

A review of the taxonomy and DNA barcoding of the *Diamesa latitarsis* group (Diptera: Chironomidae: Diamesinae), with a description of two new species

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Abstract

Two new species, *Diamesa sangecha* **sp. nov.** and *D. demavendensis* **sp. nov.**, are described from the Caucasus and Iran, respectively. An overview of the current taxonomy of the *Diamesa latitarsis* group is also provided, integrating both morphological and molecular genetic data. This combined approach expands the morphological criteria traditionally used to define the *D. latitarsis* group. In addition, twelve new mitochondrial COI DNA barcodes were generated and analyzed in conjunction with all publicly available sequences of the *D. latitarsis* species group retrieved from NCBI GenBank and BOLD. Molecular data independently confirm the distinct species boundaries of *D. demavendensis* **sp. nov.** and *D. sangecha* **sp. nov.** Furthermore, the first DNA barcode for *D. tsxhomelidzei* Kownacki *et* Kownacka reveals a close phylogenetic affinity to *D. caucasica* Kownacki *et* Kownacka, exhibiting a minimal interspecific K2P distance of 1.90%. Finally, we critically discuss the problematic cryptic species boundaries within the *D. lindrothi*–*D. goetghebuerei* complex.

Key words: Diptera, Chironomidae, Diamesinae, *Diamesa latitarsis* group, taxonomy, DNA barcoding

Introduction

In the subfamily Diamesinae, the genus *Diamesa* Meigen is the most species-rich, with over 100 described species (Ashe & O'Connor 2009). Some members of this genus form groups of morphologically and genetically related species. We have recently revised the *Diamesa steinboeckii* group (Makarchenko *et al.* 2022 a–b). In the present paper, we provide an overview of the taxonomy of the *Diamesa latitarsis* species group. Its taxonomy was examined in detail by Serra-Tosio (1968) and later by Montagna *et al.* (2016a), which included six species in this group: *D. goetghebuerei* Pagast, *D. latitarsis* (Goetghebuere), *D. laticauda* Serra-Tosio, *D. lindrothi* Goetghebuere, *D. modesta* Serra-Tosio, and *D. wuelkeri* Serra-Tosio. The main morphological characteristics of adult males for inclusion in this group of species were the presence of two stronger preapical setae on the inner margin of each gonocoxite and two more or less developed appendages (a superior and inferior volsellae or inferior volsella) on each gonocoxite (Serra-Tosio 1968). Later, the species *D. valkanovi* Sæther from Norway (Sæther 1968), as well as *D. caucasica* Kownacki *et* Kownacka, *D. tsxhomelidzei* Kownacki *et* Kownacka, *D. martae* Kownacki, *D. achipseensis* Makarchenko, Semenchenko *et* Palatov, *D. elbrusica* Makarchenko, Semenchenko *et* Palatov from the Caucasus and *D. sahandensis* Namayandeh *et* Ibrahimy from Iran were added to this group (Kownacki & Kownacka 1973, 1980; Mohammadi *et al.* 2021; Makarchenko *et al.* 2023; Makarchenko & Palatov 2024).

DNA barcoding based on the mitochondrial cytochrome c oxidase subunit I (COI) gene has proven to be a highly effective tool for species delimitation and identification within the genus *Diamesa* Meigen, including the *Diamesa latitarsis* group (e.g., Montagna *et al.* 2016b; Lencioni *et al.* 2021, Makarchenko *et al.* 2022a–c, 2023, Dvorak *et al.* 2024, Lencioni *et al.* 2024). The monophyly of the *latitarsis* species group, in various species combinations, has

consistently received moderate or strong statistical support across different geographic scales. This robustness has been demonstrated both by studies utilizing single COI DNA barcodes (Montagna *et al.* 2016a; Lencioni *et al.* 2021; Makarchenko *et al.* 2023; Dvorak *et al.* 2024) and by multi-locus phylogenetic reconstructions (Semenchenko *et al.* 2020; Lencioni *et al.* 2024). In the vast majority of molecular and faunistic works focusing on European *Diamesa*, the *latitarsis* group is conventionally represented by only three widespread species: *D. goetghebueri*, *D. latitarsis*, and *D. modesta* (Montagna *et al.* 2016a,b; Lencioni *et al.* 2021, 2024; Dvorak *et al.* 2024). Similarly, recent applications of DNA barcoding to the rich and highly endemic *Diamesa* fauna of the Caucasus region did not reveal any para- or polyphyletic anomalies within the *latitarsis* cluster (Makarchenko *et al.* 2023).

We also present an updated molecular phylogeny, along with the formal description and taxonomic re-evaluation of species within the *D. latitarsis* group based on an expanded global dataset. Our primary goals are to: (1) formally describe two new species of the group discovered from the high-altitude streams of Iran and Central Asia; (2) review the taxonomic status of the Iranian *D. sahandensis*, clarifying its true molecular boundaries and reassigning previously misidentified larval barcodes; and (3) critically evaluate the cryptic species boundaries within the highly problematic *D. lindrothi*–*D. goetghebueri* complex based on public BOLD metadata and new reference sequences from the Caucasus.

Material and methods

The material was preserved in 96% ethanol and mounted in polyvinyl lactophenol. The morphological terminology and abbreviations used generally follow Sæther (1980). For some structures of the hypopygium, namely in the key and some diagnoses, the terminology of Montagna *et al.* (2016a, p. 319) is used. 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®.

Holotypes and paratypes as well as other material are deposited in the Bioresource Collection (reg. number 2797657) of 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 extraction was isolated from tissue samples using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) or ExtractDNA Blood & Cells (Evrogen, Moscow, Russia) in strict accordance with the manufacturer's recommended protocols. The resulting purified DNA was eluted in a final volume of 100 µl. For molecular analysis, a standard 650 bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the universal primer pair LCO1490 and HCO2198 (Folmer *et al.* 1994).

The PCR products were subsequently purified using exonuclease I (ExoI) and alkaline phosphatase (FastAP) (Thermo Fisher Scientific Inc., USA). Bidirectional sequencing was performed on an ABI 3130xl Genetic Analyzer (Applied Biosystems) using the BigDye Terminator v3.1 Cycle Sequencing Kit. Comprehensive technical details regarding the specific thermal cycling regimes, reaction mixtures, and sequencing parameters strictly follow the methodologies described in our previous studies (Makarchenko *et al.* 2022a–c, 2023; Semenchenko & Makarchenko 2025).

To reconstruct a molecular phylogeny and perform species delimitation, we assembled a comprehensive COI dataset focusing on the *D. latitarsis* group. The matrix incorporated our newly generated DNA barcodes alongside all relevant reference sequences available in the Barcode of Life Data Systems (BOLD) and NCBI GenBank. A strict sampling strategy was used for the phylogenetic tree reconstruction to prevent artificial clustering and optimize tree topography without overcrowding the final phylogeny. Specifically, we retained all sequenced specimens from our collection, but for the public reference data from BOLD, sequences for each distinct Barcode Index Number (BIN) were systematically downsampled to the sequence with the longest COI sequence. Concurrently, for the K2P genetic distance calculations (performed in MEGA7, Kumar *et al.* 2016) and the Assemble Species by Automatic Partitioning (ASAP, Puillandre *et al.* 2021) species delimitation analysis, we utilized the full, unreduced dataset encompassing all available sequences, including both our newly published data and all molecular records retrieved from GenBank and BOLD.

Detailed supplementary metadata for all analyzed specimens, including global geographic distribution, lifecycle stages, sample counts, and original identifiers, are comprehensively synthesized and cross-referenced in Table 1.

Molecular species boundaries within the *D. latitarsis* complex were independently assessed using the Assemble Species by Automatic Partitioning (ASAP) web tool (Puillandre *et al.* 2021) based on Kimura 2-parameter (K2P) nucleotide distances. The analytical parameters utilized for selecting the optimal nucleotide substitution models, as well as the advanced algorithmic settings for the maximum likelihood and Bayesian phylogenetic reconstructions, are identical to those thoroughly detailed in Makarchenko *et al.* (2023) and Semenchenko & Makarchenko (2025). The best models for the first, second, and third codon position of COI was SYM+I (Zharkikh 1994), F81+I (Felsenstein 1981), and GTR+I+G (Tavare 1986)

The newly generated COI nucleotide sequences obtained during this study have been deposited in the NCBI GenBank database under the accession numbers PZ535955, PZ679320–PZ679327, and PZ694942–PZ694944.

TABLE 1. Geographic distribution and lifecycle stages of the investigated species and BINs in the *Diamesa latitarsis* species group with verified taxonomy

Species	BIN	Life Stage / sex	Max intraspecific K2P distance (%)	Specimens Count	Geographic Distribution (Countries: Regions/Provinces)	Identified by	Alternative species names in BOLD
<i>D. achipseensis</i>	BOLD: AEZ6179	Imago male	-	1	Russia: North Ossetia–Alania	Makarchenko E.A.	
<i>D. caucasica</i>	BOLD: AEZ6179	Imago male	1.07	14	Russia: North Ossetia–Alania, Krasnodar Region	Makarchenko E.A.	
<i>D. demavendensis</i> sp. nov.	BOLD: AFA2698	Imago male	1.38	4	Iran: Mazandaran Province, Russia: Kabardino-Balkaria, North Ossetia–Alania	Makarchenko E.A.	
<i>D. elbrusica</i>	BOLD: AFA2540	Imago male	0.00	3	Russia: Kabardino-Balkaria	Makarchenko E.A.	
<i>D. goetghebuerei</i>	BOLD: ADJ8799	Larvae	1.23	6	Austria: Tyrol, Italy: South Tyrol, Trento	Dvorak M., Lencioni V.	
<i>D. goetghebuerei</i>	BOLD: ADK0949	Larvae, Imago male	1.23	16	Austria: Tyrol, Italy: Trento, Bergamo Switzerland: Valais	Dvorak M., Lencioni V., Widmann C.	<i>Diamesa lindrothi</i>
<i>D. goetghebuerei</i>	BOLD: ADK3040	Larvae, Imago female	0.92	5	Austria: Tyrol, Italy: Trento, Switzerland: Valais	Dvorak M., Lencioni V., Widmann C.	
<i>D. goetghebuerei</i>	BOLD: AER0139	Larva	-	1	Austria: Tyrol	Dvorak M.	
<i>D. latitarsis</i>	BOLD: AAC7191	Larvae, Imago male, Imago female	2.35	42	Austria: Tyrol, Denmark: Faroe Islands, Germany: Bavaria, Iceland: Sudurland, Italy: Bolzano, Sondrio, Trento, Norway: Oppland, Hedmark, Russia: North Ossetia–Alania, Spain: Huesca, United Kingdom: Scotland	Doczkal D., Dvorak M., Stur E., Lencioni V., Makarchenko E.A.	<i>Diamesa bertrami</i>
<i>D. lindrothi</i>	BOLD: AAC4741	Larvae, Pupa, Imago male	0.80	12	Norway: Innlandet, Oppland	Stur E.	
<i>D. lindrothi</i>	BOLD: AFA7447	Imago male, Mature pupa	0.33	5	Russia: Kabardino-Balkaria, Karachay-Cherkessia	Makarchenko E.A.	
<i>D. martae</i>	BOLD: AGO4884	Imago male	0.87	2	Russia: North Ossetia–Alania	Makarchenko E.A.	
<i>D. modesta</i>	BOLD: ADJ8709	Larvae	1.10	55	Austria: Tyrol, Italy: Brescia, Sondrio, South Tyrol, Trento	Dvorak M., Lencioni V.	<i>Diamesa</i> sp., <i>Diamesa bertrami</i> , <i>Diamesa steinboeckii</i>

