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CHIRONOMUS Journal of Chironomidae Research

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Front page layout: Chironomid in title from photograph by Steve Marshall, Graphic design by Kolbjørn Skarpnes, NTNU Information Division.

Front page photo: Holotype male of *Coelotanypus validus* in Baltic amber. Deposited in Zoologisches Staatssammlung München. Photo: Viktor Baranov.

Editorial

A new dawn for chironomids in freshwater biomonitoring?

Non-biting midges are extraordinarily well-suited as environmental indicators in freshwater biomonitoring; numerous studies by those in our community support this claim. Nevertheless, our favorite organisms are excluded from many national monitoring programs due to their complex taxonomy, insufficiently described immatures, and resource demanding identification. For example, only a handful of European countries regularly use chironomid species in classification of water body ecological status for the EU Water Framework Directive (Weigand et al. 2019, supplement 2).

The use of molecular tools in species identification, especially DNA barcoding, is likely to change this. Numerous papers have shown that chironomids (as well as most other freshwater macroinvertebrates) can reliably be identified to species by DNA barcoding given that there is a high-quality barcode reference library. There are a few exceptions, like recently evolved species and species influenced by horizontal gene transfer (hybridization), where DNA barcoding does not work satisfactorily. But such cases are much less common in aquatic than terrestrial taxa where the endosymbiont *Wolbachia* complicates the genetic relationship between populations of closely related species (Smith et al. 2012). Of greater importance for freshwater biomonitoring might be that species previously regarded to be eurytopic actually consist of distinct genetic lineages that have different habitat preferences (Beermann et al. 2018). The ability to recognize and genetically identify cryptic species with potentially different environmental preferences can increase the resolution in biological monitoring, especially with species rich and widely distributed taxa such as Chironomidae.

DNA metabarcoding makes it possible to sequence and identify hundreds of species from bulk- or environmental samples at the same time. Thus, the time-consuming process of sorting and morphologically identifying specimens from benthic samples can be reduced, and more localities surveyed. This may also enable more biological replicates to be taken, thereby increasing the statistical power in biological assessments. Our knowledge as biologists and chironomid experts will still be needed to interpret results and detect errors, because a list of species names is of limited value if it is not proofed and put into an ecological context. Our expertise is also needed to improve existing quality metrics (indices) by incorporating chironomid data, describe the diversity, and to curate the barcode reference library.

Although progress has been made in recent years, DNA metabarcoding, for several reasons, does not provide reliable data on species abundances (Elbrecht and Leese 2015). Thus, if DNA metabarcoding is to be used regularly in freshwater biomonitoring, water quality indices that rely on presence/absence data must be preferred. Although a transfer to presence/absence data might not result in changed ecological state classifications (Buchner et al. 2019), there is room for improvement in the indices used. The obvious way would be to incorporate more species-level information on species-rich indicator taxa and thereby increase the resolution and explanatory power. Chironomidae is here the excellent group of choice for freshwater biological monitoring (Czechowski et al. 2020).

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CHIRONOMUS Journal of Chironomidae Research No. 32, 2019: 4-24. Current Research.

CLUNIO BOUDOURESQUEI SP. N. AND *THALASSOSMITTIA BALLESTAI* SP. N., TWO TYRRHENIAN MARINE SPECIES OCCURRING IN SCANDOLA NATURE RESERVE, WEST CORSICA (DIPTERA: CHIRONOMIDAE)

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http://zoobank.org/4E98B6AF-1BFF-4C12-BCF0-C416B600CA94

Abstract

Clunio boudouresquei sp. n. and *Thalassosmittia ballestai* sp. n. are diagnosed and described based on associated material of male adults, pharate male adults and pupal exuviae recently collected in the marine littoral zone of Scandola Nature Reserve (Cala Litizia, Punta Palazzu, Focolara Bay, West Corsica). While *C. boudouresquei* sp. n. is described as male and female adults and pupal exuviae, *T. ballestai* sp. n. is described only as male adult and pupal exuviae. On the basis of some atypical characters found in the male adult and pupal exuviae, both *C. boudouresquei* sp. n. and *T. ballestai* sp. n. appear to belong, to a local Tyrrhenian element. Biological cycles of both new species are closely related to alternation between marine and terrestrial ecological conditions, which are strongly reinforced during spring tides of lunar rhythms. Larval stages of both new species are typically marine shore dwellers of the intertidal zone along seacoasts of the Tyrrhenian sub-region, where the biological and ecological quality of marine coastal habitats are being seriously damaged by various anthropogenic activities. In the latter sites, the *Lithophyllum byssoides* (Lamarck) Foslie pavements (trottoirs, encorbellements) are actually threatened by the massive proliferation of Sea Mussels (Mytilidae), which predominate when changes in water quality and level of pollution become increasingly high. The two new species are considered as pertinent biological indicators of the marine coastal habitats around the Tyrrhenian Region, in particular, those delimited by the mid-littoral large bio-constructions of the red calcified alga *L. byssoides*, where the larvae of *C. boudouresquei* sp. n. are exclusively confined. Such threatened species are considered biogeographic Tyrrhenian representatives and indicators of global warming and local climate change in the region, particularly to a rising sea level. Comments on the taxonomic position, ecology and geographical distribution of the new species are given.

Introduction

Recent investigations of marine chironomids conducted in the protected area of Scandola Nature Reserve (West Corsica), allowed us to sample fully developed pharate, adults, pupae and pupal exuviae of two new species, which belong to the genera *Clunio* Haliday, 1855 and *Thalassosmittia* Strenzke & Remmert, 1957. The two new species (*C. boudouresquei* sp. n. and *T. ballestai* sp. n.) were previously reported by Moubayed-Breil et al. (2013) and Moubayed-Breil & Ashe (2012) as, *Clunio* sp. 1 and *Thalassosmittia* sp. 1. Worldwide the genus *Clunio* is known from all geographic regions, while *Thalassosmittia* is restricted the Neotropical, Nearctic, Palaearctic and Afrotropical regions.

Data on the taxonomy and geographical distribution of known valid species of these two genera from Europe and some neighbouring areas show that there are about 25 species for the genus *Thalassosmittia* and about 10 species for the genus *Clunio* (Saunders 1928; Storå 1936; Tokunaga 1936; Strenzke & Remmert 1957; Strenzke 1960; Sæther 1969; Morley & Ring 1972; Neumann 1976; Sæther 1977; Heimbach 1978; Michailova 1980a, 1980b; Coffman et al. 1986; Cranston et al. 1989; Langton 1991; Wang & Sæther 1993; Neumann et al. 1997; Sasa & Suzuki 1999a, 1999b; Yamamoto 2004; Langton & Pinder 2007; Tasdemir 2010; Sæther & Andersen 2011; Ashe & O'Connor 2012; Kaiser & Heckel 2012; Sæther & Spies 2013; Andersen & Pinho 2014; Moubayed-Breil & Ashe 2012; Andersen et al. 2013; Moubayed-Breil et al. 2013; Moubayed-Breil & Ashe 2016; Yamamoto et al. 2019). Consequently, the description of *C. boudouresquei* sp. n. and *T. ballestai* sp. n. increases worldwide the total number of described species to 26 for the genus *Thalassosmittia* and to

11 for the genus *Clunio*.

In this paper, *C. boudouresquei* sp. n. and *T. ballestai* sp. n. are described and diagnosed based on associated material recently collected in the marine littoral zone of Scandola Nature Reserve (Focolara Bay) located in western Corsica. While *C. boudouresquei* sp. n. is described as male and female adults and pupal exuviae, *T. ballestai* sp. n. is described only as male adult and pupal exuviae. On the basis of some atypical characters found in the male adult (shape of head, palpomeres, apodemes, tergite IX, inferior volsella, gonostylus) and pupal exuviae (distribution pattern of armament on tergites and sternites), *C. boudouresquei* sp. n. and *T. ballestai* sp. n. appear to belong, each to a local Tyrrhenian marine element.

Larval stages of both new species are typically marine shore dwellers of the intertidal zone along seacoasts, where dense populations are often reported. In particular, those of *C. boudouresquei* sp. n. are exclusively confined to the intertidal habitats delimited by the mid-littoral large bio-constructions (pavements, 'trottoirs') of the 'long-living' red calcified alga *Lithophyllum byssoides* (Lamarck) Foslie, which are locally present in both western Corsica (Punta Palazzu) and southern France. In addition, the latter pristine habitats are considered to be microrefugia for a well-diversified biocoenosis including marine and semi-terrestrial taxa/species. The two new species are considered as pertinent indicators of the environmental quality of seacoasts around the Tyrrhenian Region (insular and continental Provinces), where changes in biological and ecological conditions of the intertidal zone are presumably being the result of human activities and global warming in this region.

Material and methods

Material composed of adults, pharate adults and pupal exuviae belonging to both *C. boudouresquei* sp. n. and *T. ballestai* sp. n. was collected using standard methods: troubleau net for the benthos (larvae and pupae) and individuals floating on the surface of the water; Brundin drift nets towed behind a boat for pharates, pupae and drifted pupal exuviae; sweep net for flying adults. Additional inorganic material composed of plastics (micro- + macroplastics) and pellet tar (0.5-5 to 10-15 mm), was also collected during the fieldwork, which were especially of great interest for useful comments on both biological and ecological quality of environment. Male adults were preserved in 80-85% ethanol, then cleared of musculature in 90% lactic acid (head, thorax, abdomen and anal segment) for 60 to 80 minutes; this 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 when the anal point and tergite IX were removed. 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 were preserved in 85% ethanol for an eventual DNA analysis. Morphological terminology and measurements follow those of Sæther (1980), Langton (1991) and Langton & Pinder (2007) for the imagines and pupal exuviae. Taxonomic remarks on some related known species from Europe with comments on the ecology and geographical distribution of the two new species are given.

Description

Clunio boudouresquei Moubayed-Breil, sp. n.

Clunio sp. 1, in Moubayed-Breil & Ashe (2012), Moubayed-Breil et al. (2013).

http://zoobank.org/3B0274A6-5460-4D84-8028-2BF8903468B9

Material examined

Holotype. France, West Corsica, Scandola Nature Reserve, Focolara Bay, Cala Litizia, bio-constructions of the red calcified marine alga *L. byssoides* of Punta Palazzu locality (Fig. 10), 42° 21′ 25″ N, 8° 34′ 0″ E; 1 male pharate adult, leg. J. Moubayed-Breil, 03.VI.2015. Locality No. 31 in Moubayed-Breil & Ashe (2012); locality No. 30 in Moubayed-Breil et al. (2013). Marine water temperature: 10-12°C (min.), 22-24°C (max.).

Paratypes (all leg J.M-B.): 2 male adults, 1 female adult, 6 pupal exuviae (4 males and 2 females), same locality as for holotype, 03.VI.2015.

Holotype (mounted on 1 slide) and 2 pupal exuviae (1 male and 1 female) are deposited in the collections of the Zoologische Staatssammlung München (ZSM), Munich, Germany. Additional paratypes are deposited in the senior author's collection.

Diagnostic characters

Based on some characters found in the male adult (vertex with lateral projections, typical morphology of inferior volsella and both basal and caudal apodemes, presence of megaseta on gonostylus), *C. boudouresquei* sp. n. appears to belong to a local Tyrrhenian marine element. However, this new species can be distinguished from other European *Clunio* species by the blow listed characters.

Male adult: Vertex with two lateral triangular projections; antenna 10-segmented, last flagellomere longer than the 3 preceding segments; sensilla chaetica present on tibia and ta1 of PI-PIII; tergite VIII with a distinct elongate ellipse-like ridge located antero-medially, midline area with 6 short setae; apical expansion of tergite IX distinctly convex at apex; caudal apodeme with 5-6 curved claw-like tubercles; inferior volsella wider at base and narrowing distally; gonostylus unusually bearing a black fingernail-like megaseta, apex ending with a single finger-like tubercle.

Female adult: Eyes densely haired, temporals 2 including 1 inner and 1 outer vertical; clypeus semi-circular, bare; antenna 7-segmented, last flagellomere elongated, segments 6 and 7 each with 1 tubular sensilla chaetica; palpomere 2, globular, with 3 sensilla clavata distally and 1 long fine seta; tarsomere ta_1 of PI and PII is half long as ta_1 of PIII; sensilla chaetica present on tibia and tarsomere ta_1 of PI, PII and PIII; sternite VIII with 22-24 setae; dorsomesal lobe of gonapophysis VIII convex medially and projecting; apodeme lobe swollen in its postero-median part; 2 stout inwardly directed setae present on each side of gonapophysis VIII; seminal capsules sub-oval; tergite IX oval, markedly divided, with 20-22 setae; gonocoxite weekly developed; cercus sub-rectangular.

Pupal exuviae: Antero-median area of frontal apotome and thorax with wrinkles; frontal setae present on distal part of frontal apotome; dorsocentrals Dc_1-Dc_2 and Dc_3-Dc_4 located close together; anterior transverse rows of spines interrupted on tergite II; posterior transverse rows of hooks present on sternites V-VII.

Etymology: the new species is named '*boudouresquei'* in honour of our colleague Ch-Fr. Boudouresque (University of Sciences, Luminy, Marseille), who is still active in studying the biology and ecology of the Mediterranean marine flora and fauna including those of the protected area of Scandola Nature Reserve. As he always did in past, he keeps working on developing projects to preserve the marine protected area of Scandola Nature Reserve, which represents a precious and valuable inheritance area.

Male adult

(n = 5, 2 pharates; Figs 1c-h, 2a-d, 3a-b)

Total length 2.70-2.90 mm. Wing length 1.35-1.40 mm, TL/WL = 2-2.10. General colouration contrasting brown to dark brown. Head and antennae dark brown; thorax contrasting light brown to brown with dark brown mesonotal stripes; wing pale translucent; legs brown to dark brown; tergites I-VII brownish, tergite VIII and anal distinctly contrasting light brown to dark brown.

Head. Eyes sub-circular without dorso-median extension, densely hairy with long and short pin-like hairs; hairs absent on inner lateral eye margin, outer posterior margin lacking setae. Vertex (Fig. 1c, dorsal; Fig. 1d, ventral) with 2 triangular lateral expansions; temporals 2 consist only of 2 outer verticals, postorbitals absent. Antenna 10-segmented, about 500 µm long, lacking plume; segment 1-2 (Fig. 1e), segment 1 globular, segment 2 145 µm long, linearly elongated; segment 2-9 globular, nearly sub-equal (30-40 µm long); ultimate flagellomere (Fig. 1f) 105 µm long, about 40 µm maximum width, as longer than the 3 preceding segments, thumb-like shaped; sensilla chaetica present on segments 1 to 8; antennal groove reaching segment 2; AR 0.27. Palp (Figs 1g-h) 2-segmented, lacking sensilla clavata; left palpomeres 1-2 respectively 25 and 55 µm long, palpomere 2 ending with a long finger-like expansion; right palpomeres 1-2 (Fig. 1h), first one indistinct, second one sub-rectangular to square-like shaped, side about 25-30 µm long. Clypeus semicircular and bare. Thorax. Antepronotum (Fig. 1c) weakly developed with joined lobes. Antepronotals 3; acrostichals 4-5 starting close to antepronotum; dorsocentrals 5 in 1 row; prealars 2; scutellum with 8 setae. Wing. Brachiolum with 1 seta; number of setae on veins: R, 7; R_{2+3}, 6-7; remaining veins and squama bare. Legs. Femur of PI-PIII broad (100-110 µm maximum width); tibial spurs distinctly conspicuous and curved at apex, length (µm): PI, 40; PII, 65; PIII, 55. Tarsomeres ta_3 and ta_4 of PI and PIII (45 and 40 µm long) shorter than tarsomere ta_5 (75 and 65) as in Table 1; SV of PIII (9.48) is much higher than in PI and PII. Sensilla chaetica present on tibia and tarsomere ta_1 of PI-

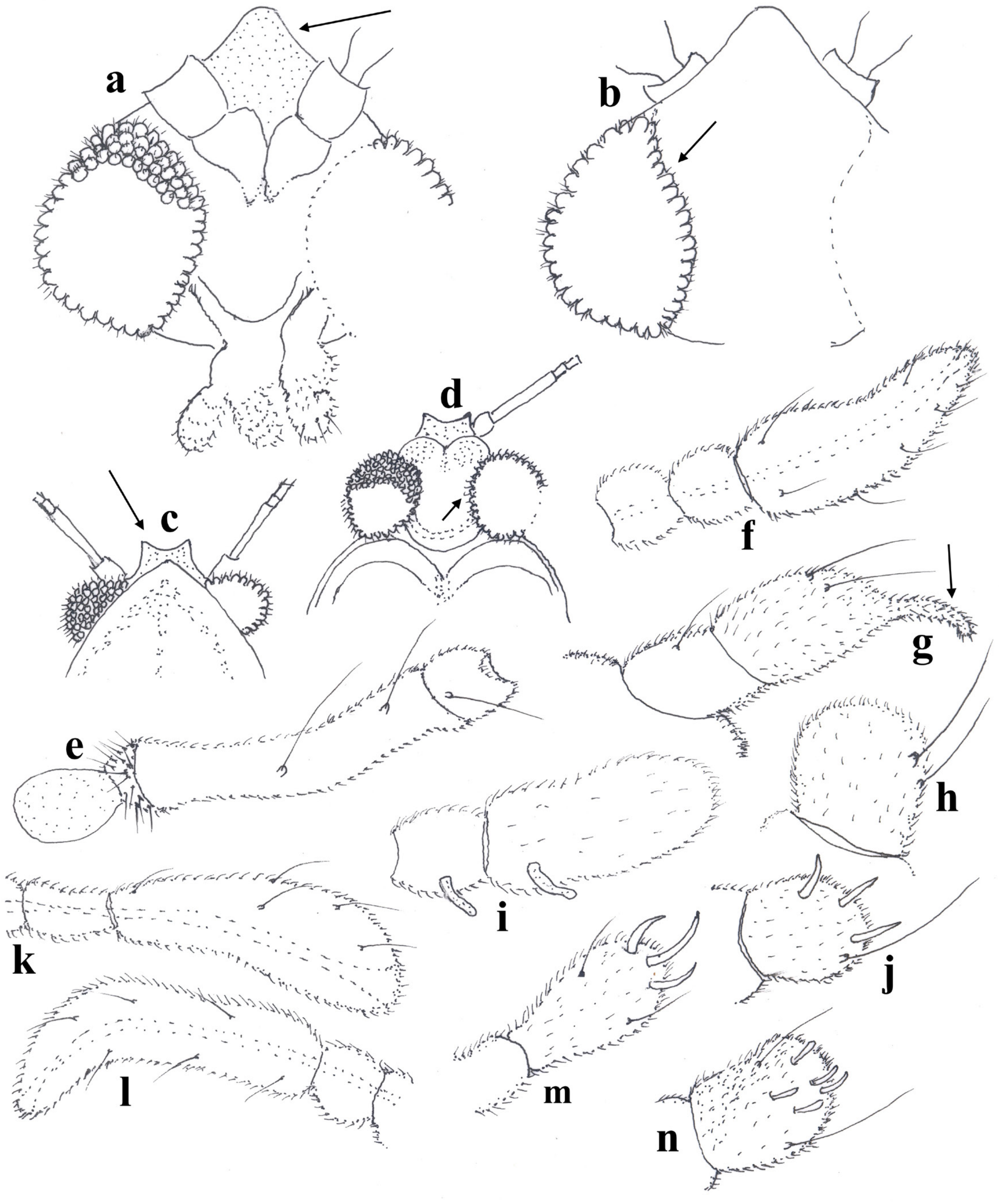


Figure 1. Male and female adults of *Clunio* spp. Male adult. Head in dorsal and ventral view of: a-b) *C. marinus;* c-d) *C. boudouresquei* sp. n. *C. boudouresquei* sp. n.: e) antenna, segments 1-3; f) segments 8-9 and last flagellomere; g) palp, left and right (h). Female adult of *C. boudouresquei* sp. n.: i) segment 6 and last flagellomere; j) palp. Male adult. Segment 9 and last flagellomere of: k) *C. boudouresquei* sp. n.; l) C. marinus. Male adult. Palp of: m) *C.* sp. 2; n) *C. marinus*.

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

Abdomen. Hypopygium in dorsal and ventral view as in Figs 2a-b (Fig. 2a, dorsal; Fig. 2b, ventral, with tergite IX removed). Laterosternite absent. Tergite VIII with a distinct elongate ellipsoidal ridge located antero-medially, midline area bearing 6 short setae (3 on each side). Tergite IX is *Clunio*-type, without anal point; dorsal side (Fig. 2a) densely covered with macrotrichia-like setae in reclinate pattern (orally directed), postero-median area with about 40 short setae about 15 µm long; ventral side (Fig. 3b) with a semi-circular posterior lamella covered with macrotrichia. Ventral side of hypopygium (Fig. 1b) includes 4 distinct apodemes (basal, axial, lateral and caudal) which can be detailed as: basal apodeme (= sternapodeme) 140 µm maximum width, T-like shaped (Figs 1b, 3a) with anterior side concave (occasionally convex as in Fig. 3c); axial apodeme about 320 µm long, ending with a bi-lobed semi-circular apical expansion (Figs 2a, 3b); lateral apodeme (= phallapodeme) 250 µm long, inwardly bent distally; caudal apodeme (Fig. 2b) distinctly branched on each lateral side, composed of 2 connected parts, basal one is rectangular brush-like shaped, posterior one consists of 5-6 grouped claws of typical structure. Gonocoxite about 600 µm long, 250 µm maximum width, distal inner area with dense group of long and short setae; inferior volsella 220 µm long, 15-20 µm width medially, located distally, conical and densely covered with short and upwardly directed setae. Gonostylus (Figs 2c-d) inversed triangle-like shaped, arched with acute posterior angle and projecting backwards posteriorly; thicker at base, much thinner in median and distal parts; length (in µm of sides): basal one about 20, concave one 25, convex one 17; apical angle (Fig. 2d) with 1 single characteristic finger-like tubercle; crista dorsalis well-developed, consists of 2 unequal lobes occupying the entire length of gonostylus; megaseta tooth-like shaped and conspicuous, nearly as high as wide (12-15 µm), represents an unusual character in the genus *Clunio*.

Female adult

(n = 2, 1 pharate; Figs 1i-j, 4a-e)

Small sized species. Total length 1.65-1.70 mm. General shape is *Clunio* female-type. Colouration as in the male adult except for the thorax, which is less dark. Antennae light brown; legs brownish with blackish claws. Abdominal tergites and anal segment contrasting brown to dark brown. Head. Eyes densely hairy, sub-circular without dorsomedian extension, hairs absent on inner lateral eye margin, outer posterior margin lacking setae. Temporals 2, including 1 inner and 1 outer vertical. Clypeus semi-circular, bare. Antenna 7-segmented, about 200 µm long; last flagellomere (Fig. 1i) 60 µm long, elongated and lobe-like; segments 6 and 7 each with 1 tubular sensilla chaetica; antennal groove reaching segment 2; AR 0.43. Palp (Fig. 1j) 2-segmented; segment 1, indistinct; palpomere 2, globular about 20 µm long bearing 3 sensilla clavata distally and 1 long fine seta. Thorax. Chaetotaxy indistinct. Legs. Tibia of PI, PII and PIII nearly equal (185, 180, 185 µm long); length (µm) of tibial spurs of: PI, 40; PII, 65; PIII, 5. Tarsomeres ta_1-ta_5 of PI and PII equal in size as in Table 2; tarsomeres ta_1 and ta_5 of PI and PIII are globular and equal in size (40 µm long each); tarsomere ta_1 of PI and PII (40 µm long) is half long as ta_1 of PIII (85 µm). Femur of PI is much wider (90 µm) than in PII-PIII (70 and 60); tibia of PIII is wider (55 µm) than in PI and PII (45 µm each); tarsomere ta_1 of PII is much wider (50 µm) than in PI and PIII (27 µm each); tarsomere ta_1 of PII is wider (55µm) than in PI and PIII (30 µm each). LR value (Table 2) of PIII (0.46) is much higher than those of PI and PII (0.22 each); SV value (Table 2) of PI and PII (10.75 and 11) is about twice of PIII (5.35). Sensilla chaetica present in low number on tibia and tarsomere ta_1 of PI, PII and PIII. Length (in µm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in Table 2.

Abdomen. Anal segment (dorsal, Fig. 4a; ventral, Fig. 4b) 280 µm long, 260 µm maximum width at base, 130 µm wide at caudal part. Genitalia in dorsal and ventral view as illustrated in Figs 4b-e. Notum about 140 µm long with separate rami; on each side the rami are connected to a sternal

Table 1. Male adult of *Clunio boudouresquei* 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	475	615	160	50	45	40	75	0.26	5.95	6.81	0.90
PII	555	555	200	95	115	55	70	0.36	3.91	5.55	0.75
PIII	610	575	125	50	45	40	65	0.22	6.55	9.48	0.80

Table 2. Female adult of *Clunio boudouresquei* 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	245	185	40	25	20	15	40	0.22	4.7	10.75	0.80
PII	260	180	40	25	20	15	40	0.22	4.8	11	0.85
PIII	270	185	85	35	40	20	45	0.46	3.86	5.35	0.90

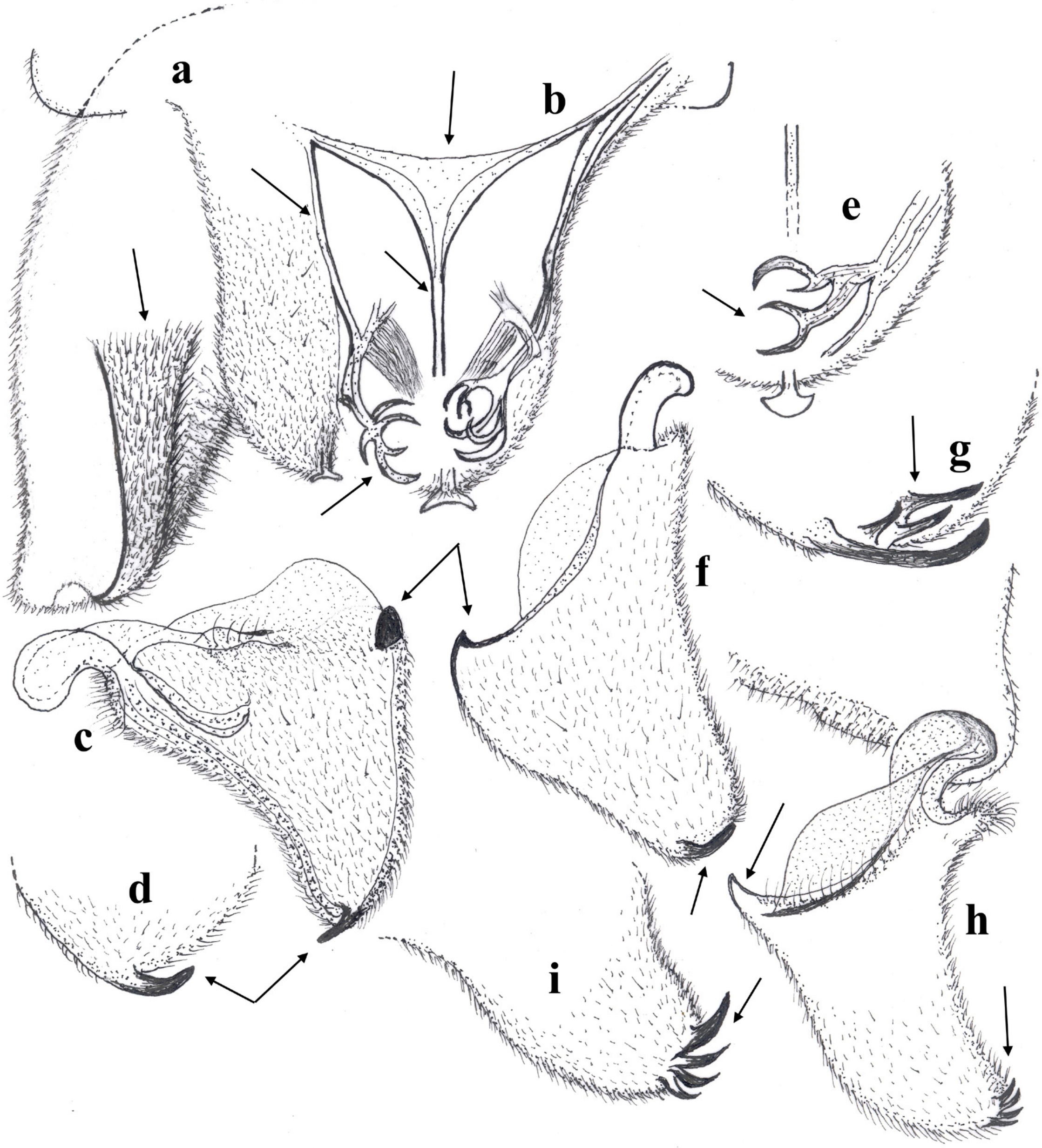


Figure 2. Male adult of *Clunio* spp. *C. boudouresquei* sp. n.: a) hypopygium, tergite IX and inferior volsella, dorsal; b) basal, lateral, axial and caudal apodemes, ventral view; c) gonostylus dorsal; d) gonostylus, apical part. *C. mediterraneus*: e) caudal apodemes of hypopygium, ventral; f) gonostylus, dorsal. *C.* sp. 1: g) apex of gonostylus. C. *marinus*: h) gonostylus, dorsal; i) apical part.

axial apodeme. Sternite VIII with 22-24 setae (11-12 on each side of the notum). Gonapophysis VIII (Figs 4b-d): dorsomesal lobe (Fig. 4d) convex medially and projecting in both proximal and apical parts; ventrolateral lobe directed downwards, broader basally and narrowing posteriorly; apodeme lobe (left, Fig. 4c) distinctly swollen in its postero-median part. Presence of 2 stout inwardly directed setae on each side of gonapophysis VIII between base of sternite VIII and ventrolateral lobe. Seminal capsules 70 µm long, 40 µm maximum width, sub-oval and well-sclerotized medially. Spermathecal ducts with loops and separate openings. Tergite IX (Fig. 4e) egg-like shaped, markedly divided, with 20-22 setae (10-11 on each side). Gonocoxite (Figs 4a-b) weekly developed but widely extended, bearing 8-9 setae. Cercus (Fig. 4b) sub-rectangular, normally developed and projecting upwards.

