

***Pseudorthocladius ledoarei* sp. n., a crenophilous species occurring in a cold karstic stream from eastern France [Diptera, Chironomidae]**

by Joel MOUBAYED-BREIL

Freshwater & Marine biology, 10 rue des Fenouils, F- 34070 Montpellier, France
joelmb34@free.fr

Keywords: *Pseudorthocladius ledoarei* sp. n., Diptera Chironomidae, cold karstic stream, E-France, conservation.

Male adult of *Pseudorthocladius ledoarei* sp. n. is diagnosed and described based on material collected by a sweep net (J. Le Doaré leg) in the upper basin of La Goutte karstic stream, located in a Beech grove down to the Oudar spring (Etau Forest, Gex, eastern France, alt. 880-900 m). A combination of some distinguishing characters found in the male adult (low value of AR 0.76; tergite IX cup-shaped, median area with 4 setae and 2 characteristic small spines located laterally on one side; anal point sub-triangular with 11 long setae; virga horseshoe-shaped; inferior volsella semi-circular, inner margin with one single seta on midline area; crista dorsalis well-developed and occupying the distal half of gonostylus) allowed us to consider *P. ledoarei* sp. n. as a local biogeographical representative of the eastern mountainous area of France. In this paper a *curtistylus*-group is emended and diagnosed, which includes: *P. filiformis* (Kieffer, 1928), *P. curtistylus* (Goetghebuer, 1921), *P. curtistylus* (sensu ALBU 1966, known from Romania), *P. rectangilobus* Caspers & Siebert, 1980, *P. ledoarei* sp. n., 2 undescribed species (*P. sp. 2* and *P. sp. 4*) and other known *Pseudorthocladius* species from the Palaearctic and Oriental Regions. Currently, the genus *Pseudorthocladius* Goetghebuer, 1943 is represented in Europe by 8 species, of which only 5 were recorded from continental France: *P. berthelemyi* Moubayed, 1990; *P. cranstoni* Sæther & Sublette, 1983; *P. curtistylus* (Goetghebuer, 1921); *P. filiformis* (Kieffer, 1928) and *P. cristagus* Stur & Sæther, 2004 (recently reported from France by MOUBAYED et al. 2019). The new species is only known, from its type-locality, which is delimited by the rhithral of La Goutte stream. Taxonomic remarks and comments on the ecology of the new species are provided.

***Pseudorthocladius ledoarei* sp. n., une espèce crénophile connue d'un ruisseau karstique froid de l'est de la France [Diptera, Chironomidae]**

Mots-Clés: *Pseudorthocladius ledoarei* sp. n., Diptera Chironomidae, ruisseau karstique froid, France-est, conservation.

L'adulte mâle de *Pseudorthocladius ledoarei* sp. n. est diagnostiqué et décrit à partir d'un matériel collecté au filet entomologique (leg. J. Le Doaré) dans le bassin supérieur du ruisseau karstique La Goutte, situé dans une hêtraie en aval de la source de l'Oudar (Forêt de l'Etau, Gex, est-France, alt. 880-900 m). Une combinaison des caractères distinctifs de l'adulte mâle (faible valeur de l'AR 0.76 ; tergite IX en forme de coupe, aire médiane avec 4 soies et 2 petites épines caractéristiques localisées latéralement sur un même côté ; pointe anale sub-triangulaire avec 11 longues soies ; virga en forme de fer-à-cheval ; volselle inférieure semi-circulaire, paroi interne munie d'une seule soie médiane; crista dorsalis bien développé, occupe la moitié distale du gonostyle) nous a permis de considérer *P. ledoarei* sp. n. comme un élément biogéographique local, représentant des régions montagneuses de l'est de la France. Dans le présent travail, un groupe *curtistylus* est proposé et diagnostiqué. Il comprend : *P. curtistylus* (Goetghebuer, 1921), *P. curtistylus* (sensu ALBU 1966, connu de Roumanie), *P. filiformis* (Kieffer, 1928), *P. rectangilobus* Caspers & Siebert, 1980, *P. ledoarei* sp.

n., 2 espèces encore non décrites (*P. sp. 2* and *P. sp. 4*, connues de France), ainsi que d'autres espèces connues des régions Paléarctique et Orientale. Le genre *Pseudorthocladius* Goetghebuer, 1943 est représenté en Europe par 8 espèces dont seulement 5 sont recensées de France continentale : *P. berthelemyi* Moubayed, 1990; *P. cranstoni* Sæther & Sublette, 1983; *P. curtistylus* (Goetghebuer, 1921); *P. filiformis* (Kieffer, 1928) et *P. cristagus* Stur & Sæther, 2004 (réemment cité de France par MOUBAYED et al. 2019). Jusqu'à présent, *P. ledoarei* sp. n. n'est connu que de sa localité-type délimitée par le rhithral du ruisseau de La Goutte. Des données taxonomiques et des commentaires sur l'écologie de la nouvelle espèce sont présentés.

