

Heleniella helvetica sp. n., a cold stenothermic species inhabiting the upper Rhône catchment in central Switzerland [Diptera, Chironomidae, Orthoclaadiinae]

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Abstract

A description of the male adult of *Heleniella helvetica* sp. n. is provided based on material collected in pristine lotic habitats delimited by the crenal and rhithral of the cold stenothermic springfed stream, which is located in the upper Rhône catchment (Mutt stream, central Switzerland, altitude 1800-2600 m). *H. extrema* Albu, 1972 represents the nearest species to *H. helvetica* sp. n. from which it can be separated by the following combination of characters: chaetotaxy of the thorax; low value of the BR ratio; distribution pattern of setae on tergite IX and anal point; anal point broad basally, parallel-sided medially and bearing dorsal setae; inferior volsella long nose-like lobe projecting downwards; gonostylus twisted, macroseta unusually inserted medially on a distinct cylindrical and projecting prominence. *H. extrema* and *H. helvetica* sp. n. currently belong to the emended *extrema*-group, which includes two additional new undescribed species both confined to glacier habitats: *H. sp.1*, known from the Mutt stream located in central Switzerland (altitude 2200-2400 m); *H. sp. A*, known from the Eastern Pyrenees (France, altitude 2200-2300 m). Taxonomic remarks, key to male adults of known *Heleniella* species from Europe and comments on the ecology and geographical distribution of the new species are given.

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Introduction

The genus *Heleniella* Gowin, 1943 includes exclusively rheophilic species mainly encountered in lotic habitats delimited by the upper and middle basins of cold mountain streams. Based on knowledge provided on the taxonomy, geographical distribution and ecology of the known *Heleniella* species from Europe and the Palaearctic Region (Brundin, 1956; Albu, 1966, 1972; Serra-Tosio, 1966; Reiss, 1968; Ringe, 1976; Rossaro, 1988; Coffman, 1986; Laville & Serra-Tosio, 1996; Andersen & Wang, 1997; Lods-Crozet *et al.*, 2001; Knispel *et al.*, 2003; Langton & Pinder, 2007; Ashe & O'connor, 2012; Lods-Crozet, 2012; Moubayed-Breil & Ashe, 2012; Sæther & Spies, 2013; Moubayed-Breil, unpublished data, 2016), the genus *Heleniella* comprises worldwide 11 valid species of which only four are reported from Europe: *H. extrema* Albu, 1972; *H. dorieri* Serra-Tosio, 1966; *ornaticollis* (Edwards, 1929); *H. serratosioi* Ringe, 1976. In this paper a description of the male adult of *H. helvetica* sp. n. is provided based on a material collected in the first major tributary (Mutt stream) of the upper Rhône River (central Switzerland). According to Lods-Crozet (1998), two *Heleniella* species (*H. ornatocollis* and *H. serratosioi*) were currently known from Switzerland. Consequently, the description of *H. helvetica* sp. n. currently increases the total number in the genus to 12 valid species worldwide and to three for this country. Additional material, collected between 1996 and 1998 in the upper area of the Mutt stream (altitude 2400-2600 m) and composed of male adults stored as slides or preserved in ethanol 85% of *Heleniella* species, revealed the presence of a fourth species of *Heleniella* (labelled as *H. sp. 1*), which enriched and updated our knowledge on this genus from central Switzerland.

H. extrema represents the nearest species to *H. helvetica* sp. n. The fact that the type material of *H. extrema* still not found and may be lost (M. Spies, pers. com.), the following taxonomic comparison is only based on the description and figures of the male adult provided in Albu (1972). The main common characters in the male adult of *H. helvetica* sp. n. and *H. extrema* are: tergite IX with circular margin; anal point sub-triangular to triangular; gonostylus twisted; megaseta inserted medially on a projecting prominence. However, the male adult of the new species can be separated from *H. extrema* and other related members of *Heleniella* genus by the following combination of characters: - wing length 1.65-1.85; chaetotaxy of the thorax; low value of the BR ratio (PI, 1.83; PII, 1.50; PIII, 1.60); - tergite IX lacking setae on its median area; - anal point broad basally, parallel-sided medially and bearing dorsal setae; phallapodeme broader basally and medially with a hammer-like base; - inferior volsella long nose-like shaped and strongly projecting downwards; gonostylus broad and distinctly twisted, macroseta inserted unusually on a distinct cylindrical and projecting prominence placed medially. *H. extrema* and *H. helvetica* sp. n. cur-