Pupal exuviae

(n = 10, 7 males and 3 females; Figs 5a, 5c, 5e-f)

Total length 2.85-3.15 mm. Colouration contrasting dark brown to yellow brown, wrinkles present on antero-median area of frontal apotome and tho-

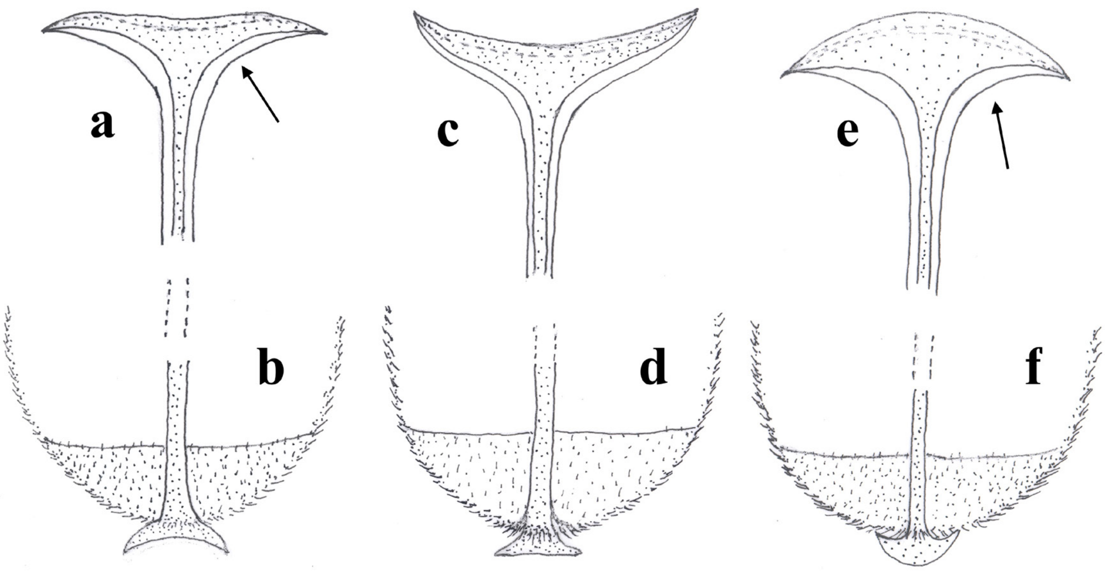


Figure 3 a-f). Male adult of *Clunio* spp. Axial apodeme and distal part of tergite IX of: a-b) *C. boudouresquei* sp. n.; c-d) *C. mediterraneus*; e-f) *C.* sp. 1.

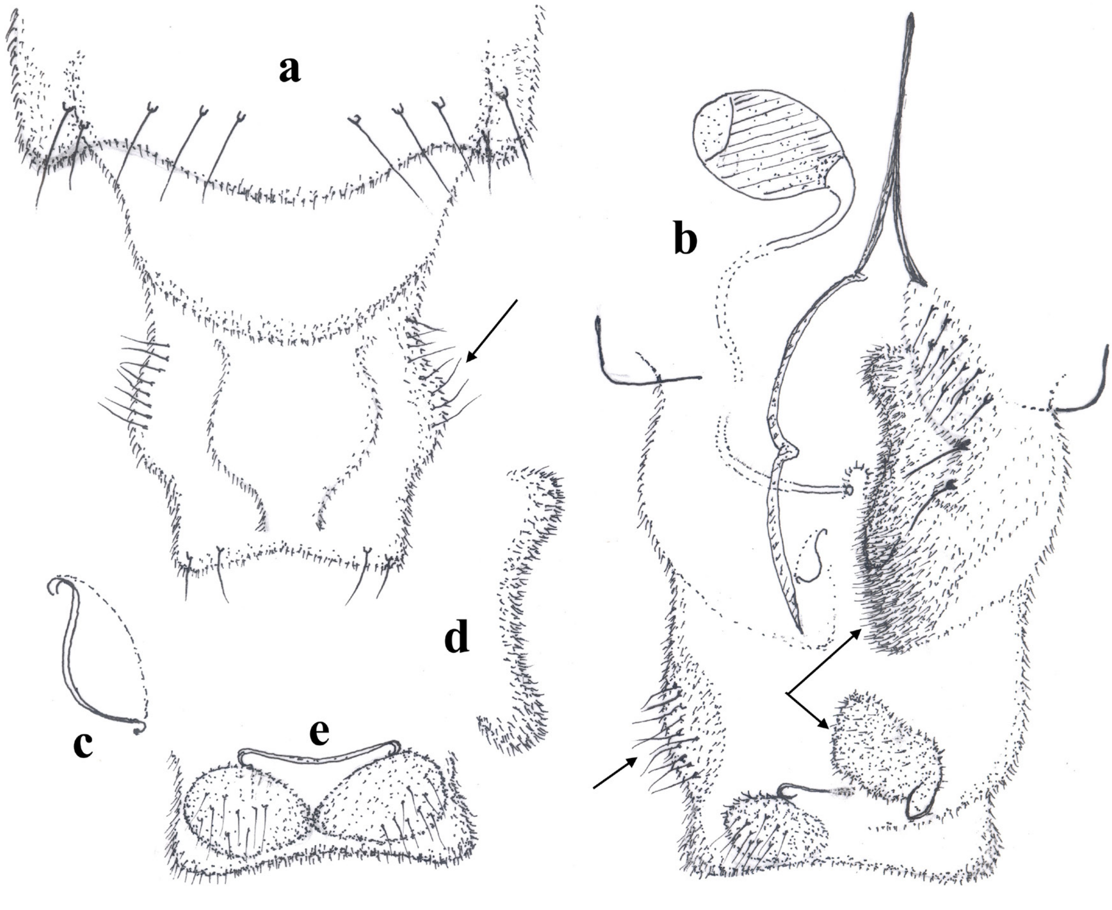


Figure 4. Female adult of *Clunio boudouresquei* sp. n. a) tergites VIII-IX with gonocoxites, dorsal; b) genitalia with gonapophysis VIII, sternite VIII, seminal capsule, gonocoxite, tergite IX and cercus; c) apodeme lobe; d) dorsomesal lobe; e) tergite IX, dorsal.

rax; abdomen and anal segment brownish. Cephalothorax. Frontal apotome broadly trapezoidal, frontal setae about 40 µm long, inserted posteromedially, distance between frontal setae 50 µm. Median antepronotals nearly subequal (30-35 µm long), lateral antepronotal absent; precorneals subequal, about 50 µm long, insertion arranged I, triangle. Dorsocentrals Dc_1-Dc_2 and Dc_3-Dc_4 located close together; Dc_4 subequal (about 10-15 µm long), Dc_3 and Dc_4 sub-equal (about 40 µm long); distance (in µm) between: Dc_1 to Dc_2 30, Dc_2 to Dc_3 80, Dc_3 to Dc_4 10.

Abdomen. Armament, chaetotaxy, distribution pattern of shagreen and details of armament on tergites and sternites II-VII as in Figs 5a, 5c, 5e-f. Tergite I and sternites I-III bare, sternite IV occasionally with 1-2 rows of small spines (Fig. 5a). Conjunctives of tergites III-VII and sternites V-VII with one transverse row of hooks, which are smaller on sternites, conjunctive on segment VIII composed only of short posteriorly directed spines. Antero-median transverse rows of spines present on tergites II-VII, sparsely present and interrupted medially on tergite II, becoming denser and more extensive on tergites III-VI. Pedes spurii A and B absent; apophyses on tergites and sternites absent. Number and distribution pattern of lateral setae on segments I-VIII: 2 on segment I; 2/3 on II-VII; 3 on VIII. Anal segment is *Clunio*-type, genital sac 490-500 µm long, 70 µm maximum width, ending each with 1 pointed tubercle.

Larva

Known but not described.

Differential diagnosis

Male adult and pupal exuviae of *C. boudouresquei* sp. n. are compared to those of known *Clunio* species from seacoasts of Europe and neighbouring areas, based on material collected by the senior author in Corsica, continental France, Italy, Spain except for Bulgaria (Varna seashores, leg. P. Michailova). Some relevant specific features found in the male adult and pupal exuviae will easily separate the new species from other members of *Clunio* by the following combination of characters:

Male adult: Frontal area of head bearing 2 apical projections (Figs 1c-d), is differently shaped in *C. marinus* (Figs 1a-b); last flagellomere narrowed apically (Fig. 1f), is widely clubbed in *C. mediterraneus* (Fig. 1k) and linearly curved in *C.* sp. 1 (Fig. 1l); typical long finger-like expansion of left palp (Fig. 1g), is absent in both *C.* sp. 2 (Fig. 1m) and *C. marinus* (Fig. 1n); caudal apodeme composed of basal brush-like and 5-6 apical claws (Fig. 2b), is lacking basal brush and less branched apically in *C. mediterraneus* (Fig. 2e); megaseta present on gonostylus (Fig. 2c), is absent in *C. mediterraneus* (Fig. 2f) and *C. marinus* (Fig. 2h); apex of gonostylus with only one single finger-like tubercle (Figs 2c-d), while consists of several unequal tubercles in both *C.* sp. 1 (Fig. 2g) and *C. marinus* (Figs 2h-i); basal and apical parts of axial apodeme (Figs 3a-b), are differently shaped in *C. mediterraneus* (Figs 3c-d and *C.* sp. 1 (Figs 3e-f).

Male pupal exuviae: Transverse row of hooks present on sternites V-VII (Figs 5a, 5e-f), only present on sternites V-VI in *C. mediterraneus* (Figs 5b, 5g-h); anteromedian rows of spines on tergite II interrupted medially and sparse (Fig. 5c), is continuous and more dense in *C. mediterraneus* (Fig. 5d).

Ecology and remarks

The immature stages of *Clunio* spp. are typically marine dwellers of the intertidal zone along the littoral and mid-littoral zones of rocky shores, sometimes in association with populations of *Mytilus* spp. In some species (in particular those associated with *Lithophyllum* beds) the emergence of the adults is synchronized with the lunar cycle (Neumann 1976, Neumann et al. 1997, Kaiser & Heckel 2012). The biological cycle (reproduction and emergence) of *C. boudouresquei* sp. n. is closely related to the typology of the intertidal zone including alternation between submerged marine habitats and terrestrial ecological conditions, which are strongly reinforced during spring tides of lunar rhythms (new and full moon).

The pavements, ‘trottoirs’ of *L. byssoides* represent a combination of habitats that typically characterize the intertidal zone of the protected area of Scandola Nature Reserve. They mainly consist of a pristine combination of habitats considered to be microrefugia for a dense and diversified community of marine, semi-aquatic and semi-terrestrial species, including members of several closely integrated dipteran families (Chironomidae, Ceratopogonidae and Dolichopdidae). The newly described species is encountered in the marine mid-littoral zone of Punta Palazzu (Fig. 6), where larval stages occur exclusively within the large bio-constructions of the ‘long-living’ red calcified alga *L. byssoides*, which clearly delimit alternate cycles of both submerged and terrestrial habitats. In addition, the bio-concretions of Punta Palazzu are currently considered as the largest *Lithophyllum* beds in Europe, where valuable knowledge on the biology (growth rate) and ecology of the algal communities are documented by Verlaque (2010).

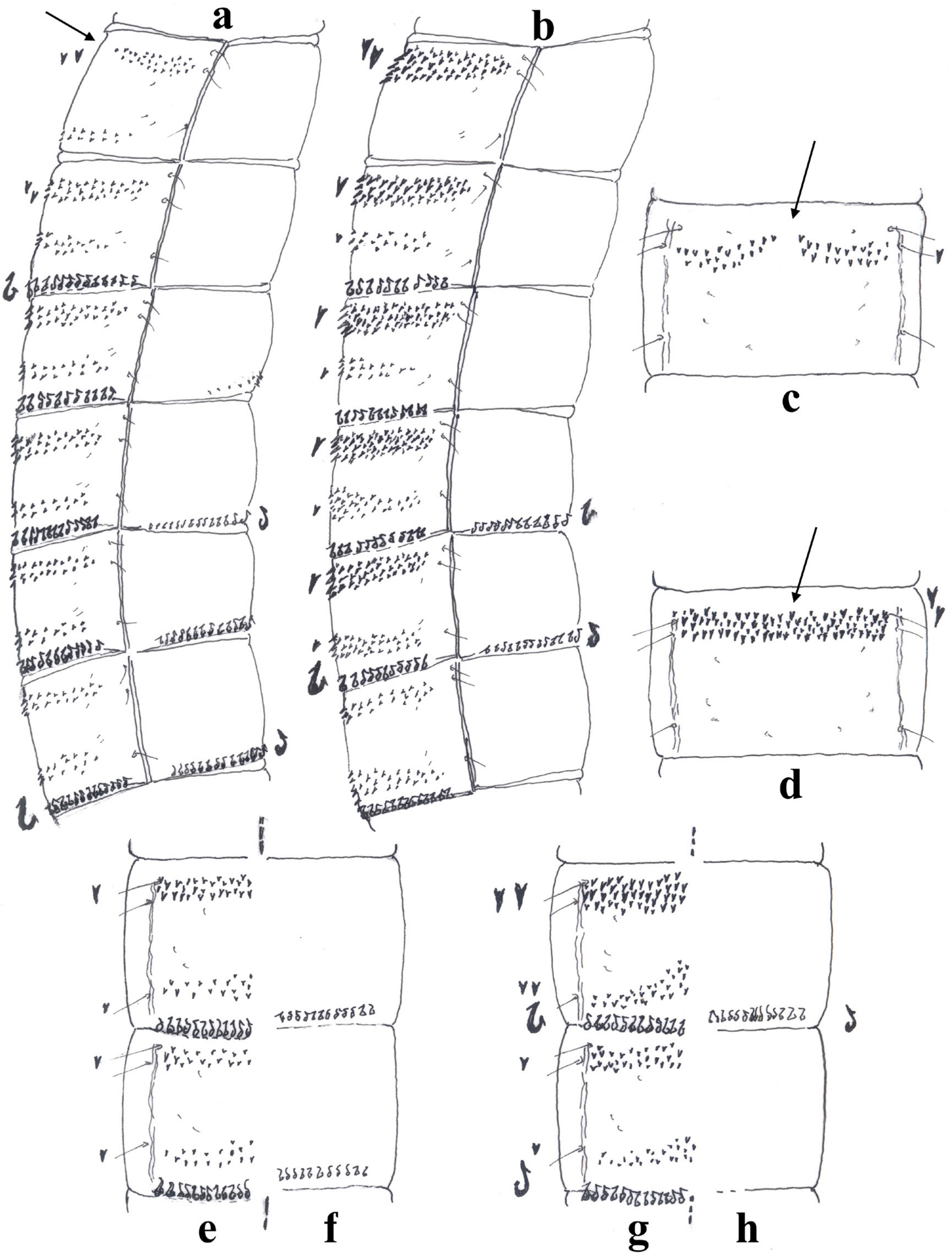


Figure 5. Pupal exuviae of *Clunio* spp. Distribution pattern, chaetotaxy and details of armament of abdominal segments II-VII of: a) *C. boudouresquei* sp. n.; b) *C. mediterraneus*. Tergite II (dorsal) of: c) *C. boudouresquei* sp. n.; d) *C. mediterraneus*. Tergite VI-VII (dorsal and ventral) of: e-f) *C. boudouresquei* sp. n.; g-h) *C. mediterraneus.*

While the biological and ecological quality of *L. byssoides* rims are still well-preserved at Punta Palazzu and Port-Cros Island (Figs 6-7), other similar marine sites located along the coastal Mediterranean ecosystem of continental France are becoming extinct, or have been deeply damaged and degraded (Figs 8-9) during the last four decades by human activities, including ecotourism and release of toxic chemical pollutants (e.g., HAP, PCB, abundance of macro- and microplastics). In addition, the *L. byssoides* beds delimited by the latter endangered sites, are heavily threatened by a massive proliferation of an invasive Mytilidae species (*Mytilus galloprovincialis* Lamarck, 1819). This sea mussel significantly predominates when changes in water quality and level of pollution become increasingly high (seashores at Banyuls, SW-France, Figs 8-9), where populations are intensely enlarging and reinforcing their potential expansion in occupying up to 70-80% of the living *L. byssoides* original cover. Such situations are also highlighted in southern France by Blanfuné et al. (2019) for the 'Canopy-forming Seaweeds' of *Cystoseira mediterranea* Sauvageau, 1810, where an important decline of local populations with risk of extinction are reported; Linares et al. (2010) and Garragou et al. (2017) report a similar scenario for the Mediterranean Red Coral, suggesting that constructive plans and management measures for conservation and preservation of autochthonous Tyrrhenian elements must be implemented. Consequently, in some of the Tyrrhenian mid-littoral coastlines (Punta Palazzu, Port-Cros, Banyuls), some relevant and vulnerable *Clunio* species are closely confined to the *Lithophyllum* beds, and therefore their loss would be clearly indicative of a combination of anthropogenic impacts and global warming in this geographical region. Such relict Tyrrhenian species are considered as potentially biogeographic representatives and biological indicators of local climate change (in particular, the rise of sea level), which strongly affect both sustainability and viability of the *Clunio* populations.

Geographical distribution

Geographical distribution of known *Clunio* species from European seacoasts (Ashe & O'Connor 2012) and the Tyrrhenian sub-region is given in in Figure 10. *Clunio boudouresquei* sp. n. '⚙' is abundant at the type-locality of Punta Palazzu (Scandola Nature Reserve, West Corsica); weakly represented in southern France (Port-Cros and Porquerolles Islands, Cassis, Banyuls). Occurrences of *C. boudouresquei* sp. n. in southern France indicate that it may be more widespread in other geographical areas of the Tyrrhenian sub-region (insular and continental Provinces), and therefore can be expected from the seacoasts of some neighbouring countries like Italy and Spain. *Clunio marinus* '✻' is found along all Atlantic seacoasts in Europe including France, Germany, England, Iceland, Ireland, Italy, Madeira, Netherland, Norway,

Figure 6. Large bio-constructions of the red calcified alga *Lithophyllum byssoides* at Punta Palazzu (Scandola Nature Reserve, W-Corsica). Type locality of *Clunio boudouresquei* sp. n. Photo J. Moubayed-Breil.

Figure 7. Bio-constructions of the red calcified alga *Lithophyllum byssoides* at Port-Cros Island (SE-France). Photo S. Ruitton.

Figure 8. Threatened bio-constructions of *Lithophyllum byssoides* by the massive proliferation of Sea Mussels at Banyuls Seashores (SW-France): level-1. Photo J. Moubayed-Breil.

Figure 9. Threatened bio-constructions of *Lithophyllum byssoides* by the massive proliferation of Sea Mussels at Banyuls Seashores (SW-France): level-2. Photo J. Moubayed-Breil.

Spain and Sweden. *Clunio mediterraneus* '✶' is widespread in the Mediterranean Basin: Southern France (Cerbère, Banyuls, Sète, Carry, Marseille, Cassis, Port-Miou, Hyère, Porquerolles, Port-Cros, Nice), northern and western Corsica, the Balearic Islands, Italy, Spain, Turkey, Croatia (the Adriatic Sea). *Clunio ponticus* Michailova, 1980 '✪' is only recorded from the Black Sea (Varna, Bulgaria).

Thalassosmittia ballestai Moubayed-Breil, sp. n.

Thalassosmittia sp. 1, in Moubayed-Breil & Ashe (2012), Moubayed Breil et al. (2013).

http://zoobank.org/0C9795ED-02C6-47BB-A8A1-190480BE9EEA

Material examined

Holotype. France, West Corsica, Scandola Nature Reserve, Focolara Bay (Fig. 14), 42° 21′ 25″ N, 8° 34′ 0″ E; 1 male pharate adult, leg. J. Moubayed-Breil, 03.VI.2015. Locality No. 31 in Moubayed-Breil & Ashe (2012), locality No. 30 in Moubayed-Breil et al. (2013). Marine water temperature: 10-12°C (min.), 22-24°C (max.).

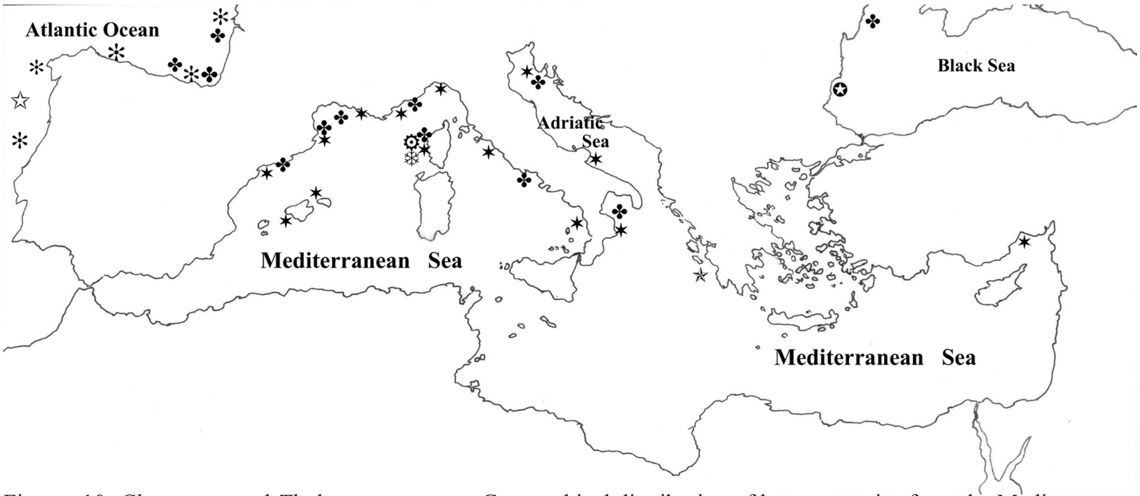
Paratypes (all leg J.M-B.): 3 pupal exuviae (2 males and 1 female), same locality as for holotype, 03.VI.2015.

Holotype (mounted on 1 slide) and 2 pupal exuviae (1 male and 1 female) are deposited in the collections of the Zoologische Staatssammlung München (ZSM), Munich, Germany. Additional paratypes are deposited in the senior author's collection.

Diagnostic characters

Though the pupal exuviae of *T. ballestai* sp. n. apparently shows a close morphological resemblance with that of *T. thalassophila* (distribution pattern of armament on tergites and shape of anal lobe), some relevant specific characters found in the male adult (shape of tergite IX, anal point and inferior volsella) will sufficiently separate the species described here from other related members of genus *Thalassosmittia* by the below listed characters.

Male adult: Temporals with 3 inner and 3 verticals; last flagellomere of antenna distinctly clubbed, abruptly narrowing distally and bearing a brush of curved sensilla chaetica; antennal groove reaching segments 2, AR 0.72; lobes of antepronotum widely opened; antepronotals absent. Brachiolum with 1 seta, veins and squama bare. Tarsomere ta$_5$ of PI-PII wider and rounded apically; spurs present on tarsomeres ta$_1$-ta$_4$; sensilla chaetica present on tibia and tarsomeres ta$_1$-ta$_4$ of PI-PIII. Tergite anal band absent on tergite IX; anal point drop-like shaped and bearing a rounded setiferous lobe at



Figures 10. *Clunio* spp. and *Thalassosmittia* spp. Geographical distribution of known species from the Mediterranean basin and neighbouring seacoasts: *C. boudouresquei* sp. n.,⚙; *C. marinus*, ✻; *C. mediterraneus*, ✶; *C. ponticus,* ✪; *T. atlantica*, ✩; *T. ballestai* sp. n., ❄; *T. thalassophila*, ✤.

base; virga with 2 closely grouped spines; inferior volsella rounded lobe-like shaped with bifid basal margin; gonostylus distinctly swollen at base and thinner distally when viewed laterally.

Pupal exuviae: Frontal apotome with sub-cylindrical tubercles; dorsocentral Dc$_1$distances between dorsocentral Dc$_1$ vestigial about 5-7 µm long; Dc$_1$ and Dc$_2$ separated by 25 µm, Dc$_2$ and Dc$_3$ by 70 µm; transverse row of hooks and orally directed pins present on conjunctives of sternites IV/V-VII/VIII is occasionally absent on IV/V; anal lobe sub-trapezoidal, genital sac distinctly swollen distally and bearing an apical finger-like tubercle. Pedes spurii A and Pedes spurii B absent. Anal lobe sub-trapezoidal to sub-triangular, bearing 2 subequal macrosetae on dorsal side; genital sac swollen distally, bearing a projecting outwards tubercle.

Etymology: the new species is named '*ballestai'* in honour of our colleague Laurent Ballesta (Andromède Océanologie, Carnon, South France) who is still an active marine biologist studying and preserving the Mediterranean marine fauna and flora including the protected area of Scandola Nature Reserve, which represents a precious and valuable inheritance area.

Male adult

(n = 2, 1 pharate; Figs 11a, 11c-h, 12a)

Total length 1.55-1.60 mm. Wing length 1.05-1.10 mm. Colouration variable in general, contrasting from light brown to brown even to greenish; Head, antenna, halters and legs light brown; thorax with mesonotal stripes contrasting light brown to brown; anal segment and inferior volsella distinctly contrasting brown to hyaline; wing translucent. Head. Eyes bare between ommatidia, nearly circular with dorso-median extension; inner lateral margin bare. Vertex and coronal area (Fig. 11a) distinctly triangular and orally projecting, basal and median margins of coronal triangle much thicker than distal part, coronal setae absent. Temporals uniserial including 3 inner and 2 outer verticals. Clypeus semicircular to sub-rectangular, with about 16-18 setae in 3-4 rows. Palp-5 segmented, segments 1-2 fused, length (µm) of palpomeres 1-5: 15, 25, 47, 63, 80; third palpomere with 2 sensilla clavata and 3 sensilla coeloconica located distally. Antenna 550 µm long; last flagellomere 230 µm long, distinctly clubbed distally, distal half abruptly narrowing and bearing a brush of curved sensilla chaetica; antennal groove reaching segments 2; AR 0.72. Thorax. Lobes of antepronotum (Fig. 11c) widely opened; antepronotals absent; acrostichals 26 in 1-2 rows; dorsocentrals 9 in 1 row; prealars 3; supraalars absent. Humeral pit indistinct. Scutellum semicircular, with 4 uniserial setae. Wing. Brachiolum with 1 seta. Veins and squama bare. Legs. Tarsomere ta$_5$ of PI-PII wider in its distal part and rounded apically; tibial spurs of PI-PIII spiniform; spurs present on tarsomeres ta$_1$-ta$_4$. Sensilla chaetica present in low number on tibia and tarsomeres ta$_1$-ta$_4$ of PI-PIII: on ta$_1$-ta$_4$ (proximally and distally); only distally on tibiae. Length (µm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in Table 3.

