# Orthocladius (Orthocladius) montisdei sp. n. and O. (O.) queyrassianus sp. n., two new species with reduced antennae from continental France [Diptera, Chironomidae]

by Joel MOUBAYED<sup>1</sup>, Peter H. LANGTON<sup>2</sup>, Gennaro COPPA<sup>3</sup> & Pierre CLÉVENOT<sup>4</sup>

<sup>1</sup>Freshwater & Marine biology, 10 rue des Fenouils, F - 34070 Montpellier, France
<sup>1</sup>Corresponding author E-mail: <u>chirojmb@free.fr</u>
<sup>2</sup>16 Irish Society Court, Coleraine, Co. Derry, BT52 IGX, Northern Ireland
<sup>3</sup>1, rue du Courlis, F - 08350 Villers-sur-Bar, France
<sup>4</sup>TERÉO Alpes du Sud, 1 impasse Sixtine, F - 05000 Gap, France

Keywords: Orthocladius genus, new species with reduced antennae, France, conservation.

The male adult with reduced antennae of two new *Orthocladius* species, subgenus *Orthocladius* (*O. montisdei* sp. n. and *O. queyrassianus* sp. n.) is described based on material collected in two cold petrifying to chalky springs: one located in the Ardennes department (chalky spring, Forest of Mont-Dieu, NE-France, alt. 180-280 m), the other in the Hautes-Alpes department (petrifying spring, Massif of Queyras, alt. 1300-1500 m). The main distinguishing characters of the two male adults of *Orthocladius* with female-like antennae are: 6 antennal segments in *O. montisdei* sp. n., only 5 in *O. queyrassianus* sp. n. The description of these two recently discovered species increases the total number worldwide of known male *Orthocladius* with reduced antennae to three and the number of *Orthocladius* (*O.*) species for France to 15. Taxonomic notes and comments on the ecology and geographical distribution of the two new species are provided.

# *Orthocladius (O.) montisdei* sp. n. et *O. (O.) queyrassianus* sp. n., deux nouvelles espèces crénophiles aux antennes réduites de France [Diptera, Chironomidae]

Mots-Clés: Orthocladius genus, nouvelles espèces avec antennes réduites, France, conservation.

L'adulte mâle avec antennes réduites de deux nouvelles espèces d'*Orthocladius*, sous-genre *Orthocladius* (*O. montisdei* sp. n. et *O. queyrassianus* sp. n.) est décrit à partir d'un matériel collecté dans deux sources pétrifiantes froides situées dans le département des Ardennes (source crayeuse, forêt de Mont-Dieu, NE-France, alt. 180-280 m) et celui des Hautes-Alpes (source pétrifiante, Massif du Queyras, alt. 1300-1500 m). Les principaux caractères distinctifs des adultes mâles d'*Orthocladius* avec des antennes de femelle sont définis par la présence de 6 segments antennaires chez *O. montisdei* sp. n. et seulement 5 chez *O. queyrassianus* sp. n. La description de ces deux espèces récemment découvertes porte à trois le total mondialement connu des mâles d'*Orthocladius* à antennes réduites, et à 15 le nombre des espèces d'*Orthocladius* pour la France. Des données taxonomiques et des commentaires sur l'écologie et la distribution géographique des deux nouvelles espèces sont présentés.

## 1. Introduction

Worldwide, the genus *Orthocladius* (subgenus *Orthocladius*) is represented by about 63 described species (BRUNDIN 1947, 1956, SÆTHER 1980, COFFMAN et al. 1986, CRANSTON et al. 1989, SOPONIS 1983, 1990, LANGTON & CRANSTON 1991, LINDEGAARD 1995, ROSSARO et al. 2003, SPIES & SÆTHER 2004, SÆTHER 2005, LANGTON & PINDER 2007, ASHE & O'CONNOR 2012, SÆTHER & SPIES 2013, MOUBAYED-BREIL & GARRIGUE 2021). Among them, 13 species are currently reported from continental France. Consequently, the description of *O. montisdei* sp. n and *O. queyrassianus* sp. n. increases the total number in the genus *Orthocladius* (*Orthocladius*) to 15 from this country.

