

**On the genus *Heleniella* Gowin, 1943 (*extrema*-gr.).
I. *H. gaultieri* sp. n., a crenophilous species confined
to glacial streams in Eastern Pyrenees
[Diptera, Chironomidae, Orthoclaadiinae]**

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Keywords: *Heleniella gaultieri* sp. n., Diptera Chironomidae, glacial springs and streams, Tech River (E-Pyrenees), conservation.

The male adult of *Heleniella gaultieri* sp. n. (= *H. sp. A*, in MOUBAYED-BREIL & LODS-CROZET 2016) is described based on material collected in some glacial springs and streams delimited by the upper basin of the River Tech (Eastern Pyrenees, France). The new species belongs to the *extrema*-group (recently emended by MOUBAYED-BREIL & LODS-CROZET 2016), which comprises two valid species (*H. extrema* Albu, 1972; known from Retezat Massif, Carpathians, Romania; *H. helvetica* Moubayed-Breil & Lods-Crozet, 2016; known from the Swiss Alps) and four undescribed taxa (*H. sp. 1*, *H. sp. 2*, *H. sp. A*, *H. sp. B*). Additional taxonomic notes on some taxa belonging to the *extrema*-gr include: illustrations of some parts of the male adult of *H. sp. 1* and *H. sp. 2*; male pupal exuviae of *H. sp. B* (morphotype, known from the Swiss Alps) is briefly described and figured. It apparently belongs to *H. helvetica* or *H. sp. 1*. The current description of *H. gaultieri* sp. n. increases the total number in the genus to three valid species from Eastern Pyrenees. Geographical distribution of the new species is restricted to some glacial springs and streams delimited by the protected area of the Nature Reserve of Prats-de-Mollo-la-Preste (altitude 1800-2200 m). Taxonomic remarks on related *Heleniella* species, key to known male adults of the *extrema*-group from Europe with comments on the ecology and geographical distribution of the new species are provided.

Sur le genre *Heleniella* Gowin, 1943 (gr. *extrema*). I. *H. gaultieri* sp. n., espèce crénophile confinée aux ruisseaux glaciaires des Pyrénées-Orientales [Diptera, Chironomidae, Orthoclaadiinae]

Mots-clés : *Heleniella gaultieri* sp. n., Diptera Chironomidae, sources et ruisseaux glaciaires, rivière Tech (Pyrénées-Orientales), conservation.

L'adulte mâle d'*Heleniella gaultieri* sp. n. (= *H. sp. A*, dans MOUBAYED-BREIL & LODS-CROZET 2016) est décrit à partir d'un matériel collecté dans des sources et ruisseaux glaciaires délimités par le bassin supérieur de la rivière Tech (Pyrénées-Orientales, France). La nouvelle espèce appartient au groupe *extrema* (récemment mis en évidence par MOUBAYED-BREIL & LODS-CROZET 2016) qui comprend deux espèces valides (*H. extrema* Albu, 1972; connue du Massif de Retezat, Carpates, Roumanie; *H. helvetica* Moubayed-Breil & Lods-Crozet, 2016; connue des Alpes Suisses) et quatre taxons encore non décrits (*H. sp. 1*, *H. sp. 2*, *H. sp. A*, *H. sp. B*). Des données taxonomiques supplémentaires sur des taxons apparentés au groupe *extrema* incluent: des illustrations de certaines parties des mâles d'*H. sp. 1* et *H. sp. 2*; une brève description de l'exuvie nymphale mâle d'*H. sp. B* (morphotype, connu des Alpes Suisses). La présente description de *H. gaultieri* sp. n. porte à trois le nombre d'espèces valides connues des Pyrénées-Orientales. Jusqu'à présent, *H. gaultieri*

sp. n. est exclusivement confinée aux sources et affluents glaciaires que couvre le bassin supérieur de la rivière Tech (Réserve Naturelle de Prats-de-Mollo-la-Preste, altitude 1800-2200 m). La position taxonomique avec une clé d'identification des adultes mâles d'espèces connues d'Europe (du groupe *extrema*) est commentée, tout comme l'écologie et la distribution géographique de la nouvelle espèce.

1. Introduction

The genus *Heleniella* Gowin, 1943 includes exclusively oxybiontic and rheophilic species mostly encountered in pristine mountain springs and streams. The knowledge provided on the taxonomy, geographical distribution and ecology of the known *Heleniella* species from Europe and the Palaearctic Region was documented by: BRUNDIN 1956, ALBU 1966, 1972, SERRA-TOSIO 1966, REISS 1968, RINGE 1976, CRANSTON et al. 1989, SERRA-TOSIO & LAVILLE 1996, ANDERSEN & WANG 1997, LANGTON & PINDER 2007, ASHE & O'CONNOR 2012, MOUBAYED-BREIL & ASHE 2016, SÆTHER & SPIES 2013, MOUBAYED-BREIL & LODS-CROZET 2016. The genus *Heleniella* comprises worldwide 12 valid species of which only five are reported from Europe: *H. extrema* Albu, 1972; *H. dorieri* Serra-Tosio, 1966; *H. helvetica* MOUBAYED-BREIL & LODS-CROZET, 2016; *H. ornatcollis* (Edwards, 1929); *H. serratosioi* Ringe, 1976.

