

## ***PSEUDORTHOCLADIUS IMMEZENSIS* SP. N., A NEW RELICT SPECIES INHABITING THE MACUN HIGH-ALPINE STREAM, SWISS ALPS (DIPTERA: CHIRONOMIDAE)**

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### **Abstract**

Male adult of *Pseudorthocladius immezensis* sp. n is diagnosed and described based on material collected in the high alpine cirque of the Macun area (Immez Lake basin, alt. 2616 m a.s.l.). A combination of morphological characters found in the male adult has allowed us to consider this new species as a local biogeographical representative of the eastern part of the Swiss Alps: coronal suture with lateral extension, low antennal ratio (AR 0.30); distal half of wing hairy; anal point broadly triangular with a characteristic enlarged base; inner apical margin of gonocoxite truncate; superior volsella large lobe-like; inferior volsella double, dorsal lobe nose-like and distinctly bent apically; virga present, with 4 spines; crista dorsalis absent. A differential diagnosis is given, in which some distinguishing morphological characters are summarised. Comments on the ecology and geographical distribution of the new species are also provided.

### **Introduction**

The relatively harsh environmental conditions, such as long winters, thick snow and ice cover and low temperatures prevail in alpine freshwaters. These limiting conditions contribute to the settlement of a highly specialized fauna (especially insects) (Ward 1994). Moreover, the insular nature of alpine regions constrains the distribution of species colonizing and inhabiting alpine streams and lakes (Hieber et al. 2005). In alpine headwaters, Chironomidae dominate most stream assemblages in terms of abundance (Lods-Crozet et al. 2001, Ilg and Castella 2006, Robinson et al. 2016, Alther et al. 2019). In the remote area of the high-alpine cirque of Macun (Swiss National Park), rare and climate sensitive species colonize spring-fed streams, ponds and lake shores (Lods-Crozet et al. 2012). New chironomid species have been re-

cently described from here (Moubayed-Breil and Lods-Crozet 2018).

Larval populations of the genus *Pseudorthocladius* Goetghebuer, 1943 include exclusively rheophilic species mainly encountered in lotic habitats (small waterfalls in particular) delimited by the upper and middle basins of cold mountain streams. Based on knowledge provided on the taxonomy, geographical distribution and ecology of the known *Pseudorthocladius* species from Europe and the Palaearctic Region (Goetghebuer 1938, Brundin 1947, 1956, Albu 1966, Lehmann 1971, Caspers and Siebert 1981, Sæther and Sublette 1983; Cranston et al. 1989, Ashe and O'Connor 2012, Moubayed 1990, Schnell 1991, Stur and Sæther 2004, Langton and Pinder 2007, Makarchenko and Makarchenko 2012, Sæther and Spies 2013, Ren et al. 2014, Moubayed-Breil 2020), worldwide, there are 53 valid species, 10 of which are reported from Europe and only two from Switzerland: *P. berthelemyi* Moubayed, 1990 and *P. curtistylus* (Goetghebuer, 1921).

In this paper, *P. immezensis* is described as new to science and its morphology compared to the morphologically similar *P. cristagus* Stur & Sæther, 2004; *P. curtistylus*; *P. filiformis* (Kieffer, 1921) and *P. pilosipennis* Brundin, 1956. A differential diagnosis that highlights morphological characteristics and relevant distinguishing characters is given.

### **Material and methods**

Male adults of *P. immezensis* sp. n were collected using a Malaise trap, preserved in 80-85% ethanol and cleared of musculature in 90% lactic acid (head, thorax, abdomen and anal segment) for about 60 to 80 minutes. The specimens were checked under a binocular microscope after 20 minutes to determine how the clearing was progressing. When clearing was complete, the specimens were washed in two baths of 70% ethanol to ensure that all traces of lactic acid were removed.

The studied material was mounted in polyvinyl lactophenol. Before the final slide mountings in dorsal view, the hypopygium including tergite IX and anal point, the gonocoxite and the gonostylus, were viewed ventrally and laterally, in order to examine and draw all the necessary details of the species, from both sides. For a better examination of the specific features of the hypopygium, the anal point and tergite IX were removed and the hypopygium was illustrated in a lateral view separately. Remaining part of the abdomen and the halteres are preserved in 85% ethanol for an eventual DNA analysis. Morphological terminology, abbreviations and measurements follow those of Sæther (1980) and Langton and Pinder (2007).

