

# ***Orthocladius (Or.) massanus* sp. n. a new crenophilous species inhabiting karstic springs in Eastern Pyrenees [Diptera, Chironomidae]**

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Keywords: *Orthocladius massanus* sp. n., Diptera Chironomidae, Eastern Pyrenees, conservation.

Male adult and pupal exuviae of *Orthocladius (Or.) massanus* sp. n. are diagnosed and described based on material collected in a karstic spring delimited by the middle basin of the Massane River (altitude 350 m, Eastern Pyrenees, S-France). A combination of some atypical characters found in the male adult and pupal exuviae (unusual shape of anal point, virga, gonocoxite, inferior volsella and gonostylus; apical margin of thoracic horn undulated, PSB II conspicuous, conjunctives III/IV-V/VI with rows of chitinous blackish granules) allowed us to consider *O. massanus* sp. n. as a local biogeographic representative of the Eastern Pyrenees. Taxonomic remarks and comments on the ecology of the new species are provided.

***Orthocladius (Or.) massanus* sp. n., une nouvelle espèce crénophile connue de sources karstiques des Pyrénées-Orientales [Diptera, Chironomidae]**

Mots-Clés: *Orthocladius massanus* sp. n., Diptera Chironomidae, Pyrénées-Orientales, conservation.

L'adulte mâle et l'exuvie nymphale d'*Orthocladius (Or.) massanus* sp. n. sont décrits à partir d'un matériel collecté dans une source karstique délimitée par le bassin moyen de la rivière Massane (altitude 350 m, Pyrénées-Orientales). Une combinaison de certains caractères atypiques de l'adulte mâle et de l'exuvie nymphale (forme inhabituelle de la pointe anale, virga, gonocoxite, volselle inférieure et gonostyle; marge apicale de la corne thoracique ondulée, PSB II robuste, conjunctives III/IV-V/VI avec des rangées de granules chitineuses noirâtres) a permis de considérer *O. massanus* sp. n. comme un élément biogéographique local, représentatif des Pyrénées-Orientales. Des données taxonomiques et des commentaires sur l'écologie de la nouvelle espèce sont présentés.

## **1. Introduction**

At present, the genus *Orthocladius* (subgenus *Orthocladius*) includes about 62 valid species worldwide (GOETGHEBUER 1940-1950, THIENEMANN 1944, BRUNDIN 1947, 1956, TOKUNAGA 1964, SÆTHER 1980, ROSSARO 1982, COFFMAN et al. 1986, CRANSTON et al. 1989, SOPONIS 1990, LANGTON 1991, LANGTON & CRANSTON 1991, SASA & OKAZAWA 1992, LINDEGAARD 1995, CALDWELL 1998, ROSSARO & CASALEGNO 2001, ROSSARO et al. 2003, SPIES & SÆTHER. 2004,

SÆTHER 2005, LANGTON & PINDER 2007, ASHE & O'CONNOR 2012, SÆTHER & SPIES 2013, MOUBAYED-BREIL & ASHE 2016, MOUBAYED-BREIL 2020). Among them, 21 species are reported from Europe. This description increases the total number in the genus *Orthocladius* (*Orthocladius*) to 13 valid species from continental France.

In this paper, the male adult and pupal exuviae of *Orthocladius* (*Orthocladius*) *massanus* sp. n. are diagnosed and described based on material collected in a karstic spring delimited by the middle basin of the Massane River (altitude 350 m, Eastern Pyrenees, S-France). Based on some atypical characters found in the male adult and pupal exuviae (unusual shape of virga, inferior volsella and gonostylus; apical margin of thoracic horn with undulation, PSB II conspicuous, conjunctives III/IV-V/VI with rows of blackish granules) allowed us to consider *O. massanus* sp. n. as a local biogeographic representative of the Eastern Pyrenees.