Six taxa/species of the genus *Heleniella* Gowin, 1943 (*extrema*-group), are currently reported from glacial springs and streams located in high mountains of Europe: *H. extrema* (Retezat Massif, Carpathians, Romania); *H. helvetica*, *H. sp. 1* and *H. sp. B* (Swiss Alps); *H. sp. A* and *H. sp. 2* (Eastern Pyrenees: Carlit Massif and upper basin of the River Tech).

In this paper, the male adult of a new species, *Heleniella gaultieri* sp. n. (= *H. sp. A*, in MOUBAYED-BREIL & LODS-CROZET 2016), is diagnosed and described based on material collected in glacial springs and streams delimited by the upper basin of the River Tech (Nature Reserve of Prats-de-Mollo, altitude 1800-2200 m, E-Pyrenees). In addition, the single male pupal exuvia of *H. sp. B* (Swiss Alps) is briefly described, while some parts of the male adult of both *H. sp. 1* and *H. sp. 2* are illustrated. Currently, the genus *Heleniella* is represented by five taxa/species from continental France (SERRA-TOSIO & LAVILLE 1991): *H. dorieri*, *H. ornatcollis*, *H. serratosioi*, *H. sp. A* and *H. sp. 2*. The description of *H. gaultieri* sp. n. increases this number to six from this country.

2. Material and methods

The examined material was collected using some 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 imagines. Material of male adults were preserved in 80% ethanol, then cleared of musculature in 90% lactic acid (head, thorax, abdomen and anal segment) for about 60 to 80 minutes, which 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 (dorsally) of the type and paratype material, the hypopygium including the IXth tergum, the anal point, the gonocoxite and the gonostylus, were viewed ventrally and laterally to examine and draw from both sides all the necessary details of the species. In particular, the ventral view of hypopygium was illustrated when anal point and tergite IX were removed.

Part of the abdomen and the halteres of the male adult are preserved in 85% ethanol for an eventual DNA analysis. Morphological terminology and measurements follow those of SÆTHER (1980) and LANGTON & PINDER (2007) for the imagines, and LANGTON (1991) for pupal exuviae.

3. Descriptions

Heleniella gaultieri Moubayed-Breil, sp. n.

Material examined

Holotype. France, Eastern Pyrenees. 1 male adult, leg. P. Gaultier, glacial springs and streams, upper basin of the River Tech (Eastern Pyrenees), (42.428° N; 2.361° W); altitude 1800-2200 m, 17.VIII.2016.

Paratype (leg. J. Mb-Br). 1 male adult, same locality and data.

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

Etymology: the new species is named “*gaultieri*” in honour to Pascal Gaultier who remains active as a curator of the Nature Reserve of ‘Prats-de-Mollo-la-Preste’ in contributing to preserving the environment and species confined to aquatic habitats in this wonderful protected mountain area of Eastern Pyrenees.

Diagnostic characters

H. gaultieri sp. n. belongs to the *extrema*-group, which was recently emended by MOUBAYED-BREIL & LODS-CROZET (2016) based, in particular, on the unusual shape of the gonostylus, which is twisted and contorted with megaseta located medially on a cylindrical to cup-like projecting and retractable prominence. However, the new species is separated from other related species of the *extrema*-group by the following characters found in the male adult: coronals absent; temporals about 23 setae; anteprenotals 28-30 setae; postnotum with 8-9 setae placed proximally; halteres with 4 apical setae; tergite IX semi-circular, bearing a distinct hump clearly visible in lateral view, anal tergite bands present medially, posterior margin with about 26 setae in one row; anal point drop-like and constricted medially, with about 12-14 setae including 12 placed laterally (6 on each side of its base) and 2 dorsally; inferior volsella broadly triangular with a finger-like distal part, which is distinctly projecting downwards; phallapodeme distinctly spherical to hammer-like at base; virga about 120-125µm long, consists of 3 linearly elongated and separate rods; gonostylus slightly twisted and contorted, bearing only one long seta on posterior margin; megaseta inserted medially on a cylindrical prominence, which is retractable and orally projecting.

Male imago

(n = 2; Figs 1, 5, 10, 14, 18, 22-27, 29-30)

Big sized *Heleniella* species. Total length 2.40-2.45 mm. Wing length 1.85-1.90 mm. General colouration brownish to dark brown. Head, antenna, thorax and anal segment brown to dark brown; legs brownish; abdomen brown, anal segment dark brown.