## Results

### *Pseudorthocladus immezensis* sp. n.

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**Material examined.** Holotype, Switzerland. 1 male adult, Malaise trap, leg. B. Lods-Crozet. Macun cirque, streamlet and rheocrenes, left shore of Immez Lake (46°43'39.678" N, 10°07'55.764" E); alt. 2616 m a.s.l., 27.VII.2013. Environmental data from inlet of Immez Lake (after Lods-Crozet et al. 2012): crystalline water, conductivity 5.9 µS/cm; temperature (min-max, 3.9-19.5 °C; mean, 11.6 °C), pH 6.7.

Paratype. 1 male adult, leg. B. Lods-Crozet, same date and locality as for holotype.

Holotype (mounted on 1 slide; GBIFCH 00597051) is deposited in the collections of the 'Musée cantonal de Zoologie, Palais de Rumine, 6 place de la Riponne, CH-1014 Lausanne (MZL), Switzerland. Paratypes, Switzerland. 1 male adult, mounted on 1 slide, is deposited in the collection of the senior author.

**Etymology.** The name '*immezensis*' refers to the Immez Lake basin where the type-material was collected.

### Diagnostic characters

*P. immezensis* sp. n. can easily be distinguished from other related species by the following combination of characters. Head. Base and median part of coronal suture with atypical lateral and median extensions; temporal setae 10-11; antenna 1280 µm long, last flagellomere about 300 µm long,

apical seta absent, AR 0.30. Sensilla coeloconica absent on palpomere 3. Clypeus sub-trapezoidal, with 8 setae. Thorax. Lobes of anteprenotum in contact; humeral pit half ellipse-like. Wing. Distal half of membrane densely covered with macrotrichia (hairy cells are:  $r_{4+5}$ ,  $m_{1+2}$ ,  $m_{3+4}$ ; cells cu and an bare); squama with 5 setae. Legs. Sensilla chaetica present on tarsomeres  $ta_1$ - $ta_5$  of PI-PIII. Abdomen. Tergite IX broad basally, narrowed distally. Anal point markedly enlarged at base, with 17 setae mostly located close to the lateral margin. Virga with 4 spines. Gonocoxite truncate in its inner apical margin. Superior volsella, large lobe-like. Inferior volsella double: dorsal lobe nose-like with smooth inner margin; ventral lobe low. Gonostylus without posterior projection, caudal margin rounded; crista dorsalis absent.

## Description

### Adult male

(n = 2; Figs 1A, D, G-H, J, I, M; 2A-H)

Small to medium sized species. Total length (TL) 2.25 mm, wing length (WL) 1.35 mm; TL/WL = 1.67 (n = 1). General colouration is ranging from pale brown to dark brown; head, thorax and antenna brown; thorax brown with dark brown mesonotal stripes; legs uniformly brown; abdomen and anal segment brown.

Head (n = 2). (Fig. 1A). Eyes bare, midline of the frontal area slightly concave, frontal tubercles low; base and median part of coronal suture with outwards and inwards extensions; coronal setae present; temporal setae 10-11 including 8-9 inner and 2 outer verticals, postorbitals absent. Palp 5-segmented, length (in µm) of segments: 15, 30, 45, 48, 55; segments 1-2 fused, segment 2 bulbous; palpomere 3 (Fig. 1D) with 3 sensilla clavata, sensilla coeloconica absent. Clypeus (Fig. 1G) sub-trapezoidal, with 8 setae in 2 rows. Antenna 1280 µm long, last flagellomere about 300 µm long, apex distinctly clubbed, apical seta absent, antennal groove reaching segment 3, AR 0.30.

Thorax. Lobes of anteprenotum (Fig. 1H) thinner basally and not gaping, lateral anteprenotals 5, located apically; acrostichals 9 in 1-2 rows, starting at some distance from anteprenotum; dorsocentrals 9 in 1-2 rows; prealars 4 in 1 row; supraalars absent; humeral area (Fig. 1I) with contrasting brownish granulation, humeral pit half ellipse-like, parapsidal fork composed of micro-granulation; scutellum (Fig. 1J) broad, heart-like with 6 thin setae in 1 row.

