and BOLD:ADL2286 BINs is assigned to *Parametriocnemus* sp.

Acknowledgements

We thank Dr. Brigitte Lods-Crozet for her critical reading of the manuscript. We also thank Dr. Torbjørn Ekrem and the anonymous reviewer for the very constructive remarks, corrections, and suggestions made during the evaluation of this work.

References

Ashe, P. and O'Connor, J.P. 2012. *A world catalogue of Chironomidae (Diptera). Part 2. Orthocladiinae (Section A and B)*. Irish Biogeographical Society & National Museum of Ireland, 968 p.

Baranov, V., Lin, X., Hübner, J. and Chimeno, C. 2024. Uncovering the hidden diversity of non-biting midges (Diptera, Chironomidae) from central Namibia, using morphology and DNA barcodes. - *African Invertebrates* 65: 13-36. DOI: https://doi.org/10.3897/AfrInvertebr.65.111920

Brodin, Y. 2025. *Procladius* (Diptera, Chironomidae) of Europe and a global view. - *Zootaxa* 5591 (1): 1-127. DOI: https://doi.org/10.11646/zootaxa.5591.1.1

Brundin, L. 1956. Zur Systematik der Orthocladiinae (Dipt., Chironomidae). - *Reports of the Institute of Freshwater Research Drottningholm* 37: 5-185.

Carew, M.E. and Hoffmann, A.A. 2015. Delineating closely related species with DNA barcodes for routine biological monitoring. - *Freshwater Biology* 60: 1545-1560. DOI: https://doi.org/10.1111/fwb.12587

Cranston, P.S., Oliver, D.R. and Saether, O.A. 1989. The adult males of Orthocladiinae (Diptera: Chironomidae) of the Holarctic region - keys and diagnoses. In Wiederholm, T. (Ed.) *Chironomidae of the Holarctic region. Keys and diagnoses. Part 3. Adult males*. Entomologica Scandinavica Supplement No. 34, pp. 165-352.

Edwards, F.W. 1929. British non-biting midges (Diptera, Chironomidae). - *Transactions of the Entomological Society of London* 77: 279-430.

Ekrem, T., Willassen, E. and Stur, E. 2007. A comprehensive DNA sequence library is essential for identification with DNA barcodes. – *Molecular Phylogenetics and Evolution* 43: 530-542.

Ekrem, T., Stur, E., Orton, M.G. and Adamowicz, S.J. 2018. DNA barcode data reveal biogeographic trends in Arctic non-biting midges. - *Genome* 61: 787-796.

Freeman, P. 1954. Chironomidae (Diptera) from Western Cape Province. - *Proceeding of the Royal Entomological Society of London (B)* 23: 172-180.

Goetghebuer, M. 1932. *Faune de France. Vol. 23. Diptères Chironomidae IV (Orthocladinae, Corynoneurinae, Clunioninae, Diamesinae)*. Editions Lechevalier, 204 p.

Goetghebuer, M. and Lenz, F. 1940. Tendipedidae (Chironomidae) 13g) Subfamilie Orthocladiinae. In Lindner E. (ed.) *Die Fliegen der Palearktischen Region* 137: 1-24.

Gouin, F. 1956. *Parametriocnemus stylatus* K. et *Pm. arciger* K., (Dipteres Nemat., Chironomidae) deux espèces ou deux variétés? *Bulletin de l'Association Philomathique d'Alsace et de Lorraine* 9: 194-198.

Goujon, M., McWilliam, H., Li, W., Valentin, F., Squizzato, S., Paern, J. and Lopez, R. 2010. A new bioinformatics analysis tools framework at EMBL-EBI. - *Nucleic Acids Research* 38: W695-W699.

Inkscape Project 2020. Inkscape. Retrieved from https://inkscape.org

Kieffer, J.J. 1925. Nouveaux représentants de la tribu des Orthocladariae (Diptères Chironomides). - *Annales de la société scientifique de Bruxelles* 44: 382-388.

Kownacki, A. and Zosidze, R. 1973. *Parametriocnemus stylatus adzharicus* n. ssp. (Chironomidae, Diptera). - *Bulletin de l'Académie Polonaise des Sciences* 21: 127-130.

Langton, P.H. and Pinder, L.C.V. 2007a. *Keys to the adult male Chironomidae of Britain and Ireland*. Volume 1. Freshwater Biological Association, 239 p.

Langton, P.H. and Pinder, L.C.V. 2007b. *Keys to the adult male Chironomidae of Britain and Ireland*. Volume 2. Freshwater Biological Association, 168 p.

Lehmann, J. 1979. Chironomidae (Diptera) aus Fliessgewässern Zentralafrikas (Systematik, Ökologie, Verbreitung und Produktionbiolo-

gie). I. Teil: Kivu-Gebiet, Ostzaire. - *Spixiana* Supplement 3: 1-144.

Li, X., Lin, X. and Wang, X. 2013. New species and records of *Parametriocnemus* Goetghebuer from China (Diptera, Chironomidae). - *ZooKeys* 320: 51-62. DOI: https://doi.org/10.3897/zookeys.320.4927

Lin, X., Stur, E. and Ekrem, T. 2015. Exploring genetic divergence in a species-rich insect genus using 2790 DNA barcodes. – *PloS ONE.* 10: e0138993. DOI: https://doi.org/10.1371/journal.pone.0138993

Lin, X., Stur, E. and Ekrem, T. 2017. DNA barcodes and morphology reveal unrecognized species in Chironomidae (Diptera). - *Insect Systematics & Evolution* 49: 329-398.

Ratnasingham S, Wei C, Chan D, Agda J, Agda J, Ballesteros-Mejia L, Ait Boutou H, El Bastami Z M, Ma E, Manjunath R, Rea D, Ho C, Telfer A, McKeowan J, Rahulan M, Steinke C, Dorsheimer J, Milton M, Hebert PDN. 2024. BOLD v4: A Centralized Bioinformatics Platform for DNA-Based Biodiversity Data. In DNA Barcoding: Methods and Protocols, pp. 403-441. Chapter 26. New York, NY: Springer US, 2024.

Sæther, O.A. 1969. Some nearctic Podonominae, Diamesinae, and Orthocladiinae (Diptera: Chironomidae). - *Bulletin of the Fisheries Research Board of Canada* 170: 1-154.

Sæther, O.A. 1977. Female genitalia in Chironomidae and other Nematocera: morphology, phylogenies, keys. - *Bulletin of the Fisheries Research Board of Canada* 197: 1-209.

Sæther, O.A., Ashe, P. and Murray, D.A. 2000. Family Chironomidae. In Papp, L. and Darvas, B. (Ed.) *Contributions to a manual of palearctic Diptera*. Science Herald, pp. 113-334.

Sasa, M. and Kikuchi, M. 1995. *Chironomidae* (Diptera) of Japan. University of Tokyo Press, Tokyo, 334 p.

Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Soding, J., Thompson, J.D. and Higgins, D.G. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. – *Molecular Systems Biology* 7: 539. DOI: https://doi.org/10.1038/msb.2011.75

Song, C., Wang, Q., Zhang, R., Sun, B. and Wang, X. 2016. Exploring the utility of DNA barcoding in species delimitation of *Polypedilum* (*Tripodura*) non-biting midges (Diptera: Chironomidae). - *Zootaxa* 4079: 534-550. DOI: https://doi.org/10.11646/zootaxa.4079.5.2

Sublette, J.E. 1967. Type specimen of Chironomidae (Diptera) in the Cornell University collection. - *Journal of the Kansas Entomological Society* 40: 477-564.

Waterhouse, A.M., Procter, J.B., Martin, D.M.A, Clamp, M. and Barton, G.J. 2009. Jalview Version 2 - a multiple sequence alignment editor and analysis workbench. Bioinformatics 25(9): 1189-1191. DOI: https://doi.org/10.1093/bioinformatics/btp033

Widmann, C. and Bächli, G. 2022. Première observation de *Drosophila mercatorum* Patterson & Wheeler, 1942 en Suisse (Diptera: Drosophilidae). - *Entomo Helvetica* 15: 65-72.

Widmann, C., Lods-Crozet, B. and Brodin, Y. 2023. Première documentation pour la Suisse de *Procladius (Holotanypus) culiciformis* (Linnaeus, 1767) (Diptera: Chironomidae). - *Entomo Helvetica* 16: 165-170.

Article submitted 11. December 2024, accepted by Torbjørn Ekrem 27. May 2025, published 8. June 2025

New distributional records and biogeographic considerations on *Pampacladius gaucho* Donato, Zanotto Arpellino and Siri (Chironomidae: Orthocladiinae)

Mariano Donato[1, 2], Juan Pablo Zanotto Arpellino[1] & Augusto Siri[1,2]

[1]*Instituto de Limnología 'Dr. Raúl A. Ringuelet' (ILPLA) CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) CCT–La Plata. Boulevard 120, Casco Urbano, B1900 La Plata, Buenos Aires, Argentina. E-mails: marianodonato@ilpla.edu.ar, zanottojp@ilpla.edu.ar, augusto@ilpla.edu.ar*

[2]*Facultad de Ciencias Naturales y Museo (FCNyM – UNLP). Avenida 122 y 60 – La Plata, Buenos Aires, Argentina.*

Abstract

New records of the recently established genus *Pampacladius*, together with the confirmation that Roback and Coffman's Genus 2 sp. is conspecific with *P. gaucho*, extend its range to the Austral region and South American transition zone. Specimens from Patagonia were measured and are presented in a comparative table. Intraspecific variation of *P. gaucho* is described and biogeographic considerations based on the new records are discussed.

Introduction

Donato et al. (2024) described a new genus of Orthocladiinae from Argentina. The genus *Pampacladius* Donato, Zanotto Arpellino and Siri was based on one species from Pampean biogeographic province in Central Argentina. Molecular analysis places the new genus closely related to genera distributed in Austral areas, mainly Australia and New Zealand.

In a personal communication, our colleague Martin Spies kindly reported us that a specimen he collected on January 18, 1986 in Tierra del Fuego (Argentina) from Rio Ewan Sur at the bridge of Ruta Nacional 3 just south of Puente Justicia, using a Brundin-driftnet, was a pharate adult male with pupal exuviae belonging to *Pampacladius*, quite likely even to the same species. In addition, Dr. Spies suggested that the pupal morphotype of the Orthocladiinae "Genus 2" from Bolivia and Peru of Roback and Coffman (1983) resembles *Pampacladius*.

Following Dr. Spies' comments, collection material from La Plata Museum (MLP) and the Instituto de Limnología 'Dr. Raúl A. Ringuelet' (ILPLA) was examined for additional specimens. Adult males previously labelled as *Botryocladius* sp. were found and, upon examination, identified as *Pampacladius gaucho*.

This study establishes the pupa of *P. gaucho* and Genus 2 sp. of Roback and Coffman (1983) as the same entity, provides measurements of specimens from new localities, and presents biogeographic considerations.

Material and methods

Specimens of *P. gaucho* deposited in MLP and ILPLA were measured. All measurements are given in µm unless otherwise stated, and values are rounded to the nearest 5 µm unless specified otherwise. Measurements are presented as ranges and summarized in Tables 1 and 2, which include data from the original description as well as new measurements of male specimens collected in the Río Negro and Tierra del Fuego provinces, Argentina. Morphological terminology and measurement standards follow Sæther (1980).

Results

Pampacladius gaucho Donato, Zanotto Arpellino and Siri, 2024

Material examined. ARGENTINA: 3 ♂; slide mounted in Canada Balsam, Río Negro, General Roca, in the shores of Negro River; -39.1105556 -67.6136111, 230 m asl; 19.07.2005; sweep net; leg. M. Donato. 3 ♂; slide mounted in Canada Balsam; Tierra del Fuego; A° s/n en R Comp. B; -53.90166667 -67.93230556; 03.21.2005; 03.12.2005; sweep net; leg. M. Donato and G. Spinelli.

The males of *P. gaucho* from the new localities did not show differences in qualitative characters such as the head, hypopygium, and thorax. However, quantitative characters of body parts (Table 1) and leg measurements (Table 2) showed slight

differences with respect to the original description, being those of the Patagonian specimens bigger and larger than those of the Pampean specimens. The mean annual temperature is 4.5°C in the Tierra del Fuego locality, 16°C in General Roca (Río Negro), and 18.4°C at the type locality of the genus. No clear increase in the measurements of meristic characters along a latitudinal gradient was observed, and thus, no clinal variation from North to South could be established. Pampean specimens are smaller than the Patagonian ones, but among the latter, those belonging to Río Negro province tend to be larger than those from Tierra del Fuego.

It is well known that temperature significantly affects chironomid developmental and growth rate, and therefore their adult body size (Pinder 1986). Some studies have documented a negative correlation between adult body size and temperature (Oliver 1971, Pinder 1986, Maier et al. 1990, Sæther and Andersen 1996, Kobayashi 1998, Frouz et al. 2002, McKie and Cranston 2005, Baek et al. 2012). However, this is not a general rule, as another study has found species in which this correlation does not hold (Wonglersak et al. 2021). Although the number of specimens analysed in this study is limited, the findings are consistent with most papers on this subject.

As was mentioned above, the specimens from MLP and ILPLA collections were labelled as *Botryocladius* sp. The genus *Botryocladius* Cranston and Edwards is a transantarctic element distributed in Australia, Argentina, and Chile. The male adult of this genus shares with *Pampacladius* the bare eyes; wings fully developed, with weakly extended costa, finely punctate and lacking macrotrichia on membrane, with setose squama; legs with strong lateral spines on spur, with comb; and thorax with few hooked/scalpellate acrostichals in mid-thorax, without pleural setae. Of the four South American species described of *Botryocladius*, the male is known in only one (Cranston & Edward 1999). The identification of the specimens as *Botryocladius* was based on the presence of many shared characteristics, while those that differed were interpreted as representing one of the males of the three known species of the genus at the pupal stage. Although the male of *Botryocladius edwardsi* has a highly developed virga and an inferior volsella that is less pronounced than in *P. gaucho*, the Australian species exhibit variation in virga development, with the possibility of its absence. Therefore, it is crucial to consider this issue when identifying future male specimens, particularly those of unknown *Botryocladius* males.

Based on the available evidence, the two genera can be distinguished as follows: South American *Botryocladius* has a weak dorsomedial extension of the eye, a sinuate Cu_1 vein, hypopygium with a strong virga, and a weak, bare anal point. In contrast, *Pampacladius* lacks a dorsomedial extension, has an almost straight Cu_1 vein, hypopygium without a virga, and the anal point with a short apical part, reduced to a hump with a posterior elongation.

From the description and illustrations provided by Roback and Coffman (1983), the pupa of Genus 2 sp. belongs to this species.

Table 1. Comparison of measurements from the original description and specimens from the new localities in *Pampacladius gaucho* Donato, Zanotto Arpellino and Siri, male, $n = 3$ for each new locality, except when otherwise stated.

