# *Limnophyes knispelae* sp. n. and *L. sartorii* sp. n., two new crenophilous species from the Swiss Alps (Chironomidae, Orthocladiinae)

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# Abstract

Limnophyes knispelae sp. n and L. sartorii sp. n. are diagnosed and described based on material collected in the high Alpine valley of the Rhône river and the cirque of Macun in the Swiss National Park (alt. 1800-2616 m a.s.l.). The first new species is described only as male adult, while the second as male adult and pupal exuviae. Main distinguishing characters found in the male adult of L. knispelae sp. n. L. sartorii sp. n. are: clypeus shield-like shaped; humeral pit typically circular with 3-4 or 9 lanceolate setae; virga strong spinelike; preepisternals vary from 4-6 to 4-8. The pupal exuviae of L. sartorii sp. n. is characterized by having frontal apotome domed; thoracic horn reduced to nose-like tubercle; posterior area of tergites II-VI with 1-7 rows of minute hooks; segment VIII with 5 lateral setae; inner posterior margin of anal lobe straight.

A combination of the latter relevant morphological characters has allowed us to consider each of the two new species as a local biogeographic representative of the Swiss Alps. Currently, the genus *Limnophyes* Eaton, 1875 is represented in Switzerland by 15 species. Consequently, the description of *L. knispelae* sp. n and *L. sartorii* sp. n. increases the total number in the genus to 17 known valid species from this country. Discussions and a differential diagnosis on the two new species are given, in which some morphological affinities and distinguishing characters with other related congeners are detailed and highlighted. Comments on the ecology and geographical distribution of the new species are also provided.

# Introduction

In general, larval populations of the genus *Limnophyes* Eaton, 1875 occur mainly in semiterrestrial, terrestrial to riparian habitats encountered in wetlands (pools, peat bogs, wet meadows) or in bordering parts of streams and rivers. On the ba-

sis of knowledge provided on the taxonomy, geographical distribution and ecology of the known *Limnophyes* species from Europe (Brundin 1947, 1956, Sæther 1975, Cranston 1979, Sæther 1980, Cranston et al. 1989, Sæther 1990, Langton 1991, Wang and Sæther 1993, Lindegaard 1995, Langton and Moubayed 2001, Langton and Pinder 2007, Moubayed-Breil and Ashe 2011, Moubayed-Breil 2013, Ashe and O'Connor, 2012, Sæther and Spies 2013), the genus comprises about 35 known valid species.

In this paper, two new *Limnophyes* species (*L. kn-ispelae* sp. n and *L. sartorii* sp. n.) are described on the basis of material recently collected in the Swiss Alps (high Alpine valley of the Rhône River and the cirque of Macun in the Swiss National Park, alt. 1800-2616 m a.s.l.). *L. knispelae* sp. n is described only as male adult, while *L. sartorii* sp. n. is described as both male adult and pupal exuviae.

## Methods and materials

Examined material of L. knispelae sp. n and L. sartorii sp. n. was collected using Malaise trap and drift nets. Male adults were preserved in 80-85% ethanol and cleared of musculature in 90% lactic acid (head, thorax, abdomen and anal segment) for about 60 to 80 minutes. The specimens were checked under a binocular microscope after 20 minutes to determine how the clearing was progressing. When clearing was complete, the specimens were washed in two baths 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 in dorsal view, the hypopygium including tergite IX and anal point, the gonocoxite and the gonostylus, were viewed ventrally and laterally, in order to examine and draw all the necessary details of the species, from both sides. For a better examination of the specific features of the hypopygium, the anal point and tergite IX were removed and the hypopygium was illustrated in a lateral view separately. Remaining part of the abdomen and the halters are preserved in 85% ethanol for an eventual DNA analysis. Morphological terminology, abbreviations and measurements follow those of Sæther (1980) and Langton and Pinder (2007) for the imagines and Langton (1991) for pupal exuviae.