Wing (n = 2). (Fig. 1M). Brachiolum with 1 seta; subcosta reaching the fork of radius, distribution

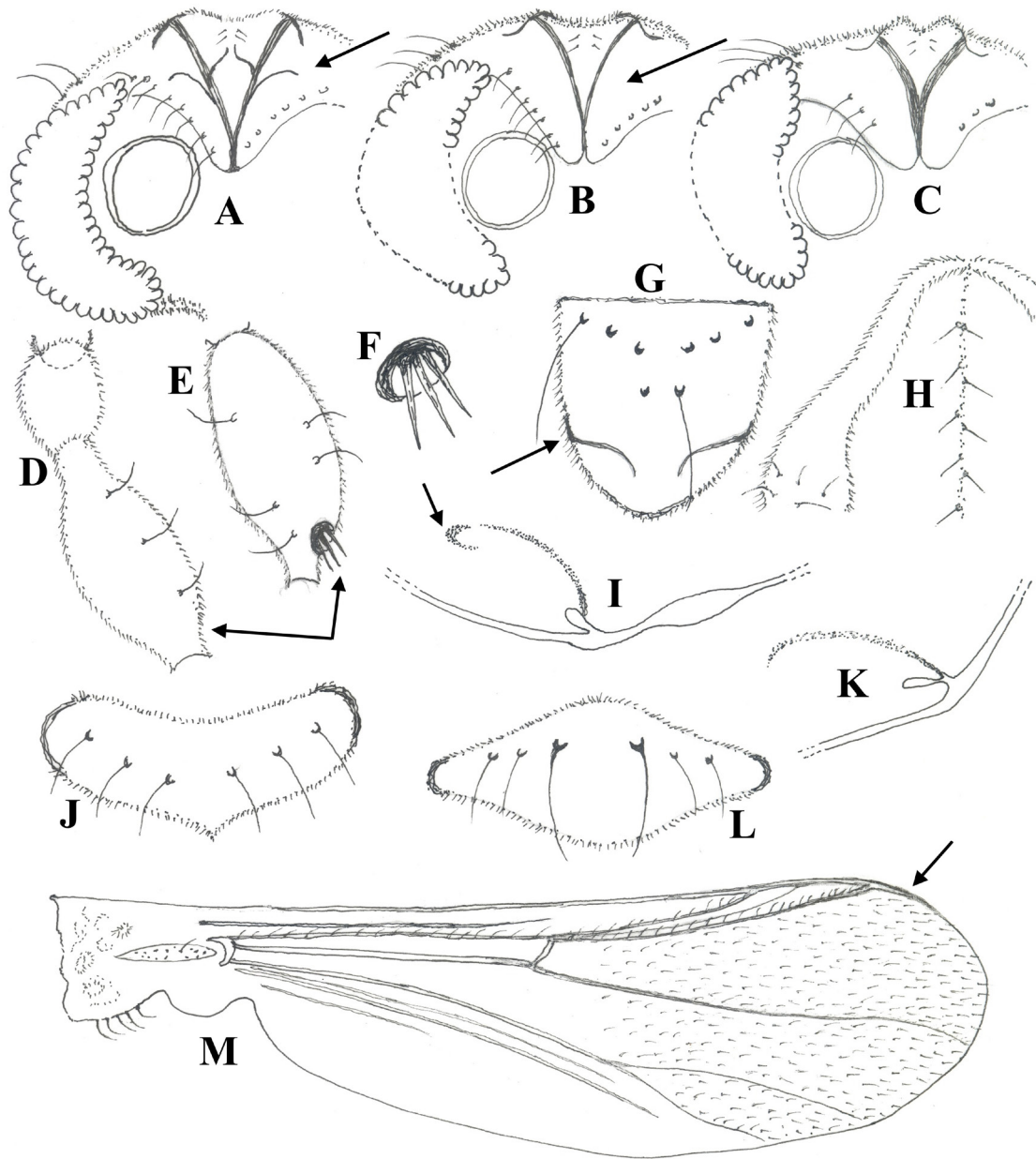


Figure 1. Male imago of *Pseudorthocladius* spp. A) Head (left side, dorsal), frontal area, vertex and temporal setae of: A) *P. immezensis* sp. n.; B) *P. curtistylus*; C) *P. sp. 1.* D) *P. immezensis* sp. n., palpomeres 2-3. E-F) *P. curtistylus*: palpomere 3 and sensilla coeloconica. *P. immezensis* sp. n.: G) clypeus; H) lobes of anteprenotum; I) humeral area; J) scutellum. *P. curtistylus*: K) humeral pit; L) scutellum. *P. immezensis* sp. n.: M) wing. The arrows indicate some distinguishing characters.

Table 1. Length ( $\mu\text{m}$ ) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs ( $n = 1$ ).

