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ORIGINAL ARTICLE

Taxonomic notes on the genus *Chaetocladius* (*laminatus*-group) (Diptera: Chironomidae, Orthocladiinae) II. Descriptions of *C. bitusiki* sp. n. and *C. mantetensis* sp. n., two relict species inhabiting cold alpine springs and streams

Joel Moubayed-Breil[1] · Peter Bitušík[2]

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Abstract
Two new species of the genus *Chaetocladius* s. str. (*C. bitusiki* sp. n. and *C. mantetensis* sp. n.) are diagnosed and described based on material collected in some cold springs and small streams located at high altitude (1940–2300 m) in the High Tatra Mountains (Slovakia) and Eastern Pyrenees (France). The first one is described as male adult and pupal exuviae, while the second is described as male and female adults and pupal exuviae. Both *C. bitusiki* sp. n. and *C. mantetensis* sp. n. key to the *laminatus*-group for known *Chaetocladius* species on the basis of some characters found in the male adult: virga composed of three typical parts, inferior volsella sub-triangular to sub-circular, narrow to broad lobe, bearing or lacking a posterior marsupial-like pouch and an apical contrasting protuberance; gonostylus triangular, projecting posteriorly as a pointed or rounded apex. Taxonomic remarks on related *Chaetocladius* species, key to known male adults of the *laminatus*-group from Europe with comments on the ecology and geographical distribution of the new species are given. The two new species can be regarded as typical relict representatives of cold springs and streams at high altitudes. Such species could be used as relevant biological indicators of the global warming and local climate change.

Keywords Diptera · Chironomidae · *Chaetocladius* genus · New species · Pyrenees · Tatra Mountains · 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), Sæther (1969), Pankratova (1970), Sæther (1990), Caspers (1987), Cranston et al. (1989), Moubayed (1989), Langton (1991), Makarchenko and Makarchenko (2001, 2003, 2004, 2006a, b, 2007, 2009, 2011a, b, 2013a, b, c), Langton and Pinder (2007), Zelentsov (2007), Langton and Armitage (2010), Stur and Spies (2011), Ashe and O'Connor (2012), Kobayashi (2012); Wang et al. (2012); Spies and Sæther (2013); Makarchenko et al. (2014); Moubayed-Breil and Dia (2017); Rossaro et al. (2017); Moubayed and Lods-Crozet (2018); Makarchenko et al. (2018). Worldwide, the genus *Chaetocladius* Kieffer, 1911 comprises about 70 species of which, only about 40 are reported from Europe.

In this paper, *C. bitusiki* sp. n. and *C. mantetensis* sp. n. are diagnosed and described based on a material of pharate adults and pupal exuviae recently collected in some alpine cold springs and streams located in the High Tatra Mountains (Slovakia) and the Eastern Pyrenees (France).

Both species belong to the *laminatus*-group as emended by Moubayed-Breil (2017) for known *Chaetocladius* species from Europe and neighbouring geographical areas that has currently included five species: *C. laminatus* Brundin, 1947, *C. coppai* Moubayed-Breil 2017, *C. guisseti* Moubayed, 2017 and *C. elisabethae* Makarchenko and Makarchenko, 2018 and *C. purbeckensis* Langton and Armitage, 2010.

Here we provide taxonomic position with notes to ecology and geographical distribution of both newly described species

✉ Peter Bitušík
peter.bitusik@umb.sk

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

[2] Faculty of Natural Sciences, Matej Bel University, Tajovského 40, 97401 Banská Bystrica, Slovakia

and a key of known males of *Chaetocladius laminatus*-group from Europe.

Material and methods

Material composed of pharate adults and pupal exuviae was collected with hand drift nets, Surber sampler and sweep net and preserved in 80% ethanol. In laboratory, the adult musculature was cleared in 90% lactic acid (head, thorax, abdomen and anal segment) for about 60 to 80 min, but can be left overnight at room temperature without any detrimental effect or damage. The specimens were checked under a binocular microscope after 20 min 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 when anal point and tergite IX were removed.

Part of the abdomen and the halters of the male adults were preserved in 85% ethanol for an eventual DNA analysis. Morphological terminology and measurements follow those of Sæther (1980), Langton (1991) and Langton and Pinder (2007) for the imagines and pupal exuviae.

Chaetocladius bitusiki Moubayed-Breil, sp. n

Diagnostic characters

Based on some distinguishing characters found in the male adult, *C. bitusiki* sp. n. keys in particular close to *C. laminatus*, *C. dissipatus* (Edwards, 1929), *C. holmgreni* (Jacobson, 1998) and *C. lopatinskiy* Makarchenko et al., 2017. However, the new described species can be separated from other related *Chaetocladius* species by the following combination of characters. Clypeus broadly semi-circular to cup-like shaped, wider medially with 8 setae placed in 3 rows; palpomere 3 with 3 sensilla clavata and 3 sensilla coeloconica; lobes of antepronotum not gaping and distinctly narrowing medially at base; humeral pit semi-circular to ellipse-like without contrasting spots and surrounded by brownish granulation. Tergite IX bearing a distinct rounded hump located distally; anal point triangular and sharply pointed; virga consists of 3 separated parts including 1 median part (bearing 3 small equal spines) and 2 lateral symmetrically elongated parts (each with 1 orally directed claw medially). Gonocoxite truncate apically, with a distinct tubercle located apically close to the articulation of gonostylus; inferior volsella large triangular, bearing an apical distinct protuberance and lacking a posterior marsupial pouch-like lobe. Gonostylus massive in general and typically triangular, markedly projecting posteriorly to a rounded bare and hyaline apex; anterior margin bearing a row of short and long spines ending in 2 characteristic large teeth of nearly similar size, which project orally, smooth and located close to the megaseta; median area with a distinct curved dorsal rows of short spines reaching the base of megaseta; crista dorsalis well developed and smooth, widely extended and occupying the length of the gonostylus.

Male adult

(*n* = 1 male adult; Figs. 1, 2, 3, 4, 5, 6
= sp. B in Moubayed-Breil (2017).

