Chaetocladius berythensis sp. n., C. callauensis sp. n., C. guardiolei sp. n. and C. parerai sp. n., four relict species inhabiting glacial springs and streams in eastern Pyrenees and Lebanon (Diptera: Chironomidae)

Joel Moubayed1 and Peter H. Langton2

¹ Freshwater & Marine Biology, 10 rue des Fenouils, F-34070 Montpellier, France.

E-mail: joelmb34@free.fr. corresponding author

²University Museum of Zoology Cambridge, Downing Street, Cambridge, UK CB2 3EJ.

Address for correspondence: 16 Irish Society Court, Coleraine, Co. Derry, BT52 IGX, Northern Ireland.

E-mail: langtonph@gmail.com

http://zoobank.org/8977CB47-5899-4241-ABB6-BA8A90D9FCFA

Abstract

Four new species of the genus Chaetocladius s. str. (C. berythensis sp. n., C. callauensis sp. n., C. guardiolei sp. n. and C. parerai sp. n.) are diagnosed and described based on material collected in some glacial springs and small streams located at high altitude (1800-2300 m). While C. berythensis sp. n. is described only as male adult from the upper basin of the Beirut River (Lebanon Mount, Western range, Mount Sannine), C. callauensis sp. n. C. guardiolei sp. n. and C. parerai sp. n. are described as male adult accompanied by descriptions of tentatively associated male pupal exuviae from the upper basin of the Mantet River and Soques stream (eastern Pyrenees, West France). Additional taxonomic notes including illustrations and brief descriptions of 10 taxa/species based on male adults, male pharate adults and pupal exuviae collected in glacial springs, peat bogs and streams located at high altitude are provided. On the basis of characters in the male adult, only C. berythensis sp. n. keys to the laminatus-group, which is reported for the first time from the Levantine sub-region. Based on the unusual shape of both inferior volsella and gonostylus, the four new Chaetocladius species each belong to local biogeographic elements: C. berythensis sp. n. to a 'Levantine element', while C. callauensis sp. n., C. guardiolei sp. n. and C. parerai sp. n. belong to a 'Pyrenean element'. All of the four new species are considered to be relict representatives of glacial helocrenes and cold stenothermic streams. Such species may be biological indicators and biogeographic representatives of global warming and local climate change.

Introduction

Knowledge on the taxonomy, geographical distribution and ecology of the known *Chaetocladius* s. str. species from Europe and neighbouring areas

are included in Goetghebuer (1940-1950), Brundin (1947, 1956), Pankratova (1970), Caspers (1987), Cranston et al. (1989), Moubayed (1989), Sæther (1990), Langton (1991), Makarchenko & Makarchenko (2001, 2003, 2004, 2006a-b, 2007, 2009, 2011a-b, 2013a-b, 2018), Langton & Pinder (2007), Zelentsov (2007), Langton & Armitage (2010), Stur & Spies (2011), Ashe & O'Connor (2012), Kobayashi (2012); Wang et al. (2012); Sæther & Spies (2013), Makarchenko et al. (2014), Moubayed-Breil (2017), Moubayed-Breil & Dia (2017), Rossaro et al. (2017), Moubayed-Breil & Lods-Crozet (2017), Makarchenko et al. (2018), Moubayed-Breil & Bitusik (2019). Worldwide, the genus Chaetocladius Kieffer, 1911 comprises about 75 species of which, only about 42 are reported from Europe.

In this paper, we provide diagnoses and descriptions of four new species of the genus Chaetocladius s. str.: C. berythensis sp. n., C. callauensis sp. n., C. guardiolei sp. n. and C. parerai sp. n. The descriptions are based on material composed of male adults and male pharate adults collected in some glacial springs and small streams located at high altitude (1800-2300 m). Chaetocladius berythensis sp. n. is described only as male adult collected in the upper basin of the Beirut River (Mount Lebanon, Western range, Mount Sannine), while C. callauensis sp. n., C. guardiolei sp. n. and C. parerai sp. n. are described as male adult and their tentatively associated male pupal exuviae occurring in the upper basin of the Mantet River and Soques stream (eastern Pyrenees, SW France). Additional taxonomic notes including illustrations and brief descriptions of ten taxa/species composed of six named species and four morphotypes are also given. These are based on male adults, male pharate adults and pupal exuviae collected between 1996 and 2013 in glacial mountain springs, peat bogs and streams.

Material and methods

Material composed of adults, pharate adults and pupal exuviae belonging to both C. berythensis sp. n. and C. callauensis sp. n. was collected using standard methods: Surber net for the benthos (larvae and pupae); Brundin drift nets for pharates, pupae and drifted pupal exuviae; troubleau net for individuals floating on the surface of the water; sweep net for flying adults. Material of male adults was preserved in 80-85% ethanol, then cleared of musculature in 90% lactic acid (head, thorax, abdomen and anal segment) for 60 to 80 minutes, but 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 in dorsal view, 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. In particular, the ventral view of hypopygium was illustrated omitting the anal point and tergite IX. For a better examination of the specific features and more accurate description of the various taxonomic details of the pupa, the pupal abdomen was mounted not only in dorsal and ventral view, but separately in lateral view, which facilitates proper examination and illustration of all the relevant taxonomic characters.

The proximal part of the abdomen and the halteres of the male adult are preserved in 85% ethanol for an eventual DNA analysis to be done. Morphological terminology and measurements follow those of Sæther (1980, 1985), Langton (1991) and Langton & Pinder (2007) for the imagines and pupal exuviae.

Results and descriptions

Chaetocladius berythensis sp. n.

http://zoobank.org/12387DA9-9A16-45B7-8E4E-06DAA726D9A2

Material examined. Holotype. 1 male adult, Lebanon, Mount Sannine, glacial springs and small waterfalls located in the upper stream of the Beirut River, 33° 95' 00" N, 35° 88' 00" E, altitude 1800-2000 m a.s.l., 05.VIII.2005, leg. J. Moubayed. Water calcareous, conductivity about 320 μS/cm;

temperature 8-10 °C during summer, about 4-6 °C in winter and spring.

Paratype Lebanon. 1 male adult, leg. J. Moubayed-Breil. Same locality and date as for holotype.

Holotype (male adult, mounted on 1 slide) is deposited in the collections of the National Museum of Ireland, Kildare Street, Dublin 2, Ireland. Paratype is deposited in the collection of the senior author.

Etymology. The new species is named 'berythensis' after the name in Greek 'Berythus', which has given the name to the river Beirut.

Diagnostic characters

Based on the unusual shape of both virga and inferior volsella C. berythensis sp. n. keys to the laminatus-group as emended in Moubayed-Breil (2017). However, the new species can be separated from other related Chaetocladius species by the following combination of characters. Clypeus broadly cup-shaped with semi-circular median lobe distally, with 14 setae in 4 rows; palpomere 3 with 5 sensilla clavata and 11-12 short and truncate atypical sensilla coeloconica. Lobes of antepronotum widely gaping and parallel-sided medially; humeral pit ellipsoid without contrasting spots. Tergite IX with a truncate hump medially, clearly seen in lateral view; anal point triangular and sharply pointed. Virga consists of 3 separate parts including a median part (bearing 4 small equal spines) and 2 lateral symmetrically elongated parts (each with 1 orally directed claw medially). Gonocoxite rounded apically; inferior volsella large marsupial pouch-like lobe, bearing a contrasting protuberance proximally. Gonostylus massive, nearly rectangular, markedly projecting posteriorly at a rounded bare and hyaline apex; anterior margin sinuous, bearing 6-7 long setae; both dorsal and ventral median area with a distinct curved row of short spines reaching the base of megaseta. Crista dorsalis moderately to well-developed occupying about the length of the gonostylus, clearly visible in dorsal, ventral and lateral view; consisting of a series of smooth lobes which are lower proximally and terminates in a larger tooth located close to the megaseta.

Description

Male adult

(n = 2 male; Figs 1A-N, 2A-C; associated material unknown)

Large. Total length 4.00-4.10 mm. Wing length 2.35-2.40 mm, TL/WL = 1.70. General colouration contrasting brown to blackish. Head dark brown;

antennae pale brown; thorax contrasting brown to dark brown, mesonotal stripes blackish; wing pale; legs brown to dark brown; tergites I-VII brownish, tergite VIII and anal segment dark brown.

