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Smittia solominae sp. nov. (Diptera: Chironomidae: Orthocladiinae), living on ice of high mountain glaciers of the Elbrus Region (North Caucasus)

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Abstract

Illustrated morphological description with a study of DNA barcoding and biology of adult male, pupa and larva of *Smittia solominae* **sp. nov.**, living on the ice surface of glaciers at an altitude of about 3000 m above sea level in the Elbrus region of the North Caucasus is given. DNA barcoding provided support that the new species unique within genus *Smittia.* The average interspecific distances between *S. solominae* **sp. nov.** and other *Smittia* from BOLD above 12% that correspond to species level.

Key words: Diptera, Chironomidae, Orthocladiinae, *Smittia,* new species, DNA barcoding, glaciers, North Caucasus

Introduction

The genus *Smittia* Holmgren, 1869 is represented in the world chironomid fauna at least by 83 valid species (Ashe & O'Connor 2012), most of which are terrestrial or semi-aquatic. Despite numerous publications (Brundin 1947; Caspers 1988; Rossaro 1988; Wang 1999; Rossaro & Delettre 1992; Rossaro & Lencioni 2000; Rossaro & Orendt 2001; Moller Pillot 2008; Caldwell & Jacobsen 2015; Moubayed-Breil & Mary 2021; etc.), the taxonomy and systematics of this genus is not sufficiently studied and its revision at the morphological and molecular genetics levels is necessary.

In this report we provide a morphological description with a study of DNA barcoding of adult male, pupa and larva of *Smittia solominae* **sp. nov.**, living on the ice surface of glaciers at an altitude of about 3000 m above sea level in the Elbrus region of the North Caucasus. Also, original data on the biology of the new species are given. It has been demonstrated that DNA barcodes have proven successful in species delimitation and life stage association in orthoclads (Silva & Wiedenbrug 2014; Prat *et al.* 2018; Makarchenko *et al.* 2017, 2018, 2019; Lin *et al.* 2020; etc.)

Materials and methods

Material was collected in Republic of Kabardino-Balkaria (North Caucasus) in July 2022 and July-August 2023 and preserved in 96% ethanol for DNA-analysis, in 70% ethanol for further study of morphology. The adult males, pupae and larvae were slide-mounted in polyvinyl lactophenol. The terminology follows Sæther (1980).

The photographs were taken using an Axio Lab.A1 (Karl 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®

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Photoshop® software. Scanning Electron Microscopy (SEM) image was made using a Vega3 Tescan microscope in the Yu. A. Orlov Paleontological Museum of the Paleontological Institute of the Russian Academy of Sciences, Moscow.

Holotype and paratypes of the new species, as well as all other material, are deposited in the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia (FSCEATB FEB RAS).

Total genomic DNA was extracted from thorax with the ExtractDNA Blood & Cells (Evrogen, Moscow, Russia). Amplification was performed in 10 µl reactions containing 6.0 µl ddH2O, 0.5 µl of each 10 µM universal primer LCO1490 and HCO2198 (Folmer *et al.* 1994), 2 µl of 5X ScreenMix-HS DNA polymerase (Evrogen, Moscow, Russia), and 1 µl of DNA template. DNA was amplified under the following conditions: 95°C for 1 minute, 35 cycles of denaturation (95°C for 30 s), annealing (48°C for 30 s), and extension (72°C for 60 s), followed by 72°C for 5 minutes. Amplified DNA was visualized on a 1.5 % TBE gel and purified with Exonuclease I (ExoI) and Thermosensitive Alkaline Phosphatase (FastAP) (Thermo Fisher Scientific Inc., USA). Sequencing reactions had a total volume of 10 µl and included 10 pmol of each primer, 0.7 µl of purified amplicon and reagents of BigDye terminator v3.1 cycle kit. Sequencing reactions were purified with ethanol precipitation and sequenced on a ABI 3500 DNA Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at the Federal Scientific Center of the East Asia Terrestrial Biodiversity (Vladivostok, Russia). Sequences were assembled using MEGA7 (Kumar *et al.* 2016) and FinchTV and submitted to GenBank under accession numbers PP133053-PP133060. Also, MEGA7 was used for calculated inter- and intraspecific COI p-distances.