In this paper, the male adult of *O. montisdei* sp. n and *O. queyrassianus* sp. n. are diagnosed and described based on material collected in two cold petrifying to chalky springs located in the Ardennes department (NE-France, Forest of Mont-Dieu, alt. 180-280 m) and the Hautes-Alpes department (Massif of Queyras, alt. 1300-1500 m).

## 2. Material and methods

The studied material was collected by an Actinic Light Trap (Photo 1 right) and using aerial sweep netting. Preserved male adults in 80% ethanol, were cleared of musculature in 90% lactic acid (head, thorax, abdomen and anal segment) for about 60 to 80 minutes (this can be left overnight at room temperature without any detrimental damage). When clearing was complete the specimens were washed in two changes of 50-60% ethanol to ensure that all traces of lactic acid were removed. The holotypes were mounted in polyvinyl lactophenol. Before the final slide mountings, the hypopygium including the tergite IX and the anal point were viewed ventrally and laterally to examine and draw all the necessary details. Terminology and measurements follow those of SÆTHER (1980) and LANGTON & PINDER (2007).

## 3. Descriptions

## Orthocladius (Orthocladius) montisdei Moubayed & Langton, sp. n.

#### Material examined

Continental France. Holotype. One male adult; chalky cold spring and stream, Forest of Mont-Dieu, Ardennes Department, NE-France (49° 32' 25" N; 4° 53' 13" E), altitude 180-280 m (Photo 1 left); 8.V.2022, leg. G. Coppa.

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'.

**Etymology**: the name '*montisdei*' refers to the well-known Forêt de Mont-Dieu in North eastern France, where the type-material of *O. montisdei* was collected.



Photo 1. Type-locality of *O. montisdei* sp. n. (left), arrows indicate the moss carpets. Actinic light trap (right).

Photo 1. Localité-type d'*O. montisdei* sp. n. (gauche), les flèches indiquent les tapis de mousse. Piège lumineux par lampe actinique (droite).

## **Diagnostic characters**

*O. montisdei* sp. n. can easily be recognized by a combination of some unusual characters found in the male adult: Head. Coronal triangle reduced; tubercles present on the face below the antennal insertions; temporals consist of 12 inner and 3 outer verticals (inner setae located in 2 separate groups); Antenna reduced, with only 6 segments, last flagellomere narrowed to apex, not clubbed, antennal groove not overreaching base of last flagellomere; palpomere 3 with 3 pre-apical pin-like sensilla coeloconica. Thorax. Dorsal antepronotals 4 including a much longer inner one. Hypopygium. Anal point sharply pointed, lateral setae 6 located only at base (3 on each side). Basal junction of gonocoxites with pubescent digitiform ridges; virga absent; superior volsella large lobe-like; inferior volsella triangular to nose-like, broad anteriorly, gradually narrowing distally; anterior part of aedeagal lobe long, reaching base of gonocoxites; distal part of gonocoxite with 5 stout inwardly projecting setae; nose-like. Gonostylus of *Orthocladius*-type, rectangular when viewed at an acute angle, with rounded posterior margin when viewed laterally and at an obtuse angle; anterior edge linear, crista dorsalis absent.



Photo 2. *O. montisdei* sp. n. Ventral view of the male adult. Photo 2. *O. montisdei* sp. n. Mâle adulte en vue ventrale.

#### Description

(n = 1, male adult; Figs 1A-H, 2A-E; Photos 2-3)

Large *Orthocladius* species. Total length 3.75 mm. Wing length 2.35 mm. TL/WL = 1.60. General colouration (Photo 3) brownish to yellowish with contrasting pale brown to dark brown mesonotal stripes; legs and anal segment brown to dark brown; dorsal and ventral side of hypopygium with faint sclerotization.