Head (Fig. 1). Eyes pubescent, inner eye margin bare; Temporals consist of 23 setae including 21 inner and 2 outer verticals. Clypeus trapezoidal, with 17 setae in 4-5 rows. Palp 5-segmented;

first palpomere well developed; length (in μm) of palpomeres: 10, 50, 80, 95, 125; sensilla clavata present on distal part of palpomere 3. Antenna 13-segmented, about 940 μm long; antennal groove clearly visible, beginning on segments 3 and reaching ultimate flagellomere; last flagellomere 400 μm long, moderately clubbed apically, with sensilla chaetica apically; remaining segments 540 μm long; AR 0.74.

Thorax (Figs 5, 10, 14). All thoracic setae are decumbent except for the dorcentrals, which are arising from a distinct pale spots (Fig. 10); acrostichals absent; lobes of antepnotum gaping, antepnotum with 28-30 setae; dorsocentral area (Fig. 10) densely covered with setae (about 50 setae ending in one row); prealars 19-20 in 2-3 rows; preepisternals about 53; halteres (Fig. 14) 350 μm long, with 4 setae located apically in one row; scutellum densely covered with setae (up to 45 in 3-4 rows); postnotum (Fig. 5) with 8 setae (on each side). Halteres (Fig. 14) 350 μm long bearing 4 setae in 1 row, located apically on median area. Wing. Brachiolum with one seta; distribution of setae on veins: R, 7-8; R₁, 0; R₂₊₃, 5-6; R₄₊₅, 0; remaining veins bare; squama bare. Legs. Length (in μm) of tibial spurs: PI (80); PII (55, 40); PIII (65, 45); tibial spur of PI distinctly long, thin and projecting upwards apically; the low value of the BR ratio (1.30) for PI apparently represents a relevant distinguishing character. Length (μm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs:

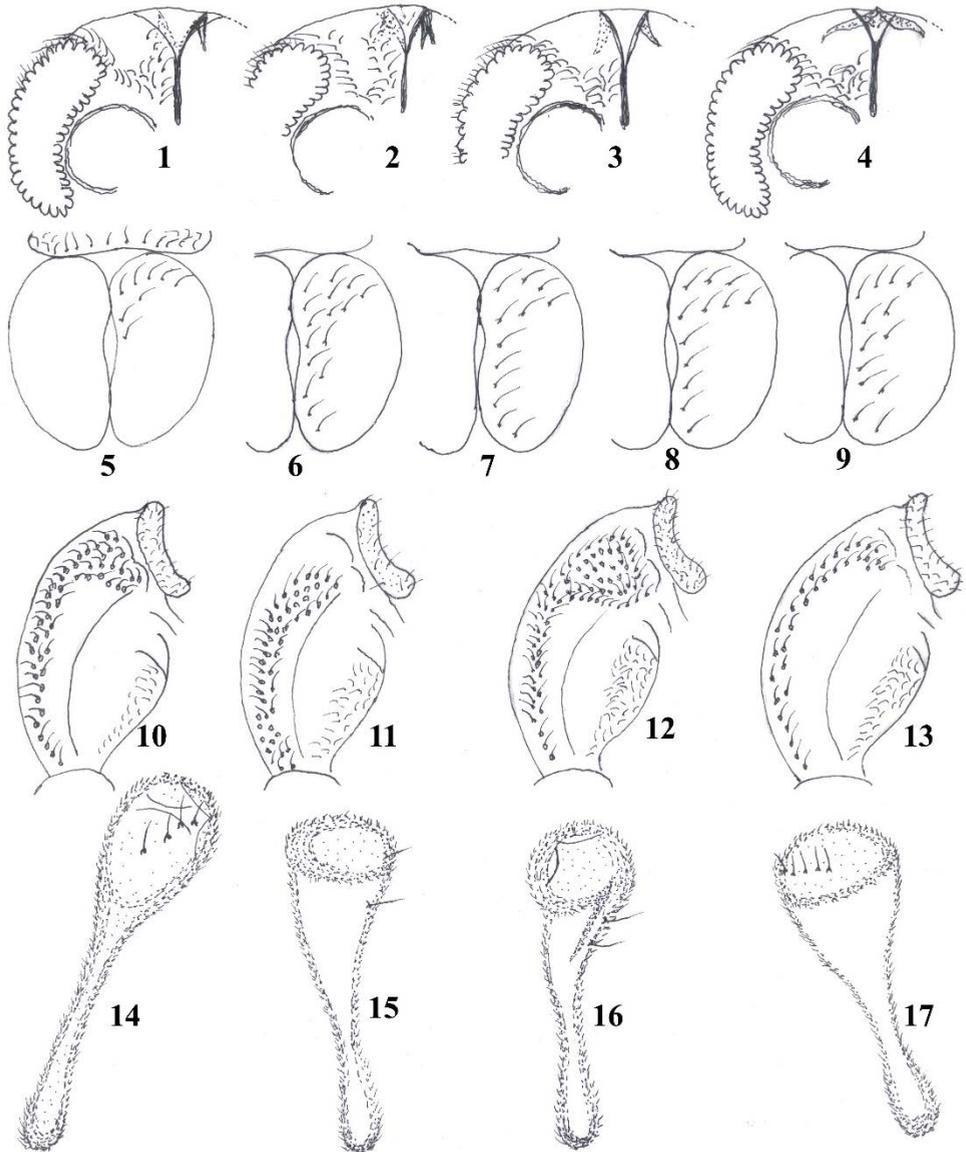
	fe	ti	ta₁	ta₂	ta₃	ta₄	ta₅	LR	BV	SV	BR
PI	695	805	640	270	185	110	100	0.80	3.22	2.34	1.30
PII	750	720	360	190	135	95	90	0.50	3.59	4.08	1.80
PIII	780	825	485	255	175	95	85	0.59	3.43	3.31	2.40