In addition to our own data COI barcodes from the Barcode of Life Data System (http://www.boldsystems.org/, accessed on 09 December 2023) of each unique barcode index number (BIN) corresponding to described *Smittia* species were added to the dataset. In turn, to search for possible conspecific species to *S. solominae* **sp. nov.**, we used BLAST in Genbank (blast.ncbi.nlm.nih.gov) and Animal Identification in BOLD (https://v3.boldsystems.org/index.php/IDS_OpenIdEngine).

Bayesian analysis was carried out on the obtained dataset in MrBayes v3.2.7 (Ronquist *et al.* 2012) using Markov Chain Monte Carlo (MCMC) randomization. Four Markov chains (three heated chains, one cold) were run for 5 million generations, with the first 25% of sampled trees discarded as burn-in. Strict clock model (brlenspr=clock: uniform) were used to obtain an ultrametric tree. PartitionFinder 2.1.1 (Lanfear *et al.* 2012) is used to select the best-fit partitioning scheme and models separately for each codon position of COI gene.

Taxonomy

Smittia solominae Makarchenko, Semenchenko *et* Palatov, sp. nov.

http://zoobank.org/NomenclaturalActs/37B17E4B-BAC8-4F65-B567-476C8C7A48BE

(Figs. 1–20)

Type material. Holotype, adult male, RUSSIA, North Caucasus, *Kabardino-Balkarian Republic*, Elbrus Region, Bashkara Glacier, collection from ice under stones, alt. 2603 m a.s.l., 1.VIII. 2023, 43.206675 N, 42.723542 E, leg. D. Palatov. Paratypes, 7 adult males, 4 pupae, 8 larvae, the same data as holotype; 5 adult males, 1 pupa, 7 larvae, the same data as holotype, except Bezingi Glacier near Barankosh alpine site, alt. 2750 m a.s.l., 25.VII. 2022, 43.064789 N, 43.087328 E., leg. D. Palatov; 4 larvae, the same data as holotype, except Kashkatash Glacier, collection from ice under stones, alt. 2625±5 m a.s.l., 43.209886 N, 42.683997 E, 23.VII. 2023, leg. D. Palatov.

Derivatio nominis. The species is named after the Russian glaciologist and paleoclimatologist Dr. Olga Nikolaevna Solomina (Institute of Geography, Russian Academy of Sciences), who devoted her entire life to studying the glaciers of the Greater Caucasus.