1. Introduction

The genus *Pseudorthocladius* Goetghebuer, 1943 includes exclusively rheophilic species mainly encountered in lotic habitats (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 Palaeartic Region (GOETGHEBUER 1938, BRUNDIN 1947, 1956, SÆTHER 1969, LEHMANN 1971, CASPERS & SIEBERT 1980, SÆTHER & SUBLETTE 1983, CRANSTON et al. 1989, MOUBAYED 1989, 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 2016, MOUBAYED-BREIL & ASHE 2016, MOUBAYED et al. 2019), there are worldwide about 52 valid species of which 8 are reported from Europe and only 5 from France.

The male adult of *P. ledoarei* sp. n is described on the basis of material collected by a sweep net in the upper basin of La Goutte cold karstic stream located in a Beech grove down to the Oudar spring (Gex, Etau forest, altitude 880-900 m, eastern France, leg. J. Le Doaré). A combination of some characters found in the male adult, allowed us to key the new described species close to *P. curtistylus* (Goetghebuer, 1921), *P. curtistylus* (sensu ALBU 1966, known from Romania), *P. filiformis* (Kieffer, 1928) and *P. rectangilobus* Caspers & Siebert, 1980. In this paper, a *curtistylus*-group is emended and diagnosed, which includes the 3 previously cited species and some other known members of the genus from the Palaeartic and Oriental Regions. Currently, the genus *Pseudorthocladius* is represented in continental France by 5 species: *P. berthelemyi* Moubayed, 1990; *P. cranstoni* Sæther & Sublette, 1983; *P. cristagus* Stur & Sæther, 2004 (recently reported from France by MOUBAYED et al. 2019); *P. curtistylus* (Goetghebuer, 1921) and *P. filiformis*. Consequently, the current description of *P. ledoarei* sp. n. increases the total number in the genus to 6 valid species from this country.

2. Material and methods

Material composed of male adults of *P. ledoarei* sp. n was collected using a sweep net, then were 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, which can be left overnight at room temperature without any detrimental effect or damage. The specimens were checked under a binocular microscope after 20 minutes in lactic acid to determine how the clearing was progressing. When clearing was complete the specimens were washed in two changes 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 of the type and paratype material (dorsal), the hypopygium including tergite IX and anal point, the gonocoxite and the gonostylus, were viewed ventrally and laterally to examine and draw from both sides all the necessary details of the species. For a better examination of the specific features and more accurate description of the male adult, various taxonomic details in particular, the hypopygium was illustrated in lateral view separately, which facilitates proper illustration of all the relevant taxonomic characters. Part of the abdomen and the halteres of the male adult are preserved in 85% ethanol for an eventual DNA analysis. Morphological terminology and measurements follow those of SÆTHER (1980) for the imagines.

3. Taxonomic notes

The following taxonomic notes are based on examined material of male adults collected in France, Corsica, Italy, Spain, Morocco, Algeria and Lebanon. They include a combination of some additional relevant characters (coronal area, antenna, humeral pit, wing, legs, tergite IX, anal point, inferior volsella, gonostylus), which supplement previous taxonomic data from the literature about some known *Pseudorthocladius* species. However, for a better comprehensive knowledge and more reliable description of the gonostylus and crista dorsalis the latter are being much clearly observed when viewed at acute and right angle (Figs 5, 17, 27, 30, 34, 37).

3.1. Examined species

Pseudorthocladius berthelemyi Moubayed, 1990

(Figs 21-24)

2 male adults, Lys stream, central Pyrenees, alt. 99-1100 m, 23.VII.1987; 1 male adult, Valescure stream, Var department, SE-France, alt. 450 m, 23.III.1995; 1 male adult, Aissi River, Algeria, alt. 800-900 m, 15.III.1994; 2 male adults, Baillaurie karstic spring, eastern Pyrenees, S-France, alt. 150 m, 07.VI.2012.

Coronal setae present; antenna, last flagellomere 225-235 μ m, apical seta present on last flagellomere, AR 0.60-0.70; lobes of pronotum gaping; humeral pit ellipsoid, hyaline, not contrasting and lacking granulation; wing, squama with 7-8 setae; legs, sensilla chaetica present in particular on apical part of tibia and tarsomeres ta₂-ta₃ of PI-PIII; tergite IX and anal point (Fig. 21), tergite IX broadly cup-like with 11-12 setae located on median and posterior areas; anal point semi-circular, with 8-9 setae; sternapodeme and phallapodeme (Fig. 24), sternapodeme semi-circular, coxapodeme is distinctly thick, lateral sternapodeme ending with a long pointed spine; virga absent; inferior volsella large lobe-like, bearing 2 setae on inner margin; gonostylus (at right angle, Fig. 23) massively elongated, markedly projecting posteriorly in a characteristic rounded apex, crista dorsalis well-developed, conspicuous and occupying the entire anterior side of gonostylus.

Pseudorthocladius curtistylus (Goetghebuer, 1921)

(Figs 36-38)

2 male adults, Yammouneh karstic cold helocrenes, E-Lebanon, alt. 1420 m, 15-17.IV.1982; 1 male adult + 2 male pharate adults, Shlefa rhithral, Bekaa plain, eastern Lebanon, alt. 1350 m, 16.IV.1982. 2 male adults + 1 male pharate adult, Massane River, rhithral at the upper basin, alt. 750-800m, 05.VI.2012. 1 male adult + 2 male pharate adults, Tech River, E-Pyrenees, alt. 1150 m, 03.VI.2005.