Head (Figs 1A-B). Eyes bare, hairs absent on inner lateral margin; coronal triangle (Fig. 1A) reduced, coronals absent; characteristic facial tubercles (Fig. 1B); temporals 11-12 including 8-9 inner (located in 2 groups) and 3 outer verticals. Antenna (Figs 1C-D, Photo 2) 6-segmented (reduced), 350  $\mu$ m long; last flagellomere 130  $\mu$ m long, markedly narrowing towards apex, with numerous sensilla chaetica, presence of a notch (Fig. 1D) close to basal part; antennal groove (Fig. 1D) starting at base of last flagellomere; AR 0.60. Palp 5-segmented, segments 1-2 fused; length (in  $\mu$ m) of segments: 45, 65, 140, 140, 185; segments 3 and 4 subequal; palpomere 3 (Fig. 1E) with 1 sensilla clavata and 3 pre-apical pin-like sensilla coeloconica (located on both sides). Clypeus (Fig. 1F) trapezoidal, anterior side longer, 130  $\mu$ m long, with 13 setae in 3 rows.

Thorax. Lobes of antepronotum (Fig. 1G) not gaping; dorsal antepronotals 4, inner one longer than the 3 outer; median antepronotals 1, lateral antepronotals 6; acrostichals 21 in 1-2 rows, starting close to antepronotal lobes; dorsocentrals 12 decumbent in 1 row; prealars 7 uniserial; humeral pit absent; scutellum with 10 setae inserted in 1 row (5 on each side of the midline); preepisternum bare. Wing. Brachiolum with 2 setae. Subcosta overreaching fork of Cu; costal extension 45  $\mu$ m long. Number and distribution of setae on veins: R, 23-24; R<sub>1</sub>, 16-17; R<sub>4+5</sub>, 45-47 in 1-2 rows; remaining veins bare; squama with 14 setae in 1-2 rows. Legs. Tibial spurs present on PI-PIII; length (in  $\mu$ m) of spurs: 35 (PI); 35, 40 (PII); 80, 35 (PIII). Sensilla chaetica present on tibia and tarsomeres ta<sub>1</sub>-ta<sub>5</sub> of PI-PIII. Length ( $\mu$ 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	970	1065	745	505	340	225	145	0,70	2,29	2,73	1,60
PII	995	970	465	295	220	120	130	0,48	3,18	4,23	2,00
PIII	1005	1140	655	365	275	160	130	0,58	3,01	3,28	2,35

" 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."



Photo 3. Male adult of *O. montisdei* sp. n.: anal point (left), hypopygium in ventral view (right). Photo 3. Adulte mâle d'*O. montisdei* sp. n. : pointe anale (à gauche), hypopyge en vue ventrale (à droite).

Abdomen. Hypopygium as in figures 2A-B (2A, dorsal; 2B, ventral, with tergite IX and anal point omitted) and Photos 2, 3. Tergite IX about 175  $\mu$ m long, 200  $\mu$ m maximum width, broadly semi-circular, slightly narrowing distally, dorsally (Figs 1H, 2A; Photo 3, left) without hump, posterior margin rounded; caudal part with 9 dorsal setae located close to base of anal point. Anal point (Figs 1H, lateral; 2A, dorsal; Photo 3, left) about 65  $\mu$ m long, 100  $\mu$ m maximum width at base, triangular and sharply pointed apically, overreaching tip of superior volsella, with 6 lateral setae located at base (3 on each side). Laterosternite IX with 12 setae (6 on each side). Sternapodeme and phallapodeme (Fig. 2B; Photo 3, right); transverse sternapodeme slightly rounded, lateral expansion with pointed apex; phallapodeme nearly sickle-shaped, anteriorly elongated, about 40  $\mu$ m long, digitiform, reaching basal junction of gonocoxites, aedeagal lobe

