


Taxonomy of *Diamesa steinboeckii* group (Diptera: Chironomidae: Diamesinae), with description and DNA barcoding of new species.

I. Subgroups *steinboeckii* and *longipes*

EUGENYI A. MAKARCHENKO^{1*}, ALEXANDER A. SEMENCHENKO² & DMITRY M. PALATOV³



¹Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, 100 let Vladivostoku 159, 690022 Vladivostok, Russia

 makarchenko@biosoil.ru;  <https://orcid.org/0000-0003-2765-8729>

²Laboratory of Ecology and Evolutionary Biology of Aquatic Organisms, Far Eastern Federal University, Suhanova St. 8, 690950 Vladivostok, Russia

 semenchenko_alexander@mail.ru;  <https://orcid.org/0000-0001-7207-9529>

³A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Leninskij prosp. 33, 119071 Moscow, Russia

 triops@yandex.ru;  <https://orcid.org/0000-0002-8826-9316>

*Corresponding author

Abstract

The results of a revision of the *Diamesa steinboeckii* group, namely subgroups *steinboeckii* and *longipes* (Diptera, Chironomidae, Diamesinae) are presented. Illustrated descriptions of the adult male of *D. dragani* **sp. nov.** from Sayan Mountains, *D. kownackii* **sp. nov.** from Wrangel Island, *D. maisaraensis* **sp. nov.** from Pamir, *D. marinskiyi* **sp. nov.** from Tian-Shan, *D. zagrosica* **sp. nov.** from Iran, *D. moubayedi* **sp. nov.** from Lebanon are provided with redescriptions of *D. steinboeckii* Goetghebuer from Pamir, French and Swiss Alps, *D. sakartvella* Kownacki et Kownacka from Caucasus and *D. praecipua* Sæther et Willassen from Himalayas. Taxonomic remarks with data on the ecology and biogeography of the investigated species are given. In addition to taxonomic information, 658-bp fragments of the mitochondrial cytochrome oxidase subunit I gene from the eight species are presented and the results of DNA barcoding are discussed. Previous investigations focused to integrative taxonomy and molecular analysis of the *Diamesa* species collected in various mountain regions such as Alps, Tien Shan and Pamir. In the present work, we focused on the principle of considering morphological groups, giving more emphasis respect to the division in geographical areas.

Key words: Diptera, Chironomidae, *Diamesa steinboeckii* group, taxonomy, DNA barcoding, Sayans, Central Asia, Alps, Caucasus

Introduction

The chironomids of *Diamesa steinboeckii* group from the subfamily Diamesinae live in cold streams in high mountains and in arctic regions of the Northern Hemisphere. Adult males of all species included in this group are characterized by the presence of antennae with reduced setae of the plume and mainly consisting of 6–8, very rarely of 13 flagellomeres as well as by long legs, very strong hypopygium with a typical internal skeleton, including a triangular or sometimes trapezoidal transverse sternapodeme, a peculiar form of fallapodeme, usually without inferior volsellae, but only with a small superior volsellae at the base of the gonocoxite, and eyes strongly haired. The wings of males and females of some species can be either normally developed or brachypterous.

Diamesa steinboeckii group includes at least 23 species which according to Kownacki (1980) and our preliminary data for convenience can be classified into four subgroups: ***Diamesa davisi* subgroup** (*D. davisi* Edwards, *D. amplexivirilia* Hansen, *D. alpina* Tokunaga, *D. tokunagai* Makarchenko et Yamamoto, *D. sonora* Willassen, *D. lupus* Willassen, *D. serratosioi* Willassen and *D. saetheri* Willassen), ***Diamesa longipes* subgroup** (*D. longipes* Goetghebuer, *D. sakartvella* Kownacki et Kownacka, *D. kenyae* Freeman, *D. ruwenzoriensis* Freeman, *D. freemani* Willassen et Cranston, *D. kohshimai* Sæther et Willassen, *D. yalavia* Sæther et Willassen, *D. praecipua*

Sæther et Willassen, *D. loeffleri* Reiss and *D. khumbugelida* Sæther et Willassen), ***Diamesa leona* subgroup** (*D. leona* Roback, *D. leoniella* Hansen, *D. starmachi* Kownacki et Kownacka and *D. japonica* Tokunaga) and ***Diamesa steinboeckii* subgroup** with single species *D. steinboeckii* Goetghebuer (Freeman 1964; Reiss 1968; Kownacki 1980; Makarchenko 1981; Willassen 1985; Willassen & Cranston 1986; Sæther & Willassen 1987; Makarchenko & Yamamoto 1995; Rossaro & Lencioni 2015; Montagna *et al.* 2016; Lencioni *et al.* 2021)..

The material at our disposal will allow a revision all the four subgroups of *Diamesa steinboeckii* group, but in the present article only the results of investigation of *D. steinboeckii* and *D. longipes* subgroups are presented. Illustrated descriptions of the adult male *D. dragani* **sp. nov.** from Sayan Mountains, *D. kownackii* **sp. nov.** from Wrangel Island, *D. maisaraensis* **sp. nov.** from Pamir, *D. marinskiyi* **sp. nov.** from Tian-Shan, *D. zagrosica* **sp. nov.** from Iran, *D. moubayedii* **sp. nov.** from Lebanon and redescriptions of *D. steinboeckii* Goetghebuer from Pamir, French and Swiss Alps, *D. sakartvella* Kownacki et Kownacka from Caucasus and *D. praecipua* Sæther et Willassen from Himalayas are provided. Both morphological data and DNA sequences of mitochondrial cytochrome c oxidase subunit I gene (COI) will be used in species delimitation, with the exception of *D. kownackii* **sp. nov.** from Wrangel Island and *D. steinboeckii* Goetghebuer from Pamir for which material for DNA barcoding was not available. Taxonomic remarks with data on the ecology and biogeography of the investigated species are given.

DNA barcoding has shown to be effective as an approach for the global assessment of species diversity (Hebert *et al.* 2003a, b). Integrated taxonomy and DNA barcoding of the genus *Diamesa* Meigen was performed for various mountain ranges, such as the Alps (Montagna *et al.* 2016, Lencioni *et al.* 2021), as well as the Tien Shan and Pamir (Makarchenko *et al.* 2018). A short sequence of gene mitochondrial cytochrome oxidase subunit I is used as marker for species-level identification, because proved to be useful in taxonomy of the genus *Diamesa* (Montagna *et al.* 2016).

The aim of this work is to study taxonomy with DNA barcoding of two subgroups *D. steinboeckii* and *D. longipes* collected from different mountain ranges of Palearctic.

A detailed analysis of the Diamesinae phylogeny using a multilocus approach will be published in future papers

Materials and methods

The adults of chironomids were preserved in 96% ethanol for DNA-analysis and in 70% ethanol for further study of morphology. It should be noted that only adult males were used for the morphological study, whereas in some cases pupae and larvae were used for the DNA barcoding. The results of morphological studies of pupae and larvae of some species have been published earlier (Serra-Tosio 1971, 1974; Rossaro 1980; Makarchenko 1981; Langton & Visser 2003; Rossaro & Lencioni 2015).

The material was slide-mounted in polyvinyl lactophenol following the recommendations of Moubayed and Langton (2019). The terminology follows Sæther (1980) with our additions concerning the anal tergal bands, the types (X or Y) of which we used to diagnose the males of *Diamesa steinboeckii* group. Anal tergal bands of X type are represented by anterolateral, median and posterolateral bands, Y-type only by anterolateral and median bands (Figs. 3, 29).

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® Photoshop® software.

Holotypes 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 of chironomids using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) followed the manufacturer's instructions. A DNA fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using the polymerase chain reaction (PCR) with the primers LCO1490 and HCO2198 (Folmer *et al.* 1994). The template profile was as follows: 94.0°C for 5 min; 35 cycles at 94.0°C for 30 sec, 50.0°C for 30 sec, and 72.0°C for 60 sec; with a final extension at 72.0°C for 5 min. PCR reaction had 10 µL of final volume, containing 5 µL of Go Taq Green Master Mix (Promega Corp, Madison, WI, USA), 0.5 µM of each primer, and 1 µL of eluted DNA. The PCR product was purified using Exonuclease I and Thermosensitive Al-

kaline Phosphatase (ThermoFisher Scientific, Waltham, MA, USA) and sequenced for both directions. Sequencing reaction was performed using BigDye® Terminator v3.1 Cycle Sequencing Kits and run on an ABI 3130xl Genetic Analyzer Sequencer (Applied Biosystems, Foster City, CA, USA).

Forward and reverse sequences are manually assembled and edited using Finch TV and MEGA 7 (Kumar *et al.* 2016). Based on the Kimura-2-Parameter (K2P) model are calculated inter- and intraspecific genetic distances using MEGA7 (Kumar *et al.* 2016). ABGD analysis (www.wabi.snv.jussieu.fr/public/abgd/abgdweb.html, Puillandre *et al.* 2012) is used for species delamination and establish taxonomic status of sequenced specimens, using relative gap width ($X = 1.0$) and intraspecific divergence (P) values between 0.005 and 0.100 with the K2P model according to Song *et al.* (2018). We used all available COI sequences of genus *Diamesa* in GenBank and BOLD systems for ABGD analysis (Approximately 2400 sequences were available at the time of publication).

To reconstruct the phylogenetic tree, on the contrary, we used only the sequences we obtained. According to BOLD systems 60 BIN numbers are currently known for the genus *Diamesa*, therefore we used only the obtained sequences to reduce the size of the tree, except three sequences of *D. steinboeckii* (LN897604, MT843170, LN897593) which were added to the 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 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 was SYM+G (Zharkikh 1994), F81+I (Felsenstein 1981) and HKY+G (Hasegawa *et al.* 1985) respectively. Bayesian phylogenetic analyses was 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 is visualized in FigTree v. 1.4.4.

The obtained sequences have been deposited in GenBank under numbers OM867238–OM867269.

Taxonomy

Diamesa steinboeckii subgroup

Diamesa dragani Makarchenko *et* Semenchenko, sp. nov.

<http://zoobank.org/NomenclaturalActs/B3C5EFC4-7AE3-4419-B2AF-DD40873B467B>

(Figs. 1–8, 30, 68)

Type material. Holotype, adult male, RUSSIA: Republic of Khakassia, Tashtypskyi District, Sayan Mountains, unnamed stream of Bolshoi On River basin, altitude 2063 m above sea level, 4.X.2020, N 51.714667, E 89.884500, leg. S. Dragan. Paratypes: 6 adult males, the same data as holotype.

Derivatio nominis. The species is named in honor of Dr. Dragan Sergey Viktorovich from Katanov Khakas State University (Abakan, Republic of Khakassia, Russia) who collected type material in Sayan Mountains.

Description

Adult male ($n = 7$, except when otherwise stated). Total length 2.1–2.5 mm. Total length/wing length 0.81–1.20.

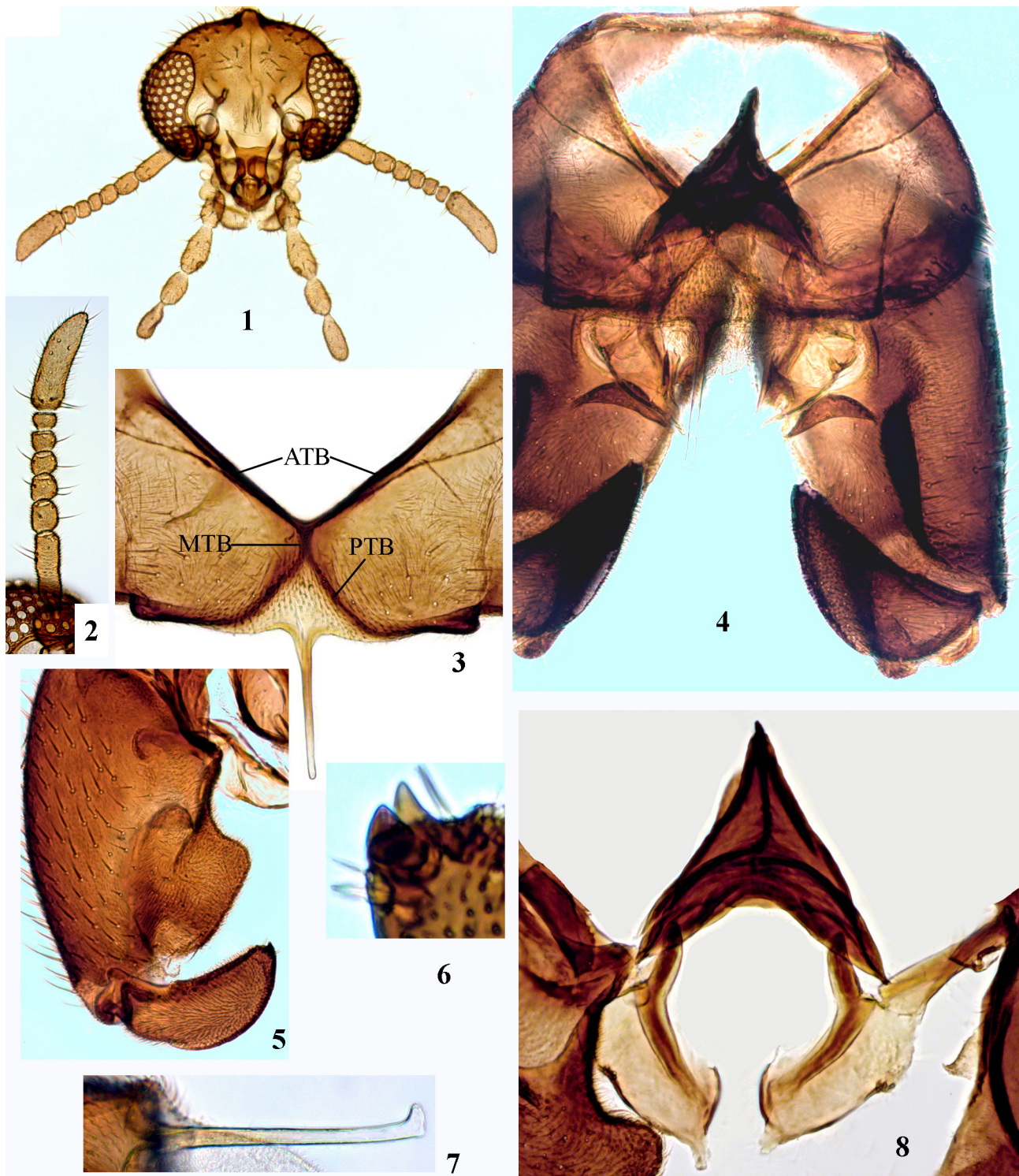
Coloration. Dark brown to black. Head, thorax and abdomen dark brown to black. Legs black. Wings grey, with brownish veins.