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV	BR
PI	520	525	265	170	115	65	60	0.51	3.20	3.94	2.10
PII	505	485	255	160	115	60	65	0.53	3.11	3.88	2.50
PIII	530	550	315	180	155	70	65	0.57	2.97	3.43	3.25

of setae on veins: R, 16-17; R<sub>1</sub>, 11-12; remaining veins bare; costal extension about 25-30  $\mu\text{m}$ ; distal half of membrane densely covered by macrotrichia which are clearly visible at 125-200X; hairy cells are: r<sub>4+5</sub> (150-160), m<sub>1+2</sub> (80-85), m<sub>3+4</sub> (about 40); cells cu and an bare; squama with 5 setae.

Legs ( $n = 1$ ). Femur of PI and PIII nearly subequal (520 and 530  $\mu\text{m}$  long); length (in  $\mu\text{m}$ ) of tibial spurs: PI, 40; PII, 35 and 40; PIII, 30 and 45; longest seta of tibial comb 35  $\mu\text{m}$  long. Sensilla chaetica present only on apical part of tibia of PI-PII, are more abundant on tarsomeres ta<sub>1</sub>-ta<sub>5</sub>. Length (in  $\mu\text{m}$ ) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in table 1.

Abdomen. Hypopygium ( $n = 2$ ) in dorsal and ventral view as in Figs 2A-B. Tergite IX (Figs 2A, E) about 135-140  $\mu\text{m}$  maximum width at base, occupying the entire width of segment IX, large cup-like basally, distal part subtriangular and extremely narrowing; dorsal side linear and lacking hump as shown in lateral view (Fig. 2E); setae absent on median and posterior area. Anal point (Figs 2A, E) with a characteristic enlarged base, distal part distinctly subtriangular (clearly visible in lateral view, Fig. 2E), presence of 17 setae (12 located close to the lateral margin and 5 on dorsomedian area). Laterosternite IX with 12-14 setae (6-7 on each side). Sternapodeme and phallapodeme as in Fig. 2B, transverse sternapodeme semi-circular and orally projecting, lateral expansion well developed; phallapodeme sinuous, thicker in its median part. Virga (Figs 2A, D) composed of 4 spines including 3 long and 1 short (located at base). Gonocoxite (Figs 2A-B, F) 155  $\mu\text{m}$  long, 65  $\mu\text{m}$  maximum width, apical part 25-30  $\mu\text{m}$  wide, inner apical margin truncate. Superior volsella (Figs 2C, F) well-developed, large lobe-like as illustrated in dorsal (Fig. 2A) and lateral view (Fig. 2F). Inferior volsella (Figs 2A, C, E) about 40-45  $\mu\text{m}$  long, 25  $\mu\text{m}$  maximum width, consists of double lobes: dorsal one nose-like with smooth inner margin, apex distinctly bent downwards, median area with a distinct cluster of short setae; ventral one low triangular lobe. Gonostylus (Figs 2A, G-H) 70  $\mu\text{m}$  long, 20  $\mu\text{m}$  maximum width, without posterior projection, posterior margin rounded bearing a distinct notch distally clearly visible when viewed at acute and right angle; crista dorsalis absent. HR = 2.22. HV = 3.22.

**Female adult, pupal exuvia and larva:** unknown.

### Differential diagnosis

Morphological differences between *P. immezensis* sp. n. and morphologically similar congeners (namely *P. cristagus*; *P. curtistylus*; *P. filiformis*; *P. pilosipennis*) are highlighted in the following differential diagnosis.

