

***Pseudorthocladius queretanus* sp. n., a crenophilous  
species inhabiting a cold stream  
in Eastern Pyrenees, France  
[Diptera, Chironomidae, Orthoclaadiinae]**

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Keywords: *Pseudorthocladius queretanus* sp. n., Diptera Chironomidae, E-Pyrenees, conservation.

Male adult of *Pseudorthocladius queretanus* sp. n is described based on associated material (pharate adults and pupal exuviae) collected in the upper basin of the Queret stream (National Nature Reserve of Mantet, Eastern Pyrenees, altitude 1500-1700 m). A combination of some distinctive characters (frontal tubercles absent, unusual shape of inferior volsella) allowed us to consider this new species as a 'relict Pyrenean element' belonging to a pertinent biological indicator of cold mountain streams. Its loss would be biogeographically indicative of the global warming and local climate change. Currently, there are 9 reported *Pseudorthocladius* species from Europe, of which 6 (7 including *P. queretanus* sp. n.) are known from France: *P. berthelemyi* Moubayed, 1990; *P. cristagus* Stur & Sæther, 2004; *P. curtistylus* (Goetghebuer, 1921); *P. filiformis* Kieffer, 1928; *P. ledoarei* Moubayed-Breil, 2020 and *P. pilosipennis* Brundin, 1956. At present, the new species is only reported from its type-locality. A taxonomic position based on distinguishing characters and morphological affinities is highlighted. A modified key to known male adults from France and comments on the ecology and geographical distribution of the new species are also provided.

***Pseudorthocladius queretanus* sp. n., une espèce crénophile connue d'un ruisseau froid des Pyrénées-Orientales, France [Diptera, Chironomidae, Orthoclaadiinae]**

Mots-Clés: *Pseudorthocladius queretanus* sp. n., Diptera Chironomidae, Pyrénées-Orientales, conservation.

L'adulte mâle de *Pseudorthocladius queretanus* sp. n. est décrit à partir d'un matériel associé (nymphe et exuvies nymphales) collecté dans le bassin supérieur du Queret, dans la Réserve Naturelle Nationale de Mantet (Pyrénées-Orientales, alt. 1500-1700 m). Une combinaison des caractères distinctifs de l'adulte mâle (absence de tubercules frontaux, forme atypique de la volselle inférieure) permet de considérer cette nouvelle espèce comme un élément pyrénéen relique, pertinent indicateur biologique de ruisseaux froids de montagne. Sa disparition serait une véritable indication biogéographique du réchauffement global et du changement climatique local. Actuellement, le genre *Pseudorthocladius* Goetghebuer, 1943 est représenté en Europe par 9 espèces dont 6 (7 en incluant *P. queretanus* sp. n.) sont recensées de France: *P. berthelemyi* Moubayed, 1990; *P. cristagus* Stur & Sæther, 2004; *P. curtistylus* (Goetghebuer, 1921); *P. filiformis* Kieffer, 1928; *P. ledoarei* Moubayed-Breil, 2020 et *P. pilosipennis* Brundin, 1956. *P. queretanus* sp. n. n'est connu, jusqu'à présent, que de sa localité-type. Sa position taxonomique, basée sur des caractères distinctifs et des affinités morphologiques, est mise en évidence. Une clé de détermination modifiée pour les adultes

mâles connus de France, accompagnée de commentaires sur l'écologie et la distribution géographique de la nouvelle espèce est également fournie.

## 1. Introduction

Larval populations of the genus *Pseudorthocladius* Goetghebuer, 1943 include exclusively rheophilic species mainly encountered in lotic habitats (riffles and waterfalls rich in bryophytes 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 neighbouring area (GOETGHEBUER 1938, BRUNDIN 1947, 1956, SÆTHER 1969, LEHMANN 1971, CASPERS & SIEBERT 1980, SÆTHER & SUBLETTE 1983, CRANSTON et al. 1989, MOUBAYED 1990, SCHNELL 1991, LAVILLE & SERRA-TOSIO 1996, STUR & SÆTHER 2004, LANGTON & PINDER 2007, ASHE & O'CONNOR 2012, MAKARCHENKO & MAKARCHENKO 2012, SÆTHER & SPIES 2013, REN et al. 2014, MOUBAYED-BREIL & ASHE 2016, MOUBAYED & TISSOT 2019, ROSSARO et al. 2019, MOUBAYED-BREIL 2020a, 2020b; MOUBAYED-BREIL & LODS-CROZET 2021), there are worldwide 54 valid species of which 9 are reported from Europe and only 6 (7 including *P. queretanus* sp. n.) from France: *P. berthelemyi* Moubayed, 1990; *P. cristagus* Stur & Sæther, 2004; *P. curtistylus* (Goetghebuer, 1921); *P. filiformis* Kieffer, 1928; *P. ledoarei* Moubayed-Breil, 2020 and *P. pilosipennis* Brundin, 1956.