Abdomen. Hypopygium in dorsal, ventral and lateral view as in Figs 11e-f, 12a (Fig. 11e, dorsal; Fig. 11f, ventral with tergite and anal point removed; Fig. 12a, lateral). Tergite IX semicircular, posterior area with a dark circular setiferous band located close to the base of anal point, which is

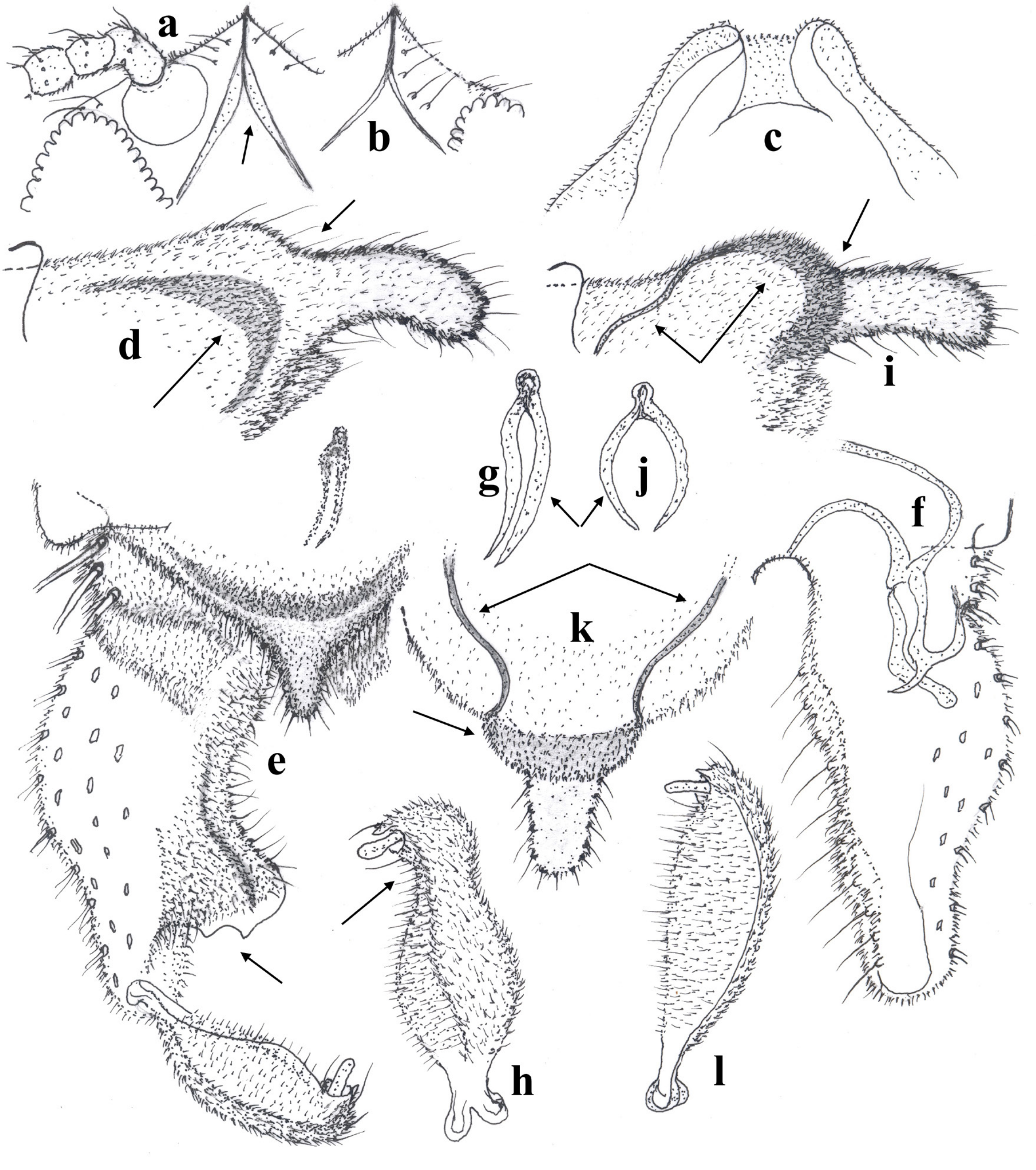


Figure 11. Male adult of *Thalassosmittia* spp. Vertex with coronal triangle of: a) *T. ballestai* sp. n.; b) *T. thalassophila*. *T. ballestai* sp. n.: c) lobes of antepronotum; d) tergite IX and anal point, lateral; e) hypopygium, dorsal; f) hypopygium, ventral view with tergite and anal point removed); g) virga; h) gonostylus, lateral. *T. thalassophila*: i) tergite IX and anal point, lateral; j) virga; k) anal point, dorsal; l) gonostylus, lateral.

clearly visible in lateral view (Fig. 11d). Anal point (Figs 11d, 11e, 12a) about 40-45 µm long, 70-75 µm maximum width at base; broadly triangular at base and drop-like shaped in its remaining part; lateral and apical margins densely covered with setae. Laterosternite IX with 6 setae (3 on each side). Sternapodeme, transverse sternapodeme and phallapodeme as in Fig. 11f, basal part orally projecting, basal part of coxapodeme semicircular and projecting inwards. Virga (Figs 11e, 11g) about 40 µm long, consists of 2 long curved spines. Gonocoxite about 265 µm long, 75 µm maximum width, much wider at base, narrowing distally to a rounded apex, inner margin with 10-11 stout setae. Inferior volsella large lobe-like shaped, rounded apically with posterior margin distinctly bi-lobed, apical and caudal parts contrasting and hyaline. Gonostylus in dorsal (Fig. 11e) and lateral view (Fig. 11h) 105 µm long, 30 µm maximum width; swollen medially and less wide distally when view laterally; posterior margin sinuous and swollen medially; crista dorsalis low and widely extended

Table 3. Male adult of *Thalassosmittia ballestai* 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	350	445	205	110	85	50	60	0.46	3.28	3.89	2.10
PII	460	420	245	135	80	60	50	0.58	3.46	3.59	2.80
PIII	495	430	215	115	75	60	55	0.50	3.74	4.30	2.60

is clearly visible only in dorsal view; megaseta present and well-developed.

Pupal exuviae

(n = 5, 2 males and 3 females; Figs 12c, 12e-k, 13a, 13e)

Total length 1.65-1.70 mm. General colouration contrasting brown to dark brown; frontal apotome with anterior half covered with fine wrinkles; antero-median area of cephalothorax and suture of thorax markedly rugulose and wrinkled; abdomen yellowish to pale, anal lobe and genital sac brown to dark brown. Cephalothorax as in Figs 12c and 13a; frontal apotome (Fig. 13a) triangular with pointed lateral expansions; frontal tubercles about 40-45 µm high, sub-cylindrical and well-developed, frontal setae about 70 µm long, separated by 25-30 µm. Thorax as in Fig. 12c. Median antepronotal nearly subequal (90 and 80-85 µm long), lateral antepronotal and prealars absent; precorneals 85, 80 and 90 µm long. Dorsocentrals consist of 3 unequal setae, length (in µm) of Dc_1-Dc_3: Dc_1, vestigial about 5-7; Dc_2, 55; Dc_3, 70; distance (in µm) between: Dc_1 to Dc_2 25, Dc_2 to Dc_3 70.

Abdomen. Armament, chaetotaxy and distribution pattern of shagreen with details of armament on tergites and sternites: III-VIII (Fig. 12e); V-VII (Figs 12f-k). Tergite I and sternites I-IV bare. Anterior transverse rows of spines present on tergites II-VIII, those on tergites VII-VIII are smaller and less extensive; posterior transverse rows of spines present on tergites III-VIII, becoming gradually more extensive on VI-VIII. Conjunctives of tergites III/IV-VII/VIII and sternites IV/V-VII/VIII with rows of hooks and orally directed pin-shaped setae (Figs 12e, 12h-k), those on sternite IV/V are occasionally absent (Fig. 12g). Caudo-lateral area of tergites and sternites II-VII with a group of short spines (Figs 12e-k). Pedes spurii A and PSB absent. Number and distribution pattern of lateral setae on segments I-VII, 2, postero-lateral seta on segments V-VI forked; segment VIII with 3 setae located distally. Anal segment in dorsal and ventral view as in Fig. 13e; anal lobe sub-trapezoidal to sub-triangular, 130-135 µm long, 135-140 µm minimum width at base, 185-190 µm maximum width at apex, a rounded patch of short spine present medially, apex bearing a finger-like tubercle which is projecting outwards; genital sac 220 µm long, distinctly swollen distally and overreaching apical margin of anal lobe by 70-75 µm; macrosetae consist of 2 subequal setae, about 90-95 µm long, separated by about 35-40 µm.

Larva

Unknown.

Differential diagnosis

Only the pupal exuviae of *T. ballestai* sp. n. directly key close to those of *T. thalassophila*, while the male adult is quite different and likely belongs to a local 'Tyrrhenian element'. On the basis of some relevant specific characters found in the male adult and pupal exuviae, *T. ballestai* sp. n. is compared, as male adult, to that of *T. thalassophila* and, as pupal exuviae, to other undescribed morphotypes collected in Corsica, continental France, Italy and Spain. However, *T. ballestai* sp. n. is easily distinguished from other related species or taxa/species of *Thalassosmittia* by the following combination of characters.

Male adult: Head with 3 inner verticals (Fig. 11a), is bearing 4 in *T. thalassophila* (Fig. 11b); tergite anal band absent on tergite IX (Figs 11d-e), is conspicuously present in *T. thalassophila* (Figs 11i, 11k); anal point drop-like shaped and much wider at base (Fig. 11e), is parallel-sided with semicircular basal part in *T. thalassophila* (Fig. 11k); virga with 2 closely grouped spines (Figs 11e, 11g), consist of 2 inwardly curved spines and horseshoe-shaped in *T. thalassophila* (Fig. 11j); inferior volsella rounded lobe-like shaped with bifid basal margin (Figs 11e, 12a), is divided in 2 separate parts in *T. thalassophila* (Fig. 12b); gonostylus in lateral view (Fig. 11h), is differently figured in *T. thalassophila* (lateral, Fig. 11l).

Male pupal exuviae: Frontal apotome with sub-cylindrical tubercles (Fig. 13a), are smaller and fused apically in *T. thalassophila* (Fig. 13b), weakly domed in *T. marinus* (Fig. 13c) and conical in *T.* sp. 1 (Fig. 13d); distances between dorsocentral Dc_1 to Dc_2 and Dc_2 to Dc_3 are respectively

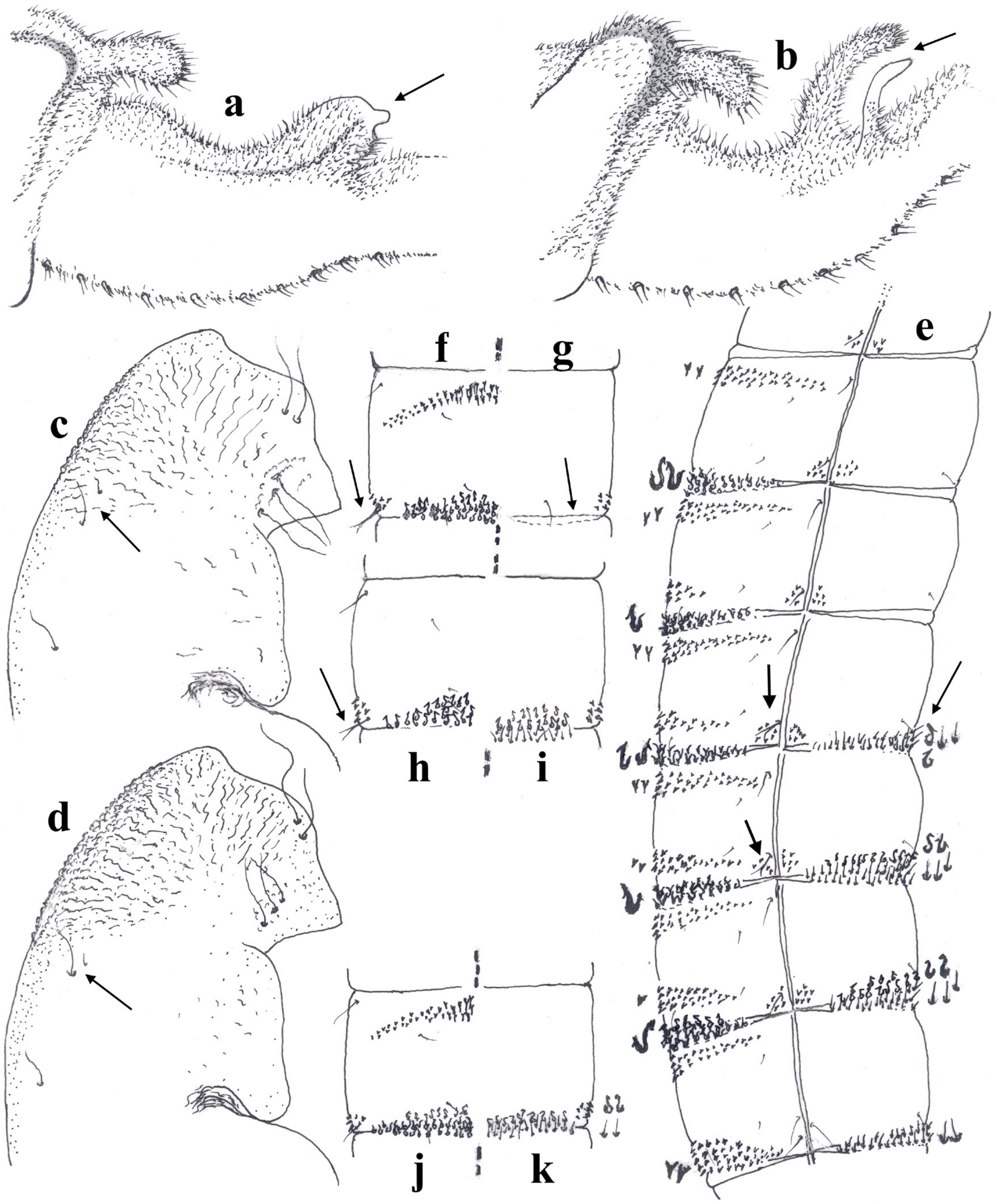


Figure 12 a-k). Male adult and pupal exuviae of *Thalassosmittia* spp. Anal point and inferior volsella in lateral view of: a) *T. ballestai* sp. n.; b) *T. thalassophila*. Cephalothorax of: c) *T. ballestai* sp. n.; d) *T. thalassophila*. *T. ballestai* sp. n.: e) chaetotaxy and details of armament on abdominal segments III-VIII; f-g) tergite V in dorsal and ventral view; h-i) tergite VI in dorsal and ventral view; j-k) tergite VII in dorsal and ventral view.

25 and 70 (Fig. 12c), while are 15 and 55 in *T. thalassophila* (Fig. 12d); transverse row of hooks and orally directed pins present on conjunctives of sternites V/VI-VII/VIII (Fig. 12e) or occasionally absent on IV/V (Fig. 12g), is regularly present on conjunctives of sternites IV/V-VII/VIII of *T. thalassophila* (couplet 196 in Langton 1991); anal lobe (Fig. 13e) sub-triangular to sub-trapezoidal, is distinctly triangular in *T. thalassophila* (Fig. 13f), semicircular in *T. marinus* (Fig. 13g), rectangular in *T.* sp. 1 (Fig. 13h) and sub-circular in *T.* sp. 2 (Fig. 13i); genital sac (Fig. 13e) distinctly swollen distally with an apical finger-like tubercle apically, is differently figured in *T. thalassophila* (Fig. 13f).

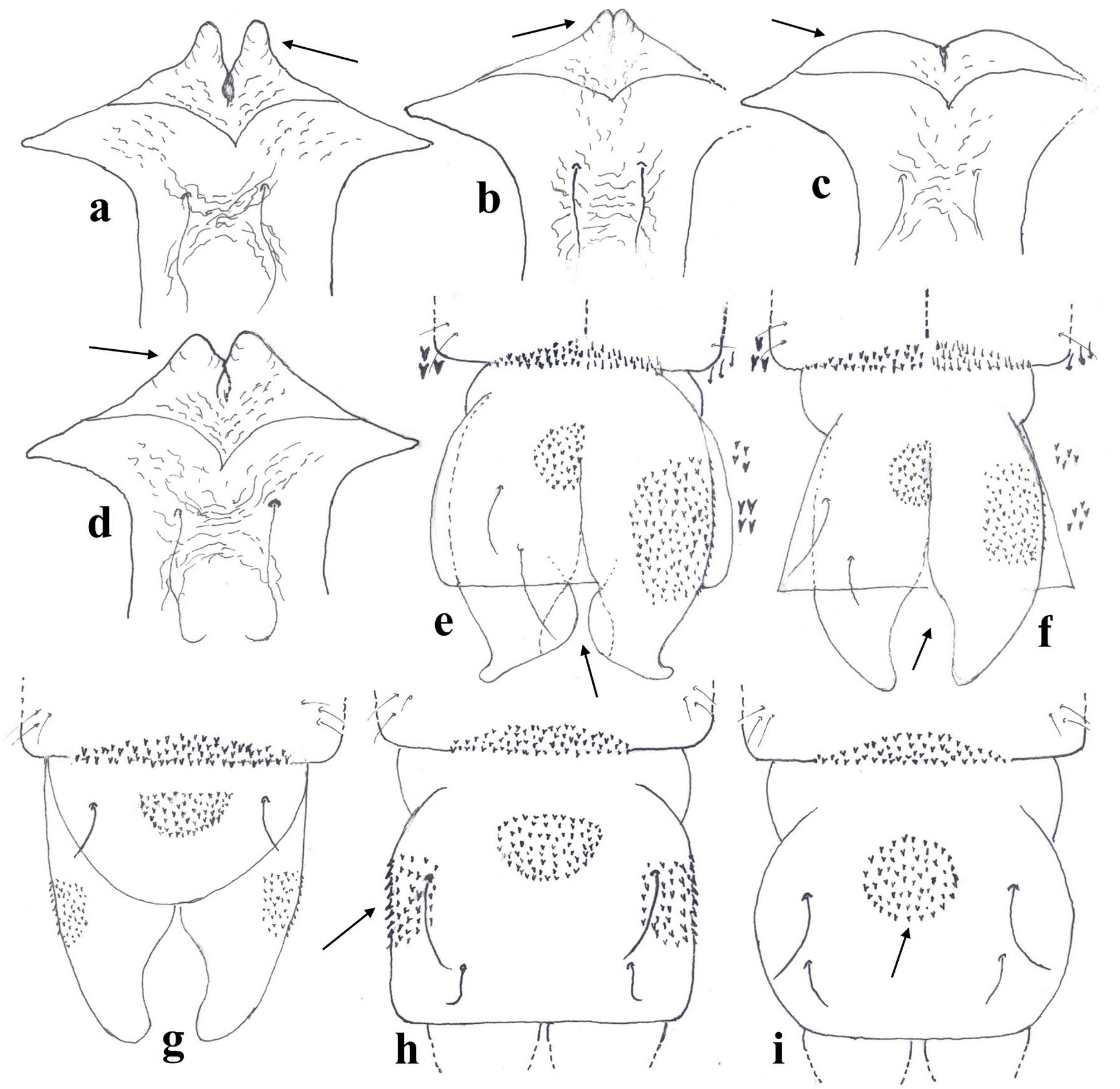


Figure 13. Pupal exuviae of *Thalassosmittia* spp. Frontal apotome of: a) *T. ballestai* sp. n.; b) *T. thalassophila*; c) *T. marinus*; d) *T.* sp. 1. Caudal armament of segment VIII and anal segment of: e) *T. ballestai* sp. n., dorsal and ventral; f) *T. thalassophila*, dorsal and ventral. Caudal armament of segment VIII and anal segment of: g) *T. marinus*; h) *T.* sp. 1; i) *T.* sp. 2.

Ecology and remarks

The examined material of *T. ballestai* sp. n. (male adults, male pharate adults and pupal were collected in the type locality of Focolara Bay (Fig. 14) located in Scandola Natural Reserve, western Corsica. Additional material including associated larval stages is needed to determine and confirm the ecology of the new described species. While the marine intertidal zone at Focolara Bay is better preserved during winter and spring periods, it still heavily degraded, as other seacoasts around the Mediterranean Basin, by the impact of pollution and ecotourism activities, which highly increase each year between June and September.

The increasing abundance of plastics along the coastal ecosystem, estuarine zones and the littoral marine environment of Corsica (Bastia, Ajaccio, Porto, etc.) including those of Scandola Nature Reserve, has been observed since the last three decades. Inorganic matter, composed of pellet tar and plastics, collected in both drift and troubleau nets (as shown and detailed in Figs 15-16), has become more dominant major threat to all types of marine organisms. Small particles of plastics, consisting of several forms of both macro- and micro-particles of 0.5-5 to 10-15 mm size, are systematically found from the surface and water column to the seabed sediment and beach, where marine organisms (especially deposit feeders and detritivores)

Figure 14. The Bay of Focolara at Scandola Nature Reserve, western Corsica. Type locality of *Thalassosmittia ballestai* sp. n. Photo J.M. Dominici.

ingest them. Therefore, these pollutants may adversely impact species that inhabit intertidal zones that cannot adapt to changing conditions via behavioural plasticity; *T. ballestai* may be among these species. Consequently, a major challenge in marine environmental disciplines (evolutionary biology, ecology, conservation) is to better understand and predict how these sensitive species will respond to the impact of human activities and the rise of sea level, which is directly related to the global warming.

Geographical distribution

Geographical distribution of known *Thalassosmittia* species from European seacoasts (Ashe & O'Connor 2012) and the Tyrrhenian sub-region is given in figure 10. *Thalasosmittia atlantica* '✩' is known only from the western Atlantic seacoasts including the Canary Islands (type-locality), Madeira, Spain and Portugal. *Thalasosmittia ballestai* sp. n. '❄' is common and abundant in the seashores of Focolara Bay (West Corsica). The records of *T. ballestai* sp. n. (pupal exuviae) from some seashores in southern France need to be confirmed by the presence of associated material composed of adults and pupae. *Thalasosmittia thalassophila* '✤' is present along the Atlantic and some Mediterranean seacoasts in Europe including: France, England, Germany, Greece, Ireland, Italy, Netherland, Romania and Spain.

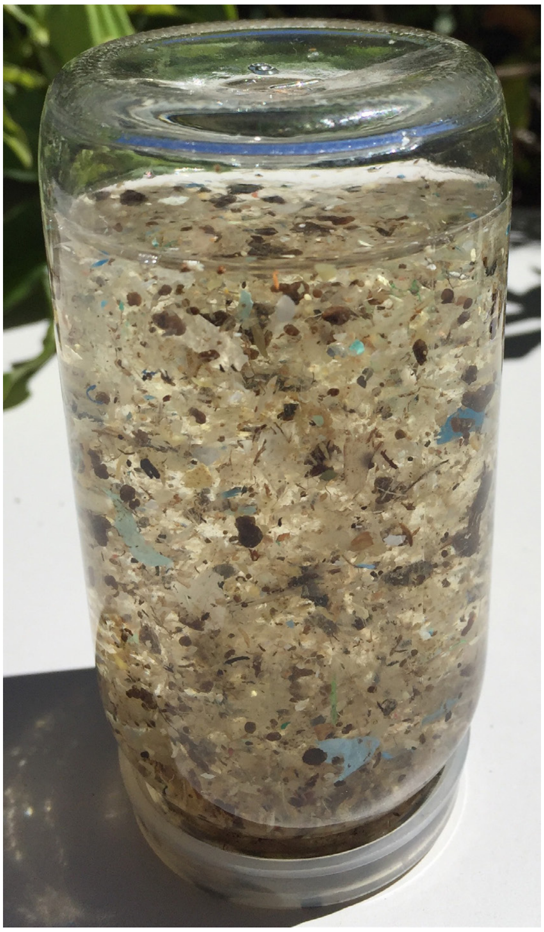


Figure 15. Plastics in the form of 'Macro- and Micro-particles' (1-5 to 10-15 mm size), collected at Focolara Bay, Scandola Nature Reserve. Photo J. Moubayed-Breil.

Acknowledgements

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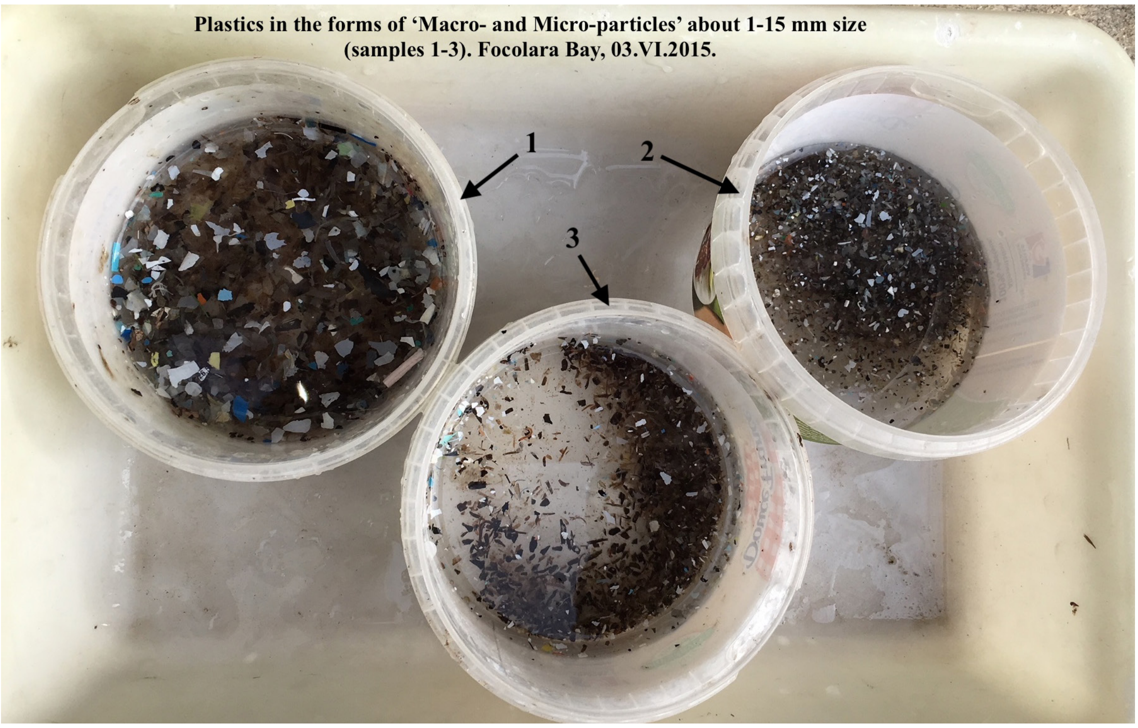

Figure 16. Plastics in the form of 'Macro- and Micro-particles' (1-5 to 10-15 mm size). Focolara Bay, Scandola Nature Reserve. Photo J. Moubayed-Breil.

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 two new species.

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AN ENIGMATIC CHIRONOMIDAE (DIPTERA): FIRST LARVAL DESCRIPTION FOR *NANDEVA* WIEDENBRUG, REISS AND FITTKAU AND EVIDENCE FOR PHYLOGENETIC POSITION IN TANYTARSINI

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Abstract

The previously unknown larva of the genus *Nandeva* Wiedenbrug, Reiss and Fittkau (Chironomidae: Chironominae) is suggested to be a candidate from tropical northern Australia belonging to the tribe Tanytarsini. The basis for the assumption is DNA analysis showing maximum values of Bayesian posterior probability and Bootstrap support for an exclusive sister relationship of the unreared larva with a male of *Nandeva* from South America. The monophyly of *Nandeva* has high support as a member of the subtribe Tanytarsina, although its precise relationships within this subtribe lack support. Larval morphology is described and illustrated, and conforms to the subtribe Tanytarsina in tribe Tanytarsini. The historical tempo is discussed in relation to fossils and dating of pertinent nodes and the term 'pseudo-gondwanan' is used to described an extant restricted austral range, but with fossils showing a wider past distribution in the north.