Head. Eyes bare between ommatidia, hairs absent on inner lateral eye margin, outer posterior margin with a few short setae. 12 temporals including 7 inner (uniserial), 3 outer verticals and 2 postorbitals. Coronal area (Fig. 1A) with a distinct median tubercle. Palp 5-segmented, first and second segments are fused and unequal, segments 3 and 4 subequal; length (µm) of palpomeres: 18, 45, 145, 147, 235; palpomere 3 (Figs 1B-C) with 5 sensilla clavata and 11-12 unusually short and truncate atypical sensilla coeloconica. Clypeus (Fig. 1D) about 115 µm maximum height and 140 maximum width, broadly cup-shaped with median semi-circular lobe distally, with 14 setae in 4 rows. Antenna 1100-1130 µm long, 13-segmented; ultimate flagellomere 675 µm long, distinctly clubbed distally and bearing a dense brush of curved sensilla chaetica apically, pre-apical seta absent; antennal groove beginning on segments 3-4 and reaching ultimate flagellomere; AR 1.80. Thorax. Lobes of antepronotum (Fig. 1E), distinctly gaping and moderately thick medially, ventral part with 6 lateral antepronotals placed near the lower margin; humeral pit (Fig. 1F) ovoid and lacking contrasting spots; 23 short acrostichals located about 110 µm from antepronotum; dorsocentrals 14 in 1-2 rows; prealars 5 in 1 row; humeral pit ovoid to semi-circular, contrasting whitish to brown and lacking spots. Wing. Brachiolum with 1 seta; membrane densely covered with coarse punctuation; number of setae on veins: R, 0; R₁, 17; R₂₊₃, 1; remaining veins bare; squama with 14-15 setae in 1 row. Legs. Tarsomeres ta, of PI and PII equal in length (185 µm) as well for ta_s of PI and PIII (140 µm); tibial spur of PI spiniform. Length (µm) of tibial spurs of: PI, 65; PII, 35 and 25; PIII, 70 and 30; longest seta of tibial comb 50 µm long. A few sensilla chaetica present (proximally and distally) on tibiae and tarsomeres ta,-ta, of PI-PIII. Length (µm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in Table 1.

Hypopygium in dorsal view (Fig. 1I), ventral view

with tergite IX and anal point omitted as in Fig. 1J. Tergite IX broadly semi-circular, with a distinct dorsal truncate hump medially which is visible when tergite IX is viewed laterally (Figs 1G-H), dorsal setae (Figs 1I, 2A) include 15 located on the antero-median part and 18-20 postero-medially close to the posterior margin (9-10, in 2 curved rows placed on each side of the base of anal point). Anal point (Figs 1G-H, 1I, 2A) 55-60 µm long, 35-40 μm maximum width at base, triangular, sharply pointed apically and markedly wider at base, with bare and hyaline apex; 8-9 setae present both dorsally and laterally on anal point base. Latero-sternite IX with 10-11 setae inserted laterally (5-6 on each side). Sternapodeme and phallapodeme Fig. 1J, phallapodeme sickle-shaped anteriorly. Virga (Fig. 1I) consists of 3 separate parts which include: a median part with 4 small sub-equal teeth, 2 lateral parts which are elongated and bear 1 median claw-like projection orally directed. Gonocoxite (Figs 1I-J, 1L) 160 μm long and 125 μm maximum width, rounded apically, ventral margin slightly swollen basally, with 9 stout setae; inferior volsella in dorsal (Figs 1I, K) and lateral view (Fig. 1L) with a wide marsupial pouch-like lobe, and apical contrasting protuberance located proximally. Gonostylus (Figs 1M, dorsal; 1N, lateral; 2B, ventral) 110 µm long and 65-70 µm maximum width, massively rectangular, markedly projecting posteriorly at a rounded bare and hyaline apex; anterior margin sinuous, bearing 6-7 long setae; dorsal and ventral median area with a distinct curved row of short spines reaching the base of the megaseta. Crista dorsalis moderately developed occupying nearly the length of the gonostylus, clearly visible in dorsal, ventral and lateral view, of a series of smooth lobes, which are lower proximally and ending with a larger distal tooth located close to the megaseta.

Chaetocladius callauensis sp. n.

<u>http://zoobank.org/8A3665D5-6D2A-461E-AC81-</u> AC15EFAA376C

Material examined. Holotype. 1 male adult, France, eastern Pyrenees, Mantet Nature Reserve, upper basin of the River Mantet, Ressec glacial stream, Callau acid helocrenes springs and peat

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

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
PI	1030	1120	720	385	300	185	140	0.64	2.84	2.99	1.40
PII	1020	1000	450	225	210	185	130	0.45	3.29	4.49	2.40
PIII	1150	1185	670	370	300	165	140	0.57	3.08	3.49	2.20

bogs, 42° 28' 38" N, 02° 18' 26" E, altitude 2000-2300 m a.s.l., 05.VIII.2010, leg. J. Moubayed. Water crystalline, conductivity 30-40 μ S/cm, pH 5.5-5.7: temperature 8-10 °C. during summer. about

3-5 °C in winter and spring.

Paratypes. 3 tentatively associated pupal exuviae (2 males and 1 female), same locality and date

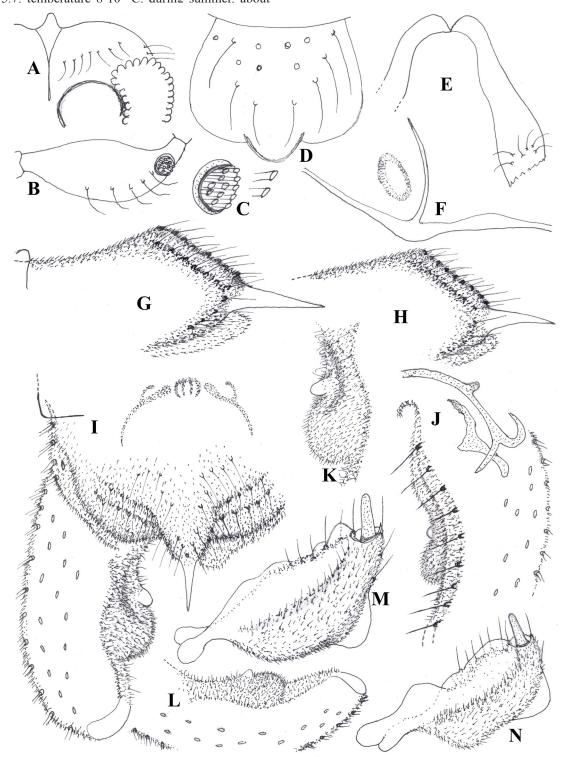


Figure 1. Male adult of *Chaetocladius berythensis* sp. n. A) head, frontal area (right side) with temporal setae; B) palpomere 3 with sensilla clavata and sensilla coeloconica; C) details of sensilla coeloconica; D) clypeus; E) lobes of antepronotum; F) humeral pit; G-H) two aspects of anal point and tergite IX in lateral view; I) hypopygium, dorsal; J) hypopygium, ventral; K) right inferior volsella; L) gonocoxite and inferior volsella, lateral; M) gonostylus, dorsal; N) gonostylus, lateral.

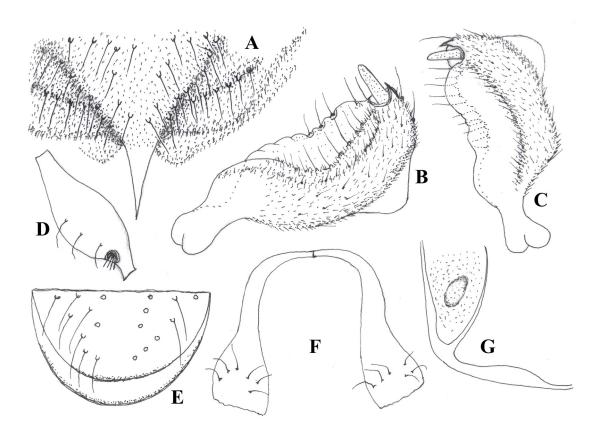


Figure 2. Male adult of *Chaetocladius* spp. *C. berythensis* sp. n.: A) anal point, dorsal; B) gonostylus, ventral; C) gonostylus, lateral. *C. callauensis* sp. n.: D) palpomere 3; E) clypeus; F) lobes of antepronotum; G) humeral pit.

as for holotype; 1 male adult, 2 tentatively associated male pupal exuviae, Font des Soques glacial spring and stream at Mantet Nature Reserve. 1 male adult and 1 male pharate adult, alt. 2000-2100 m, 05.08.2010, leg. J. Moubayed-Breil. Water crystalline, conductivity 20-30 μ S/cm, pH 5.5-5.7; temperature 8-10 °C during summer period.