medially broadly enlarged. Gonocoxite as in Figs 2A-B; 275  $\mu$ m long, 110  $\mu$ m maximum width; basal junction with digitiform pubescent inward projections; apex rounded; distal part of inner margin (Fig. 2A) with a cluster of 5 stout setae in 1 row. Superior volsella (Fig. 2A) about 40  $\mu$ m as long as wide at base, well-developed large lobe-like. Virga absent. Inferior volsella (Fig. 2A; Photo 3, right) 70  $\mu$ m long, 25  $\mu$ m maximum width, dorsally strongly projecting, nose-like, distinctly swollen at base and gradually narrowing distally. Gonostylus (Figs 2C-E, 3 aspects) 135  $\mu$ m long, 35-40  $\mu$ m maximum width; anterior edge markedly linear; posterior margin rounded when viewed at an obtuse angle (Fig. 2C) or laterally (Fig. 2E); apically (Fig. 2D) right angled when viewed at an acute angle; crista dorsalis absent; megaseta well-developed. HV (total length divided by length of gonostylus X 10) = 2.78; HR (length of gonocoxite divided by length of gonostylus) = 2.04.



Figure 1. Male adult of *O. montisdei* sp. n. A: head, frontal area, dorsal; B: facial tubercles; C-D: antenna and last flagellomere; E: palpomere 3 with sensilla coeloconica; F: clypeus; G: lobes of antepronotum with acrostichals; H: tergite IX and anal point, lateral view. The arrows indicate some distinguishing characters.

Figure 1. Imago mâle d'O. montisdei sp. n. A : tête, aire frontale, vue dorsale; B : tubercules faciaux ; C-D : antenne et dernier flagellomere ; E : palpomère 3 et sensilla coeloconica ; F : clypéus ; G : lobes de l'antépronotum et soies acrostichales ; H : tergite IX et pointe anale, vue latérale. Les flèches indiquent quelques caractères distinctifs.



Figure 2. Male adult of *O. montisdei* sp. n. A) hypopygium in dorsal view with basal junction of gonocoxites, superior and inferior volsella; B) apodemes and gonocoxite, ventral; C) gonostylus at obtuse angle; D) gonostylus at acute angle; E) gonostylus, lateral. The arrows indicate some distinguishing characters.

Figure 2. Imago mâle d'*O. montisdei* sp. n. A) hypopyge en vue dorsale avec junction basale des gonocoxites, volselles supérieure et inférieure; B) apodèmes et gonocoxite, vue ventrale; C) gonostylus à angle obtus; D) gonostylus à angle aigu; E) gonostylus, vue latérale.

Les flèches indiquent quelques caractères distinctifs.

Female, pupal exuviae and larva: unknown.

## Orthocladius (Orthocladius) queyrassianus Moubayed & Langton, sp. n.

## Material examined

Continental France. Holotype. 1 male adult; Southern Alps, Queyras Massif, Hautes-Alpes Department, SE-France (44° 45' 7.31" N, 6° 46' 47.88" E), petrifying karstic cold spring, alt. 1300-1500 m (Photos 4-5); 11.IV.2022, leg. P. Clévenot.

Holotype (mounted on 1 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'.

**Etymology**: The name 'queyrassianus' refers to the Queyras Alpine Massif (SE-France) where the type-material of *O. queyrassianus* sp. n. was collected.



Photo 4. Type-locality of *O. queyrassianus* sp. n. Arrows indicate the moss carpets. Photo 4. Localité-type d'*O. queyrassianus* sp. n. Les flèches indiquent les tapis de mousse.