Coronal setae present; antenna, last flagellomere 240-250 mm, apical seta present on last flagellomere, AR 0.70-0.85; lobes of pronotum gaping; humeral pit absent, humeral area occasionally with contrasting brownish granulation; wing, squama with 7-9 setae; legs, sensilla chaetica present on apical part of tibia and tarsomeres ta_2 - ta_3 of PI-PIII; tergite IX, broadly cup-shaped with 7-9 setae located caudally near base of anal point; anal point triangular, with 8-10 setae; virga, apparently present, consisting of minute grouped spinules; inferior volsella triangular, bent downwards and bearing 3 apical setae on inner margin; gonostylus with variable posterior margin mostly rounded, sinuous as in ALBU (1966, Fig. 5), crista dorsalis present, well-developed and distinctly protruding as in ALBU (*ibid.*, Fig. 5) and SÆTHER & SUBLETTE (1983, Fig. 37E).

***Pseudorthocladius* sp. 2** (cf. *filiformis* Kieffer, 1928): E-Pyrenees
(Figs 28-30)

1 male pharate adult, Massane River, middle basin, rhithral, alt. 300-350 m, 17.V.1986; 1 male adult, Massane River, upper basin, rhithral, alt. 600-650 m, 05.VI.2012; 1 male adult + 1 male pharate adult, Bail-laurie karstic spring, E-Pyrenees, S-France, alt. 150 m, 07.VI.2012.

Coronal setae present; antenna, last flagellomere 270-280 mm, apical seta present on last flagellomere, AR 0.85-1.0; lobes of pronotum widely-opened; humeral pit present, ellipsoid, hyaline and lacking granulation; wing, squama with 5-6 setae; legs, sensilla chaetica present in particular on apical part of tibia and tarsomeres ta_2 - ta_3 of PI-PIII; tergite IX and anal point (Fig. 28), tergite IX broadly cup-like, distal half large capital U-like, with 2-3 setae located close to base of anal point; anal point semi-circular to large lobe-like, with 5-8 setae located apically; virga, as in *P. curtistylus*, consists of minute short spinules; inferior volsella (Fig. 29) triangular to nose-like, hyaline and lacking setae on inner margin; gonostylus (Fig. 30), crista dorsalis, low to well-developed, occupying the entire anterior side of gonostylus.

***Pseudorthocladius similis* Freeman, 1953**: Africa, Lebanon
(Fig. 35)

2 male adults, Labwé karstic springs, cold helocrenes, Bekaa plain, eastern Lebanon, 11.IX.1980.

Coronal setae present; antenna, last flagellomere 165-175 mm, apical seta present on last flagellomere, AR 0.45-0.65; lobes of pronotum gaping; humeral pit ovoid, hyaline and lacking granulation; wing, squama with 4-6 setae; legs, sensilla chaetica present in particular on apical part of tibia and tarsomeres ta_2 - ta_3 of PI-PIII; tergite IX, broadly cup-like with 4-5 dorsal setae located caudally near base of anal point; anal point lobe-like with 5-6 setae; virga, well-developed, consists of one conspicuous triangle-like virgal spine; inferior volsella triangular, bent downwards, inner margin with 3 apical setae; gonostylus, crista dorsalis weakly-developed to indistinct.

3.2. The *curtistylus*-group: diagnostic characters

The *curtistylus*-group is emended here for the first time on the basis of some main characters found in the male adult of known *Pseudorthocladius* species from France, Germany, Spain, Morocco, Algeria and Lebanon (*P. curtistylus*, *P. filiformis*, *P. rectangilobus*, *P. similis* Freeman, 1953, *P. sp. 2* and *P. sp. 3*). It includes also some recently described *Pseudorthocladius* species from the Far Eastern Russia and China (*P. insularis* Makarchenko & Makarchenko, 2012; *P. multisetus* Makarchenko & Makarchenko, 2012; *P. binarius* Ren, Lin & Wang, 2014; *P. paucus* Ren, Lin & Wang, 2014). In the following, a diagnosis character of the *curtistylus*-group is refined by including a combination of the most relevant features of the *curtistylus*-group.

Male adult

Head. Frontal area with or without lateral tubercles; coronal setae present or absent. Antenna, last flagellomere with a single apical seta, AR low in general (0.25 to 0.85 for known species from Europe) but is higher (1.0 to 1.40) for some known species from the Oriental Region and Far East Russia. Palp, palpomere 3 with or without sensilla clavata, sensilla coeloconica always present. Thorax. Lobes of anteprepronotum widely gaping, humeral pit present or absent, when present it is ellipsoid, occasionally replaced by a curved contrasting band of brownish granulation, humeral area bare or with brownish granulation. Wing, membrane without setae, squama with 4-6 to 7-11 setae in general (*P. ledoarei* sp. n., with only 2). Legs, sensilla chaetica present in particular on apical part of tibia and tarsomeres ta₂-ta₃ of PI-PIII. Tergite IX, semi-circular or broadly cup-like with distal half large capital U-like, setae on caudal part present or absent. Anal point triangular, semi-circular or large lobe-like, with short or long setae located on median area and apical margin, or only on apical margin. Inferior volsella large lobe-like, triangular or sub-rectangular, inner margin setose (with 1, 2 or numerous setae) or bare. Gonostylus, slender in general, posterior margin rounded or sinuous; crista dorsalis well to weakly-developed, when present it occupies the entire anterior side of gonostylus or its distal half.