Holotype (male adult mounted on 1 slide) is deposited in the collections of the National Museum of Ireland, Kildare Street, Dublin 2, Ireland. Paratypes are deposited in the senior author's collection

Etymology. The new species is named 'callauensis' after the protected glacial helocrene springs and peat bogs area of Mantet Nature Reserve, which is located at high altitude (2000-2300 m) in the Eastern Pyrenees (SW-France) where the type material was collected.

Diagnostic characters

Based on the atypical shape of the inferior volsella *C. callauensis* sp. n. appears to belong to a separate group within the genus *Chaetocladius*. However, this new species is also distinguished from

other known Chaetocladius species in having: semi-circular clypeus with 2 distal margins; lobes of antepronotum not gaping, distinctly thinner and parallel-sided medially; humeral pit ellipsoidal, surrounded by dense contrasting brownish granulation; tergite IX without dorsal hump; virga weakly-developed, consisting of 2 sinuous fine spines; gonocoxite rounded apically, ventral margin with 2 broad lobes; inferior volsella composed of 2 subequal parts, proximal one rectangular, consisting of a contrasting smooth lobe which is hyaline and bare, distal one rounded and densely covered with setae; gonostylus slender, thinner proximally, bulbous and thicker in its distal half, posterior margin rounded; crista dorsalis wide, lower proximally, becoming higher and more conspicuous close to the megaseta.

Description

Male adult

(n = 3; Figs 2D-G, 3A-G)

Large. Total length 3.90-4.00 mm. Wing length 2.70-2.75 mm; TL/WL 1.07. General colouration brown to dark brown. Head and antennae brown;

thorax contrasting brown to dark brown, mesonotal stripes distinctly dark brown, humeral pit brownish with contrasting granulation; wing pale; legs brown; tergites I-VIII brownish, anal segment brown to dark brown.

Head. Eyes bare between ommatidia, hairs absent on inner lateral eye margin, a few short setae present on outer posterior margin. Temporals consist of 11 setae including 9 inner and 2 outer verticals; palpomere 3 (Fig. 2D) with 3 sensilla clavata and 4 sensilla coeloconica; clypeus (Fig. 2E) 115 μm long, and 210 µm maximum width, semi-circular, bearing 18 setae in 4-5 rows. Antenna 1100 µm long; last flagellomere 565 µm long, clubbed distally, covered with a dense brush of curved sensilla chaetica apically, pre-apical seta absent; antennal groove beginning on segment 2 and reaching ultimate flagellomere; AR 1.06. Thorax. Anteprontum (Fig. 2F), lobes weakly-developed at base and not gaping, distinctly thinner and parallel-sided medially, with 6 lateral antepronotals; acrostichals 16-17; dorsocentrals 12-13; prealars 4-5; humeral pit (Fig. 2G) ellipsoid, surrounded by dense contrasting granulation. Wing. Brachiolum with 1 seta; membrane densely covered with coarse punctuation; number of setae on veins: R, 7; R₁, 10; remaining veins bare; squama with 19-23 setae in 1 row. Legs. Tibial spur of PI spiniform. Length (μm) of tibial spurs of: PI, 65; PII, 35 and 20; PIII, 70 and 30; longest seta of tibial comb 50 µm long. Sensilla chaetica few (proximally and distally) on tibia and tarsomeres ta,-ta, of PI-PIII. Length (µm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in Table 2.

Hypopygium as in dorsal (Fig. 3A) and lateral view (Fig. 3F), ventral view with tergite IX and anal point omitted as in Fig. 3 B. Tergite IX broadly semi-circular, wider at base and narrowing posteriorly up to its 1/4th distal part; dorsal setae 26-28 include 10-12 located above the base of anal point and 14-16 close to the posterior margin (located in in 2 curved rows and placed on each side of the base of anal point); dorsal hump absent (Fig. 3G). Anal point in dorsal (Figs 3A, C) and lateral view (Fig. 3G) 75 μm long, about 135 μm maximum width at base, markedly wider at base, parallel-sided in its distal part and rounded apically, bare and hyaline part about 25 μm long; 8-9 setae are

present both dorsally and laterally. Latero-sternite IX with 11 setae inserted laterally (5-6 on each side). Sternapodeme and phallapodeme (Fig. 3B), phallapodeme distinctly wider anteriorly. Virga (Fig. 3A) weakly-developed, consists of 2 sinuous spines about 25 µm long. Gonocoxite (Figs 3A-B, F) 300-330 μm long and 125 μm maximum width, rounded apically, ventral margin bi-lobed, with 10 stout setae; inferior volsella in dorsal (Fig. 3A) and lateral view (Fig. 3F) well-developed, composed of 2 sub-equal lobes, proximal one rectangular and contrasting, smooth on its inner part which is hyaline and bare, distal lobe densely covered with short setae. Gonostylus at acute angle (Fig. 3D) and at right angle (Fig. 3E) 155 µm long and 40 um maximum width, slender, thinner proximally, becoming bulbous and thicker in its distal half, posterior margin rounded; megaseta about 12 µm long; crista dorsalis extending from proximal part of gonostylus to the megaseta, a wide lobe proximally, becoming higher and more conspicuous in its distal half close to the megaseta.

Chaetocladius guardiolei sp. n.

http://zoobank.org/1792C721-E5BC-464D-AAA1-70637DA544E1

Material examined. Holotype. 1 male adult, France, eastern Pyrenees, Prats-De-Mollo Nature Reserve, upper basin of the Tech River, altitude 1800-2000 m a.s.l., 05.VI.2000, leg. J. Moubayed. Water siliceous, conductivity 40-50 µS/cm; pH 5.3-5.5; temperature 6-12 °C.

Paratypes. 1 male adult, as holotype except upper basin of Mantet River, Callau glacial springs and peat bogs, 05.08.2008; 2 tentatively associated male pupal exuviae, same locality and date as for holotype.

Holotype (male adult, on 1 slide) is deposited in the collections of the National Museum of Ireland, Kildare Street, Dublin 2, Ireland. The paratypes is deposited in the collection of the senior author.

Etymology. The new species is named 'guardiolei' in honour to Olivier Guardiole, who is still active as an 'Assistant-Curator' at Prats-De-Mollo Nature Reserve (Eastern Pyrenees) in contributing to preserving the aquatic environment and species confined to the preserved area.

Table 2. Male adult of *Chaetocladius caullauensis* sp. n. Length (μ m) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs.

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
PI	970	1085	760	510	360	250	165	0.70	2.19	2.70	1.20
PII	1050	1060	540	350	270	190	160	0.51	2.73	3.91	1.30
PIII	1120	1230	830	440	320	215	165	0.68	2.79	2.83	1.60

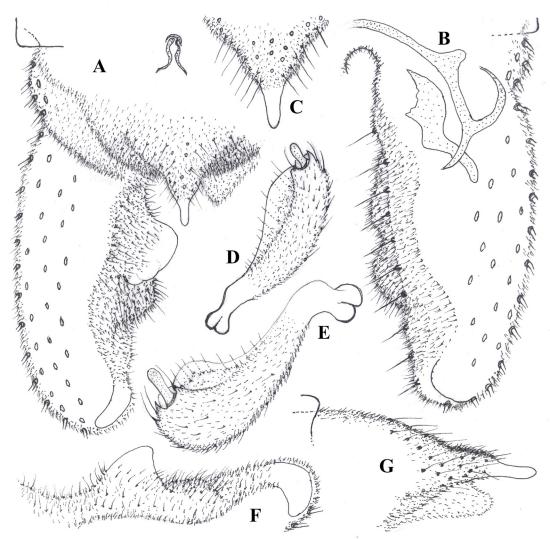


Figure 3. Male adult of *Chaetocladius callauensis* sp. n. A) hypopygium, dorsal; B) hypopygium, ventral; C) anal point, dorsal; D) gonostylus, acute angle; E) gonostylus, obtuse angle; F) gonocoxite and inferior volsella, lateral; G) anal point and tergite IX in lateral view.