“LR = Length of tarsomere ta₁ divided by length of tibia (ti); BV = Combined length of femur (fe), tibia and ta₁ divided by combined length of tarsomeres ta₂-ta₅; SV = Ratio of femur plus tibia to tarsomere ta₁; BR = Ratio of longest seta of ta₁ divided by minimum width of ta₁, measured one third from apex.”

Abdomen. Hypopygium in dorsal, ventral and lateral view (Figs 18, 22-23, 29). Tergite IX semi-circular, median area with a characteristic anal tergite bands, posterior margin with one single row of setae (about 26) in one row. Anal point (Figs 18, 22, 29) 38-40 μm long, maximum width 40-45 μm at base, minimum width 15 μm at median part, drop-like, constricted medially, with about 12-14 setae including 12 placed laterally (6 on each side of its base) and 2 dorsally. Phallapodeme and sternapodeme as in Fig. 23; sternapodeme not orally produced, lateral sternapodeme slightly curved inwards basally; phallapodeme 70-80 μm long, broadly linear, spherical to hammer-like at base. Virga (Fig. 24) 125 μm long, nearly rod-like shaped, consists of 3 separate long rods. Gonocoxite (Figs 22-23, 29) about 205 μm long, rounded to nearly truncate apically. Inferior volsella 60-65 μm long, about 25 μm wide medially, sub-triangular with finger-like apex, densely covered with short setae. Gonostylus (Figs 25, 27, 29-30) in dorsal (Fig. 25), ventral (Fig. 26) and lateral view (Figs 29-30; 83-85 μm long, unusually twisted and contorted; posterior margin bearing 1 long seta clearly visible in obtuse angle; crista dorsalis absent; megaseta 18-20 μm long, inserted medially on a cylindrical to cup-like prominence, which is retractable and orally projecting.