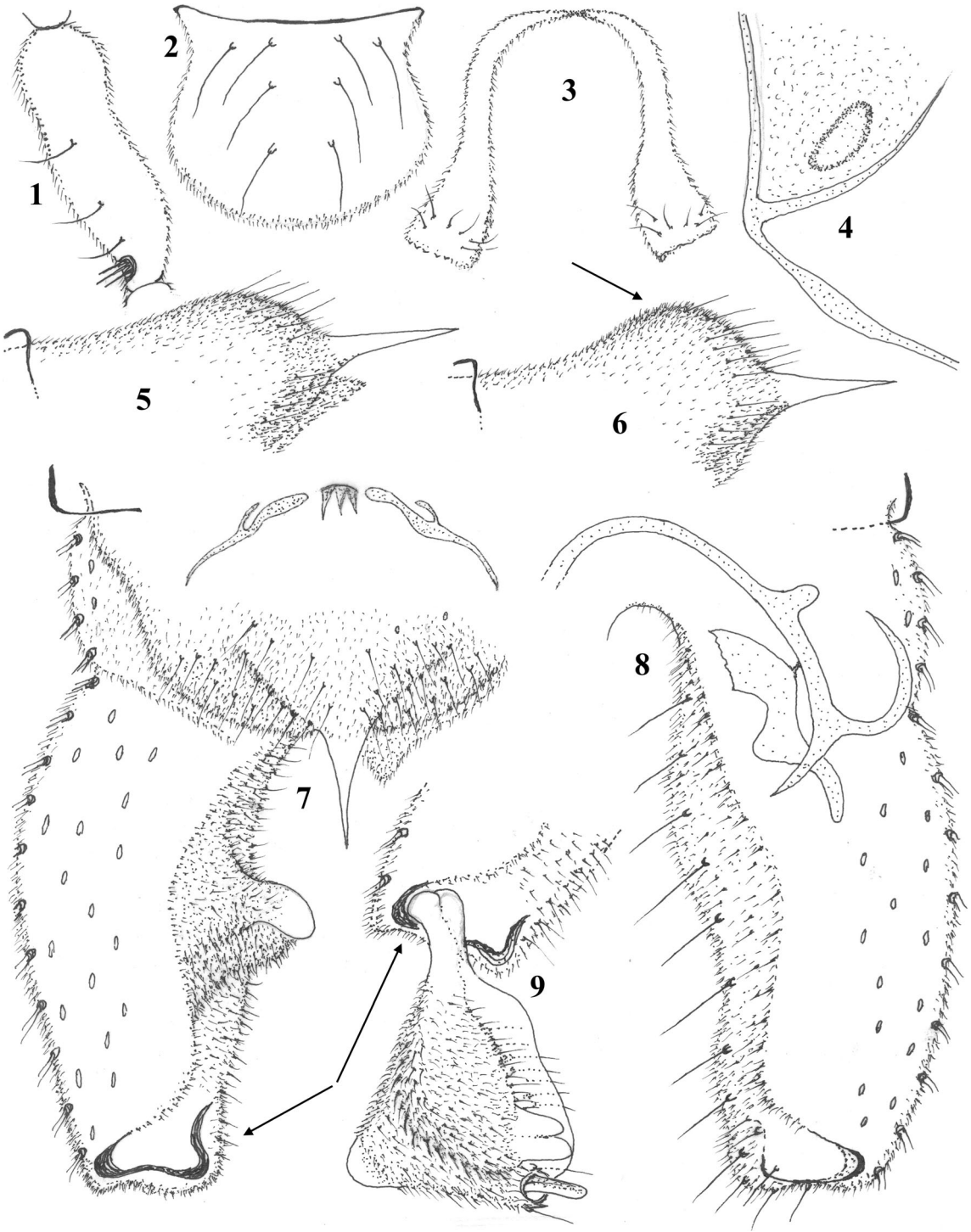
Medium to large species. Total length 3.80–3.90 mm. Wing length 1.20–1.30 mm. General colouration contrasting brown to dark brown to blackish. Head dark brown; antennae pale brown; thorax contrasting brown to dark brown, mesonotal stripes distinctly dark brown; wing pale; legs brown to dark brown; tergites I–IV brownish, tergites V–VIII and anal segment dark brown.

Head Eyes bare between ommatidia, hairs absent on inner lateral eye margin, outer posterior margin covered with few hairs. Temporals consist of 13 setae including 9 inner (uniserial) and 4 outer verticals. Palp 5-segmented, first and second segments are fused and unequal; length (μm) of segments: 30, 35, 45, 75, 115; palpomere 3 (Fig. 1) with 3 sensilla clavata and 3 sensilla coeloconica. Clypeus (Fig. 2) about 120 μm maximum high and 135 maximum width, nearly semi-circular to cup-like shaped, with 8 long setae placed in 3 rows. Antenna 1185–1200 μm long, 13-segmented; ultimate flagellomere 530–535 μm long, distinctly clubbed distally and bearing a dense brush of curved sensilla chaetica apically, apex (Fig. 1) lacking pre-apical seta; antennal groove beginning on segments 3–4 and reaching ultimate flagellomere; AR 1.21–1.22.

Thorax Lobes of antepronotum in contact and distinctly narrowing medially (Fig. 3), with 6 setae placed apically; humeral pit (Fig. 4) semi-circular to ellipsoid shaped, whitish and surrounded by dense granulation; 8 short acrostichals located far distance from antepronotum; dorsocentrals 13 in 1–2 rows; prealars 5 in 1 row; humeral pit ovoid to semi-circular, contrasting whitish to brown, lacking spots and surrounded by dense brownish granulation.

Wing Brachiolum with 1–2 setae; number of setae on veins: R, 17; R_1, 2–3; remaining veins bare; squama with 14–15 setae in 1 row.

Legs Length (μm) of tibial spurs of: PI, distinctly spiniforme, 75; PII, 25 and 35; PIII, 65 and 25; longest seta of tibial comb 60 μm long. Sensilla chaetica few (proximally and distally) on: tibia and tarsomeres ta_1-ta_5 of PI; tarsomeres ta_1-ta_5 of PII and



Figs. 1–9 Male adult of *Chaetocladius* spp. *C. bitusiki* sp. n.: **1** palpomere 3; **2** clypeus; **3** lobes of antepronotum; **4** humeral pit. Tergite IX and anal point (in lateral view) of: **1** *C. bitusiki* sp. n.: **6** *C. laminatus*. *C. bitusiki* sp. n.: **7** hypopygium in dorsal (with virga) and **8** ventral view (tergite IX and anal point removed); **9** distal part of gonocoxite and gonostylus, dorsal. Details on the measurements are provided in the text

PIII. Length (μm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in Table 1.

Hypopygium in dorsal, ventral and lateral view (Figs. 5, 6, 7, 8, 9); ventral view with tergite IX and anal point removed as in Fig. 8. Tergite IX broadly semi-circular, bearing a rounded hump located on distal part which is visible only when tergite IX is viewed laterally (Figs. 5–9), 16–18 setae are located posteriorly and close to the posterior margin. Sternapodeme and phallapodeme (Fig. 8), phallapodeme sickle-shaped and broader basally. Anal point (Figs. 5–7) triangular, sharply

Table 1 Male adult of *Chaetocladius bitusiki* 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	710	690	470	280	230	175	130	0.68	2.30	2.98	2.00
PII	680	710	320	205	180	140	120	0.45	1.77	4.34	1.60
PIII	760	810	440	280	230	170	130	0.54	2.48	3.57	3.20

LR – length of tarsomere ta_1 divided by length of tibia (ti); BV – combined length of femur (fe), tibia (ti) 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

pointed apically and straight in lateral view; 22 setae are present on both dorsal and lateral part (11 on each side). Virga consists of 3 separate parts: median one with 3 small subequal teeth, the 2 remaining parts are elongated laterally and bear 1 median claw-like appendage orally directed. Gonocoxite truncate apically with a distinct broad blackish tubercle located near the base of gonostylus, ventral margin slightly swollen medially; inferior volsella sub-circular to lobed in shape, 35–40 μm maximum length, 25–30 μm maximum width, contrasting, with a nose-like apex and a small distal marsupial pouch-like lobe. Gonostylus triangular, bulbous to massive medially, markedly projecting posteriorly in a rounded bare and hyaline apex; posterior margin nearly straight; anterior margin bearing a row of short and long spines terminating in 2 characteristic large smooth teeth of nearly similar size (about 15 μm long), which project orally and placed close to the megaseta; median area with a distinct curved rows of short spines extending from the posterior part and reaching the base of megaseta; crista dorsalis well developed, widely extended and occupying the length of the gonostylus.