In this paper, only the male adult of *P. queretanus* sp. n. is described based on associated material (pharate adults and pupal exuviae) collected in the Queret cold stream (Eastern Pyrenees, alt. 1500-1700 m).

## 2. Material and methods

Male adults of *P. queretanus* sp. n. were collected using sweep and drift nets, then were preserved in 80-85% ethanol. Additional data on the methodology of mounting and conservation of the type and paratype material are provided as documented by MOUBAYED & TISSOT 2019 and MOUBAYED-BREIL & LODS-CROZET 2021. Morphological terminology and measurements follow those of SÆTHER (1980) and LANGTON & PINDER (2007) for the imagines.

## 3. Description

### *Pseudorthocladius queretanus* Moubayed, sp. n.

#### Material examined:

Holotype. Continental France. One male adult, leg J. Moubayed, Queret stream, helocrene, riffles and waterfalls rich in bryophytes, National Nature Reserve of Mantet, E-Pyrenees (42° 28' 38" N, 02° 18' 26" E); altitude 1500-1700 m, 05.VII.2008. Environmental data of water: water crystalline, conductivity about 20-30  $\mu\text{S}/\text{cm}$ , pH 5.3-5.7; temperature about 3-5 °C during winter and spring, 8-10 °C during summer.

Holotype (mounted on one slide) is deposited in the collections of the 'Musée cantonal de Zoologie, Palais de Rumine, 6 place de la Riponne, CH-1014 Lausanne (MZL), Switzerland'.

Paratype: one male pharate adult, same locality and date as for holotype, preserved in ethanol 85%, is deposited in the collection of the senior author.

**Etymology:** the name '*queretanus*' refers to the Pyrenean stream of Queret (National Nature Reserve of Mantet, Eastern-Pyrenees, France), where the type-material was collected.

### Diagnostic characters

The male adult of *P. queretanus* sp. n. is easily distinguished by the unusual shape of its inferior volsella, which is composed of 2 fused dorsal lobes. However, the following combination of characters will separate the new species from other members of the genus. Frontal area without tubercles; basal margin vertex rounded, coronal setae present; temporal setae 9 (6 inner and 3 outer verticals). Antenna 915  $\mu\text{m}$  long; AR 1.51. Palpomere 3 with 5 sensilla clavata and one apical sensilla coeloconica. Clypeus semicircular, with 18 setae. Lobes of anteprenotum widely gaping; humeral pit absent. Anal lobe of wing well-developed, membrane not hairy, costal extension 35  $\mu\text{m}$  long, squama with 25-26 setae. Sensilla chaetica present on tibia and tarsomeres  $\text{ta}_1$ - $\text{ta}_5$  of PI, only on  $\text{ta}_1$ - $\text{ta}_5$  of PII-PIII. Tergite IX wider at base, narrowed medially, distal part half-ellipse-like. Anal point widely semi-circular, with 16-18 setae in 2 arched rows. Virga with 3 curved spines. Gonocoxite rounded apically; inferior volsella typically double, anterior lobe subrectangular with bare inner margin, posterior lobe semicircular, covered with setae. Gonostylus slender, without posterior projection; crista dorsalis well-developed, occupying distal half of gonostylus.

### Male imago

(n = 2; Figs 1A-H, 2A-H)

Large to middle sized species. Total length (TL) 2.45 mm, wing length (WL) 2.10 mm; TL/WL = 2.14. General colouration contrasting brown to dark brown; head and antenna dark brown; thorax brownish with dark brown mesonotal stripes; legs brownish; abdomen brownish; anal segment brownish and slightly contrasting.