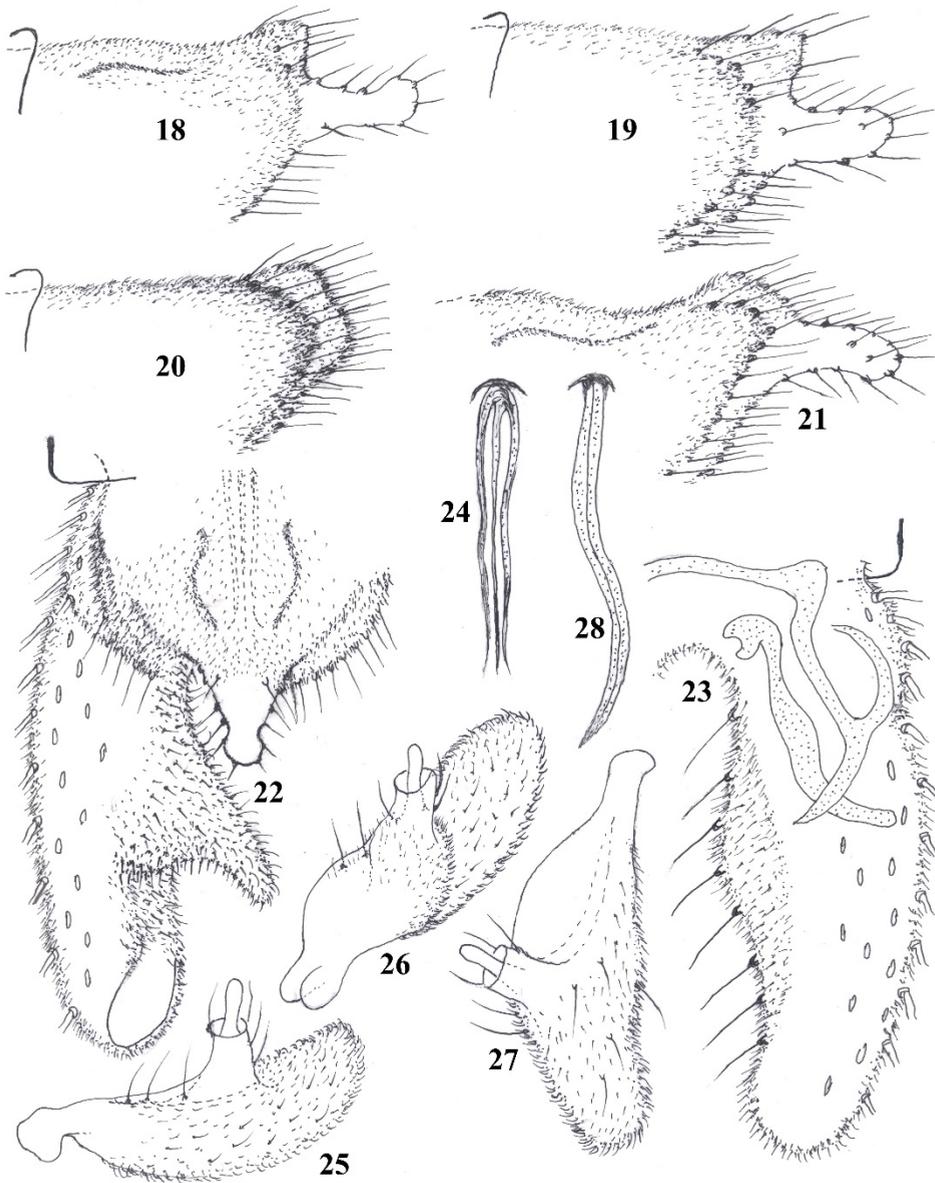
Larva

Unknown.



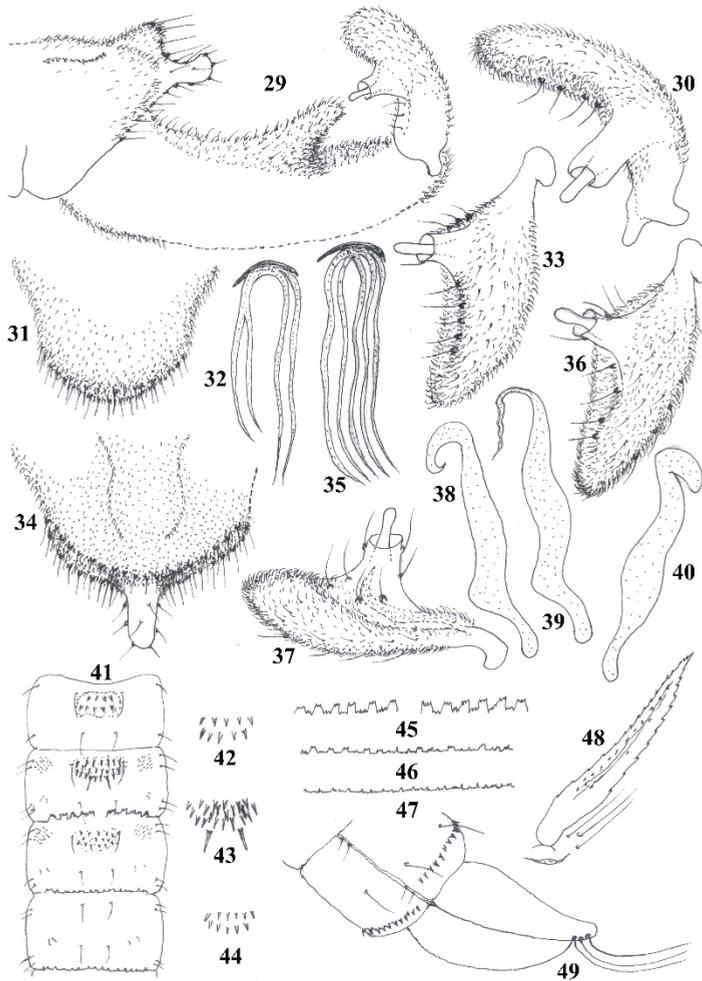
Figures 1-17. Male imago of *Heleniella* spp. Head of: *H. gaultieri* sp. n. (1); *H. helvetica* (2); *H. sp. 1* (Swiss Alps, 3); *H. sp. 2* (E-Pyrenees, 4). Distribution pattern of setae on postnotum of: *H. gaultieri* sp. n. (5); *H. helvetica* (6); *H. sp. 1* (7); *H. sp. 2* (8); *H. extrema* (9). Distribution pattern of dorsocentrals and prealars on thorax of: *H. gaultieri* sp. n. (10); *H. helvetica* (11); *H. sp. 1* (12); *H. sp. 2* (13). Halteres of: *H. gaultieri* sp. n. (14); *H. helvetica* (15); *H. sp. 1* (16); *H. sp. 2* (17).