4. Description

Pseudorthocladius ledoarei Moubayed, sp. n.

Material examined.

Holotype. Continental France. 1 male adult, leg J. Le Doaré; La Goutte karstic stream at a Beech grove, Etauf Forest, Gex, down to Oudar spring, (46° 21' 31" N, 06° 03' 07" E); altitude 880-900 m, 23.VII.2007. Environmental data of water: T°C (minima, 4-6, maxima, 18-20); calcareous, conductivity about 300 µS/cm.

Holotype (mounted on one slide) is deposited in the collections of the Zoologische Staatssammlung (ZSM), Munich, Germany.

Etymology

The new species is named '*ledoarei*' in honour to my colleague Jacques Le Doaré, who remains an active entomologist studying the taxonomy and biogeography of Plecoptera species of France.

Diagnostic characters

Though the male adult of *P. ledoarei* sp. n. shows some morphological similarities with members of the *curtistylus*-group (as emended and diagnosed previously), the new species can be easily distinguished from other related *Pseudorthocladius* species by the following combination of characters.

Head. Frontal area with lateral triangular tubercles; vertex rounded basally and orally projecting, coronal setae absent; antenna, apical seta present on last flagellomere, antennal groove reaching segment 3, sensilla chaetica present on segment 2 and last flagellomere, AR 0.76; palp, palpomere 3 without sensilla clavata, sensilla coeloconica present apically; clypeus top-like, with 12 long setae in 4 rows. Thorax. Lobes of anteprepronotum widely gaping; humeral area with contrasting brownish granulation, humeral pit absent; presence of a characteristic curved long band composed of brownish granulation. Wing, membrane without setae; squama only with 2 setae. Legs, sensilla chaetica present in low number on apical part of tibia and tarsomeres ta₂-ta₃ of PI-PIII. Abdomen. Tergite IX, broadly cup-like, distal half capital U-like, median area with 5 setae located close to

base of anal point, presence of 2 characteristic small spines laterally on one side (clearly visible in both dorsal and lateral view); anal point sub-triangular, with 11 long setae. Virga horseshoe-like. Gonocoxite, inferior volsella semi-circular, inner margin with 1 single seta. Gonostylus slightly projecting posteriorly, posterior margin rounded proximally and sinuous distally; crista dorsalis well-developed, occupying distal half of gonostylus.

Male imago

(n = 1; Figs 1-20)

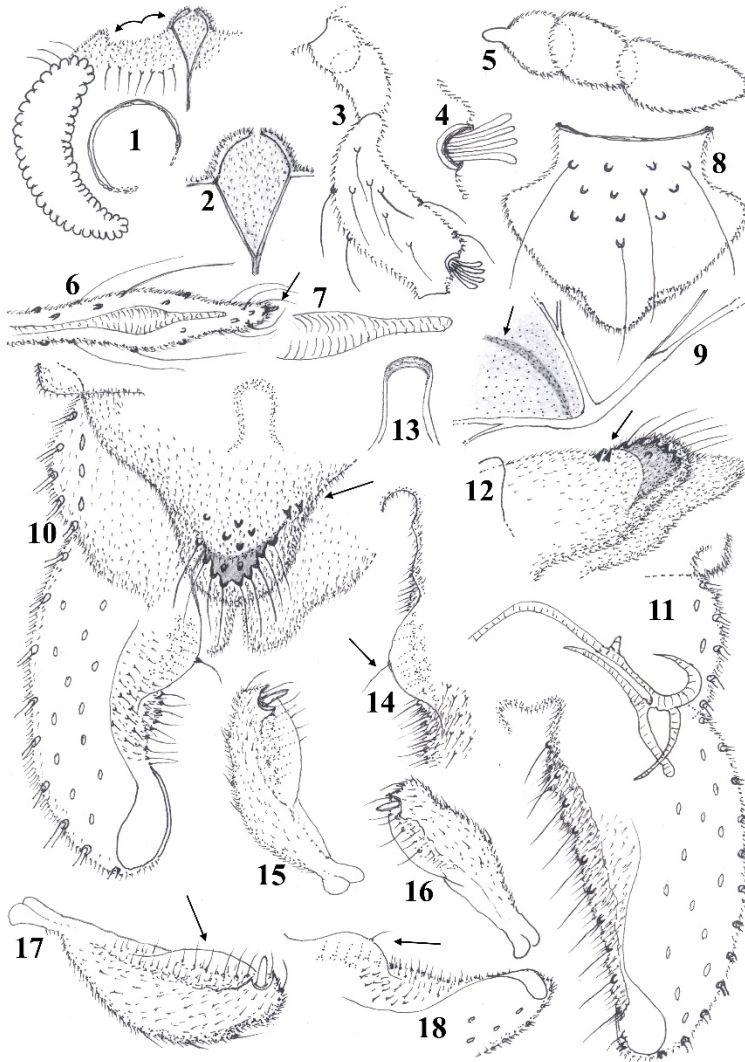
Small sized species. Total length (TL) 2.43 mm, wing length (WL) 1.30 mm; TL/WL = 1.87. General colouration contrasting brown to dark brown head and thorax. Antenna brown yellowish; thorax brownish with dark brown mesonotal stripes; legs yellowish; abdomen yellowish, anal segment yellowish with dark brown anal point.