## **Results and Descriptions**

## Limnophyes knispelae sp. n.

# https://zoobank.org/75D501E6-7BD7-4DDA-A32C-0D55ADB744A9

## Material examined

Holotype, Switzerland. 1 male adult, Malaise trap, leg. B. Lods-Crozet. Rhône River, upper stream (46°34'17.421" N, 8°22'49.460"E); alt. 1800 m a.s.l., 24.IX.1998. Environmental data of water (after Knispel and Castella 2003): crystalline water, conductivity range: 2.9-17.8  $\mu$ S/cm, mean: 10.5  $\mu$ S/cm; temperature (max 3.9 °C; mean, 2.3 °C).

Paratypes. 3 male adults, leg. B. Lods-Crozet, same date and locality as for holotype.

Holotype (mounted on 1 slide; GBIFCH00617929) and 1 paratype (mounted, each on 1 slide) are deposited in the collections of the Musée cantonal de Zoologie, Palais de Rumine, 6 place de la Riponne, CH-1014 Lausanne (MZL), Switzerland. 2 paratypes, male adults are deposited in the collection of the senior author.



Figure 1. Male adult of *Limnophyes knispelae* sp. n. A) clypeus; B) palpomere 3; C) lobes of antepronotum; D) humeral pit with dorsocentrals and prealars; E) humeral area with humeral pit; F-G) distribution pattern of preepisternals; H) tergite IX and anal point in lateral view. The arrows indicate some distinguishing characters.



Figure 2. Male adult of *Limnophyes knispelae* sp. n. A) hypopygium in dorsal view; B) apodemes; C-E) virga, three different aspects; F) gonostylus at acute angle; G) distal part of gonostylus; H) gonostylus at obtuse angle. The arrows indicate some distinguishing characters.

## Etymology

The name '*knispelae*' is given in honour to Sandra Knispel, who did her PhD thesis on an alpine glacier-fed alluvial system (Rhône River basin, Swiss Alps). She keeps working as consultant in aquatic ecology and is a Swiss specialist on Plecoptera.

## **Diagnostic characters**

Male adult of L. knispelae sp. n can be distinguished from some related congeners on the basis of the following combination of characters. Head. Temporals 6 (3 inner and 3 outer verticals); clypeus shield-like shaped, with 15 setae; palpomeres 3 with 2 sensilla clavata and 1 pin-like sensilla coeloconica; antenna 770 µm long, last flagellomere 320 µm long, AR 0.82. Thorax. Lobes of antepronotum not gaping, sinuous medially, antepronotals (6 dorsal, 2 median, only 3 lateral); acrostichals 4; dorsocentrals 17 including 11 decumbent setae located medially and 6 lanceolate setae (2 located proximally and 4 distally); humeral pit typically circular, basal part consists of a characteristic sclerotized crescent-like bearing 4 lanceolate humerals in 1 row; preepisternals 4-5 located on anterior

part. Wing, squama with 3 setae. Legs. Sensilla chaetica abundant on tarsomeres  $ta_1$ - $ta_5$  of PI-PIII, present only on apical part of tibiae. Abdomen. Tergite IX without hump. Anal point well-developed, downwardly bent. Virga consists of 3 stout spines, median one much longer. Transverse sternapodeme rounded, orally projecting; aedeagal lobe of phallapodeme slender. Gonocoxite with 2 characteristic setae on distal part. Inferior volsella triangular. Gonostylus linearly elongate, apex well-sclerotized and inwardly curved; anterior side well domed, densely covered with short setae; megaseta well developed, located pre-apically.

## Adult male

# (n = 4; Figs 1A-H, 2A-H)

Total length, TL 3.25 mm. Wing length, WL 1.55 mm; TL/WL = 2.10. General colouration pale brown to dark brown; head and antenna dark brown. Thorax brown with dark brown mesonotal stripes; humeral pit contrasting brown to dark brown. Legs brown. Abdomen brownish, anal segment brown to dark brown.