Character	Original description	Río Negro specimens	Tierra del Fuego specimens
Total length	2.22–2.95	3.35–3.62 (2)	2.92–3.06 (2)
Wing length	1.40–1.70	1.86–2.20	1.86–1.90
Total length/wing length	1.52–1.84	1.65–1.68 (2)	1.57–1.61 (2)
Wing length/length of profemur	2.83–3.09	3.1–3.14	3.14–3.30
Ultimate flagellomere	371–442	544 (1)	477–497
AR	0.88–1.03	1.15 (1)	1.04–1.16
Inner verticals	0	1–2 (2)	0
Outer verticals	0–1	1–2	1–2 (2)
Postorbitals	2–3	2	2–3 (2)
Clypeus setae	7–11	4–6	8–11 (2)
Tentorium length	130–148	146–178	142–160 (2)
Tentorium wide	24–30	30–48	28 (2)

Character	Original description	Río Negro specimens	Tierra del Fuego specimens
Stipes length	110–134	124–185	120–124 (2)
Palpomere length I	22–36	30–44	28–32
Palpomere length II	34–48	50	40–44
Palpomere length III	68–74	80–90	74–76
Palpomere length IV	70–90	96–102	86–94
Palpomere length V	80–96	100–116	106–120 (2)
Antepronotal lateral setae	0–2	0–1	0–2
Dorsocentrals	5–7	6–7	7 (2)
Acrostichals	4–8	2–4 (2)	2–3
Prealars	3–4	3–4	3
Scutellars	5–9	5–6	6–7 (2)
VR	1.32–1.45	1.34–1.54	1.34–1.4
C extension	14–36	0–20	0–32
Setae on R	0–3	2–6	1–2
Setae on R_1	0	0	0–1
Squamals	7–12	7–12	9–11
Tibial spur PI	44–76	46–63	54–60
Tibial spur PII 1	16–18	20–24	20–22
Tibial spur PII 2	18–24	24–26	24–28
Tibial spur PIII 1	12–16	13–22 (2)	–
Tibial spur PIII 2	38–50	52–60	54 (2)
Width at apex of front tibia	28–36	38–40	34–44
Width at apex of middle tibia	30–36	33–44	32–50
Width at apex of hind tibia	34–44	45–56	44–44
Setae on comb III	13–14	12–14	13
Sensilla chaetica on tarsomere 1 PII	0–1	0–1	0
Sensilla chaetica on tarsomere 1 PIII	3–6	2–3	3–4
Setae on tergum IX	9–15	13–20	12–13
Setae on laterosternite IX	4–6	5–6	4–6
Anal point length	12–20	22–40	20–24
Phallapodeme length	48–62	60–72	50–60
Transverse sternapodeme length	72–94	80–98	74–82
Gonocoxite length	164–218	204–232	204–232
Inferior volsella ending	0.20–0.25	0.20–0.33	0.17–0.24
Gonostylus length	82–92	92–96	86–90
Megaseta length	8–14	14	12 (2)
HR	2–2.37	2.17–2.42	2.27–2.58
HV	2.71–3.35	3.64–3.77 (2)	3.24–3.40 (2)

Table 2. Comparison of lengths and proportions of leg segments from the original description with respect to specimens from the new localities in *Pampacladius gaucho* Donato, Zanotto Arpellino and Siri, male, n = 3 for each new locality.

Character	Original description	Río Negro specimens	Tierra del Fuego specimens
fe P_1	473–600	600–694	576–592
fe P_2	505–639	615–742	608–615
fe P_3	552–679	686–757	655–679
ti P_1	592–757	750–915	718–757
ti P_2	513–631	647–789	631–647
ti P_3	615–765	797–963	789–797
ta_1 P_1	363–450	466–552	426–458
ta_1 P_2	252–300	316–395	308–323
ta_1 P_3	308–371	379–466	371–395
ta_2 P_1	237–284	323–355	284–316
ta_2 P_2	150–174	213–237	181–189
ta_2 P_3	205–245	260–300	245–252
ta_3 P_1	174–213	245–260	205–229
ta_3 P_2	110–142	158–174	150
ta_3 P_3	166–189	213–237	197–213
ta_4 P_1	110–126	142–166	134–142
ta_4 P_2	63–79	87–103	79–95
ta_4 P_3	87–110	110–134	110
ta_5 P_1	79–95	95–110	95–103
ta_5 P_2	79–150	79–110	79–95
ta_5 P_3	79–95	95–103	95
LR P_1	0.57–0.61	0.60–0.64	0.59–0.60
LR P_2	0.46–0.49	0.49–0.51	0.49–0.50
LR P_3	0.47–0.51	0.48	0.47–0.50
BR P_1	2.32–2.52	2.25–2.42	2.25–2.39
BR P_2	2.82–3.32	2.94–3.13	2.97–3.19
BR P_3	2.71–3.07	2.71–2.83	2.76–2.88
SV P_1	2.98–3.12	2.82–2.91	2.95–3.04
SV P_2	4.03–4.33	3.83–4	3.90–4.03
SV P_3	3.70–4.03	3.69–3.92	3.70–3.89

Discussion

New records of *Pampacladius gaucho* from the biogeographic provinces of Puna, Monte, and Patagonia extended its distribution to the South American transition zone (SAtz) and the Austral region, respectively (Fig. 1). The phylogenetic analysis obtained (Donato et al. 2024) showed that *Pampacladius* is part of a group with ancestral distribution in South America. The other genera that form the group all have distributions in continents that were part of Gondwana excepting some widespread genera and dated from 76.4 Mya (95% HPD: 90.4–61.5 Mya) when New Zealand, Australia, South America, remained connected via Antarctica, whereas east Antarctica was adjacent to southern Australia. The clade *Pampacladius*, is related to the South American *Echinocladius* Cranston; the New Zealand taxa *Paulfreemania pictipennis* (Freeman), *Naonella* Boothroyd, and *Tonnoirocladius commensalis* (Tonnoir); and the Australian taxa genus 'Australia' and Orthocladiinae 'FNQ2'. The new distributional pattern of *P. gaucho* in the Austral region and SAtz contribute to strengthen the phylogenetic relationships and biogeographic affinities obtained. Other genera with a similar distributional pattern, differing in

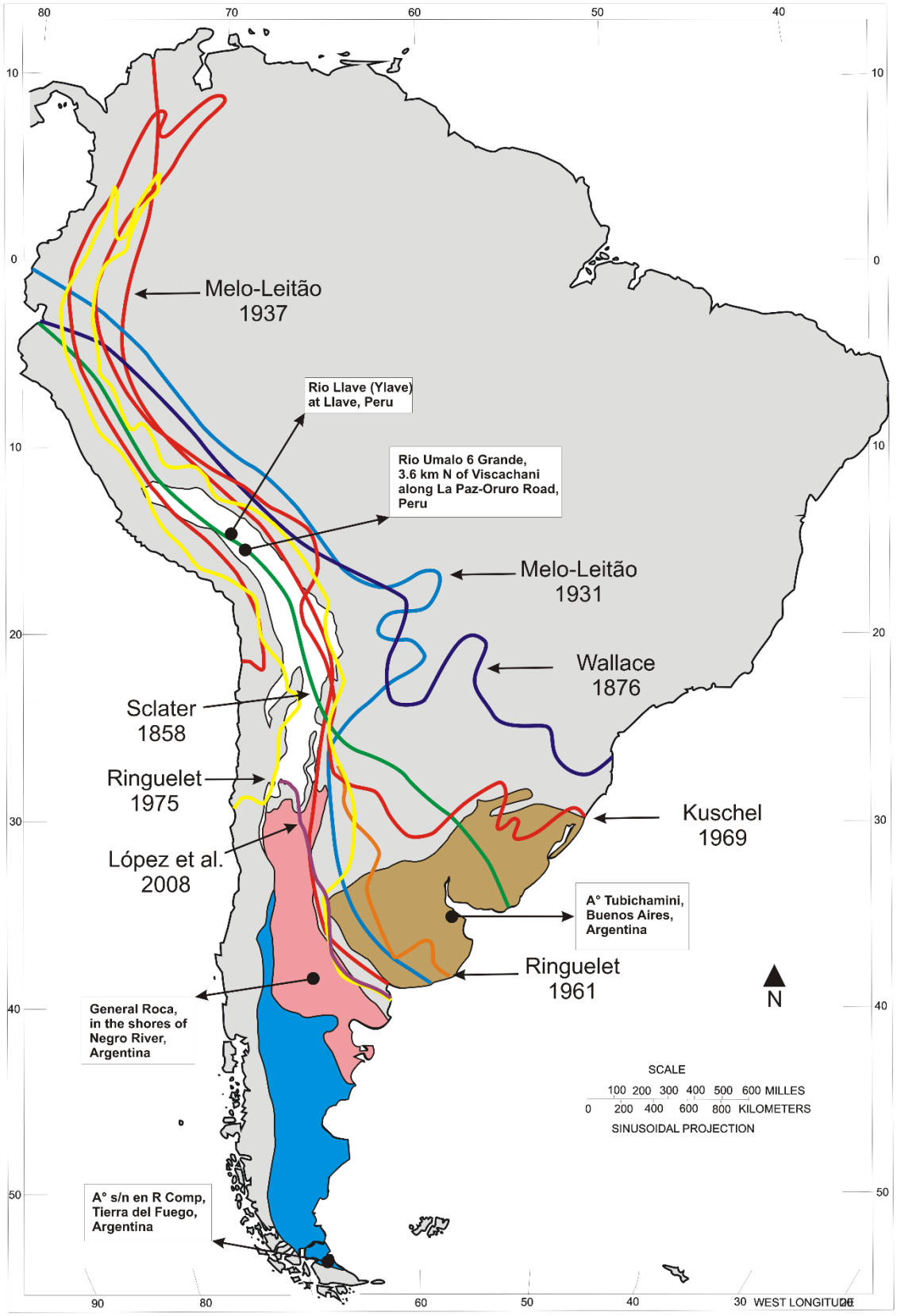


Figure 1. Lines drawn by different authors to delimit the Guiana-Brazilian and Andean-Patagonian subregions (modified from Morrone 2004). The black circles show the type locality of the genus and new localities where *Pampacladius gaucho* was collected. Those biogeographic provinces cited in the text were drawn: Puna (white), Pampean (brown), Monte (pink), Patagonian (blue).

the extent of distributional areas occupied in the SAtz and Neotropics are *Barbadocladius* Cranston and Krosch, South American *Paraheptagyia* Brundin, *Parapsectrocladius* Cranston, South American *Parochlus* Enderlein, South American *Podonomus* Philippi, South American *Riethia* (Kieffer), South American *Stictocladius* Edwards, and *Physoneura* Ferrington and Sæther.

The concept of biogeographic regions started with Buffon, who first observed in 1761 that the large mammals of the tropical regions of the Old World and the New World are quite different. However, the world-wide system of biogeographic regions was founded by Augustin de Candolle in 1820 based on the distribution of plants (Cox 2001). Several authors after de Candolle, proposed modifications to the plant based biogeographical regions until present day. Zoogeography had been developing from the early nineteenth century onwards, but in a different way than plant geographers (for a complete historical revision see Cox 2001).

Since the mid-nineteenth century, there have been numerous attempts to systematize the distribution patterns of the Latin American and Caribbean biota in a diverse number of biogeographic regions, subregions and provinces. Although these biogeographic classifications differ in the criteria used to delimit the areas (e.g., geographic, paleontological, faunal and/or floristic), the different authors generally implicitly recognize that the units of their schemes represent historical entities (for a complete historical revision see Morrone 2001).

South America is a composite area, with southern South America closely related to the southern temperate areas (Australia, Tasmania, New Zealand, New Guinea, and New Caledonia), and tropical South America closely related to Africa and North America (Crisci et al. 1991). Because of this, it is understandable that the different biogeographic schemes proposed for South America differ at the boundaries between these two regions (Fig. 1). The diverging interpretations in the limits of the Neotropical and the Andean regions are understandable based on unequal selection and evaluation of animal groups considered (Fittkau 1969). Morrone (2004, 2006) proposed the transition zones, located at the boundaries between biogeographic regions representing areas of biotic overlap, which are promoted by historical and ecological changes that allow the mixture of different biotic elements. In transition zones, pronounced changes in species richness and/or high spatial replacement of species occur. The South American transition zone (SAtz) comprises North Andean Paramo, Puna, Coastal Peruvian Desert, Prepuna, and Monte provinces. This area of change in the geographic distribution areas of species in South America coincides with the arid diagonal that marks the transition between phytogeographic regions characterized by a temperate-cold climatic regime in the southwest (Andean region and South American transition zone) and a subtropical one in the northeast (the Chaco subregion) (Morrone 2006). The cold-adapted chironomids that are generally widely distributed in the southern part of the continent only occur above 1700 to 1800 m in the tropical Andean region, corresponding to the lower limit of the cloud forest zone (Fittkau and Reiss 1979).

Although the Pampean province belongs to the Chacoan subregion, the presence of several Andean–Patagonian species, such as *Allocladius globosus* (Mauad et al. 2013), *Podonomus tehuelche* and *P. quinquesetosus* (Podonominae) (Siri & Donato 2012), along with new records of Brazilian lineages like *Pseudosmittia adunca* and *P. joaquimvenancioi* (Fuentes & Mauad 2015), highlights the complexity of this biogeographical unit, influenced by shifts in the latitudinal position of the arid diagonal zone from the Pleistocene to the present driven by glaciations, as evidenced by the rich fossil record of mammals. During glacial periods, the Pampean province experienced arid and cold climatic conditions, which led to the northward displacement of Patagonian mammal fauna (Donato et al. 2024, and references therein).

Acknowledgments

This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) under Grant [PIP 1010] and Universidad Nacional de La Plata (UNLP), Proyectos de Ciencia y Técnica under Grant [11/N914].

References

Baek, M.J., Yoon, T.J. and Bae, Y.J. 2012. Development of *Glyptotendipes tokunagai* (Diptera: Chironomidae) Under Different Temperature Conditions. - *Environmental Entomology* 41: 950–958. DOI: https://doi.org/10.1603/EN11286

Cox, B. 2001. The biogeographic regions reconsidered. - *Journal of Biogeography* 28: 511-523. DOI: https://doi.org/10.1046/j.1365-2699.2001.00566.x

Cranston, P.S. and Edward, D.H.D. 1999. *Botryocladius* gen. n.: a new transantarctic genus of Orthocladiine midge (Diptera: Chironomidae). - *Systematic Entomology* 24: 305–333. DOI: https://doi.org/10.1046/j.1365-3113.1999.00082.x

Crisci, J.V., Cigliano M.M., Morrone J.J. and Roig Juñent S. 1991. Historical biogeography of southern South America. - *Systematic Zoology* 40: 152-171. DOI: https://doi.org/10.2307/2992254

Donato, M., Zanotto Arpellino, J.P., Marin Viegas, V.S. and Siri, A. 2024. *Pampacladius*, a new genus of Orthocladiinae (Diptera: Chironomidae) and comments on the Gondwana biogeography. - *Systematics and Biodiversity* 22 (1): 2413946. DOI: https://doi.org/10.1080/14772000.2024.2413946

Fittkau, E. J. 1969. The fauna of South America. In: *Biogeography and ecology in South America*, vol. 2, p. 624-650.

Fittkau, E.J. and Reiss, F. 1979. Die zoogeographische Sonderstellung der neotropischen Chironomiden (Diptera). - *Spixiana* 2: 273–280.

Frouz, J., Ali, A. and Lobinske, R.J. 2002. Influence of Temperature on Developmental Rate, Wing Length, and Larval Head Capsule Size of Pestiferous Midge *Chironomus crassicaudatus* (Diptera: Chironomidae). - *Journal of Economic Entomology* 95: 699–705. DOI: https://doi.org/10.1603/0022-0493-95.4.699

Fuentes, M. C., and Mauad, M. 2015. *Pseudosmittia adunca* & *P. joaquimvenancioi* (Diptera: Chironomidae), first records from Argentina. - *Revista de La Sociedad Entomologica Argentina* 74: 89–91.