Head. Eyes bare, lateral inner margin not hairy; frontal area (Figs 1-2) with 2 triangular tubercles located laterally, vertex rounded basally and orally projecting, coronal area without coronal setae; temporal setae 10, including 7 inner and 3 outer verticals, postorbitals absent. Palp 5-segmented, length (in μm) of segments 1-5: 28, 45, 90, 125, 165; palpomeres 1-3 (Figs 3-4) with segments 1 and 2 fused; segment 5 linearly elongated; palpomere 3 lacking sensilla clavata, sensilla coeloconica present pre-apically as in figure 3. Antenna 660 μm long, last flagellomere 285 μm long, apex distinctly-clubbed (Fig. 6) bearing one apical seta; sensilla chaetica present on segment 2 and apex of last flagellomere, which is bearing a brush of longer sensilla chaetica; antennal groove reaching segments 2, apical part (Fig. 7) cylindrical; AR 0.76. Clypeus (Fig. 8), top-shaped, with 12 long setae 75-85 μm long, inserted in 4 rows. Thorax. Lobes of anteprenotum widely-opened, thicker basally, lateral anteprenotals 5 located apically; acrostichals 8 in 1-2 rows, starting distance from anteprenotum; dorsocentrals 9 in 1-2 row; prealars 4 in one row; supraalars absent; humeral area (Fig. 9) with contrasting granulation, humeral pit absent, presence of a long curved band of small granulation; scutellum with 8 setae in one row. Wing. Brachiolum with one seta; distribution of setae on veins: R, 13-14; R₁, 5-6; R₂₊₃, 17-18, remaining veins bare; extended costa 45 μm long; squama with 2 setae. Legs. Tibial spur of PI distinctly spiniforme; length (in μm) of tibial spurs of: PI, 50; PII, 25 and 30; PIII, 30 and 95; longest seta of tibial comb 40 μm long; sensilla chaetica present on tibia and tarsomeres ta₂-ta₅ of PI, PII and PIII; sensilla mostly located apically on tibiae and tarsomeres. Length (in μm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in table 1.

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
PI	715	595	365	280	175	115	95	0.61	2.52	3.59	1.70
PII	555	565	225	125	85	55	65	0.40	4.08	4.98	3.40
PIII	605	665	350	190	165	85	80	0.53	3.12	3.63	1.60

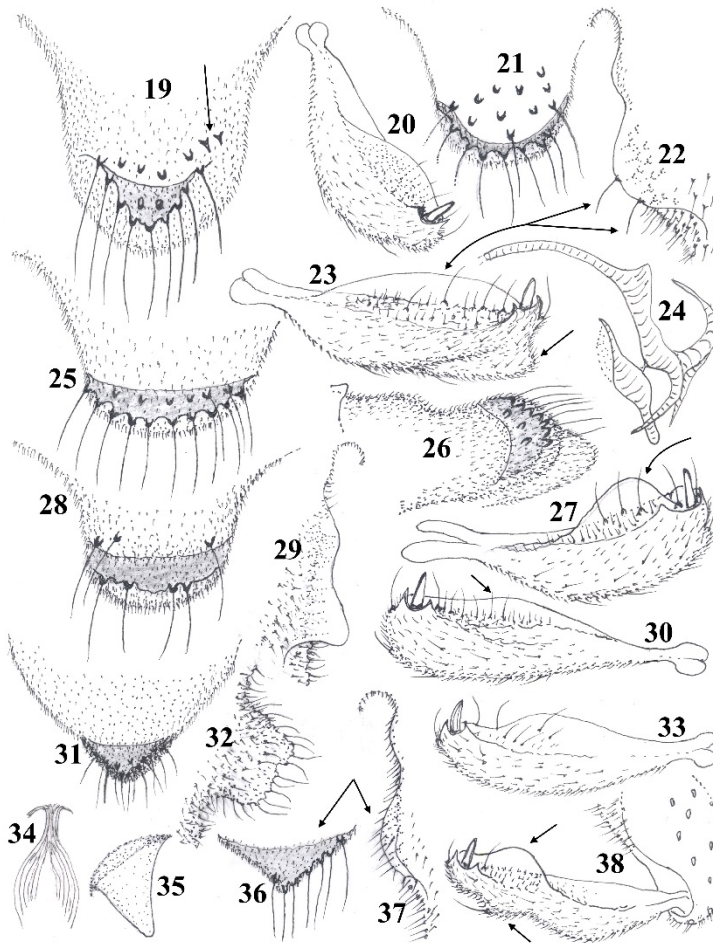
Table 1. Male adult of *Pseudorthocladius ledoarei* sp. n. Length (μm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs.