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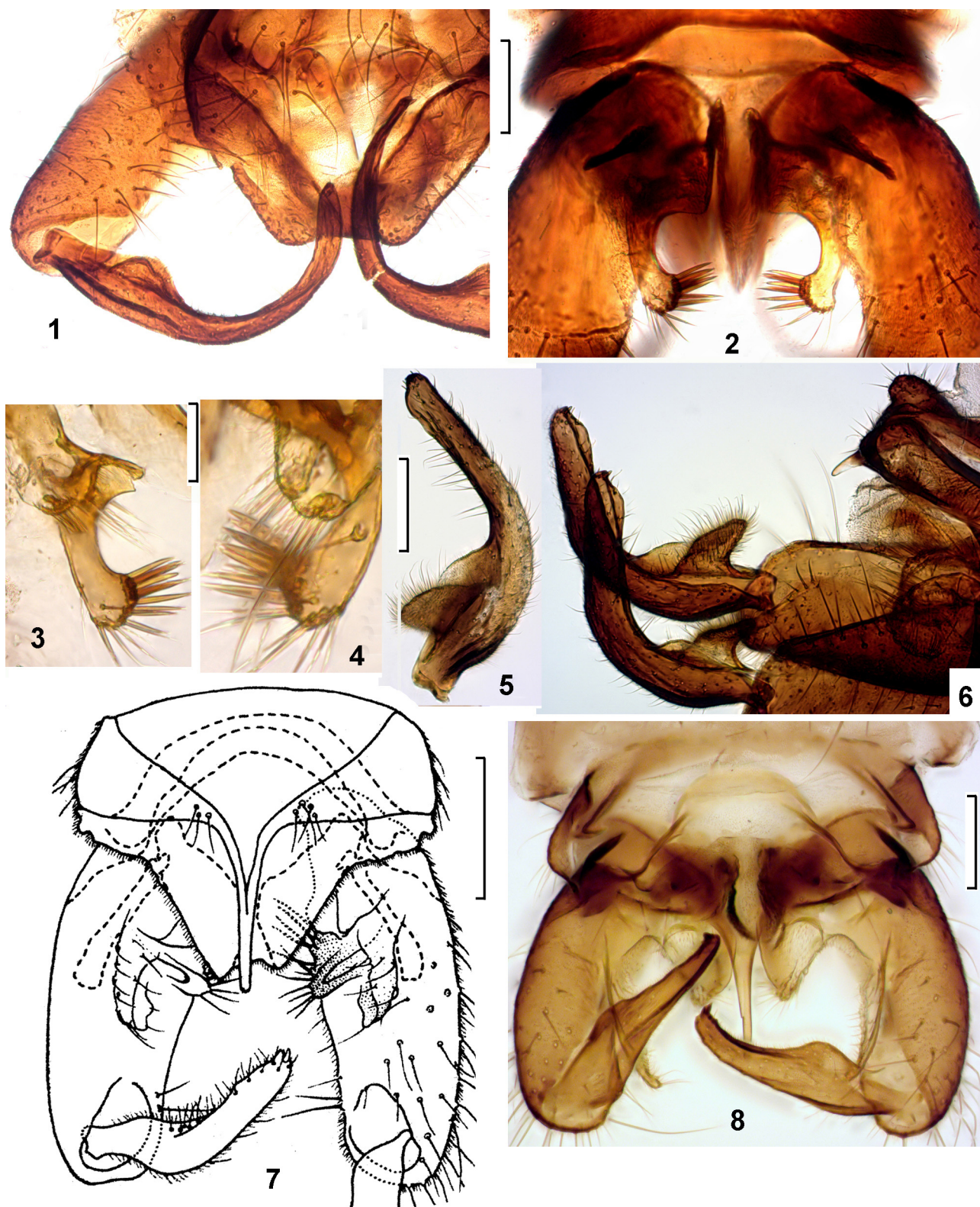
TABLE 1. (Continued)

Species	BIN	Life Stage / sex	Max intraspecific K2P distance (%)	Specimens Count	Geographic Distribution (Countries: Regions/Provinces)	Identified by	Alternative species names in BOLD
<i>D. sahandensis</i>	BOLD: ACD7013	Pupa male, larva	0.48	2	Iran: Alborz Province, Mazandaran Province	Javaherkalam, A., Makarchenko E.A.	<i>Diamesa</i> <i>latitarsis</i>
<i>D. sangecha</i> sp. nov.	Not available	Mature Pupa, Pupa, Larva, Imago male	1.54	5	Kyrgyzstan: Chüy region, China: Xinjiang Uygur Autonomous Region	Makarchenko E.A.	
<i>D. tskomelidzei</i>	Not available	Imago male	-	1	Russia: Karachay-Cherkessia	Makarchenko E.A.	

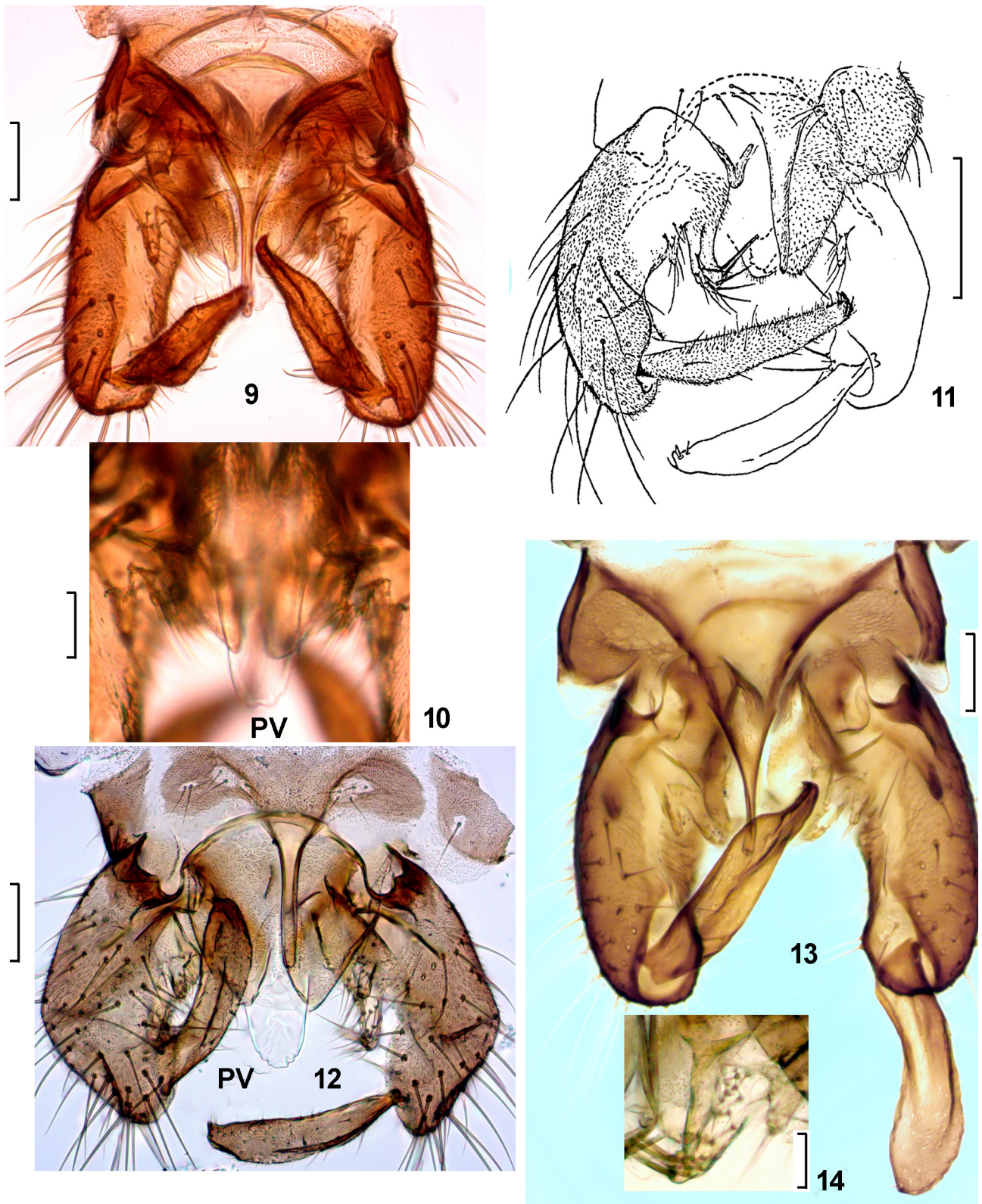
Key to species of *Diamesa latitarsis* group based on adult males

1. Anal point short, 20–64 µm long 2
- Anal point long, 90–128 µm long 6
2. Gonostylus narrow crescent-shaped; SVo with a large pedestal 3
- Gonostylus of different shape, SVo without pedestal 4
3. Eyes bare, not extended dorsomedially. AR 0.70–0.74. IVo absent (Figs 1–4)
..... *D. achipseensis* Makarchenko, Semenchenko *et* Palatov
- Eyes hairy, with short wedge-shaped dorsomedial extension. AR 0.83–0.92. IVo present (Figs 20–25)
..... *D. sahandensis* Namayandeh *et* Ibrahimi
4. Antenna with highly reduced plume of setae. Transverse sternapodeme rounded-triangular, with pointed median part.
Gonostylus black-brown, relatively narrow, slender, somewhat swollen in median part (Figs 16–19)
..... *D. martae* Kownacki
- Antenna with developed plume of setae. Transverse sternapodeme arched. Gonostylus of different shape. 5
5. Gonostylus in basal third or half widened along inner margin and with wedge-shaped appendage directed at angle backwards.
Anal point 40–48 µm long (Figs 5–6) *D. caucasica* Kownacki *et* Kownacka
- Gonostylus with expansion in middle part. Anal point 25–30 µm long (Fig. 15) *D. laticauda* Serra-Tosio
6. Antenna with slightly reduced plume of setae 7
- Antenna with normal plume of setae 8
7. Gonostylus nearly straight and very long, subequal in width along entire length. Inferior volsella broad, flat, rounded
apically. AR 0.71–0.73 (Figs 52–54) *D. elbrusica* Makarchenko, Semenchenko *et* Palatov
- Gonostylus less long, slightly curved in the basal third and slightly expanded distally, with a rounded apex and megaseta 8
µm long Inferior volsella yellow, wide in basal 1/3 and finger-like in distal 2/3. AR 0.88–0.94
..... *D. tschmelidzei* Kownacki *et* Kownacka (Figs 47–48)
8. Inferior volsella with a small, dorsolateral flat or finger-like projection and two stronger, ventromedial spine- and seta-
bearing projections 9
- Inferior volsella with dorsolateral lobe very reduced or elongated, but never digitiform or flat; ventromedial lobe without
spine or seta bearing projections 10
9. Dorsolateral lobe of inferior volsella finger-like, short but visible (Figs 9–14) *D. lindrothi* Goetghebuer
- Dorsolateral lobe of inferior volsella large and flat (Fig. 7) *D. goetghebueri* Pagast
10. Dorsolateral lobe of inferior volsella very small, difficult to see (Fig. 51) *D. wuelkeri* Serra-Tosio
- Dorsolateral lobe of inferior volsella well developed 11
11. Dorsolateral and ventromedial lobes of inferior volsella well separated; dorsolateral lobe more elongated 12
- Dorsolateral and ventromedial lobes of inferior volsella almost fused; dorsolateral lobe less elongated, only a slightly
longer than ventromedial lobe, wider (Fig. 46) *D. modesta* Serra-Tosio
12. AR 0.9–1.0; gonostylus short, 156–176 µm long, slightly curved, convex in the middle, tapering distally and beak-shaped
subapically (Figs 37–40) *D. sangecha* **sp. nov.**
- AR > 1; gonostylus longer (192–204 µm), and narrow, slender, almost straight, may be expanded in the basal third along
the inner edge, not beak-shaped subapically 13
13. AR 1.3–1.6; gonostylus expanded in the basal third along the inner edge (Fig. 8) *D. latitarsis* (Goetghebuer)
- AR 1.83–2.13; gonostylus relatively narrow, slender, somewhat swollen in median part
..... *D. demavendensis* **sp. nov.** (Figs 41–45)

Comments for key. For some species of the *D. latitarsis* group, we used the data given in the key to the genus *Diamesa*, compiled by Montagna *et al.* (2016a). Due to insufficient data, the species *D. valkanovi* Sæther is not included in the key.