Pupal exuviae

n = 3 (2 males and 1 female; Figs. 32, 34, 36, 38, 40, and 42).
= *Chaetocladius* sp. B in Moubayed-Breil (2017).

Entirely yellowish to pale brown with brownish posterior transverse rows of spines on tergites and sternites; frontal apotome more wrinkled medially than in the anterior area; cephalothorax with faint wrinkles near base of thoracic horn and between Dc_2 and Dc_3; abdomen, anal segment and genital sacs a little darker than the remaining parts of the exuviae.

Total length 3.85–3.95 mm.

Cephalothorax. Frontal apotome slightly domed, cephalic tubercles low and weak, frontal setae 55 μm long, inserted on prefrons ventral to antennal sheath, distance between setae about 30 μm. Antepronotals consist of 2 median antepronotals (160 and 105 μm long) and 1 lateral antepronotal bristle-like seta (65 μm long). Thoracic horn (Fig. 36) 205–210 μm long, 30–40 μm maximum width, wider medially and slightly narrowing distally with a rounded apex; densely toothed on one side; precorneals include 1 thick of 150 μm long and 2 thinner sub-equal of 90–95 μm long each. Dorsocentrals (Fig. 34) consist of 3 subequal setae-like (Dc_1, Dc_3 and Dc_4) of 40–50 μm long and 1 shorter (Dc_3 about 35 μm long) which is thinner and bristle-like; Dc_1 and Dc_2 well separated, Dc_3 and Dc_4 placed close together; distance between: Dc_1–Dc_2 45 μm, Dc_2–Dc_3 and Dc_3–Dc_4 about 15–15 μm.

Armament and distribution pattern of shagreen and points, posterior transverse rows of spines, chaetotaxy and lateral setation of abdominal segments IV and VIII as in Figs. 38 and 40. Field of points present on tergites I/II–VIII, and sternites III/IV–VIII; posterior rows of spines present on tergites II–VIII and sternites III/IV–VII; spines on tergites 18–23 μm long, are smaller on sternites (10–12 μm long, as in Moubayed-Breil 2017, Fig. 61); distal part of sternites IV–VIII bearing rows of short spines of variably projecting (orally and posteriorly). Shape of lateral setae on segments I–VIII are of 3 types: a-type – spine-like, b-type – setae-like, c-type – bristle-like. Pedes spurii A and B and apophyses absent. Chaetotaxy and distribution pattern of armament on segments IV and VIII as in Figs. 38 and 40. Distribution pattern of lateral setae on segments I–VII: I (2, b-type); II–VII (Fig. 38; 4 setae including 2 a-type, 1 b-type and 1 c-type); segment VIII (Fig. 40) with 3 lateral setae including 2 setae b-type and 1 c-type. Anal segment (Fig. 42) 290–300 μm long, 340–350 μm maximum width. Male genital sac 225 μm long, 100 μm maximum width, overreaching apical margin of anal lobe by 40–45 μm, bearing 2 distinct apical lobes, inner one larger than the outer. Anal macrosetae (AM_1–AM_3) subequal about 70–80 μm long, spine-like, distal half slightly bent downwards; proximal spines AM_1 and AM_2 are separated by 65–70 μm, AM_2 and AM_3 by 20 μm.

Material examined

Holotype 1 male pharate adult, leg. P. Bitušík; Slovakia, the High Tatra Mountains, Nefcerka Valley, short, ca 90 m long stream originating as rheocrene from screes and flowing into glacial lake as a small waterfall, altitude 1940–1960 m (49°10′ 07.20“ N, 20°00′51.65“ E), 23.08. 2008.
Paratypes 3 pupal exuviae (2 males, 1 female), leg. P. Bitušík. The same locality and data as for holotype.

Environmental data of the type locality: bottom substrate composed mainly of granodiorite rocks covered by mosses, stable discharge and temperature regime with annual average water temperature 2.1 °C and summer maximum reaching 5.0 °C (Hamerlík 2007).

Holotype (male adult and its pupal skin, mounted on 2 slides) is deposited in the collections of the National Museum of Ireland, Kildare Street, Dublin 2, Ireland. Remaining paratypes are deposited in the collection of the

senior author. Type material was preserved in 80% alcohol, and later mounted in polyvinyl lactophenol. For each adult, the head, thorax and abdomen were cleared in 90% lactic acid before mounting on slides.

Etymology

The new species is named *bitusiki* after Peter Bitušík who has devoted his entire professional career to studying of chironomids, their ecology, distribution and their potential as bio-indicators of recent and past environmental changes.

Chaetocladius mantetensis Moubayed-Breil, sp. n

Diagnostic characters

Based on the shape of the inferior volsella *C. mantetensis* sp. n. lies close to *C. laminatus*, *C. dissipates, C. holmgreni*. However, this new species can be easily distinguished from other related *Chaetocladius* species in having: palpomere 3 nearly linear, truncate apically and bearing 4 sensilla clavata and 4 sensilla coeloconica; clypeus semi-circular. Lobes of antepronotum not gaping and distinctly thinner medially; humeral pit ellipsoid with dense contrasting spots; tergite IX with a truncate hump located medially (clearly visible when viewed laterally). Anal point triangular and sharply pointed; virga well developed, consists of 3 separated parts including 1 median part (with 3 small equal spines) and 2 lateral symmetrically elongated parts (each bearing a posteriorly directed median claw). Gonocoxite rounded apically; inferior volsella large lobe-like shaped, bearing an inner apical contrasting protuberance, a distinct marsupial pouch-like lobe is present posteriorly. Gonostylus massive and triangular, markedly projecting posteriorly in a rounded bare and hyaline apex; anterior margin bearing a row of short and long spines ending in 3 characteristic broad teeth of nearly similar size, which project orally and are located close to the megaseta; median area with a distinct curved dorsal rows of short spines reaching the base of megaseta; crista dorsalis well developed and smooth, wide and occupying the length of the gonostylus.