Diagnostic characters

Based on the shape of the inferior volsella C. guardiolei sp. n. appears to key close to the following species: C. suecicus (Kieffer, 1916), C. longivirgatus Stur & Spies, 2011 and C. subalpinus Rossaro, Magoga & Montagna, 2017. However, this new species can be distinguished from related members of Chaetocladius species in having: clypeus circular; lobes of antepronotum well gaping, distinctly thicker medially; humeral pit ellipsoidal, bearing contrasting brownish granulation; tergite IX with a rounded dorsal hump; anal point triangular and sharply pointed apically, basal part cup-shaped with well-sclerotized lateral margin; virga welldeveloped, consisting of several long fine spines; inferior volsella well-developed, composed of 2 contrasting parts including a long finger-like expansion and a semi-circular small pouch-like lobe;

gonostylus slender and thinner proximally, becoming bulbous distally; crista dorsalis absent; megaseta well-developed.

Description

Male adult

(n = 2; Figs 4A-J)

Large. Total length 4.00-4.10 mm. Wing length 2.35-2.45 mm; TL/WL 1.70. General colouration dark brown to blackish. Head and antenna dark brown; thorax contrasting dark brown to blackish, mesonotal stripes blackish; humeral pit brownish with contrasting dark spots; legs dark brown; membrane of wing pale brown, veins and squamal area dark brown; tergites I-VIII brownish, anal segment dark brown.

Head. Eyes bare between ommatidia; temporals of 10 inner verticals, outer verticals absent; palpomere 3 (Fig. 4A) with 4 sensilla clavata and 4 sensilla coeloconica; clypeus (Fig. 4B) nearly circular, with 7 setae in 3 rows. Antenna 1000 µm long; last flagellomere 560 µm long, slightly clubbed distally, narrowing apically with numerous curved sensilla chaetica, pre-apical seta absent; antennal groove beginning on segment 3 and reaching ultimate flagellomere; AR 1.27. Thorax. Anteprontum (Fig. 4C) well-developed, lobes distinctly gaping and wider medially, with 6 lateral antepronotals; acrostichals 23-25 in 1-2 rows; dorsocentrals 14-15 in 1-2 rows; prealars 6 in 1 row; humeral pit (Figs 4D-E) ellipsoid, with contrasting dark spots. Wing. Brachiolum with 1 seta; membrane densely covered with coarse punctuation; number of setae on veins: R, 12; R₁, 1; remaining veins bare; squama with 9 setae in 1 row. Legs. Tarsomeres ta and ta₅ of PII sub-equal; sensilla chaetica present on: tibia and tarsomeres ta,-ta, of PI, tibia and tarsomeres ta,-ta, of PII-PIII.

Hypopygium. Tergite IX and anal point (dorsal) with sternapodeme and phallapodeme as in Fig. 4F; tergite IX broadly trapezoidal, wider at base with rectangular distal half; 16-18 dorsal setae including 10-11 located laterally on each side of the base of anal point and 6-7 close to the base of anal point; dorsal hump semi-circular, clearly visible in lateral view (Fig. 4G). Anal point in dorsal (Fig. 4F) and lateral view (Fig. 4G) 85 µm long, 40-45 µm maximum width at base, cup-shaped at base, distal half triangular and sharply pointed apically, lateral margin of basal part well-sclerotized, distal part (about 35 µm long) bare and hyaline; 6-7 setae present on both dorsal and lateral sides. Latero-sternite IX with 10-11 setae inserted laterally (5-6 on each side). Sternapodeme and phallapodeme (Fig. 4F), phallapodeme distinctly wider anteriorly. Virga (Figs 4F, I) well-developed, consists of 6-7 long spines about 30-40 µm long. Gonocoxite swollen basally and rounded apically: ventral margin bearing a weakly elongated lobe and 10 stout setae. Inferior volsella (Fig. 4H) welldeveloped, composed of a triangular lobe which ends in a finger-like hyaline and bare expansion; distal lobe small pouch-like, densely covered with

setae. Gonostylus in dorsolateral view as in Fig. 4J, slender, thinner proximally, becoming bulbous and thicker in its distal half, posterior margin distinctly rounded; anteriorly with 2-3 rows of setae; crista dorsalis absent; megaseta about 18 μ m long, well-developed.

Chaetocladius parerai sp. n.

http://zoobank.org/7D2E5D1E-12A5-47E6-8877-C095BCEFF523

Material examined. Holotype. 1 male adult, France, eastern Pyrenees, Mantet Nature Reserve, Soques glacial springs and stream, 42° 28' 38" N, 02° 18' 26" E, altitude 2000 m a.s.l., 05.VIII.2008 leg. J. Moubayed. Water crystalline, conductivity 30-40 μS/cm, pH 5.5-5.7; temperature 8-10 °C, during summer, about 3-5 °C in winter and spring.

Paratypes. 1 male adult, same locality as for holotype; 3 tentatively associated male pupal exuviae, same locality and date as for holotype.

Holotype (male adult mounted on 1 slide) is deposited in the collections of the National Museum of Ireland, Kildare Street, Dublin 2, Ireland. Paratypes are deposited in the senior author's collection.

Etymology. The new species is named 'parerai' in honour to Josep Parera, who is still active as an 'Assistant-Curator' at the Mantet-Py Nature Reserve (Eastern Pyrenees) in contributing to preserving the aquatic environment and species confined to the preserved area.

Diagnostic characters

C. parerai sp. n. keys close to C. guardiolei sp. n. based, in particular, on a similarly shaped inferior volsella. However, this new species is easily separated in having: lobes of antepronotum thinner at apex and gaping; humeral pit ovoid with contrasting brown granulation; tergite IX with a weakly rounded dorsal hump; anal point triangular, wider at base and parallel-sided distally, with rounded apex; virga well-developed, consists of 5 curved fine spines; inferior volsella well-developed and distinctly-contrasting, basal part long finger-like, bent downwards, caudal part a large semi-circular lobe densely covered with short setae; gonostylus

Table 3. Male adult of *Chaetocladius guardiolei* sp. n. Length (μm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs.

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
PI	825	870	560	345	240	160	115	0.64	2.62	3.03	2.55
PII	785	930	375	240	195	125	120	0.40	3.07	4.57	3.30
PIII	1120	1045	605	340	285	155	120	0.58	2.97	3.41	3.65

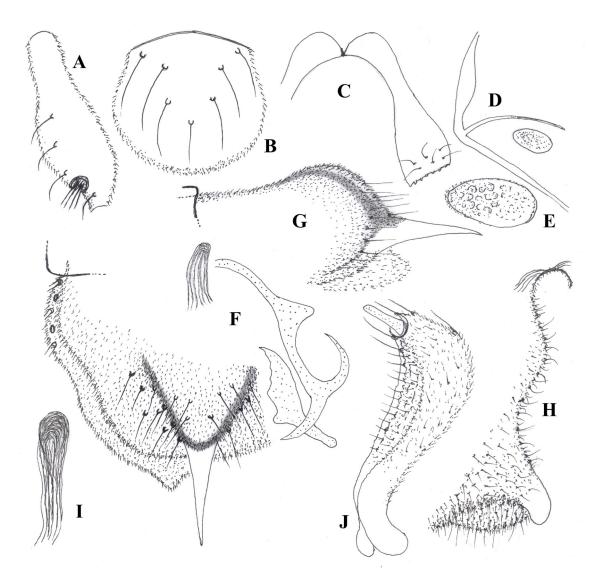


Figure 4. Male adult of *Chaetocladius guardiolei* sp. n. A) palpomere 3; B) clypeus; C) lobes of antepronotum; D-E) humeral pit, two aspects; F) hypopygium, anal segment and apodemes; G) anal point and tergite IX in lateral view; H) inferior volsella; I) virga, another aspect; J) gonostylus, dorsolateral.

slender, thin proximally, becoming much thicker distally, posterior margin with a small rounded and bare apical expansion; crista dorsalis absent; megaseta well-developed.

Description

Male adult

(n = 1, Figs 5A-F)

Small to medium sized *Chaetocladius* species. Total length 3.75 mm. Wing length 2.45 mm; TL/WL 1.53. General colouration contrasting brown yellowish to brown. Head brownish; antennae pale brown; thorax contrasting brown to dark brown, mesonotal stripes distinctly dark brown, humeral pit brownish with contrasting granulation; wing

pale; legs yellow to yellowish brown; tergites I-VIII brownish, anal segment contrasting brown to dark brown.