Head. Eyes bare, frontal tubercles absent; coro-

Table 1. Lengths (in µm) and proportions of leg segments of *Limnophyes knispelae* sp. n., adult male (n=1)

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV	BR
PI	565	675	330	215	190	95	85	0.49	0.68	3.76	1.60
PII	615	510	265	160	110	75	85	0.52	3.23	4.25	2.00
PIII	650	705	385	195	165	95	85	0.55	3.22	3.52	2.35

nals 4; temporals 5 including 3 inner and 2 outer verticals, postorbitals absent. Antenna 775  $\mu$ m long, last flagellomere about 300  $\mu$ m long, apex clubbed, apical seta absent, antennal groove reaching segment 3, AR 0.63. Clypeus (Fig. 1A) 100  $\mu$ m long, 140  $\mu$ m maximum width at base and 70  $\mu$ m at median part; typically shield-like shaped, smoothly rounded distally, apex rounded; with 15 setae in 3 rows. Palp 5-segmented; segments 1-2 fused; palpomeres 2-3 (Fig. 1B), palpomere 3 with 2 distal sensilla chaetica and 1 pin-like pre-apical sensilla coeloconica; length (in  $\mu$ m) of segments: 25, 35, 85, 75, 135; fourth segment shorter than the third.

Thorax. Lobes of antepronotum (Fig. 1C) not gaping, with sinuous edges; dorsal antepronotals 12 (6 on each side), median antepronotals 2, lateral antepronotals atypical (consist of 3 setae); acrostichals 6 in 1 row, starting at some distance from lobes of antepronotum. Half area of thorax with dorsocentrals as in Figs 1D-E; dorsocentrals 17 including 2 lanceolate setae (located proximally), 11 decumbent setae (located medially) and 4 lanceolate setae (located distally). Humeral pit typically circular (diameter, about 75 µm), with a characteristic crescent-like inner edge where 9-10 lanceolate humerals are located in 1 row. Prealars 7 in 1 row, located in 2 groups (4 proximal and 3 distal); supraalars (Figs 3G-K) vary from 4 to 5 setae located on anterior side; scutellum with 6 setae in 1 row.

Wing. Brachiolum with 1 seta; membrane with dense punctuation visible at 200X; subcosta overreaching the fork of radius, costal extension 40  $\mu$ m long. Distribution of setae on veins: R, 5-6, located on proximal part; remaining veins bare; squama with 3 setae.

Legs. Length (in  $\mu$ m) of tibial spurs: PI, 50; PII, 25; PIII, 55; pseudospurs absent. Only few sensilla chaetica present apically on tibia of PI-PIII, are abundant on tarsomeres ta<sub>1</sub>-ta<sub>5</sub>. Length (in  $\mu$ m) and proportions of leg segments as in Table 1.

Abdomen. Hypopygium in dorsal view as in Fig. 2A; apodemes as in Fig. 2B. Tergite IX (Figs 1H, lateral; Fig. 2A, dorsal) broadly rectangular, posterior margin rounded laterally; dorsal side lacking hump. Anal point (Fig. 1H, lateral; Fig. 2A,

dorsal) 30 µm long, 75 µm maximum width at base, distinctly semicircular, with 17-19 setae. Laterosternite IX with 10-12 setae (5-6 on each side). Sternapodeme and phallapodeme as in Fig. 2B; transverse sternapodeme rounded, orally projecting; phallapodeme slender, pointed basally. Virga (Figs 1C-E, 3 aspects) 30-35 µm long, composed of 3 unequal stout spines, median one much longer. Gonocoxite (Fig. 2A) 240 µm long, distal half with 2 stout inwardly projecting setae; apex broadly rounded. Superior volsella weaklydeveloped. Inferior volsella (Fig. 2A) triangular, apex rounded. Gonostylus (Figs 1F-H, 3 aspects) about 100 µm long, 15-18 µm maximum width, apex well-sclerotized, pointed and inwardly turned over; anterior side strongly swollen, densely covered with short setae; anterior area densely covered with short curved setae; crista dorsalis absent; megaseta well-developed. HV = 3.25; HR = 2.40.

Female adult, pupa and larva: unknown.

# Limnophyes sartorii sp. n.

# https://zoobank.org/34BA34DB-8A35-4FFD-B4FC-2593BC1DA575

# Material examined

Holotype, Switzerland. 1 male pharate adult, collected by drift net, leg. B. Lods-Crozet. Macun cirque (Swiss National Park), streamlet and rheocrenes, left shore of Immez Lake (46°43'39.678", 10°07'55.764"E); alt. 2616 m a.s.l.; 27.VII.2013.