Head. Eyes bare, lateral inner margin not hairy; frontal area (Fig. 1A) without tubercles, vertex rounded basally, not orally projecting, coronal setae present; temporal setae 9, including 6 inner and 3 outer verticals, postorbitals absent. Palp 5-segmented, length (in  $\mu\text{m}$ ) of segments 1-4 (segment 5 missing): 40, 45, 160, 165; segments 1 and 2 fused; palpomere 3 (Fig. 1B) with 5 sensilla clavata, and 1 spatulate apical sensilla coeloconica (Fig. 1C). Antenna 915  $\mu\text{m}$  long, last flagellomere 550  $\mu\text{m}$  long; apex distinctly-clubbed bearing a brush of sensilla chaetica, apical seta absent; antennal groove reaching segment 2; AR 1.51. Clypeus (Fig. 1D), semi-circular, with 18 setae, located in 3 rows. Thorax. Lobes of anteprenotum (Fig. 1E) widely-gaping, thinner basally, lateral anteprenotals 5; acrostichals 7 in 1-2 rows; dorsocentrals 10, uniserial; prealars 4 in one row; supraalars absent; humeral area (Fig. 1F) not contrasting, humeral pit absent, parapsidal fork weakly-developed, notopleural suture swollen on one side; scutellum with 12 setae in one row (6 on each side of the midline). Wing. Brachiolium with one seta; distribution of setae on veins: R, 10-12;  $\text{R}_1$ , 2-3; remaining veins bare; extended part of costa 35  $\mu\text{m}$  long; squama with 25-26 setae. Legs. Length (in  $\mu\text{m}$ ) of tibial spurs: PI, 60; PII and PIII, 45. Pseudospurs present on PI-PIII: PI (30, 25  $\mu\text{m}$  long on  $\text{ta}_1$ ), PII-PIII (25, 30 on  $\text{ta}_2$ - $\text{ta}_3$ ). Longest seta of tibial comb about 100  $\mu\text{m}$  long. Sensilla chaetica present on tibia and tarsomeres  $\text{ta}_1$ - $\text{ta}_5$  of PI, only on  $\text{ta}_1$ - $\text{ta}_5$  of PII and PIII. Length (in  $\mu\text{m}$ ) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in the following table (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	845	805	625	370	275	190	135	0.78	2.72	2.64	1.80
PII	755	685	385	225	130	105	105	0.39	3.23	3.74	1.40
PIII	760	855	440	290	210	125	115	0.52	2.78	3.67	1.60

"LR = length of tarsomere ta<sub>1</sub> divided by length of tibia (ti); BV = combined length of femur (fe), tibia and ta<sub>1</sub> divided by combined length of tarsomeres ta<sub>2</sub>-ta<sub>5</sub>; SV = ratio of femur plus tibia to tarsomere ta<sub>1</sub>; BR = ratio of longest seta of ta<sub>1</sub> divided by minimum width of ta<sub>1</sub>, measured one third from apex."

Abdomen. Hypopygium in dorsal, ventral and lateral view as in figures 2A-B, H. Tergite IX about 150  $\mu$ m long, maximum width 170  $\mu$ m at base, 80  $\mu$ m wide posteriorly; much wider at base, abruptly narrowed medially, caudal part half ellipse-like. Anal point (Fig. 2A) widely semi-circular, with 16-18 setae located close to the posterior margin along 2 arched rows. Laterosternite IX with 14 setae (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 basal part. Virga (Figs 2A, C) composed of 3 curved spines including 1 shorter located medially. Gonocoxite (Figs 2A-B, H) 175  $\mu$ m long, 70  $\mu$ m maximum width; apical part rounded; inner margin linearly elongate, bearing 11 long stout setae (Fig. 2B).