rently belong to the *extrema*-group, which is emended as well in this paper as in that of Moubayed-Breil (unpublished data, 2016) according to the unusual shape of the gonostylus. Moreover, the *extrema*-group includes two additional new undescribed species both confined as well to glaciers: *H. sp.1*, known from Muttbach Mountains located in C-Switzerland (altitude 2200-2400 m); *H. sp. A*, known from the Eastern Pyrenees (France, altitude 2200-2300 m).

Geographical distribution of the new described species is currently restricted to the upper Rhône catchment in the Mutt stream valley where the genus is reported for the first time up to 2000 m high. Morphological terminology and measurements follow Sæther (1980) for the imagines. Taxonomic remarks, key to male adults of known *Heleniella* species from Europe, discussion and comments on the ecology and geographical distribution of the new species are given.

Study sites and chironomid sampling

The Mutt stream (length: 3600 m) originates from the Mutt glacier (area 0.6 km², altitude 2582-3000 m a.s.l.) is the major tributary of the Rhône River in this upper catchment (46° 33'N, 8° 24'E). It joins the Rhône River at the upper limit of a floodplain and contributes approximately 10% (0.62 m³.s⁻¹ in 1997) to the Rhône discharge (Knispel & Castella, 2003). The upper parts of the Mutt catchment are composed of moraines and bare rocks. The dominant rocks are gneiss and amphibolites. Alpine grasslands are found in the lower parts while *Salix* spp. and *Alnus viridis* thickets, together with herbaceous alpine vegetation, are predominant in the floodplain (Lods-Crozet *et al.*, 2001).

Five sampling stations were located along the Mutt stream, extending from the glacier snout to the confluence with the Rhône along a gradient of altitude from 2600 to 1800 m a.s.l. Another station was located on a small springfed tributary at 2500 m a.s.l. The stations were investigated three times in 1996-97 during the three major annual hydrological phases: in June during snowmelt, in August during ice melt and in September at low water level. The field protocols followed closely those established in the EU-project 'AASER' (Brittain & Milner, 2001; Lods-Crozet *et al.*, 2001).

Ten sampling points were chosen randomly within each site. Depth, flow velocity and bed sediment composition were recorded at each of the 10 points before benthic sampling. The fauna was then collected into a 250-µm-mesh pond net at these points by kick sampling within a 30 x 30-cm area for 30 s. Adult material was collected using Malaise traps which were set up for a minimum of five days.

Material of male adult were cleared of musculature in 90% lactic acid (head, thorax, abdomen and anal segment) for about 60 to 80 min, which can be left overnight at room temperature without any detrimental effect or damage. The specimens were checked under a binocular microscope after 20 min in lactic acid to determine how the clearing was progressing. When clearing was complete the specimens were washed in two changes of 70% Ethanol to ensure that all traces of lactic acid were removed. Compared to clearing with potassium hydroxide, or other clearing solutions, no deterioration of the typical *original* structure is reported by using lactic acid. All examined material was mounted in polyvinyl lactophenol, remaining material including paratypes were preserved in 70% Ethanol. The eye on one side has been dissected from the head, which ensures that the hairs on the inner margin of eye are more clearly visible.

Before the final slide mountings (dorsally) of the type and paratype material, the hypopygium including the IX tergum, the anal point, the gonocoxite and the gonostylus, were viewed ventrally and laterally to examine and draw in both side all the necessary details of the species.

Description

Heleniella helvetica sp. n.

Material examined: Holotype. Switzerland. Upper catchment of the Rhône River, springfed stream (tributary of the Mutt stream), crenal, altitude 2600 m, 1 male adult, leg. B. Lods-Crozet, 17.09.1997.

