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Morphological redescription and DNA barcoding of *Diamesa longipes* Goetghebuer, 1941 (Diptera: Chironomidae: Diamesinae) from the Swiss Alps

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Abstract

Morphological redescription of *Diamesa longipes* Goetghebuer with using syntype from the Ötztal Alps (Tyrol) as well as the adult male and pupa from the Zermatt valley (canton Valais) downstream of the Gornera glacier (Swiss Alps) is provided, and DNA barcoding for specimens from Swiss Alps is studied. Based on the morphology and DNA barcoding using 658-bp fragments of the mitochondrial cytochrome oxidase subunit I, indicate that *D. longipes* is closely related to *Diamesa zagrosica* Makarchenko et Semenchenko from Iran. Mean K2P interspecific distances between *D. longipes* and *D. zagrosica* were 4.72% which based on four species' delimitation approaches (BIN BOLD, ASAP, mPTP, GMYC), confirms that these are valid species. Comments on the geographical distribution and ecology of this rare species in the glacial streams of the Swiss Alps are also given.

Key words: Diptera, Chironomidae, Diamesinae, *Diamesa longipes*, syntype, redescription, morphology, DNA barcoding, Alpine mountains, Switzerland.

Introduction

High alpine regions are shaped by glaciers and their connected streams, leading to environments that are difficult to access and therefore little studied. Glacier-fed streams are typically cold, turbulent, turbid, unstable, and thus create harsh conditions for life. Among invertebrates, non-biting midges of the genus *Diamesa* Meigen, 1835 (Diptera, Chironomidae, Diamesinae) can represent up to 100% of the fauna in the uppermost sites of glacier-fed streams, called kryal habitats (Lods-Crozet *et al.* 2001; Lencioni & Rossaro 2005). The genus *Diamesa* is known to survive in extreme environments such as those characterized by freezing and scarcity of food (Moller-Pillot 2014).

In the Alps, at least 25 *Diamesa* species were recorded (Lencioni *et al.* 2021; Lods-Crozet 2024). Among this genus, members of the *Diamesa steinboecki* group are the best adapted and the first invertebrates to colonise the headwaters. Adult males of most species are characterised by the presence of short antennae with reduced setae of the plume, long legs, a very strong hypopygium with a typical internal skeleton, and strongly hairy eyes. The wings of males and females of some species in this group can be either normally developed or brachypteric (Makarchenko *et al.* 2022). In the Alpine region, the *D. steinboecki* group, with subgroups, includes 3 species: *Diamesa steinboecki* Goetghebuer, *D leona* Roback (=*D. starmachi* Kownacki *et* Kownacka), and *D. longipes* Goetghebuer (Lencioni *et al.* 2021; Makarchenko *et al.* 2022; Lods-Crozet 2024; Semenchenko *et al*. 2024). The first two species are quite common and well described, unlike *D. longipes*, which is very rare and was described by Goetghebuer (1941) from a single adult male collected in the Ötztal Alps (Tyrol), at an altitude of 2400 m above sea level. Later, this species was also redescribed from a single adult male by Kownacka & Kownacki (1975) from Saas Fee (canton Valais) in the Swiss Alps and included in the *D. longipes* subgroup of the *D. steinboecki* species group (Kownacki 1978; Makarchenko *et al.* 2022). In addition, this species was also reported from the Italian Alps, where it was found

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near Avio Lake (Adamello Mountains) at an altitude of 2000 m above sea level (Montagna *et al.* 2016; Rossaro *et al.* 2019). However, the description of the adult male of this species is not detailed enough and, in our opinion, requires a redescription, as well as a comparison with other species of the *D. longipes* subgroup. Here, we provide a morphological redescription of the male and pupa and DNA barcoding of *D. longipes* from the Zermatt valley (canton of Valais) downstream of the Gornera glacier. We also compare this species to other members of the *D. longipes* subgroup.

When redescribed the adult male of *D. longipes*, we used, as far as possible, the syntype of this species, which is deposited in the Royal Belgian Institute for Natural Sciences (Brussels) and is in poor condition (Figs 1–3).

Materials and methods

Adult males and pupae were collected in the Swiss Alps in 2023 and 2024, using kick and drift nets. The material was preserved in 96% ethanol for DNA analysis and in 80% ethanol for further study of morphology. The adult males and pupae were slide-mounted in Euparal. Adult male mounted on one slide (GBIFCH01217140) and 3 pupae are deposited in the collections of the Muséum cantonal des sciences naturelles, Naturéum, département de zoologie, 6 place de la Riponne, CH-1014 Lausanne, Switzerland. To clarify the species status of the adult male from the Swiss Alps, a syntype is deposited in the Royal Belgian Institute for Natural Sciences (Brussels) was used. The morphological terminology and abbreviations used below generally follow Sæther (1980).

Photographs were taken using an Axio Lab.A1 (Carl Zeiss) microscope with an AxioCam ERc5s digital camera and an Olympus SZX16 stereomicroscope with an Olympus DP74 digital camera, and then stacked using Helicon Focus software. The final illustrations were post-processed for contrast and brightness using Adobe® Photoshop® software.