Male adult

($n = 4$ male adults; Figs. 10, 11, 12, 13, 14, 16, 17, 18, 19, 20) = sp. A in Moubayed-Breil (2017).

Large sized *Chaetocladius* species. Total length 3.90–4.00 mm. Wing length 1.25–1.35 mm. General colouration contrasting brown to dark brown to blackish. Head dark brown; antennae pale brown; thorax contrasting dark brown to blackish, mesonotal stripes distinctly dark brown to blackish, humeral pit brownish with contrasting blackish spots; wing pale; legs brown to dark brown; tergites I–IV brownish, tergites V–VIII and anal segment dark brown.

Head Eyes bare between ommatidia, hairs absent on inner lateral eye margin, outer posterior margin with few hairs. Temporals consist of 11 setae including 7 inner and 4 outer verticals; palpomere 3 (Fig. 11) with 4 sensilla clavata and 4 sensilla coeloconica placed distally; clypeus (Fig. 12) broad semi-circular shaped bearing 8 long setae in 4 rows. Antenna 1180–1200 μm long; last flagellomere (Fig. 10) 750 μm long, without pre-apical seta; antennal groove reaching segment 3.

Thorax Anteprontum (Fig. 19) well developed, lobes not gaping, with 6 lateral antepronotals located apically; acrostichals 21–22 in 1–2 rows, starting at a short distance from the antepronotum; dorsocentrals 11–12 in 1 row; prealars 4; humeral pit (Fig. 20) 55 μm long, 25 μm maximum width, oval to sub-oval, bearing distinct contrasting spots.

Wing Brachiolum with 1 seta. Number of setae on veins: R, 17–19; R_1, 5–6; remaining veins bare. Squama with 11–12 setae in 1 row.

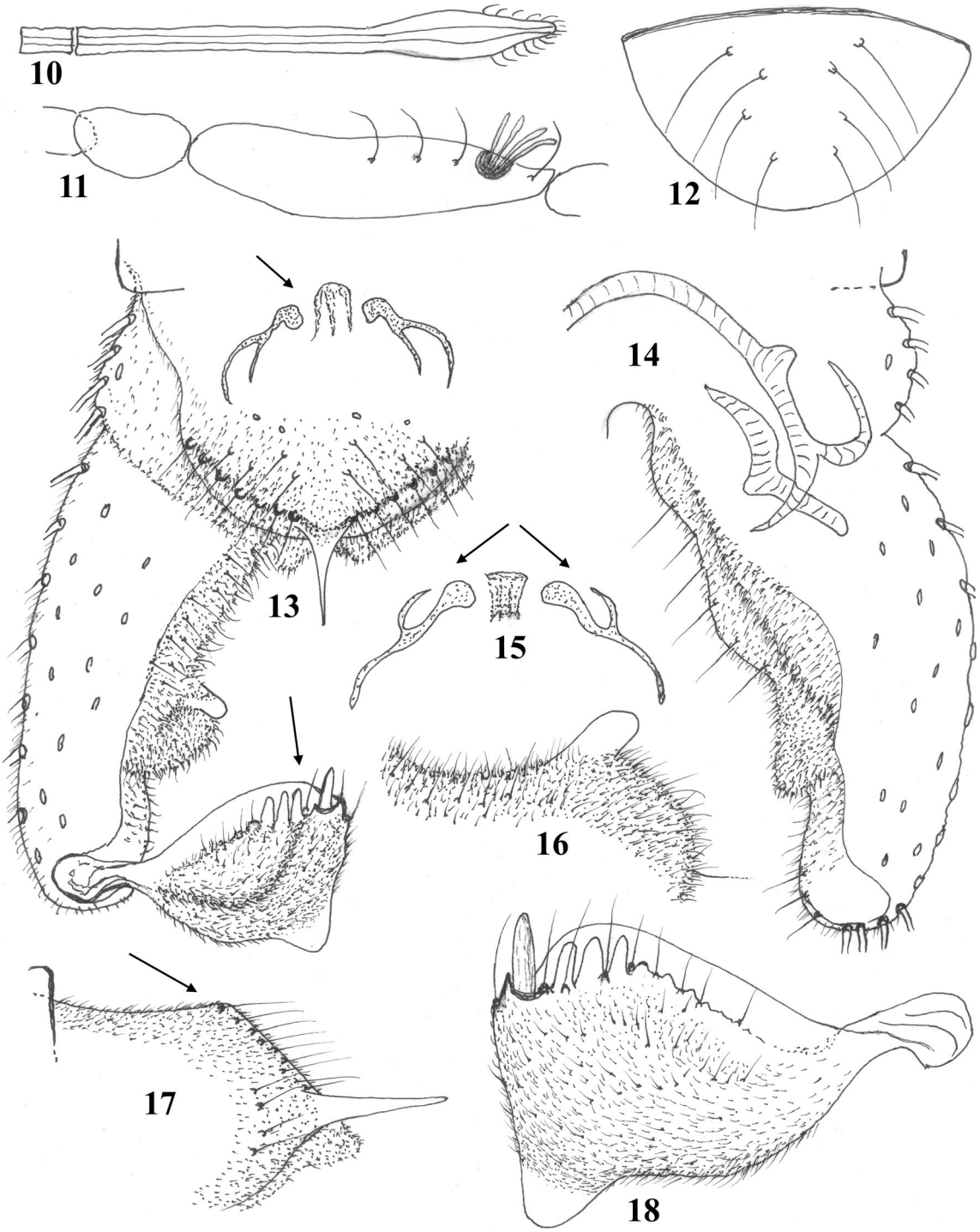
Legs Femur and tibia of PII sub-equal (each 1080 and 1090 μm long); sensilla chaetica present on tibia and tarsomeres ta_1–ta_5 of PI, PII and PIII. Length (μm) of tibial spurs: 45 (PI); 35 and 25 (PII); 65 and 25 (PIII). Length (μm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in Table 2.