Introduction

Since the 'Holarctic Chironomidae' project (Wiederholm 1983, 1986, 1989), it has been desirable to incorporate morphology of immature stages in descriptive studies, faunal guides and classifications. Continuing unknown immature stages often are associated with atypical and under-sampled life histories such as terrestrial habitats or immersed wood (e.g. Cranston 2003, 2006) and/or from infrequently surveyed and remote places, such as New Zealand's subantarctic islands (e.g. Sublette and Wirth 1980). Amongst such genera with the larva unknown yet clearly aquatic is *Nandeva* Wiedenbrug, Reiss and Fittkau (1988) that is documented from diverse lotic pupal exuviae but until now with no associated larva.

Nandeva, based on the genotype *Nandeva gaucha* Wiedenbrug, Reiss and Fittkau, was described from the male imago and pupa from Rio Grande do Sul (southern Brazil), plus two species based solely on distinctive pupal exuviae from the Amazon basin (Brazil) and southern Chile, respectively (Wiedenbrug et al. 1998). Further undescribed species suggest an extensive distribution in southern America, with an adult known from as far north as Panama.

Near simultaneously, this 'neotropical' genus was found in tropical northern Australian streams, with the sole species described as *Nandeva fittkaui* by Cranston (1999). Although all adults were pharate, associated pupae had the diagnostically posterior hook rows on tergites II-V, and lacked a thoracic horn, frontal setae, pedes spurii A and B, spur / comb on posterior segment VIII, and anal lobe fringe. Pupal exuviae were intercepted in drift from 6 streams in north-eastern Queensland between latitudes 16° to 18°S. Despite intensive conventional searching using kick and drift nets and breaking immersed wood, no candidate for the larval *Nandeva* was found.

Neotropical *Nandeva* diversity increased subsequently with two species described from São Paulo State, Brazil, together with a new pupal / adult association by Sæther and Roque (2004) who also keyed males of the then 5 described species. Additional species diversity was revealed by Andersen *et al.* (2011) in describing adults of a second species from Chile, another from Rio de Janeiro state in Brazil, with range extending to Mexico and Venezuela. Epler (2017) added Costa Rica to the meso-American distribution of the widely distributed species *Nandeva latiloba* Sæther and Roque. However, despite reports from aquatic ecological and biomonitoring researchers, no larval association was found (see e.g. Sæther and Roque 2004).

Because the larva remained unknown, its morphology could not be incorporated into phylogenetic studies. This was problematic as the adult male and pupa are morphologically very divergent (highly apomorphic) compared to prospective relatives. Regarding phylogeny, originally Wiedenbrug et al. (1988) stated only that the adult male belonged to the tribe Chironomini and, although keying to *Pagastiella*, *Nandeva* differed substantially in details of phylogenetic significance. An early phylogenetic estimate was based on morphology in a matrix of 118 characters from all life-history stages for 60 genera of Chironominae, with larval features for *Nandeva* treated as 'missing data' (Cranston 1999). Analysed under strict parsimony, *Nandeva* fell within the tribe Chironomini, associated with Gondwanan-distributed genera *Nilodosis* Kieffer, *Imparipecten* Freeman, *Fissimentum* Cranston and Nolte and 'K1' (subsequently *Xylochironomus* Cranston). Some trivially less-parsimonious trees placed *Nandeva* as sister to Tanytarsini, or even embedded within the tribe. This latter placement found support from Sæther and Roque (2004), who reinterpreted wing vein RM as a continuation of direction of R_{4+5}, diagnostic of tribe Tanytarsini. With this character state score emended in a revised matrix, analysed under a range of weighting schemes, Sæther and Roque (2004) proposed several positions for *Nandeva*, including as sister to, or within Tanytarsini, but with various different internal relationships. The position of *Nandeva* was argued as near or within tribe Tanytarsini based on male characters from the only known fossil species, *N. pudens*, from Fushun amber (early Eocene, NE China) by Giłka et al. (2016). Some morphology seemed driven by shared reductions likely to have been convergent, as suggested also by Cranston (1999). No proposed relationships had significant statistical support.

Detection of an Australian larva collected for molecular phylogenetic studies and linked by DNA sequences with an adult of *Nandeva* from Brazil, now allows association, and description of the larva. This provides better understanding of the phylogenetic relationships and the tempo of diversification.

Material and methods

Diverse and specifically targeted taxa of phylogenetic significance were sought for molecular phylogenetic studies across the Chironomidae (Cranston et al. 2011). Immature stages were obtained for ease of collection and relevance in aquatic biomonitoring programs. From a tropical submontane stream, larvae were recovered from semi-immersed leaf litter packs by coarse-sieving (1 cm mesh) to remove larger material, and then using a 125µ mesh sieve to retain larvae. Samples were inspected live in the field under dissector microscope and larvae 'of interest' were placed individually in rearing vials. The larva subsequently recognised as possibly belonging to *Nandeva*, died prior to metamorphosis and 100% isopropanol was added to the vial.

The Brazilian adult was collected into ethanol in a light trap located at Fazzara Creek, the type-locality for both *Nandeva latiloba* Sæther and Roque and *N. strixinorum* Sæther and Roque (2004).

Treatment of material for molecular study and protocols to obtain and align sequences and phylogenetic analyses were as reported in Cranston et al. (2011) and Krosch and Cranston (2012). Subsequent analyses with increased taxon sampling from tribe Tanytarsini follow Krosch et al. (2019). For the larva of *Nandeva*, DNA sequences (GenBank accessions in parentheses) were obtained for a mitochondrial protein-coding gene (*COI*, HQ440940), a ribosomal gene (*18S*, HQ440625), and two sections of the nuclear protein-coding gene (*CAD*, HQ440478, HQ440312). For the adult, only *CO1* was obtained (HQ440941).

Specimens are vouchered (MV) as microscope slides in the Australian National Insect Collection, CSIRO, Canberra, Australia (ANIC).

Results

Taxonomy

Nandeva Wiedenbrug, Reiss and Fittkau 1998

Type-species: *Nandeva gaucha* Wiedenbrug, Reiss and Fittkau 1998, by original designation.

Larval material examined: 1L, AUSTRALIA, QUEENSLAND, Mt. Lewis, Mary Ck, 16°35.2´S 145°17.5´E, 4.ix.2005, P.S. Cranston, MV FNQ9-3 (ANIC).

Adult ♂, BRASIL, São Paulo, São Carlos, Córrego do Fazzari, 21°58'S 47°53'W, 10.viii.2007, F.O. Roque, MV BRZNAND (ANIC).

Description (Fig. 1).

Small, colour in life unknown. Body length 3 mm., head length 250 µm, postmentum 90 µm. Dorsal head comprising frontoclypeal apotome with straight anterior margin, separate labrum and labral sclerites 3-5 distinct. Antenna (Fig. 1a) with pedestal undeveloped, 5-segmented, with swollen basal segment, segments decreasing successively in length from 2nd to 5th; length (in µm) 58, 20, 13, 11, 6; Antennal ratio 1.1. Lauterborn organs prominent, equal to length of 3rd antennal segment, both apical / subapical on 2nd segment, one slightly retracted compared to the other. Style long. Ring organ near middle of segment 1, seta present. Blade extends well beyond flagellum, accessory blade very short. Labrum (Fig. 1b): SI plumose with fused bases; SII plumose, arising from small pedestals, SIII short, slender; SIV short, normally developed. Seta premandibularis simple, stout. Labral lamellae pectinate with c. 25 teeth. Pecten

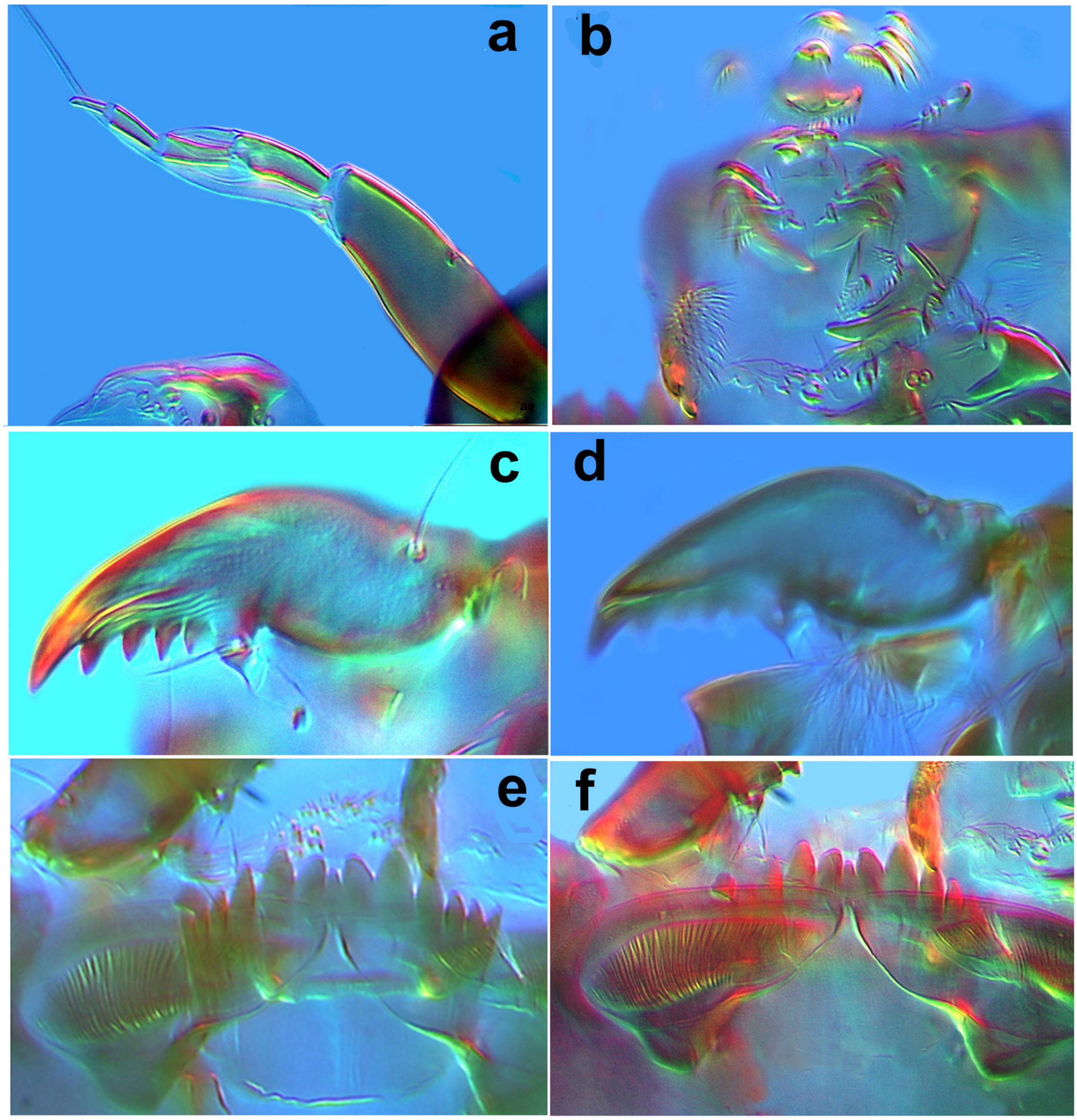

Figure 1. *Nandeva fittkaui* Cranston, putative larva. a. Antenna, b. Labrum, c. Mandible ventral, c. Mandible ventral, e. Mentum, with teeth enhanced, f. Mentum with ventromental plates enhanced.

epipharyngis tripartite, each tooth pectinate with 5-6 teeth distally. Premandible 40 µm long, with 3 teeth, with beard. Mandible (Figs 1c, d) 70 µm long, with stout dorsal tooth; apical tooth 2x length of one of 3-4 inner teeth; all teeth pale brown. Pecten mandibularis absent. Seta subdentalis inserted dorsally, narrow, reaching middle inner tooth. Seta interna multi-branched, inserted on dorsal surface. Mentum (Figs. 1e, f) 62 µm wide, with all teeth pale brown. Median (ventro-) mentum with three teeth, the central one recessed, smaller; with 5 pairs of lateral teeth, declining in size; pointed outermost tooth lies somewhat displaced laterally relative to the inner. Ventromental plates (Fig. 1f) 60 µm wide, distinctively subovoid, in contact medially, with smooth anterior margin, striae concentrated in medio-lateral area and absent from median sector. Setae submenti apparently simple. Body lacks lateral or ventral tubules. Segment 11 dorsomedially without appendages or hump. Procercus simple, unpigmented, arising directly from membrane, with few short anal setae.

Taxonomic and Ecological Comments

Although there are no formal diagnoses for the tribe Tanytarsini, these can be derived from Sæther (1977) including also for subtribes Tanytarsina and Zavreliina, and from keys such as Epler *et al*. (2013) for Holarctic larvae. The larva proposed here as belonging to *Nandeva* conforms to tribe Tanytarsini in the subfamily Chironominae, largely based on features of the labro-epipharynx. Notably the labral SI and SII setae are plumose, the bases of S1 setae are fused and SII are on pedestals. The antenna is 5 segmented with antennal seta, with sessile, opposite Lauterborn organs not on pedestals. The ventromental plates are in near median contact, diagnostic of subtribe Tanytarsina. Within this subtribe, the recessed median mental tooth and the mandible with dorsal tooth and seta subdentalis appearing to be inserted dorsally (which is seen otherwise only in tribe 'Pseudochironomini') appear to be diagnostic.

The Australian larva is associated with South American *Nandeva* at 89% similarity of sequence from molecular evidence (Cranston et al. 2011, Krosch et al. 2019). The larval collection site is a 2nd order creek at 1000 m. above sea level in tropical north Queensland. All other Australian records of *Nandeva* are from the characteristic pupal exuviae from streams in Far North Queensland including from Mary Creek that is the larval locality (Cranston 1999). No such larvae have been found in any other stream from which this pupal type derives, despite intensive sampling for over two decades. Circulating photographs of the Australian larvae amongst South American colleagues has not elicited recognition and this larval type remains unknown there.

Phylogeny

Seeking data for an extensively sampled molecular phylogenetic reconstruction, Cranston et al. (2011) obtained representatives across all available major branches of the Chironomidae. Survey strategy often targeted larvae with distinctive morphologies (see Cranston et al. 2011, including Supplementary Material) and most were unreared. Amongst these was a solitary larva from immersed leaf litter from Mary Creek at 1000 m. elevation above sea level on Mount Lewis on the Atherton Tableland of tropical northern Queensland, which is the type-locality of *Nandeva fittkaui.* Amongst material collected by colleagues was an adult *Nandeva* from the Fazzari Stream in Sao Carlos, Brazil was made available to us by Fabio Roque. As shown in Cranston et al. 2011: fig. 2), the sequence data from the isolated Australian larva and the Brazilian adult midge form a tight cluster, allowing the inference that they were each other's closest relatives, likely to be congeners. Phylogenetic analyses (loc. cit.) implied that *Nandeva* was sister to Riethia, represented by four specimens, belonging to only 2 species (Cranston 2019). Although the subtending node lacked support, at the next level the sister group was proposed as tribe Tanytarsini (Cranston et al. 2010: node C5, with 100% support). Although polythetic, morphological taxonomic concepts of tribe and subtribes thus find strong support from molecular analyses (Cranston et al. 2011; Krosch et al. 2019). Further elaborations of expanded morphological matrices and analyses under various forms of parsimony have shown *Nandeva* as belonging within the well-circumscribed Tanytarsini (Andersen et al. 2011) - in an arrangement found in some slightly suboptimal molecular results. Now a recent analysis combines new and existing molecular data, from more intensive sampling within Tanytarsini and greatly expanded by addition of *Pseudochironomus* and many more species of *Riethia* (Krosch et al. 2019). The analysis (Fig. 2, modified from Krosch et al. 2019, fig. 1) now clearly locates *Nandeva* within Tanytarsini, with 100% Bayesian Posterior Probability (PP) and .93 Bootstrap support (BS)), and within subtribe *Tanytarsina* (99% PP, .85 BS). *Nandeva* attaches as sister to the clade comprising (*Sublettea* (*Paratanytarsus* + *Micropsectra*), but with weak support of only 90% (PP). Contrary to previous predictions, addition of larval characters to the morphological matrix and re-analysed using parsimony, does not strengthen support for any of the previously inconsistent proposed relationships (Cranston pers. obs.).

Tempo of diversification

The major difference between analyses of the tempo of diversification in Cranston et al. (2010, 2011) and Krosch et al. (2019) is the use of new and different fossils to calibrate the BEAST analyses. Well-dated, newly studied, amber fossils include those assigned to Tanytarsini, including *Tanytarsus* (Giłka 2011; Giłka et. al. 2013; Zakrzewska and Giłka 2014, 2015b; Zakrzewska et al. 2016; Zakrzewska et al. 2018) and to *Nandeva* (Giłka et al. 2016). Temporal analysis (Fig. 2, red # below line is median age in Ma (million years before present) shows two highly-supported splits, between subtribes Tanytarsina and Zavreliina of 74 Ma (with range 61.1—90.8 Ma) and the later split between *Cladotanytarsus* + *Tanytarsus* and remaining members of the subtribe at 56 Ma (51.3—63.8 Ma). Although the node connecting *Nandeva* within Tanytarsini is unsupported, a date (and range around this node) of 42 Ma (33.2—49.6 Ma) can be postulated. This is expected, as we had applied the Eocene calibration fossil *Nandeva pudens* (Giłka et al. 2016) to the stem at 49.5 Ma, a youngest age attributed to Fushun amber, with the value of 42 Ma applying to the crown age.

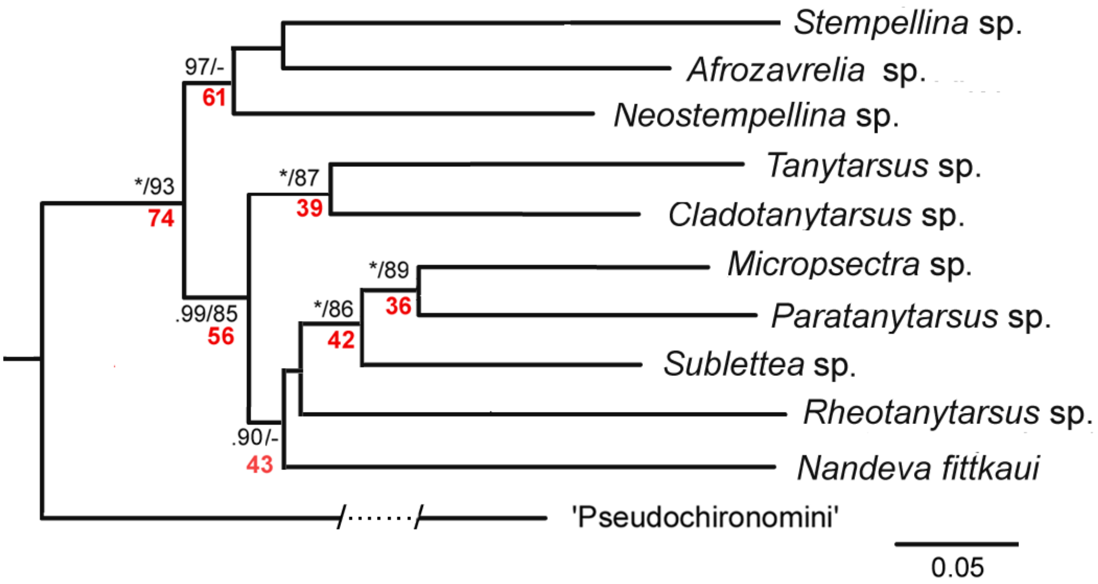

Figure 2. Bayesian dated tree for Tanytarsini, 'Pseudochironomini', terminals reduced from Krosch et al. (submitted 2019). Above node, black # Support statistics. * - maximum Bayesian posterior probability (1.00), - = maximum bootstrap (100), .xx/yy Bayesian posterior probability / bootstrap value. Below node, red # is mean date in Ma (million years before present). .... indicates attenuated distance.

Thus stem Tanytarsini originated in the late Cretaceous and commenced radiation as the extant tribe (crown group) in the end Cretaceous with the progenitors of the two subtribes. Subsequent radiation into extant genera of Tanytarsini took place through the Palaeogene, although fossil evidence is lacking, with most median dates of splits in the Palaeocene and Eocene. Sampled extant genera including *Nandeva* are postulated as existing crown groups by the late Eocene, which is in keeping with the fossil evidence.

Discussion

Studies of the Chironomidae have elucidated southern hemisphere ('austral') biogeography, commencing with Brundin's seminal works (1963, 1965, 1966). Although Brundin used 'manual' Hennigian phylogenetics and somewhat subjective temporal reconstructions, studies substantially confirm Brundin's vicariance-based reconstructions, notably Cranston *et al.* (2011) across the whole family and for subfamilies Podonominae (Cranston et al. 2010), Orthocladiinae (Krosch et al. 2011) and Tanypodinae (Krosch et al. 2017). Well-dated reconstructions recover a tempo of midge evolution that tracks Gondwanan fragmentation, even involving southern Africa that allows inference of a vicariance date of > 100 Ma (Cranston et al. 2011). Younger patterns include New Zealand (e.g. Krosch and Cranston 2013), but most vicariant taxa are separated from South America - Australian connection via Antarctica (van der Ende et al. 2017).

Calculations of the tempo of diversification relative to earth history have been greatly enhanced since Brundin's time. A cadre of insect palaeontologists are describing well-preserved and carefully dated fossils, particularly from amber-preserved specimens dating back up to 100 Ma. With respect to this study, Giłka, Zakrzewska and colleagues continue to provide valuable data on Eocene amber inclusions. In addition to dated and taxonomically informative 'calibration points', are improvements in phylogenetic and temporal diversification analyses, based on molecular data using both Maximum Likelihood and Bayesian methods of analysis.

In a dated phylogeny (Cranston et al. 2011) both sampled *Nandeva* were monophyletic, with the stem node (although as sister to *Riethia*) dated at 47 Ma (30—67 Ma) (unpublished supporting information). Note that this analysis did not use *Nandeva* in calibration. The South American *Nandeva* had to be excluded from the analysis of the Krosch et al. (submitted 2019) analysis of the tempo of diversification because its molecular representation was only by COI. However, with or without the S. American *Nandeva*, and independent of close calibration points in Tanytarsini, including for *Nandeva*, the median date for stem *Nandeva* converges on early-mid Eocene, which is the date of the fossil *N. pudens*.

The locations and diversity of extant *Nandeva* imply concordance with timing for south American vicariance from Australia via Antarctica which was temperate and connected until the opening of the Drake Passage at c. 30 Ma, and the final break from Australia via the Antarctica landbridge, along the South Tasman Rise at c. 45 Ma (van der Ende et al. 2017). However, that this represents a "southern disjunct" or "amphinotic" (Cranston 2005) distribution may be tempered by *Nandeva* occurring in more tropical streams as well as the usual cool temperate distributions of such clades. Although Amorim and Santos (2017) demonstrate that Brazil's Southern Atlantic Forest contains amphinotic elements, and *Nandeva* are found there, it is clear that they occur also in truly tropical streams.

Of greater significance is the undeniable presence of *Nandeva* in early Eocene Fushun (Palaearctic China) and the fossil genus *Eonandeva* (possibly stem *Nandeva*) from slightly younger Baltic amber (Zakrzewska and Giłka 2015b). Such ancient presence of stem groups of 'gondwanan' crown taxa, are discussed by Giłka et al. (2016) for Chironomidae. Other examples include termites of the family Mastotermitidae, the sister group to the remaining extant termites, is known from the Miocene to Eocene of Europe, Mexico, and the Dominican Republic and perhaps Cretaceous fossils in the Palearctic is reduced now to *Mastotermes darwiniensis*, a pest in northern Australia. The bulldog ants (subfamily Myrmeciinae), now restricted to Australia, indubitably include fossil taxa from Argentina and the Baltic. The biting midge *Austroconops* (Ceratopogonidae, Leptoconopinae) abundant in Lebanese and other Cretaceous ambers, is represented now by two species in Western Australia. Differential extinction in the northern hemisphere with survival in the south, seems to be frequent, giving rise to a 'pseudo-gondwanan' distribution (Cranston and Gullan 2005). *Nandeva* appears to qualify for this term.

One more issue remains to be addressed here, that the Australian *N. fittkaui* differs from the studied neotropical species in enough features that could encourage allocation to a different genus to the neotropical species (Andersen et al. 2011). Some structures are difficult to confirm due to Australian adult material all being in a pharate state. As documented by Andersen et al. (2011) differences include the absence of lateral antepronotals, the bare squama, spatulate anal point and superior volsella with more basal setae and strong microtrichia. In the female genitalia the ventrolateral lobe is strongly microtrichiose and in the same plane as the dorsomesal lobe, the spermathecal ducts are nearly straight, a floor seems absent, and the setae of tergite IX are divided into two groups. The pupal tergites VI–VII lack the antero-median patches of stronger spinules seen in the Neotropics. We still lack non-teneral adults for confirmation of the adult differences, yet can be sure that even if a new rank is 'needed' for *N. fittkaui*, it surely will be sister to the neotropical clade, and inferences here concerning evolution and biogeography will be unaffected.

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A PROTOCOL FOR RECORDING BEHAVIORAL ACTIVITY DURING LONGEVITY STUDIES OF ADULT CHIRONOMIDAE

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Abstract

A protocol for quantifying behaviors of adult Chironomidae during lab-based longevity studies is described and definitions of 12 behaviors are provided. Initial data for field-collected adults from on snow adjacent to three streams during winter, split into batches and incubated at three different temperature treatments, are summarized and show that frequencies of behaviors vary by stream and treatment. Implications of behaviors relative to reproductive fitness of adults is proposed, and changes to the protocol for use in future studies is described.

Introduction

Adults of the winter-emerging chironomid, *Diamesa mendotae* Mutkowski, have been shown to be long-lived when incubated at low air temperatures (Bouchard *et al.* 2006a, 2006b, Ferrington *et al.* 2010), including exposures to extended sub-freezing temperatures (Anderson *et al.* 2013, Mazack *et al.* 2015) and capable of walking on snow or resuming flight after landing on snow. It has been proposed that the long duration of the adult portion of the life-cycle provides increased fitness during winter, allowing extended opportunities to mate on snow, and for females to return to the water surface to complete oviposition (Ferrington *et al.* 2010). However, it is uncertain how meaningful it is to extrapolate lab-based longevity studies to fitness benefits under field conditions and, despite having a longer duration of the adult portion of the life-cycle, it is not possible within the design of previous longevity studies to determine how long the female remains healthy enough to effectively navigate back to the water to oviposit.

Longevity studies of *Drosophila melanogaster* and *Ceratitis capitata,* both species that feed as adults, have shown age-specific and lifetime behavior patterns (Carey *et al.* 2006, Jones and Grotewiel 2011), and the patterns have been quantified using well-defined behavioral categories that are recorded over the adult life span (Connolly and Tully 1998, Carey *et al.* 2006). Specific behaviors have been shown to be associated with longer life spans (Zhang *et al.* 2006), functional senescence (Grotewiel *et al.* 2005), or are effective predictors of time to death (Papadapoulos *et al.* 2002), which are all essential for understanding the evolution of life cycle dynamics. Studies of predictability and hierarchy of behavioral sequences can inform understanding of how insects interact with the proximate conditions of their environment and provide insight into potential fitness attributes associated with the evolution of specific behaviors (Carey 2003, Berman *et al.* 2016).

Most species of chironomids are considered to be non-feeding and short-lived as adults. Energy and nutrients for mating and reproduction are accumulated by the larval stages (Tokeshi 1995) and there is an energetic, and corresponding fitness cost, associated with increased longevity and delayed reproduction by adults. Consequently, in many species adults are often able to mate and oviposit within minutes of emerging. Swarming behaviors, as a mechanism for enhancing encounter efficiency and short-range mate recognition among adults, have been described and implications for reproductive success proposed (Neems *et al.* 1992), however, little is known about the corresponding mechanisms and behaviors of obligate or facultative surface-mating species. It seems plausible to assume that if a longer adult life span in a winter-emerging species has increased fitness attributes, specific behaviors would have age-specific or hierarchical relationships that off-set the energetic consequences of delayed reproduction in non-feeding species.