Paratypes (all leg. B. L-C). Switzerland. Same locality as holotype and Mutt stream, 6 male adults, leg. B. Lods-Crozet, 19.09.1997.

Holotype (on 1 slide) (GBIFCH 00190363) and 2 male paratypes (mounted on 2 slides) (GBIFCH 00190364 + GBIFCH00190365) are deposited in the collections of the 'Musée cantonal de Zoologie, Palais de Rumine, 6 place de la Riponne, CH-1014 Lausanne, Switzerland. Remaining paratypes are deposited in the collection of the senior author's. Type material was preserved in 75% alcohol, and later mounted in polyvinyl lactophenol. For each adult, the head, thorax and abdomen were cleared in 90% lactic acid before mounting on slides.

Etymology: the new species is named *helvetica* after the name of 'Swiss confederation' given in Italian of the country of Switzerland, where the type material was collected.

Diagnostic characters

Adult male: Small sized species with a brownish general colouration. *H. helvetica* sp. n. closely resembles *H. extrema*. They are considered as sister species, which can be easily distinguished from all other members of the *Heleniella* genus in having an unusual twisted gonostylus, which is bearing a median prominence where is inserted the megaseta. However, the new species is separated from *H. extrema* and from the remaining *Heleniella* species by the following characters found in the male adult: - AR 0.50-0.51; thorax with 33-35 anteprenotals, 40-45 dorsocentrals, 24-26 prealars, 16-17 episternals, 45-50 preepisternals and 42-44 scutellars; wing length 1.65-1.85; low value of the BR (PI, 1.83; PII, 1.50; PIII, 1.60); - virga very long (145-150 µm long), consisting of 2 long filaments; - tergite IX with semicircular margin, which is bearing 10-12 setae (5-6 on each side of the anal point); anal point conical, broad at base and parallel-sided medially; - inferior volsella long nose-like, projecting and bent downwards; - gonostylus unusually twisted and contorted, truncate proximally, broader and sub-rectangular distally with a truncate apex, megaseta placed medially on a cylindrical to cup-like projecting prominence, crista dorsalis absent.

Male imago

(n = 5; Figures 1-3, 5-8, 11-15)

Small sized species. Total length 2.10-2.20 mm. Wing length 1.65-1.85 mm. General colouration brown to dark brown with blackish head; antenna brown to dark brown; thorax brown to dark brown; mesonotal stripes distinct; legs light brown to brown, tarsomere of PI, PII and PIII darker than remaining tarsomeres; abdomen brown to dark brown, anal segment dark brown.

Head (Figure 1). Eyes pubescent, inner eye margin bare; proximal part of eye enlarged, distal part narrowed downwards. Temporals consist of about 33-35 setae including 21-22 inner verticals and 10-11 postorbitals; distal area of the eye bare. Palp 5-segmented; first palpomere weakly developed; length (µm) of palpomeres 20, 50, 75, 85, 115; sensilla clavata present on distal part of third segment. Antenna 13-segmented, 750-760 µm long; sensilla chaetica present on segments 2-3 and 13; antennal groove indistinct, beginning on segments 4-5 and reaching ultimate flagellomere; last flagellomere nearly clubbed apically, 250-260 µm long, remaining segments 500-510 µm long; AR 0.50-0.51. Thorax (Figures 2-3). All thoracic setae are decumbent except for the dorsocentrals, which are arising from distinct pale spots. Acrostichals absent. Humeral pit as in Figure 2. Anteprenotum densely covered with setae (33-35), anteprenotal lobes in close contact; dorsocentral area densely

covered with setae (about 55 setae in 1-3 rows), prealars 24-27 in 2-3 rows, episternals about 45-50, preepisternals about 40-45. Scutellum with 44 setae placed in 3-4 rows rows. Wing. Brachiolum with 2 setae. Distribution of setae on veins: R, 13-14; R₁, 6-7; remaining veins bare. Squama bare. Legs. The low value of the BR ratio (1.50-1.83) apparently represents a consistent distinguishing character (PI, 1.83; PII, 1.50; PIII, 1.6). Tarsomere 5 of PI weakly shorter than tarsomere 4. Pulvilli apparently absent. Average length (μm) and proportions of legs:

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
PI580	690	375	245	155	105	95	95	0.54	2.74	1.79	1.83
PII665	615	290	170	125	83	92	92	0.47	4.34	4.41	1.50
PIII675	710	385	195	165	88	91	91	0.54	4.28	3.60	1.60

Abdomen. Hypopygium in dorsal, ventral and lateral view (Figures 5-8). Tergite IX broad and circular, anteromedian area bare, posterior area with 8-10 setae (4-5 placed on each side of the base of the anal point), posterior margin broadly expanded and bearing 10-12 setae (5-6 on each side of the anal point). Anal point (Figures 5, 7, 8, 11) 23-25 μm long, maximum width 20-21 μm at base, minimum width 9-11 μm at apex, broad at base and narrowed distally, apex rounded in both dorsal (Figures 5, 11) and lateral view (Figures 7-8); presence of 3 setae on dorsal side and 6 on lateral side (3 on each side). Phallapodeme and sternapodeme as in Figure 6. Sternapodeme arc-like orally produced, lateral sternapodeme nearly vertical; phallapodeme 90-95 μm long, broadly linear basally and medially, hammer-like at base. Virga (Figures 5, 12) 145-150 μm long, consists of 2 fused long filaments elongated

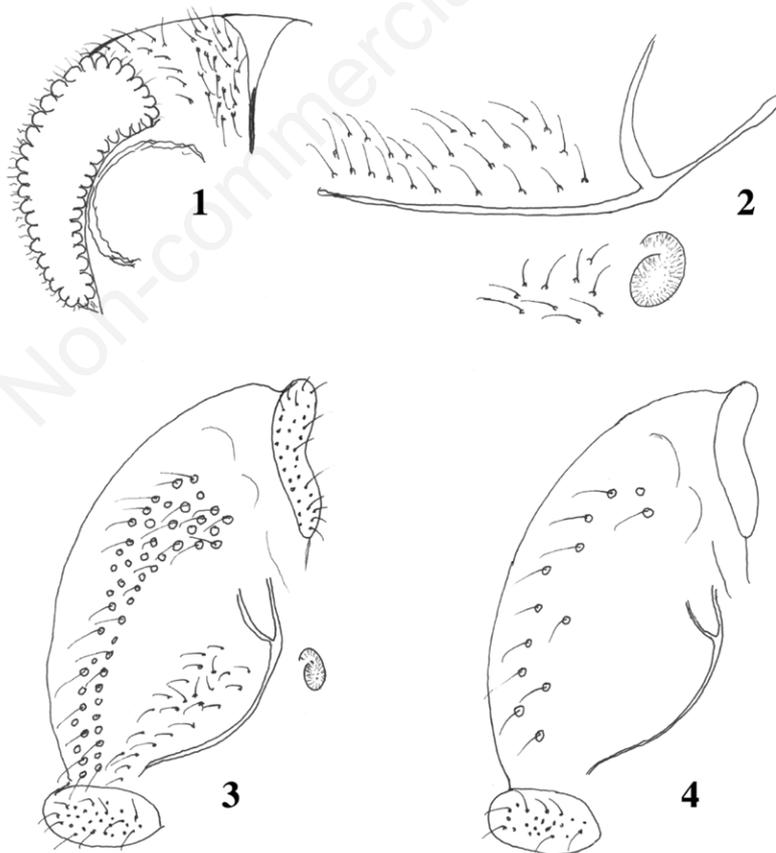
vertically. Gonocoxite 180-185 μm long, 45-50 μm wide, apex rounded to nearly truncate; basal margin sinuous, inferior volsella 75-80 μm long, maximum width 75 μm , minimum width 9 μm ; long nose-like lobe shaped, rounded apically, bearing 5-7 setae dorsally and ventrally on distal part. Gonostylus (Figures 5, 8, 13-15) 75 μm long, maximum width 30 μm , unusually shaped, twisted and contorted; crista dorsalis absent. Megaseta 18 μm long, inserted medially on a cylindrical to cup-like prominence projecting on the anteromedian part of the gonostylus; the prominence is about 23 μm high and probably retractable.