Description

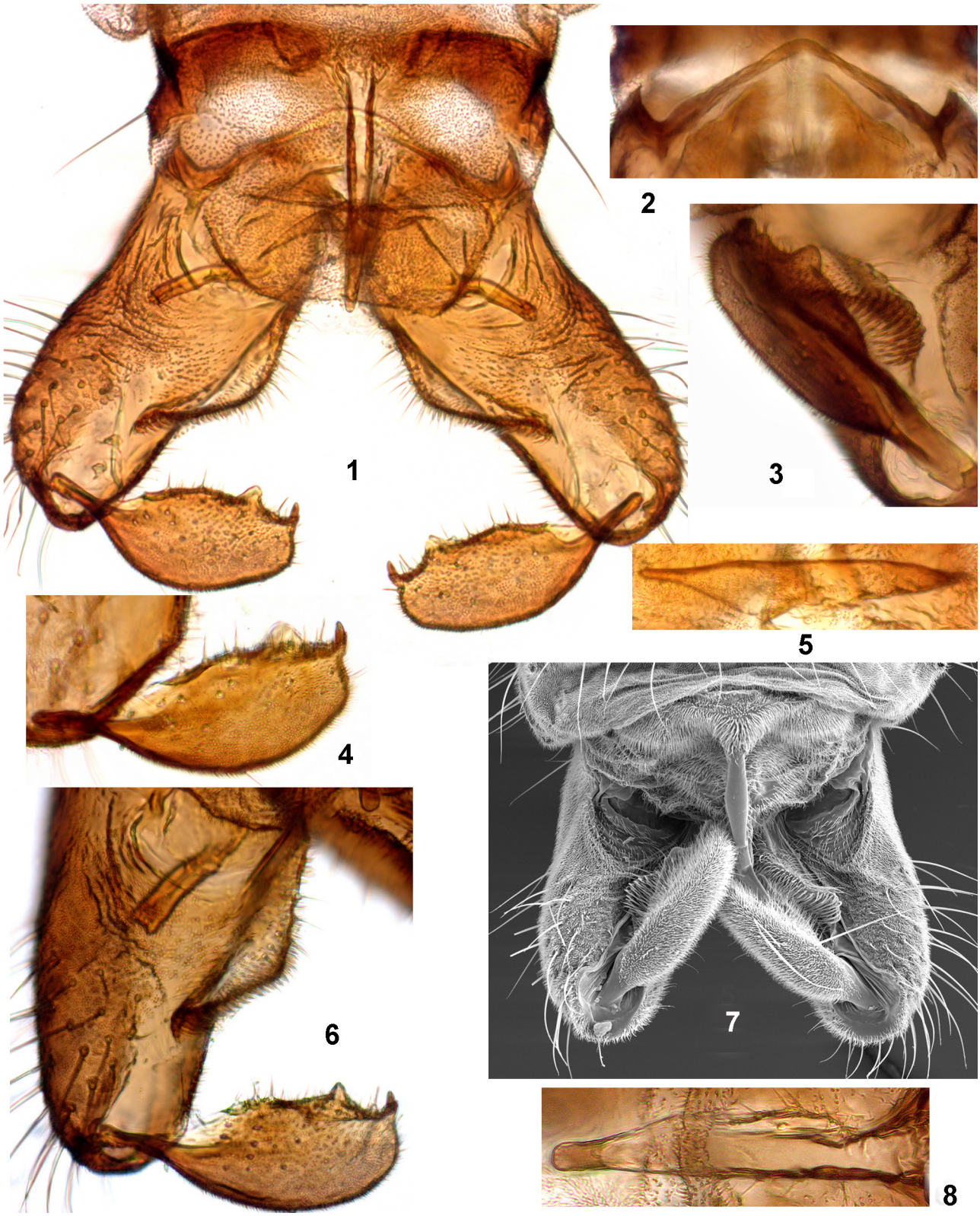
Adult male (n = 5, except when otherwise stated). Total length 2.0–3.4 mm. Total length/wing length 0.96–1.55.

Coloration. Dark brown to black. Wings greyish.

Head. Eyes reniform, hairy. Temporal setae including 4 inner verticals and 5–6 outer verticals. Clypeus with 7–13 setae. Antenna with 13 flagellomeres and normal plume of setae, 148–213 µm long; terminal flagellomere

with 1 subapical seta, 36–44 µm long; AR 0.86–0.91. Palpomere length (µm): 20–24, 36, 76–92, 56–72, 80–108. Head width/palpal length 1.5–1.8.

Thorax. Antepronotum with 3–4 ventrolateral setae. Acrostichals 5–6, 4–6 µm long; dorsocentrals 11–16, 72–92 µm long; prealars 4–7, 56–60 µm long. Scutellum with 9–12 setae, 48–68 µm long (in 1 row).



FIGURES 1–8. Adult male of *Smittia solominae* **sp. nov. 1, 7,** hypopygium in dorsal view; **2,** transverse sternapodeme; **3–4,** gonostylus; **5,** anal point in lateral view; **6,** gonocoxite and gonostylus; **8,** anal point in dorsal view.

Wing. Length 2.08–2.2 mm, width 0.56–0.58 mm. Costal extension 80–82 μm long. Anal lobe slightly reduced, rounded. Squama without setae. R with 4–7 setae, R_1 with 0–1 seta, R_{4+5} without setae. R_{2+3} unclearly expressed.

Legs. Spur of front tibia 36–64 μm long. Spurs of mid tibia 20–22 μm long. Spurs of hind tibia 44–48 μm and 20–44 μm. Hind tibial comb with *ca* 16 setae. Length (μm) and proportions of leg segments are as in Table 1.