A well-defined and consistently applied protocol is necessary to record and quantify behavioral patterns. The lab methods used for determining longevity at predetermined, nearly constant, air temperatures consists of daily checks of adults confined individually in one-dram vials to determine when adults die (Ferrington *et al.* 2010). Routine observations of fly activity in the vials over the duration of earlier experiments have revealed several different behaviors, and recently a protocol has been developed to record behaviors as the flies age. The intent of this publication is to define twelve behaviors observed during the longevity studies, and to demonstrate the frequencies of behaviors as a function of incubation temperatures and across three different collection sites. Although I have only developed and applied this protocol in longevity studies of *Diamesa mendotae*, I anticipate that it can be applied more generally in comparative longevity studies among Chironomidae.

Methods and materials

Adults of *Diamesa mendotae* were all scooped from on snow into one-dram vials along with a small amount of snow. When incubated at temperatures above freezing the snow melts and forms a layer of water in the bottom of vials to provide moisture and prevent desiccation. Vials were placed in small coolers partially filled with snow to keep vials from warming on sunny days. Coolers were returned to lab and adults were sorted by sex and briefly observed under dissecting scope to confirm that they were not damaged during collection, then were randomly assigned to differing treatment categories based on predetermined incubation temperatures of 6 °C., 18 °C, and room temperature set at 24 °C. Flies assigned to both the 6 °C. and 18 °C. treatments were incubated in a Sanyo MIR-154 Incubator with temperature fluctuations of +/- 1.5 degrees, which closely matched the +/- 2 °C. fluctuations in room temperature. The number of specimens per treatment category depended on the number and sex ratios of flies collected in the field and details are provided in Table 1. The field and incubation protocols are consistent with those used in our previous studies of longevity of this species (e.g. Ferrington et al. 2010).

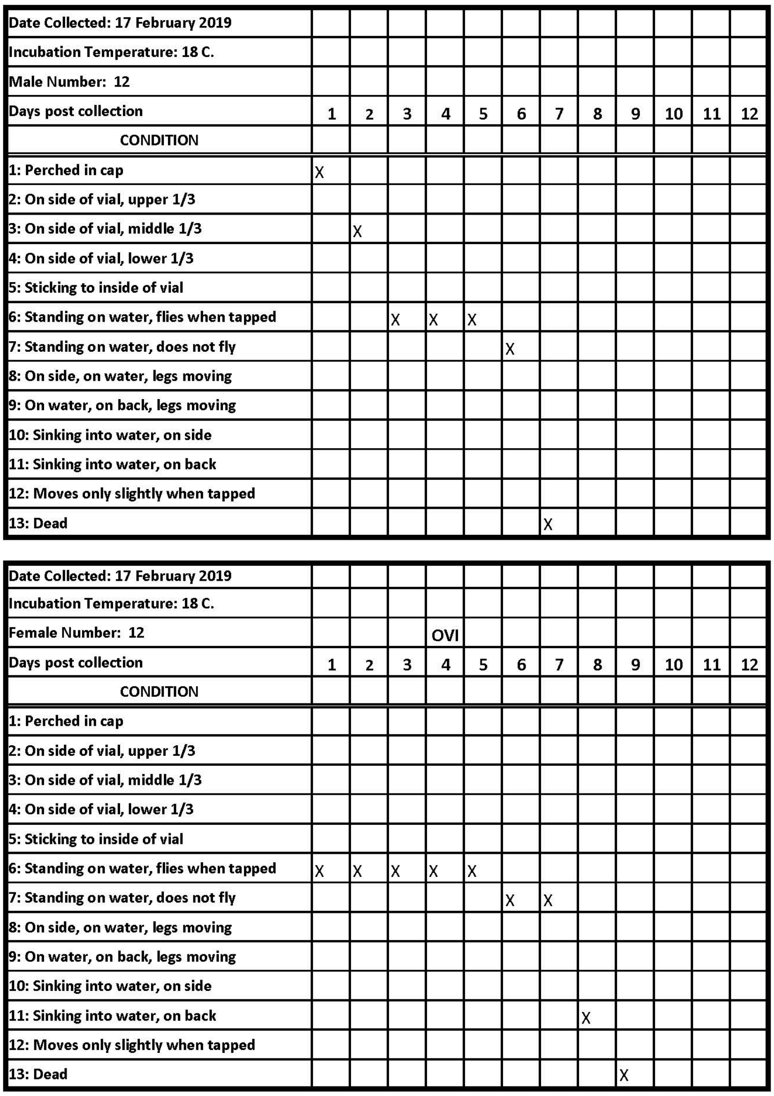
All vials were placed in small cardboard boxes with numbers added to the cap of the vial so that records of behaviors could be traced over time for each fly (Figure 1). Care was taken to not jostle the box and to carefully remove each vial to observe the initial behavior. Initial behaviors were recorded within 15 seconds of removing a vial from the box using a dissecting microscope set at 6x magnification, at approximately the same time each day. However, as flies aged it was often necessary to tap the vial or increase the observation time up to 30 seconds in order to determine if flies were still alive.

An electronic data sheet in EXCEL format was developed for each fly. After determining the initial behavior of the fly in a vial, an "X" was entered into the corresponding cell in the electronic data sheet. Twelve individual behaviors were incorporated into the data sheet based on the most active or prominent behaviors of flies during the initial

Figure 1. Photograph of cardboard box used to organize numbered vials during longevity studies.

Table 1. Summary of field collections and numbers of *Diamesa mendotae* used in each longevity treatment temperature. A total of 353 flies were analyzed (219 males and 134 females).

Locality	Date DD/MO/YR	Incubation Temperature	Number of Males/Females	Maximum longevities (days) Males/Females
Hay Creek	17/02/19	24 °C	19/20	4/6
Hay Creek	17/02/19	18 °C	18/20	12/12
Hay Creek	17/02/19	6 °C	17/19	28/30
Trout Brook	14/02/19	24 °C	29/13	5/2
Trout Brook	14/02/19	18 °C	29/13	10/8
Trout Brook	14/02/19	6 °C	29/13	27/28
Valley Creek	23/02/19	24 °C	26/12	4/4
Valley Creek	23/02/19	18 °C	26/12	7/5
Valley Creek	23/02/19	6 °C	26/12	23/19



Date Collected: 17 February 2019												
Incubation Temperature: 18 C.												
Male Number: 12												
Days post collection	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
CONDITION												
1: Perched in cap	X											
2: On side of vial, upper 1/3												
3: On side of vial, middle 1/3		X										
4: On side of vial, lower 1/3												
5: Sticking to inside of vial												
6: Standing on water, flies when tapped			X	X	X							
7: Standing on water, does not fly						X						
8: On side, on water, legs moving												
9: On water, on back, legs moving												
10: Sinking into water, on side												
11: Sinking into water, on back												
12: Moves only slightly when tapped												
13: Dead							X					

Date Collected: 17 February 2019												
Incubation Temperature: 18 C.												
Female Number: 12				**OVI**								
Days post collection	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
CONDITION												
1: Perched in cap												
2: On side of vial, upper 1/3												
3: On side of vial, middle 1/3												
4: On side of vial, lower 1/3												
5: Sticking to inside of vial												
6: Standing on water, flies when tapped	X	X	X	X	X							
7: Standing on water, does not fly						X	X					
8: On side, on water, legs moving												
9: On water, on back, legs moving												
10: Sinking into water, on side												
11: Sinking into water, on back								X				
12: Moves only slightly when tapped												
13: Dead									X			

Figure 2. Example of data sheet and daily records of behavior for a male and female collected from on snow, 17 February 2019, adjacent to Hay Creek, Goodhue County, MN. The female oviposited in the vial on Day 4, post collection.

assessments. Each behavior was assigned a number. Oviposition date was also entered into the data sheet, as was observations of mites on flies and nematodes observed in the water. See Figure 2 as an example of behaviors recorded for a male and a female that oviposited while incubated at 18 °C.

Flies were preserved with 95% ethanol and locality labels with fly number were placed individually in vials unless the female oviposited. Females that oviposited were transferred to another vial before preservative and labels were added so that eggs could continue to be checked for embryogenesis or larval hatch. Males were checked under higher magnification after preservation to confirm species identity. We currently cannot reliably determine females to species, but assume they are all *Diamesa mendotae*. All preserved flies have been stored in -80 degree C. freezer for subsequent evaluation.

Results

Definitions of behaviors

1) Perched in cap – Adults displaying this behavior tend to remain at rest in the cap when checked, but seem to be the most active when disturbed, often flying for several seconds or more before landing on the inside of the vial or within the cap. Flies landing on the side of the vial generally walk quickly up the inside of the vial and resume their position within the cap. Not all flies exhibit this behavior, and adults obtained from field collections vary substantially in the percent of individuals that perch within the cap.

2) On side of vial, upper 1/3 – This behavior is defined relative to the upper-most part of the body of the fly. In most cases the flies rest with the head up, which serves as the point for determining position on the inside of the vial. When disturbed, they fly or walk quickly up or down the inside of the vial, but rarely land or stand on the water.

3) On side of vial, middle 1/3 – As with behavior 2, this behavior is defined by the upper-most part of the body, usually the head. When disturbed flies walk or fly within the vial, usually landing on the side, but occasionally standing briefly on the water, then walking back up the inside of the vial.

4) On side of vial, lower 1/3 – This behavior is also measured by the upper-most body part. In contrast to behaviors 2 and 3, flies sometimes orient horizontally or with head up, but often have one or more legs in contact with the water surface. When disturbed they fly but quickly land, or rapidly flutter wings while walking on the inner surface of the vial, or gliding in circles on the water before again resting on the side of the vial. Some adults walk on the inside of the vial and resume resting in the middle or upper 1/3 of the vial.

5) Sticking to inside of vial – Adults exhibiting this condition are generally females that have begun to extrude eggs (but not into the water) or males that appear to have a drop of fluid extruded from near the bases of the gonocoxites (presumably spermataphore). This behavior was coded as 5 because adults that become stuck to the side of the vial are often in the middle or lower 1/3 of the vial (not commonly at the water surface) and often vigorously fan their wings and/or move their legs, sometimes for several days. However, few actually detach from the vial and resume other behaviors before dying. In some instances, adults attempt grooming behaviors with the forelegs and spread fluids to the wings or legs which then can become stuck to the vial.

6) Standing on water, flies when tapped – In contrast to behaviors 1- 3, flies exhibiting this behavior commonly rest on the water, but readily fly when the vial is tapped on the stage of the dissecting microscope. After flying briefly, flies commonly return to the water surface or land on the side, generally in the middle or lower 1/3. If not disturbed again, flies remain relatively motionless, or engage in grooming behavior, especially if touching the inside of the vial. If disturbed again by tapping, the flies typically resume flying.

7) Standing on water, does not fly – In contrast to behavior 6, flies exhibiting this behavior do not fly when the vial is tapped for up to 15 seconds. This behavior, however, has several different outcomes that were observed in the longevity experiments, and it seems useful and informative to sub-divide the definition of this behavior in future experiments. Although the behaviors are likely to be part of a general decline in vigor, by providing definitions, it will be possible to score individuals in subsequent experiments according to the three new definitions provided here. Behavior 7 (a) is defined as individuals that do not fly when disturbed, but fan their wings rapidly and glide in circles or bounce along the water surface before coming to rest, either on the water surface or in the lower 1/3 of the inner surface of the vial. After coming to rest, individuals often engage in grooming behaviors. Behavior 7 (b) consists of short, gentle bursts of fluttering the wings when tapped, but little or no gliding, and rapidly return to standing on the water or in association with the inside of the vial. In some cases, the wings may not be fully folded over the abdomen after fluttering, but are held at an acute angle to the abdomen, or one wing may be fully folded but the other remains at a sharply acute (or less sharply acute) angle to the abdomen. Behavior 7 (c) consists of the adult not fanning or fluttering the wings but walking or remaining motionless on the water. As with Behavior 7 (b), both wings can be fully folded over the abdomen, or one or both wings can remain at an angle to the abdomen.

8) On side, on water, legs moving – Individuals exhibiting this behavior appear to have lost equilibrium and cannot remain standing on the water. Often the adult can still gently flutter the wings and move their legs rapidly while on their side, but no coordinated flying or standing occurs. Some individuals partially or fully upright themselves but are uncoordinated and slip back onto their sides. Wings are usually at an acute angle to the abdomen, not fully folded at rest over the abdomen.

9) On water, on back, legs moving – In contrast to behavior 8, individuals on their back typically do not flutter their wings or temporarily upright themselves. Wings are often at or near a 90 degree angle to the abdomen, and their upper surfaces are usually fully in contact with the water surface. In this position, legs may be moving rapidly or not, and are usually not contacting the surface of the water.

10) Sinking into water, on side – The ability of adults to remain on the water surface in behaviors 8 or 9 is likely to be a function of waxes secreted onto the epicuticle. Volatilization of waxes and decomposition by microbes can degrade the waxes and reduce the hydrophobic nature to the exoskeleton. It is possible that decreased wax production associated with aging reduces the replenishment of waxes, resulting in adults loosing hydrophobicity and sinking into the water during later stages of incubation. Individuals sinking into the water while on their sides generally do not flutter wings, and leg movement is greatly reduced as water pulls up onto the cuticle.

11) Sinking into water, on back – Individuals exhibiting this condition generally are ones that transitioned to it from behavior 9. Typically water will pull up onto the sides of the thorax and/or abdomen, and in the case of males the plume setae can become entrained in the water film or fully sink into the water.

12) Moves only slightly when tapped – Adults exhibiting this behavior may have transitioned from behaviors 8-11 and may be on their side on the surface of the water, or sinking into the water to varying degrees, but not actively moving unless the vial is tapped on the stage of the dissecting microscope, and generally stop moving when tapping is discontinued. Adults transitioning to this behavior from behaviors 1-7 often look very healthy and may be on the water surface near the meniscus with the side of the vial, or even partially sticking to the vial, and also only move in response to tapping. No attempts to fly or walk occur. In some cases the only visible indications that the adult is still alive are subtle movements of the palps and/or twitching of legs, leg segments, antennae, gonocoxite, gonostylus or cercus

13) Dead – In some instances it is easy to discern that the individual is dead. Adults transitioning to death from behaviors 1-7 often have legs or wings at unusual angles to the body, presumably as rigor mortis results in muscle tissue contracting and distorting the distal portions at odd angles to the more proximal parts of the joint or segment. In addition, there can be very striking and conspicuous asymmetries in the orientation of legs, wings, antennae and/or palps. These adults also are often floating on the water surface and readily are displaced when tapping the vial. Adults transitioning from 8-11, however, often do not exhibit legs or wings at unusual angles or high degrees of asymmetry, but often show some degree of microbial growth, usually fungal, that can be detected at higher magnification. When an adult has been presumed to be dead, the observation time is often increased before recording the individual as dead.

Adult Longevities

Maximum longevities of males and females as a function of collection stream and incubation temperatures are shown in Table 1. Longevities of males incubated at 24 °C. ranged from 4-5 days versus 2-6 days for females. At 18 °C. male longevities ranged from 7-12 days compared to 5-12 days for females. Longevities of both males and females were longest at 6 °C. incubation, ranging from 23-28 and 19-30 days, respectively.

Scoring of Behaviors

Daily assessments of individual behaviors were recorded for 353 flies (Table 2). Quantification of behaviors as a function of collection locality and incubation temperatures is shown in Table 2. A total of 2,744 behaviors were recorded, including 228 for incubations at 24 °C., 666 at 18 °C. and 1,731 at 6 °C. Figures 3-5 show histograms of frequencies of behaviors exhibited by batches of flies collected at different localities and held at the three different incubation temperatures.

Discussion

The behaviors defined in this study are based on earlier routine observations of the types of activities of field collected adults exhibit when observed in vials during longevity experiments under lab conditions at predetermined intervals after col-

Table 2. Summary of numbers of behavioral observations of *Diamesa mendotae* obtained for each longevity treatment temperature.

Locality	Incubation Temperature	Number of observations (including "Dead")	Number of observations (excluding "Dead")
Hay Creek	24 °C	159	120
Hay Creek	18 °C	287	249
Hay Creek	6 °C	675	644
Trout Brook	24 °C	106	64
Trout Brook	18 °C	229	187
Trout Brook	6 °C	629	587
Valley Creek	24 °C	82	44
Valley Creek	18 °C	150	112
Valley Creek	6 °C	427	389

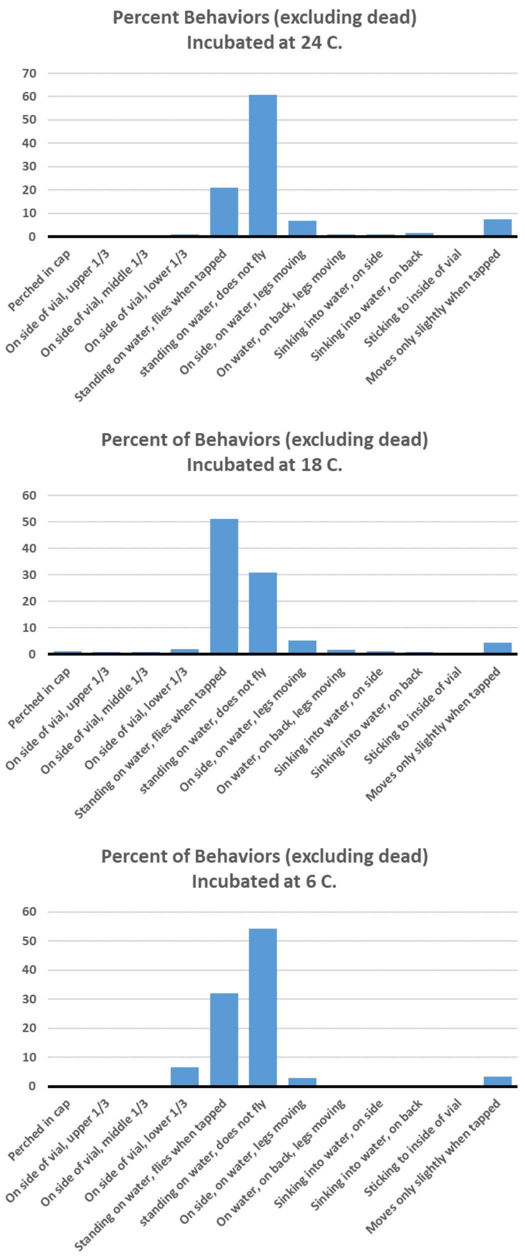


Figure 3. Percent of behaviors recorded by adult flies collected from Hay Creek during longevity experiments with adults incubated at different temperatures.

lection. Differing behaviors were recorded in lab notes, but until recently no attempts were made to standardize definitions for the behaviors, and no prior attempts were made to score the behaviors in a systematic manner. The results presented in this paper thus represent the first application of the protocol. As defined here, all behaviors can readily be assessed within 15 seconds among recently collected batches of adults. However, as longevity studies progress and adults age, it is often necessary to increase the time observing the specimens in order to determine if they are alive, but relatively non-responsive, or dead.

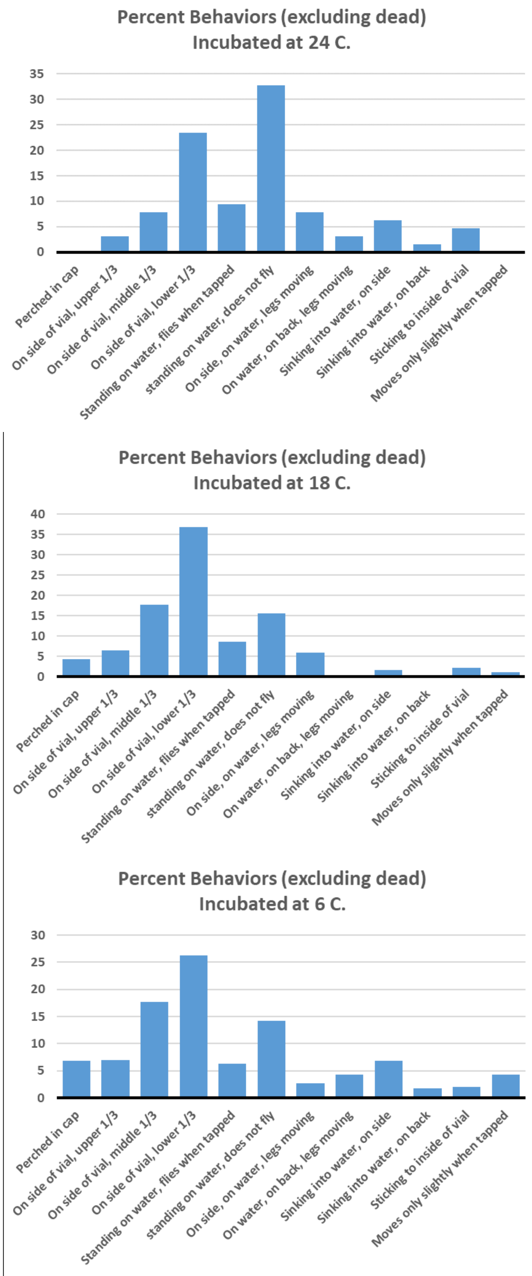


Figure 4. Percent of behaviors recorded by adult flies collected from Trout Brook during longevity experiments with adults incubated at different temperatures.

Behavior 7, ***Standing on water, does not fly***, requires further comment and will be subdivided in future studies. Although the behaviors are likely to be part of a general decline in vigor, I anticipate that it will be possible to unambiguously score individuals in subsequent experiments according to the three new definitions provided here. Behavior 7 (a) is distinctive in that individuals that do not fly when disturbed fan their wings rapidly and glide in circles or bounce along the water surface before coming to rest. This behavior can persist for several or many seconds, often continuing for the entire 15 second observation interval.

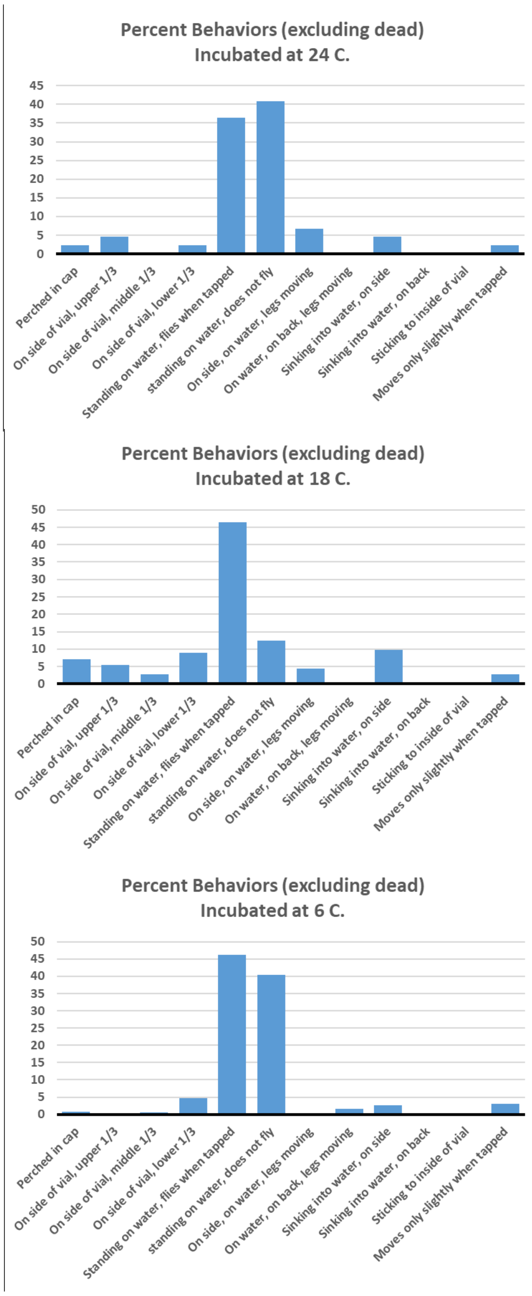


Figure 5. Percent of behaviors recorded by adult flies collected from Valley Creek during longevity experiments with adults incubated at different temperatures.

Behavior 7 (b), which is defined as consisting of short, gentle bursts of fluttering the wings when tapped, differs qualitatively from 7 (a), but in most cases it is combined with little or no gliding activity, and adults rapidly return to standing on the water or in association with the inside of the vial, often within 3-4 seconds after tapping the vial. As noted earlier, in some cases the wings may not be fully folded over the abdomen, but are held at an acute angle to the abdomen, or one wing may be fully folded but the other remains at an angle to the abdomen.

Behavior 7 (c) consists of the adult not fanning or fluttering the wings but walking or remaining motionless on the water. To assess this condition it is necessary to observe the adult for the entire 15 second interval, and repeatedly tap the vial on the stage of the microscope. Similar to behavior 7 (b), both wings can be fully folded over the abdomen, or one or both wings can remain at an angle to the abdomen and adults rarely attempt to maintain contact with the inner surface of the vial. Adults often are very unstable, shaking or quivering while attempting to remain upright on the water.

Division of behavior 7 into three distinct behaviors will add to the sensitivity to future study of transitions in behavior from ones with high activity to lower activity, and will lead to better interpretation of fitness states, transitions to higher morbidity, and quantification of age-related impairment in locomotor and other behaviors as shown for *Drosophila* by Jones and Grotewiel. (2011). The revised protocol will have 14 behavioral categories.

As mentioned in the introduction, it is uncertain how meaningful it is to extrapolate lab-based longevity studies to fitness benefits under field conditions. Despite having a longer duration of the adult portion of the life-cycle, if the female does not remain healthy enough to effectively navigate back to the water to oviposit, her reproductive potential is lost. Using the new definitions to record behaviors, it is likely that females exhibiting behaviors 7b through 12 are not capable of returning to the stream. By contrast, females on a snow bank, but in close proximity to the stream, may still have potential to fan their wings sufficiently to glide across snow (behavior 7a) and navigate to the open water to oviposit.

Although not quantified in earlier longevity studies, previous routine observations of collections from differing streams and under different field temperature conditions have indicated there are small to substantial differences in the frequencies of behaviors, especially behaviors 1-6. As shown here (Figures 3-5), there are differences in the frequencies of behaviors 1-7 for the three collections, but the differences are most conspicuous when comparing the frequencies of behaviors 1, 2 and 3 for adults from Hay Creek versus Trout Brook. Based on the limited number of field collections that have been quantified using the protocol, it is not yet possible to offer an interpretation for the population-to-population variability of lab behaviors. However, Carey (2011) has shown population differences in longevities and behavior patterns among wild-caught populations of six medfly global biotypes. Although speculative, it is possible that population differences in cold-adaptedness of *Diamesa mendotae* could influence the lab expressions of adult behaviors.

Acknowledgements

Many members of the Chironomidae Research Group have contributed to the long term success of our studies of the cold-adapted life cycle of *Diamesa mendotae*. In addition, most colleagues and guests visiting our lab during winter over the past twenty-five years have been introduced to a "winter collecting experience" of picking adults from on snow adjacent to trout streams in Minnesota or Kansas. The list of persons contributing to this research through field-collecting of adults on snow is far too long to include all their names. However, I would like to extend my gratitude to all for your help, and hope the winter experience was pleasurable. Facilities and incidental support for this research was provided by the Department of Entomology at the University of Minnesota. Grant support from the Legislative-Citizen Commission on Minnesota Resources (LCCMR Project # 03i) and Minnesota Agricultural Experiment Station Project (MAES 17-031) to the author.