Total genomic DNA was extracted using the BioSprint 96 extraction robot (Qiagen Inc., Hilden, Germany), following the supplier's instructions. The non-destructive protocol described in Vuataz *et al.* (2011), which enables post-extraction morphological study of specimens, was implemented. We then amplified a 658-bp fragment at the 5' end of the mitochondrial cytochrome c oxidase subunit I gene, corresponding to the standard animal barcode region, using the LCO1490 and HCO2198 primers (Folmer *et al.* 1994). Polymerase Chain Reaction (PCR) was conducted in a volume of 25 µl, consisting of 5 µl (unknown concentration) of template DNA, 1.3 µl (10 µM) of each primer, 0.2 µl (25 mM) of dNTP solution (Promega), 5 µl of 5X buffer (Promega) containing 7.5 mM of $MgCl_2$, 2.5 µl (25 mM) of additional $MgCl_2$, 0.5 µl (10 mg/ml) of BSA (Roche), 0.2 µl (0.2 µl of this polymerase was added because the concentration was 5 U/µL) (1U) of Taq polymerase (Promega), and 9.2 µl of sterile ddH_2O. (=25.2 µl I think there was exactly 9 µl of ddH_2O). Optimized PCR conditions included initial denaturation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min, with final extension at 72°C for 7 min. Purification of PCR products and automated sequencing were carried out in Microsynth (Balgach, Switzerland) using ABI3730XL (Applied Biosystems).

To reconstruct a phylogenetic tree and delimit species, we obtained a dataset including the two sequences obtained in this study, 17 specimens from Austria, Tyrol, belonging to the same BIN (BOLD:AER0117) as well as other members of the *D. longipes* subgroup (Semenchenko *et al.* 2024) including *D. zagrosica* Makarchenko *et* Semenchenko, *D. moubayedi* Makarchenko *et* Semenchenko, *D. sakartvella sakartvella* Kownacki *et* Kownacka, and *D. sakartvella gidanica* Makarchenko, Semenchenko *et* Palatov belonging to the. usedBarcodes from *D. caucasica* Kownacki *et* Kownacka were included as an outgroup. PartitionFinder 2.1.1 (Lanfear *et al.* 2012) was used to select the best-fit partitioning scheme and models separately for each codon position of protein-coding genes using the greedy algorithm with linked branch lengths for the corrected Bayesian Information Criterion as the optimality criterion for model selection. The best models for the first, second, and third codon position of COI were K80 (Kimura 1980), F81+I (Felsenstein 1981), and HKY+G (Hasegawa *et al.* 1985), respectively. Bayesian phylogenetic analyses were carried out using Markov Chain Monte Carlo (MCMC) randomization in MrBayes v3.2.7 (Ronquist *et al.* 2012). Four Markov chains (three heated chains, one cold) were run for 10 million generations, with the first 25% of sampled trees discarded as burn-in. Moreover, trace files of BI analysis were visually inspected in Tracer 1.7 (Rambaut *et al*. 2018), and then the tree was visualized in FigTree v. 1.4.4.

Species delimitation for the dataset was provided using distance-based approaches (BIN BOLD, ASAP) and tree-based approaches (mPTP and GMYC). The Barcode Index Number (BIN) employs varied distance metrics to

establish a persistent registry for life molecular operational taxonomic units in the Barcode of Life Data System (BOLD, www.boldsystems.org) (Ratnasingham & Hebert 2013). Assemble Species by Automatic Partitioning (ASAP) analysis was implemented on the website (https://bioinfo.mnhn.fr/abi/ public/asap/asapweb.html, Puillandre *et al.* 2021) with K2P distances. Tree-based approaches Multi-rate Poisson tree processes (mPTP, Kapli *et al.* 2017) and general mixed Yule-coalescent (GMYC, Fujisawa & Barraclough 2013) were run on the web servers (https://mptp.h-its.org/ and https://species.h-its.org/gmyc/) respectively using default parameters. The input ultrametric tree for GMYC was constructed using BEAST (Drummond *et al.* 2012). Settings were as follows: Strict clock, TN93+G nucleotide substitution model (Tamura & Nei 1993), Yule speciation process model (Gernhard 2008), and MCMC chain using 100 million generations. The obtained sequences of *D. longipes* from this study have been deposited in GenBank under numbers PX097363–PX097364.

FIGURES 1–3. Syntype of *Diamesa longipes* Goetghebuer, 1941. **1,** label; **2,** total view of adult male; **3,** hypopygium in dorsal view (photos by Tim Laebens and Camille Locatelli).

Taxonomy

Diamesa steinboecki group

Diamesa longipes subgroup

Diamesa longipes Goetghebuer
(Figs 4–10, 16, 20, 24)

Diamesa (*Brachydiamesa*) *longipes* Goetghebuer, 1941: 1.
Diamesa longipes Goetghebuer; Kownacka & Kownacki 1975: 39; Ashe & O'Connor 2009: 281; Montagna *et al.* 2016: 322.
[non] *Diamesa longipes* Tshernovskij, 1949: 105 (described by larva from Georgia).

Type material. Syntype: adult male, **Ötztal Alps (Tyrol)**, altitude 2400 m above sea level, 1940, leg. Heinz Janetschek. Deposited in Royal Belgian Institute of Natural Sciences, Brussels; N 18.073 (Figs 1–3).