FIGURES 1–8. Adult males of *Diamesa achipseensis* Makarchenko, Semenchenko *et* Palatov (1–4), *D. caucasica* Kownacki *et* Kownacka (5–6), *D. goetghebueri* Pagast (7) and *D. latitarsis* (Goetghebuer) (8). 1, 7–8, hypopygium, in dorsal view; 2, basal part of hypopygium from ventral side; 3–4, superior volsellae 5, gonostylus; 6, hypopygium, in lateral view. Scale bars are as follows: Figs 1–2, 5–6, 8—50 μ m, Figs 3–4—20 μ m, Fig. 7—100 μ m. Figs 1–4 after Makarchenko *et al.* (2024); Figs 5–6 after Makarchenko *et al.* (2022c); Fig. 7 after Serra-Tosio (1971).



FIGURES 9–14. Adult males of *Diamesa lindrothi* Goetghebuer from Caucasus (9–10, 12), Alps (11) and Norway (13–14). 9, 11, 12–13, hypopygium, in dorsal view; 10, pars ventralis. PV—pars ventralis. Scale bars are as follows: Figs 9, 12–13—50 μ m, Fig. 11—100 μ m, Figs 10, 14—20 μ m. Fig. 11 after Serra-Tosio (1971).

Taxonomy

Diagnoses and descriptions

Diamesa achipseensis Makarchenko, Semenchenko *et* Palatov

Diamesa achipseensis Makarchenko, Semenchenko *et* Palatov, 2024: 478; Ghaderi *et al.* 2024: 81.

Remarks. This species was described by single adult male (Makarchenko *et al.* 2024) from Southwest Caucasus. It is most closely related to *D. sahandensis* from Iran but is clearly distinguished by a number of features given in the key. Below we prepare short diagnosis for comparison with other species of *D. latitarsis* group.

Material examined. Adult male (holotype), RUSSIA: Caucasus, Krasnodar Region, Adler District, Sochi urban district, Sochi National Park, Achipse River, 3 km North of the Estosadok Village, alt. 600 m above sea level, 12.IV.2024, 43.707194 N 40.262828 E, leg. D. Palatov.

Diagnosis.

Adult male. Total length 3.2 mm. Total length/wing length 0.83. Eyes bare and not extended dorsomedially. Clypeus with 10 setae. Antenna with 13 flagellomeres and slightly reduced plume of setae; AR 0.70–0.74. Head width/palpal length 1.02. Anteprepronotum with 6–7 ventrolateral setae. Dorsocentrals 14–15, prealars 11–12, scutellars 34. Wing length 3.84 mm. Costal extension 82 μ m long. Anal lobe outline rounded. Squama with 34 setae in 2–3 rows. Hind tibial comb with 18 setae. LR1 0.67, BV1 3.66, SV1 2.70.

Hypopygium (Figs 1–4). Tergite IX narrow, with 16–18 setae on one side of the anal point; anal point 40 μ m long, bending under tergite IX, dark brown in basal 2/3 and light grey in distal 1/3, with rounded apex. Transverse sternapodeme wide rectangular, with rounded outer angles. Aedeagal lobe knife-shaped, 180 μ m long and 48 μ m wide, weakly chitinized; phallapodeme sclerotized, 120–128 μ m long. Gonocoxite without inferior volsella, basally with superior volsella consisting of ventral and dorsal parts; ventral part located on stalk and subapically with 18–20 needle-shaped setae, dorsal part rounded, with some long setae. Gonostylus narrow and crescent-shaped, slightly expanded basally, its surface with short setae, *ca* 8 μ m long, along the inner edge with thin setae, 40–48 μ m long; apex with short megaseta, 12 μ m long. HR 1.16.

Pupa and larva. Unknown.

Distribution. Known only from the type locality in Achipse River of the Southwest Caucasus.

Diamesa caucasica Kownacki *et* Kownacka

Diamesa caucasica Kownacki *et* Kownacka, 1973: 131; Langton & Wisser 2003: 17; Ashe & O'Connor 2009: 272; Makarchenko *et al.* 2022c: 446.

Remarks. This species was described by Kownacki & Kownacka (1973) and redescribed by Makarchenko *et al.* (2022c) from the Caucasus. Below we present a diagnosis of adult male based on our material and according to Kownacki & Kownacka 1973.

Material examined. RUSSIA: 3 adult males, Republic of North Ossetia–Alania, Irafsky District, North Caucasus, Uruk River, about 12 km from Mastocete Glacier, 03.I.2018, alt. 1829 m a.s.l., 42.900183 N, 43.585233 E, leg. D. Palatov; 5 adult males, the same locality, except Tanandon River, about 8 km from Tanancete Glacier, 03.I.2018, alt. 1718 m a.s.l., 42.89535 N, 43.604733 E, leg. D. Palatov; 3 adult males, the same locality, except Alagirsky District, Skazdon River, about 3.5 km from Skazsky Glacier, 27.VII.2019, alt. 1943 m a.s.l., 42.784167 N, 43.903667 E, leg. D. Palatov; 6 adult males, the same locality, except Tseydon River near Vertical Hotel, about 7.5 km from Tseysky Glacier, 07.V.2022, 42.79266 N, 43.92005 E, leg. D. Palatov.

Diagnosis

Adult male. Total length 2.9–6.0 mm. Total length/wing length 0.83–0.92. Eyes bare and not extended dorsomedially. Antenna with 13 flagellomeres and developed plume of setae; AR 0.91–1.22. Palpomeres 1–5 length/head width 0.94–1.0. Anteprenotum with 4–8 lateral setae. Dorsocentrals 7–12, prealars 4–6, scutellars 19–29. Wing length 3.4–3.6 mm; costa extension 115–131 μm long; anal lobe developed, squama with 21–26 setae, in 1–2 rows. Hind tibial comb with 14–15 setae. LR1 0.66–0.68, BV1 3.45–3.68, SV1 2.63–2.80.

Hypopygium (Figs 5–6). Tergite IX with 14–19 setae and located on protuberance; anal point short, 40–48 μm long and naked, in lateral view slightly curved upwards. Transverse sternapodeme trapezoidal. Superior volsella double, covered with setae. Gonostylus in basal third or half widened along inner margin and with wedge-shaped appendage directed at angle backwards, densely covered with long and fine setae; distal half or two thirds of gonostylus narrow and of the same width, covered with fine setae and ending in short megaseta. HR 0.90–1.02.

Pupa. Described by Kownacki & Kownacka (1973) and Langton & Wisser (2003).

Larva. Not described but some information available (Kownacki & Kownacka 1973).

Distribution. Endemic to the Caucasus. Known only from glacial streams of the Terek River basin in the Caucasus.

Diamesa goetghebueri Pagast

(Fig. 7)

Diamesa goetghebueri Pagast, 1947: 474; Serra-Tosio 1968: 76, 1971: 163; Langton & Wisser 2003: 21; Ashe & O'Connor 2009: 275; Rossaro & Lencioni 2015a: 126, 2015b: 66; Montagna *et al.* 2016a: 322.

Diamesa berardensis Serra-Tosio, 1964: 38.

Remarks. *D. goetghebueri* is absent from our material, so we present a brief diagnosis below based on Serra-Tosio (1964, 1968, 1971). We agree with Montagna *et al.* (2016a) that this species is difficult to distinguish from *D. lindrothi*, and that further investigation using new material and mandatory examination of the types is required. More detailed data on the *D. goetghebueri*–*D. lindrothi* complex and the problem of identifying these species are given below in the section “Phylogenetic reconstruction and discussion”.

Diagnosis

Adult male. Total length 2.5–3.4 mm. Eyes bare and not extended dorsomedially. Antenna with 13 flagellomeres and developed plume; AR 0.51–0.64. Anteprenotum with 4–7 lateral setae. Wing length 2.5 mm. LR1 0.69, BV1 3.65, SV1 2.79.

Hypopygium (Fig. 7). Tergite IX with 3–6 short setae; anal point blunt, 105 μm long and with consistently minute subterminal spine on the lower margin. Gonocoxite slightly curved; dorsolateral lobe of inferior volsella massive, well-defined but rather short, bearing a few microtrichia and numerous fairly short setae (40 μm); anterior setae more robust than posterior setae and directed mostly forward; dorsal margin forming a ridge with numerous small, short setae; ventromedial lobe of inferior volsella fairly short, flat, with its concavity facing inward, extending posteriorly beyond the level of the dorsolateral lobe. Two isolated interolateral setae are consistently present slightly anterior to the articulation of the gonocoxite. Gonostylus distinctly curved forward, pubescent, bearing a few fine, short setae; proximal half only slightly wider, with a concave anterior margin; megaseta small, 13–16 μm long, accompanied by a projection of the same size or slightly shorter.

Pupa. Described by Serra-Tosio (1968, 1971) and Langton & Wisser (2003).

Larva. Described by Rossaro & Lencioni (2015a–b).

Distribution. This species is endemic to the Alps (Serra-Tosio 1973).

Diamesa latitarsis (Goetghebuer)

(Fig. 8)

Psilodiamesa latitarsis Goetghebuer, 1921: 105.

Diamesa latitarsis (Goetghebuer, 1921); Edwards 1929: 305; Goetghebuer 1932: 175, 1939: 19; Pagast 1947: 473; Wülker 1959: 339; Serra-Tosio 1964: 34, 1968: 66, 1971: 156; Pankratova 1970: 85; Langton & Wisser 2003: 29; Ashe & O'Connor 2009: 279; Rossaro & Lencioni 2015a: 127, 2015b: 66; Montagna *et al.* 2016a: 320; Namayandeh *et al.* 2021: 403.

Remarks. The species has been described in sufficient detail. We provide a brief diagnosis of the adult male based on our material and descriptions of Serra-Tosio (1968, 1971).

Material examined. 1 adult male, NORWAY, Vaksdal Municipality, Ekse, Hoi, Ekse River, light trap, 14–16.VIII.1976, leg. G. Halvorsen. 1 adult male, Russia, Republic of North Ossetia–Alania, Irafsky District, Bilagidon River, alt. 1320 m a.s.l., 2.I.2018, 42.956483 N, 43.725633E, leg D. Palatov; 1 adult male, the same data, except Alagir District, Skazdon River at its source, near the ski lifts of the Tsey ski resort, alt. 1943 m a.s.l., 27.VII.2019, 42.784167 N, 43.903667 E, leg. D. Palatov.

Diagnosis

Adult male. Total length 4.0–4.2 mm. Total length/wing length 1.24. Eyes bare and not extended dorsomedially. Clypeus with 10–11 setae. Antenna with 13 flagellomeres and well developed plume of setae. AR 1.29–1.30. Head width/palpal length 0.9. Anteprepronotum with 9–10 ventrolateral setae. Dorsocentrals 14–15, prealars 9, scutellars 12. Wing length 3.4 mm. Costal extension 82 µm long. Anal lobe outline rounded. Squama with 38 setae in 2–3 rows. LR1 0.71, BV1 3.86, SV1 2.51.

Hypopygium (Fig. 8). Tergite IX with 10–11 setae, 40–64 µm long on one side of the anal point; anal point 120 µm long. Laterosternite IX with 8–9 setae, 36–40 µm long. Transverse sternapodeme wide and rectangular, 128 µm long and 32 µm wide, with rounded outer angles. Gonocoxite slightly curved inwards, 216 µm long; dorsolateral lobe of inferior volsella 112–120 µm long, longer than ventromedial lobe, relatively wide at the base, gradually tapering towards the apex, with short setae along the outer edge, which are longer subapically; ventromedial lobe 52 µm long, flattened and broadly triangular. Gonostylus 200–204 µm long, slightly curved in its distal half, with dense pubescence interspersed with numerous fine, short setae (15–20 µm); proximal half only slightly wider, with a slightly concave anterior margin; apex with short megaseta, 8–13 µm long, accompanied on the apical side of the segment by a smaller, darker chitinous projection; HR 1.06.

Pupa and larva. Described by Pagast (1947), Serra-Tosio (1968), Pankratova (1970), Langton & Wisser (2003), and Rossaro & Lencioni (2015b).

Distribution. Widespread in the Western Palaearctic.

Diamesa lindrothi Goetghebuer

Diamesa lindrothi Goetghebuer in Goetghebuer & Lindroth, 1931: 281; Pagast 1947: 474; Pankratova 1970: 86; Hansen & Cook 1976: 116; Langton & Wisser 2003: 31; Ashe & O'Connor 2009: 280; Montagna *et al.* 2016a: 322.