Larva: known but not described.

Taxonomic remarks

Male imaginal characters of *H. helvetica* sp. n. are nearly similar to those of *H. extrema*. These two species can be considered as sister species based on the following common characters: - both species are bearing an unusual gonostylus, which is twisted and characterized by the placement of the megaseta on its median part (Figures 5, 8 13-15 for *H. helvetica* sp.n.; Figures 10, 22 for *H. extrema*); - both species are confined to cold stenothermic high mountain streams (crystalline water for *H. helvetica* sp. n.; karstic water for *H. extrema*).

The *extrema*-group is emended here as well as in Moubayed-Breil (unpublished data, 2016) according to the unusual shape of the gonostylus, which is contorted and bearing the megaseta medially. This group currently includes *H. extrema*, *H. helvetica* sp. n. and two additional new undescribed species known from glaciers located in C-Switzerland (*H. sp. 1*) and the Eastern Pyrenees, France (*H. sp. A*). However, *H. helvetica* sp. n. is easily distinguished from other members



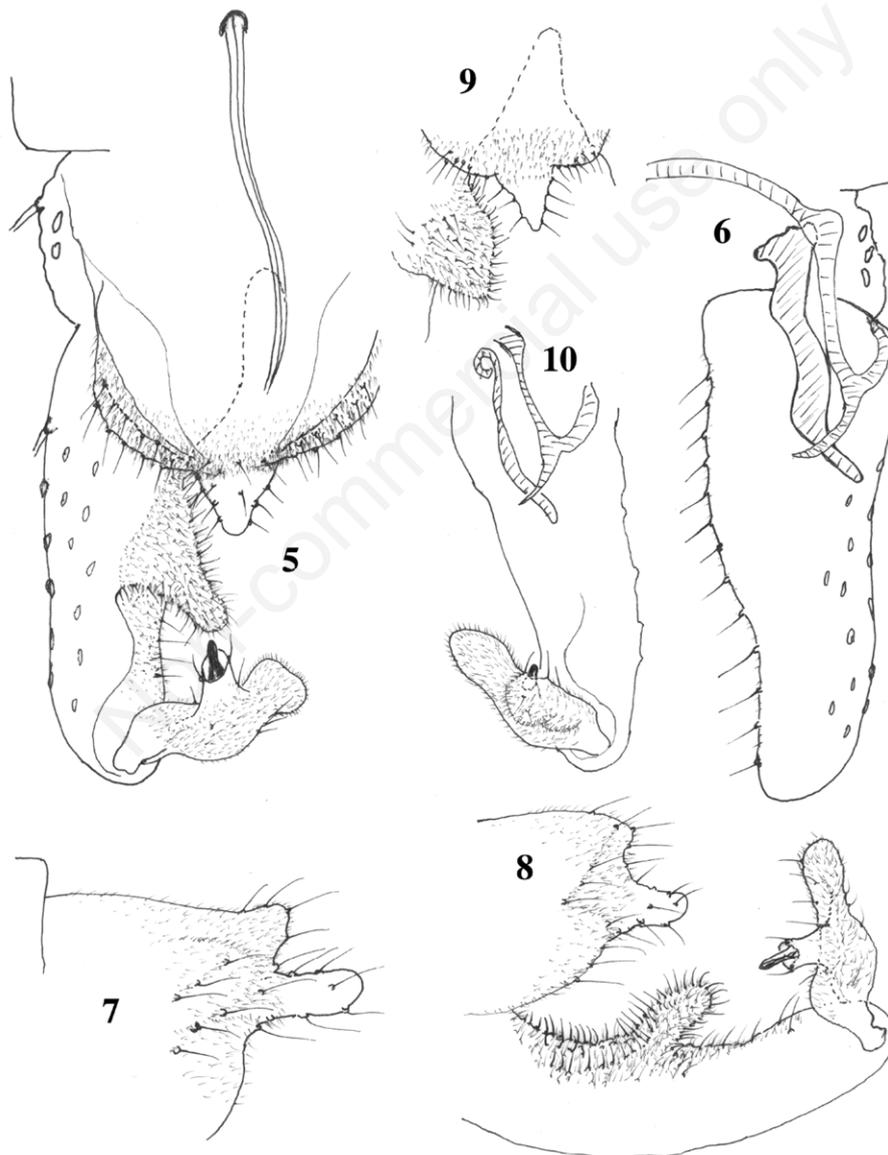
Figures 1-4. Male imago of *Heleniella* spp. *Heleniella helvetica* sp. n.: head, temporals (1); humeral pit with prealars and some episternals (2); thorax (3). *H. extrema*, thorax with dorsocentrals and scutellars, after Albu (1972, Figure 1) (4).