## 2. Material and methods

The studied material was mainly collected using a Surber net (for the benthos, larvae and pupae), supplemented by 'Troubleau' and drift nets for pupal exuviae and drowned adults floating on the surface of the water. Flying adults were hunted by aerial sweep netting. Preserved male adult in 80% ethanol, was 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 effect or 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 holotype was mounted in polyvinyl lactophenol. Before the final slide mountings, the hypopygium including the tergite IX, the 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. Terminology and measurements follow those of SÆTHER (1980) and LANGTON & PINDER (2007) for male imago, and those of SÆTHER (op. cit.) and LANGTON (1991) for pupal exuviae.

## 3. *Orthocladius* (*Orthocladius*) *massanus* Moubayed-Breil, sp. n.

### Material examined

Continental France. Holotype. 1 male pharate adult; karstic springs bordering the bed of the Massane River, Eastern Pyrenees (42° 28' 41" N, 3° 01' 26" E), middle stream, altitude 350 m (Photo 1); 11.IV.2004, leg. J. Moubayed-Breil.

Holotype (mounted on 2 slides) is deposited in the collections of the MUSE-Museo delle Scienze, Corso del Lavoro e della Scienza 3, 38122 Trento, Italy.

**Etymology:** the new species is named *massanus* after the Massane Nature Reserve located in Eastern Pyrenees (S-France) where the type material was collected.

### Diagnostic characters

#### Male adult

On the basis of some unusual characters found in the male adult, *O. massanus* sp. n. appears to belong to a separate group of *Orthocladius* species. However, the new species can easily be distinguished by a combination of characters. Head. Temporals about 11-12; clypeus semi-circular; palpomer 3 lacking sensilla coeloconica; antenna 1290 µm long, last flagellomere 815 µm

long, AR 1.93. Thorax. Lobes of antepnotum weakly gaping; acrostichals 9-10 in 1 row; dorso-centrals 11 in 1 row; prealars 4; humeral pit indistinct; squama with 38-41 setae in 1-2 rows. Legs. Sensilla chaetica present on tarsomeres ta<sub>1</sub>-ta<sub>4</sub> of PI-PIII. Abdomen. Tergite IX broadly trapezoidal; posterior margin nearly straight, with 4 setae located close to base of anal point (2 on each side). Anal point triangular and sharply pointed apically, markedly wide at base, bearing about 16-17 setae including 7-8 located on dorso-median side and 8 placed laterally (4 on each side). Gonocoxite wider at base, narrowing distally with truncate apex; presence of strong sclerotization along the ventral side; inferior volsella distinctly contrasting, thumb-like shaped, much wider at base, parallel-sided, apex inwardly bent and inwardly projecting, basal area with 3 characteristic stout setae; virga consists of 2 atypical chitinous rings. Gonostylus is unusual *Orthocladius*-type; anterior side swollen medially, posterior margin straight; posteromedian area with 3 characteristic strong setae; crista dorsalis absent; 2 typical well-developed megasetae are present on pre-apical and apical parts, curved, subequal in length with pointed apex.

#### Pupal exuviae

Among the closest pupal exuviae of *Orthocladius* species to *O. massanus* sp. n. are those of: *O. excavatus* Brundin, 1947; *O. pedestris* Kieffer, 1909 and *O. rubicundus* (Meigen, 1818). However, the new species can be separated in having: frontal apotome with 2 semi-spherical tubercles, frontal seta 160 µm long; thorax covered with fine granulation on anteromedian and posteromedian areas; antepnotals 3 including 2 lateral and 1 median; 4 dorsocentrals consist of 3 stout setae (Dc<sub>1</sub>, Dc<sub>3</sub>, Dc<sub>4</sub>) and 1 bristle-like seta (Dc<sub>2</sub>); prealar 1; thoracic horn 430-450 µm long, densely armed with points, apical margin typically undulated. Abdomen. Transverse posterior margin of tergite II armed with 2-3 rows of hooks occupying about 75% of segment width; PSB II well-developed; posteromedian transverse rows of orally projecting pin-shaped to needle-like setae present on conjunctives III/IV-V/VI, occupying about 80 to 90% of segments; characteristic posteromedian transverse rows of blackish granules is restricted to conjunctives III/IV-V/VI; anteromedian and posteromedian areas of tergites III-V with large patches of points and small to medium sized spinules, becoming bigger toward the posterior margin; tergite VI with 2 characteristic patches of spinules, median one is cup-like shaped, posterior one dome-like; apex of anal lobe with a cluster of inwardly directed long spines. Anal lobe well-narrowed towards apices and ending with long inwardly curved spines. Macrosetae distinctly curved apically. Genital sac rounded apically, overreaching caudal margin of anal lobe by 45-50 µm.