Head (Figs. 1–2). Eyes hairy, reniform. Temporal setae including 6–7 frontals, 3–6 orbitals, 5–10 verticals and 3–4 postorbitals. Clypeus with 4–6 setae. Antenna with 7 flagellomeres and reduced plume of setae (Fig. 2); number and length of these setae on 1–6 flagellomeres respectively: 1–2 (26–28 μm), 1–2 (18–32 μm), 3 (32–44 μm), 1–2 (24–32 μm), 2 (40–44 μm), 4 (40–48 μm); terminal flagellomere with 2–4 setae, 32–48 μm long in basal part and with 2 subapical setae, 20–24 μm long. Length of 1–7 flagellomeres (μm): 96–100, 40–48, 32–36, 24–28, 24–28, 20–24, 120–128; AR 0.48–0.52. Palpomere length (μm): 28–40, 68–72, 84–92, 64–68, 88–100. Palpomere 3 in distal part with sensilla capitata with diameter 12–15 μm . Head width/palpal length 1.0–1.29. Antennal length/palpal length 1.07–1.09.

Thorax. Antepronotum with 8–11 ventrolateral setae, 44–52 μm long. Dorsocentrals 4–6, 56–72 μm long; prealars 5–6, 48–60 μm long. Scutellum with 18–20 setae. AAIL with 4–5 setae, 36 μm long.

Wing (Fig. 30). Length 2.16–2.72 mm, width 0.85–0.88 mm. Costal extension 60–76 μm long. Anal lobe rounded-angular. Squama with 3–7 setae, 32–44 μm long. R and R_1 with 7–12 setae, R_{4+5} with 13–15. RM/MCu 1.8–2.0.

Legs. Spur of front tibia 36–40 μm long. Spurs of mid tibia 36–44 μm and 40–44 μm long. Spurs of hind tibia 64–72 μm and 40–44 μm long. Hind tibial comb with 16–20 setae. Length (μm) and proportions of leg segments are as in Table 1.



FIGURES 1–8. *Diamesa dragani* sp. nov., male. 1, head; 2, antenna; 3, tergite IX; 4, hypopygium in dorsal view; 5, gonocoxite and gonostylus; 6, apex of gonostylus; 7, anal point in lateral view; 8, transverse sternapodeme, phallapodeme and aedeagal lobe. ATB—anterolateral anal tergal bands, MTB—median anal tergal band, PTB—posterolateral anal tergal band.

TABLE 1. Lengths (in μm) and proportions of leg segments of *Diamesa dragani* sp. nov., male (n=5)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅
P ₁	1394–1689	1230–1394	738–787	312–344	180–197	82–98	115–131
P ₂	1279–1542	984–1132	410–459	197–213	131–148	66–98	98–115
P ₃	1394–1689	1214–1410	754–787	402–443	213–230	98	115–131

continued

	LR	BV	SV	BR
P ₁	0.56–0.60	4.46–5.25	3.56–3.92	1.5–1.7
P ₂	0.40–0.43	4.96–5.60	5.31–5.83	1.5–1.7
P ₃	0.53–0.62	3.87–4.29	3.49–4.11	1.7–2.0

Hypopygium (Figs. 3–8). Tergite IX with 8–14 setae, 16–24 μm long and with narrow (4–6 μm), pale, and naked anal point, 120–126 μm long which in lateral view apically curved upwards (Fig. 7); posterior edge of tergite IX almost straight, anal-lateral angles are rounded and slightly projecting (Fig. 3); anal tergal bands X-type; median tergal band 16–20 μm long. Laterosternite IX with 7–12 setae, 20–24 μm long. Transverse sternopodeme (TSA) triangular, peaked (Figs. 4, 8), 120–128 μm high, 156–176 μm wide at the base; TSA height/TSA width 0.70–0.77. Aedeagal lobe as in Fig. 8, weakly chitinated, apically with small digitiform process; phallapodeme sclerotized, 210–232 μm long. Gonocoxite 400–443 μm long, in middle part of dorso-internal position with small flattened transverse lobe, 98–100 μm long and 28–32 μm wide, directed backwards, and with large longitudinal lobe in middle, anterior outer margin of which is rounded or rounded-angular and slightly projecting (Fig. 5); Gonostylus 197–236 μm long, slightly curved, megaseta in form of wide terminal spine 8–12 μm long, next to it there is tooth and two setae of 12–16 length μm long (Fig. 6). HR 2.01–2.25.

Pupa and larva unknown.

Diagnosis. The male of *D. dragani* sp. nov. is most closely related in the subgroup to the *D. maisaraensis* sp. nov. and *D. marinskiyi* sp. nov., which have anal tergal bands X-type, but the new species is a little shorter than others (2.1–2.5 mm), antenna with 7 flagellomeres, AR 0.48–0.52, dorsocentrals 4–6, scutellars 18–20, femur of front leg 1394–1689 μm long, median tergal band 16–20 μm long, anal point 230–126 μm long. The males of *D. maisaraensis* sp. nov. and *D. marinskiyi* sp. nov. have the following characteristics respectively: total length 2.7–3.2 mm and 2.5–2.7 mm, antenna with 8 flagellomeres, AR 0.39–0.42 and 0.32–0.39, dorsocentrals 6–8 and 8–10, scutellars 39–50 and 44–46, femur of front leg length 1880–1960 μm and 1624–1476 μm , median tergal band length 28–32 μm and 20–24 μm , anal point length 100–120 μm and 104–116 μm .

Ecology. Adult males were collected from stones and boulders in mountain stream, located at an altitude of 2063 m, at a flow rate of 0.6 m/s, with water temperatures 0.4°C, pH 7.92, general mineralization 0.02 g/l, electrical conductivity 0.03 mS (pers. comm. by S.V. Dragan).

Distribution. Known only from the type locality in Sayan Mountains (Fig. 68).

Diamesa kownackii Makarchenko, sp. nov.

<http://zoobank.org/NomenclaturalActs/4601893D-170C-427C-8F19-AEED2CA46FB2>

(Figs. 9–14, 31, 69–70)

Diamesa steinboeckii Goetghebuer; Makarchenko *et al.*, 1980: 6; 1981: 96; 1985: 71; 2006: 261, 472, 614, misidentification.

Type material. Holotype, adult male, RUSSIA: Chukotka Autonomous Region, Wrangel Island, unnamed stream in upper part of Somnitelnaya River, altitude 205–210 m above sea level, 22.VII.1978, N 70.995783, W 179.539117, leg. E. Makarchenko. Paratypes: 52 adult males, 23 pupae, 28 larvae, the same data as holotype, except 22.VII–15.VIII.1978; 9 adult males, 14 pupae, 33 larvae, the same data as holotype, except 7–10.VIII. 1979; 5 adult males, the same data as holotype, except Khishnikov River, 17.VIII.1978, leg. E. Makarchenko; 2 adult males, Chukotka Autonomous Region, Anadyr District, northeastern spurs of the Koryak Highlands, unnamed stream of Gytgylveirgytgyn Lake basin (Velikaya River basin), 29.VII.1980, altitude ca 300 m above sea level, N 62.962222, E 171.805833, leg. E. Makarchenko.

Derivatio nominis. The species is named in honor of the Polish chironomid taxonomist and hydrobiologist Dr. Andrzej Kownacki, a leading specialist on Caucasian Diamesinae, namely of *Diamesa steinboeckii* group.

Description

Adult male (n = 10, except when otherwise stated). Total length 4.2–5.2 mm. Total length/wing length 1.27–1.75.

Coloration. Dark brown to black. Head brown. Thorax black. Legs brown. Abdomen dark brown to black. Wings grey.

Head (Fig. 9). Eyes hairy, reniform. Temporal setae including 0 frontals, 4–9 orbitals, 6–9 verticals and 5–6 postorbitals; frontals absent. Clypeus with 2–5 setae. Antenna with 8 flagellomeres and reduced plume of setae; number and length of these setae on 1–7 flagellomeres respectively: 2–3 (26–28 µm), 1–2 (18–32 µm), 3–5 (40–68 µm), 3 (32–44 µm); terminal flagellomere with 2 setae, 20–24 µm long in subapical and apical area. Length of 1–8 flagellomeres (µm): 105–118, 42–53, 42–46, 29–38, 29–38, 25–34, 29–38, 130–160; AR 0.38–0.50. Palpomere length (µm): 330–348, 64–84, 105–126, 78–92, 109–134. Palpomere 3 in distal part with sensilla capitata with diameter 16–20 µm. Head width/palpal length 1.20–1.25. Antennal length/palpal length 1.12–1.40.

Thorax. Antepronotum with 11–18 ventrolateral setae, 48–68 µm long. Dorsocentrals 7–12, 68–84 µm long; prealars 5–10, 60–76 µm long. Scutellum with 32–42 setae. AAIL without setae.

Wing (Fig. 31). Length 2.86–3.54 mm, width 0.93–1.10 mm. Costal extension 90–98 µm long. Anal lobe slightly reduced, rounded. Squama with 4–16 setae, 36–44 µm long. R and R₁ with 10–16 setae, R₄₊₅ with 8–17. RM/MCu 1.33–2.27.

Legs. Spur of front tibia 50–59 µm long. Spurs of mid tibia 46–59 µm and 42–63 µm long. Spurs of hind tibia 88–118 µm and 55–71 µm long. Hind tibial comb with 18–25 setae. Length (µm) and proportions of leg segments are as in Table 2.

TABLE 2. Lengths (in µm) and proportions of leg segments of *Diamesa kownackii* sp. nov., male (n=12)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅
P ₁	1820–2132	1664–1976	986–1190	374–440	221–281	111–160	145–200
P ₂	1612–1924	1248–1508	527–612	204–255	160–187	102–128	136–160
P ₃	1872–2236	1612–2028	1020–1190	493–544	221–323	119–160	153–200

continued

	LR	BV	SV	BR
P ₁	0.58–0.63	4.70–5.62	3.32–3.78	0.9–1.9
P ₂	0.37–0.42	5.28–6.02	5.38–6.11	0.9–1.6
P ₃	0.53–0.65	4.17–4.77	3.32–3.78	0.8–1.5

Hypopygium (Figs. 10–14). Tergite IX with 15–18 setae, 32–40 µm long and with narrow (6–8 µm), pale, and naked anal point, 76–116 µm long (Fig. 10) which in lateral view apically slightly curved down (Fig. 14); posterior edge of tergite IX slightly concave, anal-lateral angles protrude noticeably beyond edge (Fig. 10); anal tergal bands Y-type; median tergal band 76–104 µm long. Laterosternite IX with 6–10 setae, 32–48 µm long. Transverse sternopodeme (TSA) triangular, peaked (Figs. 11–12) and sometimes with spire-shaped apex (Fig. 11), 160–184 µm high, 216–268 µm wide at the base; TSA height/TSA width 0.71–0.86. Aedeagal lobe as in Figs. 11–12, weakly chitinized, leaf-shaped; phallapodeme sclerotized, 208–336 µm long. Gonocoxite 574–640 µm long, in middle part of dorso-internal position with small flattened transverse lobe, 112 µm long, 32–36 µm wide, directed backwards and with longitudinal lobe in middle, anterior outer margin of which is slightly rounded and not projecting (Fig. 13). Gonostylus 246–264 µm long, curved, megaseta in form of wide terminal spine 10–12 µm long, next to it there is tooth and two setae approximately of the same long. HR 2.0–2.53.

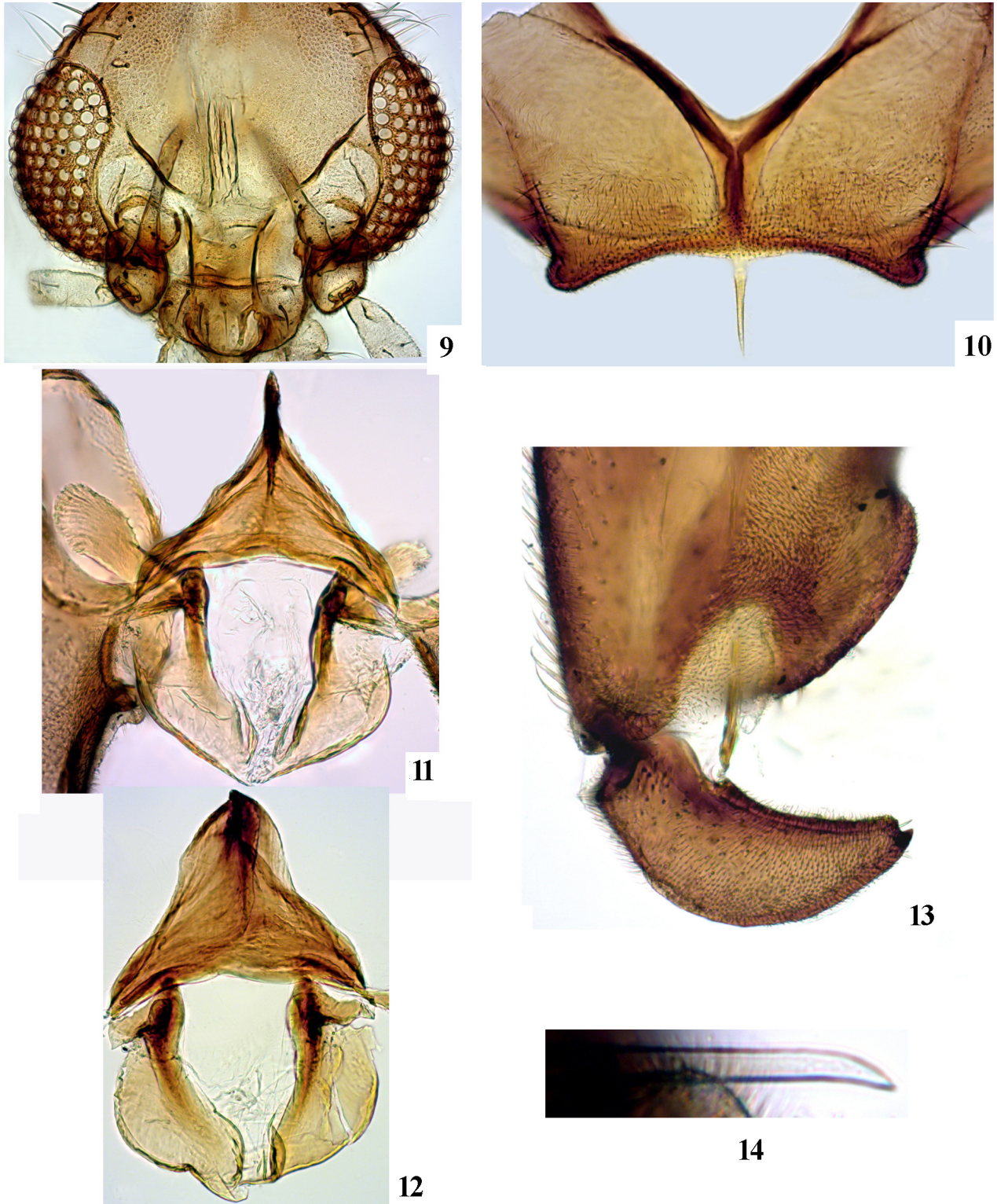
Pupa and larva were described as *D. steinboeckii* Goetghebuer by Makarchenko (1981).