“LR = length of tarsomere ta₁ divided by length of tibia (ti); BV = combined length of femur (fe), tibia and ta₁ divided by combined length of tarsomeres ta₂-ta₅; SV = ratio of femur plus tibia to tarsomere ta₁; BR = ratio of longest seta of ta₁ divided by minimum width of ta₁, measured one third from apex.”



Figures 1-18. Male imago of *Pseudorthocladius ledoarei* sp. n. Head (left side, dorsal), frontal area, vertex and temporal setae (1); details of vertex (2); palpomeres 1-3 (3); sensilla coeloconica (4); antenna, segments 1-3 (5); antenna, apex of last flagellomere (6) and antennal groove (7); clypeus (8); humeral area (9); hypopygium in dorsal (10) and ventral view (11); tergite IX and anal point, lateral (12); virga (13); right inferior volsella, dorsal (14); gonostylus in dorsal (acute angle, 15), lateral (16) and ventral view (right angle, 17); gonocoxite and inferior volsella, lateral (18).

Figures 1-18. Imago mâle de *Pseudorthocladius ledoarei* sp. n. tête (côté gauche, vue dorsale), aire frontale, vertex, et soies temporales (1); détails du vertex (2); palpomères 1-3 (3); sensilla coeloconica (4); antenne, segments 1-3 (5); extrémité du dernier segment antennaire (6) et du canal antennaire (7); clypeus (8); aire humérale (9); hypopyge en vues dorsale (10) et ventrale (11); tergite IX et pointe anale, vue latérale (12); virga (13); volsella inférieure gauche, vue dorsale (14); gonostyle en vues dorsale (angle aigu, 15), latérale (16) et ventrale (angle droit, 17); gonocoxite et volselle inférieure, vue latérale (18).



Figures 19-38. Male imago of *Pseudorthocladius* spp. *P. ledoarei* sp. n.: tergite IX and anal point, dorsal (19); gonostylus, dorsal (20). *P. berthelemyi*: tergite IX and anal point, dorsal (21); inferior volsella (22); gonostylus, ventral (right angle, 23); sternapodeme and phallapodeme (24). *P. sp. 1*: tergite IX and anal point, dorsal (25); tergite IX and anal point, lateral (26); gonostylus, ventral (right angle, 27). *P. sp. 2* (cf. *filiformis*): tergite IX and anal point, dorsal (28); inferior volsella (29); gonostylus, ventral (right angle, 30). *P. rectangilobus*, after CASPERS & SIEBERT (1980): tergite IX and anal point, dorsal (31); inferior volsella (32); gonostylus (33); virga (34). *P. similis*: virga (35). *P. curtistylus*, after ALBU (1966): anal point (36); inferior volsella (37); gonostylus (38).

Figures 19-38. Imago mâle de *Pseudorthocladius* spp. *P. ledoarei* sp. n.: tergite IX et pointe anale, vue dorsale (19); gonostyle, vue dorsale (20). *P. berthelemyi*: tergite IX et pointe anale, vue dorsale (21); volselle inférieure (22); gonostyle, vue ventrale (angle droit, 23); sternapodème et phallapodème, (24). *P. sp. 1*: tergite IX et pointe anale, vue dorsale (25); tergite IX et pointe anale vue latérale (26); gonostyle, vue ventrale (angle droit, 27). *P. sp. 2* (cf. *filiformis*): tergite IX et pointe anale, vue dorsale (28); volselle inférieure (29); gonostyle, vue ventrale (angle droit, 30). *P. rectangilobus*, d'après CASPERS & SIEBERT (1980): tergite IX et pointe anale, vue dorsale (31); volselle inférieure (32); gonostyle (33); virga (34). *P. similis*: virga (35). *P. curtistylus*, d'après ALBU (1966): pointe anale (36); volselle inférieure (37); gonostyle (38).

Abdomen. Hypopygium in dorsal, ventral and lateral view (Figs 10-18). Tergite IX about 125 μm maximum width at base and 50-55 μm in caudal part, large cup-like, distal part capital U-shaped; presence of 5 setae on median area close to base of anal point and 2 characteristic atypical small spines located laterally on one side close to base of anal point, which are clearly visible in dorsal and lateral view (Figs 10, 12, 19); anal point (dorsal, Figs 10, 19; lateral, Fig. 12) about 30 μm long and 35 μm maximum width at base, sub-triangular, with 11 setae including 2 on median area and 9 on posterior margin. Latero-sternite IX with 14-16 setae (7-8 on each side). Sternapodeme and phallapodeme as in Fig. 11, transverse sternapodeme semi-circular and orally projecting; phallapodeme linearly elongated and pointed apically. Virga (Figs 10, 13) about 30 μm long, horseshoe-like, weakly-sclerotized, consists of 4 fine and long virgal spines. Gonocoxite 180 μm long, 70 μm maximum width, rounded apically. Inferior volsella (Figs 10, 14, 18) about 50 μm long and 20 μm maximum width, semi-circular to large lobe-like, bearing 1 single seta on midline of inner margin. Gonostylus in dorsal, ventral and lateral view (Figs 15-17, 20), 93 μm long, 25-30 μm maximum width, thicker medially, slightly projecting posteriorly, posterior margin rounded proximally and sinuous distally; crista dorsalis well-developed, occupying distal half of gonostylus, clearly visible when viewed, in particular, at acute (Fig. 15) and right angle (Fig. 17). HR = 1.94. HV = 2.61.