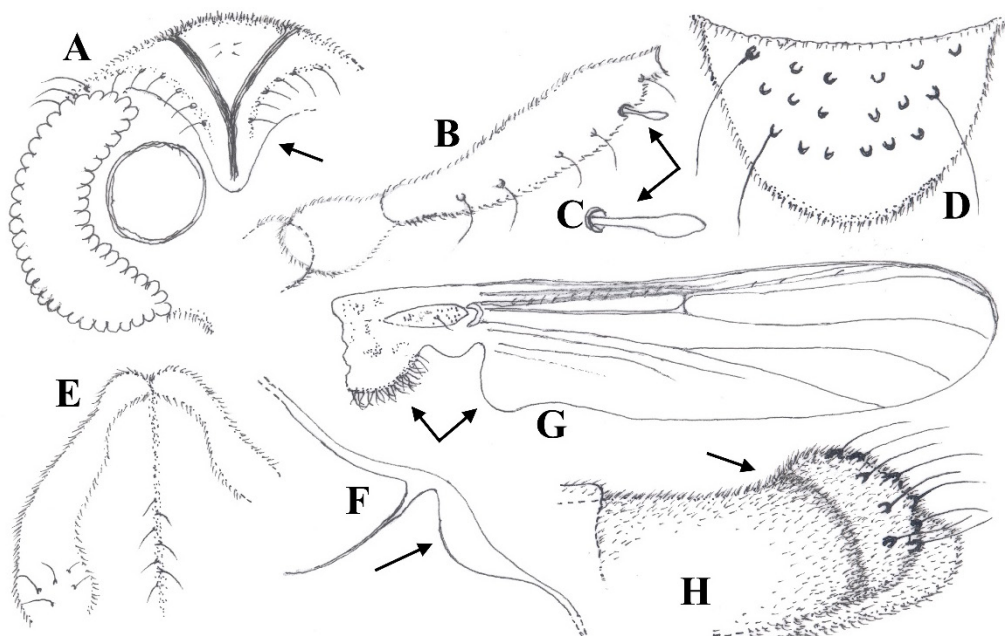


Figure 1. Male imago of *Pseudorthocladius queretanus* sp. n. Head (left side, dorsal), frontal area, vertex and temporal setae (A); palpomeres 1-3 (B); sensilla coeloconica (C); clypeus (D); lobes of antepronotum with acrostichals (E); humeral area (F); wing (G); tergite IX and anal point, lateral (H). The arrows indicate some distinguishing characters.

Figure 1. Imago mâle de *Pseudorthocladius queretanus* sp. n. Tête (côté gauche, vue dorsale), aire frontale, vertex, et soies temporales (A); palpomères 1-3 (B); sensilla coeloconica (C); clypéus (D); lobes de l'antepronotum et soies acrosticales (E); aire humérale (F); aile (G); tergite IX et pointe anale en vue latérale (H). Les flèches indiquent quelques caractères discriminants.