Kobayashi, T. 1998. Seasonal Changes in Body Size and Male Genital Structures of *Procladius choreus* (Diptera: Chironomidae: Tanypodinae). - *Aquatic Insects* 20: 165–172. DOI: https://doi.org/10.1076/aqin.20.3.165.4476

Maier, K.J., Kosalwat, P. & Knight, A.W. 1990. Culture of *Chironomus decorus* (Diptera: Chironomidae) and the Effect of Temperature on its Life History. - *Environmental Entomology* 19: 1681–1688. DOI: https://doi.org/10.1093/ee/19.6.1681

Mauad, M., Siri, A., and Donato, M. 2013. New species of *Pseudosmittia* Edwards, 1932 and new records of *Allocladius* Kieffer, 1913 (Diptera: Chironomidae, Orthocladiinae) from South America. - *Zootaxa* 3694: 445–460. DOI: https://doi.org/10.11646/zootaxa.3694.5.3

McKie, B.G. and Cranston. P.S. 2005. Size matters: systematic and ecological implications of allometry in the responses of chironomid midge morphological ratios to experimental temperature manipulations. - *Canadian Journal of Zoology* 83: 553–568. DOI: https://doi.org/10.1139/z05-051

Morrone, J.J. 2001. *Biogeografia de America Latina y el Caribe*. Manuales & Tesis SEA, Zaragoza.

Morrone, J.J. 2004. La zona de transición sudamericana: caracterización y relevancia evolutiva. - *Acta Entomologica Chilena* 28: 41–50.

Morrone, J.J. 2006. Biogeographic areas and transition zones of Latin America and the Caribbean islands based on panbiogeographic and cladistic analyses of the entomofauna. - *Annual Review of Entomology* 51: 467–494. DOI: https://doi.org/10.1146/annurev.ento.50.071803.130447

Oliver, D.R. 1971. Life history of the Chironomidae. - *Annual Review of Entomology* 16: 211–230. DOI: https://doi.org/10.1146/annurev.en.16.010171.001235

Pinder, L.C.V. 1986. Biology of freshwater Chironomidae. - *Annual Review of Entomology* 31: 1–23. DOI: https://doi.org/10.1146/annurev.en.31.010186.000245

Roback, S.S. and Coffman, W.P. 1983. Results of the Catherwood Bolivian-Peruvian Altiplano Expedition part 2. Aquatic Diptera including montane Diamesinae and Orthocladiinae (Chironomidae) from Venezuela. - *Proceedings of the Academy of Natural Sciences of Philadelphia* 135: 9–79. https://www.jstor.org/stable/4064795

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

Sæther, O.A. and Andersen, T. 1996. First Afrotropical records of *Doithrix* and *Georthocladius*, with notes on the *Pseudorthocladius* group (Diptera: Chironomidae). - *Tijdschrift voor Entomologie* 139: 243–256. https://biostor.org/reference/50061

Siri, A., and Donato, M. 2012. Two new species of *Podonomus* (Chironomidae: Podonominae) from the *decarthrus* group from Ventania hill system, Argentina. *Zootaxa* 3548: 39–54. DOI: https://doi.org/10.11646/zootaxa.3548.1.2

Wonglersak, R., Fenberg, P.B., Langdon, P.G., Brooks, S.J. and Price, B.W. 2021. Insect body size changes under future warming projections: a case study of Chironomidae (Insecta: Diptera). - *Hydrobiologia* 848: 2785–2796. DOI: https://doi.org/10.1007/s10750-021-04597-8

Article submitted 27. February 2025, accepted by Torbjørn Ekrem 18. August 2025, published 19. August 2025

Correcting a mistake: *Limnophyes stagnum* Namayandeh, Guerra & Ram, 2024 is not conspecific with *Limnophyes* sp. 14ES

Armin Namayandeh[1] Edris Ghaderi[2] and Jeffrey L. Ram[3]

[1]*Department of Environmental and Life Sciences, Trent University, 1600 West Bank Drive, Peterborough, ON, Canada. E-mail: a.namayan@taxanama.org, arminnamayandeh@trentu.ca. Corresponding author.*
[2]*Department of Fisheries Sciences, Faculty of Natural Resources, University of Kurdistan, Sanandaj, Iran. E-mail: ed.ghaderi@uok.ac.ir*
[3]*Department of Physiology, School of Medicine, Wayne State University, Detroit, MI 48201, USA. E-mail: jeffram@med.wayne.edu*

Abstract

In this communication, we report on correcting DNA barcoding records of *Limnophyes stagnum* Namayandeh, Guerra & Ram, 2024. The five sequences we submitted to BOLD and published in Namayandeh et al. (2024) under *L. stagnum* match the CO1 sequences of *Limnophyes* sp. 14ES and the two species are not conspecific. Both species were collected from the type locality of *L. stagnum* but at different times with a misassumption that both species represent a single taxon. We have corrected our records in BOLD, replacing the names of the five sequences with *Limnophyes* sp. 14ES. Additionally, we obtained and uploaded a single CO1 sequence of *L. stagnum* in BOLD and incorporated it into new molecular analyses reported here. Based on our results, the closest sequences to *L. stagnum* were those of *Limnophyes natalensis* (Kieffer, 1914). The minimum K2P distance of *L. stagnum* with *L. natalensis* was 10.0% (average 11.4%), large enough to support the delimitation of *L. stagnum* sp. nov. from *L. natalensis*. Further discussion on the morphological differences of the two species and those of *L. stagnum* and *Limnophyes* sp. 14ES are provided.

Results and Discussion

It was brought to our attention by Dr. Elisabeth Stur of NTNU that the five sequences we submitted to BOLD and published in Namayandeh et al. (2024) as sequences of *Limnophyes stagnum* Namayandeh, Guerra & Ram, 2024 match the CO1 sequences of *Limnophyes* sp. 14ES. Dr. Stur communicated to us that based on her examination, the females of *Limnophyes* sp. 14ES are morphologically different from *L. stagnum*. It is thus likely that two species of *Limnophyes* were present in Detroit's Palmer Park vernal pool when we investigated it (Namayandeh et al. 2024). We assumed that the sequences of *Limnophyes* sp. 14ES females belonged to *L. stagnum* without examining the morphology of the adult *Limnophyes* females of the specimens which were sequenced and uploaded in BOLD under the name of our new described species *L. stagnum*. Both species were collected from the type locality of *L. stagnum*, but at different times. *Limnophyes* sp. 14ES was sampled in the spring, and *L. stagnum* in the fall. We have corrected our records in BOLD and replaced the names of our sequences with *Limnophyes* sp. 14ES. In addition, we uploaded a single CO1 sequence of *L. stagnum* that we incorporated into a new molecular analysis (Fig.1).

Details of the methodology used in the molecular analysis are given in Namayandeh et al. (2024). The CO1 sequence of the *L. stagnum* specimen on which we confirmed its unique morphology has the sample ID PPA19 and the process ID DTPPA019-25 in BOLD.

The two species' most apparent differences are their coloration and chaetotaxy. *L. stagnum* is uniformly dark brown while *Limnophyes* sp. 14ES is much lighter and greyish brown. The adult females of *Limnophyes* sp. 14ES have diagonally placed preepisternals below the epimeral region, whereas the ones of *L. stagnum* have horizontally placed preepisternals close to the anapleural suture (Fig. 2A-B). Moreover, the females of *Limnophyes* sp. 14ES have a five-segmented antenna, whereas the ones of *L. stagnum* have a four-segmented antenna (Fig. 2C). Other corrections are the geographical records of *L. stagnum. Limnophyes stagnum,* for now, is only reported from the type locality whereas *Limnophyes* sp. 14ES, seems to be widespread in the Holarctic.

The NJ analyses of *Limnophyes* sequences from Pond A (i.e., *L. stagnum* and *L.* sp. 14ES) and those obtained from NCBI and BOLD produced the same tree topology (Fig. 1). The six sequences of *L.* sp. 14ES, from Pond A, clustered with three sequences identified as *Limnophyes* sp. process ID CNTIC4604-15, JSJUN346-11, and NCCA2089-11 from Ontario, Canada (Hebert et al. 2016, deWaard et al. 2019). The

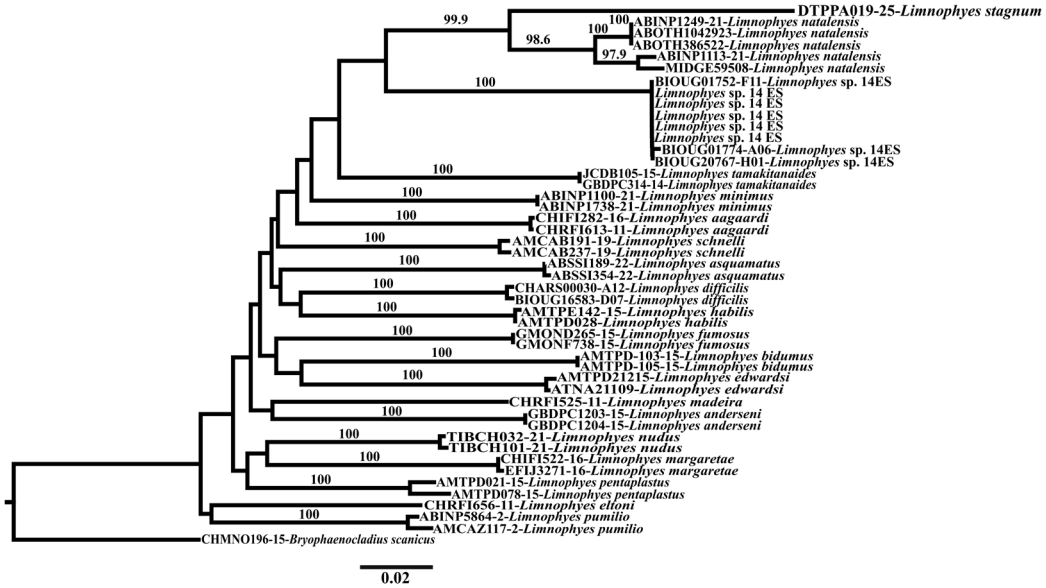


Figure 1. Neighbor-Joining (NJ) tree of *Limnophyes* Eaton species and one outgroup *Bryophaenocladius scanicus* (Brundin, 1947) inferred from the COI DNA barcode marker (658 bp). Numbers on branches represent the bootstrap value for Neighbor-Joining (NJ) using 10000 replicates; numbers < 95 were omitted.

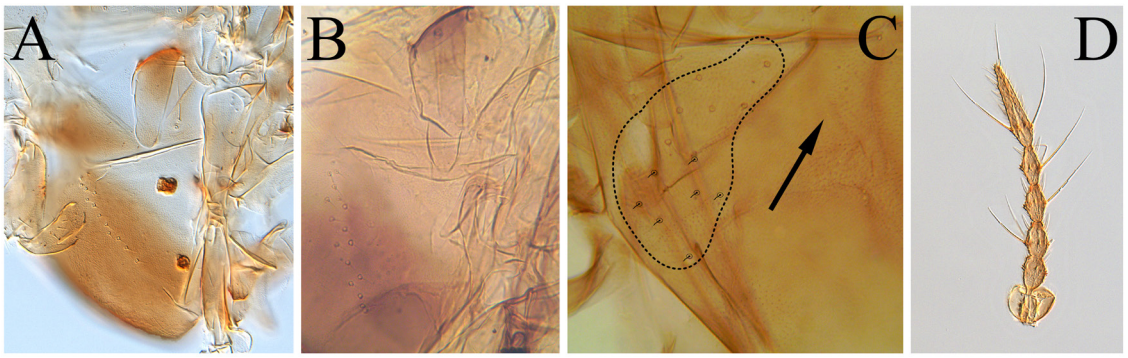


Figure 2. Comparison of some relevant female characteristics of *Limnophyes* Eaton. *Limnophyes* sp. 14ES (A, B, D); *Limnophyes stagnum* Namayandeh, Guerra & Ram, 2024 (C). A-C. Thorax preepisternals (arrow indicates the orientation of the thorax). D. Antenna. A & D specimens are from Norway; B & C specimens are from Michigan, USA. Images A & D are courtesy of E. Stur.

single sequence of *L. stagnum*, from Pond A did not cluster closely with any known sequence from BOLD or NCBI. The closest sequences to *L. stagnum* were those of *Limnophyes natalensis* (Kieffer, 1914), BOLD process id's ABINP1113-21, MIDGE595-08, ABOTH3865-22, ABINP1249-21, and ABOTH10429-23. The maximum intraspecific K2P pairwise distances calculated for the five sequences of *L. natalensis* was 1.7%. The maximum intraspecific K2P pairwise distances calculated for the six sequences of *L.* sp. 14ES, and the two sequences of *Limnophyes* sp. from Ontario were 0.05%. The minimum K2P distance of *L. stagnum*, with *L. natalensis* was 10.0% (average 11.4%). The overall mean distance of all *Limnophyes* species was 15.0%

The minimum and average interspecific distances of *L. stagnum* with *L. natalensis* are much lower than the overall mean distance of all *Limnophyes* analyzed. This may suggest that molecular methods may not clearly separate the two species. However, the minimum distance of 10.0% obtained based on the distance-based methods of K2P is large enough to support the delimitation of *L. stagnum* sp. nov. from *L. natalensis*, and it is consistent with the interspecies barcode gap in Chironomidae (Ekrem et al. 2010; Montagna et al. 2016). Additionally, the lower interspecific distances of *L. stagnum* with *L. natalensis* could stem from the fact that we only had one sequence of *L. stagnum* in our analyses, and with more sequences, this distance could vary. There is also a possibility that the two species have diverged from one another quite recently, and this has caused a low genetic divergence (Lin et al. 2015). The morphology supports that *L. stagnum*

and *L. natalensis* are two different species. The adult males of *L. stagnum* can be separated from that of *L. natalensis* by a combination of the following characteristics: Antenna 10 segmented, AR 0.8; costa extension 62 µm long; lanceolate setae absent, humerals absent, gonostylus expanded evenly from base to apex, crista dorsalis very narrow. *L. natalensis* adult males have a 12-segmented antenna with AR of 0.3–0.5; costa extension 25–53 µm long; lanceolate setae present; humerals 6; gonostylus not expanded, crista dorsalis pronounced and pointed.

Acknowledgements

Our sincere thanks to Dr. Elisabeth Stur of the Norwegian University of Science and Technology for detecting and informing us of the issues related to this species. Our sincere thanks also go to the staff and researchers at the Centre for Biodiversity Genomics, University of Guelph.

References

deWaard J. R., Ratnasingham S., Zakharov E. V., Borisenko A. V., Steinke D., Telfer A. C., Perez K. H., Sones J. E., Young M. R. and Levesque-Beaudin V. 2019. A reference library for Canadian invertebrates with 1.5 million barcodes, voucher specimens, and DNA samples. - *Scientific Data* 6: 308. DOI: https://doi.org/10.1038/s41597-019-0320-2

Ekrem, T., Willassen, E. and Stur, E., 2007. A comprehensive DNA sequence library is essential for identification with DNA barcodes. - *Molecular phylogenetics and evolution* 43(2): 530–542. DOI: https://doi.org/10.1016/j.ympev.2006.11.021

Hebert P. D. N., Ratnasingham S., Zakharov E. V., Telfer A. C., Levesque-Beaudin V., Milton M. A., Pedersen S., Jannetta P. and deWaard J. R. 2016. Counting animal species with DNA barcodes: Canadian insects. - *Philosophical Transactions of the Royal Society B: Biological Sciences* 371: 20150333. DOI: https://doi.org/10.1098/rstb.2015.0333

Lin, X., Stur, E. and Ekrem, T., 2015. Exploring genetic divergence in a species-rich insect genus using 2790 DNA barcodes. - PloS one 10(9): p.e0138993. DOI: https://doi.org/10.1371/journal.pone.0138993

Montagna, M., Mereghetti, V., Lencioni, V. and Rossaro, B., 2016. Integrated taxonomy and DNA barcoding of alpine midges (Diptera: Chironomidae). - *PLoS One* 11(3): p.e0149673. DOI: https://doi.org/10.1371/journal.pone.0149673

Namayandeh, A., Guerra, S., Islam, N., James, T., Hudson, P. L., Ghaderi, E., Yusuf, T., Vasquez, A. A. and Ram, J. L. 2024. New species and a fascinating diversity of Chironomidae (Diptera, Insecta) in and around an overlooked urban vernal pool. - *ZooKeys* 1208: 133. DOI: https://doi.org/10.3897/zookeys.1208.124495

Article submitted 5. February 2025, accepted by Torbjørn Ekrem 3. March 2025, published 3. April 2025.