Other material examined. 1 adult male, 2 pupal exuviae, **Switzerland**, canton of Valais, Matter Vispa catchment, Gornera stream, upstream of Zermatt village (GOR 2), altitude 1625 m a.s.l., 23.X.2024, 46.010092 N 7.739994 E, leg R. Bernard; 1 pupa (GBIFCH01217113) and 1 pupal exuvia, Gornera stream (GOR 1), 3 km upstream, altitude 2040 m a.s.l., 28.IX.2023 and 12.VIII.2024, 45.987456 N 7.733289 E, leg R. Bernard.

Description

Adult male (n = 1). Total length *ca* 2.2 mm. Total length/wing length 0.89.

Coloration. Dark brown to black. Head, thorax, and abdomen dark brown. Legs light brown to brown. Wings dark grey, with brownish veins.

Head (Fig. 4). Eyes hairy, reniform. Temporal setae including 8 frontals and orbitals, 12 verticals, and 8–9 postorbitals. Clypeus with 8 setae. Antenna with 8 flagellomeres and reduced plume of setae (Fig. 5); number and length of these setae on 1–7 flagellomeres respectively: 3 (20–24 µm), 2 (40 µm long), 2 (40–44 µm), 0, 2 (28–36 µm), 0, 0; terminal flagellomere with 5 setae, 72–88 µm long in basal part and with 1 subapical setae, 20 µm long. Length of 1–8 flagellomeres (µm): 84, 40, 40, 28, 28, 20, 16, 136; AR 0.53. Palpomere length (µm): 28, 64, 92, 84, 108. Palpomere 3 in distal part with sensilla capitata with diameter 20 µm. Head width/palpal length 1.09. Antennal length/palpal length 1.04.

Thorax. Antepronotum with 10 ventrolateral setae, 64 µm long. Dorsocentrals 11, 84–92 µm long; prealars 5, 72–84 µm long. Scutellum with *ca* 11 setae.

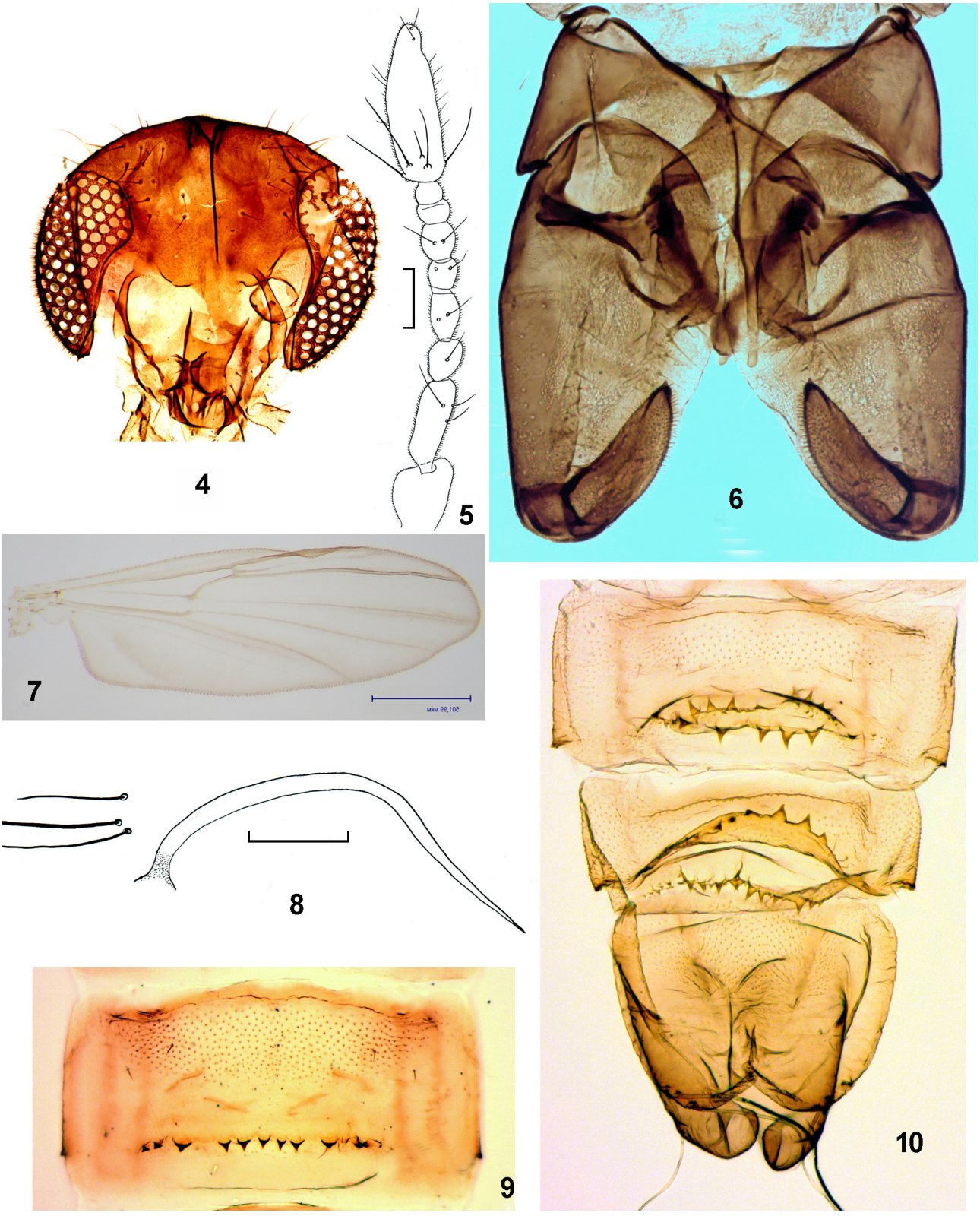
Wing (Fig. 7). Length 2.4 mm, width 0.72 mm. Anal lobe slightly reduced and angular. Squama with 7 setae, 40–60 µm long. R and R_1 with 20 setae, R_{4+5} with 3 setae (in distal part). RM/MCu 2.0–2.5.