Pupal exuviae: 1 male, same data and locality as for holotype.

Paratypes. 2 male adults (1 captured by Malaise trap and 1 by drift net as a male pupa), leg. B. Lods-Crozet. Same data and locality as for holo-type.

Environmental data from inlet of Immez Lake (Lods-Crozet et al. 2012; Robinson et al. 2016): crystalline water, conductivity 5.9  $\mu$ S/cm; temperature (min-max, 3.9-19.5 °C; mean, 11.6 °C), pH 6.7.

Holotype (mounted on 1 slide; GBIFCH00617930) and 1 paratype (mounted on 1 slide) is deposited in the collections of the 'Musée cantonal de Zoologie, Palais de Rumine, 6 place de la Riponne, CH-1014 Lausanne (MZL), Switzerland. 1 paratype, male adult, mounted on 1 slide is deposited in the collection of the senior author.

# Etymology

The name '*sartorii*' of the new species is given in honour to Michel Sartori, who is retired after being head director of the museum of Lausanne for more than 20 years. He keeps working as taxonomist on Ephemeroptera worldwide.

## **Diagnostic characters**

Male adult of *L. sartorii* sp. n is easily distinguished from all members of the genus *Limnophyes* by the shape and structure of its humeral area and distribution pattern of dorsocentrals. Additional differentiating characters are also

summarized as follows: antenna 770  $\mu$ m long, last flagellomere 320  $\mu$ m long, AR 0.82; clypeus shield-like shaped, with 15 setae; palpomeres 3 with 2 sensilla clavata and 1 pin-like sensilla coeloconica; lobes of antepronotum gaping, sinuous medially antepronotals (3 dorsal, 2 median, only 3 lateral); acrostichals 6; dorsocentrals 25-27 including 10-11 decumbent setae located medially and 15-16 lanceolate setae (5-6 located proximally and 10 distally); humeral pit typically circular, basal part consists of a characteristic sclerotized crescent-like bearing 9 lanceolate humerals in 1 row; preepisternals vary from 4-7 setae located on anterior side. Wing, squama with 3-5 setae. Legs. Sensilla chaetica abundant on tarsomeres ta<sub>1</sub>-ta<sub>5</sub> of



Figure 3. Male adult of *Limnophyes sartorii* sp. n. A) clypeus; B-C) palpomere 3 and sensilla coeloconica; D) lobes of antepronotum; E) humeral pit with dorsocentrals and prealars; F) humeral pit; G-J) distribution pattern of preepisternals; K) tergite IX and anal point in lateral view. The arrows indicate some distinguishing characters.

PI-PIII, present only on apical part of tibiae. Abdomen. Tergite IX broadly rectangular, with a low dorsal hump. Anal point semicircular, much larger at base, with 16-18 setae. Virga present, consists of 1 stout long spine. Transverse sternapodeme atypically straight; aedeagal lobe of phallapodeme typically enlarged. Gonocoxite with 3 distinct setae on distal part. Inferior volsella digitiform with 1 stout



Figure 4. Male adult and pupal exuviae of *Limnophyes sartorii* sp. n. Male adult: A) hypopygium in dorsal view; B) apodemes; C) virga, other aspect; E) gonostylus at acute angle; F) gonostylus at obtuse angle. Male pupal exuviae: G) frontal apotome; H) thoracic horn (reduced) and precorneals; I) dorsocentrals  $Dc_1-Dc_4$ ; J) segment VI in lateral view; K) details of caudal transverse rows of small hooklets; L) segment VIII and anal lobe in dorsal and ventral view; M) anal macroseta. The arrows indicate some distinguishing characters.

seta located proximally on a characteristic projecting expansion. Gonostylus narrowing distally, well domed medially and pointed apically; anterior side densely covered with setae; megaseta well developed, located pre-apically.