Hypopygium in dorsal, ventral and lateral view as in Figs. 13, 14, and 16, ventral view with tergite IX and anal point removed as in Fig. 14. Tergite IX semi-circular, bearing a truncate hump located on distal part which is clearly visible in lateral view (Figs. 5 and 6); presence of 25–27 setae close to the posterior margin and base of anal point. Sternapodeme and phallapodeme (Fig. 14), phallapodeme nearly sickle-shaped and pointed basally. Anal point (Fig. 13) triangular, sharply pointed apically and markedly straight in lateral view (Fig. 17). Virga shape diplodocus-like, consisting of 3 separate parts: median one with 3 small sub-equal teeth, the 2 remaining parts are elongated laterally and bearing 1 median claw-like appendage which is posteriorly directed. Gonocoxite 280–290 μm long, 100–110 μm maximum width, rounded apically and lacking tubercle; ventral margin (Fig. 14) bearing 2 distinct lobes located medially and distally, distal lobe triangular; inferior volsella circular to lobe-like shaped, markedly contrasting,

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

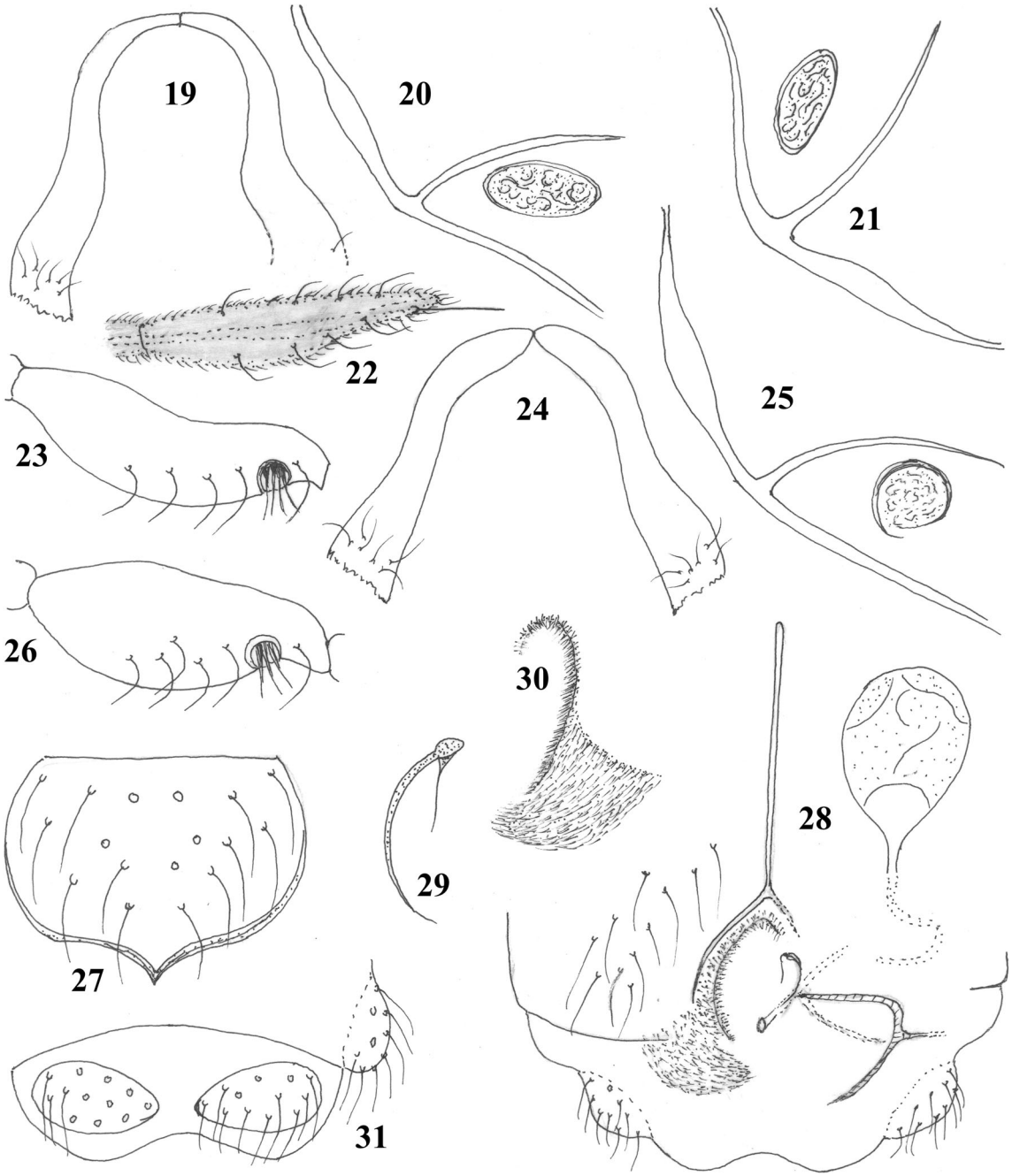
	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
PI	1070	1215	790	465	340	190	150	0,65	1,60	2,90	2,40
PII	1080	1090	510	310	240	150	135	0,47	2,00	4,25	2,17
PIII	1230	1315	760	420	330	185	150	0,58	1,80	3,35	3,15

For abbreviations see Table 1



Figs. 10–18 Male adult of *Chaetocladius* spp. *C. mantetensis* sp. n.: **10** last flagellomere; **11** palpomere 3 with sensilla clavata; **12** clypeus; **13** hypopygium in dorsal (with virga) and **14** ventral view (tergite IX and anal point removed). *C. laminatus*: **15** virga. *C. mantetensis* sp. n.: **16** inferior volsella, lateral; **17** tergite IX and anal point, lateral; **18** gonostylus, ventral. Details on the measurements are provided in the text

anteriorly with a nose-like apex, posteriorly with a large marsupial pouch-like lobe. Gonostylus (Figs. 13, 18) 120–125 μm long, 55–60 μm maximum width, broadly triangular, spherical and massive medially, markedly projecting posteriorly in a rounded bare and hyaline apex; posterior margin swollen medially; anterior margin bearing a row of short and long spines terminated by 3 characteristic large smooth teeth of nearly similar size (about 10 μm long), which project orally and are