### **Description**

#### Male adult

(n = 1, male pharate adult; Figs 1-9)

*Orthocladius* (*O.*) sp. 3: in MOUBAYED-BREIL & ASHE 2016; *Orthocladius* (*O.*) sp. 1: in MOUBAYED-BREIL 2020.

Small sized *Orthocladius* species. Total length 2.75 mm. Wing length 2.20 mm. TL/WL = 1.25. General colouration brownish with contrasting dark brown to blackish head, mesonotal strips, legs and anal segment. Head brownish with dark brown eyes and pedicels. Antenna brownish. Thorax contrasting blackish to brown; mesonotal strips and scutellum distinctly contrasting blackish to brownish. Wing pale brown. Legs brownish to blackish; femur, tibia and tarsomeres ta<sub>1</sub>-ta<sub>4</sub> of PI brownish; base of femur and tibia of PII-PIII blackish; claws of PI-PIII black. Anal segment contrasting brown to dark brown with a whitish posterior part of anal point.

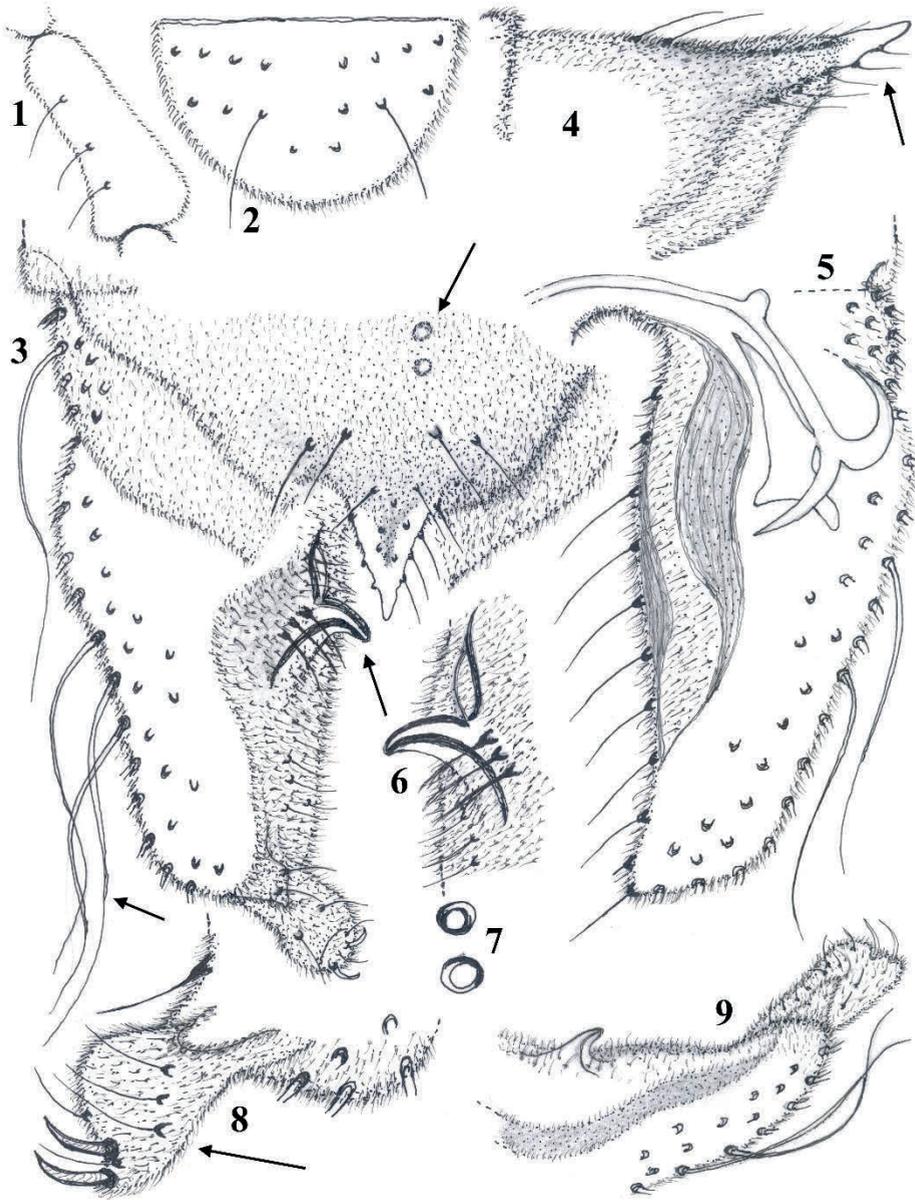
Head. Eyes bare, hairs absent on inner lateral margin; temporals consist of 11-12 including 8-9 inner and 3 outer verticals. Antenna 13-segmented, 1290  $\mu\text{m}$  long, linearly elongated; last flagellomere 815  $\mu\text{m}$  long, parallel-sided and weakly clubbed apically, with numerous apical sensillae chaetica; antennal groove clearly visible, beginning on segment 2-3 and reaching ultimate flagellomere; AR 1.93. Palp 5-segmented, segments 1-2 fused; length (in  $\mu\text{m}$ ) of segments: 155, 30, 90, 85, 110; palpomere 3 (Fig. 1) wider distally, with 3 sensilla clavata, sensilla coeloconica absent. Clypeus (Fig. 2) semi-circular, with 16 setae in 3 rows; with 3 sensilla clavata, sensilla coeloconica absent.