Head. Eyes bare between ommatidia, hairs absent on inner lateral eye margin, few short setae present on outer posterior margin. 12 temporals including 9 inner and 3 outer verticals; palpomere 3 with 3 sensilla clavata and 4 sensilla coeloconica; clypeus semi-circular, bearing 12 setae in 4 rows. Antenna 1050 µm long; last flagellomere 570 µm long, slightly clubbed distally, with numerous curved sensilla chaetica, pre-apical seta absent; antennal groove beginning on segment 3 and reaching ultimate flagellomere; AR 1.19. Thorax. Anteprontum weakly-developed, lobes gaping and thinner

at apex, with 6 lateral antepronotals; acrostichals 19 in 1 row; dorsocentrals 10 in 1 row; prealars in 1 row; humeral pit ovoid, with contrasting brown granulation. Wing. Brachiolum with 1 seta; membrane densely covered with coarse punctuation; number of setae on veins: R, 18; R₁, 0; R₂₊₃, 1; remaining veins bare; squama with 11 setae in 1 row. Legs. Tarsomeres ta₄ and ta₅ of PII sub-equal; sensilla chaetica present on: tibia and tarsomeres ta₁-ta₅ of PI, tibia and tarsomeres ta₁-ta₅ of PII-PIII.

Hypopygium in dorsal view as in Fig. 5C, ventral view (Fig. 5D) with tergite IX and anal point omitted. Tergite IX broadly rectangular, wider at base and slightly narrowing distally, posterior margin nearly straight; postero-median area with about 18 dorsal setae located around the base of anal point, 10 are inserted medially and 8 close to basal margins of anal point (4 setae on each side). Anal point in dorsal (Fig. 5C) and lateral view (Fig. 5A) about 85 μm long and 145 μm maximum width at base, with a low dorsal hump; nearly cup-shaped basally, wider at base and almost parallel-sided in its distal part, apex rounded, bare and hyaline part about 45 um long. Latero-sternite IX with 12 setae inserted laterally (6 on each side). Sternapodeme and phallapodeme (Fig. 5D), phallapodeme nearly sickleshaped. Virga (Fig. 5C) well-developed, consists of 5 curved spines. Gonocoxite with straight margin basally, apical part distinctly truncate; ventral margin with broad undulated lobes and bearing 10 stout setae. Inferior volsella (Figs 5C, F) welldeveloped and distinctly contrasting; anterior area acute triangular, apical part digitiform to long finger-shaped, markedly bent downwards and covered with short setae; caudal part consists of a semi-circular pouch-like lobe, densely covered with setae. Gonostylus in dorsal view (Fig. 5E) slender and thinner proximally, becoming thicker in its distal half; posterior margin rounded, ending with a distinct bare and rounded expansion; anterior side bearing 2 rows of setae; crista dorsalis absent; megaseta well-developed, about 15 µm long;

Taxonomic remarks

On the basis of some unusual morphological characters found in the male adult, *C. berythensis* sp. n. keys to the *laminatus*-group as emended by Mou-

bayed-Breil (2017) for known *Chaetocladius* species from Europe and neighbouring geographical areas. *Chaetocladius callauensis* likely belong to a separate group on the basis of the atypical shape of its anal point and inferior volsella, while *C. guardiolei* sp. n. and *C. parerai* sp. n. appear to belong to the *suecicus*-group based, in particular due to the shape of their inferior volsella and gonostylus.

Chaetocladius berythensis sp. n.

Though C. berythensis sp. n. shows, as illustrated in Figs 1 I-N, 2 A-C, a close morphological similarity with the laminatus-group (shape of virga, inferior volsella, and gonostylus), some other unusual relevant characters (Figs 1 B-C, G-H, M-N; 2 B-C) found in the male adult (structure of sensilla coeloconica on palpomere 3, shape of the dorsal hump on tergite IX, sinuous crista dorsalis consisting of low proximal lobes ending with a larger distal tooth located close to the megaseta, posterior margin of gonostylus with a triangular bare and hyaline expansion located medially) lead us to regard this new species to be a local glacial relict representative of the 'Levantine Province'. As reported by Moubayed-Breil (2017) and Moubayed-Breil & Bitusik (2019) and based on the previously cited characters, the laminatus-group currently includes 6 known species from Europe and neighbouring areas: C. bitusiki Moubayed, 2019; C. coppai Moubayed-Breil, 2017; C. elisabethae Makarchenko & Makarchenko, 2018; C. guisseti Moubayed, 2017; C. laminatus Brundin, 1947; C. purbeckensis Langton & Armitage, 2010. Consequently, the description of C. berythensis sp. n. increases the total number in the laminatusgroup to 7.

Chaetocladius callauensis sp. n., C. guardiolei sp. n. and C. parerai sp. n.

These three new species belong to a local 'Pyrenean element' based on the following distinguishing characters (mostly atypical) found in the male adult:

Chaetocladius callauensis sp. n.: anal point (Figs 3A, C, G) swollen basally, short and bare distally; dorsal part of inferior volsella (Figs 3 A, F) unusually rectangular, hyaline and bare apically.

Table 4. Male adult of *Chaetocladius parerai* sp. n. Length (µm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs.

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
PI	795	845	535	315	220	145	115	0.63	2.73	3.07	2.40
PII	755	895	345	215	175	120	115	0.39	3.19	4.78	2.35
PIII	910	1025	570	325	265	130	120	0.56	2.99	3.40	2.85

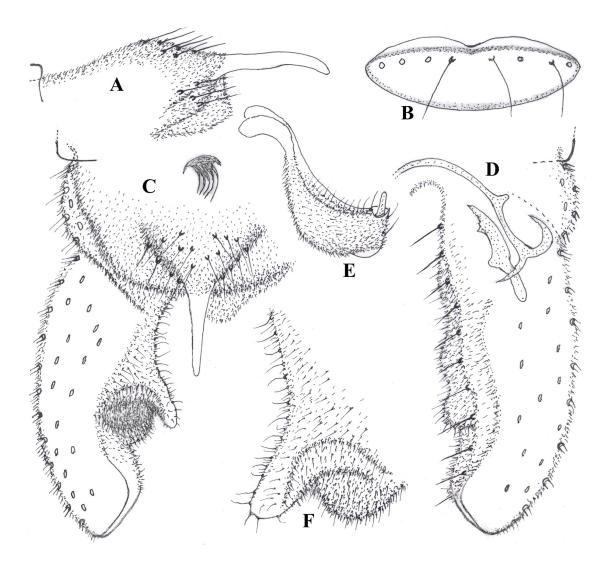


Figure 5. Male adult of *Chaetocladius parerai* sp. n. A) anal point and tergite IX in lateral view; B) scutellum; C) hypopygium, dorsal; D) hypopygium, ventral; E) gonostylus, dorsal; F) inferior volsella.

Chaetocladius guardiolei sp. n.: anal point (Figs 4 F, G) with well-sclerotized basal lateral margin, inferior volsella (Fig. 4 H) triangular and nose-like with hyaline and bare apex, gonostylus (Fig. 4 J) bulbous distally, are all differently figured in *C. suecicus* and *C. longivirgatus* (Stur & Spies 2011, Figs 6-11) and *C. subalpinus* (Rossaro 2017, Fig. 4).

Chaetocladius parerai sp. n.: dorsal part of inferior volsella (Figs 5 C, F) digitiform to long finger-like and distinctly projecting inwards, gonostylus (Fig. 5E) bearing a rounded expansion postero-apically which is hyaline and bare, differently shaped in *C. guardiolei* sp. n.

Geographical distribution

Chaetocladius berythensis sp. n. is known only from its type-locality in Lebanon (upper basin of the Beirut River, Western range, Mount Sannine). Chaetocladius callauensis sp. n., C. guardiolei sp. n. and C. parerai sp. n. are currently restricted to glacial springs, streams and peat bogs located in eastern Pyrenees. While C. guardiolei sp. n. is confined to the upper basin of the Tech River (1800-2000 m), both C. callauensis sp. n. and C. parerai sp. n. occur in the upper basin of the Mantet River and Soques stream (alt. 2000-2300 m).

Ecology

The larvae of *C. berythensis* are rheophilic and exclusively confined to glacial karstic helocrenes

with high water conductivity (Cd, up to 300 μS/ cm) and calcareous substratum. Emergence from June to early August. Associated species encountered in the same locality as the holotype include: Boreoheptagyia legeri (Goetghebuer, 1933); B. rotunda Serra-Tosio, 1983; Diamesa kasymovi Kownacki & Kownacka, 1973; D. sakartvella Kownacki & Kownacka, 1973; D. tonsa (Walker, 1856); Diamesa sp. A, near khumbugelida Willassen & Sæther, 1987; Pseudodiamesa nivosa (Goetghebuer, 1928); Chaetocladius diai Moubayed-Breil, 2017; C. melaleucus; C. perennis (Meigen, 1830); C. piger (Goetghebuer, 1913); Eukiefferiella fittkaui Lehmann, 1972; E. minor (Edwards, 1929); Heleniella sp. A, near ornaticollis (Edwards, 1929); H. sp. B, near asiatica Reiss, 1968; Metriocnemus eurynotus (Holmgren, 1838); M. hirticollis (Staeger, 1839); Thienemanniella clavicornis (Kieffer, 1911).