Adults of *P. immezensis* has a head with low frontal tubercles and atypical outward and inward extensions of the coronal suture (Fig. 1A), different from what is observed in *P. curtistylus* (Fig. 1B) and *P. sp. 1* (Fig. 1C) which belongs to an unnamed species known from the Mutt stream (upper Rhone basin, Switzerland, alt. 2100 m); sensilla coeloconica absent on palpomere 3 (Fig. 1D), present in *P. curtistylus* (Figs 1E-F); low antennal ratio (AR 0.30) compared to what is observed in *P. curtistylus* (0.84) and *P. cristagus* (about 1.40) (Sæther and Sublette 1983, Stur and Sæther 2004); humeral pit formed like a half ellipse (Fig. 1I), absent in *P. curtistylus* (Fig. 1K); scutellum large heart-like, with 6 similar thin setae (Fig. 1J), is horizontal diamond-like and bearing 2 stronger (occasionally 4) median setae in *P. curtistylus* and *P. filiformis* (Fig. 1L); distal half of wing densely haired (Fig. 1M), different from wings of *P. curtistylus* (Sæther and Sublette 1983, Figs 27C-D), *P. cristagus* (Stur and Sæther 2004, Fig. 1) and *P. pilosipennis* (Sæther and Sublette 1983, Fig. 26C); cells cu and an bare, while both are setose in *P. cristagus* and *P. pilosipennis*; basal part of anal point distinctly enlarged (Figs 2A, E), different from that of *P. curtistylus* (Fig. 2I), *P. cristagus* (Stur and Sæther 2004, Fig. 2) and *P. pilosipennis* (Sæther and Sublette 1983, Fig. 26D); virga with 4 spines (Figs 2A, D), is absent in *P. cristagus* and *P. pilosipennis* (Stur and Sæther 2004; Sæther and Sublette 1983); inferior volsella nose-like, bent downwards apically, with a distinct cluster of short setae on median area (Figs 2A, C), inferior volsella is bulbous in *P. cristagus* (Stur and Sæther 2004, Fig. 2) or large lobe-like in *P. pilosipennis* (Sæther and Sublette 1983, Fig. 26D); posterior margin of gonostylus rounded and bearing a notch distally (Figs 2A, G-H), gonostylus is linearly elongate and bearing a prominent outer heel in *P. cristagus* (Stur and Sæther 2004, Fig. 3); crista dorsalis absent (Figs 2A, G-H), is large, tooth-like in *P. cristagus* (Stur and Sæther 2004, Fig. 3).