Thorax. Lobes of antepnotum weakly gaping; antepnotals 6; acrostichals 9-10 in one row; dorsocentrals 11 in one row; prealars 4; humeral pit indistinct; scutellum with 12 setae 150-160  $\mu\text{m}$  long, inserted in one row (6 on each side of the midline); preepisternum bare. Wing. Brachio-lum with 1 seta. Number and distribution of setae on veins: R, 15-16; remaining veins bare; squama with 38-41 setae in 1-2 rows. Legs. Tibial spurs present on PI-PIII; length (in  $\mu\text{m}$ ) of spurs: 80 (PI), 45, 50 (PII), 35, 90 (PIII); pseudospurs on PII ( $ta_1$ - $ta_2$  35-40  $\mu\text{m}$ ), pseudospurs on PIII ( $ta_1$ - $ta_2$  30-50  $\mu\text{m}$ ); sensilla chaetica present only on tarsomeres  $ta_1$ - $ta_4$  of PI-PIII. Length ( $\mu\text{m}$ ) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in the following table:

	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	860	905	660	415	355	230	155	0.73	2.10	2.67	1,6
PII	815	870	460	305	235	140	140	0.53	2.62	3.70	1,7
PIII	1035	1125	640	385	275	155	155	0.57	2.89	2.79	2,1

“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.”