Legs. Spur of front tibia 32 µm long. Spurs of mid tibia 28 µm long. Spurs of hind tibia 64 µm and 44 µm long. Hind tibial comb with 10 spines. Length (µm) and proportions of leg segments are as in Table 1.

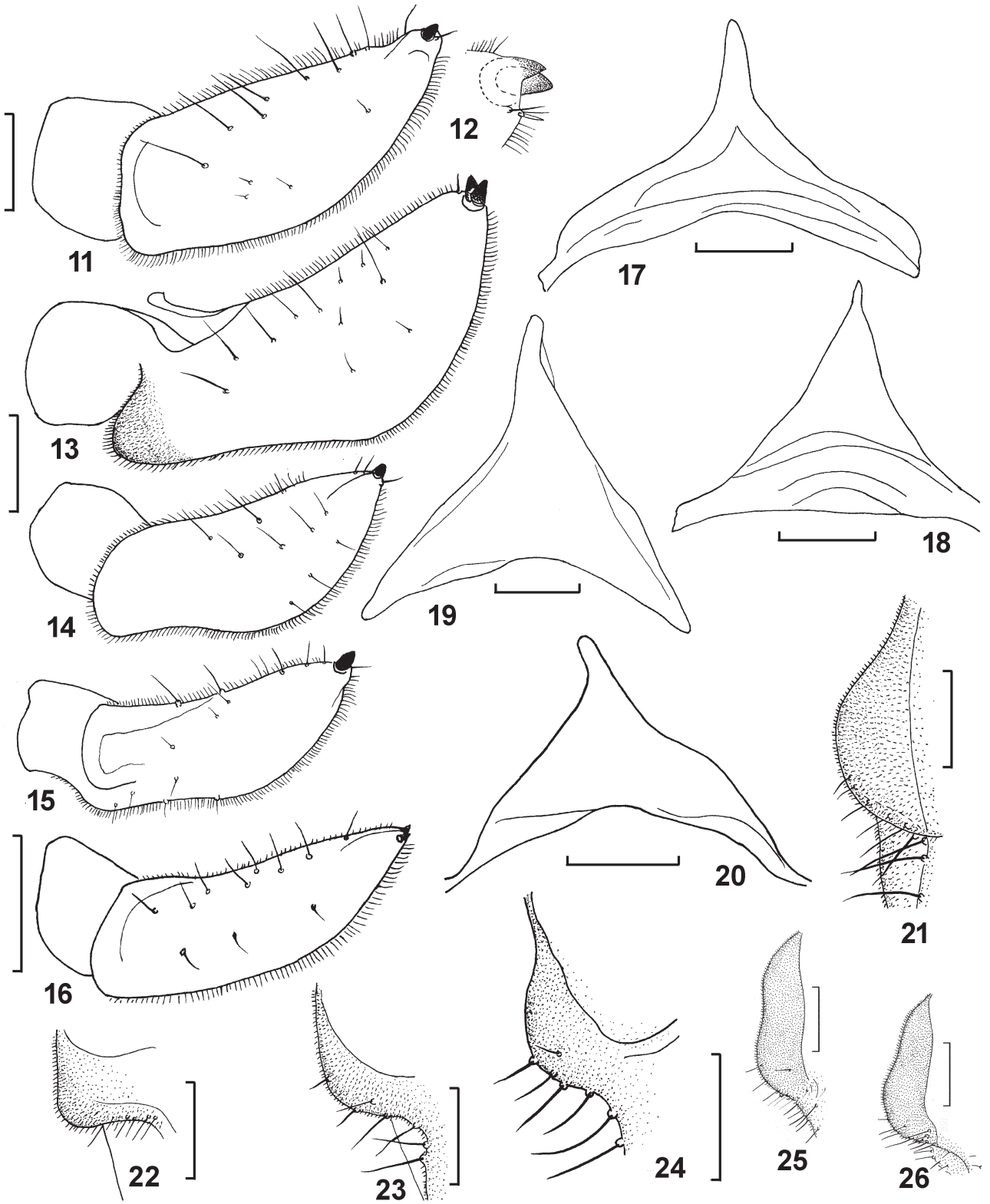
TABLE 1. Lengths (in µm) and proportions of leg segments of *Diamesa longipes* Goetghebuer, male (n = 1)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
P_1	1246	1017	804	394	213	82	98	0.79	3.90	2.81	1.0
P_2	1214	1066	476	246	148	82	98	0.45	4.80	4.79	0.9
P_3	1296	1246	853	443	213	82	98	0.68	4.06	2.98	1.1