5. Differential diagnosis

Despite the close resemblance of some morphological characters between *P. ledoarei* sp. n. and some members of the *curtistylus*-group, its male adult shows, as illustrated in figures 1 to 20 and highlighted in the description, some unusual differentiating features, which will easily separate it from other related *Pseudorthocladus* species.

Male adult

- Head with lateral tubercles on frontal area, projecting vertex and absence of coronal setae (Figs 1-2), represents an atypical combination of characters in the genus *Pseudorthocladus*; such unusual features in orthoclads are differently detected in both *Rheocricotopus* and *Smittia* genera (MOUBAYED-BREIL & KETTANI 2019, Fig. 1; MOUBAYED et al. 2019, Fig. 1);

- Antennal ratio AR 0.76, is higher in *P. curtistylus* (0.84, sensu ALBU 1966) and lower (0.45-0.63, in SÆTHER & SUBLETTE 1983);

- Tergite IX with 5 setae and 2 characteristic atypical small spines (Figs 10, 12, 19), is lacking spines in members of the *curtistylus*-group and all other *Pseudorthocladus* species;

- Anal point with 11 long setae, 2 located on median area and 9 on apical margin, is differently figured in *P. curtistylus* (Fig. 36; sensu ALBU 1966, Fig. 5), *P. berthelemyi* (Fig. 21), *P. sp. 1* (Figs 25-26), *P. sp. 2* (cf. *filiformis*, Fig. 28) and *P. rectangilobus* (Fig. 31; CASPERS & SIEBERT 1980, Fig. 2);

- Virga horseshoe shaped with 4 long spines, is bell-like with up to 10 fine virgal spines in *P. rectangilobus* (Fig. 34) or longitudinal rod-like in *P. cranstoni* (LANGTON & PINDER 2007, Fig. 185B) and *P. macrovirgatus* (SÆTHER & SUBLETTE 1983, Fig. 51D);

- Inferior volsella semi-circular, with only 1 single seta on inner margin (Figs 10-11), is triangular and bare in *P. filiformis* (Fig. 29), bearing 2 setae in *P. berthelemyi* (Fig. 22); rectangular in

P. rectangilobus (Fig. 32) and with several setae in *P. curtistylus* (BRUNDIN 1947, Fig. 30; MAKARCHENKO & MAKARCHENKO 2012, Figs 6-8);

- Crista dorsalis well-developed, occupying distal half of gonostylus (Figs 15, 16, 17, 20), is large tooth-like in *P. sp. 1* (Fig. 27) and *P. curtistylus* (Fig. 37; sensu ALBU 1966, Fig. 5) or widely extended in both *P. berthelemyi* (Fig. 23) and *P. filiformis* (Fig. 30).

6. Key to known male adults of *Pseudorthocladius* species from Europe

The current list of known valid *Pseudorthocladius* species from Europe (*Fauna Europaea*, version of SÆTHER & SPIES 2013) includes the 8 following species: *P. albiventris* (Goetghebuer, 1938); *P. berthelemyi* Moubayed, 1990; *P. cristagus* Stur & Sæther, 2004; *P. cranstoni* Sæther & Sublette, 1983; *P. curtistylus* (Goetghebuer, 1921); *P. filiformis* Kieffer, 1928; *P. macrovirgatus* Sæther & Sublette, 1983; *P. pilosipennis* Brundin, 1956 and *P. rectangilobus* Caspers & Siebert, 1980. Currently, only 5 are recorded from France: *P. berthelemyi*, *P. cristagus* (recently reported in MOUBAYED et al. 2019), *P. cranstoni*, *P. curtistylus* and *P. filiformis*. In the following key to male adults of known species from Europe, only 7 of the above 8 cited species are treated and considered, except for *P. albiventris*, which appears to key into the *curtistylus*-group, in particular close to the type-II of *P. curtistylus* (sensu SÆTHER & SUBLETTE 1983). Moreover, *P. albiventris* is considered here as a junior synonym of *P. curtistylus*. The latter two species belong to a same and one *Pseudorthocladius* species, based in particular on a similar shape of the inferior volsella, which is illustrated in: GOETGHEBUER 1938 (Fig. 2), SÆTHER & SUBLETTE 1983 (Fig. 37F), ALBU 1966 (Fig. 5), and Fig. 37.