Abdomen. Hypopygium as in Figures 3 (dorsal) and 5 (ventral, with tergite IX and anal point removed). Tergite IX 150-160  $\mu\text{m}$  long, about 300  $\mu\text{m}$  maximum width at base and 150  $\mu\text{m}$  at distal part, broadly trapezoidal, slightly narrowing distally, dorsal margin without hump, posterior margin nearly straight, with 4 setae located close to base of anal point (2 on each side). Anal point (Figs 3-4: 3, dorsal; 4, lateral) about 70  $\mu\text{m}$  long, 40 maximum width at base, reaching tip of inferior volsella, triangular and not pointed apically, with 16-17 setae including 7-8 located on dorso-median side and 8 placed laterally (4 on each side); microtrichia clearly visible in lateral view as in Fig. 4, basal part densely covered with microtrichia extending to the distal half area, dorsolateral and apical parts hyaline and bare. Laterosternite IX with 30-32 setae (15-16 on each side). Sternapodeme and phallapodeme (Fig. 5), transverse sternapodeme nearly straight and weakly projecting orally. Gonocoxite as in Figs 3, 5 and 9; 250  $\mu\text{m}$  long, 125  $\mu\text{m}$  maximum width at base of inferior volsella; distinctly wider at base, narrowing distally and distinctly truncate apically; dorsal side: inner margin (Fig. 3) linearly extended; presence of a cluster of setae on distal half, between inferior volsella and gonostylus; outer side (dorsal and ventral) with numerous long setae about 160  $\mu\text{m}$  long; ventral side (Figs 5, 9) with a large characteristic sclerotization clearly visible in ventral (Fig. 5) and lateral views (Fig. 9), inner ventral margin with 11 stout setae. Superior volsella unusually absent. Inferior volsella (Figs 3, 6) about 50  $\mu\text{m}$  long and 75  $\mu\text{m}$  maximum width at base; distinctly contrasting and atypically shaped, thumb-like much wider at base, parallel-sided toward apex and inwardly projecting; basal area with 3 characteristic stout setae.



Figures 1-9. Male imago of *Orthocladius (Or.) massanus* sp. n. Palpomère 3 (1); clypeus (2); hypopygium, dorsal (3); tergite IX and anal point in lateral view (4); hypopygium, ventral (5); inferior volsella (6); virga (7); gonostylus (8); gonocoxite and gonostylus, lateral (9).

The arrows indicate some distinguishing characters.

Figures 1-9. Imago mâle d'*Orthocladius (Or.) massanus* sp. n. Palpomère 3 (1); clypéus (2); hypopyge en vue dorsale (3); tergite IX et pointe anale en vue latérale (4); hypopyge en vue ventrale (5); virga (7); gonostylus (8); gonocoxite et gonostylus en vue latérale (9).

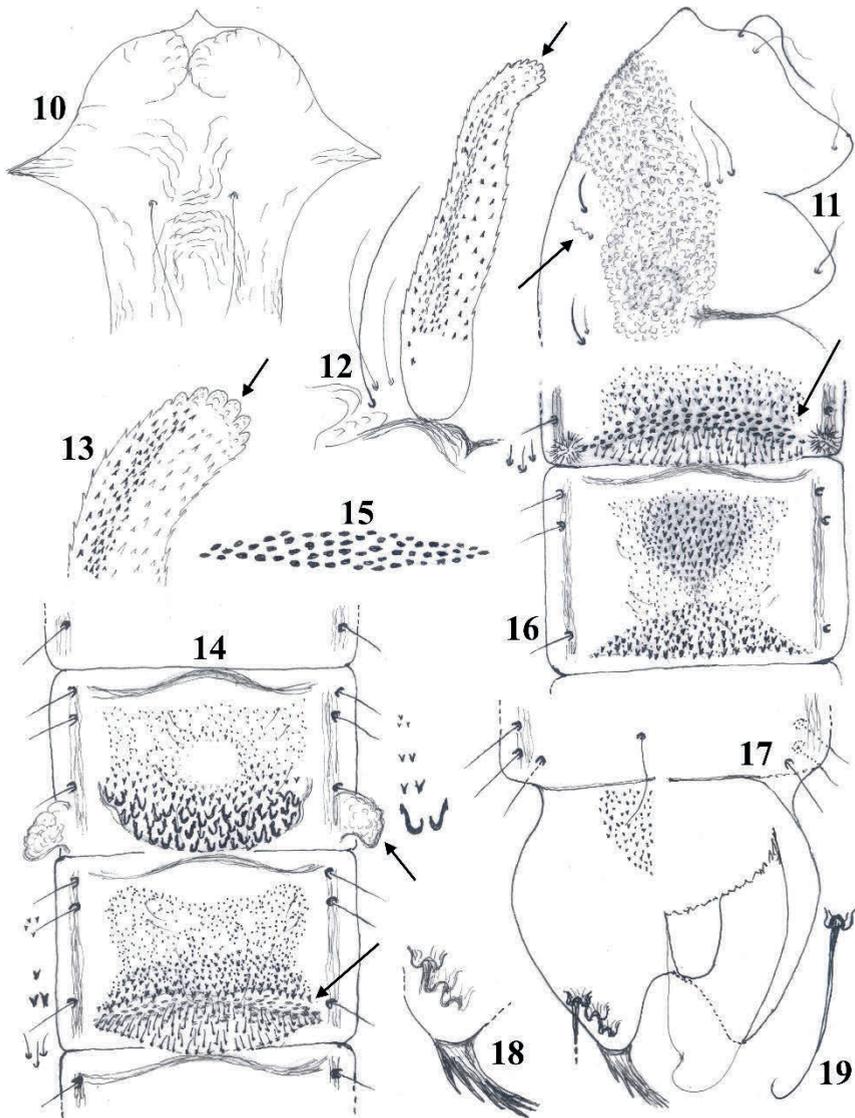
Les flèches indiquent quelques caractères discriminants.