#### **Diagnostic characters**

The male adult of *O. queyrassianus* sp. n. is easily separated from other members of the genus *Orthocladius* by the following combination of characters: Head. Coronal triangle reduced, facial tubercles absent; 10 temporals including 7 inner (in 1 row) and 3 outer verticals. Antenna reduced, with only 5 segments, last flagellomere not clubbed and narrowing apically, antennal groove starting on segment 4; palpomere 3 with 2 pre-apical pin-like sensilla coeloconica located on 1 side. Thorax. Dorsal antepronotals only 1, median antepronotals 1, lateral antepronotals 9; acrostichals 12 starting close to lobes of antepronotum. Hypopygium. Tergite IX with a dorsal hump. Anal point with pointed apex, lateral setae 10 located on base and basal half (5 on each side); virga absent; superior volsella low lob-like, broad; inferior volsella, double, dorsally elongate, nose-like, bent downwards, with short dorsal setae. Apical part phallapodeme long and sinuous, reaching base of gonocoxites, aedeagal lobe broadly swollen; basal junction of gonocoxites rounded, distal part with 5 stout inwardly projecting setae. Gonostylus, rectangular and robust, apically with a curved stout seta; crista dorsalis present, clearly visible in lateral view.

#### Description

## (n = 1, male adult; Figs 3A-G, 4A-F)

Medium sized *Orthocladius* species. Total length 3.55 mm. Wing length 2.85 mm. TL/WL = 1.25. General colouration contrasting dark brown to blackish; thorax with dark brown to blackish mesonotal strips; legs and anal segment brownish; dorsal and ventral side of hypopygium with strong sclerotization.

Head (Fig. 3A). Eyes bare; coronal triangle reduced and projecting, coronals absent, facial tubercles absent; temporals 10 including 7 inner in 1 row and 3 outer verticals. Antenna (Figs 3B-C) 5-segmented, 400  $\mu$ m long (reduced); last flagellomere 200  $\mu$ m long, not clubbed and markedly narrowing towards apex, with numerous sensilla chaetica, with a notch close to the base; antennal groove (Figs 3B-C) starting on segment 4 (overreaching last flagellomere); AR 1.0. Palp 5-segmented, segments 1-2 fused; length (in  $\mu$ m) of segments: 40, 65, 160, 135, 245; palpomere 3 (Fig. 3E) with 2 sensilla clavata and 2 pre-apical sensilla coeloconica on one side. Clypeus (Fig. 3D) trapezoidal, with 16 setae in 4 rows.

Thorax. Lobes of antepronotum (Fig. 3F) not gaping; dorsal antepronotals 1; median antepronotals 1, lateral antepronotals 9; acrostichals 12 in 1-2 rows, starting close to antepronotal lobes; dorsocentrals 9 decumbent in 1 row; prealars 5 uniserial; humeral pit absent; scutellum with 8 setae inserted in 1 row (4 on each side of the midline); preepisternum bare. Wing. Brachiolum with 1 seta. Subcosta overreaching fork of Cu; costal extension 50  $\mu$ m long. Number and distribution of setae on veins: R, 13; R<sub>1</sub>, 10-11; R<sub>4+5</sub>, 12-13 located on distal half; remaining veins bare; squama with 21-22 setae in 1-2 rows. Legs. Tibial spurs present on PI-PIII; length (in  $\mu$ m) of spurs: 50 (PI); 35, 38 (PII); 85, 35 (PIII). Sensilla chaetica present on tibia and tarsomeres ta<sub>1</sub>-ta<sub>5</sub> of PI-PIII. Length ( $\mu$ m) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in the 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	965	1125	755	475	325	190	145	0,67	2,51	2,77	2,20
PII	1055	995	485	285	210	120	135	0,49	3,38	4,23	2,40
PIII	1135	1205	685	370	275	140	150	0,57	3,24	3,42	2,50

<sup>&</sup>quot; LR = Length of tarsomere  $ta_1$  divided by length of tibia (ti); BV = Combined length of femur (fe), tibia and  $ta_1$  divided by combined length of tarsomeres  $ta_2$ - $ta_5$ ; SV = Ratio of femur plus tibia to tarsomere  $ta_1$ ; BR = Ratio of longest seta of  $ta_1$  divided by minimum width of  $ta_1$ , measured one third from apex."