TABLE 1. Lengths (in μm) and proportions of leg segments of *Smittia solominae* **sp. nov.**, male (n=4)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	607-656	738-787	328-361	197-213	131-156	98-115	82-107
P_2	672-738	672-722	238-279	131-156	98-115	82-98	90-98
P_3	771-804	771-804	361-394	180-197	131-180	82-98	90-98

TABLE 1. (continued)

	LR	BV	SV	BR
P_1	0.44-0.46	3.05-3.29	4.00-4.10	1.43
P_2	0.35-0.39	3.68-4.09	5.23-5.72	1.33
P_3	0.46-0.49	3.49-3.88	4.08-4.36	1.50

Hypopygium (Figs. 1–8). Tergite IX with 4–6 setae, ca 8 μm long, edge of Tergite IX reaches anal point in middle part (Fig. 8). Anal point bare, narrowed distally, finger-shaped (Figs. 5, 8), 120–132 μm long. Laterosternite IX with 1 long setae, 68–73 μm long (Fig. 1). Transverse sternapodeme narrow, wide triangular (Fig. 2). Phallapodeme sclerotized, 76–88 μm long. Gonocoxite 236–248 μm long; inferior volsella flat and broadly triangular, covered with setae and macrotrichia (Figs. 1, 6); superior volsella reduced. Gonostylus 144–148 μm long, in distal quarter with triangular crista dorsalis, small, 8–14 μm height; along the inner edge with 4–6 setae, 12–20 μm long; apex with macroseta, 8–12 μm long (Figs. 3–4, 6–7); HR 1.64–1.68.