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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)

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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; R_1, 17; R_{2+3}, 1; remaining veins bare; squama with 14-15 setae in 1 row. Legs. Tarsomeres ta_4 of PI and PII equal in length (185 µm) as well for ta_5 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_1-ta_5 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.

http://zoobank.org/8A3665D5-6D2A-461E-AC81-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 µS/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

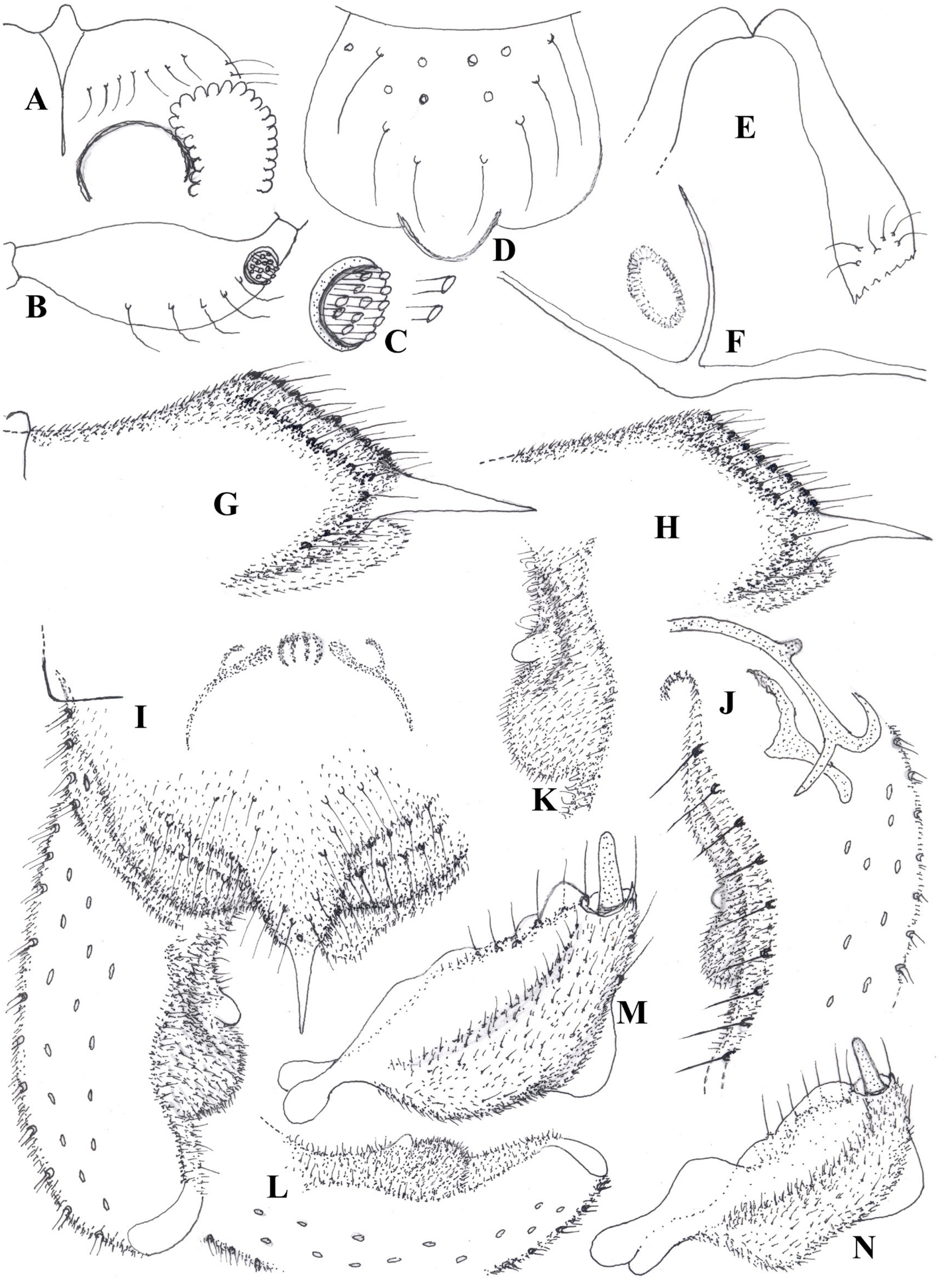

Figure 1. Male adult of *Chaetocladius berythensis* sp. n. A) head, frontal area (right side) with temporal setae; B) palpomere 3 with sensilla clavata and sensilla coeloconica; C) details of sensilla coeloconica; D) clypeus; E) lobes of antepronotum; F) humeral pit; G-H) two aspects of anal point and 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.

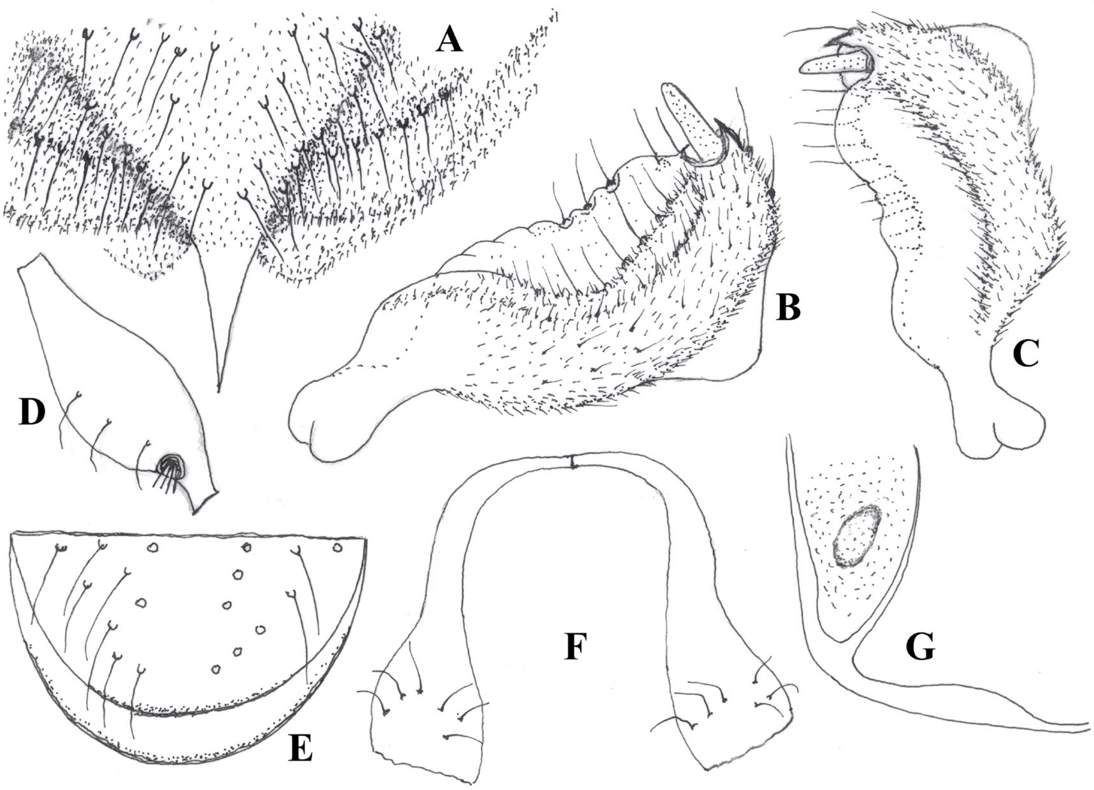

Figure 2. Male adult of *Chaetocladius* spp. *C. berythensis* sp. n.: A) anal point, dorsal; B) gonostylus, ventral; C) gonostylus, lateral. *C. callauensis* sp. n.: D) palpomere 3; E) clypeus; F) lobes of antepronotum; G) humeral pit.

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 µS/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. Antepronotum (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; R_1, 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_1-ta_5 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 µm 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 (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	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

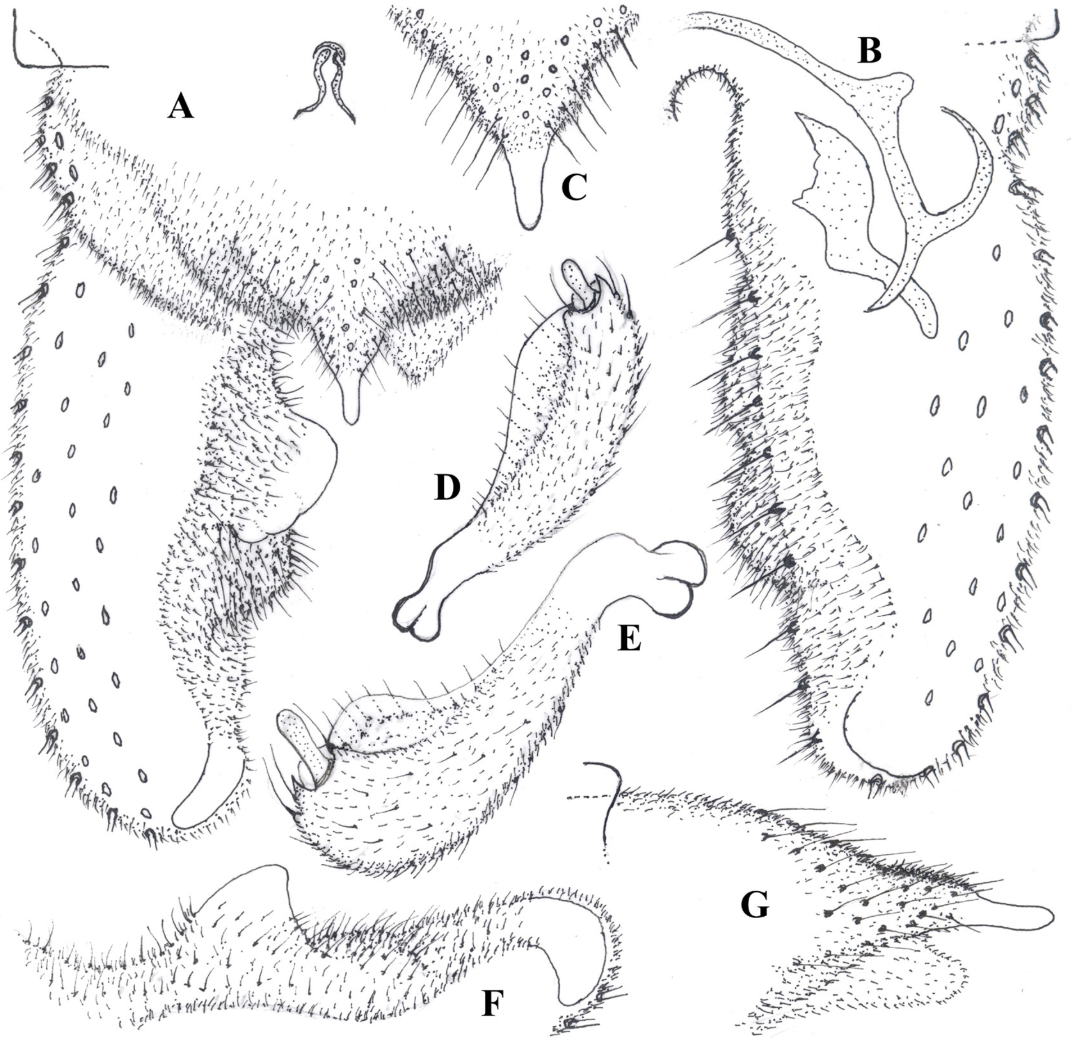

Figure 3. Male adult of *Chaetocladius callauensis* sp. n. A) hypopygium, dorsal; B) hypopygium, ventral; C) anal point, dorsal; D) gonostylus, acute angle; E) gonostylus, obtuse angle; F) gonocoxite and inferior volsella, lateral; G) anal point and tergite IX in lateral view.

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 well-developed, 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; R_1, 1; remaining veins bare; squama with 9 setae in 1 row. Legs. Tarsomeres ta_4 and ta_5 of PII sub-equal; sensilla chaetica present on: tibia and tarsomeres ta_1-ta_5 of PI, tibia and tarsomeres ta_1-ta_4 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) well-developed, 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 µm 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

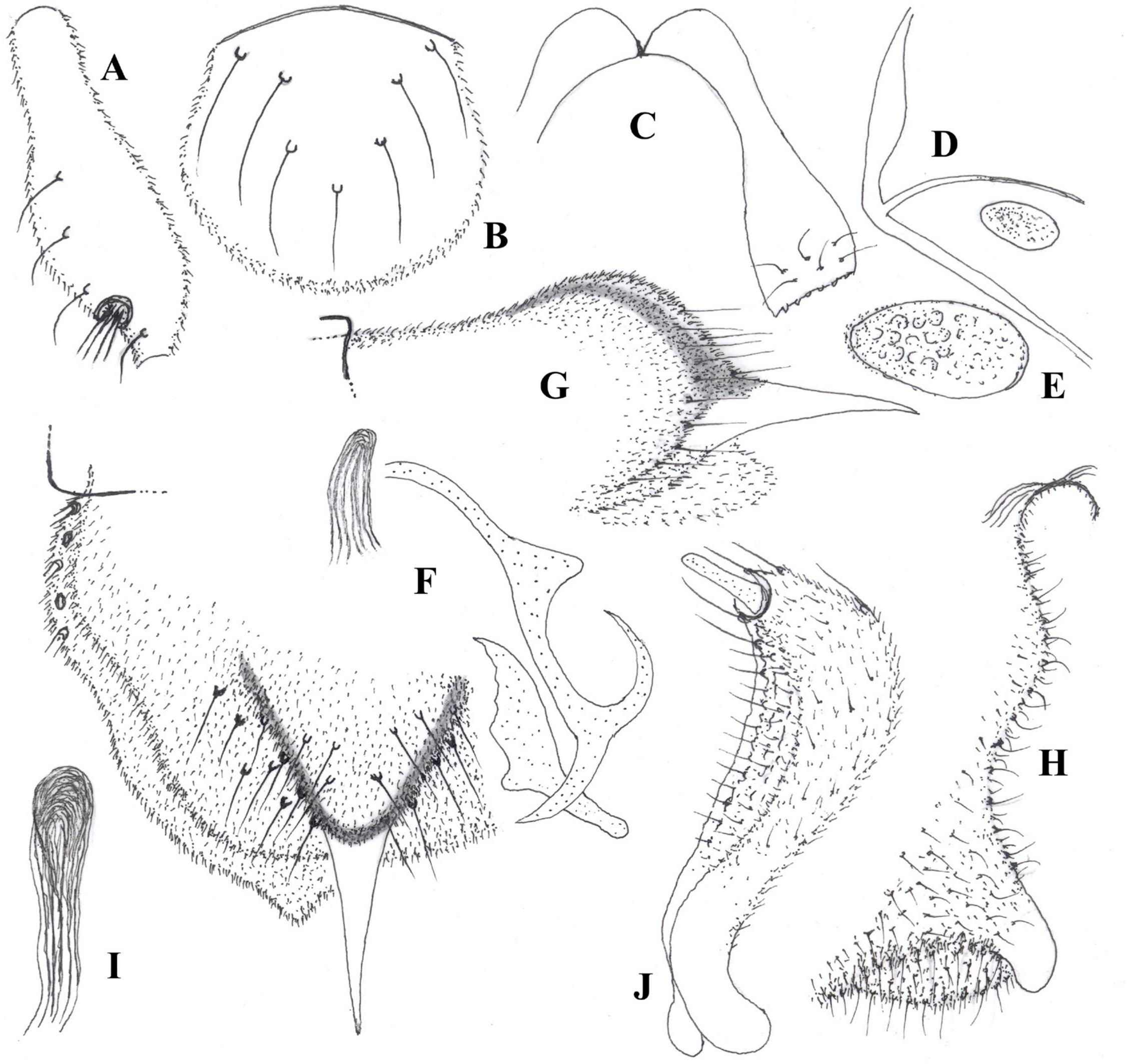

Figure 4. Male adult of *Chaetocladius guardiolei* sp. n. A) palpomere 3; B) clypeus; C) lobes of antepronotum; D-E) humeral pit, two aspects; F) hypopygium, anal segment and apodemes; G) anal point and tergite IX in lateral view; H) inferior volsella; I) virga, another aspect; J) gonostylus, dorsolateral.

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. Antepronotum 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; R_1, 0; R_{2+3}, 1; remaining veins bare; squama with 11 setae in 1 row. Legs. Tarsomeres ta_4 and ta_5 of PII sub-equal; sensilla chaetica present on: tibia and tarsomeres ta_1-ta_5 of PI, tibia and tarsomeres ta_1-ta_5 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 µm long. Latero-sternite IX with 12 setae inserted laterally (6 on each side). Sternapodeme and phallapodeme (Fig. 5D), phallapodeme nearly sickle-shaped. 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) well-developed 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 Moubayed-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 *laminatus*-group 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

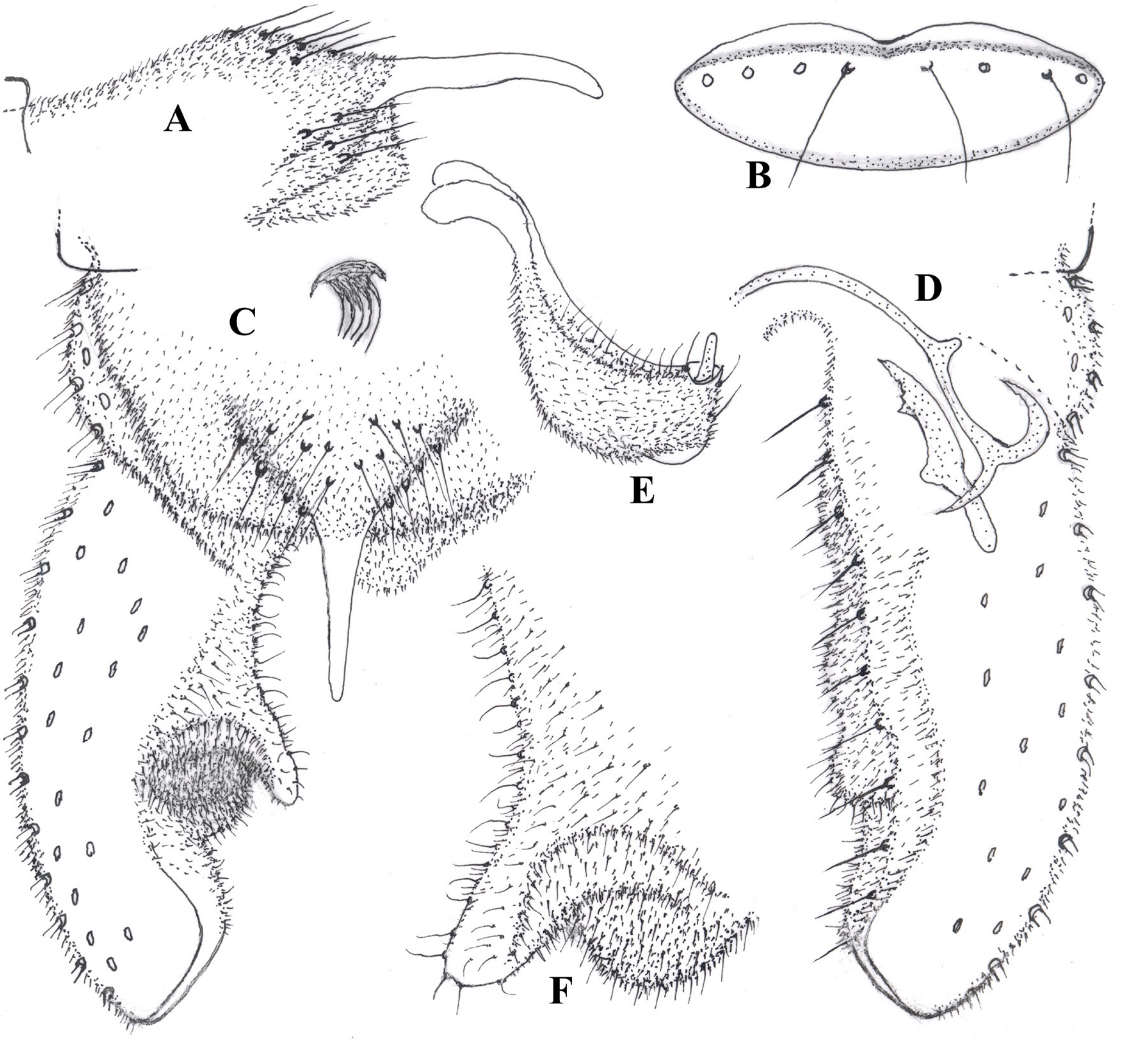

Figure 5. Male adult of *Chaetocladius parerai* sp. n. A) anal point and tergite IX in lateral view; B) scutellum; C) hypopygium, dorsal; D) hypopygium, ventral; E) gonostylus, dorsal; F) inferior volsella.

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: Dc_1-Dc_2 (d1); Dc_2-Dc_3 (d2); Dc_3-Dc_4 (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.

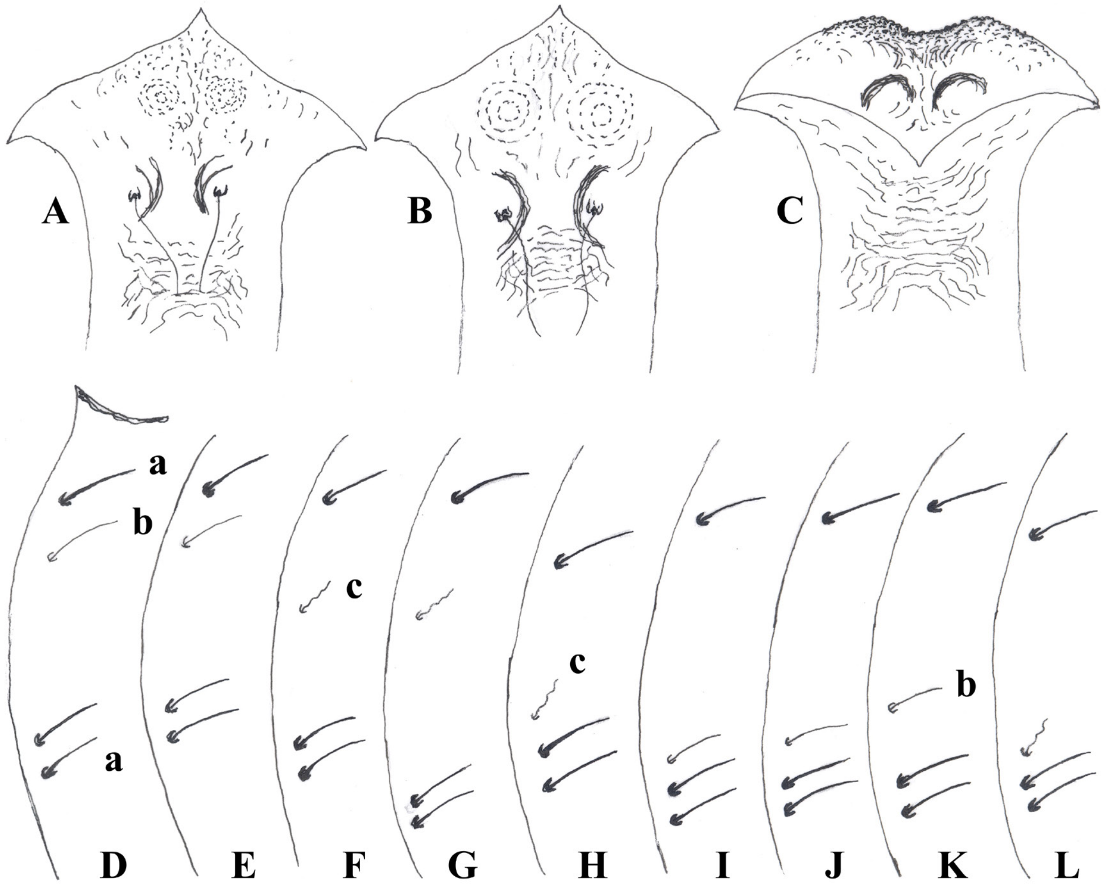

Figure 6. Pupal exuviae of *Chaetocladius* spp. Frontal apotome of: A) *C.* cf. *callauensis* sp. n.; B) *C. mantetensis*; C) *C.* cf. *guardiolei* sp. n. Distribution pattern of dorsocentral setae on thorax of: D) *C. melaleucus*; E) *C. perennis*; F) *C. guisseti*; G) *C. mantetensis*; H) *C. bitusiki*; 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. Dc$_1$, Dc$_3$ and Dc$_4$ (type-a); Dc$_2$, (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;

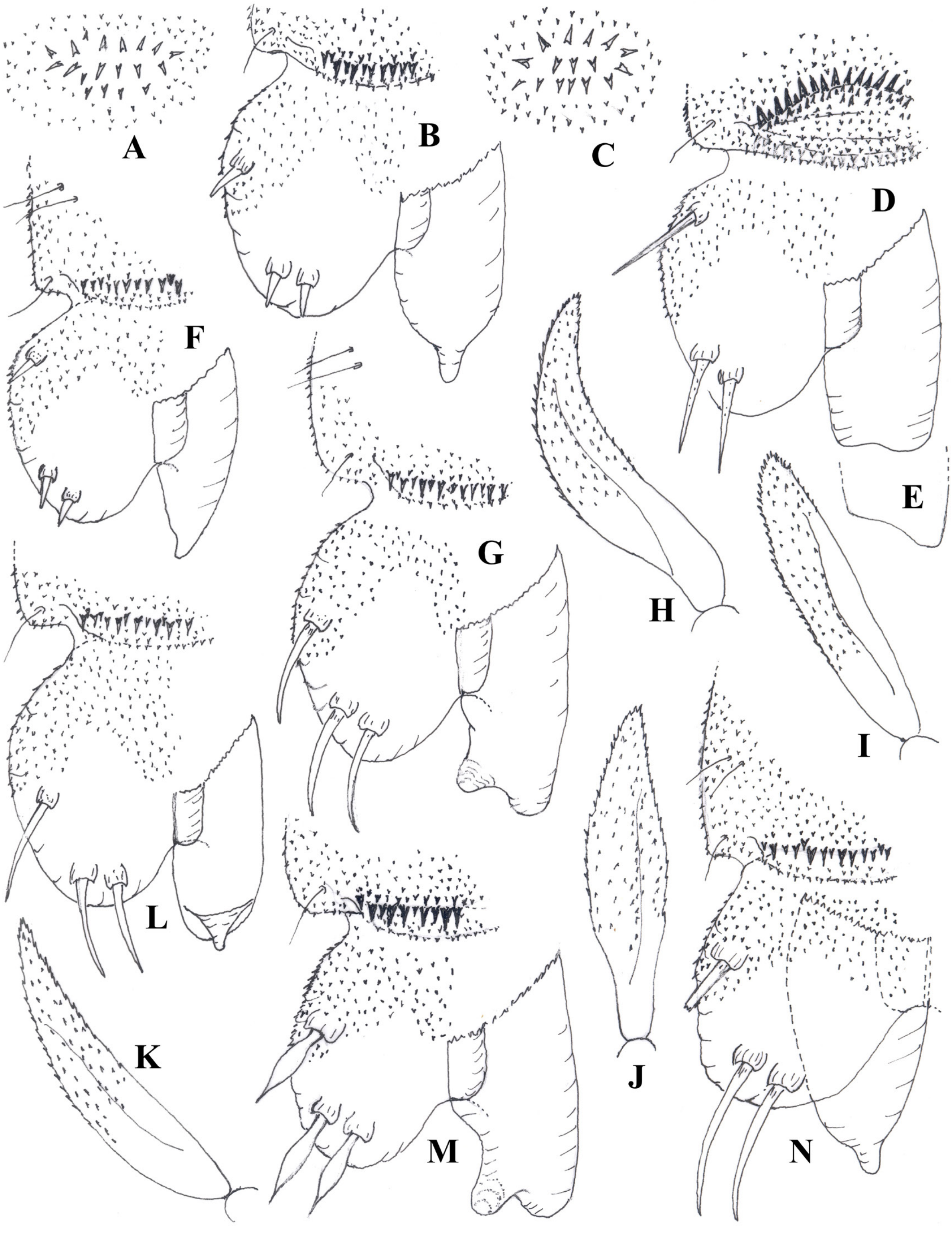

Figure 7. Pupal exuviae of *Chaetocladius* spp. Caudal area of sternite VI of: A) *C. melaleucus*; C) *C.* sp. cf. *laminatus*. Tergite VIII (caudal part, dorsal) and anal lobe of: B) *C. melaleucus*; D-E) *C.* sp. cf. *laminatus*; F) *C. dissipatus*; G) *C.* sp. cf. *guardiolei* sp. n; L) *C.* cf. *callauensis* 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. Dc_1, Dc_2 and Dc_3 well separated, Dc_3 located close to Dc_4 (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. Dc_1 located long distance from Dc_2; Dc_2 located close to Dc_3 and Dc_4 (Fig. 6H); d1 = 45, d2 = d3, 15;

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, 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 µm long ;

2. 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 µm 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. Dc_1 located far from Dc_2-Dc_4 (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. Dc_1, Dc_3 and Dc_4 (type-a); Dc_2, (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).

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First record of *Demicryptochironomus* (*Irmakia*) *neglectus* Reiss, 1988 in Slovakia

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Abstract

Here, we present the first record of *Demicryptochironomus* (*Irmakia*) *neglectus* Reiss, 1988 in Slovakia. Samples were taken using the Chironomid Pupal Exuviae Technique (CPET) from two reservoirs. Two pupal exuviae (out of the 500 ind. collected) were recorded in Kráľová reservoir and in one individual was found in Sĺňava reservoir (out of the total 514 ind. collected). Since the CPET method mostly allows species level identification of chironomids, its usage is helpful in discovering the real chironomid diversity of Slovakia. Moreover, it helps to define ecological requirements of species we have little information about, such as the present one.

