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A NEW SPECIES OF *PARAMETRIOCNEMUS* GOETGHEBUER, 1931 (DIPTERA: CHIRONOMIDAE: ORTHOCLADIINAE) FROM SWITZERLAND AND ELEVATION OF *P. STYLATUS ADZHARICUS* KOWNACKI & ZOSIDZE, 1973 TO FULL SPECIES

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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 semiaquatic, 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

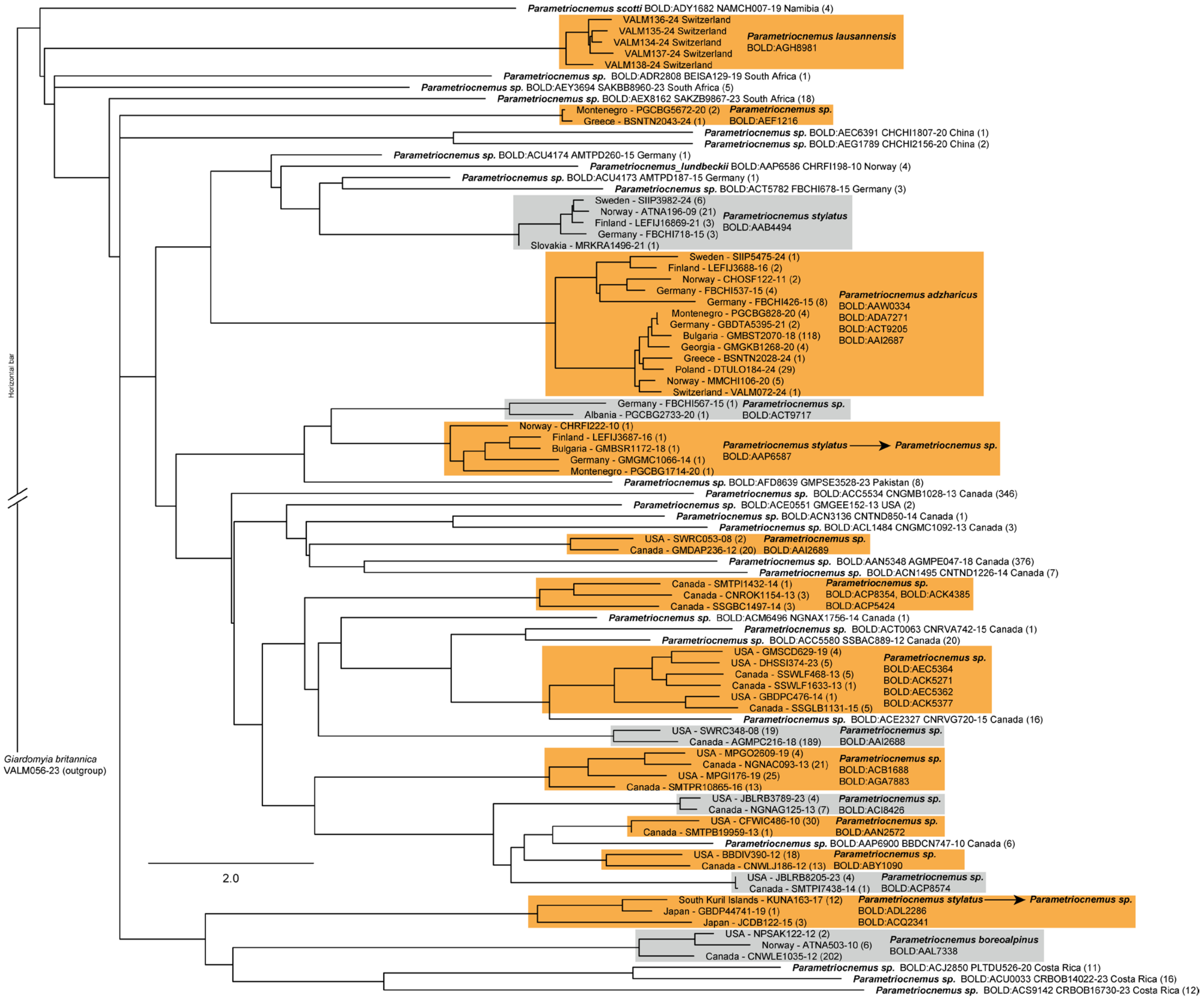

Figure 1. Parametriocnemus NJ tree based on COI sequences available in BOLD. One sequence per BIN cluster and per country was used, BOLD process ID is indicated in each terminal. For P. lausannensis, all available sequences were included. The number of available COI sequences for a given country in each 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-