Cryptotendipes usmaensis (Pagast, 1931) – a cryptic species of Chironomidae (Insecta, Diptera)

Janne Raunio[1] & Yngve Brodin[2]

[1]*Metsähallitus, Sapokankatu 2, FIN – 48100 Kotka, Finland. Corresponding author. E-mail: janne.raunio@metsa.fi*
[2]*Swedish Museum of Natural History. Department of Zoology, Box 50007, SE – 104 05 Stockholm, Sweden. E-mail: tav77ygg@gmail.com*

Abstract

We investigated the taxonomic status of *Cryptotendipes usmaensis* (Pagast, 1931), a cryptic species of Chironomidae (Insecta, Diptera), focusing on its morphological and molecular characteristics. We found discrepancies in the descriptions of adult males and pupal exuviae, suggesting the possibility of multiple species or significant polymorphism within *C. usmaensis*. Studies of recent collections from Finland revealed two distinct pupal morphotypes of *C. usmaensis* coexisting in the same habitat. DNA barcoding of adult males from Finland and other countries suggest the existence of two genetically distinct groups of the species. The findings emphasize the need for a comprehensive taxonomic review of *C. usmaensis* and suggest that species identification in some Chironomidae may require a different approach, i.e. combination of pupal morphology and molecular data, rather than relying solely on adult male morphology.

Introduction

Eight species of *Cryptotendipes* are known to occur in Europe. Two of these, *C. usmaensis* (Pagast, 1931) and *C. nigronitens* (Edwards, 1929) have males with a strongly humped tergite IX elevated far above the anal point. The males can be separated by the length of the anal point of the hypopygium using the key in Langton and Pinder (2007).

Studies of adult males and pupal exuviae assigned to *C. usmaensis* have opened a question whether it is a polymorphic species or might involve two different species (Langton and Visser 2003), further justified attention due to barcoding results from Finland present in the Barcode of Life Data Systems (BOLD, Ratnasingham et al. 2024).

Felix Pagast collected the type material of *C. usmaensis* from the Lake Usma, Latvia, where he worked during the early 1930s. Pagast's own collection may have been lost in the second World War, including the *C. usmaensis* material. Dr. Martin Spies kindly went through the large volume of *Cryptotendipes* material that is deposited in the ZMS Munich, Germany, but the collection apparently did not contain any specimen that could be regarded as a type specimen of *C. usmaensis*. There is nothing in the published literature on *Cryptotendipes* to suggest that type material of *C. usmaensis* could be stored elsewhere.

Adult males and pupae of *C. usmaensis* have been described several times since Pagast's publication in 1931, but in the absence of the type material the authors have relied on specimens from other geographic locations and habitats. As a result, descriptions differ considerably and are frequently not congruent with that of Pagast (1931).

In the following, we summarize taxonomic work on different morphotypes of *C. usmaensis* in an attempt to clarify whether there are one or more species involved. Specimens from Finland, recently collected and partly barcoded, were an important basis for the work.

Material and Methods

The data for this study were collected from various lakes and rivers in southern Finland, during different years and occasions (e.g., Raunio and Paasivirta 2008). Pupal exuviae of recently eclosed Chironomidae were collected by scooping floating debris from river and lake margins. The samples were collected with a hand net (mesh size 250 µm), especially from accumulation areas indicated by a foam and floating material, and behind obstacles (Wilson and Ruse 2005). Such samples are considered to represent chironomid taxa emerged within the past 48 hours, upstream or upwind of the sampling site (Wilson and Ruse 2005). Samples of chironomid pupal exuviae often include pharate specimens, which are useful for taxonomic studies

as they allow linking pupal exuviae to adults.

Pupal exuviae of both *Cryptotendipes* forms found in this study and a pharate male of *C.* sp. 2 have been deposited in Zoologische Statssammlung München, Munich, Germany.

Results

Pupal exuviae of Cryptotendipes usmaensis

Pagast (1931) described the pupa of *C. usmaensis* and mentioned that it was different from that of other known *Cryptotendipes* species as described by Lenz (1926). Lenz (1959) also noted the difference in the pupae. Langton and Visser (2003) separated two pupal morphotypes, one as *Cryptotendipes usmaensis* Pagast and the other one as a form called *Cryptotendipes* pe1c. The latter form was considered as an extreme variation of *C. usmaensis*. However, in Pagast's (1931) description of pupal exuviae of *C. usmaensis* the number of lateral taeniae of abdominal segments V-VIII is: 2, 3, 4, 4. Langton and Visser (2003) gave different numbers: 4, 4, 4, (3)4. Sæther (2010) separated the form *Cryptotendipes* near *usmaensis* from Pagast's (1931) species, based on different numbers of lateral taeniae on segments V-VIII and by the larger number of taeniae in the fringe of the anal lobe (17-40 vs. 13-14 that of *C. usmaensis*).

Adult male of Cryptotendipes usmaensis

As well as for the pupal exuviae, there is variation in characters in the descriptions of what is believed to be the adult male of *C. usmaensis.* The illustration of a dorsal view of the hypopygium in Goetghebuer and Lenz (1937) is very similar to Pagast's original illustration of *C. usmaensis*. The illustration in lateral view in Albu (1980) is also very similar but differs in the dorsal view particularly as the anal point is not apically expanded. Langton and Pinder (2007) adapted their illustration of the male hypopygium in a dorsal view from Pagast (1931) who showed that the adult male tergite IX is strongly humped, and that the tergite on each side of the anal point bears a low hump with three bristles (Fig. 1).

More recently, a worldwide key to adult males of *Cryptotendipes* was published by Pal and Hazra (2018) and later modified by Mukherjee et al. (2020). In couplet 6 of the key by Mukherjee et al. *C. usmaensis* is apparently considered NOT to have a high dorsal ridge. The illustration of the hypopygium in the key shows a different species to Pagast's *C. usmaensis* also by the much thinner gonostyli and the lack of lobes on tergite IX at each side of the anal point. Furthermore, in the following couplet 8, *C. usmaensis* is identified by the presence of setae on basal half of the anal point, as already mentioned in the key by Sæther (1977). However, Pagast (1931) did not mention this character. His drawing (Fig. 1) in a lateral view shows no seta on the anal point, although the drawing in a dorsal view leaves some room for different interpretations. A possible explanation is that due to the high ridge of tergite IX, lateral setae on the tergite may appear in slide mounts as if the setae are flanking the anal point. None of the hypopygia illustrations of the world's species of *Cryptotendipes* in Mukherjee et al. (2020) agrees well with Pagast's *C. usmaensis*. *Cryptotendipes lyalichi* (Zorina, 2006) is rather similar but lacks the tergite IX lobes at each side of the anal point.

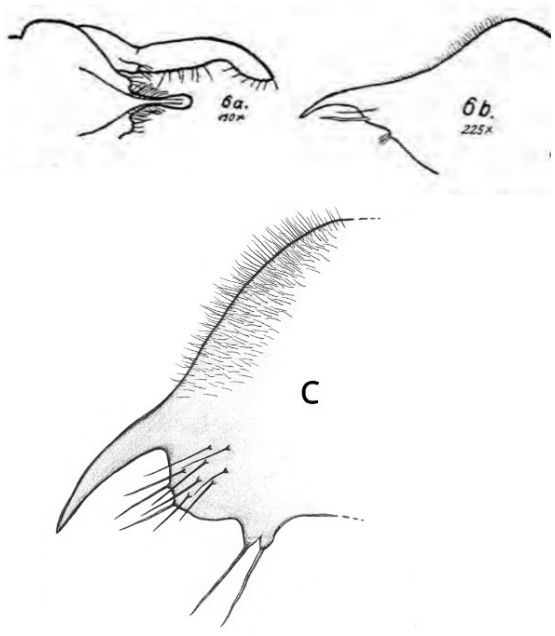


Figure 1. Hypopygium of *Cryptotendipes usmaensis* by Pagast (1931) in a dorsal view (6a) and lateral view (6b), together with our illustration in lateral view (C).

Cryptotendipes usmaensis in Finland

There are four species of *Cryptotendipes* recorded from Finland (Paasivirta 2014); *C. usmaensis* is by far the most common with larvae inhabiting mainly oligotrophic lakes. Two distinct pupal morphotypes of *C. usmaensis* have been identified among the Finnish specimens, yet adult males associated with the two pupal morphotypes could not be morphologically separated. We refer to these pupal

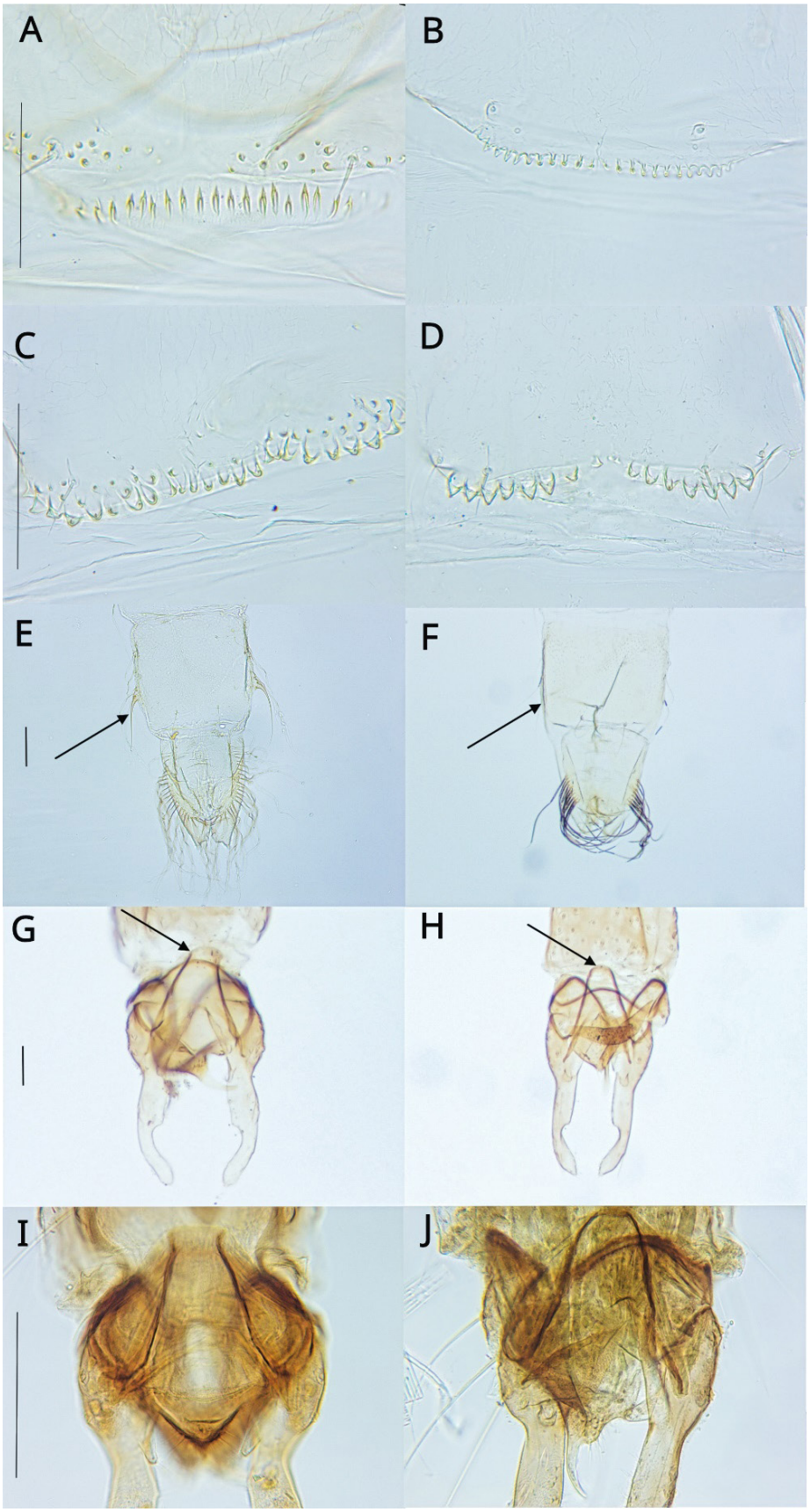


Figure 2. *Cryptotendipes* sp. 1 (A, C, E, G, I) and *C.* sp. 2 (B, D, F, H, J). Caudal hooklets of abdominal segments II present (A), absent (B); pupal exuviae tergite III (C, D); pupal exuviae tergites VIII-IX (E, F); adult male hypopygium (G-J). Arrows points to the presence (E) and absence (F) of posterolateral comb on pupal tergite VIII, and different shapes of transverse sternapodeme (G-H) of the two forms. Scale bars = 100 µm.

morphotypes as *Cryptotendipes* sp. 1 (Fig. 2A, C and E) and *Cryptotendipes* sp. 2 (Fig. 2B, D and F). Using the key by Langton and Visser 2003, *C*. sp. 1 runs to *C. usmaensis*. However, *C*. sp. 1 differs from *C. usmaensis* sensu Langton and Visser (2003) by having paired anterolateral spine patches on sternite I. It also bears larger number of taeniae in the fringe of the anal lobe than Pagast's *C. usmaensis* (Table 1). *Cryptotendipes* sp. 2, on the other hand, shows some resemblance to *C. pseudotener*, as the dorsal toothed mounds (sensu Langton and Visser 2003) or caudal hooklets (sensu Epler 2018) of abdominal tergites III-V (or III/IV alone) may be medially interrupted (see Fig. 2D). However, most specimens examined show continuous caudal hooklets. The numbers of teeth on the dorsal toothed mounds also seem to differ between the two forms (Fig. 3).

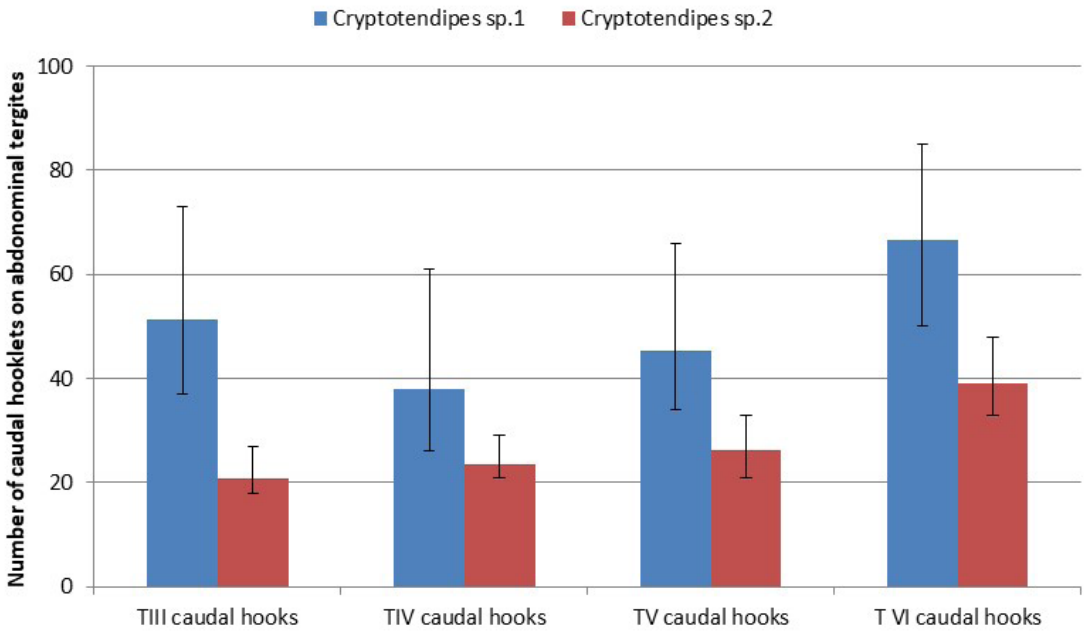


Figure 3. Numbers of hooklets on dorsal toothed mounds of abdominal tergites III-VI of pupal exuviae of the *Cryptotendipes* sp. 1 and *C*. sp. 2. Bars show the averages (n = 4 in each) and whiskers show variation (min-max).