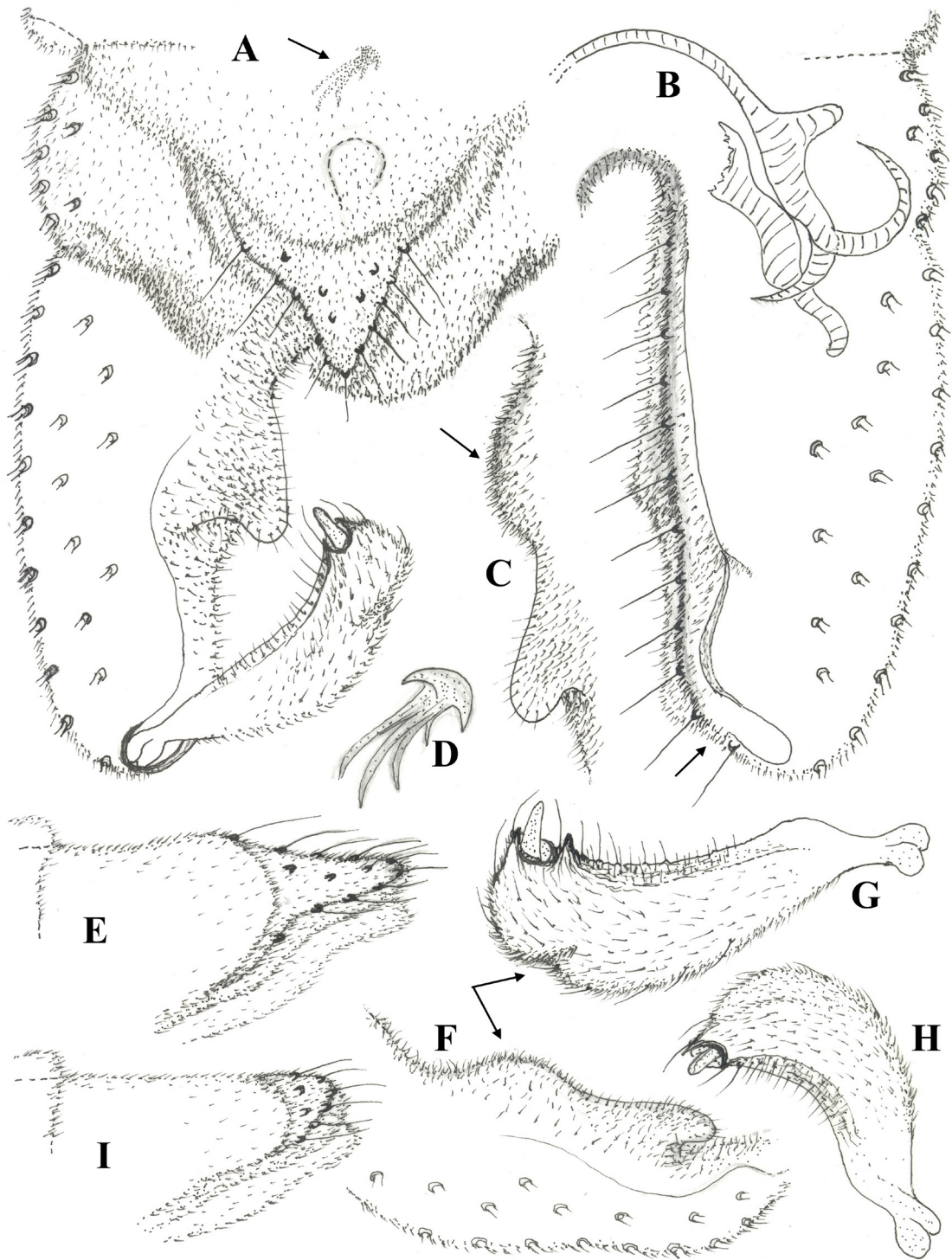


Figure 2. Male imago of *Pseudorthocladius* spp. *P. immezensis* sp. n.: A-B) hypopygium in dorsal and ventral view; C) superior and inferior volsella; D) virga; E) tergite IX and anal point, lateral; F) gonocoxite, lateral; G) gonostylus at right angle; H) gonostylus, lateral. *P. curtistylus*: I) tergite IX and anal point, lateral. The arrows indicate some distinguishing characters.

It is not feasible to provide a key to known male adult *Pseudorthocladius* from Europe until sufficient material of all species has been examined and compared.

### Ecology and geographical distribution

Only one, not associated larva of the genus *Pseudorthocladius* was collected in the inflow area of Lake Immez, where bryocolous and hygropetric habitats seem to represent the most favourable aquatic microhabitat for the larval populations. The new species likely belongs to the crenophilous community of species as documented by Lindgaard (1995) as no *Pseudorthocladius* larvae were found during extensive sampling in the lake Immez and other small lakes in the area (Lods-Crozet unpublished data; Lods-Crozet et al. 2012). Such pristine lotic habitats are considered to be microrefugia and hotspots of diversity and therefore deserve much greater consideration, protection and preservation.

Geographical distribution of the new species is currently restricted to the Alpine Swiss glacial catchments including streams and lakes delimited by the Macun cirque (Swiss National Park, eastern

Swiss Alps, alt. 2616 m, Fig. 3), but *P. immezensis* sp. n. can be expected to occur in other similar mountainous areas situated in Switzerland and neighbouring countries (Italy, France, Germany, Austria, Poland).

Chironomid species encountered in the same area, and listed by Lods-Crozet et al. (2012), Lods-Crozet (2014, unpublished data, report to the scientific commission of the Swiss National Park) and Moubayed and Lods-Crozet (2018) include: *Zavrelimyia melanura* (Meigen, 1804); *Diamesa bertrami* Edwards, 1935; *D. cinerella* Meigen, 1835; *D. nowickiana* Kownacki & Kownacka, 1975; *D. vaillantii* Serra-Tosio, 1972; *Protanypus caudatus* Edwards, 1924; *Pseudodiamesa branickii* (Nowicki 1873); *Pseudodiamesa nivosa* (Goetghebuer, 1928); *Pseudokiefferiella parva* (Edwards, 1932); *Chaetocladius castellai* Moubayed-Breil, 2018; *C. macunensis* Moubayed-Breil, 2018; *C. longivirgatus* Stur & Spies, 2011; *C. lodscozetae* Moubayed-Breil, 2018; *C. suecicus* (Kieffer 1916); *Heleniella helvetica* Moubayed-Breil and Lods-Crozet, 2016; *H. ornaticollis* (Edwards, 1929); *Corynoneura arctica* Kieffer, 1923; *E. minor*



Figure 3. Malaise trap set up close to the inlet of the Immez Lake (Macun cirque, Eastern Alps, Swiss National Park; photo J.L. Lods). The arrow indicates the larval habitat of *P. immezensis* sp. n.



(Edwards, 1929); *Orthocladius frigidus* (Zetterstedt, 1838); *Parorthocladius nudipennis* (Kieffer, 1908); *Tokunagaia rectangularis* (Goetghebuer, 1940); *Micropsectra radialis* Goetghebuer, 1939; *Paratanytarsus austriacus* (Kieffer, 1924).

The presence of *P. immezensis* sp. n. in high mountain Alpine ranges in the Swiss Alps (above 2600 m a.s.l.) highlights and confirms that some cold and glacial high mountain enclaves can contain diversity previously unknown to science. Documentation and knowledge of this fauna is important to monitor diversity and community changes caused by global warming and climate change.

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