Diagnosis. The male of *D. kownackii* sp. nov. is most closely related in the subgroup to the *D. steinboeckii*, since both have anal tergal bands Y-type, but the male of first species is larger (4.2–5.2 mm), it lacks the frontal setae of the head, posterior edge of tergite IX slightly concave and anal-lateral angles protrude noticeably beyond edge, median tergal band 76–104 µm long. The male of *D. steinboeckii* 2.0–3.5 mm long, with some frontal setae of the

head, posterior edge of tergite IX almost straight and anal-lateral angles not protrude beyond edge, median tergal band 108–116 μm long.

Ecology. Adults, pupae and larvae were collected from stones and boulders in high arctic river, located at an altitude of 200–400 m, at a flow rate of 0.5–0.8 m/s, with water temperatures 7.4–9.0°C. The density of larvae in the Somnitelnaya River was 8.3–16.7 ind./m² (July 1979) (Makarchenko & Makarchenko 1981).

Distribution. Known only from the type locality—Somnitelnaya River basin of Wrangel Island and northeastern spurs of the Koryak Highlands (Figs. 69–70).



FIGURES 9–14. *Diamesa kownackii* sp. nov., male. 9, head; 10, tergite IX; 11–12, transverse sternapodeme, phallapodeme and aedeagal lobe; 13, gonocoxite and gonostylus; 14, anal point in lateral view.

***Diamesa maisaraensis* Makarchenko et Semenchko, sp. nov.**

<http://zoobank.org/NomenclaturalActs/C386C93E-5502-4D9E-B02C-EB88357BA021>

(Figs. 15–21, 32, 71)

Type material. Holotype, adult male, TAJIKISTAN: Gorno-Badakhshan Autonomous Region, Shugnanskiy District, Pamir Mountains, Maisara River near Maisara Pass, altitude 4168 m above sea level, 1.VII.2016, N 37.483017, E 72.639133, leg. D. Palatov. Paratypes: 4 adult males, the same data as holotype.

Derivatio nominis. The species is named as *maisaraensis* after the type locality in Maisara River near the pass of the same name in the Pamir Mountains.

Description

Adult male (n = 6, except when otherwise stated). Total length 2.7–3.2 mm. Total length/wing length 0.85–1.07.

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

Head (Fig. 15). Eyes hairy, reniform. Temporal setae including 3–8 frontals, 6–8 orbitals, 7–17 verticals and 8–9 postorbitals. Clypeus with 4–7 setae. Antenna with 8 flagellomeres and reduced plume of setae; number and length of these setae on 1–7 flagellomeres respectively: 4–6 (24–28 µm), 1–2 (40 µm), 1–2 (32–40 µm), 1–2 (32–36 µm), 1–2 (24–32 µm), 2–3 (48–60 µm), 2 (48–60 µm); terminal flagellomere with 2–4 setae, 40–60 µm long in basal part and with 2 subapical setae, 16–20 µm long. Length of 1–8 flagellomeres (µm): 108–120, 42–52, 32–44, 28–36, 24–40, 24–34, 24–36, 124–142; AR 0.39–0.42. Palpomere length (µm): 40–88, 72–116, 116–132, 72–92, 120–140. Palpomere 3 in distal part with sensilla capitata with diameter 18–20 µm. Head width/palpal length 0.93–1.27. Antennal length/palpal length 0.89–1.08.

Thorax (Fig. 16). Antepronotum with 8–14 ventrolateral setae, 100–102 µm long. Dorsocentrals 6–8, 84–100 µm long; prealars 6–10, 77–100 µm long. Scutellum with 39–50 setae. AAIL with 5–10 setae, 40–88 µm long (Fig. 16).

Wing (Fig. 32). Length 3.0–3.16 mm, width 1.04–1.12 mm. Costal extension 36–60 µm long. Anal lobe rounded. Squama with 3–8 setae, 28–60 µm long. R and R₁ with 16–17 setae, R₄₊₅ with 10–19. RM/MCu 2.0.

Legs. Spur of front tibia 48–56 µm long. Spurs of mid tibia 48–60 µm and 52–64 µm long. Spurs of hind tibia 84–96 µm and 52–60 µm long. Hind tibial comb with 14–19 setae. Length (µm) and proportions of leg segments are as in Table 3.

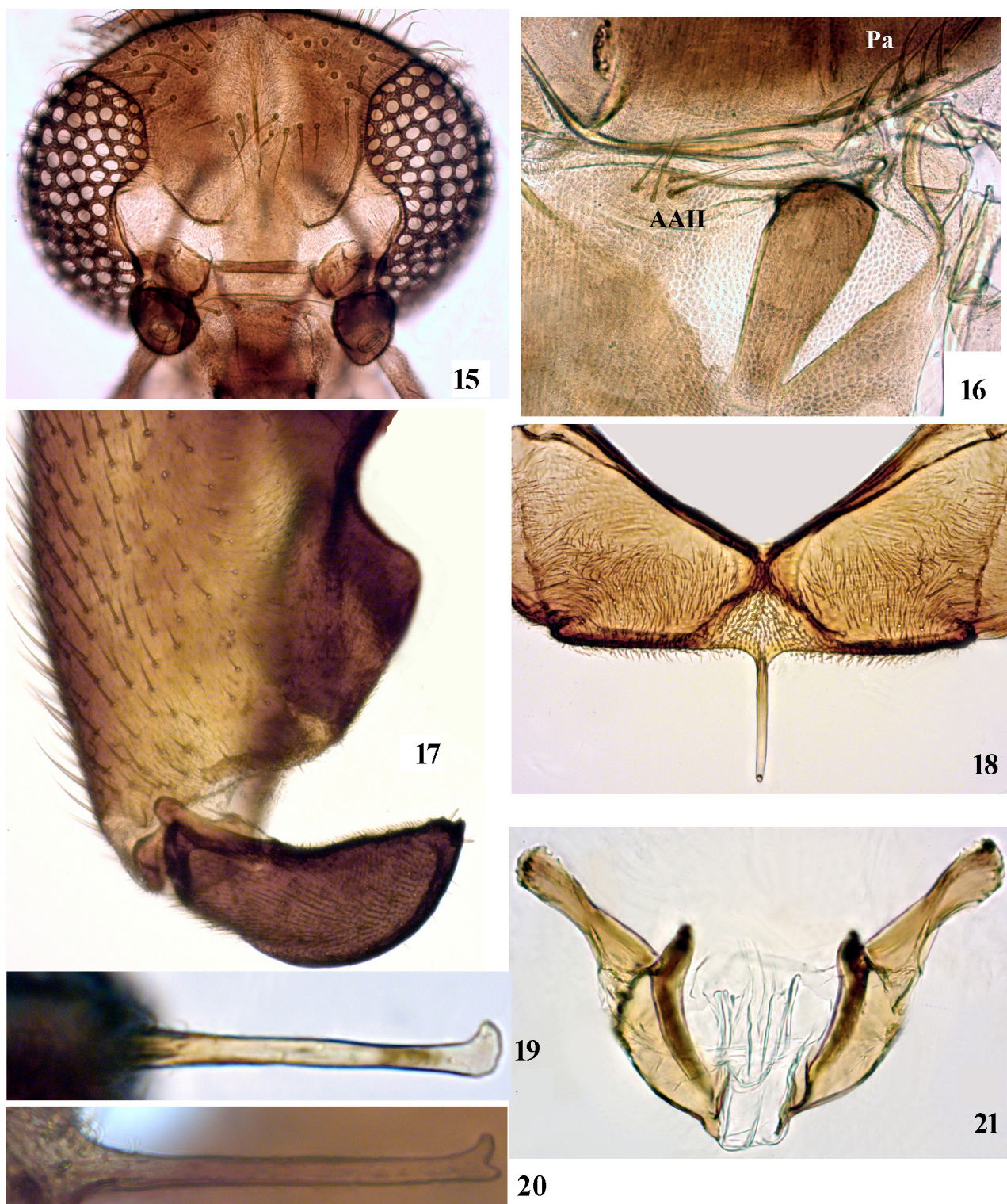
TABLE 3. Lengths (in µm) and proportions of leg segments of *Diamesa maisaraensis* sp. nov., male (n=5)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅
P ₁	1880–1960	1680–1760	1000–1040	400–440	230–280	98–120	115–160
P ₂	1673–1760	1320–1400	520–574	240–262	160–189	98–120	131–160
P ₃	1840–2000	1680–1800	1080–1160	560–580	312–320	115–120	148–160

continued

	LR	BV	SV	BR
P ₁	0.57–0.62	4.60–5.11	3.42–3.70	0.9–1.1
P ₂	0.37–0.43	5.21–5.62	5.26–6.08	0.8–0.9
P ₃	0.64–0.65	3.97–4.32	3.23–3.28	0.9–1.1

Hypopygium (Figs. 17–21). Tergite IX with 10–19 setae, 20–44 µm long and with narrow (6–6 µm), pale, and naked anal point, 100–120 µm long which in lateral view apically curved upwards and sometimes forked (Figs. 19–20); posterior edge of tergite IX almost straight, anal-lateral angles are rounded and slightly projecting (Fig. 18); anal tergal bands X-type; median tergal band 28–32 µm long. Laterosternite IX with 10–16 setae, 32–40 µm long. Transverse sternopodeme (TSA) triangular, 144–168 µm high, 180–200 µm wide at the base; TSA height/TSA width 0.73–0.89. Aedeagal lobe as in Fig. 21; phallapodeme sclerotized, 216–276 µm long. Gonocoxite 558–574 µm long, in middle part of dorso-internal position with small flattened transverse lobe, 104–112 µm long and 24–32 µm wide, directed backwards and with large longitudinal lobe in middle, anterior outer margin of which is rounded and slightly projecting (Fig. 17). Gonostylus 224–236 µm long, curved, megaseta in form of wide terminal spine 8–12 µm long, next to it there is tooth and two setae of 8–12 µm long (Fig. 6). HR 2.40–2.50.



FIGURES 15–21. *Diamesa maisaraensis* **sp. nov.**, males. **15**, head; **16**, part of thorax in lateral view; **17**, gonocoxite and gonostylus; **18**, tergite IX; **19–20**, anal point in lateral view; **21**, phallapodeme and aedeagal lobe. AAII—anterior anepisternum II, Pa—prealars.

Pupa and larva unknown.

Diagnosis The male of *D. maisaraensis* **sp. nov.** is closely related in the subgroup to the *D. dragani* **sp. nov.** and *D. marinskiyi* **sp. nov.** which have anal tergal bands X-type, but more similar to the latter because both have antenna with 8 flagellomeres, while antenna of *D. dragani* **sp. nov.** with 7 flagellomeres. Among other characteristic features of the male *D. maisaraensis* **sp. nov.** the following can be noted: AR 0.39–0.42, dorsocentrals 6–8, scutellars 39–50,

femur of front leg length 1880–1960 μm , median tergal band length 28–32 μm , anal point length 100–120 μm . The male of *D. marinskiyi* **sp. nov.** has AR 0.32–0.39, dorsocentrals 8–10, scutellars 44–46, femur of front leg length 1624–1476 μm , median tergal band length 20–24 μm , anal point length 104–116 μm .

Ecology. Adult males were collected from stones and boulders near mountain river, located at an altitude of 4168 m, at a flow rate of 0.9–1.0 m/s, with water temperatures *ca* 7–8°C.

Distribution. Known only from the type locality—Maisara River of Pamir Mountains (Gorno-Badakhshan Autonomous Region, Tajikistan) (Fig. 71).

***Diamesa marinskiyi* Makarchenko et Semenchenko, sp. nov.**

<http://zoobank.org/NomenclaturalActs/13FFB920-1638-4723-A3B4-B884C06C9D09>

(Figs. 22–25, 33, 72)

Type material. Holotype, adult male, CHINA: Xinjiang Uygur Autonomous Region, Tien Shan Mountains, Bogdo-Ula Range, Zienzan River, altitude 1923 m above sea level, 9.VII.2017, N 43.828967, E 88.172517, leg. V. Marinskiy. Paratypes: 18 adult males, the same data as holotype; 14 adult males, the same data as holotype, except Daong River, altitude 2030 m above sea level, 8.VII.2017, N 43.828967, E 88.172517, leg. V. Marinskiy.

Derivatio nominis. The species is named after the hydrobiologist Marinskiy Vadim Valerievich (Moscow State University, Russia), specialist in aquatic invertebrates of the Central Asia.

Description

Adult male ($n = 5$). Total length 2.5–2.7 mm. Total length/wing length 0.87–1.20.

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

Head (Fig. 22). Eyes hairy, reniform. Temporal setae including 8–12 frontals, 8–15 orbitals, 7–10 verticals and 5–6 postorbitals. Clypeus with 2–6 setae. Antenna with 8 flagellomeres and reduced plume of setae; number and length of these setae on 1–7 flagellomeres respectively: 4–5 (32–40 μm), 4 (24–28 μm), 1–2 (24–28 μm), 0, 1–2 (40–48 μm), 1–2 (40–48 μm), 4 (36–52 μm); terminal flagellomere with 2–4 setae, 46–48 μm long in basal part and with 2 subapical setae, 24–28 μm long. Length of 1–8 flagellomeres (μm): 100–116, 44–48, 36–40, 24–30, 28–30, 20–26, 20–24, 108–138; AR 0.32–0.39. Palpomere length (μm): 36–48, 64–68, 120–124, 84–90, 108–138. Palpomere 3 in distal part with sensilla capitata with diameter 20 μm . Head width/palpal length 1.0–1.13. Antennal length/palpal length 0.95–0.98.