Pupa (n=5). Total length 2.7–3.6 mm. Coloration brownish brown (Fig. 9). Exuviae brownish gray.

Cephalothorax. Cephalic area without warts or tubercles. Frontal setae absent. Thorax wrinkled, in anterodorsal and lateral parts slightly granulated. Thoracic horn absent. Precorneal setae lengths (μm): Pc_1–32–36, Pc_2–40–60, Pc_3–28–48. Antepronotum and mesonotum without setae.

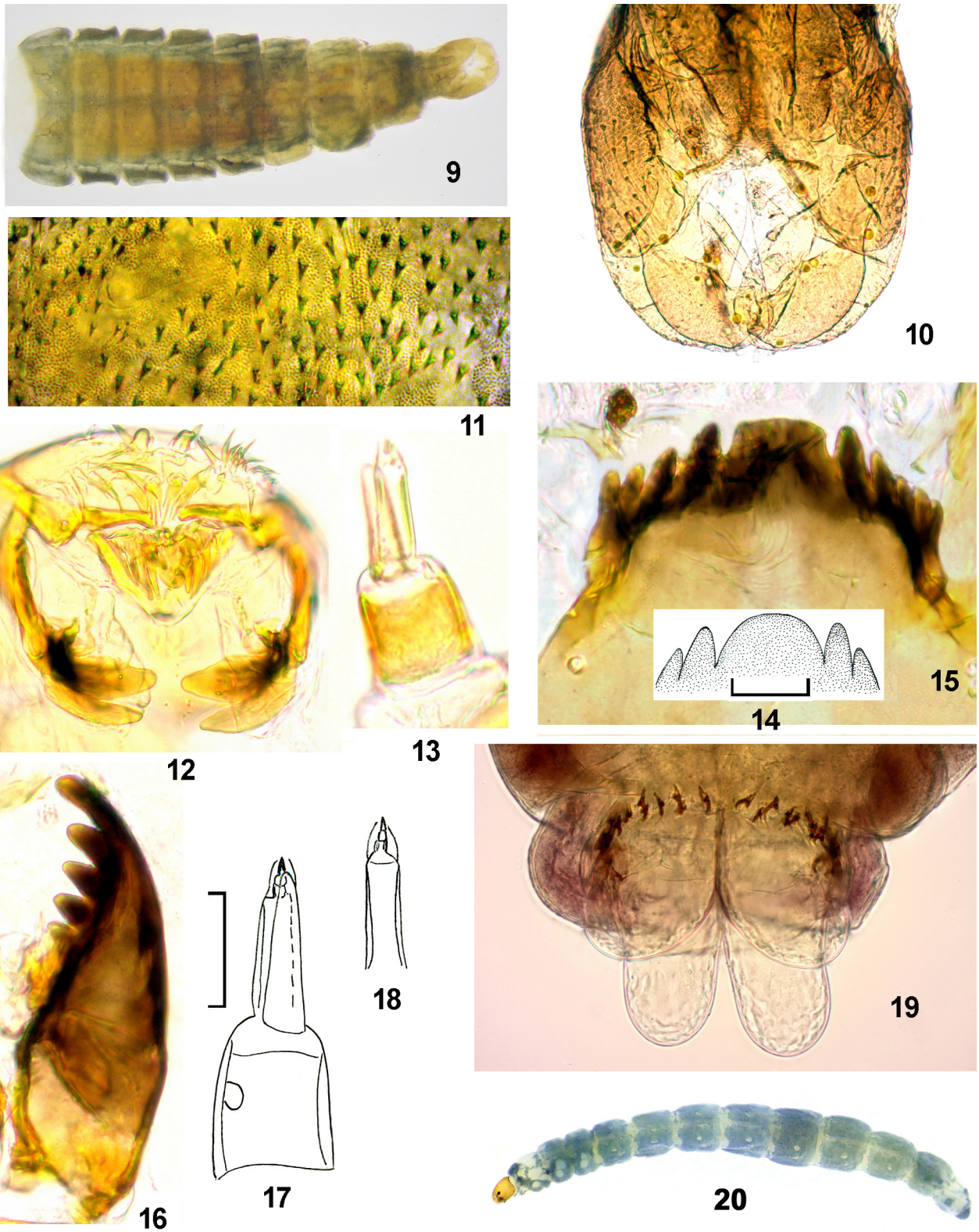
Abdomen. Tergite I bare or with some spinules in the back. Tergites II –IX evenly covered with spinules (Fig. 11); sternites V—IX with some shagreen in middle posteriorly. Apophyses absent. Pedes spurii A and B absent. Abdominal setation very weak; lateral setae segments II–VIII only 1 pair, 16–20 μm long. Anal lobe absent. Tergite IX reduced, apically rounded with posterior margin medially indented, without setae. Genital sac of male extending beyond tergite IX, without basal spinules (Fig. 10).