of the *Heleniella* genus and in particular from *H. extrema* by a combination of differentiating characters including: - AR 0.50-0.51; wing length 1.65-1.85; - distribution pattern of setae on tergite IX and anal point (Figures 5, 11), otherwise figured in *H. extrema* (Figure 9; Albu 1972, Figure 2); - anal point broadly rectangular at base and parallel-sided medially (Figures 5, 11), while it is triangular and narrowed in *H. extrema* (Figure 9) and absent in *H. sp. 1* (Figure 16); - phallapodeme broadly linear proximally and medially (Figure 16), thicker in *H. extrema* (Figure 10, 21) and sickle-like in *H. sp. 1* (Figure 19); - virga composed of 2 fused long filaments (Figures 5, 12), differently shaped in *H. sp. 1* (Figures 17-18); - inferior volsella long nose-like shaped and projecting downwards (Figures 5, 8, 15), broader and sub-rectangular in *H. extrema* (Figure 9); - gonostylus distinctly twisted (Figures 5, 8, 13-15) and bearing a cylindrical median prominence, differently figured in *H. extrema* (Figures 10, 22) and *H. sp. 1* (Figure 20).

Key to male adults of known *Heleniella* species from Europe

The following key on the male adults of known *Heleniella* species from Europe (*H. extrema*, *H. dorieri*, *H. ornatocollis*, *H. serratosioi*) is reproduced with more additional morphological details provided based on other new described species from continental France, Corsica and Lebanon (Moubayed-Breil, unpublished data, 2016).

1. Gonostylus of usual *Heleniella* type, linear, triangular or broadened distally; apex projecting, nearly right angle-like or rounded; megaseta inserted apically2
- Gonostylus unusually shaped, not of *Heleniella* type, distinctly twisted and contorted (Figures 5, 8, 10, 13-15, 22-23), bearing a distinct or indistinct cylindrical median prominence; megaseta inserted medially4
2. Inferior volsella triangular, narrowed apically and projecting down-