Thorax. Anteprenotum with 12–19 ventrolateral setae, 44–68 μm long. Dorsocentrals 8–10, 112–120 μm long; prealars 9–10, 64–72 μm long. Scutellum with 44–46 setae. AAI with 6–7 setae, 60–68 μm long.

Wing (Fig. 33). Length 2.60–2.88 mm, width 0.92–1.04 mm. Costal extension absent. Anal lobe rounded. Squama with 6–10 setae, 32–60 μm long. R and R_1 with 10–20 setae, R_{4+5} with 11–15. RM/MCu 1.6–2.2.

Legs. Spur of front tibia 44–50 μm long. Spurs of mid tibia 48–50 μm and 48–50 μm long. Spurs of hind tibia 80–84 μm and 44–48 μm long. Hind tibial comb with 19–20 setae. Length (μm) and proportions of leg segments are as in Table 4.

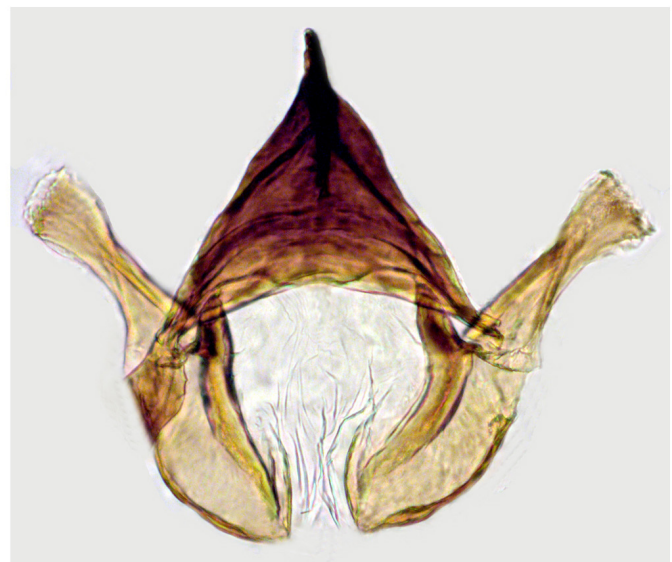
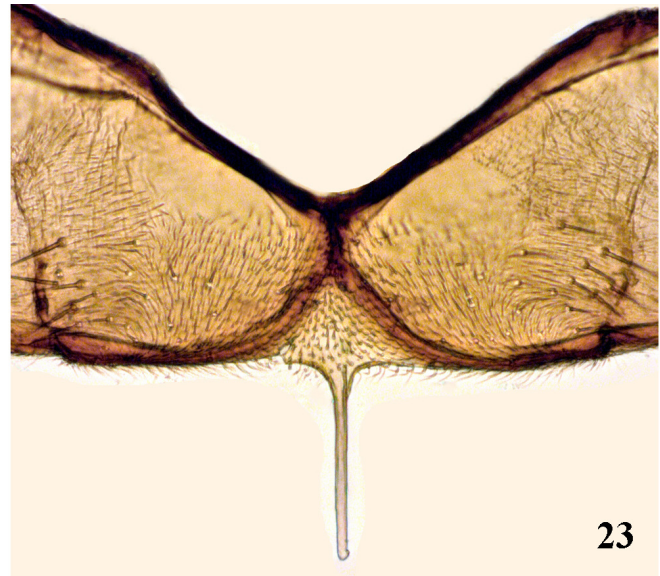
TABLE 4. Lengths (in μm) and proportions of leg segments of *Diamesa marinskiyi* **sp. nov.**, male ($n=5$)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅
P ₁	1624–1476	1230–1509	723–984	312–410	189–230	98	131
P ₂	1394–1509	1082–1246	426–558	197–221	131–148	82–98	115–131
P ₃	1525–1706	1345–1591	869–1050	476–508	262–279	98	131

continued

	LR	BV	SV	BR
P ₁	0.55–0.65	4.70–4.86	3.18–3.74	1.1–1.2
P ₂	0.39–0.45	5.07–5.85	4.94–5.85	1.0–1.1
P ₃	0.63–0.70	3.87–4.28	3.14–3.39	1.1–1.2

Hypopygium (Figs. 23–25). Tergite IX with 14–16 setae, 40–48 μm long and with narrow (8 μm), pale, and naked anal point, 104–116 μm long which in lateral view apically not curved upwards; posterior edge of tergite IX almost straight, anal–lateral angles are rounded and slightly projecting (Fig. 23); anal tergal bands X-type; median tergal band 20–24 μm long. Laterosternite IX with 10–15 setae, 40–44 μm long. Transverse sternopodeme (TSA) triangular, with spire–shape apex, 124–160 μm high, 196–208 μm wide at the base; TSA height/TSA width 0.63–0.80. Aedeagal lobe as in Fig. 25, 144–160 μm long; phallapodeme sclerotized, 108–128 μm long. Gonocoxite 459–508 μm long, in middle part of dorso–internal position with small flattened transverse lobe, 136 μm long and 32 μm wide, directed backwards and with large longitudinal lobe in middle, anterior outer margin of which is rounded and slightly projecting (Fig. 24). Gonostylus 197–212 μm long, curved, megaseta in form of wide terminal spine 12–16 μm long, next to it there is tooth and two setae of the same length. HR 2.15–2.50.



FIGURES 22–25. *Diamesa marinskiyi* sp. nov., male. 22, head; 23, tergite IX; 24, gonocoxite and gonostylus; 25, transverse sternapodeme, phallapodeme and aedeagal lobe.

Pupa and *larva* unknown.

Diagnosis. The male of *D. marinskiyi* **sp. nov.** is closely related in the subgroup to the *D. dragani* **sp. nov.** and *D. maisaraensis* **sp. nov.** which have anal tergal bands X-type, but more similar to the latter because both have antenna with 8 flagellomeres, while antenna of *D. dragani* **sp. nov.** with 7 flagellomeres. Among other characteristic features of the male *D. marinskiyi* **sp. nov.** the following can be noted: has AR 0.32–0.39, dorsocentrals 8–10, scutellars 44–46, femur of front leg length 1624–1476 μm , median tergal band length 20–24 μm , anal point length 104–116 μm . The male of *D. maisaraensis* **sp. nov.** has AR 0.39–0.42, dorsocentrals 6–8, scutellars 39–50, femur of front leg length 1880–1960 μm , median tergal band length 28–32 μm , anal point length 100–120 μm .

Ecology. Adult males were collected from stones and boulders in mountain river, located at an altitude of 1923 m and 2030 m, at a flow rate of 0.3–0.9 m/s, with water temperatures *ca* 8°C.

Distribution. Known only from the type locality—Bogdo-Ula Range of Tien Shan Mountains (Xinjiang Uygur Autonomous Region, China) (Fig. 72).

Diamesa steinboeckii Goetghebuer

(Figs. 26–29, 34–36, 73, 77)

Diamesa steinboeckii Goetghebuer, 1933: 73, 1939: 16; Pagast 1947: 487; Wülker 1959, figs. 2–4; Pankratova 1970: 83; Serra-Tosio 1971: 182, 1974: 168; Schmid 1993: 41; Langton & Visser 2003: 47; Ashe & O'Connor 2009: 285; Rossaro & Lenicioni 2015: 65; Montagna *et al.* 2016: 329.

Material examined. 1 adult male, TAJIKISTAN: Murgab District, Gorno-Badakhshan Autonomous Region, Pamir Mountains, Sasykteke River, (Sauksay River basin), altitude 3815 m above sea level, 25.VIII.1987, N 39.288347, E 72.370182, leg. D. Shcherbakov; 2 adult males, 2 mature pupae (male and female), SWITZERLAND: Valais Canton, Swiss Alps, Muttbach Stream, 16.IX.2018, altitude 2500 m above sea level, leg. B. Lods-Crozet; 1 adult male, FRANCE: cold spring, French Alps, Massif of Pelvoux, Lautaret Pass, 9.IX.1966, leg. B. Serra-Tosio; 1 mature pupa (male), 2 mature pupae (females), 2 larvae, French Alps, Massif of Aiguilles-Rouges, *Balm Stream*, 8.IX.2021, altitude 1862 m above sea level, N 45.97933, E 6.840494, leg. P. Clévenot.

Remarks. Despite the rather widespread of *D. steinboeckii* in the mountains of Europe, a detailed description of the adult male, the variability of brachypteris and macropteris forms at different altitude above sea level, was made only for the French Alps (Serra-Tosio 1971, 1973, 1974). Therefore, we found it expedient to give a brief re-description of males based on our material from the Pamir Mountains, where the species was found for the first time, and the Swiss Alps, as well as to add some data to the description of Serra-Tosio (1971, 1974) from the French Alps. Data of Serra-Tosio (1974) in our description are highlighted in bold.

Description of adult male from Pamir Mountains

Adult male (n=1). Total length 2.30 mm. Total length/wing length 3.97.

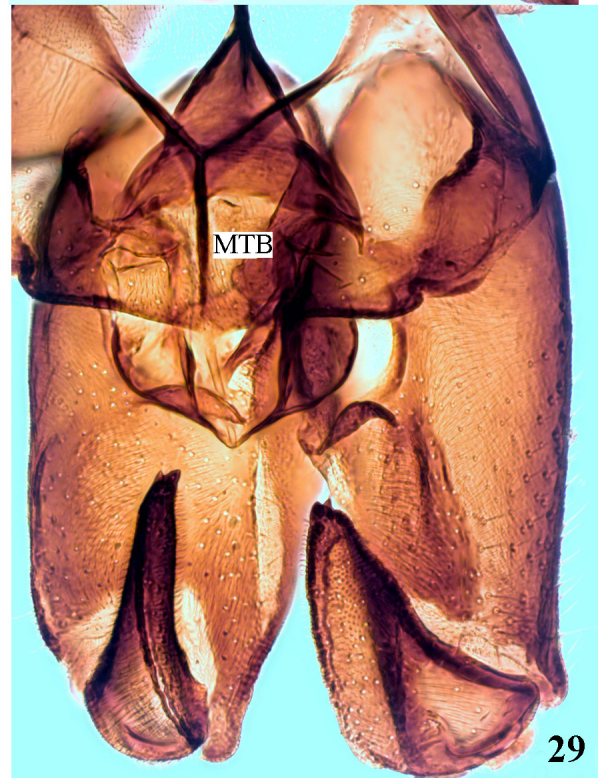
Coloration. Brown to dark brown. Wings yellowish.

Head. Eyes hairy, reniform. Temporal setae including 2 frontals, 4 orbitals, 5–6 verticals and 4 postorbitals. Clypeus with 5 setae. Antenna with 8 flagellomeres and reduced plume of setae; number and length of these setae on 1–7 flagellomeres respectively: 4 (24–28 μm), 2 (24–32 μm), 1 (28 μm), 0, 2 (20–24 μm), 0, 4 (28–36 μm); terminal flagellomere only with 2 subapical setae, 12 μm long. Length of 1–8 flagellomeres (μm): 96, 36, 32, 24, 28, 22, 24, 108; AR 0.41. Palpomere length (μm): 32, 48, 98, 60, 84. Palpomere 3 in distal part with sensilla capitata with diameter 20 μm . Head width/palpal length 1.12. Antennal length/palpal length 1.15.

Thorax. Anteprepronotum with 8 ventrolateral setae. Dorsocentrals 7, prealars 3. Scutellum with 10 setae. AAIL without setae.

Wing reduced (Fig. 36). Length 0.58 mm, width 0.18 mm. Costal extension absent. Anal lobe reduced. Squama without setae. Venation reduced. R and R₁ with 4 setae.

Legs. Spur of front tibia 32 μm long. Both spurs of mid tibia 32 μm long. Spurs of hind tibia 52 μm and 48 μm long. Hind tibial comb with 16–18 setae. Length (μm) and proportions of leg segments are as in Table 5.

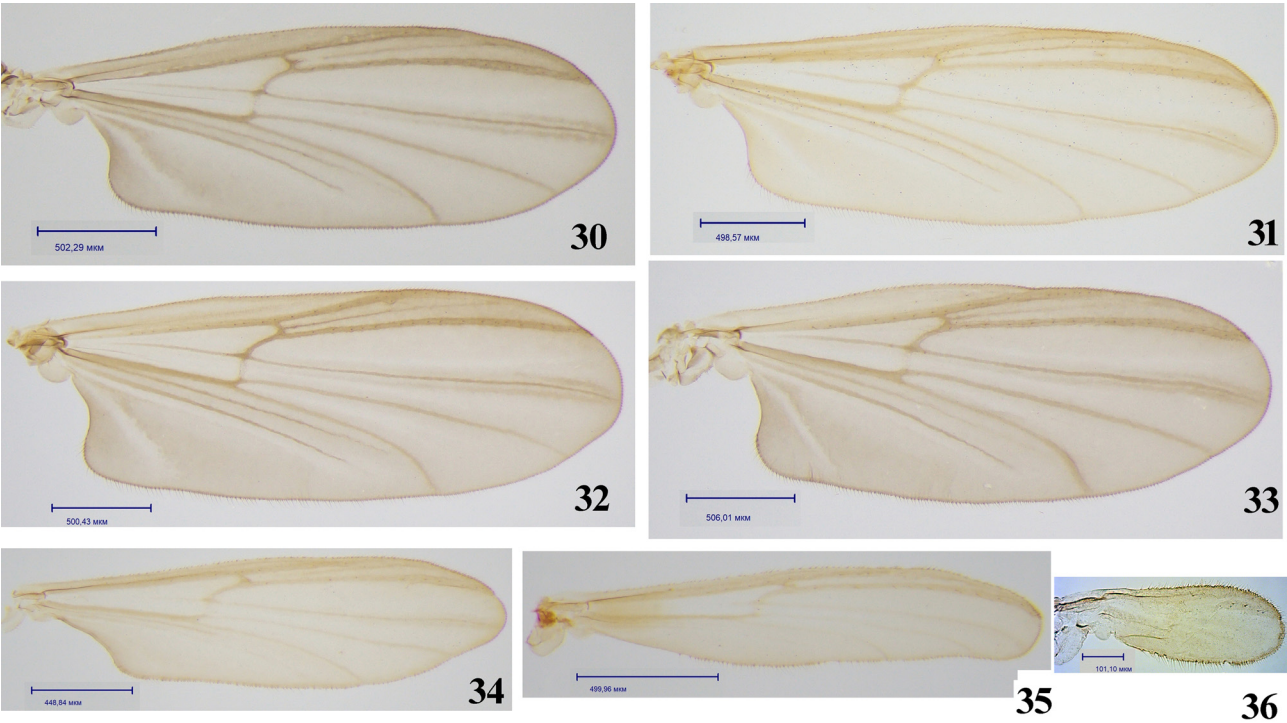


FIGURES 26–29. Males of *Diamesa steinboeckii* Goetghebuer from Pamir Mountains (**26**), Swiss Alps (**27–28**) and French Alps (**29**). **26**, gonocoxite and gonostylus; **27**, **29**, hypopygium in dorsal view; **28**, transverse sternapodeme, phallapodeme and aedeagal lobe. Designations are the same as in Figures 1–8.