Fourth instar larva (n=6, except when otherwise stated). Coloration of fixed larvae greenish-bluish, head yellow (Fig. 20). Total length 4.3–5.8 mm (n=10).

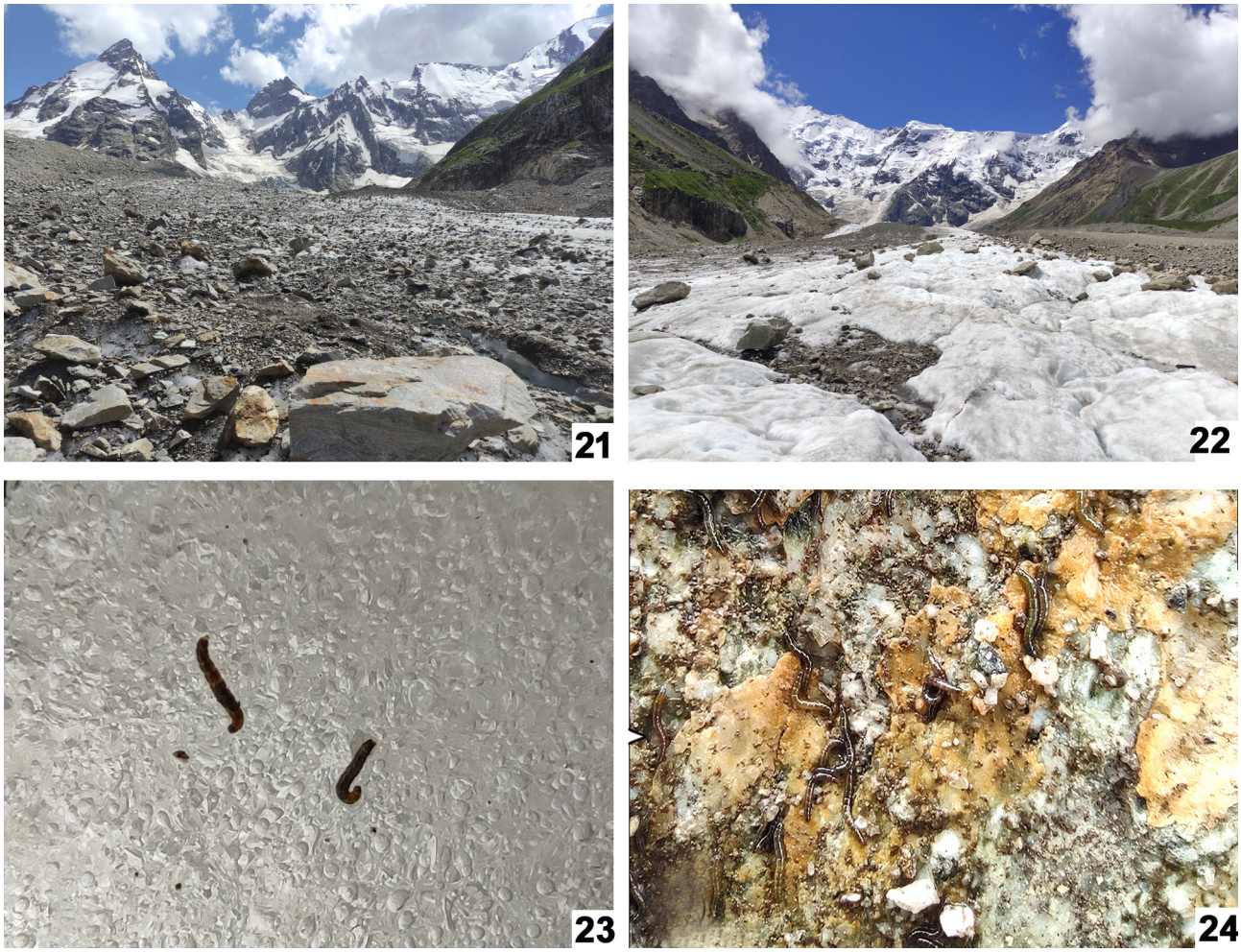
Head capsule 262–295 μm long and 230–246 μm wide; postoccipital margin black and narrow. Labral setae S_I divided into 3–4 unequal sized branches (Fig. 12); S_{II} simple. Premandible with 2 apical teeth (Fig. 12). Antenna 4 segmented, total length 54–59 μm; length of segments (μm): 25–30, 21–23, 3.3–5, 2.5–3. Antennal blade 26–31 μm long, reaches base of 4th segment; segment 2 with weak Lauterborn organs; large ring organ with a diameter of 4.5–5 μm is located in distal half of basal segment (Figs. 13, 17–18); AR 0.97–1.17. Mandible dark brown or black in distal part; total length 116–133 μm; with apical tooth and 3 true inner teeth and one false tooth; apical tooth shorter than combined width of 3 inner teeth; seta subdentalis reaches top of lower inner tooth; seta interna with 4–6 terminally serrate branches (Fig. 16). Mentum with 5 pairs of lateral teeth and wide median tooth which has a rounded or ground straight edge and is 3.4–4 times wider than first lateral tooth; ventromental plates weak (Figs. 14–15). Anterior parapods basally fused, with numerous fine spines in basal part and claws at apex. Posterior parapods reduced and bearing simple claws (Fig. 19). Procercus absent; anal tubules slightly reduced but good visible (Fig. 19).

Diagnosis. The adult male of the new species differs from all known representatives of the genus *Smittia* in the structure of the hypopygium and the following characters. Total length 2.0–3.4 mm. Wing length 2.08–2.2 mm. AR 0.86–0.91. Acrostichals 5–6, dorsocentrals 11–16, prealars 4–7, scutellars 9–12 setae in 1 row. LR_1 0.44–0.46, BV_1 3.05–3.29, SV_1 4.0–4.10. Tergite IX with 4–6 setae only along edge of anal point in middle part. Anal point bare, narrowed distally, finger-shaped. Transverse sternapodeme narrow, wide triangular. Inferior volsella flat and

broadly triangular, covered with setae and macrotrichia; superior volsella reduced. Gonostylus in distal quarter with triangular crista dorsalis; along the inner edge with 4–6 setae; apex with macroseta; HR 1.64–1.68. The pupa and larva are typical for the genus and can hardly be distinguished from other species, for most of which the preimaginal stages of development are still unknown.