Figure 3. Male adult of *O. queyrassianus* sp. n. A) head, frontal area, dorsal; B) antenna; C) segment 4 and last flagellomere; D) clypeus; E) palpomere 3 with sensilla coeloconica; F) lobes of antepronotum with acrostichals; G) tergite IX and anal point, lateral view. The arrows indicate some distinguishing characters.
Figure 3. Imago mâle d'*O. queyrassianus* sp. n. A) tête, aire frontale, vue dorsale; B) antenne; C) segment 4 et dernier flagellomère ; D) clypéus; E) palpomère 3 et sensilla coeloconica; F) lobes de l'antépronotum

et soies acrostichales ; G) tergite IX et pointe anale, vue latérale.

Les flèches indiquent quelques caractères distinctifs.

Abdomen. Hypopygium as in Figs 4A-B (4A, dorsal; 4B, ventral, with tergite IX and anal point omitted). Tergite IX about 165  $\mu$ m long, 175  $\mu$ m maximum width, broadly rectangular, posterior margin not narrowing, nearly straight; dorsally with a distinct hump clearly visible in lateral view (Fig. 3G); distally with 15 dorsal setae located close to posterior margin and base of anal point. Anal point (Figs 3G, lateral; 4A, dorsal) about 80  $\mu$ m long, 75  $\mu$ m maximum width at base, triangular with pointed apex, not reaching tip of inferior volsella; with 25 setae including 15 located at base and 10 laterally over basal half. Laterosternite IX with 14 setae (7 on each side). Sternapodeme and phallapodeme (Fig. 4B). Transverse sternapodeme rounded, lateral expansion well-developed; Phallapodeme nearly sickle-shaped; basally moderately elongated, about 30  $\mu$ m long, digitiform with sinuous inner margin, reaching basal junction of gonocoxites; aedeagal lobe broadly enlarged. Virga absent. Gonocoxite. 240  $\mu$ m long, 90  $\mu$ m maximum width, basal junction (Fig. 4C) is *Orthocladius*-type, rounded; distal part of inner margin (Fig. 4A) with 5 characteristic inwardly projecting setae in 1 row; ventrally with strong and broad sclerotization. Superior volsella (Fig. 4C) broadly low, about 50  $\mu$ m long, 25  $\mu$ m maximum width. Inferior volsella 80  $\mu$ m long, maximally wide; double, dorsally elongate thumb-like to

large nose shaped, distinctly bent posteriad, covered at base with short dorsal setae. Gonostylus (Figs 4D-F, 3 aspects) 135  $\mu$ m long, 35-40  $\mu$ m maximum width, broadly rectangular and robust; inner side swollen medially when viewed at right angles (Fig. 4D) and at an acute angle (Fig. 4F); caudal area with a characteristic stout curved seta, visible in Figs 4D-F; crista dorsalis present pre-apically, clearly visible in lateral view (Fig. 4E); megaseta well-developed. HV = 2.63; HR = 2.04.

Female, pupal exuviae and larva: unknown.



Figure 4. Male adult of *O. queyrassianus* sp. n. A) hypopygium in dorsal view; B) apodemes and basal part of gonocoxite, ventral; C) basal junction of gonocoxites, superior and inferior volsella; D) gonostylus at right angle; E) gonostylus, lateral; F) gonostylus at acute angle. The arrows indicate some distinguishing characters.

Figure 4. Imago mâle d'*O. queyrassianus* sp. n. A) hypopyge, vue dorsale; B) apodèmes et partie basale du gonocoxite, vue ventrale; C) junction basale des gonocoxites, volselles supérieure et inférieure; D) gonostylus à angle droit; E) gonostylus, vue latérale ; F) gonostylus à angle aigu.

Les flèches indiquent quelques caractères distinctifs.

## 4. Remarks

Worldwide, the only previously described species in the genus *Orthocladius* with reduced antennae is *O. ferringtoni*. Currently, the male adult with female-like antennae of two additional congeners have been recently discovered in France, hitherto undescribed: *O. montisdei* sp. n. and *O. queyrassianus* sp. n. The three species may be separated by the following key.