TABLE 5. Lengths (in μm) and proportions of leg segments of *Diamesa steinboeckii* Goetghebuer from Pamir Mountains, male ($n=1$)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
P ₁	1509	1345	754	312	156	82	115	0.56	5.43	3.79	1.0
P ₂	1279	1066	459	197	131	82	115	0.43	5.34	5.11	0.9
P ₃	1574	1296	853	459	246	82	115	0.66	4.13	3.36	1.1

Hypopygium (Fig. 26). Tergite IX with 15 setae, 16–20 μm long and with narrow (6 μm), pale, and naked anal point, 80 μm long which in lateral view apically not curved upwards; posterior edge of tergite IX almost straight, anal-lateral angles are rounded; anal tergal bands Y-type; median tergal band 112 μm long. Laterosternite IX with 7 setae, 20 μm long. Transverse sternopodeme (TSA) triangular, with spire-shaped apex, 96 μm high, 160 μm wide at the base; TSA height/TSA width 0.60. Aedeagal lobe 92 μm long; phallapodeme sclerotized, 104 μm long. Gonocoxite 404 μm long, in middle part of dorso-internal position with small flattened transverse lobe, directed backwards and with middle size of longitudinal lobe, anterior outer margin of which is rounded and not projecting (Fig. 26). Gonostylus 188 μm long, curved, megaseta in form of wide terminal spine 12 μm long, next to it there is tooth and two setae of the same length. HR 2.15.



FIGURES 30–36. Wings of *Diamesa dragani* sp. nov. (30), *D. kownackii* sp. nov. (31), *D. maisaraensis* sp. nov. (32), *D. marinskiyi* sp. nov. (33) and *D. steinboeckii* Goetghebuer from Swiss Alps (34), French Alps (35) and Pamir Mountains (36).

Description of adult male from Swiss Alps

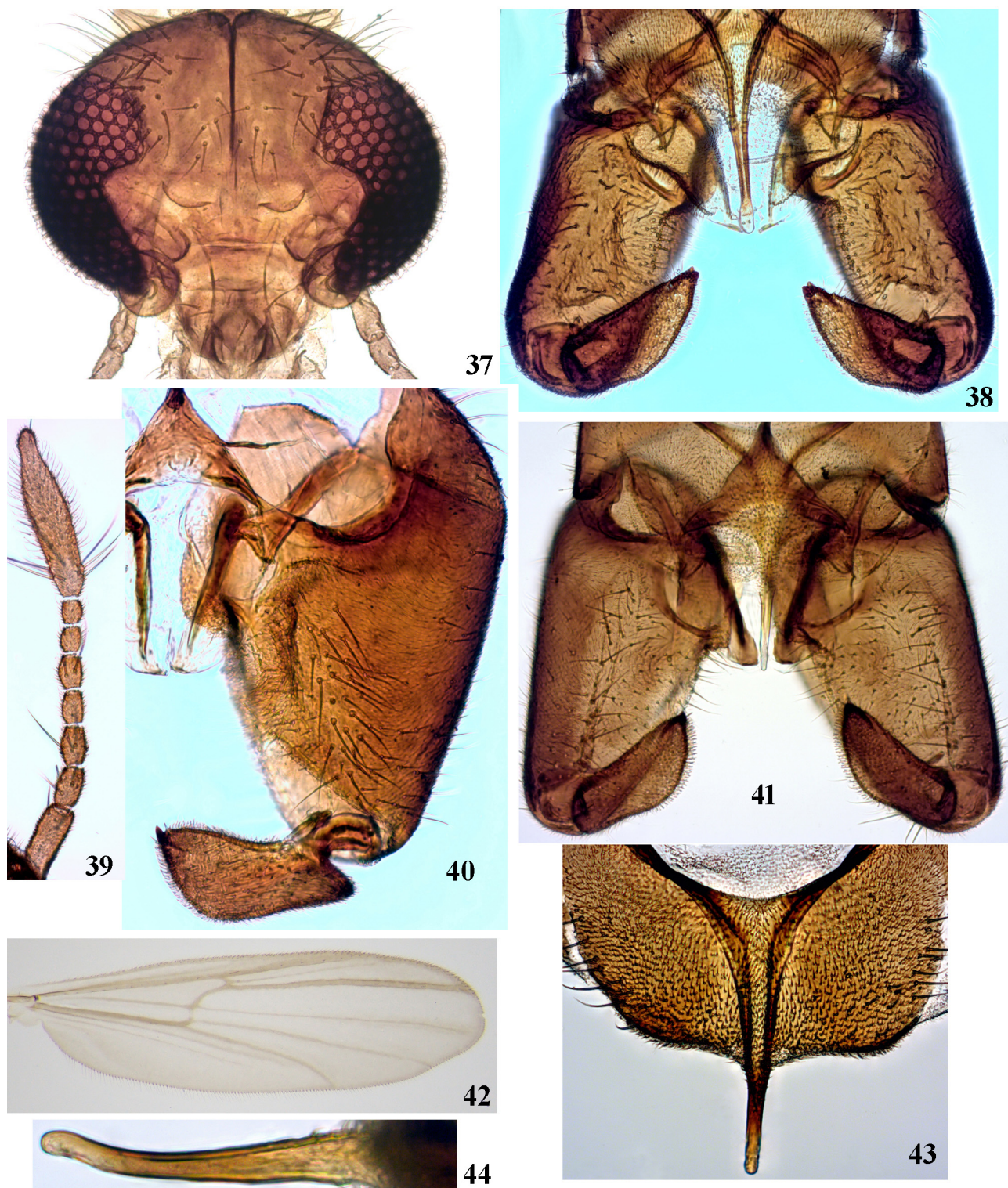
Adult male ($n = 2$). Total length 2.2–2.4 mm. Total length/wing length 1.0–1.92.

Coloration. Dark brown. Wings brownish.

Head. Eyes hairy, reniform. Temporal setae including 2–3 frontals, 3–4 orbitals, 3–5 verticals and 2 postorbitals. Clypeus with 4 setae. Antenna with 8 flagellomeres and reduced plume of setae; number and length of these setae on 1–7 flagellomeres respectively: 3 (28–36 μm), 3 (28–32 μm), 1 (32 μm), 0, 1 (40 μm), 0, 4 (48–56 μm); terminal flagellomere with 2, 48–56 μm long in basal part and with 2 subapical setae, 16–20 μm long. Length of 1–8 flagellomeres (μm): 100–108, 38–48, 32–36, 28, 26–28, 20–24, 28–36, 108–144; AR 0.38–0.50. Palpomere length (μm): 32–36, 48–60, 100–112, 68–76, 96–100. Palpomere 3 in distal part with sensilla capitata with diameter 20–24 μm . Head width/palpal length 1.0–1.13. Antennal length/palpal length 1.04–1.09.

Thorax. Anteprenotum with 15–17 ventrolateral setae, 32–40 μm long. Acrostichals 2–3, 32–40 μm long; dorsocentrals 10–11, 52–64 μm long; prealars 6, 52–64 μm long. Scutellum with 23–25 setae. AAIL without setae.

Wing slightly reduced (Fig. 34). Length 1.92–2.30 mm, width 0.46–0.59 mm. Anal lobe reduced. Squama with 4–8 setae, 28–40 μm long. Venation partly reduced; R_{2+3} invisible. R and R_1 with 4–7 setae, R_{4+5} with 8 setae. RM/MCu 2.8–3.0.



FIGURES 37–44. Males of *Diamesa zagrosica* sp. nov. (37, 41), *D. sakartvella* Kownacki et Kownacka (38, 42) and *D. moubayedii* sp. nov. (39–40, 43–44). 37, head; 38, 41, hypopygium in dorsal view; 40, hypopygium in dorsal view without tergite IX; 42, wing; 43, tergite IX; 44, anal point in lateral view.

Legs. Spur of front tibia 36–40 μm long. Spurs of mid tibia 32–44 μm and 36–44 μm long. Spurs of hind tibia 64–76 μm and 36–44 μm long. Hind tibial comb with 21 setae. Length (μm) and proportions of leg segments are as in Table 6.

TABLE 6. Lengths (in μm) and proportions of leg segments of *Diamesa steinboeckii* Goetghebuer from Swiss Alps, male (n=2)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅
P ₁	1542–1800	1460–1640	804–935	312–361	197–213	98–115	123–131
P ₂	1345–1542	1140–1230	410–426	164–180	131–148	98	115
P ₃	1566–1800	1378–1560	853–984	410–492	246–262	98	115–123

continued

	LR	BV	SV	BR
P ₁	0.55–0.57	5.21–5.34	3.68–3.73	0.9–1.1
P ₂	0.35–0.36	5.70–5.91	6.06–6.51	0.9–1.0
P ₃	0.62–0.63	4.37–4.46	3.41–3.45	0.9–1.1

Hypopygium (Figs. 27–28). Tergite IX with 18–26 setae, 10–12 μm long and with narrow, pale and naked anal point, 88–100 μm long which; posterior edge of tergite IX almost straight, anal-lateral angles are rounded and slightly projecting (Fig. 27); anal tergal bands Y-type; median tergal band 108–116 μm long. Laterosternite IX with 7–14 setae, 20–28 μm long. Transverse sternopodeme (TSA) triangular with spire-shaped apex, 128–160 μm high, 180–200 μm wide at the base; TSA height/TSA width 0.64–0.82. Aedeagal lobe as in Fig. 28, 172 μm long; phal-lapodeme sclerotized, 104–132 μm long. Gonocoxite 500–508 μm long, in middle part of dorso-internal position with small flattened transverse lobe, directed backwards and with longitudinal lobe in middle, anterior outer margin of which is rounded and not projecting. Gonostylus 224–262 μm long, curved, megaseta in form of wide terminal spine 12 μm long, next to it there is tooth and two setae of the same length. HR 1.94–2.21.

Description of adult male from French Alps (Massif of Pelvoux)

Adult male (n = 2). Total length **2.0–3.5** mm.

Coloration. Dark brown. Wings brownish.

Head. Eyes hairy, reniform. Temporal setae including 3–7 frontals, 4–5 orbitals, 10–12 verticals. Clypeus with 2–9 setae. Antenna with 8 flagellomeres and reduced plume of setae; number and length of these setae on 1–7 flagellomeres respectively: 4 (24–32 μm), 1–2 (28 μm), 2 (28–32 μm), 0, 0, 0, 3–4 (28–32 μm); terminal flagellomere with 2 setae, 32–40 μm long in basal part and with 2 subapical setae, 16–20 μm long. Length of 1–8 flagellomeres (μm): 108–120, 44–47, 36–42, 32–34, 29–32, 24–29, 24–26, 128–135; AR **0.40**–0.43. Palpomere length (μm): 32, **60**, 104–115, 72–80, **95**–96. Palpomere 3 in distal part with sensilla capitata with diameter 20 μm . Antennal length/palpal length 1.18.

Thorax. Antepronotum with **8–22** ventrolateral setae, 28–40 μm long. Acrostichals 0; dorsocentrals 6–**15**, 60–100 μm long; prealars 3–9, 36–52 μm long. Scutellum with *ca* 17 setae. AAIL with **0–4** setae.

Wing slightly reduced (Fig. 35). Length 1.8 mm (**0.5–2.7**), width 0.34–0.36 mm. Anal lobe reduced. Squama with 5 setae, 24–28 μm long. Venation partly reduced; R₂₊₃ invisible. R and R₁ with 10 setae, R₄₊₅ with 8–9 setae. RM/MCu 2.8.

Legs. Spur of front tibia 40 μm long. Spurs of mid tibia 32–36 μm long. Spurs of hind tibia 76 μm and 44 μm long. Hind tibial comb with 19 setae. Length (μm) and proportions of leg segments are as in Table 7.

Hypopygium (Fig. 29). Tergite IX with 22 setae, 8–12 μm long and with narrow, pale and naked anal point, 84 μm long which; posterior edge of tergite IX almost straight, anal-lateral angles are rounded and slightly projecting; anal tergal bands Y-type; median tergal band 116 μm long (Fig. 29). Laterosternite IX with 7 setae, 16–20 μm long. Transverse sternopodeme (TSA) triangular with spire-shaped apex, 156 μm high, 176 μm wide at the base; TSA height/TSA width 0.89. Aedeagal lobe as in Fig. 28; phallapodeme sclerotized. Gonocoxite 508 μm long, in middle

part of dorso-internal position with small flattened transverse lobe, directed backwards and with longitudinal lobe in middle, anterior outer margin of which is rounded and not projecting. Gonostylus 197 μm long, curved, megaseta in form of wide terminal spine 12 μm long, next to it there is tooth and two setae of the same length. HR 2.58.

TABLE 7. Lengths (in μm) and proportions of leg segments of *Diamesa steinboeckii* Goetghebuer from French Alps (Massif of Pelvoux), male (n=2). Data of Serra-Tosio (1974) are in bold.