Larvae of C. callauensis, C. guardiolei. and C. parerai likely occur in glacial acidic helocrenes, streams and peat bogs located in Eastern Pyrenees (altitude 2000-2300 m), where water is crystalline with siliceous substratum and a very low conductivity (Cd, 10-13µS/cm). Emergence from June to September. Associated species encountered in the same localities as for holotypes include: Diamesa aberrata Lundbeck, 1898; D. bertrami Edwards, 1935; D. bohemani Goetghebuer, 1932; D. cinerella Meigen, 1835; D. modesta Serra-Tosio, 1968; D. thomasi Serra-Tosio, 1970; D. veletensis Serra-Tosio, 1971; Pseudodiamesa branickii (Nowicki, 1873); P. nivosa (Goetghebuer, 1928); Syndiamesa edwardsi Pagast, 1947; S. hygropetrica (Kieffer, 1909); Bryophaenocladius subvernalis (Edwards, 1929); Chaetocladius guisseti Moubayed-Breil, 2017; C. laminatus Brundin, 1947; C. mantetensis Moubayed-Breil; C. suecicus (Kieffer, 1916); H. ornaticollis (Edwards, 1929); Metriocnemus eurynotus; Parametriocnemus valescurensis Moubayed & Langton, 1999; Rheocricotopus pyrenaeus Moubayed-Breil, 2018; R. thomasi Moubayed-Breil, 2016; Thienemannia gracilis Kieffer, 1909; T. valespira Moubayed-Breil & Ashe, 2013; Trissocladius orsini Moubayed-Breil & Ashe; Micropsectra alyssae Moubayed-Breil & Ashe, 2018; M. ekremi Moubayed-Breil & Ashe, 2018; M. nohedensis (Moubayed & Langton, 1996).

The four new *Chaetocladius* species appear to belong to the crenobiotic and crenophilous community of species as documented by Lindegaard (1995). Their occurrence in such preserved high mountain habitats highlights the importance of glacial springs and streams, which are considered to be microrefugia and hotspots of diversity and

endemism. Like other rare members of the genus *Chaetocladius* occurring in high mountain glacial springs and streams (French, Italian and Swiss Alps; Eastern Pyrenees), only few individuals of *C. berythensis* sp. n. and *C. callauensis* sp. n. have been collected after extensive investigation. The melting period of snow has become much shorter over the three last decades, which has greatly affected the ecological conditions of the original habitats. Consequently, the loss of such biogeographically representative and relict species would be ecologically indicative of the global warming and climate change.

Male pupal exuviae of taxa/species and morphotypes

(Figs 6A-L, 7A-N)

Many specimens of 10 taxa/species including associated male pharate adults and male pupal exuviae of Chaetocladius s. str. were collected between 1996 and 2013 in some glacial mountain springs, peat bogs and streams located at high altitude (2000-2300 m) in the eastern Pyrenees (France) and High Tatra Mountains (Slovakia). Nearly 50 associated male adults and male pupal exuviae have been examined, which allow us to provide complementary short taxonomic notes on 10 taxa/ species and morphotypes, which are briefly illustrated and described based on characters found in the male pupal exuviae. The 10 taxa/species include 6 named species (C. melaleucus; C. dissipatus; C. perennis, C. guisseti, C. bitusiki, C. mantetensis) and 4 morphotypes, 3 of which could be pupae of the above described species (C. cf. laminatus; C. cf. callauensis sp. n.; C. cf. guardiolei sp. n.; *C.* cf. *parerai* sp. n.).

Brief descriptions

The male pupal exuviae are described on the basis of a combination of 6 distinguishing characters, which are briefly summarized as follows:

- 1. Frontal setae (FS) well-developed or vestigial;
- 2. Shape of thoracic horn (TH);
- 3. Distribution pattern of dorsocentral setae with mean distance between setae: Dc₁-Dc₂ (d1); Dc₂-Dc₃ (d2); Dc₃-Dc₄ (d3);
- 4. Shape of dorsocentrals: type-a, thick setae; type-b, thin setae; type-c, bristle-like;
- 5. Oval postero-median patch of spines on sternites;
- 6. Shape of macrosetae (M) and location of the apical lobe (ApiL) on genital sac.

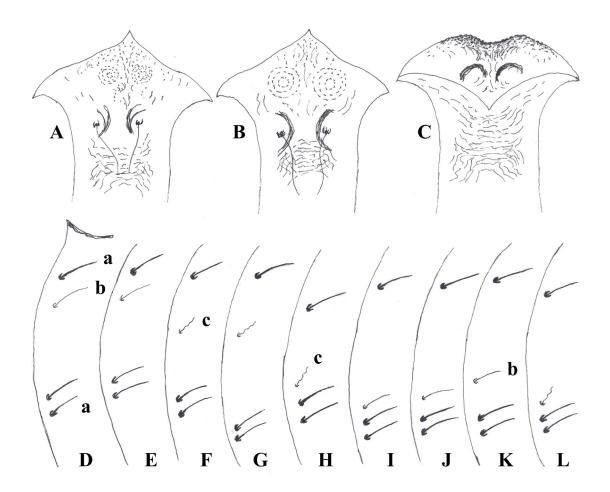


Figure 6. Pupal exuviae of *Chaetocladius* spp. Frontal apotome of: A) *C.* cf. *callauensis* sp. n.; B) *C. mantetensis*; C) *C.* cf. *guardiolei* sp. n. Distribution pattern of dorsocentral setae on thorax of: D) *C. melaleucus*; E) *C. perennis*; F) *C. guisseti*; G) *C. mantetensis*; H) *C. bitusiki*; I) *C.* cf. *laminatus*; J) *C.* cf. *callauensis* sp. n.; K) *C.* cf. *guardiolei* sp. n; L) *C.* cf. *parerai* sp. n.

Chaetocladius melaleucus

(n = 10 male exuviae including 5 male pharate adults; Figs 6D, 7A-B)

Eastern Pyrenees, SW France.

- 1. FS, well-developed, about 110 µm long;
- 2. TH, club-like;
- 3. Dc_1 located close to Dc_2 , Dc_3 close to Dc_4 (Fig. 48); d1 = d3, 10-15; d2, 65;
- 4. Dc_1 , Dc_3 and Dc_4 (type-a); Dc_2 , (type-b);
- 5. Oval postero-median patch of spines present on sternites II-VII (Fig. 7A);
- 6. M, short, conspicuous and spine-like; ApiL, finger-like, located medially (Fig. 7B).

Chaetocladius dissipatus

(n = 2 male exuviae + 2 male pharate adults; Fig. 7F)

Eastern Pyrenees, SW France.

- 1. FS, well-developed, 90-100 µm long;
- 2. TH, club-like;
- 3. Dc_1 located close to Dc_2 , Dc3 close to Dc_4 ; d1 = d3, 10-15; d2, 60-70;
- 4. Dc₁, Dc₃ and Dc₄ (type-a); Dc₃, (type-b);
- 5. Present on sternite III/IV-VII, consists of scattered spines, (Langton 1991, Fig. 63i);
- 6. M, short, conspicuous and spine-like; ApiL, indistinct (Fig. 7F; Langton 1991, Fig. 63j).

Chaetocladius perennis

(n = 5 male exuviae including 3 male pharate adults; Fig. 6E)

Eastern Pyrenees, SW France.