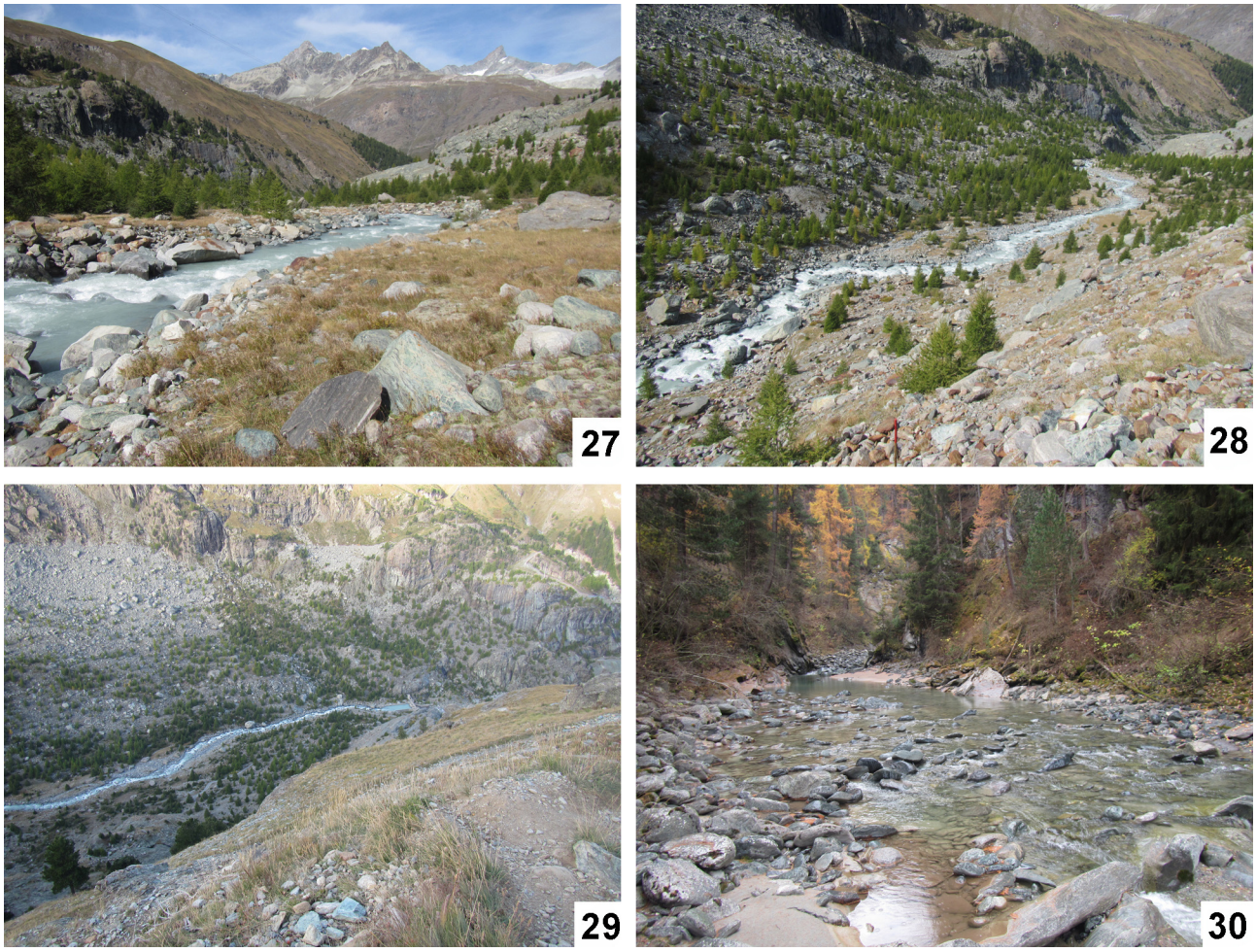
Hypopygium (Figs 6, 16, 20, 24). Tergite IX densely covered with strong macrotrichia apices of which are directed anteriorly, with 5–6 setae, 12 µm long (laterally setae longer) and with narrow (8 µm), weakly chitinized and naked anal point, 108 µm long (Fig. 6). Laterosternite IX with 5 setae, 12–16 µm long. Transverse sternapodeme (TSA) regular triangular, 76 µm high, 128 µm wide at the base (Fig. 20); TSA height/TSA width 0.59. Aedeagal lobe 140 µm long; phallapodeme sclerotized, 80–84 µm long. Gonocoxite 296 µm long; inferior volsellae rounded, along margin with 4–5 setae, 16–28 µm long (Fig. 24). Gonostylus 136 µm long, weakly curved, not expanded in distal half along the outer edge (Fig. 16); in apical part with megaseta in form of wide terminal spine, 8 µm long and tooth the same size, next to it there is two setae approximately of the same length. HR 2.18.



FIGURES 4–10. Adult male **(4–7)** and pupa **(8–10)** of *Diamesa longipes* Goetghebuer. **4,** head; **5,** antenna; **6,** hypopygium in dorsal view; **7,** wing; **8,** thoracic horn and precorneals; **9,** tergite III; **10,** tergites VII–VIII and anal segment. Scale bar: 50 µm.