FIGURES 9–20. Pupa **(9–11)** and larva **(12–20)** of *Smittia solominae* **sp. nov. 9,** abdomen; **10,** anal segment; **11,** shagreen of tergites; **12,** labrum and premandibles; **13, 17,** antenna; **14,** median teeth of mentum; **15,** mentum; **16,** mandible; **18,** 2–4 antennal segments; **19,** anal segment; **20,** total view of larva. Scale bars: 20 μm.

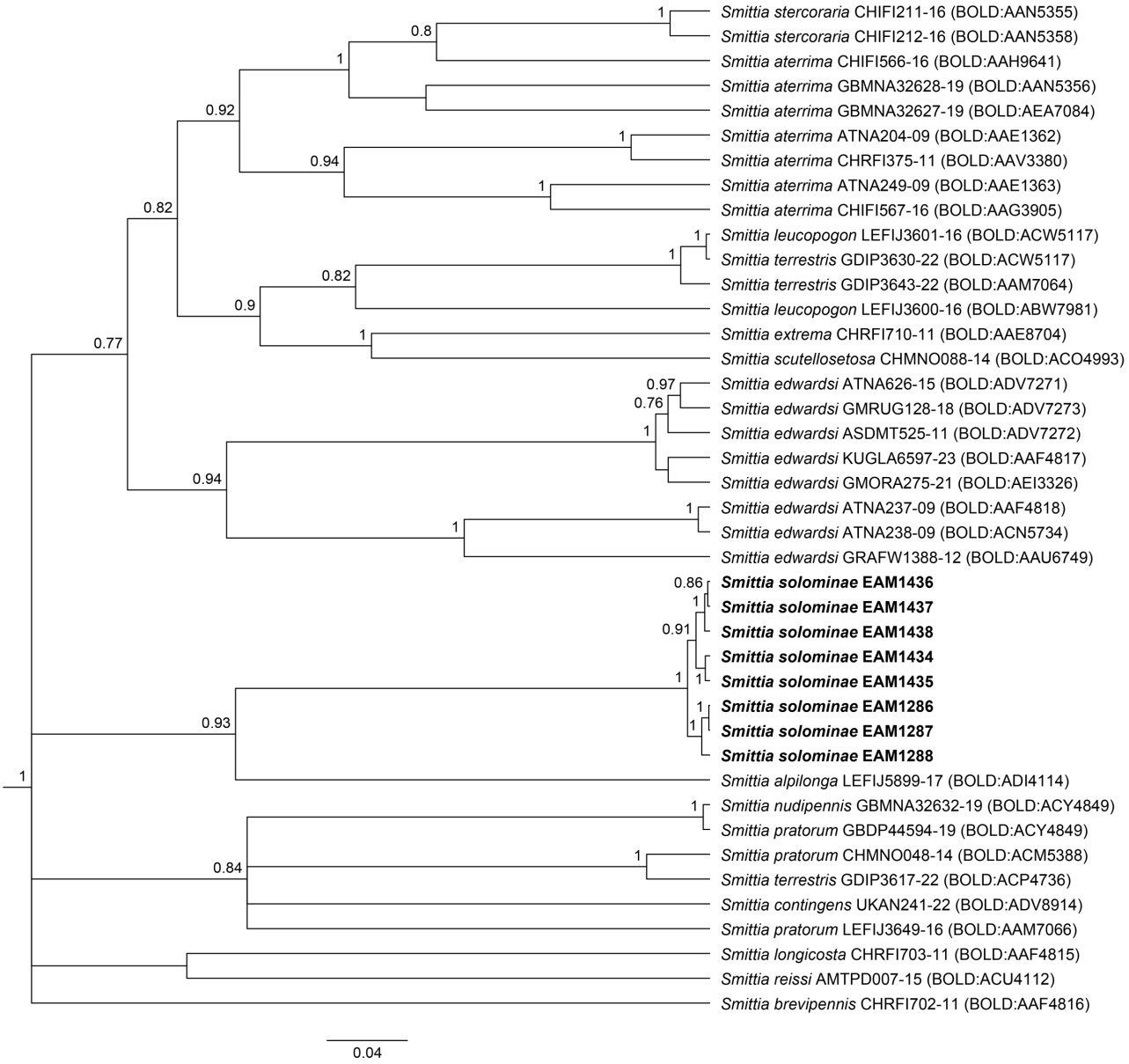


FIGURES 21–24. Habitat of *Smittia solominae* **sp. nov.** on Bashkara **(21)** and Bezingi **(22)** Glaciers. **23,** larvae crawling on the ice surface; **24,** larvae on the underside of a rock lying on the glacier.