1. FS, well-developed, about 90 μm long;

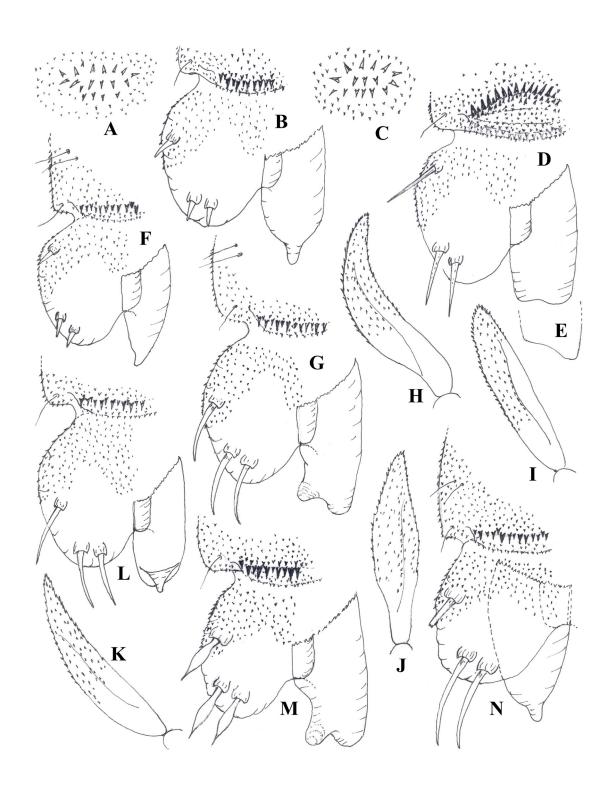


Figure 7. Pupal exuviae of *Chaetocladius* spp. Caudal area of sternite VI of: A) *C. melaleucus*; C) *C.* sp. cf. *laminatus*. Tergite VIII (caudal part, dorsal) and anal lobe of: B) *C. melaleucus*; D-E) *C.* sp. cf. *laminatus*; F) *C. dissipatus*; G) *C.* sp. cf. *guardiolei* sp. n; L) *C.* cf. *callauensis* sp. n.; M) *C.* sp. cf. *parerai* sp. n; N) *C. mantetensis*. Thoracic horn of: H) *C.* sp. cf. *laminatus*; I) *C. mantetensis*; J) *C. cf. parerai* sp. n; K) *C.* cf. *callauensis* sp. n.

- 2. TH, elongated with nearly parallel-side margins (Pankratova 1970, Fig. 144);
- 3. Dc_1 located close to Dc_2 , Dc3 close to Dc_4 (Fig. 6E); d1 = d3, 10-15; d2, 50-60;
- 4. Dc_1 , Dc_3 and Dc_4 (type-a); Dc_2 , (type-b);
- 5. Absent;
- 6. M, thorn to long spine; ApiL, finger-like, located laterally.

Chaetocladius guisseti

(n = 5 male exuviae including 2 male pharate adults; Fig. 6F)

Eastern Pyrenees, SW France.

- 1. FS (Fig. 6F), well-developed, 80-90 μm long, as in Moubayed-Breil (2017, Fig. 22);
- 2. TH, club-like (Moubayed-Breil 2017, Fig. 26);
- 3. Dc_1 and Dc_2 well separated, Dc_3 located close to Dc_4 (Fig. 50); d1, 45; d2, 50; d3, 10;
- 4. Dc_1 , Dc_3 and Dc_4 (type-a); Dc_2 , (type-c);
- 5. Present on sternites IV-VII;
- 6. M, thorn to long spine; ApiL, consists of 2 distinct expansions, outer one a larger lobe (Moubayed-Breil 2017, Figs 31-32).

Chaetocladius mantetensis

(n = 5 male exuviae including 2 male pharate adults; Figs 6B, G; 7N)

Eastern Pyrenees, SW France.

- 1. FS, well-developed, 80-90 μm long (Fig. 6B; Moubayed-Breil & Bitusik 2019, Fig. 33);
- 2. TH, club-like (Fig. 7I);
- 3. Dc₁, Dc₂ and Dc₃ well separated, Dc₃ located close to Dc₄ (Fig. 6G); d1, 45; d2, 50; d3, 10;
- 4. Dc_1 , Dc_3 and Dc_4 (type-a); Dc_2 , (type-c);
- 5. Present on sternites IV-VII;
- 6. M, thorn to long spine; ApiL, finger-like, located laterally (Fig. 7N).

Chaetocladius bitusiki

(n = 1 male pharate adult; Fig. 6H)

High Tatra Mountains, Slovakia.

- 1. FS, well-developed, 50-55 μm long (Moubayed-Breil & Bitusik 2019, Fig. 32);
- 2. TH, club-like (Moubayed-Breil & Bitusik 2019, Fig. 36);
- 3. Dc₁ located long distance from Dc₂; Dc₂ located

close to Dc_3 and Dc_4 (Fig. 6H); d1 = 45, d2 = d3, 15:

- 4. Dc₁, Dc₃ and Dc₄ (type-a); Dc₂, (type-c);
- 5. Present on sternites IV-VII;
- 6. M, thorn to long spine; ApiL, with 2 distinct rounded expansions, inner a large lobe and wider (Moubayed-Breil & Bitusik 2019, Fig. 42).

Chaetocladius cf. laminatus

Male pharate adults unknown.

(n = 3 male exuviae Figs 6I, 7C-E)

Eastern Pyrenees, SW France.

- 1. Frontal setae well-developed, about 85 μ m long ;
- TH, narrowed distally and arched medially (Fig. 7H);
- 3. Dc_1 located long distance from Dc_2 -Dc3 (Fig. 6I); d1 = 65;
- 4. Dc_1 , Dc_3 and Dc_4 (type-a); Dc_2 , (type-c);
- 5. Present on sternites III-VII (Fig. 7C);
- 6. M, thorn to long spine; ApiL, located medially (Fig. 7D).

Chaetocladius cf. callauensis sp. n.

Male pharate adults unknown.

(n = 5 male exuviae; Figs 6A, J; 7K-L)

Eastern Pyrenees, SW France.

- 1. FS, well-developed (Fig. 6A), about 75-80 μ m long;
- 2. TH, wider at base, narrowing distally with pointed apex (Fig. 7K);
- 3. Dc_1 located far from Dc_2 - Dc_4 (Fig. 7J); d1 = 110-115; d2 = d3, 15-20;
- 4. Dc_1 , Dc_3 and Dc_4 (type-a); Dc_2 , (type-b);
- 5. Present on sternites III-VII;
- 6. M, thorn to long spine; ApiL, typically located medially (Fig. 7L).

Chaetocladius cf. guardiolei sp. n.

Male pharate adults unknown.

(n = male exuviae; Figs 6C, K; 7G)

Eastern Pyrenees, SW France.

- 1. FS, vestigial (Fig. 6C), about 10 µm long;
- 2. TH, elongate, wider at base, distinctly narrowing distally with pointed apex;

- 3. Dc₁ located far from Dc₂-Dc₄ (Fig. 6K); d1, 95; d2, 35; d3, 10-15;
- 4. Dc_1 , Dc_3 and Dc_4 (type-a); Dc_2 , (type-b);
- 5. Present on sternites II-VII;
- 6. M, thorn to long spine; ApiL, located medially (Fig. 7 G).

Chaetocladius cf. parerai sp. n.

Male pharate adults unknown.

(n = 3 male exuviae; Figs 6L; 7 J, M)

Eastern Pyrenees, SW France.

- 1. FS, well-developed, about 100 µm long;
- 2. TH, club-like (Fig. 7J);
- 3. Dc_1 located far from Dc_2 - Dc_4 (Fig. 6L); d1, 110; d2 = d3, 10-15;
- 4. Dc₁, Dc₃ and Dc₄ (type-a); Dc₂, (type-c);
- 5. Present on sternites V-VII;
- 6. M, thorn to long spine; ApiL, with 2 distinct rounded expansions, inner one lobe-like, outer one wider (Fig. 7M).

Acknowledgements

The authors are grateful to their colleague Dr. T. Ekrem (NTNU University Museum, Trondheim, Norway) for his constructive suggestions, which greatly improved the manuscript. Special thanks are also due to Jade and M-Hélène Breil-Moubayed for their kind assistance in achieving the measurements of the leg ratios for the four new species.