The pupal form *Cryptotendipes* sp. 2 is recorded only from three oligotrophic lakes in SE Finland (lakes Suolajärvi, Niskajärvi and Simpeleenjärvi), while the form *Cryptotendipes* sp. 1 is more common and has been found from various locations in Finland, including rivers and lakes. There appears to be no differences in emergence periods, as both forms eclose from early June to early August. In Simpeleenjärvi, the two forms coexist.

Pagast (1931) mentioned that the pupa of *C. usmaensis* has hump-like small hooks dorsally on III-V and ventrally on III-IV in a single row, dorsally on II and VI in two rows, nowhere interrupted in the middle. This indicates that the form *C*. sp. 2 is different from Pagast's (1931) species (Figs 2B and D). In addition, paired anterolateral spine patches on sternite I of *C*. sp. 1 are absent in the form *C*. sp. 2. Perhaps the most striking difference between the two forms is the absence of posterolateral comb of VIII in *C*. sp. 2 (Table 1, Fig. 2F), which is unique among known *Cryptotendipes* species. However, the numbers of lateral taeniae on segments V-VIII appear to vary and may not be a useful character for species identification, as used e.g. by Sæther (2010) (Table 1).

We were not able to find any obvious differences in the hypopygium of the two forms, keeping in mind that the anal point of most specimens could been seen only in a lateral view due to slide mounting procedures. However, we noted that the transverse sternapodeme of *C.* sp. 1 is less sclerotized (Fig. 2G), whereas it is more distinct in *C.* sp. 2 (Fig. 2H).

Molecular data of Cryptotendipes usmaensis

In BOLD (Ratnasingham et al. 2024), there are currently only a handful of specimens identified as *C. usmaensis*. There are three specimens from Finland, three from Germany and one from Austria. Analysis of the barcode gene CO1 data of *C. usmaensis* in BOLD indicates that the specimens form two genetically distinct groups, so called BINs, separated by 3.3% which is a distance often long enough to separate species

Table 1. Key characteristics of pupal exuviae of the Finnish *Cryptotendipes* sp. 1 and *C.* sp. 2.

Taxon	St. I spine patches	T II dorsal toothed mounds	T III-V dorsal toothed mounds	Comb of VIII	Numbers of IX taeniae	T IX dorsal seta	Lateral taeniae of V-VIII
Cryptotendipes sp. 1	Present	Present (usually paired)	Continuous	Present	24.4 (20-27)	Present or absent	3-4, 3-4, 3-4, 3-5
Cryptotendipes sp. 2	Absent	Absent	Interrupted or continuous	Absent	14.5 (10-20)	Present or absent	3-4, 3-4, 3-4, 3

of Chironomidae (Song et al. 2016, Brodin 2025). There is only one specimen in BIN BOLD:AFT5715 (*Cryptotendipes* sp. 2 from Finland), while six in BIN BOLD:ADA4237. Interestingly, both groups of *C. usmaensis* are closer (among-group distances of 2.3% and 2.6%, respectively) to a group of 22 specimens from Manitoba, Canada (BIN BOLD:ACR8808), which are identified only as *Cryptotendipes* sp. (all females).

Discussion

In summary, there are several forms of *Cryptotendipes usmaensis* described as pupal exuviae and adult males. Some of the discrepancies seen in literature are likely derived from the absence of type specimens, and the fact that *C. usmaensis* appears to have many morphotypes at least regarding pupae. Data from Finland indicates that morphologically apparently very similar adult males may have distinctly different pupal exuviae, and that the differences seen in pupae are supported by the molecular data. The two forms have been found to coexist in the same lake and emerge simultaneously.

Our analyses of morphological and molecular data assigned to *C. usmaensis* did not provide enough information to conclude if there are two species involved or a single one with at least two distinct pupal exuviae forms and a long barcode distance of 3.3%. There are species of Chironomidae with as much as 10-12% intraspecific barcode distance with adult males not possible to separate morphologically (Lin et al. 2018). A more thorough taxonomic study on *Cryptotendipes usmaensi*s involving more pupal exuviae and barcoded males might provide knowledge to arrive at another conclusion. It could even challenge the fact that the current morphological concept for practically all scientifically named chironomid species is mainly tied to adult male specimens. *Cryptotendipes usmaensis* might be among the species in which the male morphology does not appear to differ sufficiently for the purpose of species identification. Thus, we may need to acknowledge that for such chironomid taxa, species identification could rely mainly on pupal exuviae and molecular data instead. Less than ten species of Chironomidae present in Europe are based originally on the pupal stage, in one or two cases with so far unknown males or females (Lehmann 1972). But above all, a review of *Cryptotendipes* in general, and particularly a re-description of *C. usmaensis* is required.

Acknowlegments

We wish to thank Martin Spies at the Zoologische Staatssammlung Munich for the help in search of type material of *C. usmaensis,* John Epler and Peter Langton for valuable conversations via emails and Lauri Paasivirta for sharing his pupal exuvial sample from the Lake Simpeleenjärvi.

References

Albu, P. 1980. Familia Chironomidae, Subfamilia Chironomidae, Diptera. Academia Republicii Socialiste Romania, Fauna Republicii Socialiste România. - *Insecta* 11: 1–320.

Brodin, Y. 2025. *Procladius* (Diptera, Chironomidae) of Europe and a global view. - *Zootaxa* 5591 (1): 1–127. https://doi.org/10.11646/zootaxa.5591.1.1

Epler, J.H. 2018. The genus *Cryptotendipes* Beck et Beck in Florida, with the description of a new species (Diptera: Chironomidae: Chironominae). - *Zootaxa* 4433 (3): 583–594. https://doi.org/10.11646/zootaxa.4433.3.12

Goetghebuer, M. and Lenz, F. 1937. *Subfamilie Tendipedinae (Chironominae)*. In: Lindner, E. (Ed) Die Fliegen der Palaearktischen Region 13c, E. Scweizerbart'sche Verlegsbuchhandlung, 260 p.

Langton, P.H. and Pinder, L.C.V. 2007. *Keys to the adult male Chironomidae of Britain and Ireland*. Freshwater Biological Association, Scientific Publication No 64, Ambleside, volume 1, 240 p., Volume 2, 168 p.

Langton, P.H. and Visser, H. 2003. *Chironomidae exuviae. A key to pupal exuviae of the West Palaearctic Region*. CD-ROM. Expert Center for Taxonomic Identification, University of Amsterdam, Amsterdam. Available from https://chironomidae-exuviae.linnaeus.naturalis.nl/ [accessed 11 April 2025]

Lehmann, J. 1972. Revision der europäischen Arten (Puppen und Imagines) der Gattung *Eukiefferiella* Thienemann. - *Beiträge zur Entomologie* 22 (7-8): 347–405.

Lenz, F. 1926. Chironomiden aus den Balatonsee. - *Archivum Balatonicum* 1(2): 129-144.

Lenz, F. 1959. Die Metamorphose der Gattung *Cryptotendipes* Lenz. - *Deutsche Entomologische Zeitschrift* 6 (1-3): 238–250.

Lin, X.-L., Stur, E. and Ekrem, T. 2018. DNA barcodes and morphology reveal unrecognized species in Chironomidae (Diptera). - *Insect Systematics & Evolution* 49: 329–398. https://doi.org/10.1163/1876312X-00002172

Mukherjee, B., Mukherjee, T. and Hazra, N. 2020. A new species of the genus *Cryptotendipes* Beck et Beck, 1969 (Diptera: Chironomidae) from India, with a world key to the males and tentative phylogenetic relationship. - *Zootaxa* 4896(2): 201–223. https://doi.org/10.11646/zootaxa.4896.2.3

Paasivirta, L. 2014. Checklist of the family Chironomidae (Diptera) of Finland. - *ZooKeys* 441: 63–90. https://doi.org/10.3897/zookeys.441.7461

Pagast, F. 1931. Chironomiden aus der bodenfauna der Usma-Sees in Kurland. - *Folia Zoologica et Hydrobiologica* 3: 199–248.

Pal, G. and Hazra, N. 2018. Two new species of the genus *Cryptotendipes* Lenz (Diptera: Chironomidae) from India with a worldwide key to the genus. - *Oriental Insects* 53 (1): 34–45. https://doi.org/10.1080/00305316.2018.1439785

Ratnasingham, S., Wei, C., Chan, D., Agda, J., Agda, J., Ballesteros-Mejia, L., Ait Boutou, H., El Bastami, Z.M., Ma, E., Manjunath, R., Rea, D., Ho, C., Telfer, A., McKeowan, J., Rahulan, M., Steinke, C., Dorsheimer, J., Milton, M. and Hebert, P.D.N. 2024. BOLD v4: A centralized bioinformatics platform for DNA-based biodiversity data. *In*: DNA Barcoding: Methods and Protocols. Chapter 26. Springer, United States, NY, New York, pp. 403–441.

Raunio, J. and Paasivirta, L. 2008. Emergence patterns of lotic Chironomidae (Diptera: Nematocera) in southern Finland and the use of their pupal exuviae in river biomonitoring. - *Fundamental and Applied Limnology* 170 (4): 291–301. https://doi.org/10.1127/1863-9135/2008/0170-0291

Song, C., Wang, Q., Zhang, R., Sun, B. and Wang, X. 2016. Exploring the utility of DNA barcoding in species delimitation of *Polypedilum* (*Tripodura*) non-biting midges (Diptera: Chironomidae). - *Zootaxa* 4079 (5): 534–550. https://doi.org/10.11646/zootaxa.4079.5.2

Sæther, O.A. 1977. Taxonomic studies on Chironomidae: *Nanocladius*, *Pseudochironomus*, and the *Harnischia* complex. - *Bulletin of the Fisheries Research Board of Canada* 196: 1–143.

Sæther, O.A. 2010. *Cryptotendipes* Lenz from Manitoba, Canada, with keys to known immatures of the genus (Diptera: Chironomidae). - *Zootaxa* 2412 (1): 1–20. https://doi.org/10.11646/zootaxa.2412.1.1

Wilson, R.S. and Ruse, L.P. 2005. A guide to the identification of genera of chironomid pupal exuviae occurring in Britain and Ireland and their use in monitoring lotic and lentic freshwaters. Freshwater Biological Association Special Publication no. 13, 176 p.

Zorina, O.V. 2006. *Cryptotendipes lyalichi* sp.n., a new species of non-biting midges (Diptera, Chironomidae, Chironominae) from the south of the Russian Far East. - *Euroasian Entomological Journal* 5 (4): 341–343.

Article submitted 8. May 2025, accepted by Torbjørn Ekrem 30. June 2025, published 19. August 2025.

Some new faunistic records of Chironomidae (Diptera) from the Republic of Kosovo

Astrit Bilalli[1], Milaim Musliu[1], Donard Geci[2], Halil Ibrahimi[2] and Armin Namayandeh[3]

[1]*Faculty of Agribusiness, University of Peja "Haxhi Zeka", Pejë, Kosovo. E-mail: astrit.bilalli@unhz.eu, milaim.musliu@unhz.eu*

[2]*Department of Biology, Faculty of Mathematics and Natural Sciences, University of Prishtina "Hasan Prishtina", Prishtinë, Kosovo. E-mail: donard.geci@uni-pr.edu, halil.ibrahimi@uni-pr.edu*

[3]*Department of Environmental and Life Sciences, Trent University, 1600 West Bank Drive, Peterborough, ON, Canada. E-mail: a.namayan@taxanama.org, arminnamayandeh@trentu.ca; corresponding author*

Abstract

In this article, we share our results regarding adult male Chironomidae collected from the Lepenc River Watershed located above the settlements of Dimcë and Dërmjak, part of the Karadak Mountain range in western Kosovo. We document 16 species and one taxon at the generic level from these habitats, with 12 species identified as new faunistic records for Kosovo.

Introduction

Despite Europe being a well-studied part of the Palearctic, a few regions in this continent remain that require further investigation into the diversity of their Chironomidae fauna, in particular countries in the Balkans and the Caucasus regions. In the Balkan Peninsula, the Chironomidae diversity of the landlocked nation of Kosovo has only recently received attention. Płóciennik (2008) expedition in Albania updated the number of known Chironomidae from this country and provided additional faunistic records from the neighbouring Balkan countries. Berlajolli et al. (2019) presented a list of the Chironomidae collected from two springs in western Kosovo as supplementary materials. Berlajolli et al. (2020) conducted a comprehensive study of 37 springs in the Bjeshkët e Nemuna Mountains, recording 43 Chironomidae taxa with clear zonation of species distribution based on their habitats. The Bjeshkët e Nemuna Mountains encompass three countries in the Balkans: Albania, Kosovo, and Montenegro. Berlajolli and Płóciennik's (2023) study of benthic invertebrates of springs in the Bjeshket e Nemuna National Park, Kosovo, established that, with 43 out of 51 taxa identified, Chironomidae are the major invertebrate component of these habitats. Płóciennik et al. (2023) presented a detailed overview of Chironomidae taxa in small freshwater systems of the Balkans, their ecology, and the environmental factors influencing their diversity. These studies overall produced 23 named species for Kosovo.

As part of the investigation into the diversity of aquatic insects from the western Balkans, Musliu et al. (2024) collected adults of Chironomidae, representing interesting findings on faunistic knowledge of Kosovo's Chironomidae. In this study, we present these findings on adult male Chironomidae collected from Lepenc River Watershed above the villages of Dimcë and Dërmjak, part of the Karadak Mountain in western Kosovo.

Material and Methods

Collection, preparation, mounting, and imagery

The adult Chironomidae specimens were sampled at night with ultraviolet pan traps. The 50 cm-diameter pan traps were filled approximately 30% with a solution of 30% water mixed with a few drops of detergent and positioned near the water source. To attract specimens, ultraviolet light sources with an eight-watt output were installed above the white pans and operated from dusk to the next morning (Fig. 1). Collected samples were immediately preserved in 80% ethanol. The adult specimens were

Figure 1. The ultraviolet light trap used for the collection of Chironomid specimens.

mounted on slides using a Canada balsam mounting medium following the procedures outlined in Epler (2001). The imagery was produced using an OMAX A3550U Camera mounted on an AMScope compound microscope. Images were stacked and compressed using Helicon Focus 8.0 and further edited in GIMP 2.10.38 software. The voucher specimens of Chironomidae are deposited at the entomology collection of the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Prishtina, Kosovo.