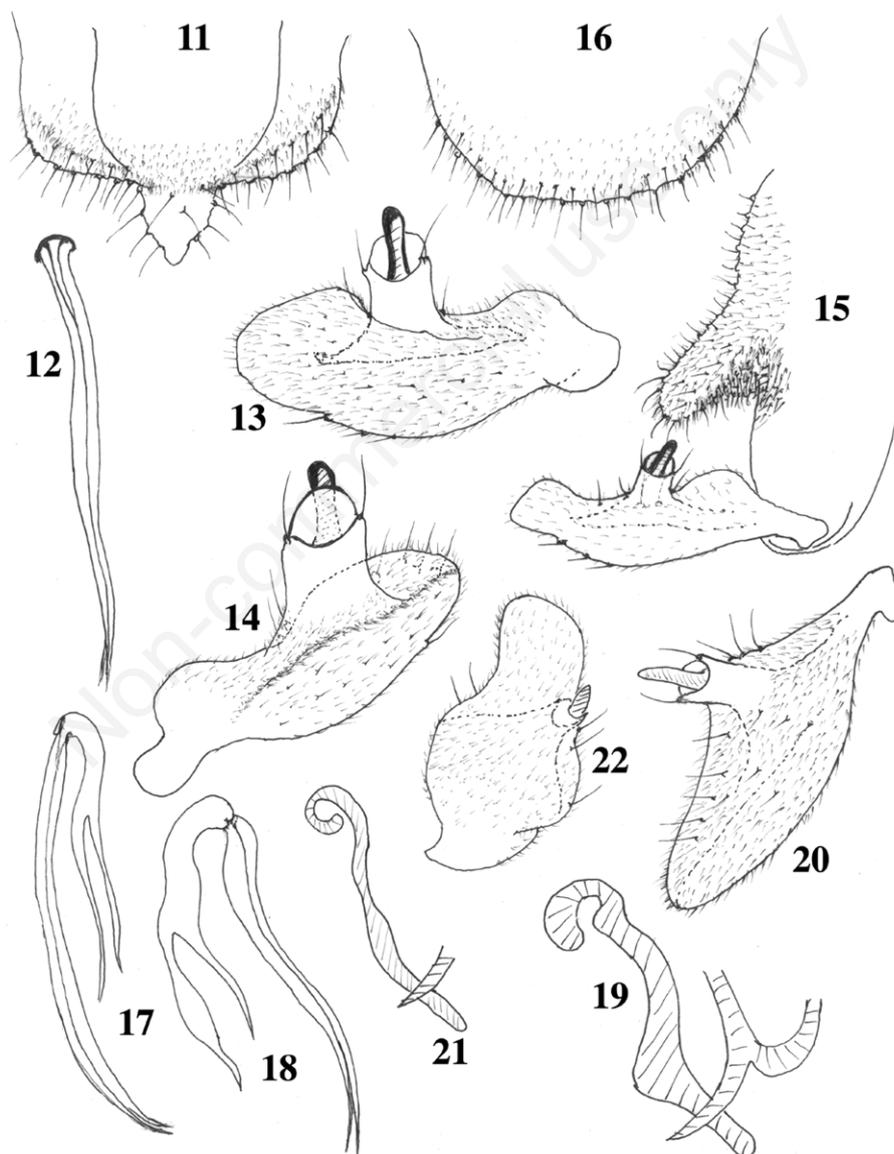
Figures 5-10. Male imago of *Heleniella* spp. *Heleniella helvetica* sp. n.: hypopygium, dorsal (5) and ventral (6); anal point in lateral view (7); anal point and gonocoxite in lateral view (8). *H. extrema*: anal point and inferior volsella, dorsal, after Albu (1972, Figure 2) (9); gonocoxite with sternapodeme, phallapodeme and right gonostylus, ventral, after Albu (1972, Figure 2) (10).

wards (Brundin, 1956, Fig. 107; Albu, 1966, Fig. 6; Reiss, 1968, Fig. 14; Ringe, 1976, Fig. 4; Langton & Pinder, 2007, Fig. 161A).....3
 - Inferior volsella rectangular, rounded apically and not projecting downwards; gonostylus linear with slender apex and not projecting; virga consists of 2 fused filaments extended vertically (Ringe, 1976, Fig. 1)*H. serratosioi* Ringe, 1976
 3. Gonostylus triangular, weakly projecting apically with a right angle to rounded apex; virga consists of 3 separated long filaments (Moubayed-Breil, unpublished data, 2016)
*H. ornaticollis* (Edwards, 1929)
 - Gonostylus not triangular, distinctly broadened distally, not projecting apically and ending with a rounded apex; virga unknown.....
*H. doriei* Serra-Tosio, 1966
 4. Inferior volsella triangular and weakly projecting (Figure 9); anal point triangular, narrowed distally with a nearly pointed apex (Figure 9); phal-

lapodeme rounded and turned over at base (Figures 10, 21); virga unknown*H. extrema* Albu, 1972
 - Inferior volsella nose-like shaped, strongly projecting and distinctly bent downwards (Figures 5, 8, 15); anal point not triangular, broad at base, slightly narrowed distally with rounded apex (Figures 5, 7-8, 11); phallopodeme hammer-like at base (Figure 6); virga 150 μ m long, consists of 2 fused long filaments (Figures 5, 12)*H. helvetica* sp. n.