References

- Ashe, P. and O'Connor, J.P. 2012. A World Catalogue of Chironomidae (Diptera) Part 2. Orthocladiinae. Irish Biogeographical Society & National Museum of Ireland, Dublin. 968 pp.
- Brundin, L. 1947. Zur Kenntnis der schwedischen Chironomiden. *Arkiv för Zoologi* 39A: 1-95.
- Brundin, L. 1956. Zur Systematic der Orthocladiinae (Diptera, Chironomidae). - Report of the Institute of Freshwater Research, Drottningholm 37: 5-185.
- Cranston, P.S. and Oliver, D.R. 1988. Aquatic xylophagous Orthocladiinae-systematics and ecology (Diptera, Chironomidae). *Spixiana*, *supplement* 14: 143-154.
- Cranston, P.S., Oliver, D.R. and Sæther, O.A. 1989. The adult males of Orthocladiinae (Diptera, Chironomidae) of the Holarctic region-Keys

- and diagnoses. *Entomologica scandinavica*, *supplement* 34: 165-352.
- Goetghebuer, M. 1940-50. Tendipedidae (Chironomidae) Subfamilie Orthocladiinae. A. Die Imagines. In: Lindner, E. (ed.): Die Fliegen der palaearktischen Region, 13g: 1-208.
- Kobayashi, T. 2012. *Chaetocladius* (s. str.) *eugenyii* sp. n. (Ditera, Chironomidae, Orthocladiinae) from Japan. *Euroasian Entomological Journal* 3 (2): 13-15.
- Langton, P.H. 1991. *A key to pupal exuviae of the West Palaeractic Chironomidae*. Privately published. Huntingdon, England, 386 pp.
- Langton, P.H. and Armitage P.D. 2010. A new species of *Chaetocladius* Kieffer (Diptera, Chironomidae) from the Dorset coast. *Dipterist Digest* 17: 103-108.
- Langton, P.H. and Pinder, L.C.V. 2007. Keys to the adult male Chironomidae of Britain and Ireland; 2 vols. Freshwater Biological Association Scientific Publication 64, 239+168 pp.
- 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.
- Makarchenko, E.A. and Makarchenko, M.A. 2004. *Chaetocladius* Kieffer (Diptera, Chironomidae, Orthocladiinae) in the Russian Far East. *Euroasian Entomological Journal* 3 (4): 311-317. [in Russian]
- Makarchenko, E.A. and Makarchenko, M.A. 2006a. *Chaetocladius* (s. str.) *amurensis* sp.n. (Diptera, Chironomidae, Orthocladiinae) from the Amur River basin (Russian Far East). *Euroasian Entomological Journal* 5 (4): 276-277.
- Makarchenko, E.A. and Makarchenko, M.A. 2006b. Subfamily Orthocladiinae. *In*: Lelej, A. (Ed.), *Key to the insects of Russian Far East. Vol. 6. Diptera and Siphonaptera. Pt 4.* Vladivostok, Dal'nauka: pp. 280-372, 482-530, 623-671. [in Russian]
- Makarchenko, E.A. and Makarchenko, M.A. 2007. New records of chironomids (Diptera, Chironomidae) in the Russian Far East. I. Subfamily Orthocladiinae. - *Euroasian Entomological Journal* 6 (3): 299-310. [in Russian]
- Makarchenko, E.A. and Makarchenko, M.A. 2009. New records of chironomids (Diptera, Chironomidae) from the Far East and bordering territories. VIII. Subfamily Orthocladiinae.

- Euroasian Entomological Journal 8 (3): 326-334. [in Russian]
- Makarchenko, E.A. and Makarchenko, M.A. 2011. Chaetocladius (s. str.) antipovae sp.n. (Diptera, Chironomidae, Orthocladiinae) from the Amur River basin (Russian Far East). - Euroasian Entomological Journal 10 (3): 383-384.
- Makarchenko, E.A. and Makarchenko, M.A.
 2013a. Chaetocladius (s. str.) chrulevae sp.n.
 (Diptera, Chironomidae, Orthocladiinae), from the Wrangel Island (Chukotka, Russian Far East). Euroasian Entomological Journal 12 (4): 400-402.
- Makarchenko, E.A. and Makarchenko, M.A. 2013b. Chaetocladius (Chaetocladius) elenae sp.n. (Diptera, Chironomidae, Orthocladiinae), a new chironomid species from the Magadan Region, Russian Far East. Euroasian Entomological Journal 12 (6): 383–384.
- Makarchenko, E.A., Makarchenko, M.A. and Semenchenko A. 2014. New or little-known species of *Chaetocladius* s. str. Kieffer, 1911 (Diptera: Chironomidae: Orthocladiinae) from the Amur River basin (Russian Far East). *Zootaxa* 4247 (3): 313-330. DOI: https://doi.org/10.11646/zootaxa.4247.3.5
- Makarchenko, E.A. and Makarchenko, M.A. 2018. The chironomids of *Chaetocladius* Kieffer and *Hydrobaenus* Fries (Diptera, Chironomidae) from collection of N.I. Zelentsov. *Euroasian Entomological Journal* 17 (3): 171-178.
- Makarchenko, E.A., Makarchenko, M.A., Semenchenko, A. and Palatov D. 2018. Morphological description and DNA barcoding of *Chaetocladius* (*Chaetocladius*) *elisabethae* sp. nov. (Diptera: Chironomidae: Orthocladiinae) from the Moscow Region. *Zootaxa* 4403 (2): 378-388. DOI: https://doi.org/10.11646/zootaxa.4403.2.9
- Moubayed-Breil, J. 2017. On the genus *Chaeto-cladius* (*laminatus*-group). I. Taxonomic notes with description of *C. guisseti* sp. n. from glacial springs and streams located in Eastern Pyrenees (Diptera: Chironomidae, Orthocladiinae). *Euroasian Entomological Journal* 16 (5): 487-500.
- Moubayed-Breil J, and Dia A. 2017. *Chaetocladius coppai* sp. n. and *C. diai* sp. n., two mountain relic species inhabiting glacial springs and cold streams (Diptera: Chironomidae, Orthocladiinae). *Zoosystematica Rossica*

- 26: 369-380. DOI: https://doi.org/10.31610/zsr/2017.26.2.369
- Moubayed, J. and Lods-Crozet B. 2018. On the genus *Chaetocladius* s. str. Kieffer, 1911 from Switzerland with descriptions of five new relic species occurring in glacial alpine springs and streams (Diptera, Chironomidae). *Alpine Entomology* 2: 15-34. DOI: https://doi.org/10.3897/alpento.2.22759
- Moubayed, J. and Bitušík P. 2019. Taxonomic notes on the genus *Chaetocladius* (*laminatus*group). II. Descriptions of *C. bitusiki* sp. n. and *C. mantetensis* sp. n., two relic species inhabiting cold stenothermic springs and streams (Diptera: Chironomidae, Orthocladiinae). *Biologia* 74: 1489–1500. DOI: https://doi.org/10.2478/s11756-019-00253-8
- Pankratova, V.Ya. 1970. Larvae and pupae of the midges of the subfamily Orthocladiinae (Diptera, Chironomidae = Tendipedidae) of the USSR fauna. Key to the USSR fauna, published by Zoological Institute of the USSR Academy of Sciences, Leningrad, Nauka 102: 1-344. [in Russian]
- Rossaro, B, Magoga, G. and Montagna M. 2017. Revision of the genus *Chaetocladius* Kieffer (Diptera, Chironomidae), 1st note: description of four new species from Italy. - *Journal of Entomological and Acarological Research* 49 (6658): 36-47. DOI: https://doi.org/10.4081/ jear.2017.6658
- Sæther, O.A. 1969. Some Nearctic Podonominae, Diamesinae and Orthocladiinae (Diptera: Chironomidae). *Bulletin of the Fisheries Research Board of Canada* 170: 1-154.
- Sæther, O.A. 1980. Glossary of chironomid morphology terminology (Diptera, Chironomidae).
 Entomologica scandinavica, supplement 14: 1-51.
- Sæther, O.A. 1990. Redescription of *Chaetocladius glacialis* (Lundström, 1915) comb. Nov. *Aquatic Insects* 12(1): 61-64. DOI: https://doi.org/10.1080/01650429009361389
- Stur, E. and Spies M. 2011. Description of *C. lon-givirgatus* sp. n., with a review of *C. suecicus* (Kieffer) (Diptera, Chironomidae). *Zootaxa* 2762: 37-48. DOI: https://doi.org/10.11646/zootaxa.2762.1.3
- Wang, Q., Kong, F. and Wang, X. 2012. *Chaeto-cladius* Kieffer (Diptera: Chironomidae) in China. *Entomologica Fennica* 23: 42-48. DOI: https://doi.org/10.33338/ef.84563

Zelentsov, N.I. 2007. A new species of chironomid, genus *Chaetocladius* (Diptera, Chironomidae) from the Novaya Zemlya Archipelago. *Entolomological Review* 87 (6): 1145-1149. DOI: https://doi.org/10.1134/S0013873807060139

Article submitted 23. March 2019, accepted by Torbørn Ekrem 2. November 2019, published 8. November 2019.