Virga (Fig.7) consists of 2 atypical chitinous rings. Gonostylus (Figs 3, 8-9) 70  $\mu\text{m}$  long, 50  $\mu\text{m}$  maximum width, is unusual *Orthocladius*-type; anterior side markedly swollen medially, posterior margin straight; posteromedian area with 3 characteristic strong setae; crista dorsalis absent; megaseta well-developed, consists of 2 typical pre-apical and apical long and curved setae (15  $\mu\text{m}$  long), which are subequal and inwardly directed. HV (total length divided by length of gonostylus X 10) = 3.93; HR (length of gonocoxite divided by length of gonostylus) = 3.57.

#### Pupal exuviae

(n = 1; Figs 11-19)

Colouration in general golden to brownish. Frontal apotome slightly wrinkled on cephalic tubercles. Cephalothorax brownish, base of the wing sheath inwardly extended forming a blackish transverse shading. Abdomen including anal segment brownish; dark brown apophyses present on tergites II-VII, which are indistinct on tergites VI-VII and sternites; muscles marks distinctly conspicuous. Small sized species. Total length 2.85 mm; abdomen length 1.75. Frontal apotome (Fig. 10) strongly domed on anterior side, lateral margin projecting laterally with pointed apex; presence of semi-spherical tubercles; frontal setae 160  $\mu\text{m}$  long, inserted on prefrons ventral to antennal sheaths and separated by 25  $\mu\text{m}$ . Cephalothorax (Figs 10-11). Frontal apotome with 2 semi-circular tubercles. Anteprenotals 3 including 2 median anteprenotals (225 and 125  $\mu\text{m}$  long) and 1 lateral anteprenotal 120  $\mu\text{m}$  long; 1 prealar 115  $\mu\text{m}$  long; 4 dorsocentrals consist of 3 stout setae ( $\text{Dc}_1$ ,  $\text{Dc}_3$ ,  $\text{Dc}_4$ ) and 1 bristle-like seta ( $\text{Dc}_2$ ); length (in  $\mu\text{m}$ ) of dorsocentrals:  $\text{Dc}_1$  and  $\text{Dc}_4$  75 (subequal),  $\text{Dc}_2$  55,  $\text{Dc}_3$  60; distance between  $\text{Dc}_1$  to  $\text{Dc}_2$  ( $d_1$ , 12  $\mu\text{m}$ ),  $\text{Dc}_2$  to  $\text{Dc}_3$  ( $d_2$ , 145  $\mu\text{m}$ ),  $\text{Dc}_3$  to  $\text{Dc}_4$  ( $d_3$ , 4-5  $\mu\text{m}$ ). Thorax covered with fine granulation on both anteromedian and posteromedian areas including the thoracic suture, those on posteromedian area are reaching base of antennal sheath and dorsocentrals  $\text{Dc}_3$ - $\text{Dc}_4$ . Thoracic horn (Figs 12-13), 430-450  $\mu\text{m}$  long, wider at base and gradually narrowing distally, densely armed with points, toothed on both sides, apical margin distinctly and typically undulated, clearly visible on Fig. 13; precorneals differently sized, respectively 205, 165 and 75  $\mu\text{m}$  long. Abdomen. Armament and distribution pattern of shagreen, patches of spinules and points, chaetotaxy and lateral setation of abdominal segments as illustrated in Figs 14-17. Tergite I bare. Transverse posterior margin of tergite II (Fig. 14) armed with 2-3 rows of stout hooks, orally projecting and occupying about 75% of segment width. Anteromedian patches of shagreen and spinules present on tergites III-VI (Figs 14, 16) are sub-rectangular and laterally extensive (gradually more extensive laterally and almost reaching muscles marks), becoming typically cup to diamond-like shaped on tergite VI (Fig. 16); size of spinules of tergites III-V becoming gradually bigger toward the posterior margin. Posteromedian transverse patches of spinules present on tergites III-VI (Figs 14-15), is well-developed on tergite VI forming a separate typical dome (Fig. 16), which is weakly connected to anteromedian patch of spinules. Posteromedian transverse rows of orally projecting pin-shaped to needle-like setae are restricted to conjunctives III/IV and V/VI, occupying about 80 to 90% of segments; typical and characteristic posteromedian transverse rows of chitinous granulation present on conjunctives III/IV to V/VI (Figs 14-16), is clearly visible on Fig. 15. Sternites I-II bare. Distribution of lateral setae on segments I-VIII: I (2), II-VII (4 located 2 anteriorly and 2 posteriorly), VIII (5); anterior lateral setae on segments II-VII are similar or differently sized (anterior one occasionally shorter). Pedes spurii B on segment II well-developed; Pedes spurii A present on sternites IV-VII, is less distinct on VI and VII. Anal segment (Figs 17-18). Anal lobe 225  $\mu\text{m}$  long, 245  $\mu\text{m}$  maximum width, well-narrowed towards apices and ending with long inwardly curved spines. Macrosetae (Fig. 19) 65-70  $\mu\text{m}$  long, distinctly curved apically. Genital sac 175  $\mu\text{m}$  long, rounded apically, overreaching caudal margin of anal lobe by 45-50  $\mu\text{m}$ .