Figures 1-17. Imago mâle d'*Heleniella* spp. Tête de: *H. gaultieri* sp. n. (1); *H. helvetica* (2); *H. sp. 1* (Alpes suisses, 3); *H. sp. 2* (4). Distribution des soies sur le postnotum de: *H. gaultieri* sp. n. (5); *H. helvetica* (6); *H. sp. 1* (7); *H. sp. 2* (8); *H. extrema* (9). Distribution des soies dorso-centrales et préalaires sur le thorax de: *H. gaultieri* sp. n. (10); *H. helvetica* (11); *H. sp. 1* (12); *H. sp. 2* (13). Balanciers de: *H. gaultieri* sp. n. (14); *H. helvetica* (15); *H. sp. 1* (16); *H. sp. 2* (17).



Figures 18-28. Male imago of *Heleniella* spp. Anal point, lateral view of: *H. gaultieri* sp. n. (18); *H. helvetica* (19); *H. sp. 1* (20); *H. sp. 2* (21). *H. gaultieri* sp. n.: hypopygium, dorsal (left, 22) and ventral (right, 23); virga (24); right gonostylus, dorsal (25); right gonostylus, ventral (acute angle, 26); left gonostylus, dorsal (obtuse angle, 27). *H. helvetica*: virga (28).

Figures 18-28. Imago mâle d'*Heleniella* spp. Pointe anale, vue latérale de: *H. gaultieri* sp. n. (18); *H. helvetica* (19); *H. sp. 1* (20); *H. sp. 2* (21). *H. gaultieri* sp. n.: hypopyge, vue dorsale (gauche, 22) et ventrale (droit, 23); virga (24); gonostyle, vue dorsale (25); gonostyle, vue ventrale (angle aigu, 26); gonostyle gauche en vue dorsale (angle obtus, 27). *H. helvetica*: virga (28).



Figures 29-49. Male imago and pupal exuviae of *Heleniella* spp. *H. gaultieri* sp. n.: hypopygium, dorsolateral (29); gonostylus, lateral (30). *H. sp. 1*: anal point, dorsal (31); virga (32); right gonostylus (obtuse angle, 33). *H. sp. 2*: anal point, dorsal (34); virga (35); right gonostylus (obtuse angle, 36); left gonostylus (37). Phallapodeme of: *H. sp. 1* (38); *H. sp. 2* (39); *H. helvetica* (40). Male pupal exuviae of *H. sp. B* (Swiss Alps): armament and chaetotaxy of sternites I-IV (41); patch of spines on sternites I-III (I, 42), (II, 43), (III, 44); armament of conjunctives: II/III (45); III/IV (46); IV/V (47); thoracic horn and precorneals (48); segment VIII and anal segment, lateral (49).

Figures 29-49. Imago mâle et exuvie nymphale d'*Heleniella* spp. *H. gaultieri* sp. n.: hypopyge, vue dorso-latérale (29); gonostyle, vue latérale (30). *H. sp. 1*: pointe anale, vue dorsale (31); virga (32); gonostyle droit (angle obtus, 33). *H. sp. 2*: pointe anale, vue dorsale (34); virga (35); gonostyle droit (angle obtus, 36); gonostyle gauche (37). Phallapodème de: *H. sp. 1* (38); *H. sp. 2* (39); *H. helvetica* (40). Exuvie nymphale mâle de *H. sp. B* (Swiss Alps): ornementation et chaetotaxie des sternites I-IV (41); groupe d'épines sur les sternites I-III (I, 42), (II, 43), (III, 44); ornementation des conjunctives: II/III (45); III/IV (46); IV/V (47); corne thoracique et soies précornéales (48); segment VIII et segment anal, vue latérale (49).

***Heleniella* sp. B**

Male pupal exuviae

(n = 1, Figs 41-49)

Material examined

Switzerland. 1 male pupal exuvia, 15.08.1999, Swiss Alps, Gletschboden stream, upper basin of the Rhône River, leg B. Lods-Crozet.

Colouration pale yellowish in general. Cephalothorax smooth without granulation; antennal sheath, thoracic horn and wing sheath pale to yellowish. Medium sized species, total length 2.70 mm. Thoracic horn (Fig. 48) 225 µm long, uniformly narrowing distally and toothed on both sides; precorneals 85-90, nearly sub-equal; dorsocentrals Dc₁-Dc₄ sub-equal (about 15µm) long. Tergites I-II bare; fields and rows of shagreen present on tergites III-VIII and sternites V-VIII; posterior transverse row of strong spines (mainly composed of pointed spines) present on: tergites II-VIII and sternites V-VIII. Distribution pattern of armament and chaetotaxy on sternites I-IV including that of conjunctives II/III, III/IV and IV/V as shown in Figs 41-44; anterolateral area of sternites II-III bearing a circular field of points; anteromedian area of sternites I-III with semi-circular patch of medium to big sized spines as illustrated in details in Figs 42-44; patch on sternite II consists of 20-23 long spines including 2 much stronger located posteriorly; patches on sternites I and III consist of 10-11 shorter spines. Armament of conjunctives II/III, III/IV and IV/V (Figs 45-47) is interrupted medially on II/III (Fig. 45) and continuous on III/IV and IV/V (Figs 46-47). Distribution pattern of posterior transverse row of spines on segment VIII and anal segment are figured in lateral view with dorsal side represented upwards (Fig. 49).