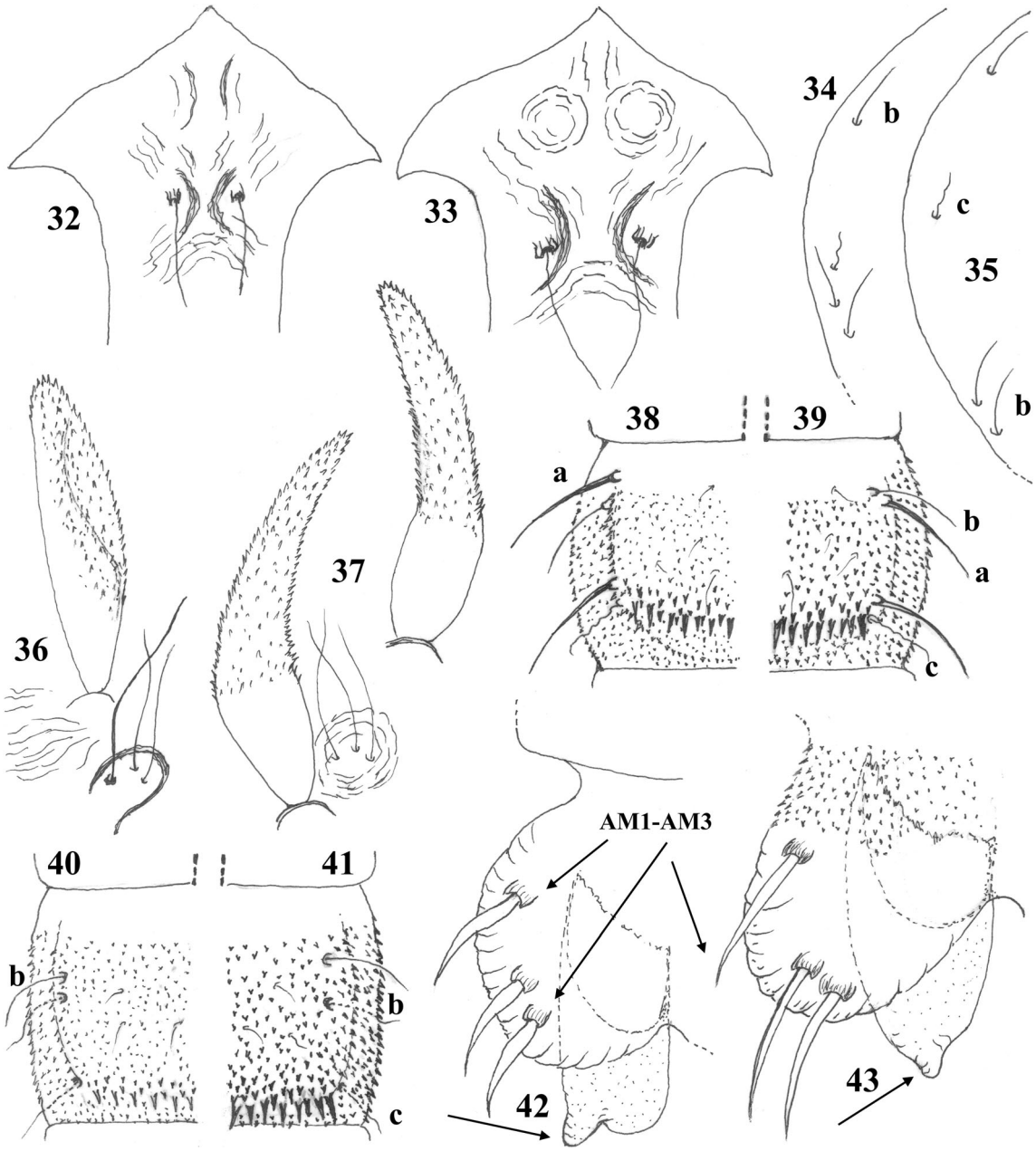
Figs. 19–31 Male and female adults of *Chaetocladius* spp. *C. mantetensis* sp. n.: **19** lobes of antepronotum of both male and female; **20** humeral pit of male and **21** female; **22** last flagellomere of antenna, female. *C. laminatus* (male adult): **23** palpomere 3; **24** lobes of antepronotum; **25** humeral pit. *C. mantetensis* sp. n. (female adult): **26** palpomere 3; **27** clypeus; **28** genitalia, ventral and dorsal view including gonapophysis VIII, sternite VIII, seminal capsule and gonocoxite; **29** apodeme lobe; **30** dorsomesal and ventrolateral lobes; **31** tergite IX and right gonocoxite, dorsal. Details on the measurements are provided in the text

placed close to the megaseta; median area with a distinct curved rows of short spines only visible dorsally and extending from the posterior part and reaching the base of megaseta, curved rows of short spines absent when gonostylus is viewed ventrally; crista dorsalis well developed, wide and occupying the length of the gonostylus.

Female adult

($n = 2$: pharate adults; Figs. 19, 21, 22, 26, 27, 28, 29, 30, 31)

Large sized species. Total length 4.00–4.10 mm. Wing length 2.08–2.10 mm. Colouration dark brown to blackish including head, antenna, thorax and tergites. Head and



Figs. 32–43 Male pupal exuviae of *Chaetocladius* spp.: **32, 34** Frontal apotome and distribution of dorsocentrals on thorax of: *C. bitusiki* sp. n.: **33, 35** *C. mantetensis* sp. n.: **36** thoracic horn of *C. bitusiki* sp. n. and **37** *C. mantetensis* sp. n. (two aspects). Chaetotaxy and distribution pattern of armament and conjunctives on segment IV and VIII of: **38, 40** *C. bitusiki* sp. n. (left); **39, 41** *C. mantetensis* sp. n. (right). Anal segment of **42** *C. bitusiki* sp. n.: **43** *C. mantetensis* sp. n. Details on the measurements are provided in the text

halteres dark brown; antenna distinctly dark brown, last flagellomere blackish (Fig. 22); thorax dark brown, mesonotal stripes blackish and contrasting; humeral pit brownish to dark brown. Abdominal tergites and anal segment dark brown to blackish.

Head Temporal setae 10–11, including 7–8 inner and 3 outer verticals, postorbitals absent. Palp 5-segmented, length (μm) of palpomeres: 35, 50, 125, 110, 175; distal part of third palpomere (Fig. 26) with 6 sensilla clavata and 4 pointed sensilla coeloconica. Clypeus (Fig. 27) 210 μm long, 275 μm maximum width, semi-circular to cup-like in shape, bearing 16 setae in 4–5 rows. Antenna 350 μm long; last flagellomere (Fig. 22) 125 μm long, proximal part clubbed on one side, distal part narrowing, surface including apex with numerous sensilla chaetica on each side, antennal groove reaching segment 3. AR 0.50.

Thorax Lobes of antepronotum (Fig. 19, as in the male) not gaping and narrowing at apex, lateral antepronotals 6 located apically; acrostichals 22–23 in 1–2 rows and starting a short distance from the antepronotum; dorsocentrals 16–17 in 1–2

rows; prealars 4, supraalars absent; humeral pit (Fig. 21) ellipsoidal, bearing contrasting darkened spots. Scutellum with 8 uniserial setae.

Wing Brachiolum with 1 seta. Number of setae on veins: R, 12; R_1, 13; R_{4+5}, 20–25; remaining veins bare. Squama with 9–13 setae in 1 row.

Legs As in the male except for the leg ratio.

Genitalia in dorsal and ventral view as illustrated in Fig. 28. Notum 155 μm long. Gonapophysis VIII including ventrolateral, dorsomesal and apodeme lobe as in Figs. 28, 29, 30: apodeme lobe (Fig. 29) arc-like shaped; dorsomesal lobe (Fig. 30) slightly curved outwards; ventrolateral lobe projecting inwards and distinctly narrowed apically. Sternite VIII with 22 setae (11 on each side of gonapophysis VIII). Seminal capsules (Fig. 28) 115 μm long, 85–90 μm maximum width, pearl-like, sclerotization partly occupying the apical area, ducts with loops and separate openings. Tergite IX and gonocoxite (Fig. 31), gonocoxite lobe-like with 9–10 setae; tergite IX nearly semi-circular, convex anteriorly and concave caudally, distinctly divided in 2 oval lobes, bearing 24 setae (12 on each side). Cercus 65–70 μm long.

Pupal exuviae

n = 6 (4 males and 2 females; Figs. 33, 35, 37, 39, 41, 43).