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅
P ₁	1720– 1830	1480– 1680	869– 925	328– 360	189– 195	98– 105	123– 130
P ₂	1427– 1585	1107– 1245	426– 440	164– 180	131– 140	95 –98	115– 130
P ₃	1680– 1820	1440– 1600	869– 960	426– 480	246– 255	98– 110	123– 150

continued

	LR	BV	SV	BR
P ₁	0.56 –0.59	5.51– 5.61	3.68– 3.79	0.9
P ₂	0.35 –0.38	5.83– 6.00	5.95– 6.43	0.9
P ₃	0.60	4.42 –4.47	3.56 –3.59	1.0

Ecology. Adult male in Pamir Mountains was found near the stream at an altitude of 3815 m, while adults, pupae and larvae in Swiss Alps were collected from stones and boulders in stream, located at an altitude of 2500 m, with water temperatures 4.7°C and conductivity 130 microS/cm (pers. comm. by B. Lods-Crozet). The ecology and ethology of the *D. steinboeckii* population from Massif Pelvoux of the French Alps is considered in the works of Serra-Tosio (1973, 1974). Mature pupae and larvae of this species for DNA-barcoding from French Alps were collected by P. Clévenot from the Massif of Aiguilles-Rouges in a stream at an altitude of 1862 m.

Distribution. Previously was known from the mountainous regions of Europe (Ashe & O'Connor 2009) (Figs. 73, 77). For Central Asia (Pamir Mountains) it is recorded for the first time.

Diamesa longipes subgroup

Diamesa zagrosica Makarchenko *et* Semenchenko, sp. nov.

<http://zoobank.org/NomenclaturalActs/678CF541-8E78-4AC4-BE01-B73D857EA04E>

(Figs. 37, 41, 48–49, 53–54, 56, 76)

Type material. Holotype, adult male, IRAN: Zagros Mountains, Lorestan Province, Aligudarz County, Dalooni Preserve Park, Dare Daei River in the alpine zone of Zagros ridge, altitude 2244 m above sea level, N 33.186067, E 49.510117, 13.VI.2019, leg. D. Palatov. Paratypes: 4 adult males, the same data as holotype; 6 adult males, the same data as holotype, except Dez (Sesar) River, 1 km to North of Sepid Dasht Town, alt. 1022 m above sea level, N 33.226117, E 48.885667, 13.VI.2019, leg. D. Palatov.

Derivatio nominis. The species is named as *zagrosica* after the type locality in Zagros Mountains of Lorestan Province, Iran.

Description

Adult male (n = 5, except when otherwise stated). Total length 2.3–2.8 mm. Total length/wing length 0.89–0.90.

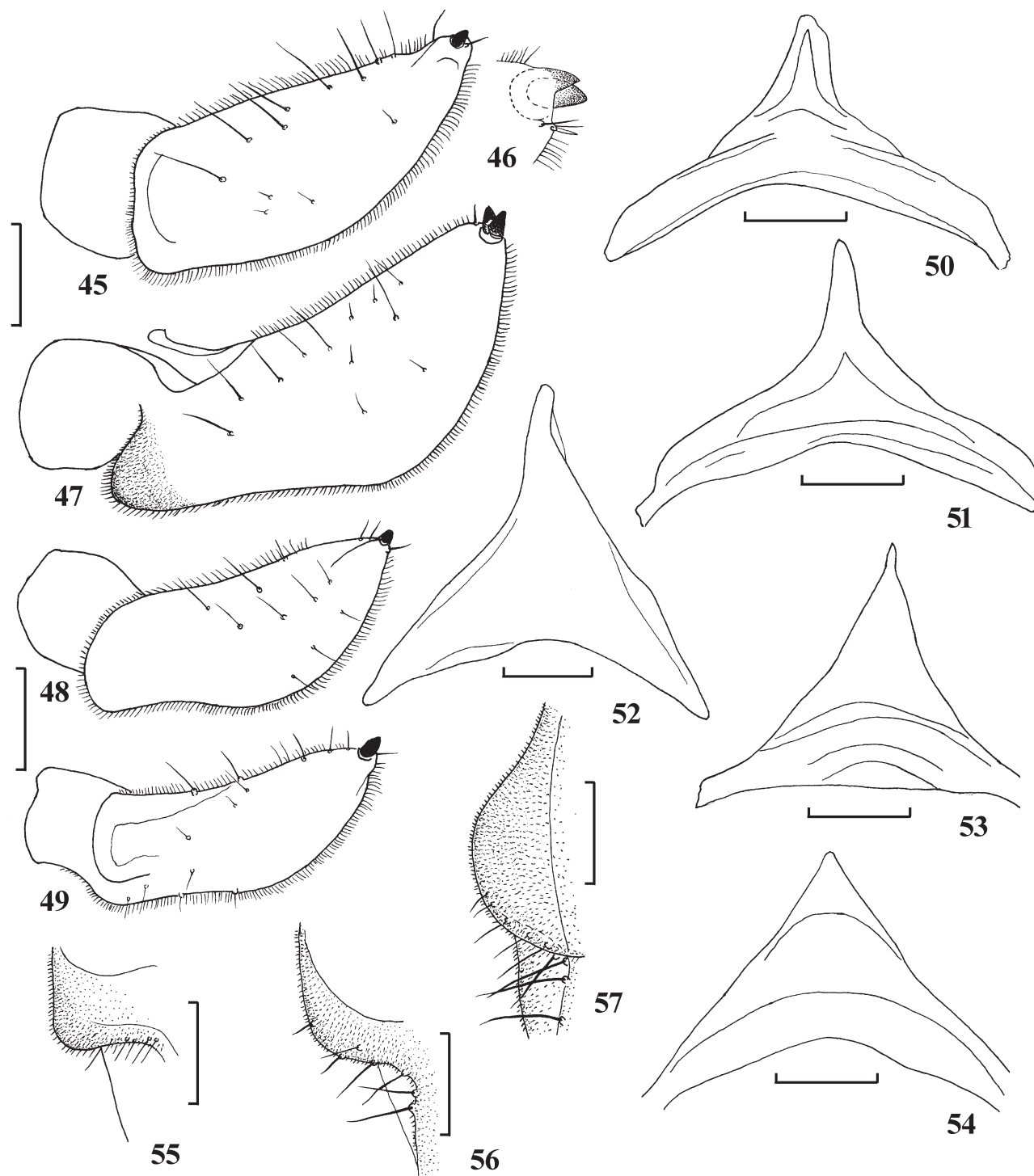
Coloration. Dark brown to black. Head, thorax and abdomen dark brown. Legs light brown to brown. Wings dark grey, with brownish veins.

Head (Fig. 37). Eyes hairy, reniform. Temporal setae including 6–7 frontals, 10–12 orbitals, 7–13 verticals and 8–9 postorbitals. Clypeus with 9 setae. Antenna with 8 flagellomeres and reduced plume of setae; number and length of these setae on 1–7 flagellomeres respectively: 1–3 (32 μm), 3 (32–36 μm), 3 (32–36 μm), 0, 2 (48–52 μm), 0; terminal flagellomere with 6 setae, 72–88 μm long in basal part and with 2 subapical setae, 28–32 μm long. Length of 1–8 flagellomeres (μm): 80–96, 44–48, 40–44, 28–36, 28–32, 22–24, 22–24, 160–164; AR 0.56–0.59. Palpomere length (μm): 44–52, 64–68, 96–100, 96–108, 136–140. Palpomere 3 in distal part with sensilla capitata with diameter 20–22 μm . Head width/palpal length 1.03–1.06. Antennal length/palpal length 0.94–0.97.

Thorax. Anteprenotum with 12–13 ventrolateral setae, 64–100 μm long. Dorsocentrals 17–20, 80–140 μm long (2 rows in front); prealars 7–10, 92–104 μm long. Scutellum with *ca* 20 setae.

Wing. Length 2.56–2.68 mm, width 0.80–0.84 mm. Anal lobe rounded. Squama with 13–16 setae, 20–48 μm long. R and R_1 with 31–37 setae, R_{4+5} with 6–9 (in distal part). RM/MCu 2.0–2.5.

Legs. Spur of front tibia 28–36 μm long. Spurs of mid tibia 36–40 μm and 40 μm long. Spurs of hind tibia 68–74 μm and 44 μm long. Hind tibial comb with 17 setae. Length (μm) and proportions of leg segments are as in Table 8.



FIGURES 45–57. Some details of male's hypopygium of *Diamesa sakartvella* Kownacki et Kownacka (45, 50–51, 57), *D. moubayedii* sp. nov. (46–47, 52, 55), *D. zagrosica* sp. nov. (48–49, 53–54, 56). 45–49, gonostylus; 46, apex of gonostylus; 50–54, transverse sternapodeme; 55–57, superior volsellae.

TABLE 8. Lengths (in μm) and proportions of leg segments of *Diamesa zagrosica* **sp. nov.**, male (n=5)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅
P ₁	1427–1476	1312–1345	918–943	476	279–295	98–107	123–131
P ₂	1410–1423	1197–1214	374–590	287–295	197–210	98–107	115–131
P ₃	1492–1509	1394–1443	1017	508–525	295–312	98–107	123–131

continued

	LR	BV	SV	BR
P ₁	0.68–0.72	3.65–3.83	2.90–3.07	1.5–2.2
P ₂	0.47–0.49	4.43–4.59	4.42–4.57	1.1–1.2
P ₃	0.70–0.73	3.72–3.75	2.84–2.90	1.1–1.2

Hypopygium (Figs. 41, 48–49, 53–54, 56). Tergite IX densely covered with strong macrotrichia apices of which are directed anteriorly, with 11–13 setae, 12–22 μm long (laterally setae longer) and with narrow (8–12 μm), weakly chitinized and naked anal point, 120–140 μm long (Fig. 41). Laterosternite IX with 9–16 setae, 24–28 μm long. Transverse sternopodeme (TSA) regular triangular, 84–108 μm high, 162–172 μm wide at the base (Figs. 53–54); TSA height/TSA width 0.49–0.67. Aedeagal lobe 136–168 μm long; phallapodeme sclerotized, 108–112 μm long. Gonocoxite 352–380 μm long; superior volsellae rounded-angular, along margin with 4–6 setae, 28–36 μm long (Fig. 56). Gonostylus 148–168 μm long, weakly curved, slightly expanded in the distal half along the outer edge (Figs. 48–49); in apical part with megaseta in form of wide terminal spine, 8–12 μm long and tooth the same size, next to it there is two setae approximately of the same length. HR 2.15–2.26.

Pupa and larva unknown.

Diagnosis. The male of *D. zagrosica* **sp. nov.** is closely related in the subgroup to the *D. sakartvella* and *D. moubayedii* **sp. nov.** from which it can be distinguished by shape and structure of transverse sternopodeme, inferior volsellae and gonostylus. Transverse sternopodeme of *D. zagrosica* **sp. nov.** is regular triangular shape (Figs. 53–54), of medium height (84–108 μm) and not very wide basally (162–172 μm), the ratio of its height to width is 0.49–0.67; superior volsellae rounded-angular (Fig. 56); gonostylus slightly expanded in the distal half along the outer edge and with small rounded projection basally (Figs. 48–49). Also, male of this species has shortest legs, femur of front leg 1427–1476 μm long. Transverse sternopodeme of *D. sakartvella* **sp. nov.** is wide triangular (Figs. 50–51), of low height (68–88 μm) and very wide basally (172–188 μm), the ratio of its height to width is 0.36–0.50; superior volsellae rounded (Fig. 57); gonostylus almost not expanded in the distal half along the outer edge and without projection basally (Figs. 45); femur of front leg 1607–1960 μm long. Transverse sternopodeme of *D. moubayedii* **sp. nov.** is triangular shape (Fig. 52), of great height (104–140 μm) and not very wide basally (148–184 μm), the ratio of its height to width is 0.80–0.89; superior volsellae angular (Fig. 55); gonostylus expanded in the distal half along the outer edge and with rounded-angular projection basally (Figs. 40, 47); femur of front leg 1920–2040 μm long.

Ecology. Adult males were collected from stones and boulders in high mountain river, located at an altitude of 2244 m, at a flow rate of 0.4–0.9 m/s, with water temperatures *ca* 4.8°C.

Distribution. Known only from the type locality—Zagros Mountains in Iran (Fig. 76).

Diamesa sakartvella Kownacki et Kownacka

(Figs. 38, 42, 45, 50–51, 57, 74)

Diamesa sakartvella Kownacki et Kownacka, 1973: 21; Langton & Visser 2003: 43; Ashe & O'Connor 2009: 284.

Material examined. 11 adult males, RUSSIA: Greater Caucasus, Republic of North Ossetia-Alania, Alagirsky District, Skazdon River near the Tsey (Tseyskoe gorge) ski resort, altitude 1943 m above sea level, N 42.784167, E 43.903667, 27.VII.2019, leg. D. Palatov.

Description

Adult male (n = 5, except when otherwise stated). Total length 2.9–3.1 m. Total length/wing length 1.04–1.19.

Coloration. Brown to dark brown. Head, thorax and abdomen dark brown. Legs brown. Wings brownish gray, with brownish veins.

Head. Eyes hairy, reniform. Temporal setae including 7–8 frontals, 7–8 orbitals, 7–9 verticals and 3–4 post-orbitals. Frontal tubercles 12 µm height and 56–60 µm width. Clypeus with 9 setae. Antenna with 8 flagellomeres and reduced plume of setae; number and length of these setae on 1–7 flagellomeres respectively: 1–2 (52 µm), 2 (60–72 µm), 2 (60–76 µm), 1–2 (76 µm), 0, 0; terminal flagellomere with 3–4 setae, 60–80 µm long in basal part and with 2 subapical setae, 28–32 µm long. Length of 1–8 flagellomeres (µm): 80, 40, 40, 32, 32, 28, 32, 164; AR 0.58. Palpomere length (µm): 40, 76, 128, 92, 112. Palpomere 3 in distal part with sensilla capitata with diameter 28 µm. Head width/palpal length 0.91. Antennal length/palpal length 1.0.

Thorax. Anteprenotum with 11–13 ventrolateral setae, 36–48 µm long. Dorsocentrals 11–18, 92–120 µm long (2 rows in front); prealars 3–7, 92–104 µm long. Scutellum with 10–12 setae.

Wing (Fig. 42). Length 2.4–2.8 mm, width 0.72–0.84 mm. Anal lobe reduced. Squama with 9–14 setae, 52–60 µm long. R and R₁ with 22–25 setae, R₄₊₅ with 5 (in distal part). Costal extension 48–68 µm. RM/MCu 1.9–2.0.

Legs. Spur of front tibia 28–32 µm long. Spurs of mid tibia equal length (32 µm). Spurs of hind tibia 68 µm and 36–40 µm long. Hind tibial comb with 24–26 setae. Length (µm) and proportions of leg segments are as in Table 9.