FIGURES 11–26. Some details of hypopygium of *Diamesa sakartvella sakartvella* Kownacki *et* Kownacka **(11, 17, 21)**, *D. sakartvella gidanica* Makarchenko, Semenchenko *et* Palatov **(25–26)**, *D. moubayedi* Makarchenko *et* Semenchenko **(12–13, 19, 22)**, *D. zagrosica* Makarchenko *et* Semenchenko **(14–15, 18, 23)** and *D. longipes* Goetghebuer **(16, 20, 24)**. **11, 13–16,** gonostylus; **12,** apex of gonostylus; **17–20,** transverse sternapodeme; **21–26,** inferior volsellae. Scale bar: 50 μm. **Figs 11–15, 17–19, 21–23** after Makarchenko *et al.* 2022, **Figs 25–26** after Makarchenko *et al.* 2023.



FIGURES 27–30. Type localities of pupae **(27–29)** and adult male **(3)** of *Diamesa longipes* Goetghebuer. **27–29,** site GOR 1 upstream; **30,** site GOR 2 (photos by Régine Bernard.

Pupa (n=2), exuviae brownish. Total length *ca* 3.6 mm.

Cephalothorax. Frontal tubercles reduced, frontal apotome with 2 setae 200–232 µm long. Thorax slightly scaly in anterior part. Thoracic horn filiform, 168–224 µm long, brown at base, yellowish distally, approximately same width (12–14 µm) up to the middle, then gradually narrows and at top with some small teeth (Fig. 8). Three brown or dark brown precorneal setae anterior to thoracic horn: Pc_1 76–84 µm, Pc_2 124–126 µm, Pc_3 40–48 µm. Antepronotum with 2 median setae and 1 lateral antepronotal.

Abdomen. Length 2.5–2.6 mm. Tergite I without shagreen or teeth. Tergites II–VIII with shagreen in anterior third or half. Sternites I–II without shagreen, sternites III–VIII with sparse shagreen and IX without shagreen. Tergite I and sternites I–III without posterior transverse row of spines. Tergites II—VIII with posterior transverse row spines, number of these spines on these tergites: 12–14, 10–12, 9–11, 9–13, 8–10, 7–9, 8–9 (Figs 9–10). Number of posterior transverse row spines of sternites IV–VIII: 13–16, 11–12, 8–11, 9–11, 20–22 (Figs 9–10). The total number of spines of the anal rows of tergites 54–65 and sternites 61–72. Segment I with 2 pairs of lateral setae, 50–56 µm long; segments III–VIII with 4 pairs of lateral setae, 60–76 µm long (L_1–L_3) and 40–48 µm long (L_4). Segments II –VIII with spine-like process on posterolateral corners. Anal lobe with 3 yellowish anal macrosetae, 200–242 µm long, slightly curved in distal part and pointed. Male genital sac extended far beyond anal lobe (Fig. 10).

Remarks. The adult male of *D. longipes* is closely related to three species in the subgroup – *D. sakartvella sakartvella* and *D. sakartvella gidanica* from Caucasus, *D. zagrosica* from Iran, and *D. moubayedi* from Lebanon. All of these species have a similar hypopygium structure, namely the shape of the gonostylus, TSA, and IVo, but the male of *D. longipes* has the lowest AR (0.49–0.53), smaller number of dorsocentral setae of the mesonotum (8–11),

a short anal point on tergite IX (108 µm), shorter gonostylus length (136 µm), and higher ratio of the length of the gonostylus to its width is 3.1. By comparing the morphological characters of these species (Table 2 and Figs. 11–15, 17–19, 21–23), as well as the results of DNA barcoding, we conclude that *D. longipes* is most closely related to *D. zagrosica*.

TABLE 2. Comparison of morphological characters of some closely related species of *Diamesa longipes* subgroup (males).

Characters	*D. longipes* (n = 3), after Goethebuer 1941, Kownacka & Kownacki 1975, and our data	*D. sakartvella sakartvella* (n = 6), after Kownacki & Kownacka 1973, Makarchenko *et al.* 2022	*D. sakartvella gidanica* (n = 4), after Makarchenko *et al.* 2023	*D. zagrosica* (n = 5), after Makarchenko *et al*. 2022	*D. moubayedi* (n = 5), after Makarchenko *et al*. 2022
Total length, mm	2.2–3.0	2.9–4.8	2.1–2.2	2.3–2.8	3.7–4.1
TL/WL	0.89	1.04–1.19	0.92–0.97	0.89–0.90	1.13–1.23
Antennal length/ palpal length	1.04	1.0	1.0–1.1	0.94–0.97	0.88–0.97
AR	0.49–0.53	0.58–0.63	0.61–0.66	0.56-0.59	0.58–0.61
Wing length, mm	2.4	2.4–3.0	2.1–2.4	2.56–2.68	3.08–3.44
Dc	8–11	15–18	10–11	17–20, 2 rows in front	20
Pa	5	3–7	3	7–10	5
Sc	*ca* 11	10–12	8–10	*ca* 20	*ca* 20
LR_1	0.62–0.79	0.62–0.68	0.63–0.65	0.68–0.72	0.64–0.70
BV_1	3.9	4.10–4.35	4.07–4.28	3.65–3.83	3.78–4.41
SV_1	2.81	3.15–3.31	3.20–3.28	2.90–3.07	3.0–3.17
Number of setae on tergite IX	5–6	17–19	5–7	11–13	7–15
Anal point, µm	108	120–156	196–220	120–140	140–160
TSA shape	Regular triangular	Wide triangular	Wide triangular	Regular triangular	Regular triangular
Gonostylus length, µm	136	148–168	160–180	160–184	200–240
Gonostylus length/gonostylus width	3.1	2.4	2.6–3.0	2.6	2.7
TSA high, µm	76	68–92	48–76	84–108	104–140
TSA wide, µm	128	172–192	152	162–172	148–184
TSA high/TSA wide	0.59	0.36–0.50	0.32–0.50	0.49–0.67	0.80–0.89
IVo shape	Rounded	Rounded	Rounded and elongated	Rounded-angular	Angular
Aedeagal lobe length, µm	140	140	-	136–168	164–176
HR	2.18	2.02–2.10	1.75–1.93	2.15–2.26	2.14–2.23