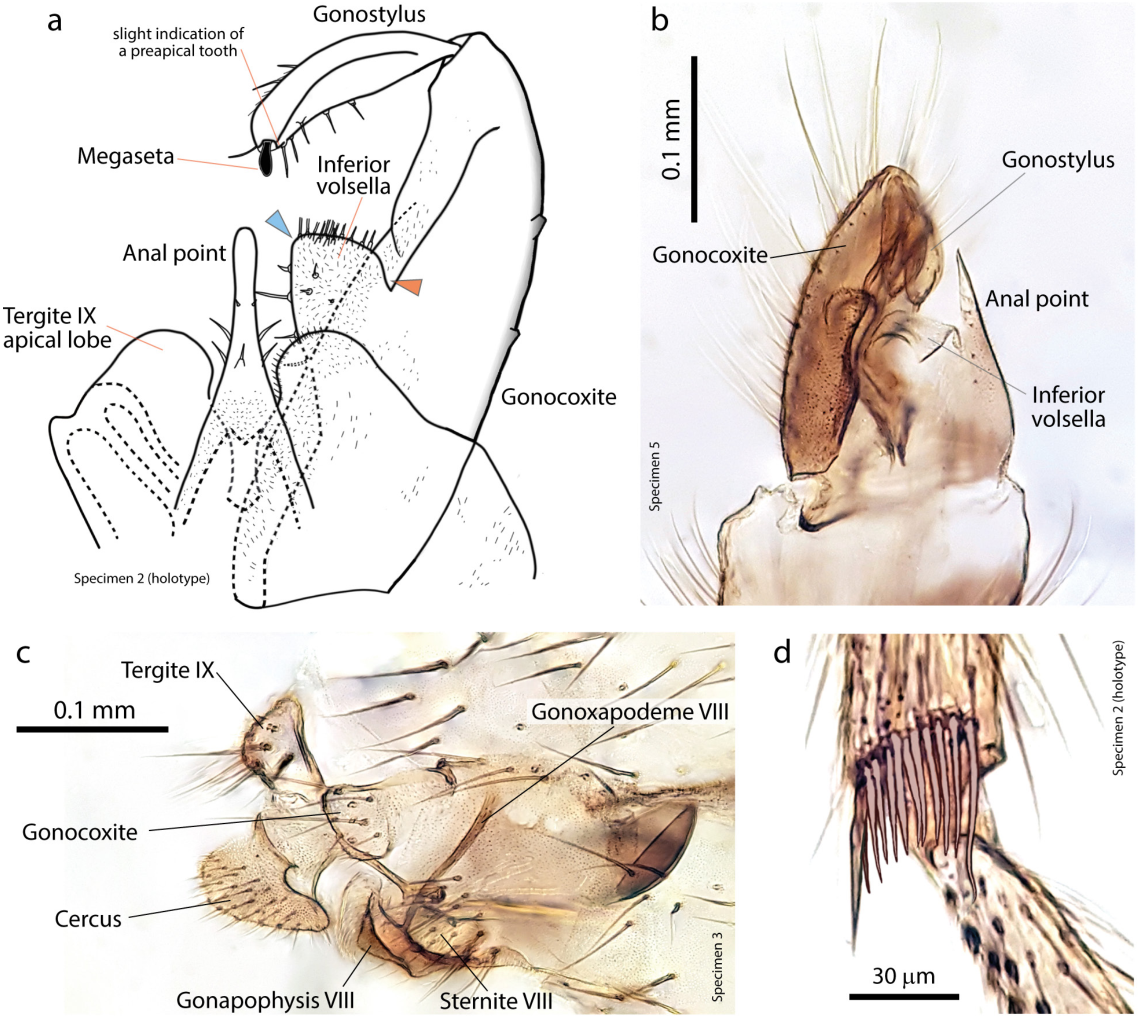

Figure 2. Genitalia and tibial comb of *Parametriocnemus lausannensis*. a, hypopygium dorsal view; b, hypopygium lateral view; c, female genitalia; d, male hind tibial comb. Orange arrowhead: distal angle (indentation between the inferior volsella and the gonocoxite), blue arrowhead: apical angle of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