= *Chaetocladius* sp. A in Moubayed-Breil (2017).

Colouration yellow brownish to pale brownish except for the posterior transverse rows of spines on tergites and sternites, which are brownish to dark brown, tergites are slightly darker than sternites when the mounted exuviae are viewed laterally. Frontal apotome strongly wrinkled in antero-median area, cephalothorax with faint wrinkles near base of thoracic horn; abdomen, anal segment and genital sacs a little darker than remaining parts of the exuviae.

Total length 4.00–4.20 mm.

Frontal apotome (Fig. 33) distinctly domed, cephalic tubercles well developed, frontal setae 75–80 μm long, inserted on prefrons ventral to antennal sheath, distance between setae about 45 μm. Thoracic horn (two aspects, Fig. 37) 225–235 μm long, 30–35 μm maximum width, wider in its proximal part, gradually narrowing distally to the apex; densely toothed on both sides; precorneals nearly sub-equal about 110 μm long. Antepronotals consist of 2 median antepronotals (135 and 105 μm long) and 1 lateral antepronotal bristle-like setae (55 μm long). Dorsocentrals (Fig. 35) consist of 3 subequal setae (Dc_1, Dc_3 and Dc_4) of 50–60 μm long and 1 shorter (Dc_3 about 35 μm long) which is thinner and bristle-like; Dc_1 and Dc_2, well separated, Dc_3 placed close to Dc4; distance between: Dc_1–Dc_2 60–70 μm, Dc_2–Dc_3 100–115 μm, Dc_3–Dc_4 10–15 μm. Thoracic horn (two aspects, Fig. 37) 225–235 μm long, 30–40 μm maximum width, wider proximally and slightly narrowing distally; densely toothed on both sides; precorneals sub-equal, about 110 μm long.

Armament and distribution pattern of shagreen and points, posterior transverse rows of spines, chaetotaxy and lateral setation of abdominal segments IV and VIII as in Figs. 39 and 41. Field of points present on tergites I–VIII, and sternites IV–VIII. Posterior transverse rows of spines present on tergites II–VIII and sternites IV–VII; spines on tergites 20–25 μm long, are smaller on sternites (13–15 μm long); distal part of sternites IV–VII bearing rows of short spines variably projecting (orally and posteriorly). Pedes spurii A and B and apophyses absent. Distribution pattern of lateral setae on segments I–VII: I with 2 setae, b-type; II–VII (Fig. 39) with 4 setae including 2 a-type, 1 b-type and 1 c-type; segment VIII (Fig. 41) with 3 setae including 2 setae b-type and 1 c-type. Anal segment (Fig. 43) 240–250 μm long, 290–300 μm maximum width. Male genital sac 225–230 μm long, 105–110 μm maximum width, overreaching apical margin of anal lobe by 65 μm, bearing only 1 apical lobe located medially. Anal macrosetae (AM_1–AM_3) subequal about 90 μm long, spine-like and nearly straight; proximal spines AM_1 and AM_2 are separated by 80 μm, AM_2 and AM_3 by 15 μm.

Material examined

Holotype 1 male pharate adult, leg. J. Moubayed-Breil; Continental France, Mantet Nature Reserve (Eastern Pyrenees), upper basin of 'Font des Soques', glacial springs and streams, altitude 2000 m, (42°28′38" N, 02°18′26" E), 05.08.2010.

Environmental data of aquatic habitat: crystalline water, conductivity 30–40 $\mu S.cm^{-1}$, pH 5.5–5.7; temperature 6–12 °C.

Paratypes 3 pharate adults (1 male and 2 females), 1 male pupal exuviae, leg. J. Moubayed-Breil, the same locality and data as for holotype. 1 male adult and 1 male pharate adult, leg. J. Moubayed-Breil, Callau acid springs and peat bogs at Mantet Nature Reserve, altitude 2000–2300 m, 05.08.2010.

Environmental data of aquatic habitat: crystalline water, conductivity 30–40 $\mu S.cm^{-1}$, pH 5.5–5.7; temperature 6–12 °C.

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

Type material was preserved in 80% ethanol, and later mounted in polyvinyl lactophenol. For each adult, the head, thorax and abdomen were cleared in 90% lactic acid and then washed in 70% ethanol before mounting on slides.

Etymology

The new species is named *mantetensis* after the protected area of Mantet Nature Reserve, which is situated in the Eastern Pyrenees (SW-France) where the type material was collected.

Taxonomic position

Based on some features found in the male adult of *C. bitusiki* sp. n and *C. mantetensis* sp. n, these two new species can be separated from other related *Chaetocladius* species on the basis of the following combination of characters.

C. bitusiki sp. n. Sensilla clavata and sensilla coeloconica on palpomere 3 (Fig. 1) are different as figured in *C. laminatus* (Fig. 23); lobes of antepronotum narrowing at apex (Fig. 3), are much thicker in *C. laminatus* (Fig. 24); humeral pit semi-circular, while is circular in *C. laminatus* (Fig. 25); gonocoxite truncate apically and bearing a distinct tubercle at apex (Figs. 7, 8, 9); caudo-anterior part of gonostylus with 2 distinct teeth located close to the megaseta (Fig. 9), bearing few setae in *C. laminatus* (Moubayed-Breil 2017, Fig. 42); crista dorsalis well developed and broad (Fig. 9), is composed of 3 low lobes in *C. laminatus* (Moubayed-Breil 2017, Fig. 42).

C. mantetensis sp. n.: Palpomere 3 (Fig. 11) nearly linear, truncate apically and bearing 4 sensilla clavata and 4 sensilla coeloconica, is clubbed distally in *C. coppai* (Moubayed-Breil and Dia 2017, Fig. 3); clypeus (Fig. 12) semi-circular; dorsal hump on tergite IX distinctly truncate when viewed laterally (Fig. 17), is absent in *C. coppai* (Moubayed-Breil and Dia 2017, Fig. 5); median claw of lateral parts of virga posteriorly directed (Fig. 13), is orally directed in both *C. bitusiki* sp. n. (Fig. 7) and *C. laminatus* (Fig. 15); posterior apex of gonostylus rounded, is pointed in *C. coppai* (Moubayed-Breil and Dia 2017, Figs. 9–10); caudo-anterior part of gonostylus with 3 characteristic smooth teeth located close to the megaseta, is bare in *C. laminatus* (Moubayed-Breil 2017, Fig. 42) and bearing only 2 teeth in *C. coppai* (Moubayed-Breil and Dia 2017, Figs. 9–10).