Male adult and larva

Unknown.

4. Taxonomic remarks

According to the atypical shape of the contorted gonostylus with megaseta located medially, *H. gaultieri* sp. n. keys directly into the *extrema*-group, which currently includes three valid species (*H. extrema*, *H. helvetica*, *H. gaultieri* sp. n.) and two additional undescribed taxa (*H. sp. 1*, known from glaciers located in N-Switzerland; *H. sp. 2*, collected in the Carlit Massif, Eastern Pyrenees). *H. helvetica* and *H. gaultieri* sp. n. can be considered as sister species in having the following common characters: an unusual gonostylus (MOUBAYED-BREIL & LODS-CROZET 2016: Figs 5, 8, 13-15 for *H. helvetica*; Figs 10, 22 for *H. extrema*); both species are confined to cold stenothermic high mountain streams with crystalline water. However, *H. gaultieri* sp. n. can be distinguished from other members of the *Heleniella* genus and in particular from related members of the *extrema*-group by a combination of differentiating characters: postnotum with 8 setae located in proximal half (Fig. 5), while bearing 11-18 setae occupying its entire surface in other members of the *extrema*-group (Figs 6-9); halteres 350 µm long (Fig. 14) bearing 4 apical setae located medially, are differently figured in *H. helvetica*, *H. sp. 1* and *H. sp. 2* (Figs 15-17); distribution pattern of setae on tergite IX and anal point (Figs 18, 22, 29); anal point drop-like and narrowed basally, while is broadly rectangular to sub-triangular at base and parallel-sided medially (MOUBAYED-BREIL & LODS-CROZET 2016, Figs 5, 11) in *H. helvetica*, and absent in *H. sp. 1* (Figs 20, 31); base of phallapodeme is spherical (Fig. 23), while is truncate in *H. helvetica* (Fig. 40) and

nearly sickle-like in both *H. sp. 1* and *H. sp. 2* (Figs 38-39); virga composed of 3 long separate rods (Fig. 24), is differently shaped in *H. helvetica*, *H. sp. 1* and *H. sp. 2* (Figs 28, 32, 35); posterior margin of gonostylus with one single long seta (Fig. 27), while is bearing 4-5 long setae in both *H. sp. 1* and *H. sp. 2* (Figs 33, 36-37). Moreover, *H. gaultieri* sp. n. can be easily separated from other related species based on the morphological features, which are summarized in the following key.

Key to male adults of known *Heleniella* taxa/species (*extrema*-gr.) from Europe

The key to male adults of known *Heleniella* (*extrema*-group) from Europe includes the following species and taxa/species: *H. extrema*, *H. gaultieri* sp. n., *H. helvetica*, *H. sp. 1* and *H. sp. 2*.

1. Gonostylus of typical *Heleniella* type, not twisted or contorted (ALBU 1966, Fig. 6; REISS 1968, Fig. 14; LANGTON & PINDER 2007, Fig. 161); apex projecting, nearly at a right angle; megaseta inserted apically *H. dorieri*, *H. ornaticollis*, *H. serratosioi*
 - Gonostylus of atypical *Heleniella* type, distinctly twisted and contorted (Figs 25-27, 29-30, 37); apex rounded and not projecting at a right angle; megaseta inserted medially on a distinct cylindrical median prominence 2
2. Inferior volsella triangular and weakly projecting; anal point triangular, narrowed distally with a nearly pointed apex (MOUBAYED-BREIL & LODS-CROZET 2016, Fig. 9); phallapodeme rounded and turned over at base (MOUBAYED-BREIL & LODS-CROZET 2016, Figs 10, 21); virga not illustrated in ALBU (1972) *H. extrema*
 - Inferior volsella finger-like shaped, strongly projecting and distinctly bent downwards (Figs 22, 29); anal point not triangular, broad at base, slightly narrowed distally with rounded apex (MOUBAYED-BREIL & LODS-CROZET 2016, Figs 5, 7-8, 11); virga consists of 2-3 or 4-5 long rod-like spines or composed of 4-5 curved long rods which are branched (Fig. 32) or not branched (Figs 24, 28, 35) 3
3. Virga linear or sinuous, sub-equal and not branched, composed of 2-3 long rod, which are fused or separate (Figs 24, 28, 35); postnotum with 8 setae, which are located proximally (Fig. 5); anal point present (Figs 22, 34); phallapodeme weakly sinuous, nearly linear and not pointed at base (Figs 23, 40) 4
 - Virga sinuous, branched or not branched, composed of 3-5 long sinuous rods, which are distinctly not fused; postnotum with 11-18 setae occupying its entire surface (Figs 6-9); anal point present or absent; phallapodeme markedly sinuous, and pointed at base (Figs 38-39) 5
4. Virga less than 130 μm long, composed of 3 long separate rods, sub-equal and linear; phallapodeme spherical to hammer-like at base (Fig. 23); posterior margin of gonostylus with only 1 long seta (Fig. 27); halteres 350 μm long, with 4 apical setae placed medially (Fig. 14) *H. gaultieri* sp. n.
 - Virga up to 150 μm long; composed of 2 long fused rods, sub-equal and sinuous (Fig. 28); phallapodeme truncate at base (Fig. 40); posterior margin of gonostylus with 4-5 long setae (Figs 33, 36-37); halteres 260 μm long, with 2 lateral setae placed apically (Fig. 15) *H. helvetica*
5. Anal point absent (Figs 20, 31); virga composed of 4 unequal sinuous and well separated rods including 2 fused and 2 branched rods (Fig. 32); phallapodeme truncate to crochet-like at