Figures 10-19. Male pupal exuviae of *Orthocladius* (*Or.*) *massanus* sp. n. Frontal apotome (10); cephalothorax (11); thoracic horn (12); apical part of thoracic horn (13); armament and chaetotaxy of abdominal segments II-III (14); rows of chitinous granules on conjunctive V-VI (15); caudal part of segment V and segment VI (16); caudal part of segment VIII and anal lobe (17); apex of anal lobe (18), macroseta (19).

The arrows indicate some distinguishing characters.

Figures 10-19. Exuvie nymphale mâle d'*Orthocladius* (*Or.*) *massanus* sp. n. Pièce frontale (10); céphalothorax (11); corne thoracique (12); partie apicale de la corne thoracique (13); ornementation et chaetotaxie des segments abdominaux II-III (14); rangées de granules chitineuses sur la conjonctive V-VI (15); partie postérieure du segment V et segment VI (16); partie postérieure du segment VIII et lobe anal (17); apex du lobe anal (18); macroseta (19). Les flèches indiquent quelques caractères discriminants.

## 4. Differential diagnosis

Based on some atypical characters found in the male adult (unusual shape of the inferior volsella, virga, gonocoxite and gonostylus), *O. massanus* sp. n. can be easily distinguished from other known *Orthocladus* (*Or.*) species. However, a similar vestigial and atypically shaped gonostylus is also observed in other genera of orthoclads, e.g.: *Chaetocladus britae* Säwedal, 1976 and *Eukiefferiella mirabilis* Serra-Tosio, 1983. Nevertheless, in the following differential diagnosis, only the pupal exuviae is treated due to some common and usual characters.