Introduction

The Chironomid Pupal Exuviae Technique (CPET; Wilson & Ruse, 2005) as a method for assessing ecological quality of water reservoirs in Slovakia started in 2009. Since then, eight new chironomid species were recorded in Slovakia using the CPET technique within the national monitoring program (Ščerbáková & Bitušík; 2013, 2015) confirming the usefulness of this method to identify chironomids in various aquatic ecosystems. This method greatly contributes, among other things, to the knowledge of the regional fauna. In the present paper we report the first record of *Demicryptochironomus* (*Irmakia*) *neglectus* for Slovakia from two reservoirs along with information on the environmental conditions of the habitat of occurrence.

Material and methods

Material was collected by drift sampling according to the Chironomid Pupal Exuviae Technique (Wilson & Ruse, 2005) and preserved with 75% ethanol. In laboratory around 500 exuviae were picked from a total amount of sampled euviae, mounted on permanent microscopic slides and identified using Langton and Wisser (2003). Sampling and processing of material was in accordance with the European standard (SFS-EN15196:2006). Material is deposited at the Department of Hydrobiology, Microbiology and Ecotoxicology, Water Research Institute in Bratislava.

Figure 1. Water reservoir Sĺňava. Photo S. Ščerbáková 03.08.2015.

Figure 2. Water reservoir Kráľová. Photo S. Ščerbáková 19.06.2013.

Samples were taken from two water reservoirs – Sĺňava and Kráľová (Figs. 1, 2), both situated at the lower stretch of the River Váh. These two reservoirs represent the lowermost situated reservoirs built within the chain of hydropower plants on the River Váh. The distance between the two studied reservoirs is 38.6 km. Details of hydromorphological conditions and measured physicochemical parameters from both reservoirs collected in the year of findings are presented in Table 1.

Table 1. Hydromorphological and physicochemical characteristics of the studied water reservoirs (WR).

Variable/site name	**Unit**	**WR Kráľová**	**WR Sĺňava**
		48°12’33.15” N	48°33’17.1” N
Coordinates		17°48’03.18” E	17°49’30.6” E
Altitude	m a. s. l.	124	158
Depth Ø	m	6.00	3.10
Area	10^6 m^2	10.90	4.10
Max. volume	10^6 m^3	65.50	12.50
Ret. time	day	1.7	0.3
Water chemistry			
Oxygen	$mg.l^{-1}$	9.00	7.62
pH	-	7.79	8.03
Temperature	°C	18.03	19.27
Conductivity	$mS.m^{-1}$	37.53	40.34
$NH^{4-}N$	$mg.l^{-1}$	0.054	0.074
$NO^{3-}N$	$mg.l^{-1}$	0.984	0.960
N total	$mg.l^{-1}$	1.436	1.510
P total	$mg.l^{-1}$	0.062	0.058
$PO^{4-}P$	$mg.l^{-1}$	0.036	0.036

Results

The following specimens of *Demicryptochironomus* (*Irmakia*) *neglectus* Reiss, 1988 (Chironominae, Chironomini) were found:

Kráľová water reservoir - 2 pupal exuviae (from total number of 500 individuals), 19. 6. 2013, coll. S. Ščerbáková, det. S. Ščerbáková; Sĺňava water reservoir - 1 pupal exuvia (from total number of 514 individuals), 3. 8. 2015, coll. S. Ščerbáková, det. V. Štillová (Fig. 3).

Demicryptochironomus (*Irmakia*) *neglectus* is a chironomid with distribution in Western Europe (Austria, Britain, Finland, French mainland, Germany, Ireland, Switzerland) and in East Palearctic (Spies and Sæther, 2013). Adults and exuviae were collected in and along streams and rivers in the alpine region, the Black Forest and the British Isles (as Pe1) (Moller Pillot, 2009 and references therein), but also in China (Yan et al., 2005).

In general, larvae of the genus *Demicryptochironomus* Lenz, 1941 are tolerant to organic pollution – CPET tolerant group D (Wilson & Ruse, 2005). They occur in lakes and rivers with sandy or muddy substrate. They are predatory and feeding especially on oligochaetes (Pinder & Reiss, 1983).

Exuviae identified by Langton (1991) have been described and illustrated in Pinder & Reiss (1986) as Chironomini Genus D. The larval stage has not yet been described.

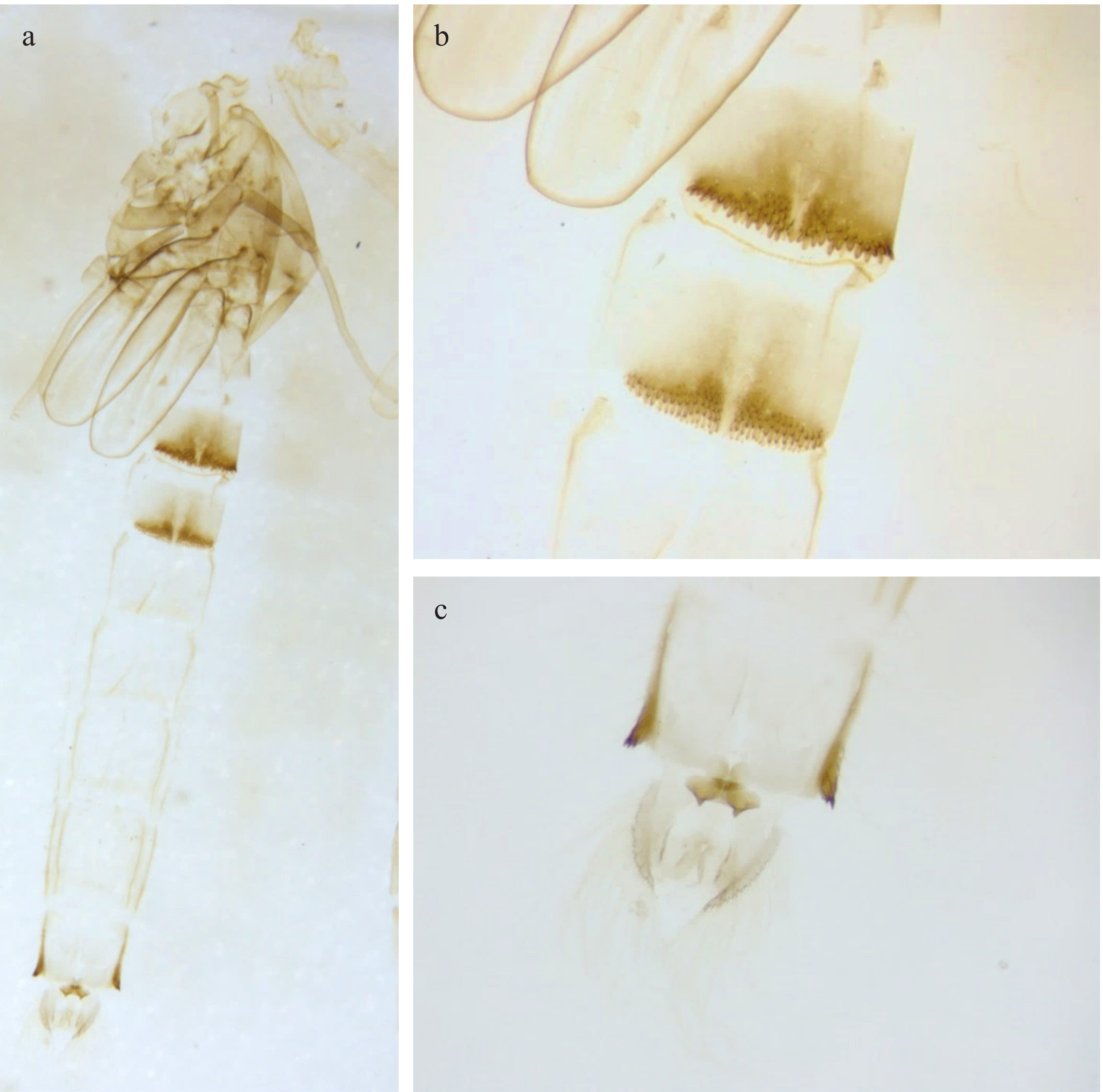

Figure 3. Photos of the exuvia of *Demicryptochironomus* (*Irmakia*) *neglectus.* a) pupal exuvia, b) 2nd and 3rd segment, c) anal combs of the 8th segment. Photo S. Ščerbáková.

Acknowledgements

Financial supports from the EU Cohesion Funds within the project Monitoring and assessment of water body status (No. 24110110158, 2012-2015 Phase II) and by grant VEGA No 1/0664/15 are acknowledged. We are grateful to Ladislav Hamerlík and Peter Bitušík, Department of Biology and Ecology, Matej Bel University for their valuable comments to the manuscript.

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Descriptions of two interesting chironomid pupae collected in Yunnan Province, China (Chironomidae: Chironominae)

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Abstract

Descriptions of two Chironomini pupal types collected in Yunnan Province are given. Among known genera, both types closely resemble that of the North American *Hyporhygma* Reiss, 1982, but the spine and spinule pattern of the dorsal abdomen does not fit within the current taxonomic boundaries, and represent an endemic component in the Oriental region. A new genus description is expected when associated adults are available.

Introduction

The tribe Chironomini has a nearly worldwide distribution and comprises about 60 genera in Holarctic region (Epler *et al.* 2013). In the Oriental region, 43 Chironomini genera were listed in the most recent catalogue (Hazra *et al.* 2016), and 28 of them were recorded from China (Yamamoto *et al*. 2018).

In an expedition along the borders of Yunnan Province, China, we collected two pupal exuviae belonging to Chironomini from macrophyte-dominated ponds. Close examination revealed that the two pupae are similar to each other in the truncated cephalic tubercle with an apical crown of many spines, and the dark anteromedian mounds bearing strong spines on the abdominal tergites II–VI, but significantly different in the body size. The pupae are distinct from other Chironomini pupae by the combination of having a cephalic tubercle with apical spines, a thoracic horn with several branches, an abdominal tergite II with a posterior band of spines and an abdominal conjunctive IV/V with many spines. Here we describe and illustrate the two pupal types under the names of taxon 1 and taxon 2 in order to promote chironomid studies in Oriental region.

Materials and Methods

Pupal exuviae were collected by a hand net (mesh size 250 µm). Specimens examined were slide-mounted in Euparal. Morphological terminology and abbreviations follow Sæther (1980) and Langton and Visser (2003). All the specimens examined are deposited in the Institute of Groundwater and Earth Science, Jinan University (EJNU), Guangzhou, China.

Description

Pupal Diagnosis.

Pupae conform mostly to the generic pupal description of *Hyporhygma* Reiss, 1982 (Pinder and Reiss 1986), except for the following characters: Antepronotals absent; thoracic horn with 6–8 branches, one of the basal branches distinctly stronger than others (Fig. 1b). Wing pad brown, with two vein stems darkened in basal 1/2; nose absent. Strong anterior spines on T II–VI located on sclerotized mounds. T II with posterior band of spines; III–VI without distinct posterior spine band of points. Conjunctive III/IV bare; IV/V with band of spinules. Paratergite II–V present or weak. Anal lobe without dorsal seta. Male genital sac extending beyond the outer margin of anal lobe.

Taxon 1

Pupa (n = 1). Total length 9.9 mm, abdominal length 7.7 mm.

Coloration: Exuviae brown, abdominal apophyses dark brown. T II–VI darkened on anteromedian mounds. T II dark on posterior spine patch.

Cephalothorax: Cephalic tubercles rounded apically, ca. 120 µm in outer diameter, central spine crown consisting of many spines 10–12 long; frontal seta weak, 20 µm long (Fig. 1a). Precorneals 2, longest ca. 100 µm. Thoracic dorsum pebbled along anterior 1/3 of median suture. Lengths of dorsocentrals 1–4 (µm): 80, 50, 60, 70. Dc1 and Dc4 stronger than Dc2 and Dc3 (Fig. 1c). Tracheal bundle simple; basal ring elliptical, longest diameter about twice as long as broad.

Abdomen (Fig. 1d): T II–VI with extensive spinulation; T VII–VIII with anterolateral spinulation. T II with anteromedian and posteromedian transverse bands of strong spines, posterior band narrowly interrupted by posterior muscle marks. T III–VI each with anteromedian spine patch laterally accompanied by sparse spines. T II with row of about 70 caudal hooklets; its row 2/3 times as long as segment width. A I with anterolateral tubercle; A II with PSB; A IV with vortex. Paratergite II–V weak or absent S II–VIII with extensive anterolateral spinulation. A V–VI with 3 Lt-setae; A VII–VIII with 4 Lt-setae. Anal comb on segment VIII with 1 large and 4 smaller yellow teeth, largest 50 µm (Fig. 1e). Anal lobe 550 µm long, with 1–2 irregular rows of 100 lateral taeniae; ALR 1.57.

Specimen Examined. Pe (female), CHINA, Yunnan Province, Xishuangbannan Dai Autonomous Prefecture, Jinghong City, Menglun Town, Xishuangbanna Tropical Botanical Garden, 19.ii.2019, H.Q. Tang.

Taxon 2

Pupa (n = 1). Abdomen length 5.0 mm, total length 6.5 mm.

Coloration: As the above taxon.

Cephalothorax: Cephalic tubercles ca. 90 µm in outer diameter central spine crown consisting of many spines 8–10 long; frontal seta hard to observe. Lengths of dorsocentrals 1–4 (µm): 50, 30, 25, 50. Dc1 and Dc4 stronger than Dc2 and Dc3. Tracheal bundle as previous taxa.

Abdomen (Fig. 1f): Spinulation pattern as previous taxa except the weak developed accompanied sparse spines lateral to anteromedian spine patch. Hook row on tergite II with 58 caudal hooklets, 2/3 of width of tergal II. Paratergite II–V present, well-developed on IV–V Anal comb VIII with 1 larger and 3 small yellow teeth, largest 45 µm long (Fig. 1g). Anal lobe 375 µm long, with one row of 60 lateral taeniae, ALR 1.58.

Specimen Examined. Pe (male), CHINA, Yunnan Province, Pu'er City, Ximeng Va Autonomous County, Mengsuo Lake, 27.viii.2014, H.Q. Tang.

Discussion

These two taxa are characterized by the pupal cephalic tubercle with an apical whirl of spines, which also occurs in *Endochironomus* Kieffer, 1918, *Endotribelos* Grodhaus, 1987*, Hyporhygma, Phaenopsectra* Kieffer, 1921 and *Sergentia* Kieffer, 1922 (Grodhaus 1987). Among these genera, the pupae of the present taxa most closely resemble those of North American *Hyporhygma* Reiss as the abdominal tergite II has a posterior transverse band of strong spines, but are distinct in the thoracic horn bearing 6–8 branches, the absence of posterior spine bands on the abdominal tergites III–VI and the bare abdominal conjunctive III/IV. In *Hyporhygma*, the pupal thoracic horn is plumose, the pupal abdomen bears posterior spine bands on the tergites III–VI and two groups of strong spines on the conjunctive III/IV (Pinder and Reiss 1986). The species group A of *Endochironomus* possesses a posterior transverse spine band on the tergite II, but lacks spines on the cephalic horn (*l.c.* p. 322).

Although those two types are similar to each other, the smaller taxon apparently represent an independent status, based on the weak lateral sparse spines and extensive spinulation on paratergite IV–V. Since the extent of morphological variations are hard to detect in small samples, we here treat the two taxa separately.

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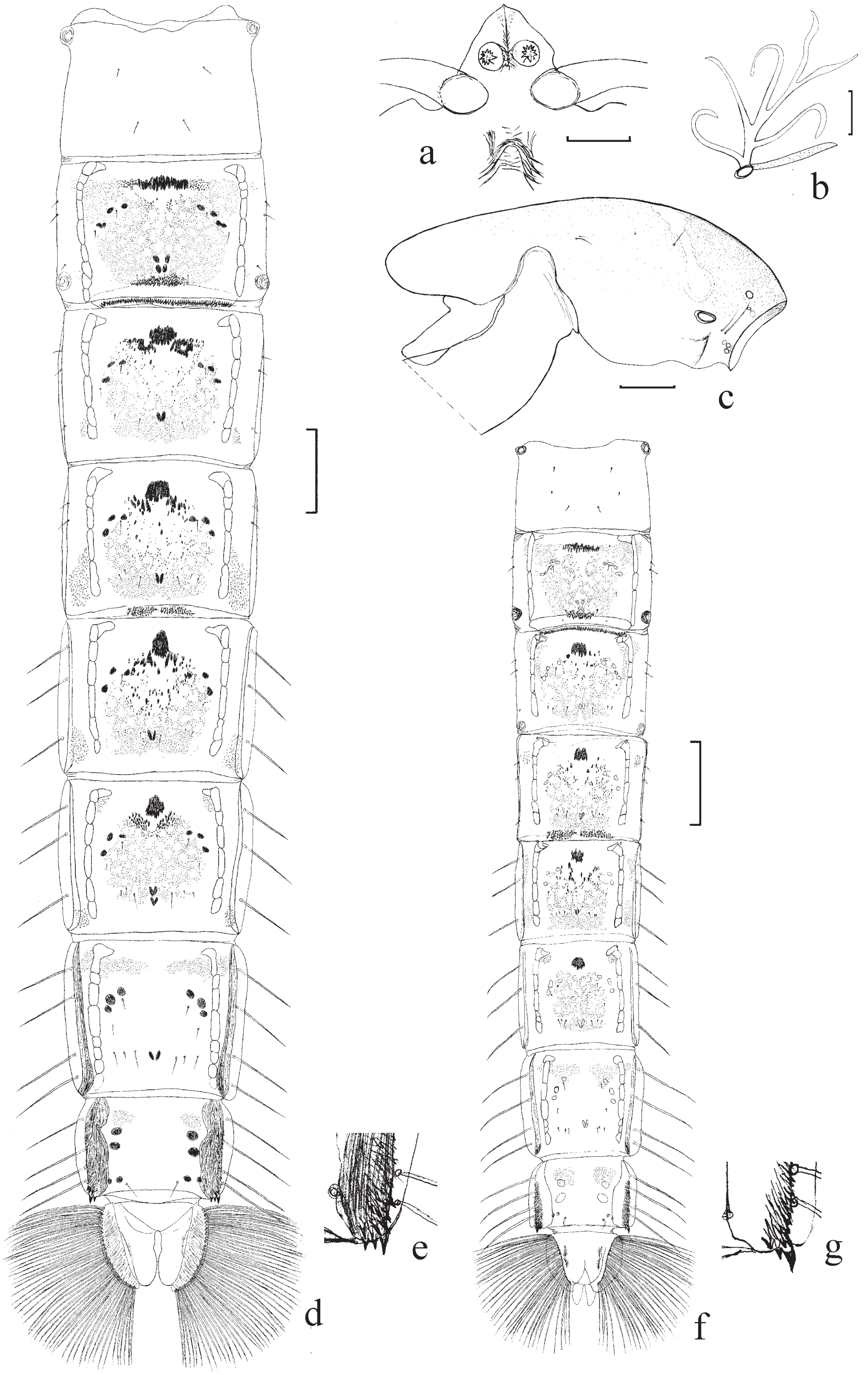

Figure 1. Taxon 1: a) frontal apotome; b) thoracic horn; c) thorax; d) abdoman dorsal view; e) posterolateral spines of segment VIII. Taxon 2: f) abdoman dorsal view; g) posterolateral spines of segment VIII. Scale bar = XX μm.

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Male description of the phytotelma-dwelling species *Polypedilum parthenogeneticum* (Chironomidae: Chironominae)

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Abstract

The male of the phytotelma-dwelling species *Polypedilum parthenogeneticum* is described from *Eryngium* L. (Apiaceae) (Corrientes province, Argentina) and is included within the subgenus *Polypedilum*. The pupal diagnosis is emended, its geographic distribution is updated, and their taxonomic relationships with other Neotropical species of *Polypedilum* are discussed.

Introduction

The genus *Polypedilum* Kieffer, 1913 occurs in all zoogeographical regions except Antarctica (Vårdal et al. 2002). The larvae of this genus are found in sediments of standing and flowing waters, with a few species mining wood or grazing on epilithic surfaces (Cranston et al. 1989; Vårdal et al. 2002). This genus is quite diverse, with more than 500 species worldwide and about 70 belong to the Neotropical region. The taxonomic classifications and the subgeneric delimitation of *Polypedilum* have been problematic and were treated in several studies (Sæther et al. 2010; Cranston et al. 2016; Yamamoto and Yamamoto 2016; Shimabukuro et al. 2019),

The species *Polypedilum parthenogeneticum* was described by Donato & Paggi (2008) based on reared material collected from the leaf axils of *Eryngium* L. (Apiaceae). All the specimens obtained were females and the parthenogenesis was confirmed in the laboratory, being the eclosion one day after the oviposition. New specimens of *P. parthenogeneticum* collected from the leaf axils of *Eryngium* sp. were reared in the laboratory and a male was obtained for the first time. Therefore, the aims of this study are to describe the male of *P. parthenogeneticum* and to provide new information on its geographic distribution, as well as to emend the pupal diagnosis of this species.

Material and methods

Microscope slides were prepared by clearing the specimen with 10% KOH; neutralization with glacial acetic acid; followed by a dehydration series of 80%, 96% and 100% ethanol and mounting in Canada Balsam. Morphological terminology and measurement standards follow Sæther (1980) and Bidawid-Kafka & Fittkau (1995); the values are rounded off to the nearest 5 µm unless otherwise stated; measurements are given in µm (except when otherwise stated) as ranges. The specimens studied are deposited in the collection of the Instituto de Limnología "Dr. Raul A. Ringuelet", Argentina (ILPLA).

Taxonomy

The subgenus *Polypedilum* defined by Sæther et al. (2010) is characterized for the lack of frontal tubercles; wing membrane without macrotrichia; R_{2+3} usually well separated from R_1; superior volsella with a long projection, usually with lateral midbasal to subapical strong setae; and the anal point is never trifid or with lateral shoulders. As Cranston et al. 2016 considered *Tripedilum* as junior synonym of *Polypedilum* s. str., the absence of frontal tubercles and the presence of outer setae in the digitiform projection of superior volsella become inconsistent to define *Polypedilum* s. str. because those characters are diagnostic for *Tripedilum* as it was stated by Sæther et al. (2010) (see also Yamamoto and Yamamoto (2016) for further discussion). We consider the inclusion of *P. parthenogeneticum* in the subgenus *Polypedilum* by a combination of characters such as bare wing membrane, long digitiform projection of superior volsella and anal point not trifid, following Shimabukuro et al. (2019). The inclusion is also supported since the larval and pupal stages of *P. parthenogeneticum* belong to *Polypedilum* s. str. Taxonomic relationships of those stages together with the female are discussed in Donato and Paggi (2008).

***Polypedilum* (*Polypedilum*) *parthenogeneticum* Donato & Paggi, 2008**

(Fig. 1)

Polypedilum parthenogeneticum Donato and Paggi 2008: 52.
Polypedilum (*Polypedilum*) *parthenogeneticum*, new combination.

Material examined. ARGENTINA: 1 male with pupal exuviae, Corrientes, Riachuelo, 27.5452754° S, 58.7159499° W, 31.xii.2018, pipette phytotelmata in *Eryngium* L. (Apiaceae), L. Baccaro (ILPLA).

Male description

Total length 2.03 mm; wing length 1.5 mm. Total length/wing length 1.35. Wing length/length of profemur 1.97.

Head. AR 1.24. Ultimate flagellomere 128 long. Temporal setae 13. Clypeus with 22 setae. Tentorium 117 long; 27 wide. Length of palp segments: 29; 31; 68; 87; 126. Third palpomere with 3 lanceolate sensilla clavata in small pit; longest 19 long.

Thorax. Dorsocentrals 11, with 3 on humeral area; acrostichals 7; prealars 3; antepronotals 1. Scutellum with 8 setae.

Wing (Fig. 1A). VR 1.15. Brachiolum with 1 seta. R with 18; R_1 with 9; R_{4+5} with 21; remaining veins bare. Squama with 3 setae. Lengths and proportions of legs in Table 1.

Legs. Scale of fore tibia: 45. Spur of middle tibia: 16 and 56; of hind tibia: 26 and 70. Width at apex of fore tibia 50; of middle tibia 49; of hind tibia 54. Lengths and proportions of legs in Table 1.

Hypopygium (Fig. 1B, C). Tergite IX with 14 medial setae. Phalapodeme length: 76. Transverse sternapodeme length: 49. Superior volsella 74 long, lateral seta lacking, basal portion of superior volsella with microtrichia and 4 inner setae. Inferior volsella slender 120 long, with 11 setae arranged in two rows. Gonocoxite length: 128. Gonostyle length: 159. HR= 0.81. HV= 1.28.

Table 1. Lengths (in μm) and proportions of legs of the male of *Polypedilum* (*Polypedilum*) *parthenogeneticum* Donato and Paggi (n = 1). Abbreviations: Femur (fe); Tibia (ti); Tarsomeres 1-5 (ta_{1-5}); Leg Ratio (LR), ratio of metatarsus to tibia; «Beinverhältnisse» (BV), combined length of femur, tibia, and basitarsus divided by combined length of tarsomeres 2-5; «Schenkel-Scheine-verhältnis» (SV), ratio of femur plus tibia to metatarsus.

	fe	Ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV
P_1	763	474	886	577	392	289	144	1.87	1.51	1.40
P_2	804	660	536	227	165	103	82	0.81	3.46	2.73
P_3	927	660	598	309	289	165	103	0.91	2.52	2.66

Remarks

The larva and pupa of this specimen fits in the original description, except that the branches of the thoracic horn present fine spinules in its tegument. This feature was corroborated also in the females previously described, and therefore the emendation of the pupal diagnosis of *P. parthenogeneticum* is established.

Geographic distribution

Through the present study, the distribution of the *P. parthenogeneticum* extends northward (about 800 km north to the type locality), with the new record located in Argentina in the Chacoan province of the biogeographic scheme of Cabrera and Willink (1973). Because of the notorious absence of male specimens on previous studies, it is possible that *P. parthenogeneticum* presents geographical parthenogenesis such that bisexual and parthenogenetic forms of the same species differ on their distributions (Vandel 1928).

Discussion

The male of *P. parthenogeneticum* most closely resembles *P.* (*P.*) *solimoes* Bidawid-Kafka (couplet 45, Bidawid-Kafka, 1996),but differs from this species by the possession of anal tergal bands type D; the superior volsella more curved, with 4 inner setae, lateral seta absent, and lower AR and LR_1. , The male of

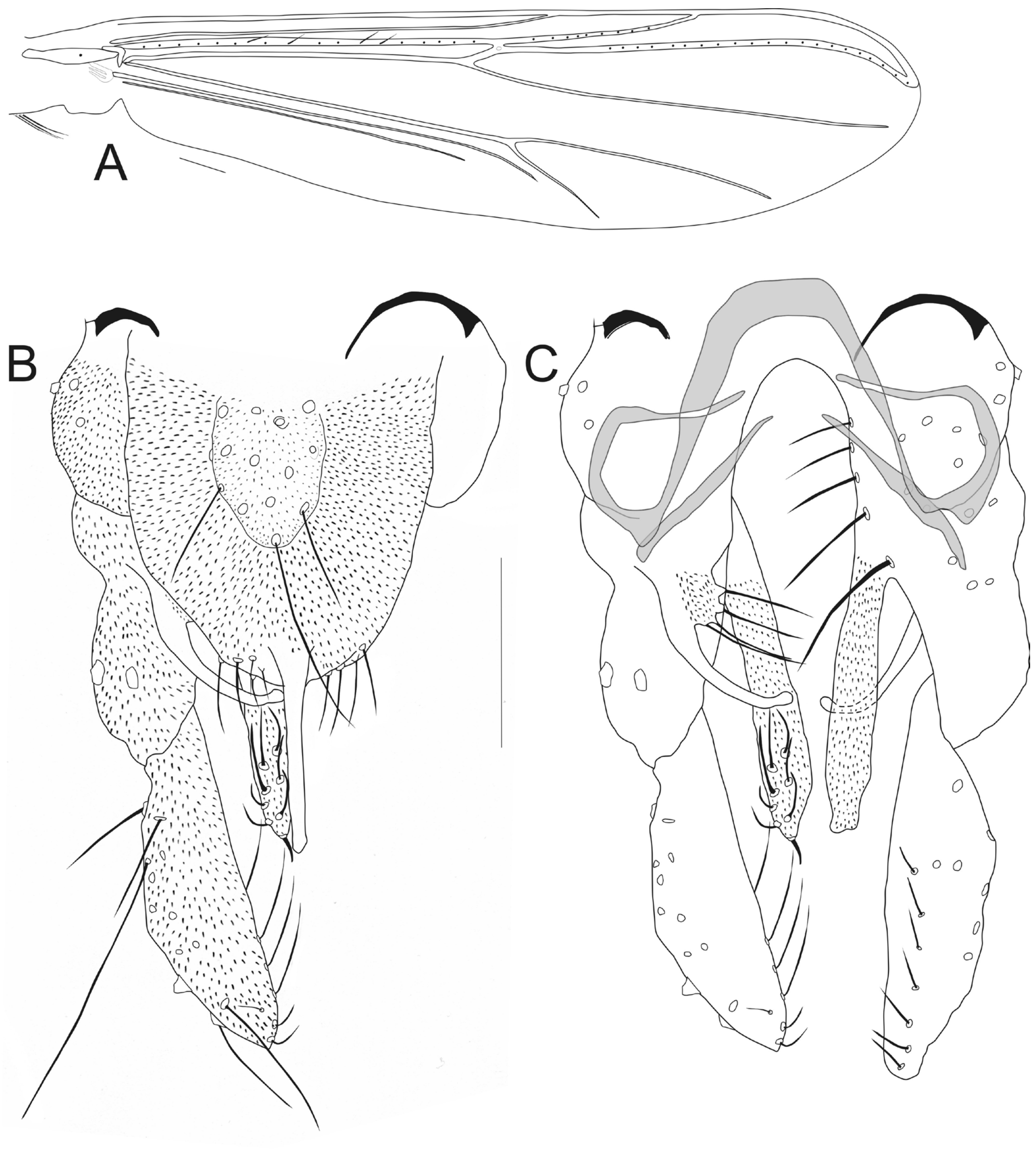

Figure 1. *Polypedilum* (*Polypedilum*) *parthenogeneticum* Donato and Paggi: A, wing; B, hypopygium, dorsal view; C, hypopygium with anal point and tergite IX removed, dorsal aspect to the left, ventral aspect to the right. Scale bar = 100 µm.

