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Morphological description and DNA barcoding of *Diamesa achipseensis* sp. nov. (Diptera: Chironomidae: Diamesinae) from Southwestern Caucasus

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Abstract

Illustrated description of adult male, as well as DNA barcoding, of *Diamesa achipseensis* **sp. nov.** in comparison with close related species *D. caucasica* Kownacki et Kownacka from Southwestern Caucasus are provided. Interspecific distances between *D. achipseensis* **sp. nov.** and *D. caucasica* are extremely low (0.64% in average) despite the identified morphological differences. High similarity of these species is new example of genetically indistinguishable of some species of the genus *Diamesa* Meigen.

Key words: Diptera, Chironomidae, Diamesinae, *Diamesa,* new species, DNA barcoding, Caucasus

Introduction

To date, eighteen species of the genus *Diamesa* Meigen have been registered for the Caucasus, five of which, *D. caucasica* Kownacki et Kownacka, *D. elbrusica* Makarchenko, Semenchenko et Palatov, *D. sakartvella* Kownacki et Kownacka, *D. tskomelidzei* Kownacki et Kownacka and *D. valentinae* Makarchenko, are endemic (Makarchenko *et al.* 2023).

In this report we provide an illustrated description of *D. achipseensis* **sp. nov.** with DNA barcoding from Achipse River (Southwestern Caucasus) which we assign to the *latitarsis* group and closely related to *D. caucasica* Kownacki et Kownacka and from which it does not differ using DNA barcoding.

Materials and methods

The adult male of new species was preserved in 96% ethanol for DNA-analysis and for further study of morphology. The material was mounted in the polyvinyl lactophenol. The morphological terminology and abbreviations used below generally follow Sæther (1980).

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

Holotype of the new species is 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 isolated from one specimen of *Diamesa achipseensis* **sp. nov.** using a Qiagen Blood and Tissue Kit (Qiagen, Hilden, Germany). Fragment of cytochrome c oxidase subunit I (COI-5P) were amplified using Go Taq Green Master Mix (Promega corp, Madison, WI, USA) and primers LCO1490 and HCO2198 (Folmer

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et al. 1994). Amplification products were purified by exonuclease I (ExoI) and alkaline phosphatase (FastAP) (Thermo Fisher Scientific Inc., USA) and bidirectionally sequenced by ABI 3500 sequencer (Applied Biosystems) using reagents BigDye terminator v3.1 cycle kit. More details about DNA extraction, PCR regime and sequence can be found in Makarchenko *et al.* (2022a, 2022b, 2023). Interspecific genetic divergence values were calculated using p-distances implemented in MEGA7 software (Kumar *et al.* 2016). The obtained sequence have been deposited in GenBank under number PP955284.

Description

***Diamesa achipseensis* Makarchenko, Semenchenko et Palatov, sp. nov.**
http://zoobank.org/NomenclaturalActs/6EE16603-172D-4F5A-BABD-49380AC31B55
(Figs. 1–11)

Type material. Holotype, adult male, RUSSIA: Caucasus, Krasnodar Region, Adler District, Sochi urban district, Sochi National Park, Achipse River, 3 km North of the Estosadok Village, altitude 600 m above sea level, 12.IV.2024, 43°42'25.9"N 40°15'46.18"E, leg. D. Palatov.

Derivatio nominis. The species is named as *achipseensis* after the type locality in the Achipse River of the Southwestern Caucasus.

Description

Adult male (n = 1). Total length 3.2 mm. Total length/wing length 0.83.

Coloration. Dark brown. Head, thorax and abdomen with hypopygium dark brown, antenna grey. Legs brown. Wings greyish.

Head. Eyes reniform, bare. Temporal setae including 6–7 preoculars, 6 inner verticals, 8 outer verticals and 8–10 postorbitals. Clypeus with 10 setae. Antenna with 13 flagellomeres and slightly reduced plume of setae; maximal length of these setae on flagellomeres 770 μm; terminal flagellomere with 1 subapical seta, 40 μm long; pedicel with 3 setae, 60–70 μm long; AR 0.70–0.74. Palpomere length (μm): 48, 100, 160, 176, 252. Palpomere 3 in distal part with sensilla capitata with diameter 12 μm. Head width/palpal length 1.02.

Thorax. Antepronotum with 6–7 ventrolateral setae. Dorsocentrals 14–15, 116–148 μm long; prealars 11–12, 120–152 μm long. Scutellum with 34 setae, 160–180 μm long.