## Adult male

(n = 3; Figs 3A-K; 4A-F)

Total length, TL 3.45 mm. Wing length, WL 1.60 mm; TL/WL = 2.16. General colouration contrasting from pale brown to dark brown; head, thorax and antenna brown. Thorax brown with dark brown mesonotal stripes; humeral pit contrasting brown to dark brown, inner part distinctly dark brown. Legs uniformly brown. Abdomen brownish, anal segment contrasting brown to dark brown.

Head. Eyes bare, frontal tubercles absent; coronal setae 4; temporal setae 6 including 3 inner and 3 outer verticals, postorbitals absent. Antenna 770  $\mu$ m long, last flagellomere about 320  $\mu$ m long, apex clubbed, apical seta absent, antennal groove reaching segment 3, AR 0.82. Clypeus (Fig. 3A) 125  $\mu$ m long, 130  $\mu$ m maximum width at base, typically shield-like shaped, smoothly rounded distally, apex rounded; with 15 setae in 3 rows. Palp 5-segmented; segments 1-2 fused; palpomeres 2-3 (Figs. 3B-C), palpomere 3 with 2 sensilla chaetica and 1 characteristic spatulate pre-apical sensilla coeloconica; length (in  $\mu$ m) of segments: 25, 45, 80, 70, 125; segment 3 longer than the fourth.

Thorax. Lobes of antepronotum (Fig. 3D) slightly gaping, with sinuous edges; dorsal antepronotals 6 (3 on each side), median antepronotals 2, lateral antepronotals only 3; acrostichals 4 in 1 row, starting at some distance from antepronotum. Half area of thorax with dorsocentrals as in Figs 3E-F, dorsocentrals 25-27 including 5-6 lanceolate setae (located proximally), 10-11 decumbent setae (located medially) and 15-16 lanceolate setae (5-6 proximal, 10 distal). Humeral pit typically circular (diameter, about 70 µm), with a characteristic crescent-like inner edge with 9 lanceolate humerals in 1 row. Prealars 8 in 1 row. located in 2 groups (5 proximal and 3 distal); supraalars (Figs 3G-K) vary from 4 to 7 setae located on anterior side; scutellum with 6 setae in 1 row.

Wing. Brachiolum with 1 seta; membrane with dense punctuation visible at 200X; subcosta not

reaching the fork of radius, costal extension  $35-40 \mu m$  long. Distribution of setae on veins: R, 4-5, located on proximal part; remaining veins bare; squama with 3-5 setae.

Legs. Length (in  $\mu$ m) of tibial spurs: PI, 45; PII, 25; PIII, 50; pseudospurs absent. Only few sensilla chaetica present apically on tibia of PI-PIII, are abundant on tarsomeres ta<sub>1</sub>-ta<sub>5</sub>. Length (in  $\mu$ m) and proportions of leg segments as in Table 2.

Abdomen. Hypopygium in dorsal view as in Fig. 4A; apodemes as in Fig. 4B. Tergite IX (Figs 4A) broadly rectangular, lateral margins nearly vertical, posterior margin straight with rounded angles; dorsal side with a weak median hump as shown in lateral view (Fig. 3K). Anal point (Fig. 4A, dorsal; Fig. 3K, lateral) 30 µm long, 75 µm maximum width at base, semicircular, with 16-18 setae. Laterosternite IX with 12-14 setae (6-7 on each side). Sternapodeme and phallapodeme as in Fig. 4B; transverse sternapodeme atypically straight, lateral expansion well-developed (horn-like); phallapodeme with a large aedeagal lobe, projecting medially. Virga (Figs 4A, C) 15-20 µm long, composed of 1 cylindrical to an acute triangular stout spine. Gonocoxite (Figs 4A-D) 145 µm long, distal half with sclerotization and 3 stout inwardly projecting setae; apical part broadly rounded. Superior volsella weakly-developed. Inferior volsella (Figs 4A, D) about 25-30 µm long, 15 µm maximum width, consists of a digitiform lobe; proximal margin with a prominent small expansion bearing a curved characteristic seta. Gonostylus (Figs 4E-F, 2 aspects) 80 µm long, 5-7 µm maximum width, slightly narrowing distally, posterior part projecting with horn-like backward process clearly visible on Fig. 4F (obtuse angle); anterior and posterior margins sinuous, medially swollen; anterior area densely covered with short curved setae; crista dorsalis absent; megaseta robust and well-developed, occasionally bifid apically. HV = 4.44; HR = 1.81.