Ecology and geographical distribution

Larvae of all members of the *Heleniella* genus are exclusively rheophilic and commonly encountered in lotic habitats delimited by the crenal and rhithral of cold streams. Male adults of *H. helvetica* sp. n. were collected in small groundwater resurgences and a springfed stream located in the upper basin of the Rhône River, Mutt stream catchment (central Switzerland, altitude 1800-2600 m). Localities



Figures 11-22. Male imago of *Heleniella* spp. *Heleniella helvetica* sp. n.: anal point and distribution pattern of setae on tergite IX (11); virga (12); right gonostylus, dorsal (13); left gonostylus, ventral (14); inferior volsella and gonostylus, dorsal (15). *H. sp. 1* (central Switzerland); tergite IX lacking anal point (16); virga, two aspects (17-18); phallopodeme (19); right gonostylus (20). *H. extrema*: phallopodeme, after Albu (1972, Figure 2) (21); left gonostylus, dorsal, after Albu (1972, Figure 2) (22).

where material was collected consist of moderate to weakly shaded pristine stenothermic stretches with cold mountain helocrenes and streams, including small waterfalls and riffles on rocky or sandy to gravely substrata. Bryocolous, hygropetric and madicolous habitats including waterfalls probably represent the most common and favoured aquatic areas for larval populations. Abiotic and biotic factors recorded along the crenal and rhithral waters are provided in Lods-Crozet (2012) as: - crystalline water characterized by a low value of the water conductivity (18-60 $\mu\text{S}/\text{cm}$); - cold stenothermic water temperature (4-10°C); - weakly shaded lotic habitats (waterfalls and riffles). The discovery of *H. helvetica* sp. n. in such preserved lotic habitats highlights the position of the headwaters in the upper Rhône catchment as a remarkable microrefugia, which is considered as an extremely important hotspot of diversity. The new species is typically rheophilic and representative of helocrenes and cold stenothermic streams. It belongs to the crenobiotic and crenophilous community of species as documented by Lindegaard (1995).

All members of the *extrema*-group including *H. extrema*, *H. helvetica* sp. n. and two additional new undescribed species (*H.* sp. 1, Switzerland; *H.* sp. A, France) are apparently confined to cold and stenothermic helocrenes and rhithral located in the vicinity of glaciers such those of the Mutt stream (crystalline water, C-Switzerland), Retezat Massif (karstic water, Carpathian Mountains, Rumania) and Carlit Massif (crystalline water, E-Pyrenees, France). The new described species is rare and sparsely distributed in the helocrenes and rhithral of some cold streams delimited by the upper Rhône catchment. In addition, the geographical distribution of *H. helvetica* sp. n. is apparently restricted to this area and therefore can be expected to occur in other similar geographic areas of the alpine mountains of Italy, Austria and France. This indicates and highlights the importance of pristine headwaters in the Alps (cold enclaves) in the preservation and persistence of autochthonous cold crenal relic species, which can be considered, in one hand as a relevant biogeographic representative and in another hand, as an eventual biological indicators of the global warming and climate change.

Chironomid species encountered in the same streams, and listed by Lods-Crozet (2012) include: *Krenopelopia binotata* (Wiedemann, 1817); *Boreoheptagyia alpicola* Serra-Tosio, 1989; *Diamesa aberrata* Lundbeck, 1898; *D. bohemani* Goetghebuer, 1932; *D. cinerella* Meigen, 1835; *D. goetghebueri* Pagast, 1947, *D. nowickiana* Kownacki & Kownacka, 1975, *D. vaillanti* Serra-Tosio, 1972; *Pseudodiamesa branickii* (Nowicki 1873), *Syndiamesa edwardsi* Pagast, 1947, *Bryophaenocladus femineus* (Edwards, 1929); *B. subvernalis* (Edwards 1929), *Chaetocladus laminatus* Brundin, 1947; *C. sueticus* (Kieffer 1916), *C. cf. longivirgatus* Stur & Spies, 2011; *Cricotopus lygropis* Edwards, 1929; *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; *Stilocladus montanus* Rossaro, 1979; *Thienemanniella gracilis* Kieffer 1909; *Thienemanniella caspersi* Sæther, 2003; *Tokunagaia rectangularis* (Goetghebuer, 1940); etc.

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