1. Membrane of wing with setae (SÆTHER & SUBLETTE 1983, Fig. 26C; STUR & SÆTHER 2004, Fig. 1).....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 (STUR & SÆTHER 2004, Figs 2-3); AR 1.38-1.43 *P. cristagus*
 - Gonostylus not projecting posteriorly, outer heel absent, inferior volsella large lobe-like; crista dorsalis absent (BRUNDIN 1956, Fig. 102; SÆTHER & SUBLETTE 1983, Fig. 26D; LANGTON & PINDER 2007, Fig. 185C); AR 1.20-1.40 *P. pilosimanus*
3. Virga rod-like, consists of long fused spines located on midline of hypopygium (SÆTHER & SUBLETTE 1983, Figs 51D, 54D; LANGTON & PINDER 2007, Fig. 185B)4
 - Virga not as above, when present it consists of minute grouped spinules5
4. Inferior volsella large thumb-like; gonostylus slender; crista dorsalis absent (SÆTHER & SUBLETTE 1983, Fig. 51D); virga with longest spine reaching 96 µm; squama with 6-8 setae *P. macrovirgatus*
 - Inferior volsella small and nose-like; gonostylus bulbous; crista dorsalis present, well-developed and occupying the proximal half; virga with longest spine reaching 105 µm (SÆTHER & SUBLETTE 1983, Fig. 54D; LANGTON & PINDER 2007, Fig. 185B); squama with 12 setae *P. cranstoni*
5. Crista dorsalis well-developed (Figs 15-17, 20, 23, 27, 30, 37)6
 - Crista dorsalis weak, occasionally absent7
6. Inner margin of inferior volsella with only 1 single seta (Figs 10, 14, 18); AR 0.76; squama with 2 setae; virga horseshoe-like; gonostylus not protruding posteriorly (Figs 15-17, 20) *P. ledoarei* sp. n.
 - Inner margin of inferior volsella with 2 setae; AR 0.65; squama with 7-8 setae; virga absent; gonostylus protruding posteriorly (Fig. 22; MOUBAYED 1990, Fig.4) *P. berthelemyi*

7. Inferior volsella triangular, inner margin without setae (Figs 29; SÆTHER & SUBLETTE 1983, Fig. 33D); anal point broadly semi-circular or sub-triangular (Fig. 28; LEHMANN 1971, Fig. 23); crista dorsalis well-developed, widely extended (Fig. 30; SÆTHER & SUBLETTE 1983, Fig. 33D); AR 0.85-1.0.....*P. filiformis*
-Inferior volsella semi-circular to lobe-like, or rectangular, inner margin with setae (Fig. 32; CASPERS & SIEBERT 1980, Figs 2-3; SÆTHER & SUBLETTE 1983, Figs 37E-F); AR 0.45-1.278
8. Anal point triangular (Fig. 36; SÆTHER & SUBLETTE 1983, Figs 37E-F; MAKARCHENKO & MAKARCHENKO 2012, Fig. 6); inferior volsella semi-circular (Fig. 37); crista dorsalis weakly-developed, occasionally present on distal half (Fig. 38; SÆTHER & SUBLETTE 1983, Fig. 37E); AR 0.45-0.63.....*P. curtistylus* (= *P. albiventris*)
- Anal point semi-circular (Fig. 31; CASPERS & SIEBERT 1980, Fig. 2); virga bell-like, composed of several virgal spines (Fig. 34); inferior volsella rectangular with numerous setae on inner margin (Fig. 32; CASPERS & SIEBERT 1980, Figs 2-3); AR 1.27; squama with 8-11 setae*P. rectangilobus*

7. Ecology and geographical distribution

P. ledoarei sp. n. occurs in cold stenothermic waterfalls delimited by the upper basin of La Goutte karstic stream where bryocolous and hygropetric habitats with fast running water represent the most favourite aquatic microhabitat 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. Geographical distribution is currently restricted to the rhithral of cold streams situated in eastern France, close to the Swiss boarder. The new species belongs to a local continental biogeographical representative, which can be expected to occur in some neighbouring areas of eastern France and other countries (Switzerland, Germany).

Associated species encountered with *P. ledoarei* sp. n. *dubius* (Meigen 1804); *Paramerina cingulata* (Walker, 1856); *Boreoheptagyia legeri* (Goetghebuer, 1933); *Diamesa hamaticornis* Kieffer, 1924; *D. insignipes* Kieffer, 1908; *Bryophaenocladus aestivus* (Brundin, 1947); *B. subvernalis* (Edwards, 1929); *Chaetocladus dentiforceps* (Edwards, 1929); *C. melaleucus* (Meigen, 1818); *Eukiefferiella devonica* Lehmann, 1972; *E. minor* (Edwards, 1929); *Metriocnemus eurynotus* (Holmgren, 1883); *Orthocladus frigidus* (Zetterstedt, 1838); *Pseudorthocladus berthelemyi* Moubayed, 1990; *Rheocricotopus effusus* (Walker, 1856); *Microtendipes pedellus* (De Geer, 776); *Polypedilum albicorne* (Meigen, 1838); *Rheotanytarsus curtistylus* (Goetghebuer, 1921); *R. distinctissimus* (Brundin, 1947).

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