Remarks. This species is considered widespread in the Western Palaearctic (Ashe & O'Connor 2009). However, no detailed morphological analysis has been carried out on populations from different parts of its range, nor has the type material from Iceland been examined. Our own material for this purpose was also limited; nevertheless, the difference in the shape of the male gonostylus among specimens from Norway, the Alps, and the Caucasus is striking. We therefore cannot exclude the possibility that we are dealing with different species, possibly cryptic ones. This hypothesis is supported by the results of the genetic analysis presented below.

Material examined. 1 adult male, NORWAY, Finse, near Blåisen Glacier, 13.VIII.1980, leg. E. Willassen. 1 adult male, RUSSIA, Kabardino-Balkaria Republic, Chereksky District, a spring stream on the right bank of the Mizhirgi River (right source of the Bezengi Cherek River), opposite the Bezengi alpine camp alt. 2150 m a.s.l., 19.VII.2022, 43.1099556 N, 43.1508944 E, leg. D. Palatov; 1 adult male, the same data, except waterfall on an

unnamed river of Mizhirgi River basin, 1.3 km SE of the Bezengi alpine camp, alt. 2410 m a.s.l., 21.VII.2022, 43.104700 N, 43.161586 E, leg. D. Palatov, 1 adult male, the same data, except Elbrus region, the valley of the Baksan River near the village “Polyana Azau” and the ski lifts to Elbrus, night light trap, alt. 2370 m a.s.l., 26.VII.2023, 43.265200 N, 42.476786 E, leg. D. Palatov; 1 adult male, the same data, except Krasnodar Krai, Sochi Urban District, Adler District, Rzhany Brook, 500 m southeast of the Rosa Khutor railway station, alt. 600 m a.s.l., 13.IV.2024, 43.673175 N, 40.285014 E, leg. D. Palatov.

Diagnosis

Adult male. Total length 2.5–4.8 mm. Total length/wing length 1.3. Eyes bare and not extended dorsomedially. Clypeus with 3–9 setae. Antenna with 13 flagellomeres and well-developed plume of setae; AR 0.9–1.33. Anteprenotum with 6 ventrolateral setae. Dorsocentrals 10–13, prealars 7–12, scutellars 12. Wing length 2.4–3.6 mm. Costal extension 82 µm long. Anal lobe outline rounded. Squama with 38 setae in 2–3 rows. LR1 0.71–0.75, BV1 3.62–3.86, SV1 2.51.

Hypopygium (Figs 9–14). Tergite IX with 10–11 setae, 40–64 µm long on one side of the anal point; anal point 120 µm long. Laterosternite IX with 8–9 setae, 36–40 µm long. Transverse sternopodeme wide and rectangular, 128 µm long and 32 µm wide, with rounded outer angles. Gonocoxite slightly curved inwards, bearing 2 very unequal lobes of inferior volsella—ventromedial and dorsolateral. Ventromedial lobe prominent, very detached from the gonocoxite, wider at its distal end, bearing numerous setae 40 µm long; distal end of this lobe with anterior setae much more robust than the others and directed rather forwards. Dorsolateral lobe very short, not extending or only slightly extending posteriorly beyond the level of the tip of the lower segment, bearing short setae, 30–35 µm long and sparse microtrichiose hairs. Remainder of the gonocoxite with long setae (max. 100–130 µm long) and 2 inferolateral setae at the joint of the gonostylus. Gonostylus from different regions has different shapes. For male from Alps, Serra-Tosio (1971) indicated that the gonostylus was straight, pubescent, bearing fine, short setae (15–25 µm long), with anterior margin always nearly straight; megaseta 9–10 µm long, light-colored, accompanied by a pointed chitinous projection, smaller and darker (Fig. 11). In our material from Norway the gonostylus has a narrowed subapical part (Fig. 13), and in material from the Caucasus there is an expansion basally along the outer edge of the gonostylus and an expansion in the middle along the inner edge (Fig. 9), as well as a slightly expanded apex (Fig. 12).

Pupa. Described by Pagast (1947), Serra-Tosio (1968) and Pankratova (1970).

Larva. Unknown.

Distribution. Widespread (?) in the Western Palearctic.

Diamesa laticauda Serra-Tosio

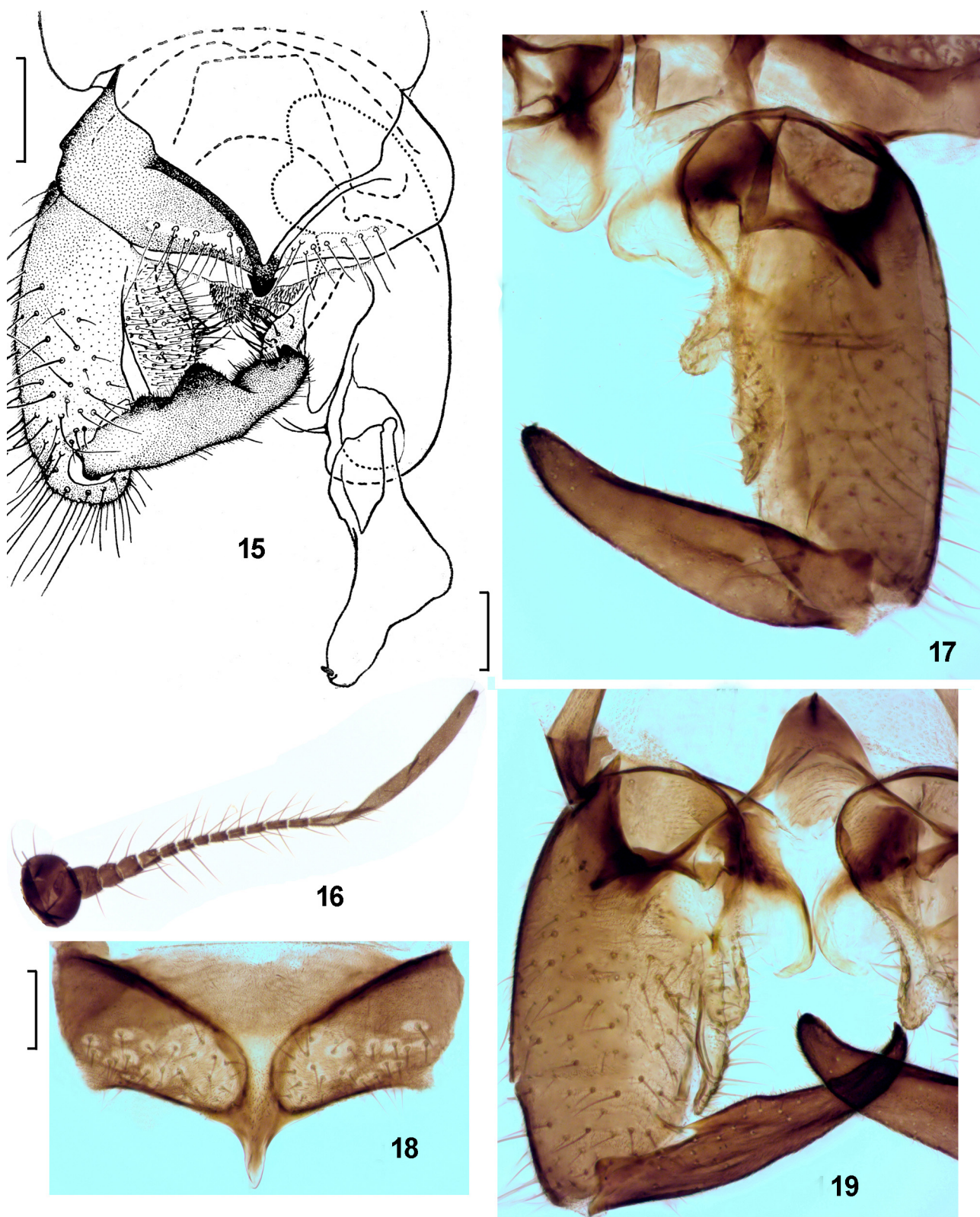
(Fig. 15)

Diamesa laticauda Serra-Tosio, 1964: 35, 1971: 178; Ashe & O'Connor 2009: 279; Montagna *et al.* 2016a: 322.

Remarks. The species is known only from the original description of the adult male by Serra-Tosio (1964, 1971) and the findings of Montagna *et al.* (2016a) in the Alps. Below we provide a brief diagnosis of this species according to Serra-Tosio (1964).

Diagnosis

Adult male. Total length 3.1–4.0 mm. Total length/wing length 0.83–0.92. Eyes pubescence and not extended dorsomedially. Antenna with 13 flagellomeres and developed plume; AR 0.98–1.2. Clypeus with 10 setae. Anteprenotum with 4–7 lateral setae. Dorsocentrals 10–15, prealars 5–7. Wing length 3.5 mm; anal lobe developed, squama with *ca* 30 setae. LR1 0.67, BV1 3.64, SV1 2.89.



FIGURES 15–19. Adult males of *Diamesa laticauda* Serra-Tosio (**15**) and *D. martae* Kownacki (**16–19**). **15**, **19**, hypopygium in dorsal view; **16**, antenna; **17**, gonocoxite and gonostylus; **18**, tergite IX. Scale bars are as follows: Fig. 15—200 μm , Figs 16–19—50 μm . Fig. 15 after Serra-Tosio (1971).

Hypopygium (Fig. 15). Tergite IX short, with 10–15 setae, 85 μm long; anal point very short (25–30 μm), broad and bluntly lance-shaped, rounded at the apex. Laterosternite IX with 15–20 setae. Transverse sternapodeme

trapezoidal. Gonocoxite in dorsal view slightly curved inwards; dorsolateral lobe of inferior volsella flat, densely covered with fine setae and extending backwards beyond 3/4 of the gonocoxite; ventromedial lobe shorter, club-shaped, covered at its distal end with robust setae. Remainder of the gonocoxite with long setae (max. 140 µm) and 2 inferolateral setae at the articulation with the gonostylus. Gonostylus with a large rounded-triangular protrusion along the inner edge, bearing a strong protuberance on its upper margin, covered with dense pubescence interspersed with numerous fine setae (max. 40 µm); in a bent boomerang shape; megaseta short (15–20 µm), black, with a chitinous projection.

Pupa. Described by Serra-Tosio (1968).

Larva. Unknown.

Distribution. Known from the Alps, Poland, and Slovakia (Ashe & O'Connor 2009).

***Diamesa martae* Kownacki**

Diamesa martae Kownacki in Kownacki & Kownacka 1980: 314; Ashe & O'Connor 2009: 281; Montagna *et al.* 2016a: 322.

Remarks. The species was described from the Austrian Alps and the Great Caucasus (Kownacki & Kownacka 1980). Later, Montagna *et al.* (2016a) refined the description of *D. martae* based on material from the Italian Alps. We present a diagnosis based on adult males from our material and the data of authors *loc. cit.*

Material examined. 1 adult male, RUSSIA: Republic of North Ossetia–Alania, Irafsky District, Tanadon River, alt. 1718 m a.s.l., 8.5, 42.89535 N, 43.604733 E, 3.I.2018, leg. D. Palatov; 1 adult male, the same data, except Alagirsky district, Tseyskoye Gorge, Tseydon River in the area of the Vertical Hotel, alt. 1755 m a.s.l., 24.VII.2021, 42.79266 N, 43.920051 E, leg. D. Palatov. 1 adult male, REPUBLIC OF ABKHAZIA: Gulripshi District, Kodor River basin, Dzshampal River near mouth of Shakuran River, 43.028589 N, 41.334719 E, 2.II.2018, leg. D. Palatov.

Diagnosis

Adult male. Total length 3.9–6.5 mm. Total length/wing length 0.87–1.05. Eyes bare and not extended dorsomedially. Antenna with 13 flagellomeres and reduced plume of setae; pedicel with 4 setae; AR 0.87–1.04. Anteprenotum with 6–12 lateral setae. Dorsocentrals 12–16, prealars 4–6, scutellars 18. Wing length 3.62–4.6 mm; costa extension 20–164 µm long; anal lobe developed, squama with 34 setae in 2–3 rows. Hind tibial comb with 16–20 setae. LR1 0.61, BV1 3.86–4.65, SV1 3.0–3.03.