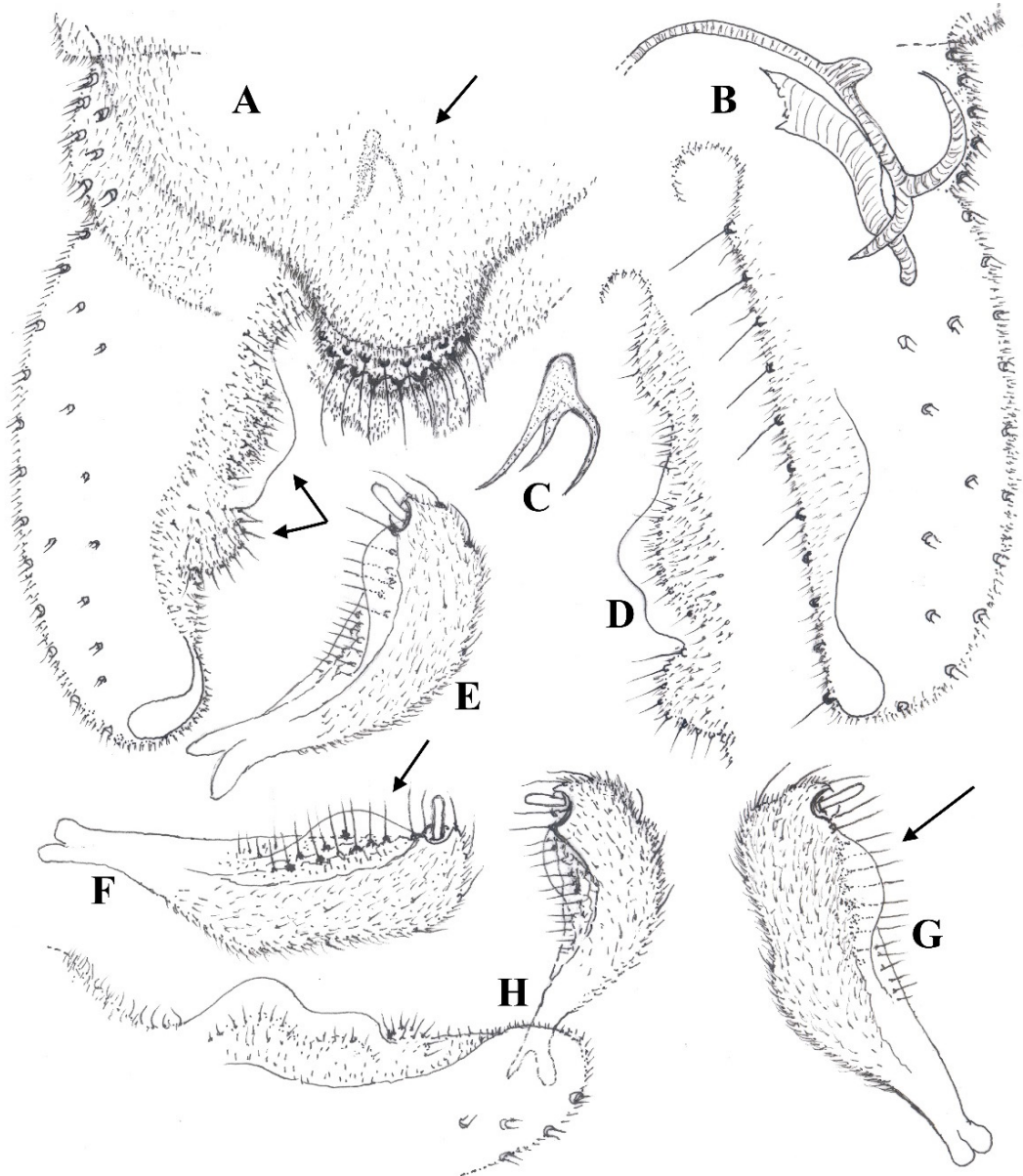


Figure 2. Male imago of *Pseudorthocladius queretanus* sp. n. Hypopygium in dorsal (A) and ventral view (B); virga (C); right inferior volsella, dorsal (D); gonostylus at acute angle, dorsal (E); gonostylus at right angle, ventral (F); gonostylus, dorsolateral (G); gonocoxite and gonostylus, lateral (H). The arrows indicate some distinguishing characters.

Figure 2. Imago mâle de *Pseudorthocladius queretanus* sp. n. Hypopyge en vues dorsale (A) et ventrale (B) ; virga (C) ; volselle inférieure droite, vue dorsale (D) ; gonostyle, angle aigu, vue dorsale (E) ; gonostyle, angle droit, vue ventrale (F) ; gonostyle, vue latérale (G) ; gonocoxite et gonostyle, vue dorso-latérale (G). Les flèches indiquent quelques caractères discriminants.

Superior volsella low lobe-like. Inferior volsella 90  $\mu\text{m}$  long, consists of 2 fused dorsal lobes as shown in Figs 2A, D (dorsal) and 2H (lateral); anterior lobe 50  $\mu\text{m}$  long and 35  $\mu\text{m}$  maximum width, subrectangular, inner margin slightly sinuous and entirely bare, inner side covered 1-2 rows of short setae; posterior lobe 40  $\mu\text{m}$  long and 20  $\mu\text{m}$  maximum width, semi-circular and densely covered with setae. Gonostylus as in Figs 2E-H (2E, acute angle; 2F, right angle, ventral; 2G, dorsolateral; 2H, lateral); 125  $\mu\text{m}$  long, 35  $\mu\text{m}$  maximum width, without posterior projection; slender when viewed at right angle as in Figs 2F-G; posterior margin rounded when viewed at acute angle and laterally (Figs 2E, H); anterior side with 1-3 rows of orally directed setae, posterior side with 3 setae including 2 located distally; crista dorsalis well-developed, consists of a large lobe occupying distal half of gonostylus, clearly visible, in particular, when viewed at acute angle (Fig. 2E) and laterally (Fig. 2H). HR = 1.40. HV = 1.96.