TABLE 9. Lengths (in µm) and proportions of leg segments of *Diamesa sakartvella* Kownacki et Kownacka, male (n=5)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅
P ₁	1607–1960	1394–1880	943–1160	459–600	295–380	100–115	131–140
P ₂	1525–1680	1214–1600	590–720	328–380	189–260	98–120	123–160
P ₃	1607–2040	1476–1840	968–1280	590–640	312–360	98–100	131–140

continued

	LR	BV	SV	BR
P ₁	0.62–0.68	4.10–4.35	3.15–3.31	1.5–1.8
P ₂	0.45–0.49	4.35–4.98	4.56–4.64	1.4–1.5
P ₃	0.66–0.76	3.97–4.16	2.75–3.18	1.5–1.8

Hypopygium (Figs. 38, 45, 50–51, 57). Tergite IX densely covered with strong macrotrichia apices of which are directed anteriorly, with 17–19 setae, 20–96 µm long and with narrow (14–16 µm), chitinized and naked anal point, 120–156 µm long. Laterosternite IX with 12–14 setae, 20–24 µm long. Transverse sternopodeme (TSA) wide triangular, 68–92 µm high, 172–192 µm wide at the base (Figs. 50–51); TSA height/TSA width 0.36–0.50. Aedeagal lobe 140 µm long (n=1); phallapodeme sclerotized, 120 µm long (n=1). Gonocoxite 372–379 µm long; superior volsellae rounded, along margin with 4–7 setae, 20–40 µm long (Fig. 57). Gonostylus 160–184 µm long, almost not expanded in the distal half along the outer edge (Fig. 45); in apical part with megaseta in form of wide terminal spine, ca 12 µm long and tooth the same size, next to it there is two setae approximately of the same length. HR 2.02–2.10.

Pupa and larva unknown.

Diagnosis. The male of *D. sakartvella* is closely related in the subgroup to *D. zagrosica* **sp. nov.** and *D. moubayedii* **sp. nov.** from which it can be distinguished by shape and structure of transverse sternopodeme, inferior volsellae and gonostylus. Transverse sternopodeme of *D. sakartvella* is wide triangular (Figs. 50–51), of low height (68–88 µm) and very wide basally (172–188 µm), the ratio of its height to width is 0.36–0.50; superior volsellae rounded (Fig. 57); gonostylus almost not expanded in the distal half along the outer edge and without projection basally (Fig. 45). Transverse sternopodeme of *D. zagrosica* **sp. nov.** is regular triangular shape (Figs. 53–54), of medium height (84–108 µm) and not very wide basally (162–172 µm), the ratio of its height to width is 0.49–0.67; superior volsellae rounded-angular (Fig. 56); gonostylus slightly expanded in the distal half along the outer edge and with small rounded projection basally (Figs. 48–49). Transverse sternopodeme of *D. moubayedii* **sp. nov.** is triangular shape (Fig. 52), of great height (104–140 µm) and not very wide basally (148–184 µm), the ratio of its height to width is 0.80–0.89; superior volsellae angular (Fig. 55); gonostylus expanded in the distal half along the outer edge and with rounded-angular projection basally (Fig. 47).

Ecology. Adult males were collected from stones and boulders at the source of a mountain river, located at an altitude of 1943 m, at a flow rate of 0.3–0.8 m/s, with water temperatures ca 7°C.

Distribution. Known only from Caucasus (Fig. 74).

***Diamesa moubayed* Makarchenko et Semenchenko, sp. nov.**

<http://zoobank.org/NomenclaturalActs/ECE88876-308D-4C72-9120-F8DECF3CD8D2>

(Figs. 39–40, 43–44, 46–47, 52, 55, 75)

Diamesa sakartvella Kownacki et Kownacka; Moubayed & Laville 1983: 222.

Type material. Holotype, adult male, LEBANON: glacial springs and small cold stream located in the upper basin of the Beirut River, Mount Sannine, N 33.9025, E 35.783889, 5.VIII.2005, altitude 2000 m above sea level, leg. J. Moubayed. Paratypes: 6 adult males, the same data as holotype.

Derivatio nominis. The species is named in honor of the French chironomid taxonomist Joel Moubayed who collected type material.

Description

Adult male ($n = 5$, except when otherwise stated). Total length 3.7–4.1 m. Total length/wing length 1.13–1.23.

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

Head. Eyes hairy, reniform. Temporal setae including 6–8 frontals, 7–9 orbitals, 5–8 verticals and 4–5 postorbitals. Frontal tubercles 28 μm height. Clypeus with 5–11 setae. Antenna with 8 flagellomeres and reduced plume of setae (Fig. 39); number and length of these setae on 1–7 flagellomeres respectively ($n=2$): 3 (32–40 μm), 3 (56–68 μm), 3 (64–68 μm), 0, 2 (60–64 μm) 0, 0; terminal flagellomere with 5 setae, 80–88 μm long in basal part and with 2 subapical setae, 20–24 μm long. Length of 1–8 flagellomeres (μm): 100–104, 44–56, 48, 36–38, 32–44, 30–36, 30–32, 200–204; AR 0.58–0.61. Palpomere length (μm): 40–44, 92–104, 140–164, 116–132, 164–180. Palpomere 3 in distal part with sensilla capitata with diameter 24 μm . Head width/palpal length 0.90–0.97. Antennal length/palpal length 0.88–0.97.

Thorax. Antepnotum with 12–14 ventrolateral setae, 52–88 μm long. Dorsocentrals 20, 72–120 μm long; prealars 5, 52–116 μm long. Scutellum with *ca* 20 setae.

Wing. Length 3.08–3.44 mm, width 0.96–1.0 mm. Anal lobe slightly reduced, angular. Squama with 24–26 setae (in 1–2 rows), 56–112 μm long. R and R_1 with 26–27 setae, R_{4+5} with 5–6 (in distal half). Costal extension 76–82 μm . RM/MCu 2.5–3.0.

Legs. Spur of front tibia 38–40 μm long. Spurs of mid tibia 40–44 μm and 40–48 μm . Spurs of hind tibia 60–68 μm and 40–44 μm long. Hind tibial comb with 21–25 setae. Length (μm) and proportions of leg segments are as in Table 10.

TABLE 10. Lengths (in μm) and proportions of leg segments of *Diamesa moubayed* sp. nov., male ($n=5$)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅
P ₁	1920–2040	1840–1960	1200–1320	560–680	320–380	98–131	156–164
P ₂	1720–1960	1520–1640	640–780	320–400	220–240	98–131	139–164
P ₃	1940–2100	1769–1960	1120–1300	600–660	340–360	123–131	164

continued

	LR	BV	SV	BR
P ₁	0.64–0.70	3.78–4.41	3.0–3.17	1.1–1.2
P ₂	0.42–0.48	3.38–4.99	4.62–5.06	1.1
P ₃	0.64–0.66	3.93–4.14	3.12–3.30	1.1–1.2

Hypopygium (Figs. 40, 43–44, 46–47, 52, 55). Tergite IX densely covered with strong macrotrichia apices of which are directed anteriorly, with 7–15 setae, 32–48 μm long and with hitinized, narrow (12–14 μm) and naked anal point, 148–160 μm long, when viewed from side it is slightly curved in distal part (Figs. 43–44). Laterosternite IX with 8–10 setae, 28–32 μm long. Transverse sternopodeme (TSA) triangular, 104–140 μm high, 148–184 μm wide at the base (Fig. 52); TSA height/TSA width 0.80–0.89. Aedeagal lobe 164–176 μm long; phallapodeme sclerotized, 140–156 μm long. Gonocoxite 490–492 μm long; superior volsellae angular, along margin with some setae (Fig. 55). Gonostylus 200–240 μm long, slightly curved, expanded in the distal half along the outer edge and

with rounded-angular projection basally (Fig. 47); in apical part with megaseta in form of wide terminal spine, 12 μm long and tooth the same size, next to it there is two setae approximately of the same length (Fig. 46). HR 2.14–2.23.

Pupa and larva unknown.

Diagnosis. The male of *D. moubayedi* **sp. nov.** is closely related in the subgroup to *D. zagrosica* **sp. nov.** and *D. sakartvella* but this species has longest body (3.7–4.1 mm); transverse sternopodeme is triangular shape (Fig. 52), of great height (104–140 μm) and not very wide basally (148–184 μm), the ratio of its height to width is 0.80–0.89; superior volsellae angular (Fig. 55); gonostylus expanded in the distal half along the outer edge and with rounded-angular projection basally (Fig. 47). Total length of *D. zagrosica* **sp. nov.** 2.3–2.8 mm; transverse sternopodeme is regular triangular shape (Figs. 53–54), of medium height (84–108 μm) and not very wide basally (162–172 μm), the ratio of its height to width is 0.49–0.67; superior volsellae rounded-angular (Fig. 56); gonostylus slightly expanded in the distal half along the outer edge and with small rounded projection basally (Figs. 48–49). Total length of *D. sakartvella* 2.9–3.1 mm; transverse sternopodeme is wide triangular (Figs. 50–51), of low height (68–88 μm) and very wide basally (172–188 μm), the ratio of its height to width is 0.36–0.50; superior volsellae rounded (Fig. 57); gonostylus almost not expanded in the distal half along the outer edge and without projection basally (Figs. 45).

Ecology. Adult males were collected in karstic springs and streams, located at an altitude of 2000 m. Water calcareous, conductivity 320–360 $\mu\text{S}/\text{cm}$, water temperature 8–10 $^{\circ}\text{C}$ during summer, about 4–8 $^{\circ}\text{C}$ in winter and spring (pers. comm. by J. Moubayed).

Distribution. Known only from the type locality—karstic springs and streams of Sannyn Mount (Central Lebanon) (Fig. 75).

Previously, this species, after the identification of B. Serra-Tosio (pers. comm. by J. Moubayed), was recorded for Lebanon as *D. sakartvella* Kownacki et Kownacka (Moubayed & Laville 1983).

Diamesa praecipua Sæther et Willassen

(Figs. 58–67, 78)

Diamesa praecipua Sæther et Willassen, 1987: 200; Ashe & O'Connor 2009: 283.

Material examined. 7 adult males, INDIA: Himalayas, Uttarakhand Province, Chamoli District, Rishi Ganga River, right tributary of the Alaknanda River near of Badrinath Town, altitude 3128 m above sea level, N 30.740733, E 79.48875, 12.V.2018, leg. D. Palatov.

Description

Adult male (n = 5, except when otherwise stated). Total length 1.8–1.9 mm. Total length/wing length 5.79–8.49.

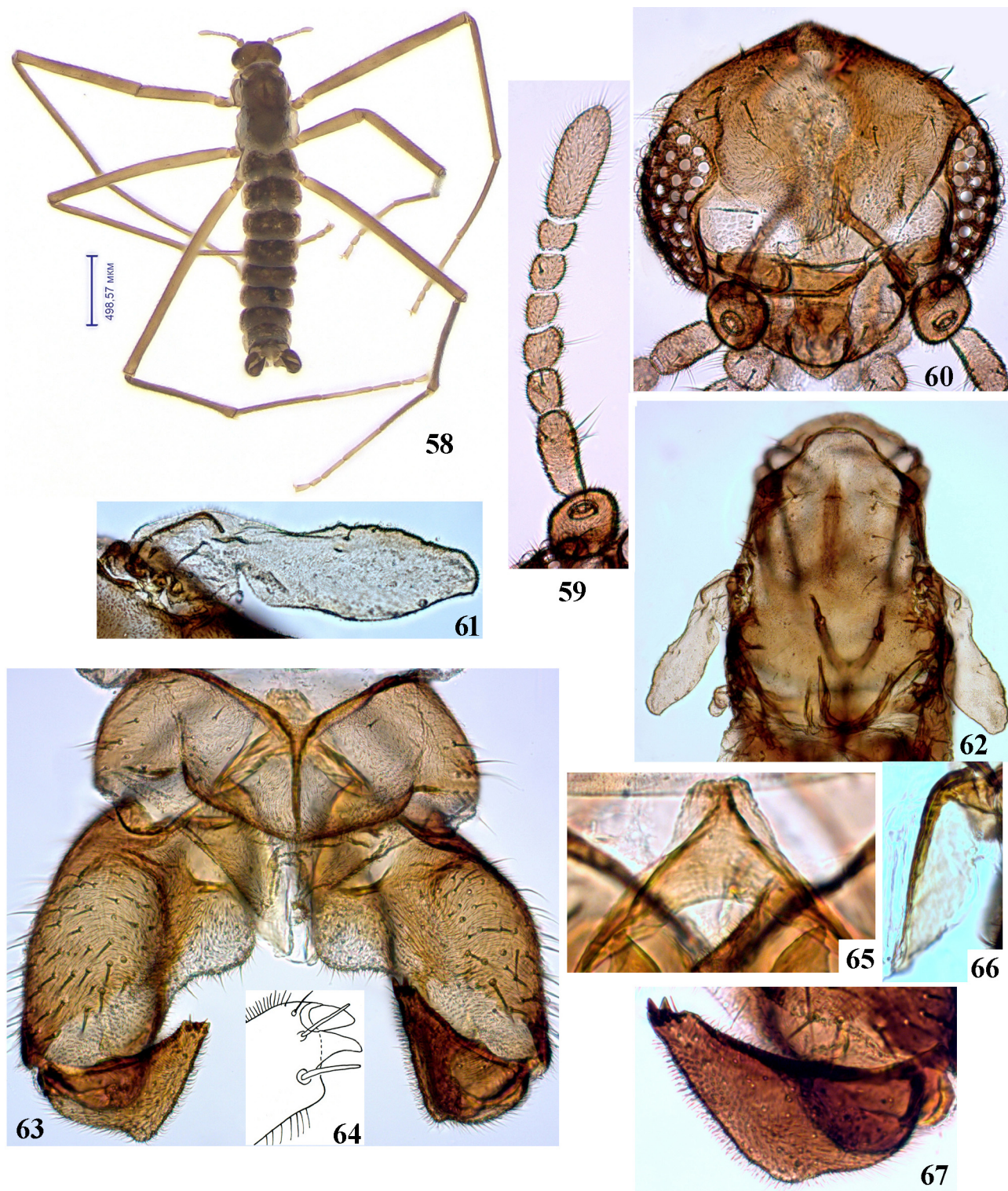
Coloration. Head, thorax and abdomen dark brown. Legs brown to light brown. Wings grey or brownish grey (Fig. 58).