base (Fig. 38); posterior margin of gonostylus with 5 long setae (Fig. 33); halteres 280 μm long, with 2 lateral setae placed apically (Fig. 16) **H. sp. 1** (Swiss Alps)

- Anal point present (Figs 21, 34); virga composed of 5 subequal and linear rods (Fig. 35), which are not branched; phallapodeme distinctly narrowed and pointed at base (Fig. 39); posterior margin of gonostylus with 4 long setae (Figs 36-37); halteres 270 μm long, with 4 apical setae placed medially (Fig. 17) **H. sp. 2** (E-Pyrenees)

5. Ecology and geographical distribution

Male adults of *H. gaultieri* sp. n. were collected in some glacial helocrenes and streams delimited by the protected area of the Nature Reserve of Prats-de-Mollo (upper basin of the River Tech, E-Pyrenees). Environmental data of water recorded along the shady crenal and rhithral of the River Tech are: siliceous water, conductivity 40-50 $\mu\text{S}/\text{cm}$; pH 5.3-5.5; temperature 6-12 $^{\circ}\text{C}$. The discovery of *H. gaultieri* sp. n. in such preserved lotic habitats highlights the upper Tech catchment, which is considered as a remarkable microrefugia and extremely important hotspot of diversity. The new species is typically rheophilic and representative of glacial springs and cold stenothermic streams. It belongs to the crenobiontic and crenophilous community of species as documented by LINDEGAARD (1995).

Geographical distribution of *H. gaultieri* sp. n. is apparently restricted to the crenal and rhithral sections of the upper Tech River, and therefore can be expected to occur in other similar areas all around the Pyrenees. This indicates and reinforces the importance of pristine headwaters and cold enclaves in the preservation and persistence of autochthonous glacial relic species, which can be considered as biological indicators of the global warming and climate change in this biogeographical region.

Associated species encountered in the same localities with *H. gaultieri* sp. n. include: *Bo-reoheptagyia legeri* (Goetghebuer, 1933); *Diamesa aberrata* Lundbeck, 1898; *D. bertrami* Edwards, 1935; *D. bohemani* Goetghebuer, 1932; *D. cinerella* Meigen, 1835; *D. modesta* Serra-Tosio, 1968; *D. thomasi* Serra-Tosio, 1970; *D. veletensis* Serra-Tosio, 1971; *Pseudodiamesa branickii* (Nowicki, 1873); *P. nivosa* (Goetghebuer, 1928); *Pseudokiefferiella parva* (Edwards, 1932); *Syndiamesa edwardsi* Pagast, 1947; *S. hygropetrica* (Kieffer, 1909); *Bryophaenocladus subvernalis* (Edwards, 1929); *Chaetocladus guisseti* Moubayed, 2017; *C. laminatus* Brundin, 1947; *C. suecicus* (Kieffer, 1916); *Eukiefferiella fittkaui* Lehmann, 1972; *H. ornaticollis* (Edwards, 1929); *H. serratosioi* Ringe, 1976; *Krenosmittia boreoalpina* (Goetghebuer, 1944); *K. camptophleps* (Edwards, 1929); *Parametriocnemus boreoalpinus* Gowin & Thienemann, 1942; *Rheocricotopus effusus* (Walker, 1856); *R. sp. 1*; *Thienemannia gracilis* Kieffer, 1909; *T. valespira* Moubayed-Breil & Ashe, 2013; *Tvetenia bavarica* (Goetghebuer, 1934).

Acknowledgements

The authors are grateful to their colleagues P. Ashe, A. Thomas and M. Brulin for their constructive suggestions and editorial comments, which greatly improved the manuscript.

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