Moreover, *C. bitusiki* sp. n. and *C. mantetensis* sp. n. can be easily separated from other related *Chaetocladius* species (*laminatus*-group) on the basis of the main distinguishing morphological features, which are summarized in the following key to known male adults from Europe and neighbouring areas: *C. laminatus, C. coppai, C. guisseti, C. elisabethae* and *C. purbeckensis.*

Key to known male adults of *Chaetocladius*, *laminatus*-group, from Europe

1. Anterior side of gonostylus rough to bumpy, lacking lobes, with two rows of setae located distally close to the megaseta (Moubayed-Breil 2017, Figs. 5, 7, 18); gonostylus projecting posteriorly with rounded apex; inferior volsella weakly developed, a narrow lobe, lacking or bearing a minute apical protuberance (Moubayed-Breil 2017, Figs. 5, 16, 20; Makarchenko et al. 2018, Figs. 1, 8–10; Langton and Armitage 2010, Fig. 2) ..... ***C. guisseti*, *C. elisabethae, C. purbeckensis***

– Anterior side of gonostylus smooth, lacking or bearing lobes (Figs. 9, 13, 18; Moubayed-Breil 2017, Fig. 42; Moubayed-Breil and Dia 2017, Fig. 6); gonostylus projecting posteriorly at a pointed or rounded apex (Moubayed-Breil 2017, Fig. 42; Moubayed-Breil and Dia 2017, Figs. 9–10); inferior volsella well developed, a triangular or broad lobe (Moubayed-Breil 2017, Fig. 42) and bearing a distinct apical protuberance **2**

2. Caudal apex of gonostylus pointed (Moubayed-Breil 2017, Fig. 42; Moubayed-Breil and Dia 2017, Figs. 9–10); crista dorsalis consists of 3 low fused lobes; inferior volsella well developed, triangular, inwardly projecting and bearing a distinct contrasting protuberance (Moubayed-Breil 2017, Fig. 42); humeral pit nearly circular (Fig. 25); hump on tergite IX lobe-like (Fig. 6) ..... ***C. laminatus***

– Caudal apex of gonostylus pointed or rounded (Moubayed-Breil and Dia 2017, Figs. 9–10; Figs. 9, 13, 18); crista dorsalis well developed and extended along the anterior margin of gonostylus; inferior volsella well developed, triangular or large lobe-like shaped, inwardly projecting and bearing or lacking a contrasting protuberance; humeral pit otherwise shaped (Figs. 4, 20–21); hump on tergite IX otherwise shaped (Figs. 5, 17) **3**

3. Caudal apex of gonostylus pointed; anterior side of gonostylus bearing 2 distinct long teeth (Moubayed-Breil and Dia 2017, Figs. 9–10); hump on tergite IX absent (Moubayed-Breil and Dia 2017, Fig. 5); median claw of lateral parts of virga posteriorly directed and diplodocus-like (Moubayed-Breil and Dia 2017, Figs. 6, 8) ..... ***C. coppai***

– Caudal apex of gonostylus rounded; anterior side of gonostylus bearing 2 or 3 long teeth (Figs. 9, 13, 18); hump on tergite IX present (Figs. 5, 17); lateral parts of virga otherwise shaped with median claw orally or posteriorly directed **4**

4. Humeral pit semi-circular and lacking contrasting spots (Fig. 4); hump on tergite IX a weak lobe (Fig. 6); lateral parts of virga with median claw orally directed (Fig. 7); apex of gonocoxite markedly truncate and bearing a characteristic tubercle (Figs. 7–9) ..... ***C. bitusiki***

– Humeral pit ovoid and with distinct bearing contrasting spots (Figs. 20-21); hump on tergite IX truncate and well developed (Fig. 17); median claw of lateral parts of virga posteriorly directed (Fig. 13); apex of gonocoxite

rounded and lacking tubercle (Figs. 13–14) ..... ***C. mantetensis***

Geographical distribution

Though *C. bitusiki* sp. n and *C. mantetensis* sp. n. apparently show closely phylogenetic affinities with the *laminatus*-group, as for *C. coppai* which is recently described from the French and Swiss Alps by Moubayed-Breil and Dia (2017); distinguishing characters found in the male adults of these three *Chaetocladius* species lead us to believe that both of the two new described species belong to a separate local 'Pyrenean and Tatra – Alpine' relict element.

While *C. bitusiki* sp. n. is known only from lotic habitats delimited by cold spring and stream located in the Tatra Mountains at altitude 1940–1960 m, the geographical distribution of *C. mantetensis* sp. n. is restricted to glacial springs, peat bogs and cold streams situated at high altitude (2000–2250 m) in the Eastern Pyrenees (France).

Ecology

Bryocolous and hygropetric habitats including waterfalls probably represent the most common sites within the crenal, cold streams and peat bogs for larval populations of both *C. bitusiki* sp. n and *C. mantetensis* sp. n. They both belong of the crenobiontic and crenophilous chironomid community as documented by Lindegaard (1995).

In the type locality of *C. bitusiki* sp. n., the chironomid assemblage was dominated by Diamesinae accounting for about 60% of all individuals, while Diamesa *tonsa/ cinerella/ vaillanti*-group, *Diamesa latitarsis* (Goetghebuer, 1921), *Pseudodiamesa branickii* (Nowicki, 1873), *Diamesa bertrami* Edwards, 1935, *Pseudokiefferiella parva* (Edwards, 1932) were the most abundant. Orthocladiinae were dominated by *Parorthocladius* sp., *Eukiefferiella fittkaui* Lehmann, 1972/ *minor* (Edwards, 1929), *Chaetocladius* spp. and *Orthocladius* (*Mesorthocladius*) *frigidus* (Zetterstedt, 1838). Despite of low abundance, *Tvetenia bavarica* (Goetghebuer, 1934) and *Smittia* sp. constituted a stable part of the assemblage. The assemblage consists of a mixture of kryal and kreno-rithral species/ taxa that underlines a uniqueness of this locality (Hamerlík et al. 2006, Hamerlík 2007).

Emergence of *C. mantetensis* sp. n. is confined from July to September. Associated species encountered in the type locality include: *B. legeri* (Goetghebuer, 1933); *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, 2017; *C. laminatus* Brundin, 1947; *C. suecicus* (Kieffer, 1916); *Eukiefferiella fittkaui* Lehmann, 1972; *Heleniella ornaticollis* (Edwards, 1929); *Krenosmittia boreoalpina* (Goetghebuer, 1944); *Parametriocnemus boreoalpinus* Gowin and Thienemann, 1942; *Rheocricotopus effusus* (Walker, 1856); *Rheosmittia spinicornis* (Brundin, 1956); *Thienemannia gracilis* Kieffer, 1909; *T. valespira* Moubayed-Breil and Ashe, 2013.

As many members of the *Chaetocladius* genus, *C. bitusiki* sp. n. and *C. mantetensis* sp. n. can be considered as relict species surviving in headwaters of European high mountain ranges. Their occurrence highlights the importance of headwater habitats as hotspots of diversity and endemism. Such pristine habitats are recently threatened by local and global environmental changes, therefore deserve much greater consideration, protection and preservation. Both the new described species can be used, together with other cold-stenothermal species, as sensitive bio-indicators for detecting of the global warming and local climate change.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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