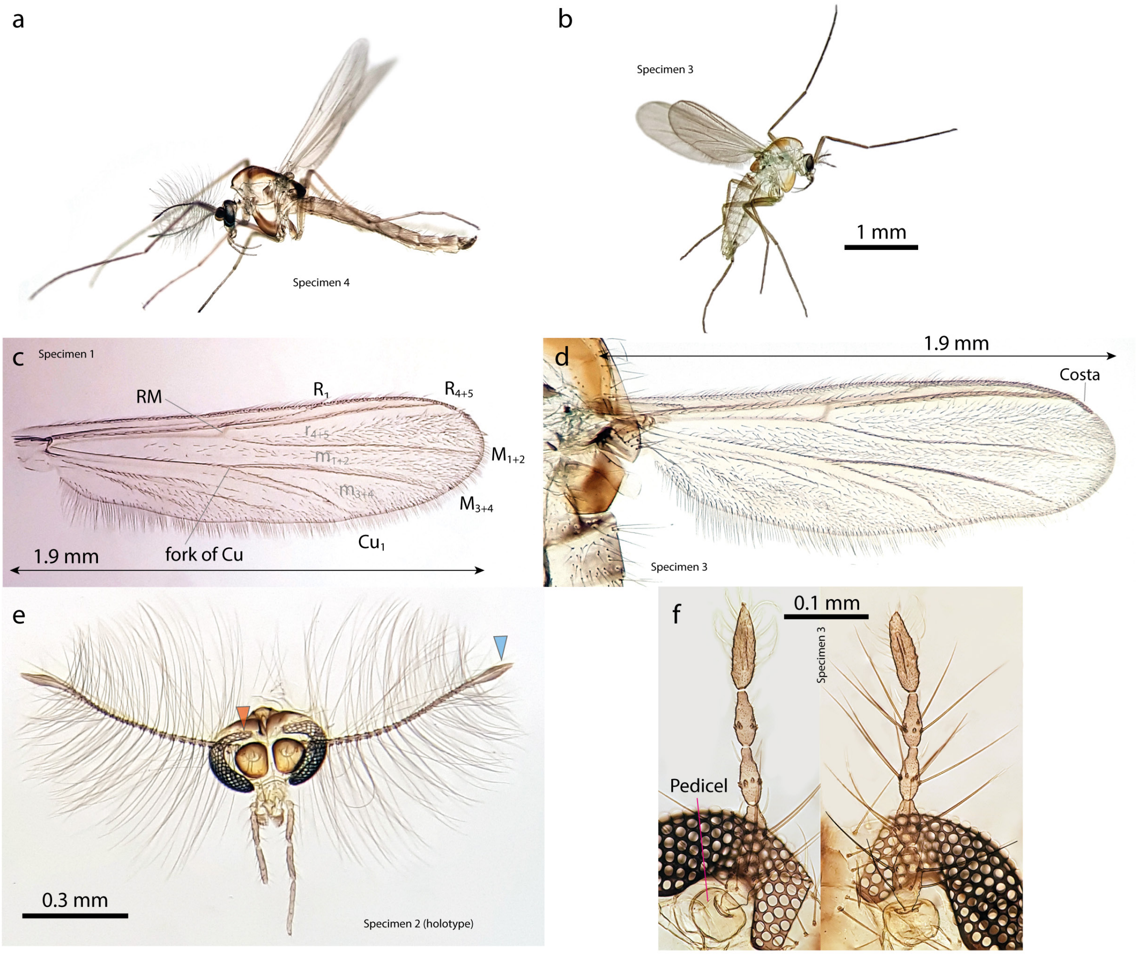

Figure 3. *Parametriocnemus lausannensis*. a, male habitus; b, female habitus; c, male wing; d, female wing; e, male head with antennae; f, female head with antennae. Orange arrowhead points to dorsal extension of the male eye. Blue arrowhead points to wedge-shaped end of male antenna.

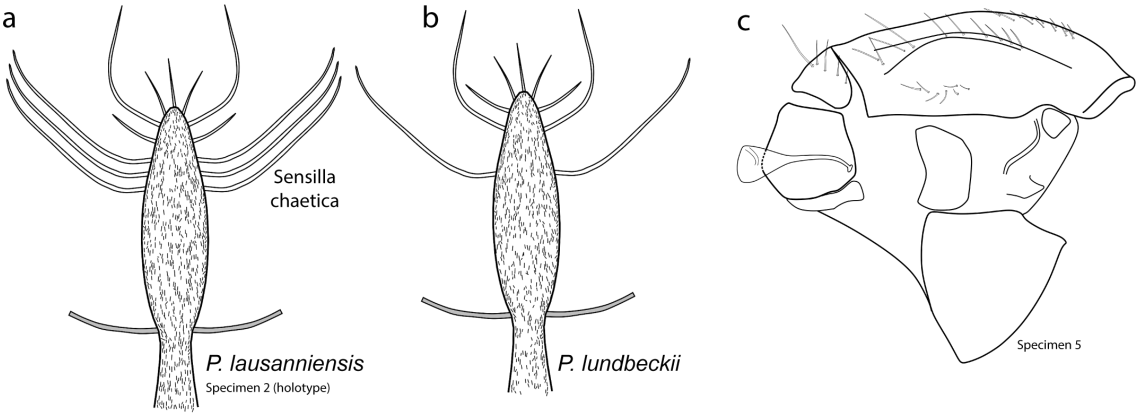

Figure 4. Male antenna apex and thorax. a, *P. lausannensis* with multiple longish sensilla chaetica; b, *P. lundbeckii* (specimen from Sweden stored in the Swedish Museum of Natural History) with fewer sensilla chaetica; c, thorax of *P. lausannensis* (male, lateral view).

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.

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