Sampling area

The sampling was carried out at two sampling sites: Dimcë (Figs 2a-b) (42.170295°N, 21.315259°E) and Dërmjak (Figs 2b-c) (42.173038°N, 21.316659°E) in Hani i Elezit Municipality, Kosovo. Both sites are located within Kosovo's Lepenc River Watershed and the Black Sea Basin. At the Dimcë sampling site, the stream bed was primarily composed of large rocks, accompanied by stones, pebbles, gravel, and a small amount of fine sediment. The streambanks were densely vegetated, with over 80% of their surface covered

Figure 2. Sampling habitats at Lepenc River Watershed, Kosovo (a-d). a-b. Dimcë, c-d. Dërmjak.

with vegetation. The riparian zone on both sides of the stream was well-preserved and largely undisturbed, contributing to the ecological stability of the area. At the Dërmjak sampling site, the streambed was primarily composed of large rocks, along with stones, pebbles, gravel, and a small amount of fine sediment. The stream banks were densely vegetated, with more than 80% of their surface covered by vegetation, and the riparian zone on both sides remained intact and undisturbed. A notable feature of this site was a small waterfall-like segment that created a deeper pool of water immediately below it, adding to its hydrological diversity.

Results

A total of 16 species, and one taxon at the generic level, were identified from 66 adult males collected from the Lepenc River Watershed, with 12 species representing new faunistic records for Kosovo (Table 1). The gallery of the newly recorded species from Kosovo is provided in Fig. 3.

Discussion

With the exception of *Limnophyes spinigus* Sæther, 1990, all other species identified in this study have a widespread Palearctic distribution. *L. spingus,* previously widely reported from Northern and Western Europe, now has two records from the Balkans, one from Montenegro in BOLD system (Sample ID: BIOUG56208-C11) and the other from Kosovo, suggesting a more widespread distribution of this species on the continent.

Although previous freshwater studies conducted in the Balkans, including Kosovo, have produced and provided significant information about the Chironomidae fauna and their ecology, actual faunistic studies

Table 1. List and record of the Chironomidae (Diptera) species collected from Lepenc River Watershed, Kosovo, 2024. NFR = New Faunistic Record, No. = Number.

Subfamily	Species	No. adult male	Location	NFR
Tanypodinae	*Procladius (Psilotanypus) choreus* (Meigen, 1804	1	Dërmjak	Kosovo
	Psectrotanypus varius (Fabricius, 1787)	1	Dërmjak	Kosovo
Orthocladiinae	*Brillia longifurca* Kieffer, 1921	2	Dimcë	Kosovo
	Cardiocladius fuscus Kieffer, 1924	9	Dërmjak	Kosovo
	Cricotopus (s.s.) bicinctus (Meigen, 1818)	17	Dimcë	
		8	Dërmjak	
	Cricotopus (Paratrichocladius) nigritus (Goetghebuer, 1938)	1	Dërmjak	Kosovo
	Eukiefferiella claripennis (Lundbeck, 1898)	1	Dërmjak	
	Eukiefferiella devonica (Edwards, 1929)	2	Dërmjak	Kosovo
		3	Dimcë	
	Limnophyes spinigus Sæther, 1990	2	Dërmjak	Kosovo
	Orthocladius (s.s.) oblidens (Walker, 1856)	1	Dërmjak	Kosovo
	Rheocricotopus (Psilocricotopus) chalybeatus (Edwards, 1929)	6	Dërmjak	Kosovo
	Tvetenia calvescens (Edwards, 1929)	4	Dimcë	Kosovo
Chironominae	*Chironomus (s.s.) riparius* (Meigen, 1804)	3	Dërmjak	
	Microchironomus tener (Kieffer, 1918).	1	Dimcë	Kosovo
	Polypedilum (Uresipedilum) convictum (Walker, 1856)	1	Dërmjak	
	Polypedilum (Tripodura) pullum (Zetterstedt, 1838)	1	Dërmjak	Kosovo
	Polypedilum (Tripodura) sp.	2	Dërmjak	

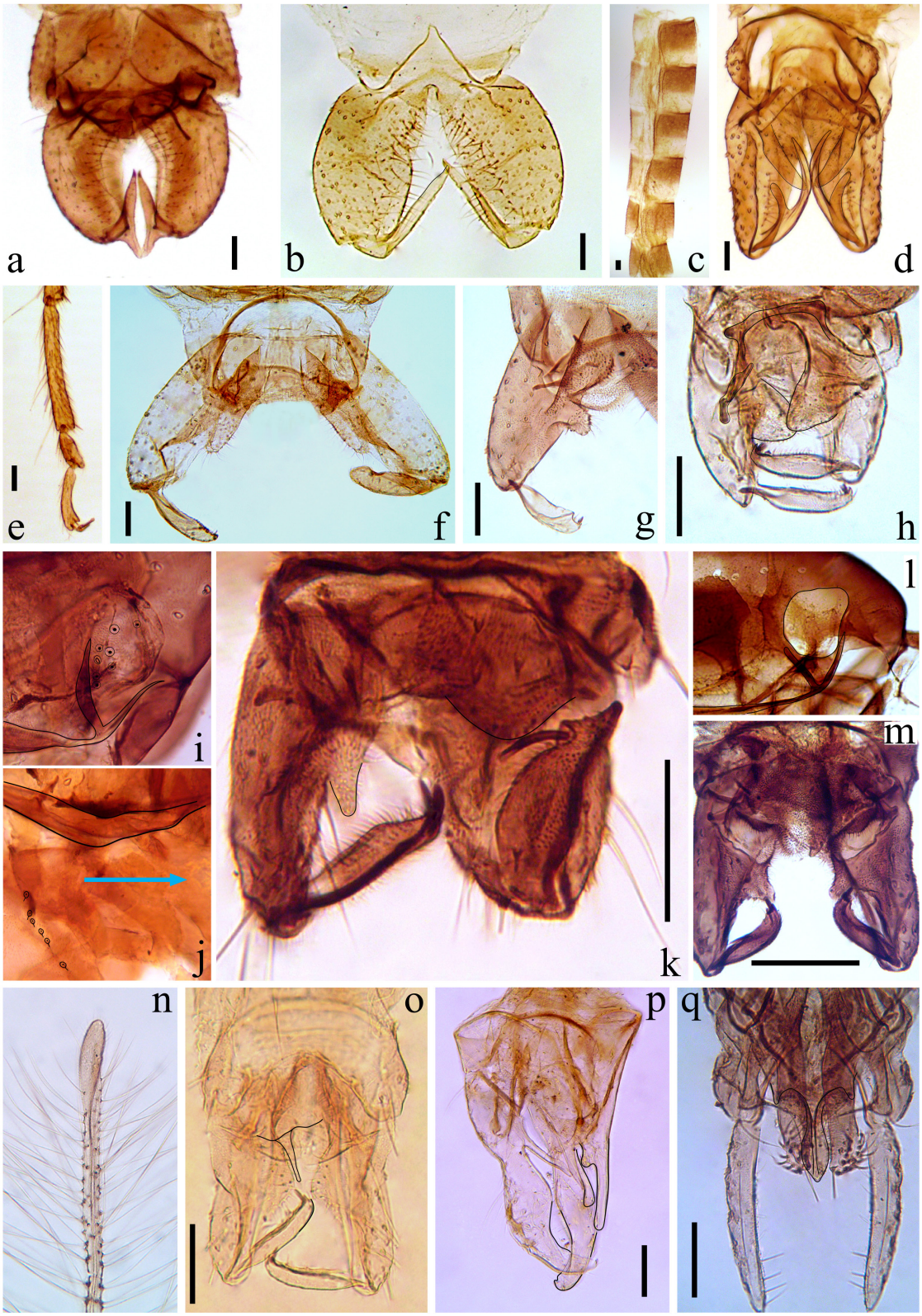


Figure 3. Gallery of the newly recorded species collected from Lepenc River Watershed, Kosovo. a. *Procladius (Psilotanypus) choreus* (Meigen, 1804), b. *Psectrotanypus varius* (Fabricius, 1787), c-d. *Brillia longifurca* Kieffer, 1921, e-f. *Cardiocladius fuscus* Kieffer, 1923, g. *Cricotopus (Paratichocladius) nigritus* (Goetghebuer, 1938), h. *Eukiefferiella devonica* (Edwards, 1929), i-k. *Limnophyes spinigus* Sæther, 1990, l-m. *Rheocricotopus (Psilocricotopus) chalybeatus* (Edwards, 1929), n-o. *Tvetenia calvescens* (Edwards, 1929), p. *Microchironomus tener* (Kieffer, 1918), q. *Polypedilum (Tripodura) pullum* (Zetterstedt, 1838). a, b, d, f-h, k, m, o-q. Hypopygium, c. Abdominal segments II–VII, e. Hind leg tarsal segments 3–5, i. Thoracic humeral setae, j. Thoracic preepisternal setae; arrow indicates the direction of thorax, l. Thoracic humeral pit, n. Apex of last antennal flagellomere. *Orthocladius (s.s.) oblidens* (Walker, 1856) was omitted due to the poor condition of the specimen. All scale bars are 50 µm.

are still necessary to assess the real richness and diversity of the species. Furthermore, to increase the taxonomic resolution of Chironomidae fauna, conducting these faunistic studies by including adults rather than just immatures would be necessary (Bitušík and Trnková, 2019). The 12 new faunistic records obtained in this study for Kosovo, from a single freshwater system, demonstrate the future potential of discovering more Chironomidae fauna in the country and the Balkans.

Acknowledgments

This study was made possible through "Refuge areas of aquatic insect endemism in Kosovo and the Balkans in function of environmental protection" granted to Halil Ibrahimi and financed by the University of Prishtina.

References

Berlajolli, V. and Płóciennik, M. 2023. A preliminary list of crenobiontic species in the Bjeshket e Nemuna National Park (Republic of Kosovo). - *Oltenia-Studii şi comunicări Ştiinţele naturii* 39 (2): 100–1003.

Berlajolli, V., Płóciennik, M., Pesić, V. and Berlajolli, X. 2020. Chironomidae of the Cursed Mountains (Bjeshkët e Nemuna National Park). BIODIVEST Conference, Craiova, Romania.

Berlajolli1, V., Płóciennik, M., Antczak-Orlewska, O. and Pešić, V. 2019. The optimal time for sampling macroinvertebrates and its implications for diversity indexing in rheocrenes – case study from the Prokletije Mountains. - *Knowledge and Management of Aquatic Ecosystem* 420(6): 1–9. DOI: https://doi.org/10.1051/kmae/2018043

Bitušík, P. and Trnková, K. 2019. A preliminary checklist of Chironomidae (Diptera) from Albania with first records for the Balkan Peninsula. - *Zootaxa* 4563: 361–371 DOI: https://doi.org/10.11646/zootaxa.4563.2.10

Epler, J.H. 2001. Identification manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of southeastern United States, including Florida. North Carolina Department of Environment and Natural Resources, Raleigh, NC and St. Johns River Water Management District, Plalatka, FL. Special Publication SJ2011-SP13 526.

Musliu, M., Bilalli, A., Geci, D. and Ibrahimi, H. 2024. First record of *Hydroptila kalonichtis* Malicky, 1972 (Trichoptera, Hydroptilidae) from the western Balkans. - *Natura Croatica* 33: 279–286. DOI: https://doi.org/10.20302/NC.2024.33.22

Płóciennik, M. (2008) New records of Chironomidae (Diptera) from Albania. - *Lauterbornia* 63: 101–108.

Płóciennik, M., Berlajolli, V., Dmitrović, D., Gligorović, B., Pešić, V. and Gadawski, P. 2023. The Chironomidae (Diptera) diversity in the Balkan Peninsula spring systems and other small water bodies. - *International Journal of Limnology* 59 (6): 1–11. DOI: https://doi.org/10.1051/limn/2023005

Article submitted 20. February 2025, accepted by Torbjørn Ekrem 20. August 2025, published 26. August 2025.

Two peculiar species of Oriental Chironomini (Diptera, Chironomidae)

Peng Xiang[1] & Hongqu Tang[2]

[1]*Life Science and Technology College, Jinan University, Guangzhou 510632, China*
E-mail: xp3124@163.com
[2]*Research Centre of Hydrobiology, Jinan University, Guangzhou 510632, China*
E-mail: thqtang@jnu.edu.cn, corresponding author

Abstract

Two strange species are described and illustrated here based on adult males collected from China and Malaysia respectively. The combination of conventional diagnostic character states is unusual in both species, and we cannot allocate either into any currently recognized genera. We propose that these represent two new genera in the tribe Chironomini. Here we adopt 'Chironomini taxon 1' and 'taxon 2' as coded unresolved names for further discussion.

Introduction

Knowledge of species diversity and zoogeographic distribution of chironomid fauna is critical for us to understand their evolutionary diversification (Brundin 1966). However, when we compare chironomid richness between major zoogeographic regions, the Oriental regional fauna, is undoubtedly the most poorly studied (Sublette and Sublette 1973, Ashe 1990, Ferrington 2008). Although recent decades have seen greater activity concerning this fauna, notably a few regional guides (Cranston 2004, Cranston and Tang 2024) and some newly confirmed wide-distribution genera (Tang and Cranston 2025). Nevertheless, there are many new findings together with undescribed taxa needing for revealing highly threatened hotspots for conservation (Sodhi et al. 2010, Hughes 2017).

During recent fieldwork in 2024, two strange midges were sorted from light traps, of which each species is represented by only a single individual. Detail examination indicated neither of them can be assigned confidently into any known genus, but can be narrowed to subfamily Chironominae, tribe Chironomini. Since the material is limited and it is hard to extract DNA or accumulate additional material in the near future, we drawn attention to these peculiar species. Establishment of taxonomic rank without any associated immature materials or evidence from molecular data is unwise. Here, two species with no generic rank are described and illustrated.

Materials and methods

Adults were collected using light traps, sorted, and mounted on slides in Euparal. Photographs were taken under an Olympus BX53 compound microscope through a mounted camera-ToupViewTM. Digital photos of different focal planes were stacked using Helicon Focus version 7. Morphological terminology and abbreviations generally follow Sæther (1980) except the base projection of the gonostylus in the taxon 2. Line drawings were aided using a drawing tube attached to an Olympus BX53. All material is deposited in the Department of Ecology, Research Centre of Hydrobiology, Jinan University, Guangdong, China (EJNU).

Taxonomy

Chironomini taxon 1

Fig. 1

Material examined. 1 male, CHINA: Macao SAR, an artificial ecology park of Alto de Coloane, near South China Herbs Garden, 22°07'N 113°33'E, 160 m, 09.x.2024, light trap, leg. JD Yin.

Diagnostic characters. Taxon 1 can be separated from others by the banded wings and legs; TIX with a pair of posterior tubercles; superior volsella bare, pad-like; inner margin of gonostylus with a field of long setae in apical 2/3.

Male (n = 1)

Total length 2.8 mm. Wing length 1.25 mm.

Generally brown in color. Wing membrane somewhat smoky, with two broad darker bands in basal 1/3 and at mid length (Fig. 1A), lacking macrotrichia. Legs banded: femur with a brown band near apex, tibia almost brown except the apex 1/5 in P_1, all tarsi of P_1 are lost. In P_2 and P_3, femur with two brown rings, one near the middle and another located in the subapex, tibia almost brown except the two terminals (Fig. 1B), all tarsi yellow.

Head. Frontal tubercle absent. Flagellomeres 1–12, 470 μm; flagellomere 13, 410 μm, AR 1.15, apex with 3–4 setae, 30–40 μm long. Lengths (μm) of Pm 1–5: 40; 30; 80; 110; 165, respectively. Temporals 8. Clypeus with 32 setae.