### Pupal exuviae

- Frontal apotome (Fig. 10) and granulation on thorax (Fig. 11), are differently shaped in *O. excavatus*, *O. pedestris* and *O. rubicundus*;

- Thoracic horn with atypical undulation on apical margin (Figs 12-13), is lacking such undulation in the 3 latter species (Figs 77B, 79F in LANGTON 1991; Fig. 1C in LANGTON & CRANSTON 1991; Figs 7, 21 in ROSSARO et al. 2003);

- Posteromedian rows of blackish rings on conjunctives III/IV to V/VI (Figs 14-16), differently figures and located in *O. excavatus*, *O. pedestris* and *O. rubicundus* (Figs 77C, 79G in LANGTON 1991; Fig. 2D in LANGTON & CRANSTON 1991; Fig. 1 in ROSSARO & CASALEGNO 2001).

**Female and larva:** unknown.

## 5. Ecology and geographical distribution

Larvae of *O. massanus* sp. n. are rheophilic and strictly confined to low-mountain springs extended along the middle basin of streams and rivers. Type material of the new species was collected in moderately shaded karstic helocrenes (permanent/temporary) with sandy to gravely substrata supplied by fresh underground water, which maintains lower annual variation of temperature. Type-locality (Photo 1) occasionally includes rocks and stones covered by submerged and emerged bryophytes and microalgae (composed in particular of dense populations of *Hildenbrandia rivularis*), which represents the favourable microhabitats for larval and pupal stages. Environmental data of water recorded along the upper rhithral of the Massane River are: calcareous water with high to moderate value of conductivity (125 to 300  $\mu\text{S}/\text{cm}$ ); pH 6.2-6.5; temperature 8-14°C. The biological and ecological quality of many karstic springs over the Mediterranean ecosystem in continental France is now heavily threatened and affected by increasingly greater risk of human activities (pollution, use of pesticides, ecotourism, canyoning, etc.). The new species belongs to the large community of crenobiontic and crenophilous species documented by LINDEGAARD (1995). Such threatened species (described after only one single male pharate adult) is believed to be a typical biological indicator of pristine helocrenes. It may be biogeographic representatives of global warming and local climate change, and therefore deserve greater consideration and conservation measures.

*O. massanus* sp. n. appears to belong to a typical Tyrrhenian element of lower helocrenes over the eastern side of the French Pyrenees. This highlights and indicates that it is likely more widespread in similar karstic springs located in southern Spain.

Associated species encountered in the same locality as *O. massanus* sp. n. include: *Paramerina vaillantii* Fittkau, 1962; *Diamesa hamaticornis* Kieffer, 1924; *D. insignipes* Kieffer, 1908; *Potthastia dominicii* Moubayed-Breil & Orsini, 2016; *Chaetocladus acuticornis* (Kieffer, 1914);

*C. melaleucus* (Meigen, 1818); *Eukiefferiella brulini* Moubayed-Breil & Ashe, 2015; *E. coerulescens* (Kieffer, 1926); *E. gracei* (Edwards, 1929); *Orthocladius pedestris* Kieffer, 1909; *O. rivulorum* Kieffer, 1909; *O. saxosus* (Tokunaga, 1939); *O. thienemanni* Kieffer, 1906; *O. vaillanti* Langton & Cranston, 1991; *Parakiefferiella pyrenaica* Moubayed, 1991; *Parametriocnemus valescurensis* Moubayed & Langton, 1999; *Paraphaenocladius pseudirritus* Strenzke, 1950; *Paratrachocladius rufiventris* (Meigen, 1830); *Rheocricotopus effusus* (Walker, 1856); *R. meridionalis* Moubayed-Breil, 2016; *R. thomasi* Moubayed-Breil, 2016; *R. tirolus* Lehman, 1969; *Thienemanniella clavicornis* (Kieffer, 1911); *Micropsectra andalusiaca* Marcuzzi, 1950.



Photo 1. Type-locality of *Orthocladius* (*Or.*) *massanus* sp. n. Arrow shows karstic lateral spring.  
Photo J. Garrigue.

Photo 1. Localité type d'*Orthocladius* (*Or.*) *massanus* sp. n. La flèche indique une source latérale.  
Cliché J. Garrigue.

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