## Key to known male adult of Orthocladius with reduced antennae

## 5. Ecology and geographical distribution

Male adults of both *O. montisdei* sp. n. and *O. queyrassianus* sp. n. were collected around petrifying springs with hard water and active formation of travertine or Tufa. Their type-localities are moderately to densely covered with bryophytes as shown in Photos 1, 4, 5. Emergence of adults is observed during April to May.



Photo 5. Type-locality of *O. queyrassianus* sp. n. Arrows indicate the tufa and moss carpets. Photo 5. Localité-type d'*O. queyrassianus* sp. n. Les flèches indiquent les tufs et les tapis de mousse.

Currently, the two new species are only reported from their type-localities: *O. montisdei* sp. n., from the Ardennes department (NE-France, alt. 180-280 m); *O. queyrassianus* sp. n. from the Hautes-Alpes department, alt. 1300-1500 m. This suggests that these two taxa are differently distributed in continental France: *O. montisdei* sp. n. is likely more widespread in similar chalky springs and streams located in north eastern France, while *O. queyrassianus* sp. n. seems to belong to a local biogeographic representative of some Alpine petrifying springs. However, the two new species belong to the large community of crenobiontic and crenophilous species documented by LINDEGAARD (1995). They may be biological indicators and therefore deserve consideration for conservation measures.

Associated species encountered in the same locality as O. montisdei sp. n. include: Heleniella ornaticollis (Edwards, 1929); Limnophyes asquamatus Soegaard Andersen, 1937; L. pentaplastus (Kieffer, 1921); Metriocnemus eurynotus (Holmgren, 1883); Paraphaenocladius impensus (Walker, 1856); Cricotopus (Paratrichocladius) rufiventris (Meigen, 1830); Pseudosmittia angusta (Edwards, 1929); P. trilobata (Edwards, 1929); Smittia aterrima (Meigen, 1818); S. pratorum (Goetghebuer, 1927); Micropsectra atrofasciata Kieffer, 1911; M. shrankelae Stur & Ekrem, 2006; Lithotanytarsus emarginatus (Goetghebuer, 1933).

Encountered species in the same locality as O. queyrassianus sp. n. are: Diamesa aberrata Lundbeck, 1889; D. cinerella Meigen, 1835; D. dampfi (Kieffer, 1924); D. lateralis (Goetghebuer, 1921); D. zernyi Edwards, 1933; Pseudodiamesa branickii (Nowicki, 1873); P. nivosa (Goetghebuer, 1928); Krenosmittia camptophleps (Edwards, 1929); Limnophyes asquamatus Soegaard Andersen, 1937; L. bidumus Sæther, 1990; L. difficilis Brundin, 1947; L. edwardsi Sæther, 1990; Metriocnemus albolineatus Meigen, 1818; M. eurynotus (Holmgren, 1883); M. hirticollis (Staeger, 1869); Orthocladius fuscimanus (Kieffer, 1908); O. frigidus (Zetterstedt, 1838); Paraphaenocladius exagitans (Johannsen, 1905); P. impensus (Walker, 1856); P. pseudirritus Strenzke, 1950; Smittia alpicola Goetghebuer, 1941; S. foliosa (Kieffer, 1921); S. nudipennis (Goetghebuer, 1913); Micropsectra shrankelae Stur & Ekrem, 2006; M. roseiventris Kieffer, 1909.