Of the species listed above, the pupa is described only for *D. sakartvella* (Kownacki & Kovnacka 1973) but this description is uninformative and does not allow a detailed comparison with *D. longipes*. However, the pupa of *D.*

sakartvella is larger, its length is 5 mm, and there are 2 precorneal setae at the thoracic horn, whrease the length of the pupa of *D. longipes* is 2.2–3.0 mm and it has 3 precorneal setae.

Distribution and ecology. According to the data given in the catalogue of Ashe & O'Conner (2009), *D. longipes* is known from Austria, Italy, Moldova, and Switzerland. In our opinion, the distributional records of this species outside the Alps are unlikely. The indication of the species' location outside the Alps apparently is connected with the identification of *D. longipes* Tshernovskij described from a larva collected in a mountain stream near Bakuriani, Georgia (Tshernovskij 1949). This species is a junior primary homonym of *D. longipes* Goetghebuer. *D. longipes* Tshernovskij was included in his key to chironomid larvae (Tshernovskij 1949) and later it was included in a key to larvae by Pankratova (1970). Both books were then widely used for identifying chironomid larvae in faunistic and ecological studies, especially in the former Soviet Union and Eastern Europe. Hence, all identifications of larvae as "*D. longipes*" should be referred to *D. longipes* Tshernovskij nec Goetghebuer.

The location of *D. longipes* is also indicated for Iran (Namayandeh *et al.* 2021). However, the record is most likely to belong to *D. zagrosica,* which was discovered from the same region (Makarchenko *et al.* 2022).

The chironomid community in the Gornera stream, the collection habitat, is almost entirely composed and dominated by the following *Diamesa* species: *D. latitarsis* (Goetghebuer), *D. wuelkeri* Serra-Tosio, *D. bohemani* Goetghebuer, *D. zernyi* Edwards, *D. cinerella* Meigen, and *D. tonsa* (Haliday). In the upstream site at 2040 m a.s.l. (GOR 1) (Figs 27–29), the flow is naturally occurring (glacial hydrological regime) and the stream flows over serpentinite bedrock with most terrestrial vegetation typical of an alpine floodplain with larch (*Larix decidua*) and alpine grassland. During the time of sampling, the water was turbid, with conductivity of 293 µS/cm, a temperature of 2.2 °C, and flow rate of 0.75–1.50 m/s (23.X.2024), and. Environmental data of Gornera downstream sampling station at 1640 m a.s.l. (GOR 2) (Fig. 30) are: section subjected to water fluctuations due to upstream water abstraction (hydroelectric installation), crystalline water, conductivity 216 µS/cm; temperature 5.8 °C, flow rate 0.25 – 0.75 m/s, and surrounding vegetation consisting of coniferous forest with mainly larch, birch (*Betula pendula*), and pine (*Picea abies*).

The adult male of *D. longipes* was collected from stones and boulders in Gornera downstream (GOR2) with a driftnet. However, it could come from upstream sites where the pupae were collected. As a result of extensive spatio-temporal sampling efforts over the past 30 years in Swiss Alpine streams (Lods-Crozet *et al.* 2001; Alther *et al*. 2019; Robinson *et al.* 2024), *D. longipes* can now be considered rare and endemic to glacial rivers in the Alpine region. As indicated by Montagna *et al.* (2016), *D. longipes* is one of the rare species in the Alps and it is categorized as a cold-stenothermal species and threatened by global warming. The morphological and molecular study of the extended populations of the rare species, such that of *D. longipes* can greatly improve our understanding of their distribution and ecology, and efforts to conserve them.

Results of DNA barcoding

We have sequenced fragments of the cytochrome oxidase subunit I 658 bp in length, from two samples, adult male (GBIFCH01217140) and pupa (GBIFCH01217113) of *D. longipes***.** Both samples shared one haplotype, so intraspecific distances were zero. Search of the obtained sequences in the BOLD system revealed 17 additional conspecific samples from Austria (Tyrol, Pitze stream, 2000–2200 m above sea level, Dr. Steffen Pauls, pers. comm.) identified to the family level (larvae) (Fig. 31). Combining these sequences, the K2P intraspecific distances increased slightly to 0.04%, based on two synonymous transitions in the COI sequence of sample GBOS1563-22 from BOLD systems.