Biology and distribution. Currently, adults, pupal and larval stages of *S. solominae* **sp. nov.** have been found on two glaciers in the Greater Caucasus mountain range within the Kabardino-Balkaria Republic: Bashkara Glacier (43.206675 N, 42.723542 E, elevation – 2600 m above sea level) and Bezingi Glacier (43.064789 N, 43.087328 E., elevation – 2750 m above sea level). Only larvae have been found on Kashkatash Glacier (43.209886 N, 42.683997 E, elevation – 2610 m above sea level), also within Kabardino-Balkaria (Figs. 21–22).

Thus, the new species inhabits the surface of large valley glaciers in the Central Caucasus at elevations ranging from 2600 m above sea level.

In all cases, *S. solominae* **sp. nov.** was found relatively far from the glacier edge, usually several hundred meters away. The larvae, pupae, and adults of this species inhabit spaces beneath large rocks that fall onto the glacier from the surrounding rocky slopes of the valleys. They have been found both on the underside of rocks directly adjacent to the ice and on the surface of the ice beneath the rocks. Adult individuals were not observed flying (possibly due to constant strong winds), although clusters of adults (including mating individuals) were found on the underside of rocks (Figs. 23–24). In some cases, the density of *S. solominae* **sp. nov.** adults and pupae on the ice reached 15–20 individuals per square meter, while larvae on the surface of rocks ranged from 0.9–2.4 individuals per square decimeter; however, their distribution on the glacier surface is highly uneven. The larvae are likely algophagous and feed on algae covering the glacier surface and rocks. Their distribution may be related to algal accumulations on the glacier surface. The presence of larvae of different ages, pupae, and adults simultaneously indicates an extended period of emergence for this species. *S. solominae* **sp. nov.** individuals were not observed outside the glacier surface. During nighttime, adults did not fly towards specially installed light traps located outside the glacier boundaries. It is possible that adult individuals of this species do not have the ability to actively move, partly due to the constant sub-zero temperatures at the surface.



FIGURES 25. Ultrametric Bayesian inference (BI) tree based on the cytochrome c oxidase I (COI) nucleotide sequence data of the genus *Smittia* Holmgren. Bayesian posterior probabilities (higher than 0.7) are given above tree nodes. Specimens obtained in this study are in bold.

Results of DNA barcoding

Overall, we have sequenced fragments of the cytochrome oxidase subunit I (658 bp in length) of 8 samples of *S. solominae* **sp. nov.** Genetic distances showed high sequence divergence between *S. solominae* **sp. nov.** and available chironomids in GenBank and BOLD. The closest sequences belonging to Chironomidae gen. sp. (BIN BOLD: ADI3692 from Canada, p-distances = 11.3%), *Orthocladius* sp. (BIN BOLD:ACR0587 from Germany, p-distances = 12.2%) and *Smittia* sp. (BIN BOLD:ACU4931 from Germany, p-distances = 12.6%). Obtained sequence divergence corresponds to interspecific level in orthoclads which averages above 10% K2P or p-distances (Silva & Wiedenbrug, 2014; Makarchenko *et al.*, 2017, 2018, 2019), however the second distance belongs to intergeneric level in case the genus *Orthocladius* (BIN BOLD:ACR0587) identified correctly. By contrast, intraspecific divergence was lower and ranged from 0.00% to 2.13 (1.26% in average).

Phylogenetic tree was reconstruct using obtained sequences of *S. solominae* **sp. nov.** and DNA barcodes of described *Smittia* form BOLD (Fig. 25). Described species and *S. alpilonga* Rossaro et Lencioni (BIN BOLD: ADI4114) formed monophyletic clade (Bayesian posterior probability = 0.93) however, genetic distances were 15,8%, which is higher than the comparison pairs above. This clade was one of five clades included in the basal polytomous node on the phylogenetic tree.

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