Hypopygium (Figs 16–19). Tergite IX with 24–25 setae; anal point short, 64 µm long, and naked. Transverse sternapodeme almost the triangular shape, 72 µm height, 96 µm wide. Phallapodeme 116 µm long, aedeagal lobe as in Fig. 17, 48 µm long, Superior volsella of gonocoxite rounded, not clearly differentiated, inferior volsella with two lobes, an anterior dorsal lobe digitiform, well separated from gonocoxite, projecting medially, and a posterior ventral lobe elongated, adhering to gonocoxite; outer surface of the gonocoxite covered with longer hairs; two strong setae on the posteromedian margin of gonocoxite. Gonostylus slender, somewhat swollen in median part, ending with apical spine, covered with short setae on bright areas. HR 1.31.

Pupa and larva. Unknown.

Distribution. Known from the Caucasus and Alps.

***Diamesa sahandensis* Namayandeh et Ibrahimi**

Diamesa sahandensis Namayandeh et Ibrahimi in Ghaderi, Namayandeh, Ibrahimi, Mohammadi, Karimian, Mansouri *et* Molodi, 2024: 80.

Diamesa latitarsis, larva, available from: <https://www.ncbi.nlm.nih.gov/nuccore/JF764767>

Remarks. Two years ago, *D. sahandensis* Namayandeh et Ibrahimi, a species in the *Diamesa latitarsis* group, was described based on a single adult male from East Azerbaijan, Iran. While studying material collected in 2019 by D.M.

Palatov in Iran, we discovered pharate males, mature pupae and larvae of this species, conducted morphological and DNA analyses, and deemed it appropriate to redescribe this species based on the new material.

Material examined. 3 pharate adult males extracted from mature pupa, 4 mature pupae, 6 larvae, IRAN: Mazandaran Province, Velayat Roud River below Dizin Village, alt. 2529 m a.s.l., 27.VI.2019, 36.0554N, 51.40843E, leg. D. Palatov.

Description

Pharate adult male (n=2). Total coloration brown to dark-brown; head, thorax, legs, and abdomen brown to dark brown; antennae light brown.

Head. Eyes hairy, with short wedge-shaped dorsomedial extension. Temporal setae including 4–5 preoculars, 16 verticals, 12–14 postorbitals. Clypeus with 8 setae. Antenna with 13 flagellomeres and slightly reduced plume of setae; terminal flagellomere with 1 subapical seta, 32 μm long; pedicel with 3 setae, 36–72 μm long. Lengths (μm) of flagellomeres 1–13: 40, 28, 36, 36, 40, 40, 36, 40, 48, 44, 48, 48, 400. AR 0.83. Palpomere lengths (μm): 62, 84, 120, 140, 180. Palpomere 3 in distal part with sensilla capitata with diameter 12 μm .

Thorax. Anteprenotum with 7–8 ventrolateral setae, 52–64 μm long. Dorsocentrals 8–12, 88–100 μm long; prealars 3–4, 8–88 μm long; scutellars 15–16, 144–160 μm long.

Wings and legs absent.

Hypopygium (Figs 20–25). Tergite IX with 14 setae on each side and; anal point 20 μm long. Laterosternite IX with 4 setae. Tergite IX with 9–14 setae and short anal point, 20–47 μm long and 29 μm wide at the base, with rounded apex. Superior volsella well developed, with a large pedestal, 71 μm long, 47 μm wide at the base, and 33 μm wide at the apex, bearing 20 robust, spine-like setae. IVo club-shaped, on a long stalk. Gonostylus 308 μm long, curved, crescent shaped, with a basiventral projection bearing 6 apical setae, 10–25, 16 μm long; ventral surface of gonostylus with long, fine setae 23–35 μm long, dorsal surface with shorter setae 5–16 μm long; megaseta 12 μm long.

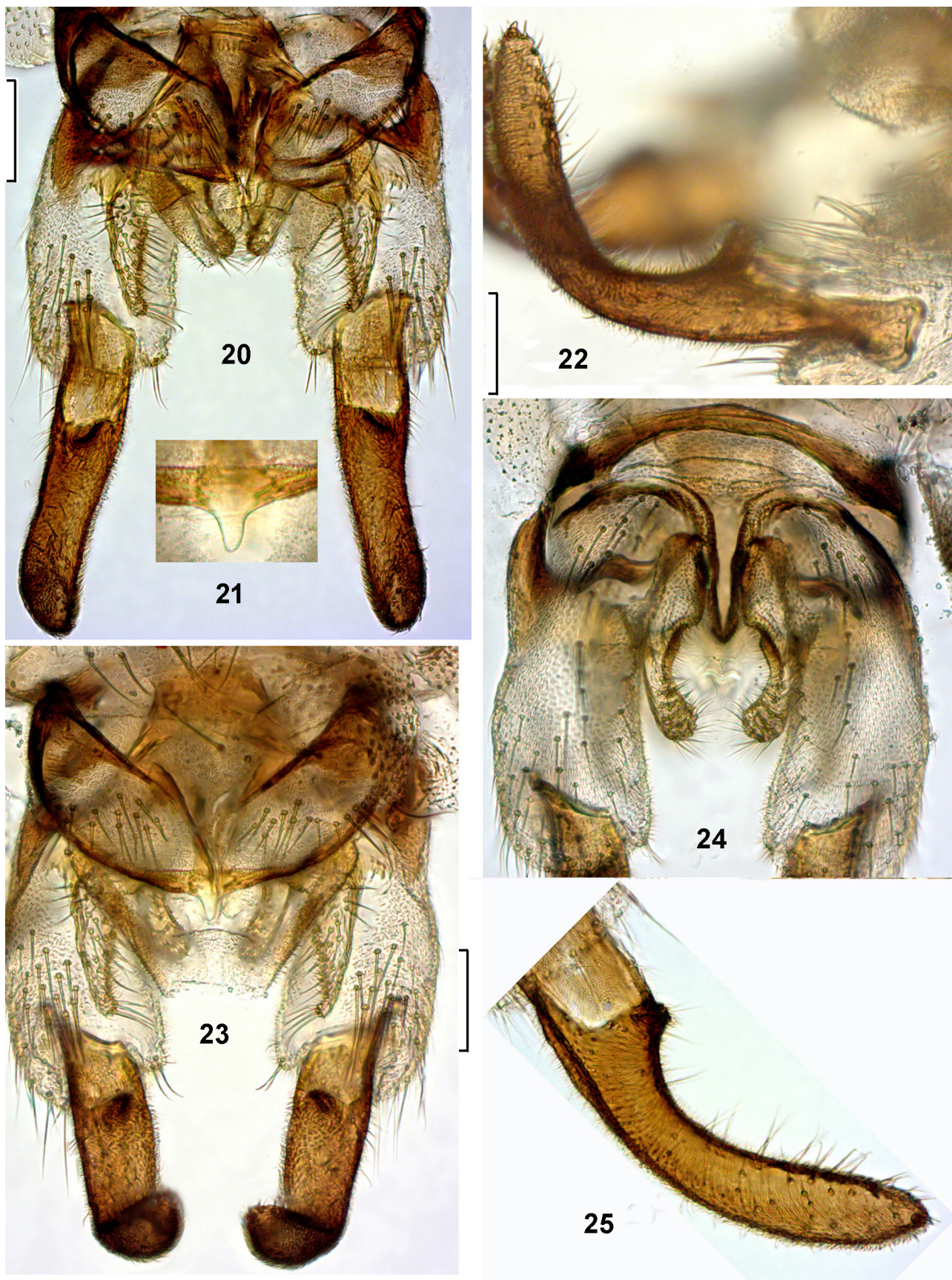
Pupa (n=2). Total length 3.2–3.4 mm.

Coloration. Cephalothorax and abdomen dark brown, brown; thoracic horn brownish with dark basal part (Fig. 26). Exuviae brownish yellow.

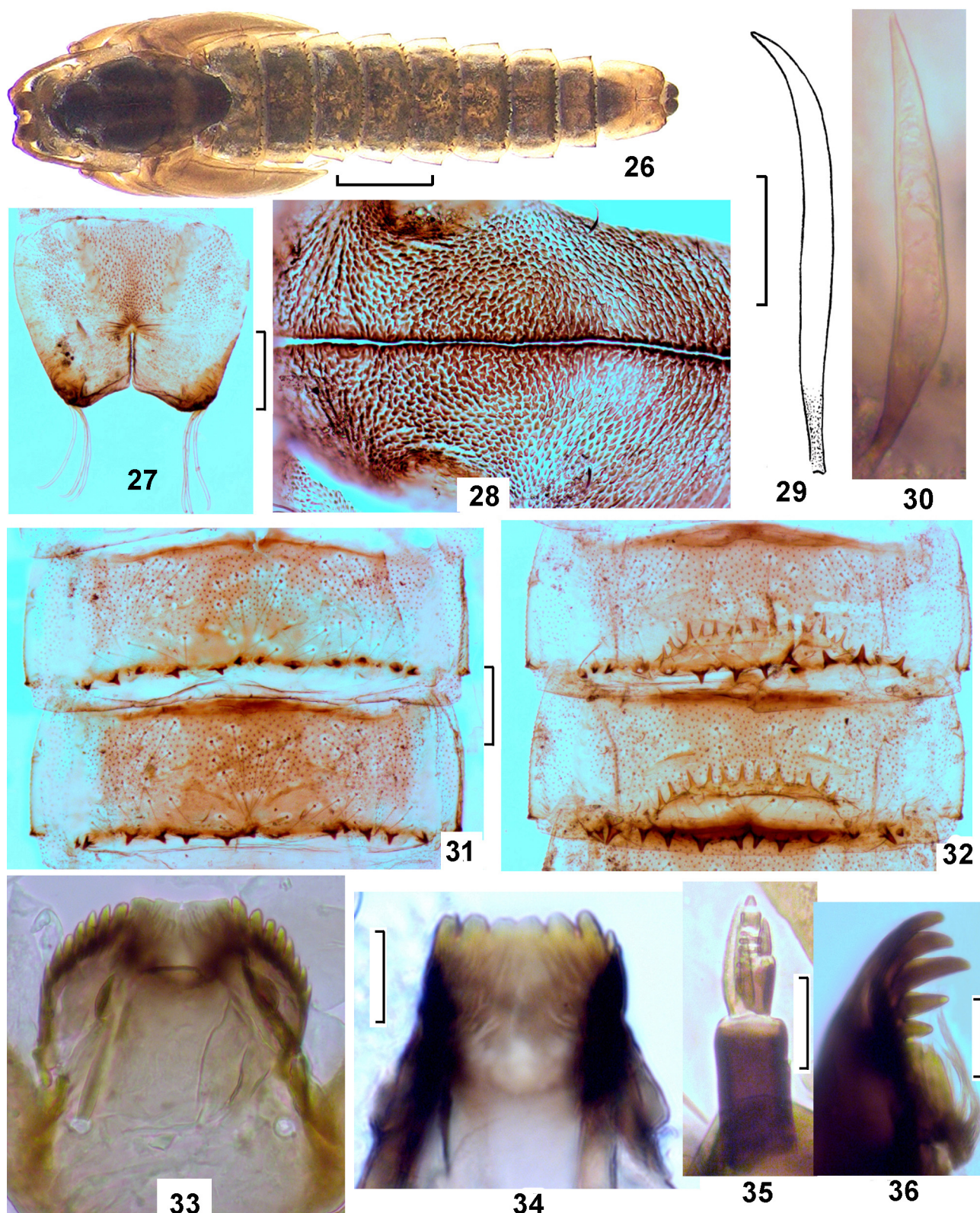
Cephalothorax. Frontal apotome granulated, with 2 setae 76–88 μm long. Thorax wrinkled in anterodorsal and lateral parts granulated (Fig. 28). Thoracic horn 128–150 μm long, almost lanceolate, without small spinules at the top (Figs 29–30). Two precorneal setae, 60–68 μm long. Anteprenotum with 2 median and 2 lateral anteprenotals. Mesonotum with 2 dorsocentrals: Dc1 strong, 249 μm long, Dc2 hair-like, 40 μm long.