#### References

- ASHE, P. & J.P. O'CONNOR. 2012. A World Catalogue of Chironomidae (Diptera). Part 2. Orthocladiinae. Irish Biogeographical Society & National Museum of Ireland, Dublin. 968 pp.
- BRUNDIN, L. 1956. Zur Systematic der Orthocladiinae (Diptera, Chironomidae). Report of the Institute of Freshwater Research, Drottningholm, 37: 5-185.
- COFMANN, W.P., P.S. CRANSTON, D.R. OLIVER & O.A. SÆTHER. 1986. The pupae of Orthocladiinae (Diptera: Chironomidae) of the Holarctic region-Keys and diagnoses. In: Wiederholm, T. (Ed.). Chironomidae of the Holarctic region. Keys and diagnoses. Part 2-Pupae. *Entomologica Scandinavica, Supplement* 28: 147-296.
- CRANSTON, P.S., D.R. OLIVER & O.A. SÆTHER. 1989. The adult males of Orthocladiinae (Diptera, Chironomidae) of the Holarctic region – Keys and diagnoses. In: Wiederholm, T. (Ed.). Chironomidae of the Holarctic region. Keys and diagnoses. Part 3-Adult males. *Entomologica Scandinavica, Supplement* 34: 164-352.
- LANGTON, P.H. & P.S. CRANSTON. 1991. Pupae in nomenclature and identification: West Palaearctic Orthocladius s.str. (Diptera, Chironomidae) revised. Systematic Entomology, 16: 239-252.
- LANGTON, P.H. & L.C.V. PINDER. 2007. Keys to the adult males of Chironomidae of Britain and Ireland. Volume 1 (Pp 1-239) and volume 2 (Pp 1-68). Freshwater Biological Association, Scientific publication, N° 64.

- LINDEGAARD, C. 1995. Chironomidae (Diptera) of European cold springs and factors influencing their distribution. *Journal of the Kansas Entomological Society, Supplement* **68** (2): 108-131.
- MAGOGA, G., M. MONTAGNA, L. MARZIALLI & B. ROSSARO. 2017. Revision of type and non-type material assigned to the genus *Orthocladius* by Goetghebuer (1940–1950), deposited in the Royal Belgian Institute of Natural Sciences (Diptera, Chironomidae). *Acta Entomologica Musei Nationalis Pragae*, 57: 723-749.
- MOUBAYED-BREIL, J. & J. GARRIGUE. 2021. Orthocladius (Or.) massanus sp. n. a new crenophilous species inhabiting karstic springs in Eastern Pyrenees (Diptera, Chironomidae). Ephemera, 22 (1): 1-10.
- ROSSARO, B., V. LENCIONI & C. CASALEGNO. 2003. Revision of West Palaearctic species of Orthocladius s. str. van der Wulp, 1874 (Diptera, Chironomidae, Orthocladiinae), with a new key to species. Studi di Scienze Naturali-Acta Biologica, 79: 213-241.
- SÆTHER, O.A. 1980. Glossary of chironomid morphology terminology (Diptera, Chironomidae). Entomologica Scandinavica, Supplement 14: 1-51.
- SÆTHER, O.A. 2005. A new subgenus and new species of *Orthocladius* van der Wulp, with phylogenetic evaluation of the validity of the subgenera of the genus (Diptera, Chironomidae). *Zootaxa*, **974**: 1-56.
- SÆTHER, O.A. & M. SPIES. 2013. Fauna Europaea: Chironomidae. In P. Beuk & T. Pape (eds): Fauna Europaea: Diptera Nematocera. Fauna Europaea version 2.6. Internet data base at <u>http://www.faunaeur.org</u> (accessed December 2020).
- SOPONIS, A.R. 1983. Orthocladius (Orthocladius) ferringtoni, n. sp., from Kansas (Diptera, Chironomidae). Journal of the Kansas Entomological Society, 56 (4): 571-577.
- SOPONIS, A.R. 1990. A revision of the Nearctic species of *Orthocladius (Orthocladius)* Van der Wulp (Diptera: Chironomidae). *Memoirs of the Entomological Society of Canada*, Ottawa, **102**: 1-173.
- SPIES, M. & O.A. SÆTHER. 2004. Notes and recommendations on taxonomy and nomenclature of Chironomidae (Diptera). Zootaxa, 752: 1-90.