**Female adult and pupal exuvia:** known but undescribed.

**Larva:** unknown.

#### 4. Differential diagnosis

The unusual shape of the inferior volsella (composed of 2 dorsal lobes) will separate *P. queretanus* sp. n. from the known European members of the genus *Pseudorthocladius*. Such atypical relevant character is hitherto similarly observed in some known species from the Nearctic Region (SÆTHER & SUBLETE 1983), namely: *P. lunatus* Sæther & Sublete, 1983; *P. tricantus* Sæther & Sublete, 1983; *P. uniserratus* Sæther & Sublete, 1983; *P. morsei* Sæther & Sublete, 1983. Among the latter cited species, only *P. uniserratus* (form B) has been recently reported from Italy (ROSSARO et al. 2019). Distinguishing morphological characters between *P. queretanus* sp. n. and other known congeners from Europe and the Nearctic Region are highlighted in the following differential diagnosis.

- Tubercles on frontal area absent (Fig. 1A), are present in *P. immezensis* Moubayed-Breil & Lods-Crozet, 2021 and *P. curtistylus* (MOUBAYED-BREIL & LODS-CROZET 2021, Figs 1A-B) as well in *P. ledoarei* (MOUBAYED-BREIL 2020b, Fig. 1);

- Antennal ratio AR 1.52, is much lower in *P. berthelemyi* (0.60-0.70, MOUBAYED 1990), *P. curtistylus* (0.45-0.84, sensu ALBU 1966 and SÆTHER & SUBLETTE 1983), *P. ledoarei* (0.76, MOUBAYED-BREIL 2020b);

- Palpomere 3 with 1 single sensilla coeloconica (Figs 1B-C), is bearing 3 in both *P. immezensis* (MOUBAYED-BREIL & LODS-CROZET 2021, Figs 1 E-F) and *P. ledoarei* (MOUBAYED-BREIL 2020b, Figs 3-4);

- Membrane of wing without setae (Fig. 1G), is hairy in *P. immezensis* (MOUBAYED-BREIL & LODS-CROZET 2021) and *P. pilosiomanus* (STUR & SÆTHER 2004, Fig. 1);

- Tergite IX lacking spines distally (Fig. 1A), is bearing 2 characteristic small spines in *P. ledoarei* (MOUBAYED-BREIL 2020b, Figs 10, 12);

- Anal point semicircular with 16-18 setae located close to the posterior margin, is differently figured in *P. berthelemyi* (MOUBAYED 1990, Fig. 4; MOUBAYED-BREIL 2020b, Fig. 25), *P. curtistylus* (ALBU 1966, Fig. 5; SÆTHER & SUBLETTE 1983, Figs 37E-F) and *P. rectangilobus* (CASPER & SIEBERT 1980, Fig. 2);

- Virga with 3 curved spines (Figs 1A, C), is differently figured in the remaining known *Pseudorthocladius* species;
- Inferior volsella atypically double (Figs 2A, D), is similarly illustrated but differently shaped in some reported Nearctic species by SÆTHER & SUBLETTE (1983) namely: *P. lunatus*, *P. morsei*, *P. tricanthus* and *P. uniserratus*;
- Crista dorsalis well-developed, occupying distal half of gonostylus (Figs 2EH), is tooth shaped in *P. curtistylus* (sensu ALBU 1966, Fig. 5) or widely extended in both *P. berthelemyi* (Fig. 23) and *P. filiformis* (Fig. 30).

## 5. Key to known male adults of *Pseudorthocladius* species from France

The total of known valid *Pseudorthocladius* species from France (*Fauna Europaea*, version of SÆTHER & SPIES 2013; MOUBAYED-BREIL 2020b) currently includes 6 species: *P. berthelemyi*; *P. cristagus* (recently reported from NE-France); *P. curtistylus*; *P. filiformis*, *P. ledoarei* and *P. pilosipennis*. A previous key to known male adults from Europe was recently performed by MOUBAYED-BREIL (2020b), in which *P. albiventris* (Goetghebuer, 1938) was considered as a junior synonym of *P. curtistylus*. Therefore, a modified key to male adults of known species from France is highlighted and proposed as follows:

1. Membrane of wing with setae ..... 2
  - Membrane of wing without setae ..... 3
2. Gonostylus projecting posteriorly into an outer heel-like; inferior volsella broadly triangular; crista dorsalis triangular and well-developed ..... *P. cristagus*
  - Gonostylus not projecting posteriorly, outer heel absent; inferior volsella large lobe-like; crista dorsalis absent ..... *P. pilosimanus*
3. Tergite IX with 2 small characteristic spines; inner margin of inferior volsella with only 1 single seta; AR 0.76; squama with 2 setae; virga horseshoe-like ..... *P. ledoarei*
  - Tergite IX without spines; inner margin of inferior volsella with or without setae; virga present or absent ..... 4
4. Inner margin of inferior volsella with 2 setae; AR 0.65; squama with 7-8 setae; virga absent; gonostylus protruding posteriorly ..... *P. berthelemyi*
  - Inner margin of inferior volsella with numerous short setae or bare ..... 5
5. Anal point broadly semicircular, triangular or sub-triangular ..... 6
  - Anal point broadly sub-triangular; crista dorsalis well-developed, widely extended (SÆTHER & SUBLETTE 1983, Fig. 33 D); AR 0.85-1.0; squama with 5-6 setae ..... *P. filiformis*
6. Inferior volsella single; anal point triangular; virga consisting of minute spinules or absent (SÆTHER & SUBLETTE 1983, Fig. 37E-F); crista dorsalis weakly-developed, occasionally present on distal half (MOUBAYED-BREIL 2020b, Fig. 37); AR 0.45-0.63; squama with 7-8 setae ..... *P. curtistylus* (= *P. albiventris*)
  - Inferior volsella double (Figs 2A, D); anal point semicircular (Fig. 2A); virga composed of 3 curved spines (Figs 2A, C); crista dorsalis well-developed, occupying distal half of gonostylus; AR 1.51; squama with 25-26 setae ..... *P. queretanus* sp. n.



Photo 1. Type-locality where the type-material of *Pseudorthocladius queretanus* sp. n. was collected. (photo C. Galy-Fajou).

Photo 1. Localité-type, où le matériel-type *Pseudorthocladius queretanus* sp. n. a été collecté. (cliché C. Galy-Fajou).

## 6. Ecology and geographical distribution

*P. queretanus* sp. n. occurs in a cold stenothermic waterfall and riffle covered with dense bryophytes where hygropetric microhabitats with fast running water represent the most favourite habitat for larval populations. The new species belongs to the crenophilous community of species as documented by LINDEGAARD (1995). Such pristine habitats are considered to be microrefugia and hotspot of diversity and therefore deserve greater consideration, protection and preservation.

At present, our knowledge on the geographical distribution of *P. queretanus* is restricted to its type-locality, which is delimited by the upper basin of the Queret high mountain stream (Mantet Natural Reserve, Eastern-Pyrenees). On the basis of some unusual morphological characters, the new species appears to belong to a local Pyrenean element, which is considered as a pertinent biological indicator of cold mountain streams. Its loss would be biogeographically indicative of the global warming and local climate change. Moreover, *P. queretanus* sp. n. can be expected to occur in some neighbouring areas of French and Spanish Pyrenees.

Associated species encountered with *P. queretanus* sp. n. include: *Diamesa veletensis* Serratosio, 1971; *D. aberrata* Lundbeck, 1898; *D. bertrami* Edwards, 1935; *D. bohemani* Goetghebuer, 1932; *Syndiamesa edwardsi* (Pagast, 1947); *Bryophaenocladius aestivus* (Brundin, 1947);



*B. subvernalis* (Edwards, 1929); *B. xanthogyne*; *Chaetocladius callauensis* Moubayed & Langton, 2019; *C. guisseti* Moubayed, 2017; *C. laminatus* Brundin, 1947; *C. melaleucus* (Meigen, 1818); *C. parerai* Moubayed & Langton, 2019; *C. suecicus* (Kieffer, 1916); *Eukiefferiella fittkaui* Lehman, 1972; *E. minor* (Edwards, 1929); *Orthocladius excavatus* Brundin, 1947; *Psilometriocnemus* sp. 1; *Rheocricotopus thomasi* Moubayed-Breil, 2015; *Trissocladius orsinii* Moubayed-Breil & Ashe, 2015; *Rheotanytarsus dactylophoreus* Moubayed-Breil, Ashe & Langton, 2012.

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