Abdomen. Tergite I with shagreen in anterior 1/3 and laterally. Tergites II–VIII with shagreen in anterior half or almost all with shagreen. Sternites I–II without shagreen, sternites III–VIII with sparse shagreen and IX without shagreen. Tergites I–VIII with posterior transverse row spines, number of these spines on tergites respectively—16–19, 10–15, 13–18, 11–15, 14–15, 12–13, 13–14, 9–10 (Figs 31–32). Number of posterior transverse row spines of sternites IV–VIII respectively—11–13, 11–16, 10–11, 8–12, 10–12; sternite III with very small spinules of posterior transverse row. Segments I–VIII with 4 pairs of lateral setae, 56–72 μm . Segments II–VIII with spine-like process on posterolateral corner. Anal lobe with 3 yellow anal macrosetae, 204–224 μm long, slightly curved in distal part and pointed (Fig. 27). Male genital sac extended beyond anal lobe (Fig. 26).

Fourth instar larva (n=4). Total length 4.7–5.5 mm. Head capsule brown, 508–541 μm long and 312–344 μm width; postoccipital margin black and wide. SI and SII short and simple, SIII with 3 short branch. Labral lamellae consisting of 5 lobes. Premandible broad, apically with 6–8 teeth. Antenna with 5 segments, length of antennal segments (μm): 33, 16, 8, 5, 3. Lauterborn organs small; style, 5–8 μm long, reaches base of fourth segment; longest branch of antennal blade, 30 μm long, reaches the apex of the fourth segment; ring organ 5–6 μm diameter located in basal quarter of first segment (Fig. 35); AR 1.03. Mandible dark brown to black, with apical tooth and 4 inner teeth, with the second from the bottom larger than the rest; apical tooth slightly longer than the first inner tooth; seta subdentalis short and wedge-shaped; seta interna with 24–27 simple branches (Fig. 36). Mentum with 2 median and 9 pairs of lateral teeth; median teeth and 2 pairs of lateral teeth are brownish yellow, other teeth brown or dark brown; ventromental plate small (Figs 33–34). Procercus dark brown, in the form of incompletely sclerotized ring, bearing 4 dark brown strong anal setae, 200–210 μm long and 1 hair-like lateral seta which is on the body, 60 μm long. Posterior parapods 1.4 times as long as last body segment.



FIGURES 20–25. Adult male of *Diamesa sahandensis* Namayandeh *et* Ibrahimi. **20, 23**, hypopygium in dorsal view; **21**, anal point; **22, 25**, gonostylus in lateral view; **24**, hypopygium in ventral view. Scale bars: 50 μ m.



FIGURES 26–36. Pupa (26–32) and fourth instar larva (33–36) of *Diamesa sahandensis* Namayandeh *et* Ibrahimi. 26, pupa in dorsal view; 27, anal lobe; 28, dorsal surface of thorax; 29–30, thoracic horn; 31, tergites II–III; 32, tergites VI–VII; 33, mentum with deformed medial teeth; 34, medial teeth of mentum; 35, antenna; 36, mandible. Scale bars are as follows: Fig. 26—500 μ m, Figs 27–28, 31–32—200 μ m, Figs 29–30, 33–34, 36—50 μ m, Fig. 35—20 μ m.

Comments. Our male specimens are morphologically consistent with the holotype of *D. sahandensis*, exhibiting only slight variations in the length of the anal point, the antennal ratio (AR), and the shape of the superior volsella. For instance, while the holotype possesses an anal point of 47 µm and an AR 0.92, our specimen measures 40 µm and 0.83, respectively. Furthermore, this study provides the first descriptions of the pupal and larval stages.

Distribution. Known only from Iran.

Diamesa sangecha Makarchenko, Semenchenko et Palatov, sp. nov.

urn:lsid:zoobank.org:act:9B45D2E7-EA9A-4D4A-A671-C01470FEF6FD

Type material. Holotype, adult male, CHINA: Xinjiang Uyghur Autonomous Region, Dabancheng District, Urumqi City, Tien Shan Mountains, Sangecha River at the first bridge, measured from above, alt. 2356 m a.s.l., 13.VII.2017, 43,676383 N, 88,2834333 E, leg. D. Palatov. Paratypes: 1 adult male, the same data as holotype; 1 pupa, 2 larvae, Kyrgyzstan, Chui Region, Tien Shan Mountains, Kyrgyz Ridge, Ala-Archa River at the mouth of the Karabulak River, alt. 2061 m a.s.l., 28.V.2016, 42.603333 N, 74.480317 E, leg. D. Palatov.

Derivatio nominis. The species is named as *sangecha* after the type locality in the Sangecha River of the Xinjiang Uyghur Autonomous Region of China. The name is a noun in apposition.

Description

Adult male (n = 2, except when otherwise stated). Total length 2.9–3.3 mm. Total length/wing length 1.04–1.06.

Coloration. Dark brown. Wings greyish, with brownish veins.

Head. Eyes bare and not extended dorsomedially. Temporal setae including 6–8 frontals, 9–12 verticals. Clypeus with 9–12 setae. Antenna with 13 flagellomeres and well-developed plume of setae; basal flagellomere with 3 setae; terminal flagellomere with 1 subapical seta, 28–30 µm long; AR 0.9–1.0. Palpomere length (µm): 40–44, 84, 102–104, 100–120, 156–160. Palpomere 3 in distal part with sensilla capitata with diameter 14–16 µm. Head width/palpal length 1.14–1.20. Antennal length/palpal length 2.29.

Thorax. Anteprepronotum with 5–6 ventrolateral setae. Dorsocentrals 10–11, 96–136 µm long; prealars 6–8, 72–80 µm long. Scutellum with 28–30 setae.

Wing. Length 3.1–3.4 mm, width 0.96–1.0 mm. Costal extension 98 µm long. Anal lobe developed, rounded. Squama with 20–23 setae, 120–148 µm long. R and R1 with 23–25 setae, R4+5 with 2–4 setae in apical part. RM/MCu 2.1–2.5.

Legs. Spur of front tibia 68–72 µm long. Spurs of mid tibia 48 µm long. Spurs of hind tibia 80 µm and 44 µm long. Hind tibial comb with 14 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 sangecha* sp. nov., male (n = 2)

	fe	ti	ta1	ta2	ta3	ta4	ta5
P1	1099–1197	1328–1492	951	459	246	98	115
P2	1230–1312	1214–1378	590–607	328	197–336	87–98	115
P3	1394–1476	1476–1640	902–1082	476–508	213–279	98	115–131

TABLE 2. (Continued)

	LR	BV	SV	BR
P1	0.70	3.68	2.55	3.3
P2	0.44–0.49	3.52–4.47	4.14–4.43	3.3
P3	0.61–0.66	3.90–4.42	2.88–3.18	3.5

Hypopygium (Figs 37–40). Tergite IX with 12–14 setae on one side of the anal point, 70–98 µm long; narrow naked anal point, 92–108 µm long, 8 µm wide. Laterosternite IX with 6 setae, 80–100 µm long. Transverse

sternopodeme in the form of a yoke for buckets, 156 μm long. Aedeagal lobe 140 μm long; phallapodeme 108 μm long. Gonocoxite 252–288 μm long; dorsolateral and ventromedial lobes of inferior volsella well separated to each other; dorsolateral lobe broad at the base and gradually narrows toward the apex, covered with short setae; ventromedial lobe wedge-shaped or rounded-wedge-shaped, covered with short setae, which longer at the margin. Gonostylus 156–176 μm long, short, slightly curved, convex in the middle, tapering distally and beak-shaped subapically, with a short megaseta. HR 1.62–1.64.



FIGURES 37–40. Adult male of *Diamesa sangecha* **sp. nov.** 37–38, hypopygium in dorsal view; 39, hypopygium in ventral view; 40, gonocoxite and gonostylus. Scale bars: 50 μm .

Pupa and larva. Unknown because specimens were fully utilized for DNA barcoding.

Diagnosis. Total length 2.9–3.3 mm, wing length 3.08–3.4 mm. Eyes bare, reniform. Clypeus with 9–12 setae. Antenna with 13 flagellomeres and developed plume of setae; AR 1.0. Head width/palpal length 1.14–1.20. Anal lobe rounded. Squama with 20–23 setae. Dorsocentrals of mesonotum 10–11, prealars 6–8, scutellars 28–30. Hind tibial comb with 14 setae. Tergite IX with 12–14 setae and narrow naked anal point, 92–108 μm long, 8 μm wide.

Transverse sternopodeme in the form of a yoke for buckets. Inner lobe of inferior volsella broad at the base and gradually narrows toward the apex, covered with short setae; outer lobe wedge-shaped or rounded-wedge-shaped, covered with short setae, longer at the margin. Gonostylus short, slightly curved, convex in the middle, tapering distally and beak-shaped subapically, with a short megaseta. HR 1.62–1.64.

The new species is closely related to *D. latitarsis*, but is clearly distinguished by the characters given in the key, as well as by molecular genetic data.

Ecology. Adult males were collected in two regions of Tien Shan Mountains on the territory of Kyrgyzstan and China. In the mountain Sangecha River (China), a flowing in a shallow semi-desert mountain valley at an alt. of 2356 m above sea level. The width is 12–16 m, substrates are large stones the flow rate is 0.3–1.2 m/s and the temperature is 7–9°C. Ala-Archa River (Kyrgyzstan) flows in a narrow rocky canyon at an alt. 2061 m above sea level. The stream width is approximately 5 m, the substrate is rocks with sand sometimes near the shore, the flow rate is 0.3–1 m/s, and the temperature is 12°C. Pupa and larvae were collected from silted sand near the shore.

Distribution. Known only from the type locality in Tien Shan Mountains.

***Diamesa demavendensis* Makarchenko, Semenchenko et Palatov, sp. nov.**

urn:lsid:zoobank.org:act:6471E1C1-CCCB-41CD-83E1-985CD38091FD

Diamesa aff. *latitarsis* Semenchenko et al. 2024: 5.

Remarks. The specimen EAM695, originally designated as *Diamesa* aff. *latitarsis* in the multi-locus phylogeny of Semenchenko et al. (2024), is securely recovered here within the *D. demavendensis* **sp. nov.** cluster, establishing its true species identity.

Type material. Holotype: adult male, IRAN: Mazandaran Province, light trap on the Elborz Ridge, under Mount Siah seng, above the Davilat Village, 25.VI.2019, alt. 2792 m a.s.l., 36.230917 N, 51.84255 E, leg. D. Palatov. Paratypes. 1 adult male, the same data as holotype; 1 adult male, RUSSIA: Republic of North Ossetia–Alania, Alagirsky District, Skazdon River, about 3.5 km from Skazsky Glacier, 27.VII.2019, alt. 1943 m a.s.l., 42.784167 N, 43.903667 E, leg. D. Palatov; 1 adult male, the same data except Kabardino-Balkaria Republic, Chereksky District, Psygansu River Valley, on the pass from the village of Verkhnyaya Balkaria, light trap near small spring, 600 m from the river, alt. 2096 m a.s.l., 27.VII.2022, 43.099922 N, 43.523308 E, leg. D. Palatov.

Derivatio nominis. The species is named as *demavendensis* after Demavend Mount, the highest peak in Iran, Western Asia, and the entire Middle East, located near its type locality.

Description

Adult male (n = 2, except when otherwise stated). Total length 4.0–4.2 mm. Total length/wing length 0.87–1.09.