# Pupa

(n = 1, pharate male; Figs 4G-M)

Total length 3.55 mm. General colouration of exuviae as in other Limnophyes, almost entirely transparent, wing sheath and cephalothorax with slight yellowish to pale tinge, apophyses and genital sac

Table 2. Lengths (in  $\mu$ m) and proportions of leg segments of *Limnophyes sartorii* sp. n., adult male (n = 1)

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV	BR
PI	535	605	310	195	125	75	85	0.51	3.02	3.68	2.20
PII	520	535	250	140	105	75	80	0.47	3.26	4.22	2.40
PIII	565	620	355	185	160	75	85	0.57	3.05	3.34	2.00

#### distinctly brownish.

Cephalothorax. Frontal apotome (Fig. 4G) broadly triangular, frontal tubercles domed; frontal setae 35-40  $\mu$ m long, markedly thin. Antepronotals 3 including 1 median (95 70  $\mu$ m long) and 2 subequal lateral antepronotals 60-65  $\mu$ m long. Thoracic horn and precorneals (Fig. 4H), thoracic horn reduced, long lobe-like shaped, precorneals 50, 55 and 85 75  $\mu$ m long. Dorsocentrals (Fig. 4I) consist of 4 subequal setae 25-30  $\mu$ m long, inserted in 2 groups (3 proximal, 1 distal); Dc<sub>1</sub>, Dc<sub>2</sub> and Dc<sub>3</sub> located close together in one longitudinal row; distance between Dc<sub>1</sub>, Dc<sub>2</sub> and Dc<sub>3</sub> about 15  $\mu$ m, Dc<sub>3</sub> and Dc<sub>4</sub> are 45  $\mu$ m distant.

Abdomen. Tergite I bare. Tergites II-VIII densely covered with chagrin, spinulae and spinous points becoming progressively more elongated at the posterior margin of each tergite. Posterior transverse rows of small hooklets, restricted to tergites II-VI, consist of 1-7 rows of minute hooks (Fig. 4J) progressively increasing on tergites VI-VIII. About 50 to 75 long spines on posterior part II-VIII are longer medially and decreasing in size laterally; chaetotaxy of segments VI and VIII as in figures 4J and 4L. Paratergites II-VII armed with minute spines, are bare on tergite VIII (Fig. 4L). Sternites I-II/III bare; sternites III/IV-V with scattered anterior patch of spinulae located on median and lateral areas, which become more extensive on sternites VI-VIII. Lateral setae (LS) on segments I-VII: I (2), II-VII (4); segment VIII (Fig. 4L) with 5 LS including 1 long anteriorly and 4 little shorter caudally of 80-85  $\mu$ m long. Apophyses weaklydeveloped. Anal segment (Fig. 4L); anal lobe 210  $\mu$ m long, 200  $\mu$ m maximum width; much wider in proximal half, narrowing distally; outer caudal margin lobe-like, inner part typically straight. Genital sac (Fig. 4L) about 200  $\mu$ m long, overreaching tip of anal segment by 25-30  $\mu$ m; apex ending with a characteristic tubercle. Anal macrosetae (Fig. 4M) 210  $\mu$ m long, not curved apically.

#### Female adult and larva: unknown.

# Discussion

#### **Systematics**

A large material of Orthocladiinae, recently collected between 1996 and 2018 in Switzerland (mostly in the Swiss Alps (Lods-Crozet 2012, Lods-Crozet et al. 2001; Knispel and Castella 2003, Robinson et al. 2016) has been identified by the first author. This enabled us to generate a list of 15 species belonging to the genus Limnophyes Eaton, 1875 namely: *Limnophyes angelicae* Sæther, 1990; *L. asquamatus* Soegaard Andersen, 1937; *L. bidumus* Sæther, 1990; *L. brachytomus* (Kieffer, 1922); *L. difficilis* Brundin, 1947; *L. edwardsi* Sæther, 1990;



Figure 5. Type-locality of *Limnophyes knispelae* sp. n. at the upper basin of the Rhône river (Gletschboden, Central Swiss Alps, alt. 1800 m; photo J.L. Lods).