Thorax. Scutal tubercle absent. Ac 16, extending to the mid-scutum. Dc 20, including 2 humerals, robust two rows after middle section. Pa 4. Scutellars consist of two rows, the anterior with three small setae, the posterior with eight strong setae.

Wing (Fig. 1A). Anal lobe obtuse, VR 1.24. R with 20 setae; R_1 with 14 setae; R_{2+3} close to R_1. R_{4+5} with 24 setae, other veins and cells bare. Squama with 3 setae.

Legs. Fore tibia apex tongue-shaped, not pointed. Mid and hind tibia with two separated combs, only outer small comb bearing a curved spur, 25–30 μm long. Pulvilli well-developed, as long as the claws, without further bifurcation. Leg segment length and proportions as Table 1.

Table 1. Lengths (μm) and proportions of legs of Chironomini taxon 1, male (n = 1).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV
P_1	680	380	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
P_2	700	560	330	190	130	80	50	0.59	3.53	3.82
P_3	750	630	470	260	200	100	70	0.75	2.94	2.94

Abdomen. Tergite VIII tapered anteriorly, triangular in shape.

Hypopygium (Figs 1C–E). Anal tergite bands well-developed, extending posteriorly to the base of anal point, not fused medially, with 8 anal median setae, distally with two proturbances, apex bearing 6–8 long setae. Anal point 55 μm long, somewhat spatulate, constricted slightly in the middle, each side with 4 setae, and 6–8 small setae ventrally. Superior volsella pediform, microtrichiose, 75 μm long and 50 μm wide in maximum. Inferior volsella rod-shaped, apex with 5–6 strong recurved long setae. Gonocoxite 125 μm long. Gonostylus 130 μm long, with a clump of long setae along the inner margin, more than 35 setae (Fig. 1E). HR 0.96.

Remarks. In the key to the males of the Holarctic Chironominae (Cranston et al. 1989), the species keys to couplet 40, as it has 3 setae on squama, with further to couplet 42 to *Stelechomyia* Reiss (now a junior synonym of *Kribiodorum* Kieffer), but the shape of the fore femur apex and the fore tibial apex are clearly different. If the number of squamals is omitted, the species keys out to couplet 47 where it keys to *Polypedilum* Kieffer. The taxon clearly has similarities with the *Polypedilum* Kieffer generic complex given the tapering abdominal segment VIII, the tibial comb pattern and well-developed gonocoxite lobes, and especially to some species of the subgenus *Tripodura* regarding the pair of caudolateral projections on the anal tergite (Zhang et al. 2016). However, some details differ from the core generic diagnosis of *Polypedilum*: e.g., there are no characteristically bifurcate pulvilli or 6–8 distinct long, even-spaced setae along the inner margin of gonostylus. Although the pulvilli are well developed, each branch with several short twigs, there are no further bifurcate branches. Furthermore, the inner margin seta of the gonostylus of current species are concentrated to the apical 2/3, bearing numerous strong setae, easily observed in lateral view (Fig. 1E). Thus, we cannot make decision on generic placement and adopt “Chironomini taxon 1” here.

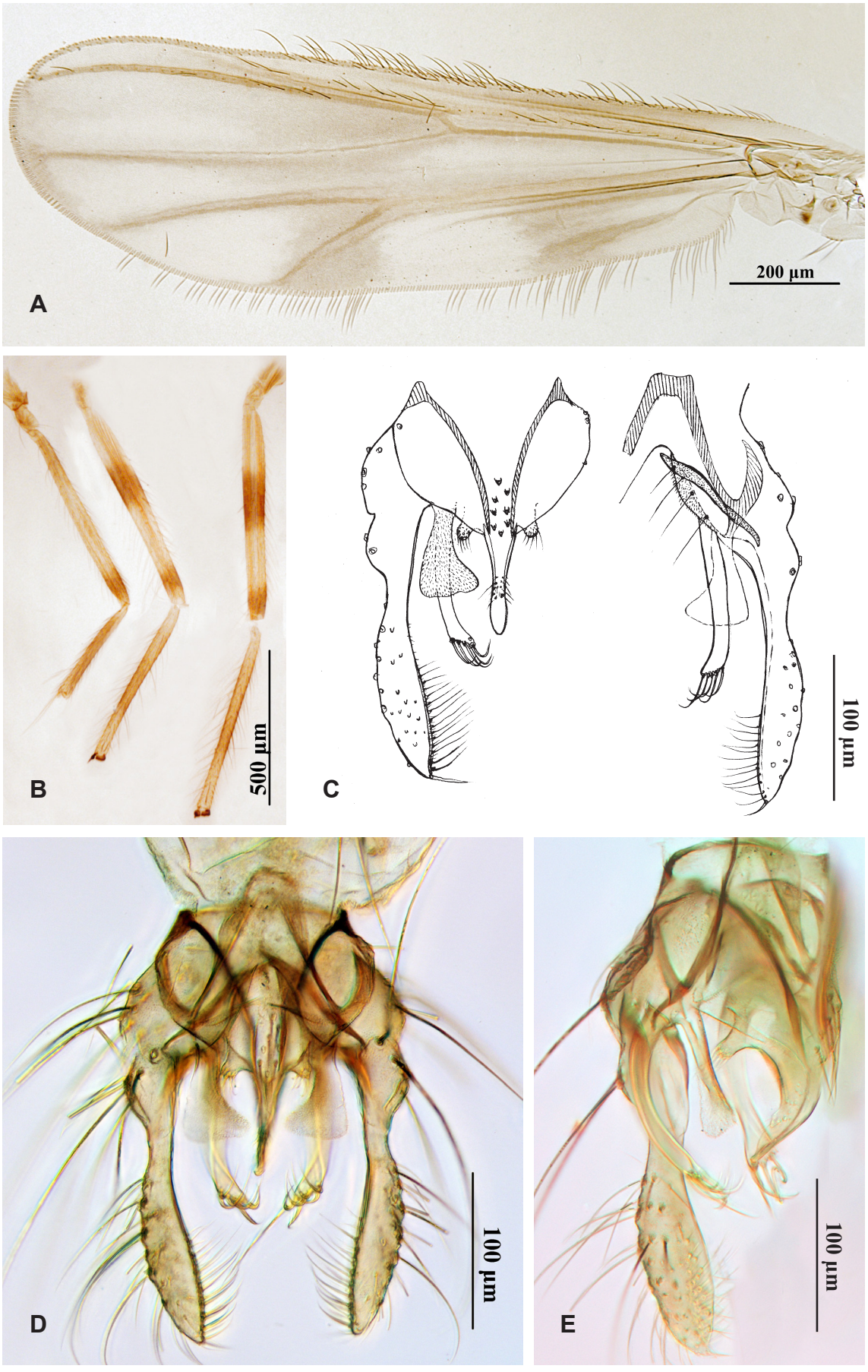


Figure 1. Chironomini taxon 1, male. A, wing; B, legs; C–E, hypopygium: C, line drawing; D, dorsal view; E, lateral view.

Chironomini taxon 2

Fig. 2

Material examined. 1 teneral male, MALAYSIA: Sabah, Kota Kinabalu, Kibunut Stream, 5°54'N 116°13'E, 120 m, 13.vii.2024, light trap, leg. H. Tang.

Diagnostic characters. Taxon 2 can be separated from others by the 11-segmented antenna, the stout gonostylus with a basal microtrichose digitiform projection, both superior volsella and inferior volsella are without microtrichia, but bearing 2 long setae.

Male (n = 1)

Total length 2.3 mm. Wing length 0.96 mm.

Generally yellowish green in color. Thorax with yellowish background, scutal vittae and postnotum brown. Wing surface without macrotrichia.

Head (Fig. 2B). Frontal tubercle absent. Flagellomeres 1–10, 420 μm; flagellomere 11, 530 μm, AR 1.26. Lengths (μm) of Pm 1–5: 20; 25; 25; 75; 95, respectively. Temporals 6. Clypeus with 12 setae.

Thorax. Scutal tubercle absent. Ac 8, starting close to antepronotum. Dc 6. Pa 2. Scutellars 6, uniserial.

Wing (Fig. 2A). Anal lobe nearly obtuse, wing venation indistinct. Wing membrane without macrotrichia, squama bare.

Legs (Fig. 2C). Fore tibia apex with a short extension, not pointed, without comb or spine. Mid and hind tibia with two separated combs, each comb bearing a short straight spur, 20 μm long. Pulvilli present, shorter than the claws. Leg segment length and proportions as Table 2.

Table 2. Lengths (μm) and proportions of legs of Chironomini taxon 2, male (n = 1).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV
P_1	450	280	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
P_2	425	330	170	80	50	25	30	0.52	5.00	4.44
P_3	450	430	270	145	135	70	50	0.63	2.88	3.26

Hypopygium (Fig. 2D–G). Anal tergite bands well-developed, extending posteriorly, not fused and connected with the base of anal point, no median tergite setae, but each side with 4–6 setae above the base of anal point. Anal point 50 μm long, nearly parallel-sided, with inner rim. Superior volsella (Fig. 2F) rod-shaped, about 40 μm long, with two small setae near apex, without microtrichia. Inferior volsella (Fig. 2G) small triangular with two points, about 20 μm by main axle, the basal apex with 2 small setae. Gonocoxite 120 μm long, with a sharp ventrolateral invasion towards the middle of gonostylus, the inner margin of gonocoxite apex slightly expanded, bearing 3–4 long setae. A pubescent digitiform projection is present at the base of gonostylus. Pars ventralis absent. HR 1.60.

Remarks. In the key to the males of the Holarctic Chironominae (Cranston et al. 1989), this taxon keys to couplet 21, but it does not resemble either *Robackia* Sæther or *Parachironomus* Lenz, as it has a reduced and stout gonostylus. If the digitiform projection at the base of gonostylus is regarded as the second branch of the gonostylus, the taxon likely falls into the *Harnischia* generic complex with a bifurcate gonostylus. The general contour of hypopygium also resembles those of some Pseudochironomini, i.e., with bare or digitus-like superior volsella and inferior volsella, plus a pubescent rod-like basal gonostylus projection, yet this taxon shows an 11-segmented antenna, fore tibial apex with no spine or comb, and a hypopygium with distinct long anal point. Those characters contradict the generic diagnosis of the tribe Pseudochironomini. In conclusion, the current taxon cannot be allocated into any known genus, perhaps representing a new genus, here a coded name "Chironomini taxon 2" is adopted here.

Acknowledgements

We thank Dr. Torbjørn Ekrem for discussions on the male hypopygium of taxon 2 and help generating the plates. We are grateful to Mr. Jiadong Yin (Sun Yat-Sen University) and Dr. Wu Han (The University of Hong Kong) who assisted in the fieldwork. The species were collected under the research permit SFC.810-4/6/1 (2023)-106 in Malaysia and the permission under Environmental Protection Bureau of Macao (DSPA: Direcção dos Serviços de Protecção Ambiental).

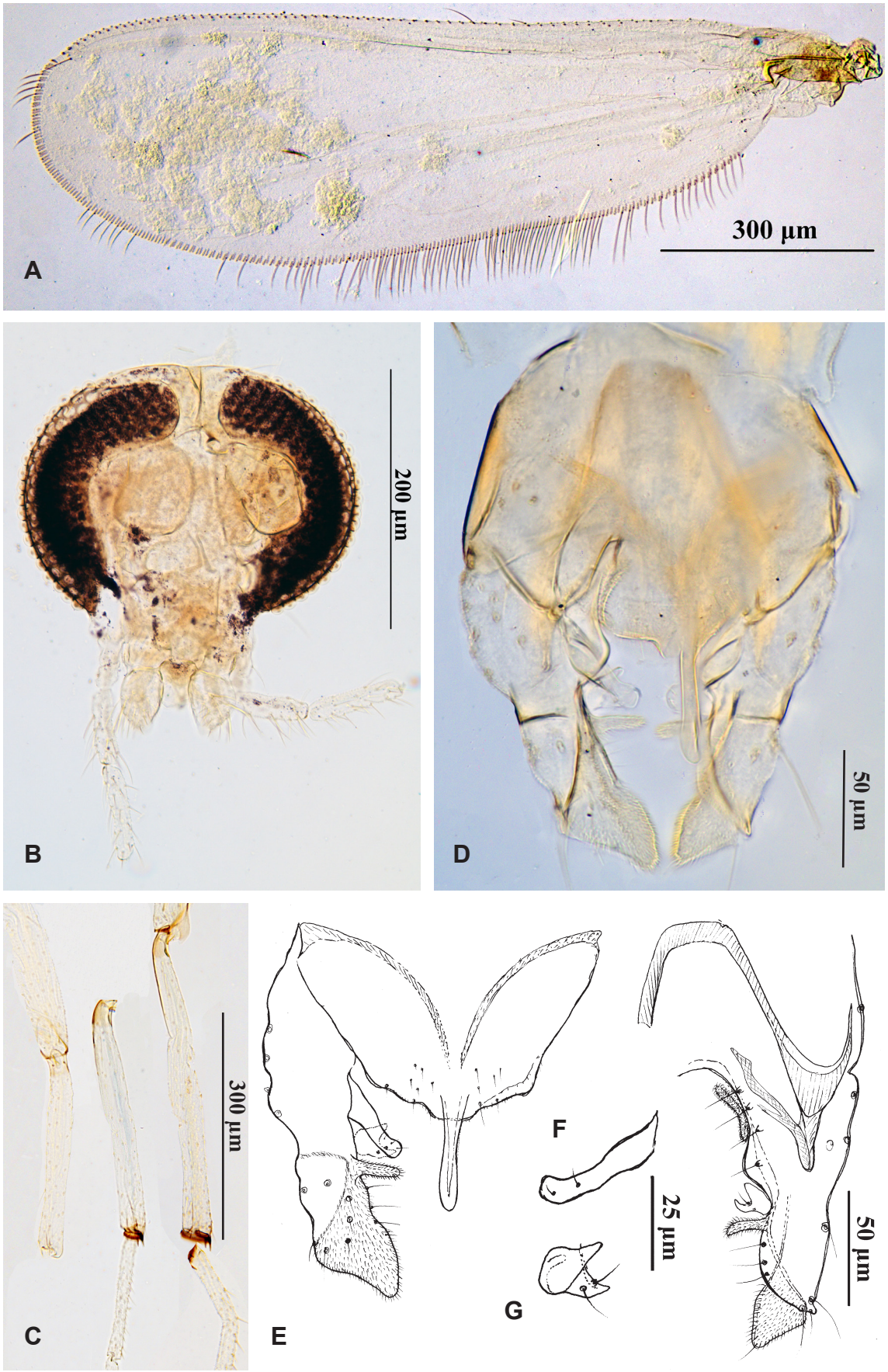


Figure 2. Chironomini taxon 2, A, wing; B, head; C, legs; D–G, male hypopygium: D, color photo; E, line drawing; F, superior volsella; G, inferior volsella.

References

Ashe, P. 1990. Chapter 22. Ecology, zoogeography and diversity of Chironomidae (Diptera) in Sulawesi with some observations of relevance to other aquatic insects. In: Knight W.J. & Holloway J.D. (eds.) *Insects and the Rain Forests of Southeast Asia (Wallacea)*. Royal Entomological Society, London, pp. 261–268.

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

Cranston, P.S. 2004. Insecta: Diptera: Chironomidae. In: Yule, C.M. & Yong, H.S. (eds.) *Guide to Freshwater Invertebrates of the Malaysian Region*. Academy of Sciences Malaysia, Kuala Lumpur, Malaysia, pp. 711–735.