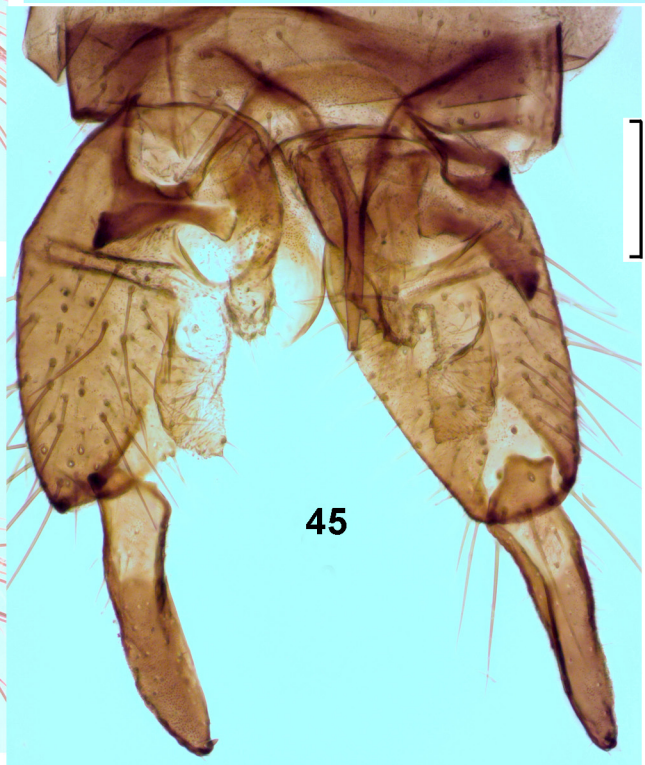
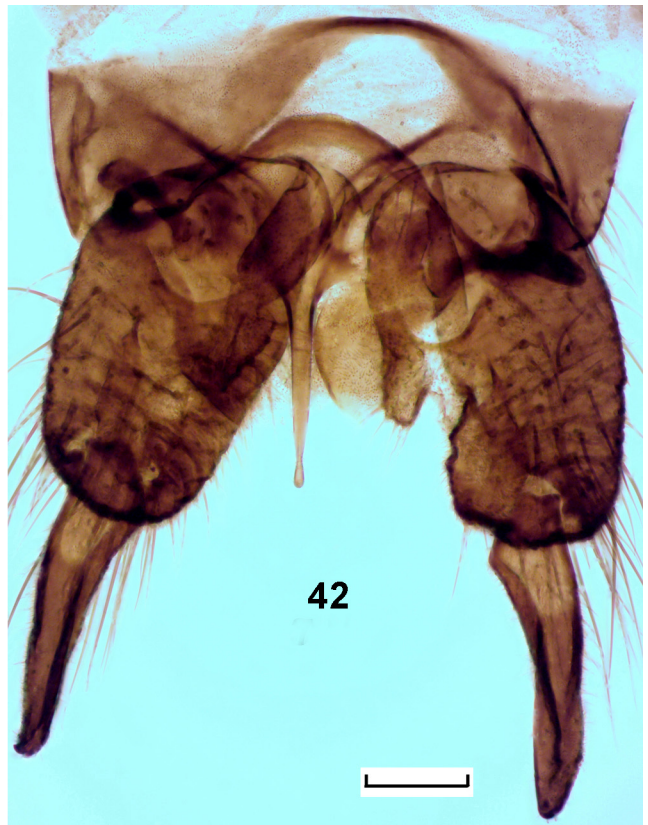
Coloration. Dark brown. Wings greyish, with brownish veins.

Head. Eyes bare and not extended dorsomedially. Temporal setae including 8–9 frontals, 13–14 verticals. Clypeus with 10–14 setae. Antenna with 13 flagellomeres and well-developed plume of setae; pedicel with 3–4 setae; terminal flagellomere with 1 subapical seta, 32–44 µm long; AR 1.83–2.13. Palpomere length (µm): 44–48, 100–108, 152–156, 164–176, 160–188. Palpomere 3 in distal part with sensilla capitata with diameter 24 µm. Head width/palpal length 0.97–1.21.

Thorax. Anteprepronotum with 6–7 ventrolateral setae, 120–192 µm long. Dorsocentrals 10–16, 120–140 µm long; prealars 9–10, 108–140 µm long. Scutellum with ca 30 setae.

Wing. Length 3.84–4.0 mm, width 1.04–1.24 mm. Costal extension 98–148 µm long. Anal lobe developed, rounded. Squama with 37–40 setae in 2–3 rows, 148–200 µm long. R and R1 with 23–28 setae, R4+5 with 2–3 setae in apical part. RM/MCu 2.5–2.7.

Legs. Spur of front tibia 84–88 µm long. Spurs of mid tibia 64–68 µm long and 64–72 µm long. Spurs of hind tibia 104–112 µm and 60–64 µm long. Hind tibial comb with 14–19 setae. Length (µm) and proportions of leg segments are as in Table 3.



FIGURES 41–45. Hypopygium in dorsal view of *Diamesa demavendensis* **sp. nov.** from North Ossetia–Alania (**41**), Kabardino-Balkaria Republic (**42**) and Iran (**43–45**). Scale bars: 50 μ m.

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

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅
P ₁	1280–1400	1640–1880	1200–1240	590–950	361–377	98–115	148–211
P ₂	1480–1520	1600–1640	680–780	402–660	213–262	98–131	115–211
P ₃	1720–1800	1760–2000	1200–1320	623–656	328–361	115–185	129–148

TABLE 3. (Continued)

	LR	BV	SV	BR
P ₁	0.66–0.73	3.35–3.69	2.43–2.65	3.4–3.5
P ₂	0.43–0.48	3.18–4.57	4.41–4.67	3.2–3.8
P ₃	0.65–0.68	3.15–3.81	2.88–2.94	3.5–3.8

Hypopygium (Figs 41–45). Tergite IX with 12–15 setae on one side of the anal point, 36–40 μm long and with narrow naked anal point, 112–120 μm long. Laterosternite IX with 9–14 setae. Transverse sternopodeme in the form of a yoke for buckets. Aedeagal lobe 136 μm long; phallapodeme 96–100 μm long. Gonocoxite 276–280 μm long; dorsolateral lobe of the inferior volsella is flat, does not reach the apex of the gonocoxite, and is sparsely covered with short setae; the ventromedial lobe is wedge-shaped, with a few short setae along the margin. Gonostylus 192–196 μm long; in the bent position, a small angular protrusion at the base along the inner edge can be observed; in the flexed position, it is only slightly expanded in the basal third; terminating in a megaseta and a tooth of the same size. HR 1.41–1.44.

Pupa and larva. Unknown.

Diagnosis. Total length 4.0–4.2 mm. Total length/wing length 0.87–1.09. Eyes bare, reniform. Clypeus with 10–14 setae. Antenna with 13 flagellomeres and well developed plume of setae; pedicel with 3–4 setae; terminal flagellomere with 1 subapical seta; AR 1.83–2.13. Head width/palpal length 0.97–1.21. Anteprenotum with 6–7 ventrolateral setae, Dorsocentrals 10–16, prealars 9–10, scutellars *ca* 30 setae. Wing length 3.84–4.0 mm. Costal extension 98–148 μm long. Anal lobe developed, rounded. Squama with 37–40 setae. Hind tibial comb with 14–19 setae. Hypopygium (Figs 41–45). Tergite IX with 12–15 setae, and narrow naked anal point, 112–120 μm long. Aedeagal lobe 136 μm long; phallapodeme 96–100 μm long. Dorsolateral lobe of the inferior volsella of gonocoxite is flat, does not reach the apex of the gonocoxite, and is sparsely covered with short setae; the ventromedial lobe is wedge-shaped, with a few short setae along the margin. Gonostylus in the bent position with a small angular protrusion at the base along the inner edge; in the flexed position, it is only slightly expanded in the basal third; terminates in a megaseta and a tooth of the same size. HR 1.41–1.44.

The new species is closely related to *D. latitarsis*, but is clearly distinguished by the characters given in the key, as well as by molecular genetic data.

Distribution. Known only from the type locality in Iran and some regions of the Caucasus.

Diamesa modesta Serra-Tosio

(Fig. 46)

Diamesa modesta Serra-Tosio, 1968: 82, 1971: 171; Langton & Wisser 2003: 36; Ashe & O'Connor 2009: 282; Rossaro & Lencioni 2015a: 127, 2015b: 66; Montagna *et al.* 2016a: 321.

Remarks. *D. modesta* is absent from our material and below we present a brief diagnosis according to Serra-Tosio (1968, 1971).

Diagnosis. Total length 3.5 mm. Eyes bare and not extended dorsomedially. Antenna with 13 flagellomeres and developed plume of setae; AR 1.3–1.7.

Hypopygium (Fig. 46). Tergite IX with about 10 setae, 50 μm long, a few similar lateral setae; moderately long anal point, with a rounded end with and apical spine. Gonocoxite slightly curved inwards; ventromedial lobe of inferior volsella massive, only slightly detached from the gonocoxite, with microtrichia and numerous short setae (25 μm), all fine; dorsolateral lobe of inferior volsella a fairly long and wide, extending posteriorly beyond the level

of the gonostylus articulation, with its concavity facing inwards, bearing numerous fine, short setae (25–35 µm) and microtrichia. The remainder of the basal segment has long setae (max. 130 µm), with two inferolateral setae near the articulation with the gonostylus. Dorsolateral and ventromedial lobes of inferior volsella almost fused. Gonostylus nearly straight, pubescent, with short, fine setae (20–35 µm); distal half slightly wider than the proximal half; anterior margin almost straight; megaseta small (10 µm), dark, accompanied by a small dark projection.

Pupa. Described by Serra-Tosio (1968, 1971) and Langton & Wisser (2003).

Larva. Described by Rossaro & Lencioni (2015a,b).

Distribution. Known from the Alps and Caucasus (Ashe & O'Connor 2009).

***Diamesa tskhomelidzei* Kownacki et Kownacka**

Diamesa tskhomelidzei Kownacki et Kownacka 1973: 135; Langton & Wisser 2003: 51; Ashe & O'Connor 2009: 287; Makarchenko & Palatov 2024: 448.

Remarks. This species was described by Kownacki & Kownacka (1973) and redescribed by Makarchenko & Palatov (2024) from the Caucasus. Below we prepare diagnosis of adult male by our material and according to Kownacki & Kownacka (1973).

Material examined. RUSSIA: 2 adult males, Republic of Kabardino-Balkaria, Elbrus District, Kashkatash Glacier, alt. 2630 m a.s.l., 23.VII.2023, 43.2098861 N, 42.6839972 E, leg. D. Palatov; 1 mature pupa (male), the same data, except Dzhankuat Glacier, Adylsu River, alt. 2500 m a.s.l., 18.VII.2023, 43.2132639 N, 42.7202917 E, leg. D. Palatov.

Diagnosis

Adult male. Total length 3.0–3.5 mm. Total length/wing length 0.97–1.03.

Eyes bare and not extended dorsomedially. Clypeus with 10 setae. Antenna with 13 flagellomeres and developed plume but number of setae is slightly reduced; AR 0.88–0.94. Head width/palp length 1.2–1.3. Anteprepronotum with 5–6 lateral setae. Dorsocentrals 6–9, prealars 5–6, scutellars 14–18. Wing length 2.9–3.6 mm; costa extension 98 µm long. Anal lobe developed; squama with 21–25 setae. Hind tibial comb with 12–14 setae. LR1 0.65–0.66, BV1 3.59–3.64, SV1 2.79–2.87.

Hypopygium (Figs 47–48). Tergite IX with 6 setae on one side of the anal point; wide triangular anal point, 100–104 µm long which in apical part is 24 µm long, pale hyaline and with seta 4–5 µm long at apex; anal tergal bands V-shaped. Inferior volsellae yellow, wide in basal 1/3 and finger-like in distal 2/3, densely covered with short setae; superior volsellae dark brown, 84–96 µm long, covered with short setae. Gonostylus slightly curved in the basal third and slightly expanded distally, with a rounded apex and megaseta 8 µm long.

Pupa. Described by Langton & Wisser (2003) and Makarchenko & Palatov (2024).