Head (Fig. 60). Eyes haired, reniform. Temporal setae including 0 frontals, 2–5 orbitals, 4–5 verticals and 7 postorbitals. Clypeus with 3–4 setae. Antenna with 7 flagellomeres and reduced plume of setae (Fig. 59); number and length of these setae on 1–6 flagellomeres respectively: 4 (28–32 μm), 2 (24–26 μm), 2 (24–40 μm), 0, 0, 0; terminal flagellomere with 2 setae, 24–28 μm long in subapical and apical area. Length of 1–7 flagellomeres (μm): 92–96, 36, 32–36, 28–32, 28, 26–28, 104–108; AR 0.41–0.43. Antennal length/palpal length 1.33–1.35. Palpomere length (μm): 28–36, 40–44, 72–84, 50–52, 56–72. Palpomere 3 in distal part with sensilla capitata with diameter 16–20 μm . Palpomeres 1–5 length/head width 0.80–0.82.

Thorax (Fig. 62). Strongly reduced. Scutellum fused with scutum. Anteprenotum with 10–15 ventrolateral setae, 40–60 μm long. Dorsocentrals 4–5, 60–64 μm long in anterior half of mesonotum; prealars 2–3, 40–56 μm long. Scutellum with 8–11 setae.

Wing (Figs. 61–62). Strongly reduced, speckled and without venation. Length 212–328 μm , width 100–104 μm . Wing membrane with 2–3 setae, 16–28 μm long.

Legs. Spur of front tibia 34–40 μm long. Spurs of mid tibia 36–40 μm and 36–44 μm long. Spurs of hind tibia 56–60 μm and 40–52 μm long. Hind tibial comb with 18–22 setae. Fore leg without pseudospurs on ta_1 ; mid leg with 10–11 pseudospurs on ta_1 , hind legs with 12–14 pseudospurs on ta_1 . Length (μm) and proportions of leg segments are as in Table 11.



FIGURES 58–67. *Diamesa praecipua* Sæther et Willassen, male. **58**, total view; **59**, antenna; **60**, head; **61**, wing; **62**, thorax; **63**, hypopygium in dorsal view; **64**, apex of gonostylus; **65**, transverse sternapodeme; **66**, aedeagal lobe; **67**, gonostylus.



FIGURES 68–74. Type localities of *Diamesa dragani* sp. nov. (68), *D. kownackii* sp. nov. (69–70), *D. maisaraensis* sp. nov. (71), *D. marinskiyi* sp. nov. (72), *D. steinboeckii* Goetghebuer (73) and *D. sakartvella* Kownacki et Kownacka (74). 68, unnamed stream of Bolshoi On River basin, alt. 2063 m a. sea l., Sayan Mountains (photo by S.V. Dragan); 69, unnamed stream of Somnitelnaya River basin, Wrangel Island (photo by E.A. Makarchenko); 70, Somnitelnaya River, upper stream, alt. 205–210 m a. sea l., Wrangel Island (photo by O.A. Khruleva); 71, Maisara River near Maisara Pass, alt. 4168 m a. sea l., Pamir Mountains (photo by D.M. Palatov); 72, Zienzan River, Tien Shan Mountains, alt. 1923 m a. sea l., Xinjiang Uygur Autonomous Region, China (photo by D.M. Palatov); 73, *Balm stream*, Massif of Aiguilles–Rouges, French Alps (photo by Pierre Clévenot); 74, Skazdon River near the Tsey (Tseyskoe gorge), alt. 1943 m a. sea l., North Caucasus (photo by D.M. Palatov).

TABLE 11. Lengths (in μm) and proportions of leg segments of *Diamesa praecipua* Sæther et Willassen, male (n=5)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅
P ₁	1673–1706	1476–1558	894–869	377	189–197	107–115	115
P ₂	1558–1624	1279–1345	607–623	262–279	156–164	90–98	107
P ₃	1673–1706	1394–1542	918	459	221–230	98–107	123–131

continued

	LR	BV	SV	BR
P ₁	0.56–0.61	5.03–5.24	3.52–3.76	0.9–1.1
P ₂	0.45–0.49	5.41–5.74	4.55–4.89	1.0
P ₃	0.60–0.66	4.38–4.54	3.34–3.54	1.1–1.2

**75****76****77****78**

FIGURES 75–78. Type localities of *Diamesa moubayedii* **sp. nov.** (**75**), *Diamesa zagrosica* **sp. nov.** (**76**), *D. steinboeckii* Goetghebuer (**77**) and *D. praecipua* Sæther et Willassen (**78**). **75**, glacial karstic spring, Sannyn Mount, alt. 2000 m a. sea l., Central Lebanon (photo by J. Moubayed); **76**, Dare Daei River, Zagros Mountains, alt. 2244 m a. sea l., Lorestan Province, Iran (photo by D.M. Palatov); **77**, Muttbach Stream, Valais Canton, Swiss Alps (photo by Jean-Louis Lods); **78**, Rishi Ganga River, right tributary of the Alaknanda River near town of Badrinath, alt. 3128 m a. s. l., Himalayas, India (photo by D.M. Palatov).

Hypopygium (Figs. 63–67). Tergite IX without anal point, with 7–16 pale setae from one side, 13–20 μm long; anal tergal bands Y-type (Fig. 63); median tergal band 84–92 μm long. Laterosternite IX with 5–8 setae, 24–32 μm long. Transverse sternapodeme trapezoidal (Fig. 65), length 52–64 μm and 144–208 μm wide in basal part. Aedeagal lobe weakly sclerotized, shape as in Fig. 66. Gonocoxite 260–304 μm long, with grey rectangular superior volsellae covered by short setae (Fig. 63); inferior volsellae absent. Gonostylus triangular, 168–176 μm long, along outer edge in middle with keel (Fig. 67); megaseta in form of wide terminal spine 12–14 μm long, next to it there is tooth and two setae of approximately same length (Fig. 64). HR 1.50–1.81.

Adult female was described by Sæther & Willassen (1987).

Pupa and larva unknown.

Remarks. Adult male from our material collected in the Indian Himalayas at an altitude of 3128 m above sea level differs from the males of this species described by Sæther & Willassen (1987) from an altitude of 5100–5600 m above sea level in small body sizes (1.8–1.9 mm), longer wings (212–328 μm), the presence of antenna with 7 flagellomeres, a smaller number of setae (7–9) on tergite IX, and a trapezoidal shape of the transverse sternapodeme. Total length of male *D. praecipua* from the original description 2.84–3.26 mm, wing length 169–195 μm , antenna with 6 flagellomeres, tergite IX with 55–69 setae, transverse sternapodeme triangular.

We consider differences in the structure of the males of these two populations as a result of individual variability associated with living at different altitude, although it is possible that these are different species and a new species is present in our material. To confirm this, it is necessary to compare these two populations at the molecular genetics level in future.

Ecology. Adult males were collected from stones and boulders near water in mountain river and near waterfall, located at an altitude of 3128 m, at a flow rate of 0.1–0.8 m/s, with water temperatures 11°C. Previously, this species was known only from the original description of adults collected in Nepal Himalaya at an altitude 5100–5600 m above sea level, where imagines with vestigial wings walked on the surface of the glacier and in small cavities beneath it. The larvae lived in meltwater drainage channels beneath the ice and fed on blue-green algae and bacteria. Insects were active at temperatures as low as -16°C (Koshima 1984, 1985; Sæther & Willassen 1987).

Distribution. Known only from high altitude of Himalayas (Fig. 78).

Results of DNA barcoding

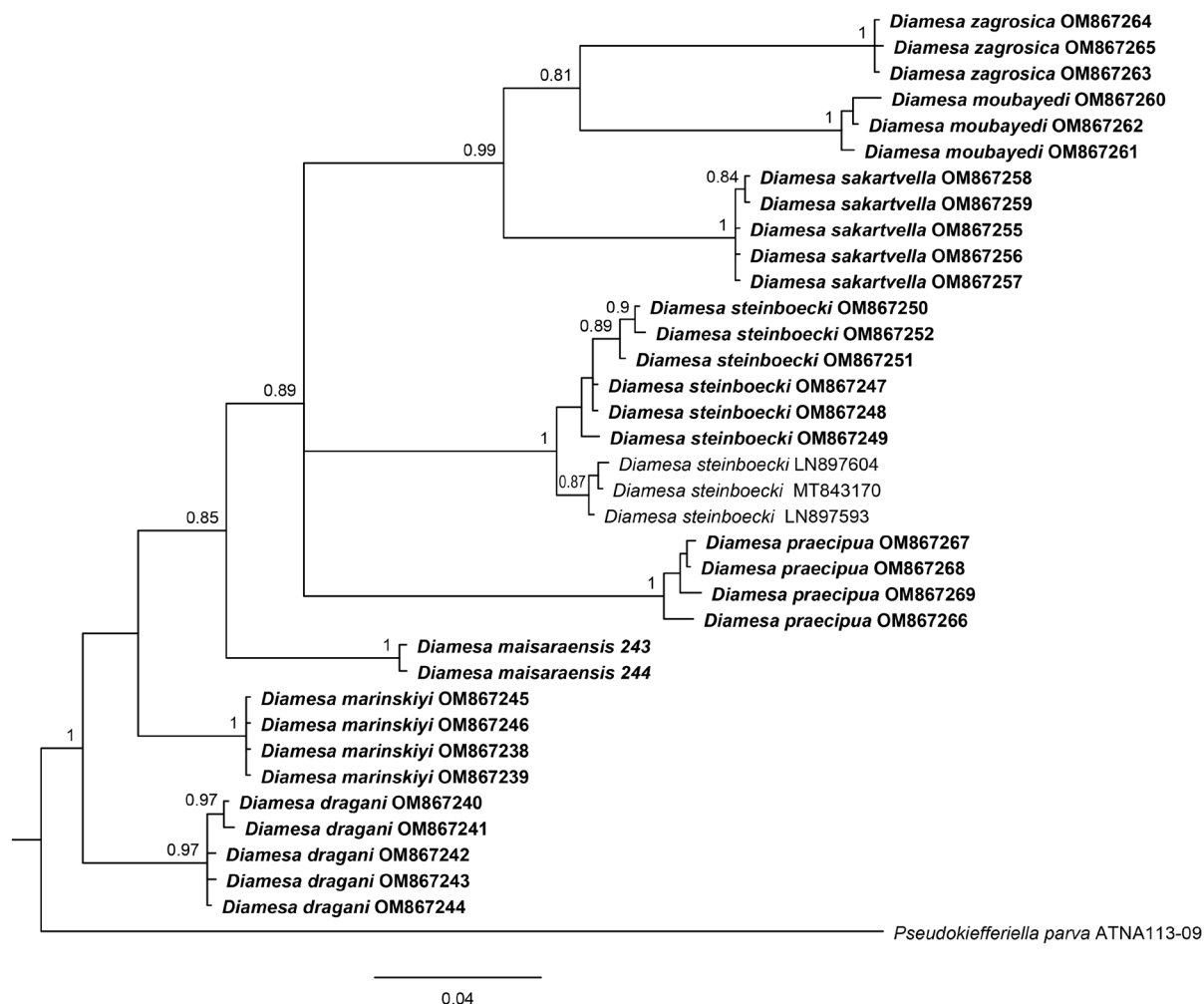
Overall, we have sequenced fragments of the cytochrome oxidase subunit I (579–658 bp in length) of 30 samples from eight *Diamesa* species. To perform the final dataset, we added two COI sequences (658 bp in length) of *D. maisaraensis* **sp. nov.** which were published in Makarchenko *et al.* (2018) as *D. steinboeckii* Goetghebuer (GenBank numbers: KY640397–KY640398).

The mean nucleotide base compositions were A 26.2%, T 39.6%, G 17.1%, and C 17.2% across all sites for all sequences. Variable and parsimony informative sites were 152 and 146 respectively most of which were synonymous transitions. The K2P sequence divergence within and between eight *Diamesa* species shown in the Table 12, the obtained interspecific K2P corresponds to species level (Montagna *et al.* 2016).

TABLE 12. Intraspecific and interspecific K2P nucleotide distances (%) of eight *Diamesa* species estimated using COI sequences.

Species	n	Intraspecific	Interspecific						
			1	2	3	4	5	6	7
1. <i>D. dragani</i> sp. nov.	5	0.37							
2. <i>D. maisaraensis</i> sp. nov.	2	0.15	6.41						
3. <i>D. marinskiyi</i> sp. nov.	4	0.00	5.08	6.45					
4. <i>D. moubayedi</i> sp. nov.	3	0.63	10.72	10.39	11.29				
5. <i>D. praecipua</i> Sæther et Willassen	4	0.87	10.39	10.88	10.49	14.53			
6. <i>D. sakartvella</i> Kownacki et Kownacka	5	0.09	10.09	8.68	9.13	9.22	12.08		
7. <i>D. steinboeckii</i> Goetghebuer	6	0.65	8.13	7.90	7.86	11.61	11.83	10.11	
8. <i>D. zagrosica</i> sp. nov.	3	0.10	9.80	9.67	10.47	10.00	13.30	8.80	9.88

ABGD analysis confirmed species independence of eight obtained *Diamesa* species. We combine COI sequences of *Diamesa* from the GenBank, BOLD systems and added the obtained data which gave 2424 sequences. ABGD analysis yielded 60 operational taxonomic units (OTU) using a 0.0070–0.0189 intraspecific divergence of COI gene. Sequences of *D. steinboeckii* (OM867247–OM867252) formed a common OTU with *D. steinboeckii* from Italian Alps deposited under GenBank numbers MT843146–MT843171, LN897593, LN897601, LN897603–LN897606, LN897618, LN897628–LN897630, LN897668.



Additional mitochondrial (COII, 16S rRNA) or nuclear loci (18S rRNA, EF-1a) allow increased node support on phylogenetic relationships (Makarchenko *et al.* 2018, Lencioni *et al.* 2021), but are not considered here. Congruence between molecular and morphological characters was obtained for most studied species, whereas DNA barcoding did not allow to delimit *D. bohemani* Goetghebuer and *D. zernyi* Edwards, as well as *D. tonsa* (Haliday), *D. hamaticornis* Kieffer, *D. cinerella* Meigen (Montagna *et al.* 2016, Lencioni *et al.* 2021). As stated in the Introduction a detailed analysis of the Diamesinae phylogeny using a multilocus approach will be published in future papers

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