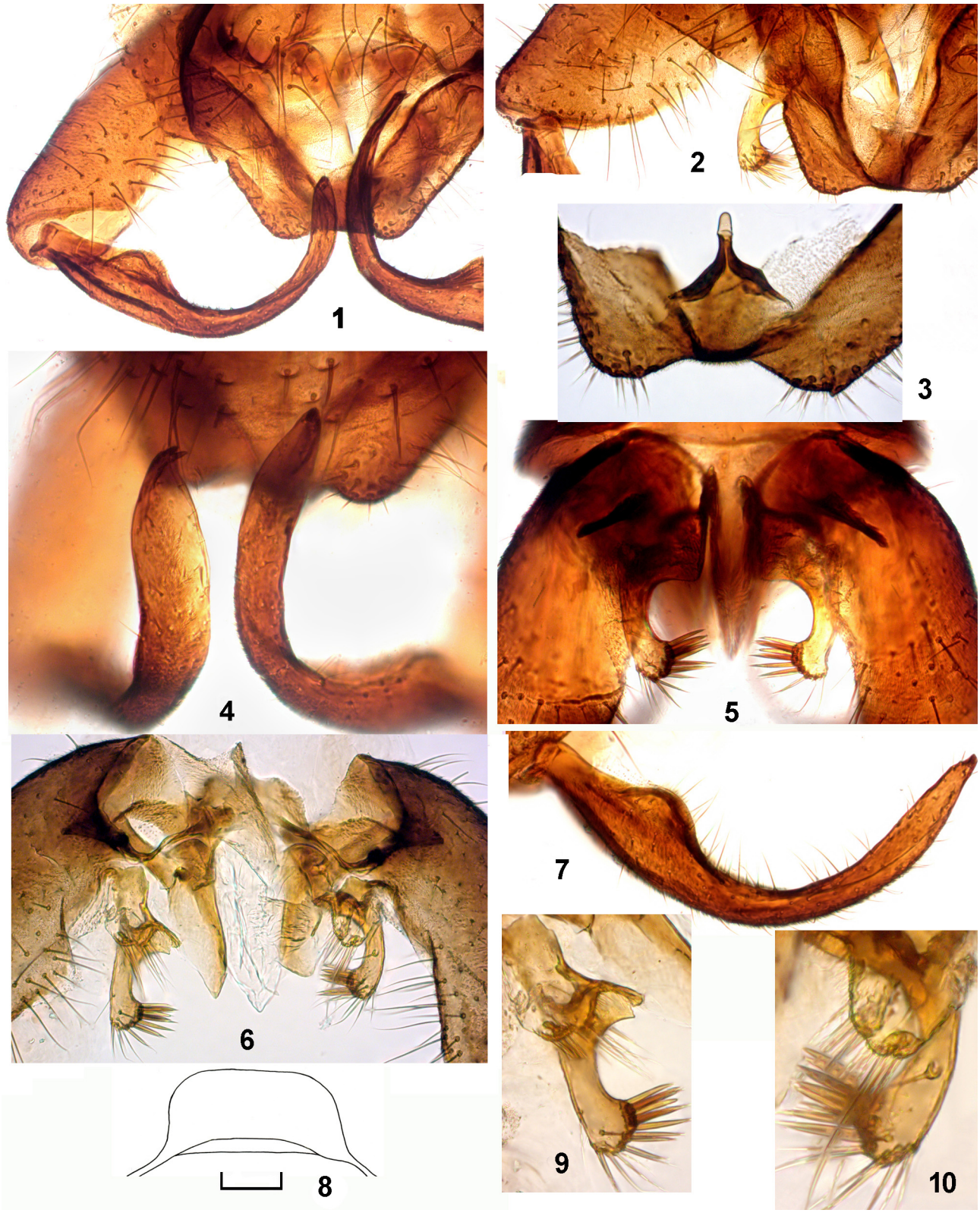
Wing. Length 3.84 mm, width 1.12 mm. Costal extension 82 μm long. Anal lobe outline rounded. Squama with 34 setae. R and R_1 with 28 setae, R_{4+5} with 3 setae in subapical part. RM/MCu 2.2.

Legs. Spur of front tibia 84 μm long. Spurs of mid tibia 60 μm and 64 μm long. Spurs of hind tibia 84 μm and 56 μm long. Hind tibial comb with 18 setae. Length (μm) and proportions of leg segments are as in Table 1.

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

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
P_1	1440	1800	1200	590	361	115	148	0.67	3.66	2.70	1.7
P_2	1591	1591	754	470	246	115	148	0.47	4.28	4.22	1.5
P_3	1800	1880	1240	574	377	115	148	0.66	4.05	2.97	2.1

Hypopygium (Figs. 1–10). Tergite IX narrow, with 16–18 setae from one side, 48–60 μm long and with anal point, 40 μm long, which bends under tergite IX, dark brown in basal 2/3 and light grey in distal 1/3, with rounded apex (Figs. 1–3). Laterosternite IX with 8–11 setae. Transverse sternopodeme (TSA) wide rectangular with rounded outer angles (Fig. 8), 56 μm high, 156 μm wide; TSA height/TSA width 0.36. Aedeagal lobe knife-shaped, 180 μm long and 48 μm wide, weakly chitinized; phallapodeme sclerotized, 120–128 μm long. Gonocoxite 476 μm long, without inferior volsellae, basally with superior volsellae consisted of ventral and dorsal parts (Figs. 6, 9–10); ventral part located on stalk 130–132 μm long and in subapical part with 18–20 needle-shaped setae, 40–44 μm long; dorsal part rounded, with some long setae (Figs. 9–10). Gonostylus 410 μm long, narrow crescent-shaped, slightly expanded basally, its surface with short setae, *ca* 8 μm long, along the inner edge with thin setae, 40–48 μm long; apex with short macroseta, 12 μm long (Figs. 1, 4, 7). HR 1.16.