L. gelasinus Sæther, 1990; L. gurgicola (Edwards, 1929); L. habilis (Walker, 1856); L. minimus (Meigen, 1818); L. natalensis (Kieffer, 1914); L. ninae Sæther, 1975; L. pentaplastus (Kieffer, 1921); L. pumilio (Holmgren, 1869) and L. punctipennis (Goetghebuer, 1919). Consequently, the description of the two new described species increases the total number in the genus of known Limnophyes species from Switzerland from 15 to 17. A combination of some common morphological characters found in the male adult (clypeus shield-like shaped; humeral pit typically circular with 3-4 or 9 lanceolate setae; virga strong spine-like; preepisternals vary from 4-6 to 4-8) indicate that these two new species are related and separate from other Limnophyes. Although phylogenetic analyses should be conducted to confirm if species groups in Limnophyes are evolutionary coherent entities, we hypothesize that they form a group defined by a set of morphological characters.

Morphological differences between the male adult of the two new species and that of morphologically similar congeners (namely: *L. asquamatus*, *L. bidumus*, *L. cranstoni* and *L. difficilis*) are: An unusual shape of the humeral pit, which is atypically circular; atypical distribution pattern of dorsocentrals and humerals; the preepisternals on anterior side of preepisternum vary from 4 to 7.

The pupa of *L. sartorii* sp. n. can be separated from those of other members of the genus by having a reduced thoracic horn, which is markedly long lobe-like shaped; the morphological details of armament on tergites; and the characteristic shape of anal lobe and genital sac. However, since the pupa of *L. knispelae* is unknown it is at present not possible to determine if these are characters are shared between the two species.

# Ecology and geographical distribution

Several male adults of the two new described species were collected in riparian habitats bordering the upper and down basins of glacial springs and cold streams over the Swiss Alps. They belong to the crenophilous community of species as documented by Lindegaard (1995) and Lencioni et al. (2000). Enriched soil in humus, deciduous woods and bark trees on the margins of rivulets and streams, represent the most favourable microhabitats for larval populations. Emergence of adults is observed between July and October.



Figure 6. Type-locality of *Limnophyes sartorii* sp. n. at the inlet of the Immez Lake (Macun cirque, Eastern Alps, Swiss National Park, alt. 2616 m; photo J.L. Lods).

*L. knispelae* sp. n. and *L. sartorii* sp. n. belong to the Helvetico-Alpine elements, which are considered as local biogeographical representatives of the Swiss Alps. Their geographical distribution is currently restricted to their type-locality, which is delimited by: the Alpine streams in the high valley of the Rhône river (central Swiss Alps, alt. 1800 m, Fig. 5) for *L. knispelae* sp. n.; the Macun cirque (Swiss National Park, eastern Swiss Alps, alt. 2616 m, Fig. 6) for *L. sartorii* sp. n. Moreover, these two new species can be expected to occur in other similar mountainous areas situated in Switzerland and neighbouring countries (Italy, France, Germany, Austria, Poland).

Associated semiterrestrial species encountered with L. knispelae sp. n. and L. sartorii sp. n. include: Hydrosmittia brevicornis (Strenzke, 1950); H. oxoniana (Edwards, 1929); Limnophyes bidumus Sæther, 1990; L. difficilis Brundin, 1947; L. gelasinus Sæther, 1990; L. habilis Walker, 1856; L. pumilio (Holmgren, 1869); Pseudosmittia albipennis (Goetghebuer, 1921); P. angusta (Edwards, 1929); Smittia alpicola Rossaro & Lencioni, 2000; S. aterrima (Meigen, 1818); S. contingens Walker, 1856; S. edwardsi Goetghebuer, 1932 ; S. foliosa (Kieffer, 1921); S. leucopogon (Meigen, 1804); S. nudipennis (Goetghebuer, 1913); S. paranudipennis Brundin, 1947; S. pratorum (Goetghebuer, 1927); S. reissi Rossaro & Orendt, 2001.

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