Cranston, P.S. and Tang, H.Q. 2024. An identification guide to the genera of aquatic larval Chironomidae (Diptera) of south-east Asia. - *Zootaxa* 5497 (2): 151–193. DOI: https://doi.org/10.11646/zootaxa.5497.2.1

Cranston, P.S., Dillon, M.E., Pinder, L.C.V. and Reiss, F. 1989. 10. The adult males of Chironominae (Diptera: Chironomidae) of the Holarctic region – Keys and diagnoses. In: Wiederholm, T. (ed.) *Chironomidae of the Holarctic region. Keys and diagnoses. Part 3. Adult males*. Entomologica scandinavica 34 (Supplement): 353–502.

Ferrington, L.C., Jr. 2008. Global diversity of non-biting midges (Chironomidae; Insecta-Diptera) in freshwater. - *Hydrobiologia* 595: 447–455. DOI: https://doi.org/10.1007/s10750-007-9130-1

Hughes, A.C. 2017. Understanding the drivers of Southeast Asian biodiversity loss. - *Ecosphere* 8 (1): e01624. DOI: https://doi.org/10.1002/ecs2.1624

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

Sodhi, N.S., Posa, M.P.C., Lee, T.M., Bickford, D., Koh, L.P. and Brook, B.W. 2010. The state and conservation of Southeast Asian biodiversity. - *Biodiversity and Conservation* 19: 317–328. DOI: https://doi.org/10.1007/s10531-009-9607-5

Sublette, J.E. and Sublette, M.S. 1973. Family Chironomidae. In: Delfinado, M. & Hardy, E.D. (eds.) *Catalogue of the Diptera of the Oriental Region, Part 1*. University Press, Honolulu, Hawaii, pp. 389–422.

Tang, H.Q. and Cranston, P.S. 2025. *Paraskusella* Cranston, 2018 and *Kribiodosis* Kieffer, 1921 (Diptera: Chironomidae: Chironomini): two new species from Borneo described, allowing incorporation into a multi-gene molecular phylogeny. - *Raffles Bulletin of Zoology* 73: 34–42. DOI: https://doi.org/10.26107/RBZ-2025-0003

Zhang, R.L., Song, C., Qi, X. and Wang, X.H. 2016. Taxonomic review on the subgenus *Tripodura* Townes (Diptera: Chironomidae: *Polypedilum*) from China with eleven new species and a supplementary world checklist. - *Zootaxa* 4136 (1): 1–53. DOI: http://doi.org/10.11646/zootaxa.4136.1.1

Article submitted 27. August 2025, accepted by Torbjørn Ekrem 2. November 2025, published 02. November 2025.

The 6th Chinese Symposium on Chironomidology

Xiao-Long Lin[1,2] & Rui-Lei Zhang[1,2]

[1]*Engineering Research Center of Environmental DNA and Ecological Water Health Assessment, Shanghai Ocean University, Shanghai 201306, China. E-mail:* lin880224@gmail.com
[2]*Shanghai Universities Key Laboratory of Marine Animal Taxonomy and Evolution, Shanghai Ocean University, Shanghai 201306, China*
[3]*College of Life Science, Nankai University, Tianjin 300071, China*

The 6th Chinese Symposium on Chironomidology was held on 13th, September, 2025 in Shanghai, China. The symposium was organized by Shanghai Ocean University.

Twenty-eight participants representing eight institutions attended the symposium, including Nankai University, Geological Museum of China, Jinan University, The University of Hong Kong, Shanghai Ocean University, Tianjin Normal University, Huanggang Normal University, and Taizhou University.

The symposium served as a significant collaborative platform for research institutions and universities to address critical issues in Chironomidae studies and related disciplines. Scientific exchanges covered multiple research areas, including morphological taxonomy, DNA barcoding, molecular systematics, zoogeography, ecology, and environmental monitoring. We anticipate that this symposium will further enhance cooperation among Chinese researchers and strengthen connections with the international academic community.

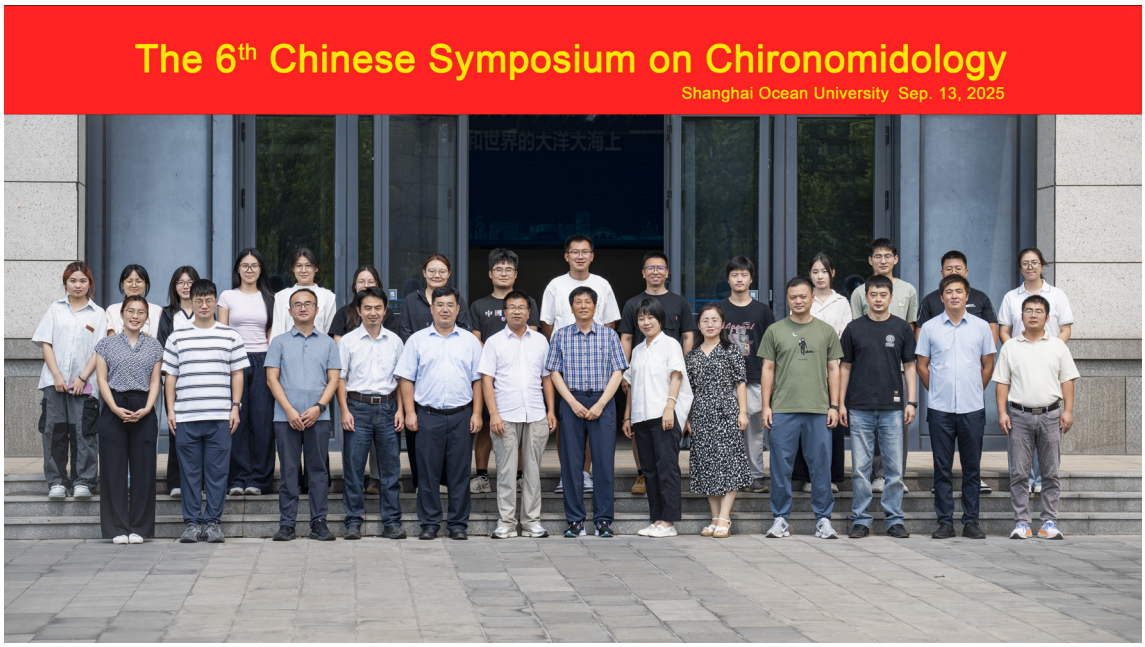
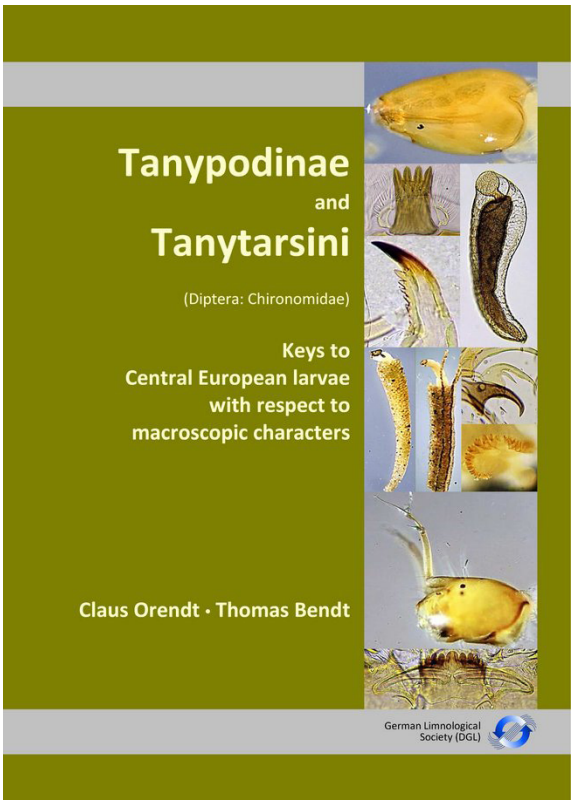


Figure 1. Group photo of participants at the 6th Chinese Symposium on Chironomidology held at Shanghai Ocean University, September 13, 2025.

New books

Tanypodinae and Tanytarsini (Diptera: Chironomidae). Keys to Central European larvae using mainly macroscopic characters.

This book on the identification of the larvae of non-biting midges of the subfamily Tanypodinae and the tribe Tanytarsini (subfamily Chironominae) is the follower of previously published works on Chironominae (tribe Chironomini) and Orthocladiinae sensu lato in 2012 and 2021. It supplies and concludes the series of illustrated guides for Chironomidae larvae. Thus, a complete work is presented for beginners and less experienced colleagues who are concerned with chironomid identification in water monitoring, science, or because of other interests. Similar to our earlier volumes on Chironomini and Orthocladiinae sensu lato, we used macroscopic characters for the identification of genera, species groups, and easily separable species as much as possible. The keys can be used in all of Europe without restriction.



With the illustration of nearly 700 photographs, this book provides an identification guide for 27 Tanypodinae genera and characters for more than 27 taxa at the species level. The identification for 15 genera and 10 species of Tanytarsini larvae is included. For each taxon identified, information on the habitat is also provided. In combination with the richly and excellently illustrated profile sheets published from the LANUK agency (https://www.lanuk.nrw.de/publikationen/arbeitsblaetter) as a reference these keys and the other mentioned are powerful tools to start and work with chironomid larval identification in Europe.

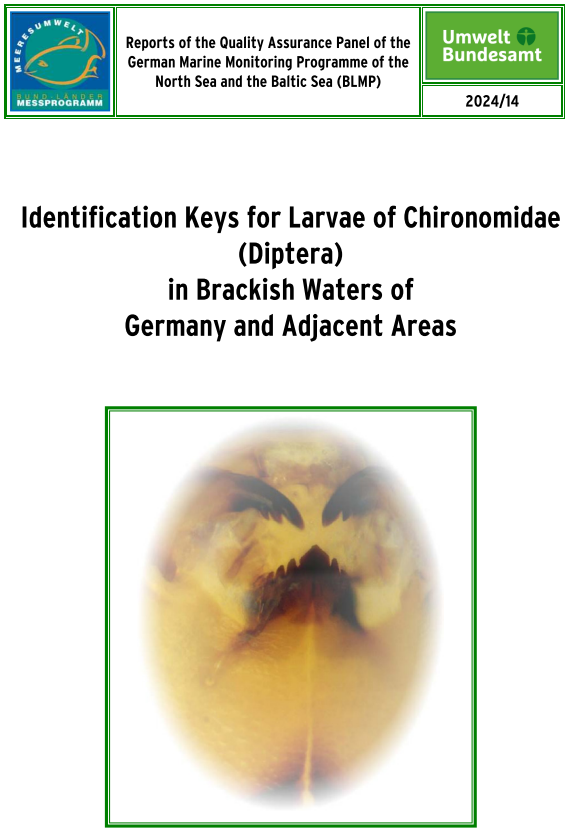
Orendt, C. & Bendt, Th. 2025. Tanypodinae and Tanytarsini (Diptera: Chironomidae). Keys to Central European larvae using mainly macroscopic characters. DGL- Tools No. 1-2025 (DGL-Arbeitshilfe 1-2025). Editor: DGL e. V. German Limnological Society), 98 p., ISBN 978-3-9827220-0-9.
Orders: geschaeftsstelle@dgl-ev.de
Price: 30.00 € + shipping costs
Preview and keys to the other chironomid subfamilies: https://hydro-bio.de/chironomidae.htm

Identification keys of Chironomidae larvae in brackish waters of Germany and adjacent areas.

The key includes about 90 taxa (genera, species groups, species) that can be identified in the larval stage and have been recorded from brackish waters in Germany and adjacent North Sea and Baltic Sea areas. The monograph comprises (1) an introductory section, including data on the relevant taxa and their ecology as well as instructions concerning methods and key operation; (2) the identification key; and (3) profile sheets with morphological and biological data on each taxon. These taxon portraits serve also for quality assurance of the identification results.

The key is built from macroscopically visible features as much as feasible. Where a compound microscope is needed, the required preparations are kept as simple as possible."

With its broad geographical scope and practical approach, this monograph is unique and of equal interest for scientists and technical offices in countries along the North and Baltic Seas. It is available in an English and a German edition."



Orendt, C., Dettinger-Klemm, A. & Spies, M. 2024.Identification keys of Chironomidae larvae in brackish waters of Germany and adjacent areas. Revised version in English language. Reports of the Quality Assurance Panel of the German Marine Monitoring Programme of the North Sea and the Baltic Sea 2024/14. Editor: Federal Environment Acency, Berlin, 242 p., ISSN 2194-7902. Price: free of charge

Order: petra.schilling@uba.de
Preview and other chironomid keys: https://hydro-bio.de/chironomidae.htm

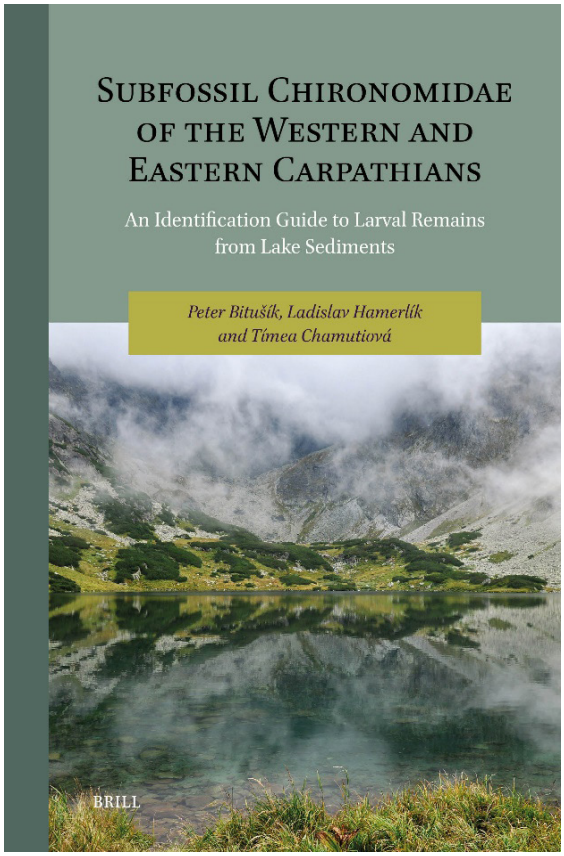
***Subfossil Chironomidae of the Western and Eastern Carpathians:* a new identification guide to larval chironomid remains of Carpathian lake-sediments**

The guide to Chironomidae larval remains from lake sediments in the Western and Eastern Carpathians is the culmination of decades of dedicated research and hundreds of field expeditions to mountain lakes in Slovakia, Poland, Ukraine, and Romania.

Nestled within the dramatic landscapes of the Carpathian Mountains, these lakes are not only strikingly beautiful but also serve as time capsules, preserving vital information for reconstructing past environmental and climate processes.

The guide provides identification keys for more than 100 chironomid taxa from six subfamilies, recorded in over 70 lakes. Each species, morphotype, and genus is documented with high-quality photographs and follows up-to-date taxonomy. The keys are preceded by a general introduction to the study area and a historical overview of chironomid research in the Carpathians. For each genus or morphotype, ecological insights and the most plausible palaeoecological interpretations are summarized.

The Subfossil Chironomidae of the Western and Eastern Carpathians is an important resource not only for chironomid experts, teachers, and students of aquatic ecology and palaeoecology, but also for ecologists and conservationists dedicated to understanding and protecting the Carpathians and their unique biodiversity.



Bitušík, P., Chamutiová, T. and Hamerlík, L. 2025. Subfossil Chironomidae of the Western and Eastern Carpathians. An Identification Guide to Larval Remains from Lake Sediments. Brill, 190p. ISBN: 978-90-04-69466-8

Available as E-Book (PDF) or hardback

Price: 150.00€

For more information please see https://brill.com/display/title/69972