FIGURES 1–10. Adult male of *Diamesa achipseensis* **sp. nov. 1–2,** part of hypopygium in dorsal view with invisible bent gonostylus; **3,** part of tergite IX in ventral view with anal point; **4,** dorsally curved gonostylus; **5,** part of hypopygium from ventral side; **6,** endoskeleton; **7,** gonostylus; **8,** transverse sternapodeme; **9–10,** superior volsellae. Scale bar: 50 µm.

Pupa and ***larva*** unknown.

Diagnosis. Total length 3.2 mm. Eyes bare. Antenna with 13 flagellomeres and slightly reduced plume of setae; AR 0.70–0.74. LR_1 0.67, BV_1 3.66, SV_1 2.70. Tergite IX narrow, with short anal point, which bends under tergite IX, dark brown in basal 2/3 and light grey in distal 1/3, with rounded apex. Transverse sternopodeme wide rectangular with rounded outer angles. Gonocoxite without inferior volaellae, basally with superior volsellae consisted of ventral and dorsal parts; ventral part located on long stalk and in subapical part with 18–20 needle-shaped setae; dorsal part rounded, with some long setae. Gonostylus narrow crescent-shaped, slightly expanded basally, its surface with short setae, along inner edge with thin setae; apex with short macroseta. HR 1.16.

The new species belongs to the *latitarsis* group and most closely related to *D. caucasica* Kownacki et Kownacka, from which it is well distinguished by structures of gonostylus and superior volsellae as well as some other characters given in Table 2.

TABLE 2. Comparison of adult males characters of *Diamesa achipseensis* **sp. nov.** and *D. caucasica* Kownacki et Kownacka

Characters	*D. achipseensis* **sp. nov.** (n=1)	*D. caucasica* Kownacki et Kownacka (n=7), Kownacki & Kownacka 1973; Makarchenko *et al.* 2022a
Total length, mm	3.2	2.97–3.5, 5–6*
Wing length, mm	4.1	3.4–3.6, 3.5*
TL/WL	0.83	0.82–0.92
Antenna	With slightly reduced plume of setae	With developed plume of setae
AR	0.70–0.74	1.0–1.22, 0.91*
Clypeals	10	7–10
Dorsocentrals	14–15	7–12
Prealars	11–12	4–6
Scutellars	34	19–29
Squamal setae	34	21–26
LR1	0.67	0.66–0.68
BV_1	3.66	3.45–3.52
SV1	2.70	2,63–2.80
Tergite IX, number of setae	16–18	14–39
Anal point length, ìm	40	40–49
Superior volsellae	Ventral part located on stalk 130–132 µm long and in subapical part with 18–20 needle-shaped setae; dorsal part rounded, with some long setae.	Ventral and dorsal parts without stalk, rounded, or elongated-rounded, almost same size, covered with long setae.
Gonostylus	Gonostylus narrow crescent-shaped, slightly expanded basally, without any appendages.	Gonostylus in basal third or half widened along inner margin and with wedge-shaped appendage directed at angle backwards.
HR	1.16	0.90–1.02

Note. Data from Kownacki & Kownacka (1973) are marked with an asterisk

Ecology and distribution. Adult male was collected near mountain large Achipse River (Fig. 11) in a wide beech-fir valley. The bottom of this river is large stones and boulders, flow rate of 0.7–1 m/s, located at an altitude of 600 m a.s.l. Known only from the type locality in Achipse River of the Southernwest Caucasus.

FIGURE 11. Locality and habitat of *Diamesa achipseensis* **sp. nov.** on Achipse River of the Southwestern Caucasus (Photo by D.M. Palatov).

Results of DNA barcoding

The single COI-5P sequence of 658 base pairs was obtained. After assembly and alignment, nucleotide frequencies across these sequences were as follows: A=26.3%, T=40.9%, G=17.1%, and C=15.7%.

The BLAST algorithm of GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) showed high similarity of the *D. achipseensis* **sp. nov.** and *D. caucasica.* The average interspecific divergence between two species were 0.64% which is acceptable for an intraspecific level (Montagna *et al.* 2016).

The genus *Diamesa* Meigen contains many indistinguishable species using DNA barcoding. *D. cinerella* Meigen, *D. hamaticornis* Kieffer, *D. hyperborea* Holmgren, *D. kasymovi* Kownacki et Kownacka, *D. lavillei* Serra-Tosio, *D. tonsa* (Haliday) on one hand and *D. bohemani* Goetghebuer, *D. vaillanti* Serra-Tosio, *D. valentinae* Makarchenko, and *D. zernyi* Edwards on the other one has extremely low interspecific distances (Ekrem *et al.* 2010, Montagna *et al.* 2016, Lencioni *et al.* 2021, Makarchenko *et al.* 2023). Such results may be a consequence of incomplete lineage sorting (Montagna *et al.* 2016). Apparently high similarity of *D. achipseensis* **sp. nov.** and *D. caucasica* is new example of genetically indistinguishable species of the genus *Diamesa*.

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