The average intraspecific pairwise K2P distances between *D. longipes* and three species and two subspecies from the *D. longipes* group – *D. zagrosica*, *D. moubayedi, D. sakartvella sakartvella,* and *D. sakartvella gidanica* were 4.72%, 7.77%, 7.15% and 7.58% respectively. This divergence corresponds to the species level for the genus *Diamesa* (Montagna *et al*. 2016; Makarchenko *et al*. 2023; Semenchenko & Makarchenko 2025). The results were confirmed by four species delimitation analyses, BOLD, ASAP, mPTP, and GMYC, that assigned *D. longipes* to a unique molecular operational taxonomic unit (Fig. 31).

Bayesian analyses confirm monophyly of *D. longipes* with high nodal support (Bayesian posterior probability, BPP = 1) (Fig. 31). The sister clade was *D. zagrosica* with high support (BPP = 1)

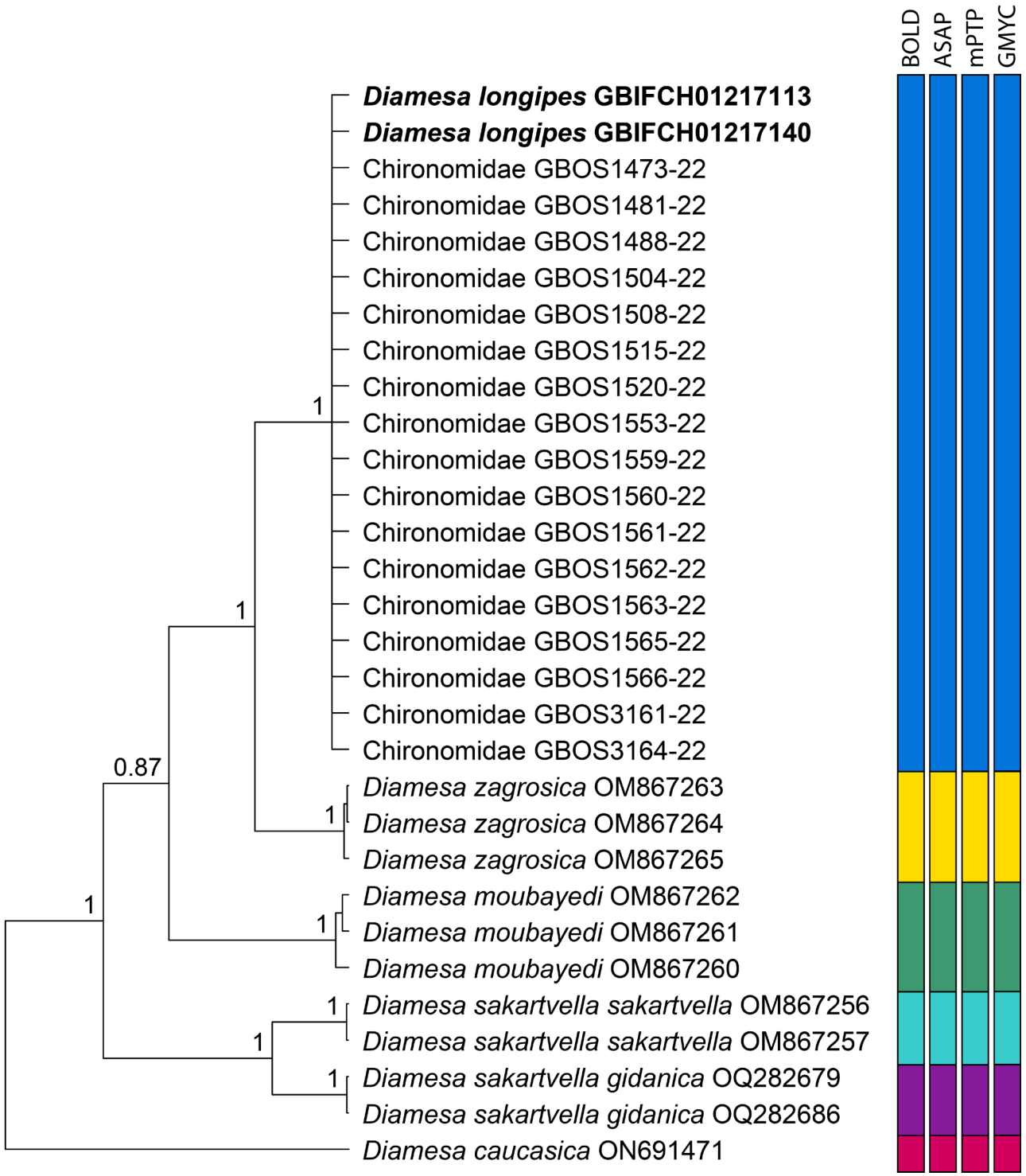


FIGURE 31. Ultrametric Bayesian inference (BI) tree based on the cytochrome c oxidase I (COI) nucleotide sequence data of the *Diamesa longipes* subgroup and *Diamesa caucasica* Kownacki *et* Kownacka as outgroup. Bayesian posterior probabilities (higher than 0.7) are given above the tree nodes. Specimens obtained in this study are in bold.

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