P. parthenogeneticum resembles *P.* (*P.*) *trigonus* Townes (in Maschwitz & Cook, 2000), but it is separable by abdominal tergites without white patches, superior volsella not large with 4 inner setae and lateral seta absent.

Within the family Chironomidae, 24 formal described species have been recorded from many types of phytotelmata (Siri and Donato 2014; Paul et al. 2014; Dantas and Hamada 2017; Siri and Donato 2018), and from these species, 6 belong to the genus *Polypedilum*. One of them is *P.* (*P.*) *solimoes* that was treated above. The species *P. pedipalpus* (Picado) is known from its original description as larva, pupa and female and is not possible to be assigned to any of the subgenera. From the remaining species, two of them belong to the subgenus *Polypedilum.* The presence of spotted wings and the superior volsella with 3 inner setae in the male and the spinules on conjunctive V-VI, lacking of median patches of shagreen in tergite III, fine and weak shagreen on tergites IV and V, and anal comb with several spines of the pupa separates the species *Polypedilum* (*P.*) *panacu* Mendes et al. 2011 from *P.* (*P.*) *parthenogeneticum*. The other species is *Polypedilum* (*Polypedilum*) *kaingangi*, described by Pinho et al. (2013). This species is clearly differentiated from *P.* (*P.*) *parthenogeneticum* by having a highly setose clypeus (63–78 to 22 in *P.* (*P.*) *parthenogeneticum*) and the superior volsella has 2 inner setae in the male; the pupa with fine and weak shagreen on tergites III, IV and V, and anal comb with several spines.

Recently the Neotropical fauna of the genus *Polypedilum* was increased by Shimabukuro et al. 2019. In that study, the authors described two species in the subgenus *Polypedilum*. The species

Polypedilum (*P.*) *caete* differs from *P.* (*P.*) *parthenogeneticum* by the possession of a long lateral seta in the superior volsella; 4–6 setae arranged in a row on inferior volsella; presence of weak marking on wings and RM distinctly darkened; and scale of fore tibia apically truncated. The other species is *Polypedilum* (*P.*) *aymbere* that is separable from *P.* (*P.*) *parthenogeneticum* by the anal point small and club-shaped; projection of superior volsella proximally enlarged, presenting a lateral seta in the middle of the projection and with 3 inner setae; and inferior volsella bearing a apical seta and 3-4 preapical.

Acknowledgements

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***Thienemannimyia* (*Thienemannimyia*) *sinogalbina* nom. n., a replacement name for *Thienemannimyia galbina* Cheng & Wang, 2009**

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http://zoobank.org/FBE357A4-BF71-499B-98F6-A3CE25D228C0

Abstract

The recent transfer of *Hayesomyia galbina* Cheng & Wang, 2006 to *Thienemannimyia* (*Hayesomyia*) created a homonymy with *Thienemannimyia* (*Thienemannimyia*) *galbina* Cheng & Wang, 2009. *Thienemannimyia* (*Hayesomyia*) *galbina* (Cheng & Wang, 2006) has priority and *Thienemannimyia* (*Thienemannimyia*) *sinogalbina* **nom. n.** is proposed as a new replacement for the junior secondary homonymy *Thienemannimyia* (*Thienemannimyia*) *galbina* Cheng & Wang, 2009.

Introduction

Cheng and Wang (2006) described *Hayesomyia galbina*, and Cheng and Wang (2009) described *Thienemannimyia galbina* based on adult males from China. According to recent morphological phylogeny of Tanypodinae, Silva and Ekrem (2016) treated *Hayesomyia* as a subgenus of *Thienemannimyia*. Hence *Thienemannimyia* (*Thienemannimyia*) *galbina* Cheng & Wang, 2009 was rendered a junior secondary homonymy.

According to Article 53.3 of the International Code of Zoological Nomenclature (ICZN 1999), *Thienemannimyia* (*Hayesomyia*) *galbina* (Cheng & Wang, 2006) has priority and a new name *Thienemannimyia* (*Thienemannimyia*) *sinogalbina* **nom. n.** is proposed for *Thienemannimyia* (*Thienemannimyia*) *galbina* Cheng & Wang, 2009.

Abbreviations

NKU: College of Life Sciences, Nankai University.

***Thienemannimyia* (*Thienemannimyia*) *sinogalbina* nom. n.**

Thienemannimyia galbina Cheng & Wang, 2009: 54, figs 14–16.

http://zoobank.org/FBE357A4-BF71-499B-98F6-A3CE25D228C0

Holotype: (NKU No. 02260) China, Fujian, Wuyishan, Wuyi Mountain Natural Conversation, Sangan, 24.iv.1993, light trap, X.H. Wang. **Paratype**: 1 male (NKU N0. 11728) China, Sichuan, Shimian County, near Nanya River, 16.vi.1996, light trap, X.H. Wang.

Distribution. China.

Etymology. The new name consists of the original species name with the prefix 'sino' indicating the currently known distribution, China.

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A newly recorded species, *Sergentia kizakiensis* (Tokunaga, 1940) (Diptera: Chironomidae), from Oriental China with DNA barcode

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Abstract

Sergentia kizakiensis (Tokunaga, 1940) is newly recorded from Oriental China. Here, adult males are redescribed and illustrated. Additionally, we provide a DNA barcode of *S. kizakiensis* from China.

Introduction

The lentic chironomid genus *Sergentia* includes 17 species known to Holarctic and Oriental regions (Papoucheva et al. 2003, Wülker et al. 1999, Yamamoto et al. 2019). Papoucheva et al. (2003) explored the phylogeny of *Sergentia* using molecular data, concluding that the genus appears to be monophyletic. Yamamoto et al. (2019) formally transferred the Japanese species *Phaenopsectra kizakiensis* to the genus *Sergentia*, documenting its synonymy. However, *Sergentia* is poorly studied in China, and with only two recorded species: *Sergentia baueri* and *Sergentia prima*. Recently, we found another *Sergentia* species from Oriental China, which was difficult to identify morphologically, especially considering the high morphological similarity among most species in the genus. Considering that DNA barcodes have proven efficient in species delimitation in chironomids (Anderson et al. 2013, Lin et al. 2015, Lin et al. 2018), we use DNA barcodes to confirm the taxonomic status of this unidentified *Sergentia* species from China.

Based on the material from China, adult males of *Sergentia kizakiensis* are redescribed and illustrated. A DNA barcode of *S. kizakiensis* from China is also included.

Material and Methods

Five adults were collected using a sweep net near an alpine lake from Oriental China. The material examined was mounted on slides, following the procedure outlined by Sæther (1969). Digital photographs were taken using a Nikon Digital Sight DS-Fi1 camera mounted on Nikon Eclipse 80i compound microscope at the Nankai University. All specimens were deposited in the College of Life Sciences, Nankai University (NKU), China. Morphological nomenclature in the description below follows Sæther (1980).

DNA amplifications of COI barcode sequences with the universal primers LCO1490 and HCO2198 (Folmer et al. 1994) followed methods outlined by Lin et al. (2015) and were carried out at the College of Fishery, Tianjin Agricultural University. PCR products were electrophoresed in 1.0% agarose gel, purified and sequenced at BGI TechSolutions Co., Lit. (Beijing, China). Raw sequences were edited and assembled in SeqMan (DNASTAR, Madison, USA), and then uploaded on Barcode of Life Data systems (BOLD) (Ratnasingham and Hebert 2007, Ratnasingham and Hebert 2013) along with collateral information and an image.

Sergentia (*Baicalosergentia*) *kizakiensis* (Tokunaga, 1940)

Pentapedilum kizakiensis Tokunaga, 1940: 290 (as subgenus *Phaenopsectra*).

Phaenopsectra kizakiensis: Sasa & Yamamoto, 1977: 313; Sasa, 1984: 54; Sasa & Hirabayashi, 1991: 110; Sasa & Suzuki, 2000: 179.

Sergentia kizakiensis: Yamamoto, 2010: 224; Yamamoto & Yamamoto, 2014: 339; Yamamoto, Suzuki & Yamamoto, 2019: 67 (as subgenus *Baicalosergentia*).

Stictochironomus kamiprimus Sasa & Hirabayashi, 1991: 110.

Material examined. 5 ♂♂ (NKU: K5B28, K5B28, K5B42, K5B44; NKU & BOLD Sample ID: K5B45), CHINA: Zhejiang Province, Taizhou City, Tiantai County, Huadingshan Mountain, 29.2523°N, 121.091°E, 1000m a.s.l., 12.IV.2011, Sweep net, leg. X.L. Lin.

Diagnostic characters. The male imago can be distinguished from known species of the genus by having wings covered with microtrichia; $LR_1 > 1$; mid tarsomere 1 with 10–14, 12 sensilla chaetica; superior volsella curbed only at tip, without lateral seta.

Description

Male (n = 5)

Total length 5.68–7.08, 6.24 mm. Wing length 3.40–4.20, 3.71 mm. Total length/wing length 1.60–1.78, 1.68. Wing length/length of profemur 2.62–2.89, 2.78.

Coloration. Head, legs and abdomen dark brown; wing brown; thorax dark brown.

Head. AR 2.49–3.38, 2.83. Ultimate flagellomere 0.98–1.19, 1.04 mm long. Temporal setae 16–21, 19 including 3–6, 4 inner verticals; 11–13, 11 outer verticals and 2–4, 3 postorbital. Clypeus with 23–38, 31 setae. Tentorium 185–215, 196 µm long, 62–80, 68 µm wide. Palpomere lengths (in µm): 70–79, 75; 70–92, 84; 189–220, 202; 198–251, 223; 253–317, 275. L: 5th/3rd 1.29–1.44, 1.36.

Wing (Fig. 1A). VR 1.02–1.03. Brachiolum with 2–3, 2 setae. R with 46–56, 52 setae, R_1 with 48–58, 53 setae, R_{4+5} with 120–130, 126 setae, M_{1+2} with 69–89, 80 setae, M_{3+4} with 54–73, 64 setae, Cu_1 with 18–31, 24 setae, remaining veins bare. Cell r_{4+5}, m_{1+2} with and m_{3+4} with numerous setae. Squama with 28–30, 28 setae.

Thorax (Fig. 1B). Dorsocentrals 17–28, 22; acrostichals 4–5, 5; prealars 6–8, 6. Scutellum with 24–30, 26 setae.

Legs. Scale on fore tibia rounded, 50–75, 60 µm long, sometimes with a small lateral spine. Mid tibia with one 28–32, 30 µm long spur; spurs of hind tibia 28–33, 30 µm and 13–16, 15 µm long. Width at apex of fore tibia 75–88, 81 µm, of mid tibia 63–90, 78 µm, of hind tibia 75–95, 83 µm. Mid tarsomere 1 with 10–14, 12 sensilla chaetica. Lengths (in µm) and proportions of legs as in Table 1.

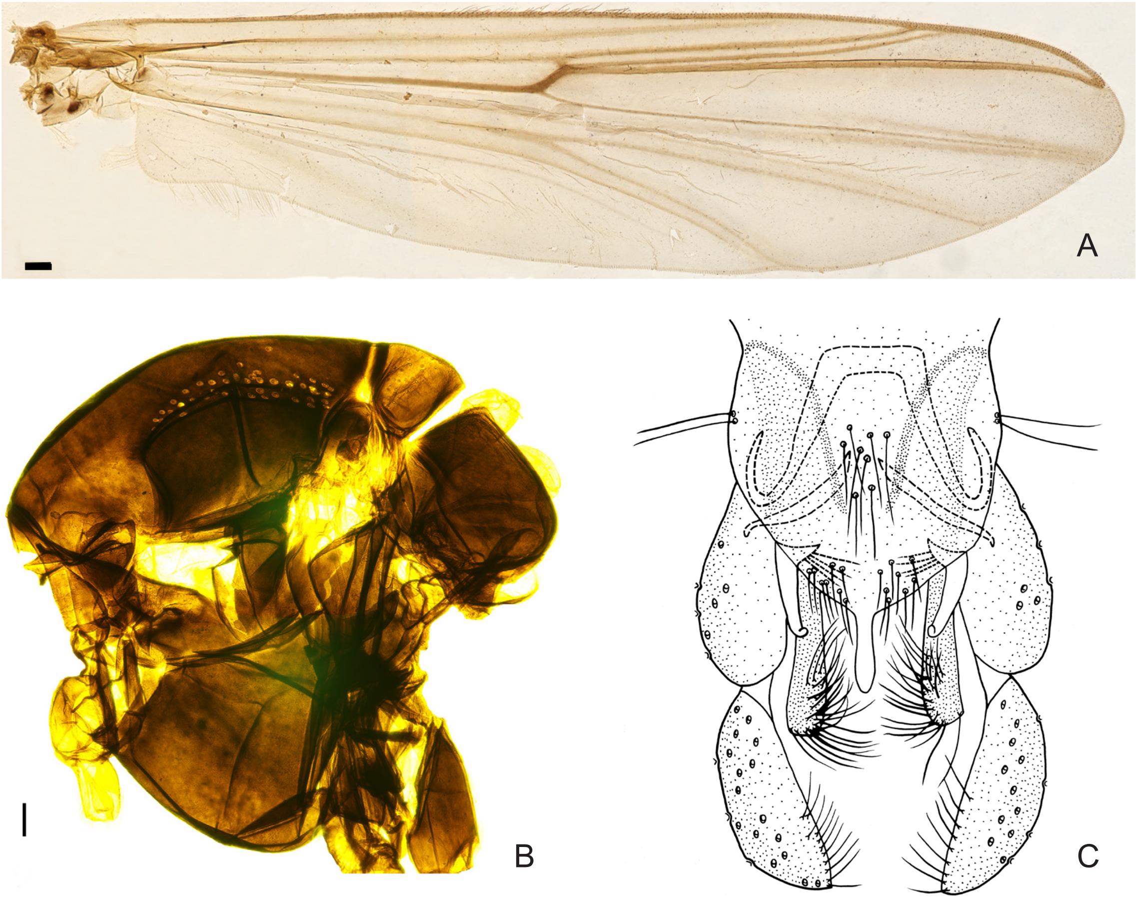

Figure 1. *Sergentia* (*Baicalosergentia*) *kizakiensis* (Tokunaga, 1940). A) wing; B) thorax; scale bar = 100 µm; C) hypopygium.

Table 1. Lengths (in µm) and proportions of legs of *Sergentia kizakiensis* (Tokunaga, 1940), male (n = 5).

	P_1	P_2	P_3
fe	1250–1475, 1335	1325–1600, 1405	1475–1775, 1575
ti	1250–1450, 1335	1250–1475, 1325	1500–1825, 1630
ta$_1$	1425–1755, 1546	725–875, 783	1075–1350, 1185
ta$_2$	875–1075, 945	500–600, 540	700–875, 743
ta$_3$	640–750, 676	380–440, 398	500–640, 552
ta$_4$	500–580, 522	240–280, 258	320–380, 340
ta$_5$	250–290, 260	160–190, 173	180–210, 195
LR	1.14–1.19, 1.36	0.57–0.58, 0.58	0.72–0.74, 0.73
BV	1.68–1.79, 1.74	2.54–2.65, 2.59	1.50–2.50, 2.40
SV	1.67–1.79, 1.71	3.50–3.59, 3.54	2.70–2.77, 2.73
BR	7.80–8.89, 8.39	2.9–7.86, 6.56	7.71–10.7, 8.86

Hypopygium (Fig. 1C). Tergite IX with 4–10, 8 median setae. Anal point with rounded apex. Laterosernite IX with 2–3, 2 setae. Superior volsella digitiform, with four inner setae, and without lateral setae. Phallapodeme 115–155, 134 µm long. Transverse sternapodeme 75–105, 85 µm long. Gonocoxite 233–273, 249 µm long. Gonostylus µm 172–216, 192 µm long. Inferior volsella straight, 145–175 µm long, with 24–30, 26 setae. HR 1.26–1.35, 1.30. HV 3.03–3.58, 3.25.

Distribution. China, Japan.

Remarks. *Sergentia kizakiensis* is redescribed here based on additional material from China. Based on morphological characters, Chinese specimens fit well with the original description (Tokunaga 1940), but a few differences are noted: Chinese specimens have a higher AR (2.49–3.38), and 3–7 inner setae on superior volsella, as compared to Japanese specimens (Sasa 1984, Sasa and Suzuki 2000), described with AR = 2.3–2.4 and superior volsella with 3–7 inner setae. *Sergentia kizakiensis* includes two DNA barcodes clustering into two BINs in BOLD: the BIN (BOLD: ADY6241) from China with a genetic divergence of 4.65% to the BIN (BOLD: ACH8232) from Japan.

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Lasiodiamesa (Podonominae, Chironomidae), first record of the genus from Slovakia

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Abstract

Here we report the first record of *Lasiodimesa* (Podonominae) in Slovakia. A single larva was collected in a small (366 m^2) and shallow (max depth 0.6 m) alpine pond located at 1,654 m a.s.l. in the High Tatra Mountains. Acidotolerant taxa such as *Psectrocladius octomaculatus*, *Zalutschia tatrica, Synendotendipes* sp. and *Tanytarsus* cf. *gregarius* were present in the community of the pond. Our finding indicates that the typical habitat of *Lasiodiamesa* larva is not necessarily a bog, but can also be a small acidic waterbody.

Introduction

Lasiodiamesa is a genus of the Podonominae subfamily confined to the northern Holarctic. The larva can be distinguished from other Podonominae by the elongate and bicolored procerci bearing strong apical setae, and by the mentum with high number (up to 14) of teeth. The labrum is less laterally compressed relative to other Podonominae (Cranston). Species of the genus are known from North America and Canada (4 species) and Fennoscandia, some with their southernmost distribution in Middle Europe. Out of the nine known species (Brundin 1966; Sæther 1967, 1969a; Wirth and Sublette 1970a), pupae are known for seven species (Brundin 1966a; Sæther 1969a). Here we present a record of a *Lasiodimesa* larva from an alpine pond in Slovakia. It is the first record of this genus in Slovakia.

Material and methods

The study pond is located in the Dolina Bielych plies valley (Fig. 1) in the north-eastern part of the Tatra Mts. (Slovakia, Central Europe), at 49.22227° N 20.22376° E. The pond is surrounded by dense growths of

Figure 1. Location of the pond (indicated by arrow) in the Dolina Bielych plies valley where the larvae of *Lasiodiamesa* was recorded.

dwarf pine (*Pinus mugo*). Basic characteristics of study pond are presented in Table 1. The pond has no inlet, nor outlet and the substrate consists of 10 % megalithal (>40 cm), 70 % macrolithal (>20 – 40 cm) and 20 % organic mud.

A combination of drift sampling and the kicking technique was used to collect larvae and pupal exuviae. Preimaginal stages of chironomids were picked, mounted on permanent slides and identified using Sæther and Andersen (2013). The material is deposited at the Department of Biology and General Ecology, Technical University in Zvolen, Slovakia. For the list of other invertebrates recorded in the pond see Table 2.

Table 1. Basic physical, chemical and hydromorphological characteristics of the surveyed pond. Environmental variables were recorded during the field works or determined in laboratory by analysis of water samples taken in time of sampling.

Characteristic	**Value**
Area	368 m^2
Altitude	1,654 m
Max. depth	0.6 m
pH	5.99
Conductivity (25 °C)	8 μS cm^{-1}
DOC	5.727 mg L^{-1}

Results

Chironomidae: Podonominae: *Lasiodiamesa* sp.

Material examined: Material: 1 larva (4 July 2013, Fig. 2a-d) out of 650 specimens collected (Table 2), leg. M. Veselská, det. L. Hamerlík.

Distribution: Four species are known from the Palaearctic (Spies and Sæther 2015). The most common member of the genus is the Holarctic *L. sphagnicola* (Kieffer 1925) recorded in 9 European countries including Scandinavia and countries from Western to Eastern Europe. *L. gracilis* (Kieffer, 1924) is known from Finland, Sweden, Poland, The Netherlands and Czech Republic (Syrovátka and Langton 2015). One species is known from Scandinavia (*L. armata* Brundin 1966) and one from Germany and Norway (*L. bipectinata* Sæther 1967). From the Palaearctic only *L. sphagnicola* is known as larva. The degree of specific differences among larvae of the genus is uncertain (Sæther and Andersen 2013), thus it is not possible to classify our record to any of the Palaeartic species.

Figure 2. Photograph of the head (a), mandible (b), mentum (c) and anal end with procerci (d) of the recorded *Lasiodiamesa* larva.

Ecology: Larvae of *Lasiodiamesa* live in bog waters and Sæther and Andersen (2013) state that in Central Europe immature stages are restricted to *Sphagnum* bogs. Interestingly, the site of our record in the Tatra Mts. is not a peat bog, however, it had slightly acidic water (pH 5.99), and we assume that due to its small size it is extremely prone to pH changes, especially during the spring snow thaw. The community composition with multiple aciodotolerant taxa such as *Zalutschia tatrica, Synendotendipes* sp. and *Tanytarsus* cf. *gregarius* confirm the acidic character of the surveyed pond and indicates that the typical habitat of *Lasiodiamesa* larva is a small acidic waterbody, however, not necessarily a bog.

Table 2. List and counts of taxa recorded in the study pond. PE refers to taxa recorded only as pupal exuviae.

Taxon name	**Abundance**
Hydracarina	
Hydracarina indet.	1
Heteroptera	
Sigara nigrolineata (Fieber, 1848)	4
Coleoptera	
Agabus sp.	3
Hydroporus melanarius Sturm, 1835	4
Hydroporus palustris (Linnaeus, 1761)	24
Hydroporus sp.	89
Trichoptera	
Limnephilus coenosus Curtis, 1834	6
Oligotricha striata (Linnaeus, 1758)	1
Diptera	
Procladius (Holotanypus) spp.	115
Zavrelimyia sp.	4
Diamesa sp.	5
Corynoneura scutellata group	27
Cricotopus (*Isocladius)* sp.	PE
Psectrocladius (s. str.) *octomaculatus* Wulker, 1956	109
Zalutschia tatrica (Pagast, 1935)	60
Micropsectra sp.	28
Synendotendipes sp.	11
Tanytarsus sp.	158
Tanytarsus cf. *gregarius* (Kieffer 1909)	PE
Lasiodiamesa sp.	1

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The 3rd Chinese Symposium on Chironomidae

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The 3rd Chinese Symposium on Chironomidae was held in Taizhou, Zhejiang Province, China organized by College of Life Sciences, Taizhou University, on November 8-11, 2019.

Thirty-five participants representing 11 institutions attended the symposium, including Huanggang Normal University, Nankai University, Jinan University, Shanghai Ocean University, South China Institute of Environmental Sciences, South-Central University for Nationalities, Taizhou University, Tianjin Agricultural University, Tianjin Normal University, Tianjin University of Science & Technology and The Geological Museum of China.

The participants shared recent research progress on Chironomidae or related field during the last two years after the 2nd symposium in Tianjin, China. Prof. Dr. Xinhua Wang, Nankai University, delivered a keynote speech entitled "The present and prospects of chironomid research in China". There were 16 oral presentations and 4 posters presented during the symposium. The scientific communication included several related research fields: morphological taxonomy, DNA barcoding, molecular systematics, zoogeography, ecology and environmental monitoring. We hope the Chinese Symposium will promote the cooperation among Chinese researchers and bridge with international colleagues.

Group photo of participants at the 3rd Chinese Symposium on Chironomidae held at Taizhou University, November 8-11, 2019.

CHIRONOMUS Journal of Chironomidae Research, No. 32, 2019: 84-85. News for the Chironomidae Community.

The 21st International Symposium on Chironomidae

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As requested by many participants during recent years, the next International Symposium on Chironomidae finally will be organized in Japan. The 21st edition of the symposium will be held in Tsukuba, expected between the 4th and the 10th of July, 2021, after the fever of the Tokyo 2020 Olympic Games has cooled down.

Tsukuba is a science city with a population of about 230 000, located approximately 50 km from Tokyo. A large number of national science institutes, including for example the Japanese Aerospace Agency (JAXA) and many private research companies, are settled in Tsukuba. For this reason, Tsukuba welcomes about 7,000 foreign residents, mostly researchers, giving the city an international ambiance.


Expo center in the middle of Tsukuba city. Photo: Richard Cornette.

The climate of Japan in summer is very hot and humid, harboring an exceptional biodiversity of insects, and of course chironomids! Thus, we have chosen to organize the symposium in early summer to avoid the hottest temperatures, which could be unpleasant to many participants. However, this period corresponds to the end of the rainy season and we should expect some rainy days during the symposium.

Tsukuba city is also famous for the twin peaks of mount Tsukuba, 877 m above sea level. Despite its low altitude, mount Tsukuba is one of the 100 most famous mountains in Japan. This is due to its sacred character since the antiquity, as for mount Fuji. The nature on mount Tsukuba, situated within the associated Shinto shrine, has always been protected and still hosts a remarkable fauna and flora.

Tsukuba is also near to lake Kasumigaura, the second largest lake in Japan. Lake Kasumigaura used to be a


Mount Tsukuba in spring. Photo: Richard Cornette.

brackish-water lagoon, but it was disconnected from Pacific Ocean and is now freshwater. Lake Kasumigaura is a place for massive swarms of Chironomids, such as *Chironomus plumosus* in the spring and formerly *Propsilocerus akamusi* in the autumn.

Traditional Japanese fishing boat on lake Kasumigaura. Photo: Richard Cornette.

Tsukuba's proximity to Tokyo facilitates the access to all touristic places around the Japanese capital. One day trips can be planned for example to the ancient and traditional city of Kamakura or to the world heritage shrines and temples of Nikko, which is also the gate for hiking in the mountains of Nikko national park.

We plan to organize the symposium through sessions focusing on different topics such as systematics, ecology, biomonitoring and recent advances in molecular biology. Tsukuba national museum of nature and science holds Manabu Sasa's collection of Chironomidae specimens, and the symposium could be an occasion to make this collection directly accessible to the expertise.

Tsukuba is located one hour from Tokyo-Narita international airport and about two hours from Tokyo-Haneda international airport by bus.

We will provide more information in the future, but please note the dates of the Symposium in your calendars.

The organizing staff is looking forward to welcome the community of researchers working on chironomids to Tsukuba. See you in 2021!

UNESCO World heritage Toshogu shrine in Nikko. Photo: Richard Cornette.