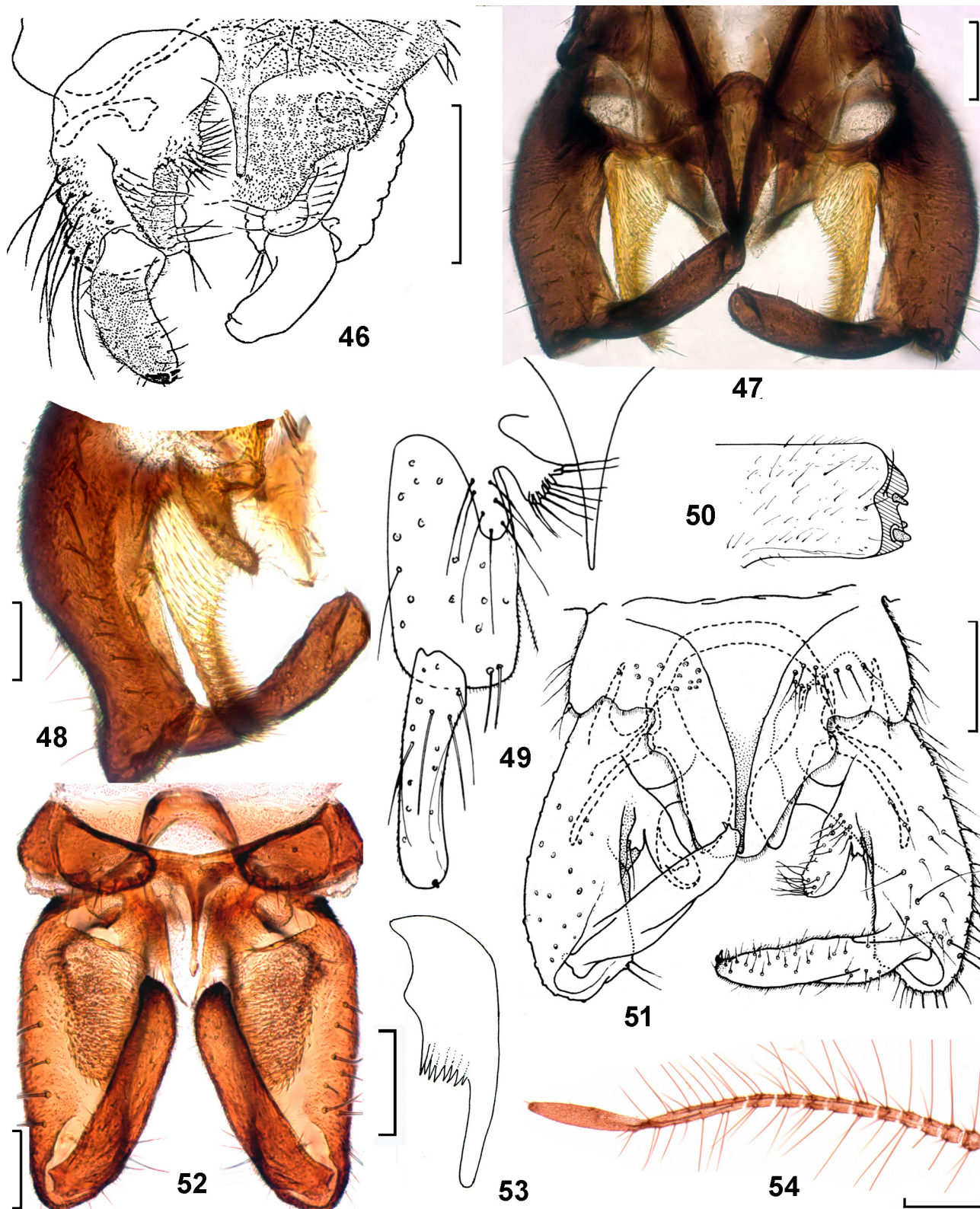
Larva. Unknown.

Distribution. Apparently endemic to the Caucasus. Known only from glacial streams of Terek River basin in the Caucasus.

***Diamesa valkanovi* Sæther**

Diamesa valkanovi Sæther, 1968: 431; Ashe & O'Connor 2009: 288.

Remarks. The species is known only from a brief original description made from adult male and female extracted from a mature pupa (Sæther 1968). Based on this, we provide a brief diagnosis of the adult male.



FIGURES 46–54. Adult males of *Diamesa modesta* Serra-Tosio (46), *D. tskhomelidzei* Kownacki *et* Kownacka (47–48), *D. valkanovi* Sæther (49–50), *D. wuelkeri* Serra-Tosio (51) and *D. elbrusica* Makarchenko, Semenchenko *et* Palatov (52–54). 46–47, 49, 51–52, hypopygium in dorsal view; 48, gonocoxite and gonostylus; 50, apical part of gonostylus; 53, aedeagal lobe; 54, flagellomeres 3–13 of antenna. Fig. 46 after Serra-Tosio (1986); Figs 47–48 after Makarchenko & Palatov (2024); Figs 49–50 after Sæther (1968); Fig. 51 after Serra-Tosio (1971); Figs 52–54 after Makarchenko *et al.* (2023). Scale bars are as follows: Figs 46, 51, 54—100 µm, Figs 47, 48, 52—50 µm, Fig. 53—20 µm.

Diagnosis

Adult male. Eyes bare. Antenna with 13 flagellomeres; AR about 1.5. Hypopygium with long anal point. Dorsolateral and ventromedial lobes of the inferior volsella of the gonocoxite are approximately the same length; the ventromedial lobe is slightly wider apically and with 8–9 long setae along the margin (Fig. 49). Gonostylus a slightly broader at base, slightly curved, with long and strong hairs, and with three minute apical spines (Fig. 50). HR *ca* 1.3.

Diamesa wuelkeri Serra-Tosio

(Fig. 51)

Diamesa wuelkeri Serra-Tosio, 1964: 36, 1968: 85, 1971: 168; Ashe & O'Connor 2009: 289; Montagna *et al.* 2016a: 322.

Remarks. This species is not present in our material. Below we provide a brief diagnosis of *D. wuelkeri* according to Serra-Tosio (1968, 1971) and Montagna *et al.* (2016a).

Diagnosis

Total length 3.5 mm. Eyes bare and not extended dorsomedially. Clypeus with 7 setae. Antenna with 13 flagellomeres and developed plume of setae; AR 0.8–0.9. Dorsocentrals 7. Wing length 3.2 mm; anal lobe developed, squama with 30 setae. LR1 0.70, BV1 3.58, SV1 2.66.

Hypopygium (Fig. 51). Tergite IX with 10 setae and long anal point, with a blunt end. Inferior volsella of gonocoxite spoon-shaped with its concavity facing outwards, bearing a number of setae on its convex face and posterior margin, and furnished on its lower margin with a small lobe with a few setae; ventromedial lobe developed and dorsolateral lobe reduced, consisting of three small tubercles, the two outer tubercles each bearing one to three setae.

Pupa. Described by Serra-Tosio (1971).

Larva. Unknown.

Distribution. Known from the Alps and Pyrenees (Serra-Tosio 1973; Rossaro & Lencioni 2015a,b).

Diamesa elbrusica Makarchenko, Semenchenko *et* Palatov

Diamesa elbrusica Makarchenko, Semenchenko *et* Palatov, 2023: 316.

Remarks. The species was described in detail based on adult male from the Elbrus region (Makarchenko *et al.* 2023). A brief diagnosis of this species is provided below.

Material examined. 6 adult males (including the holotype), RUSSIA: Republic of Kabardino-Balkaria, Chereksky district, waterfall on an unnamed river, the right tributary of the Mizhirga River, 1.3 km SE of the Bezengi alpine camp. It flows from the Gidan glacier and the Gidantau mountain, alt. 2410 m above sea level, 21.VII.2022, 43.105033 N, 43.161586 E, leg. D. Palatov.

Diagnosis

Adult male. Total length 2.3–3.0 mm. Total length/wing length 1.02–1.12. Eyes pubescent with microtrichiae, not extended dorsomedially. Clypeus with 8–12 setae. Antenna with 13 flagellomeres and reduced plume of setae (Fig. 49); AR 0.71–0.73. Head width/palpal length 1.0–1.29. Anteprepronotum with 2–3 ventrolateral setae. Dorsocentrals 8–10, prealars 4–5, scutellars 13–14 (in 2 rows). Wing length 2.44–2.68 mm. Costal extension absent. Anal lobe rounded. Squama with 11–15 setae. Hind tibial comb with 13–14 setae. LR1 0.63–0.67, BV1 3.75–4.00, SV1 2.89–3.08.

Hypopygium (Figs 52–53). Tergite IX narrow, with 8–13 setae on one side of the anal point; slender anal point,

96–100 µm long and 8–10 µm wide, slightly extended subapically. Transverse sternopodeme trapezoidal or rounded trapezoid; aedeagal lobe weakly chitinized, wide in basal 2/3, with teeth in places along outer edge, narrow finger-like in distal third (Fig. 48). Gonocoxite on inner edge with long setae; inferior volsella 116–128 µm long and 76–80 µm wide, broad and flat, rounded apically, densely covered with microtrichia and setae; superior volsella reduced. Gonostylus nearly straight and very long, subequal in width along entire length; gonostylus length/gonostylus width 4.33–4.78; apex with long macroseta. HR 1.31–1.44.

Pupa and larva. Unknown.

Distribution. Known only from the type locality in Elbrus Mount area of the Central Caucasus.

Sequencing results and genetic divergence

Following the final alignment, a clean matrix of 658 base pairs of the mitochondrial COI gene was obtained for 12 newly sequenced chironomid specimens, which were deposited in GenBank (shown in bold in Fig. 55). This newly generated dataset includes single representative sequences for *D. tskhomelidzei* and *D. sahandensis*, 2 specimens of *D. demavendensis* **sp. nov.**, 3 specimens of *D. lindrothi*, and 5 specimens of *D. sangecha* **sp. nov.** It is worth noting that a critical re-evaluation of public data revealed that several specimens of *D. demavendensis* **sp. nov.** and *D. sahandensis* had been previously uploaded to GenBank and BOLD, where they were misidentified as *D. latitarsis* or *Diamesa* aff. *latitarsis*. Concurrently, the mitochondrial DNA barcodes for *D. sangecha* **sp. nov.** and *D. tskhomelidzei* are deposited and published here for the first time.

To eliminate sample-size bias across the investigated taxa, we calculated the maximum intraspecific and minimum interspecific Kimura 2-parameter (K2P) distances for each species. The highest maximum intraspecific divergence was observed in *Diamesa latitarsis* (2.35%), based on a large dataset of 42 specimens collected from diverse European localities (Table 1). Notably high intraspecific values were also recorded for *D. sangecha* **sp. nov.** (1.54%) and *D. demavendensis* **sp. nov.** (1.38%), which is directly attributable to the significant geographic fragmentation and isolation of their respective sampling locations. For all remaining species, the maximum intraspecific distances ranged tightly between 0.00% and 1.23% (Table 1).

Our pairwise analysis revealed numerous closely related species pairs exhibiting minimum interspecific genetic distances well below the threshold that corresponds to the species level for the genus *Diamesa* and approximately equal 5% (Montagna *et al.* 2016b; Makarchenko *et al.* 2023; Semenchenko & Makarchenko 2025), corresponding to exceptionally short branch lengths on the phylogenetic tree (Fig. 55).

The first prominent well-supported cluster comprises *D. caucasica*, *D. tskhomelidzei*, and *D. demavendensis* **sp. nov.**, with minimum interspecific distances ranging from 1.90% to 3.75%. Within this complex, the lowest genetic divergence (1.90%) was found between *D. caucasica* and *D. tskhomelidzei*. Concurrently, *D. achipseensis* was found to be genetically indistinguishable from *D. caucasica* (0.00% K2P distance), sharing an identical BIN despite clear morphological diagnostic characters (Makarchenko *et al.* 2024).

The second major evolutionary group encompasses all molecular variants of *D. goetghebuerei* and *D. lindrothi*, with minimum interspecific divergence spanning from 1.38% to 4.95%. The closest distances were revealed between the *D. lindrothi* from Norway (BIN BOLD:AAC4741) and the Alpine specimens of *D. goetghebuerei* (BIN BOLD:ADK3040), a relationship visually supported by the short sister-branch topology of the tree.

Interestingly, two additional species pairs demonstrated low minimum interspecific distances, *D. elbrusica* vs. *D. caucasica* (4.55%) and *D. elbrusica* vs. *D. tskhomelidzei* (4.71%), despite occupying distinctly isolated and disjunct positions on the final phylogenetic tree.

Phylogenetic reconstruction and discussion

The comprehensive molecular phylogeny resolved five primary clades within the *D. latitarsis* group. The earliest diverging lineage was represented by *D. martaе*, which occupies a basal position relative to the rest of the group and exhibits maximum branch support (Bayesian posterior probability, BPP = 1.00; Fig. 55).

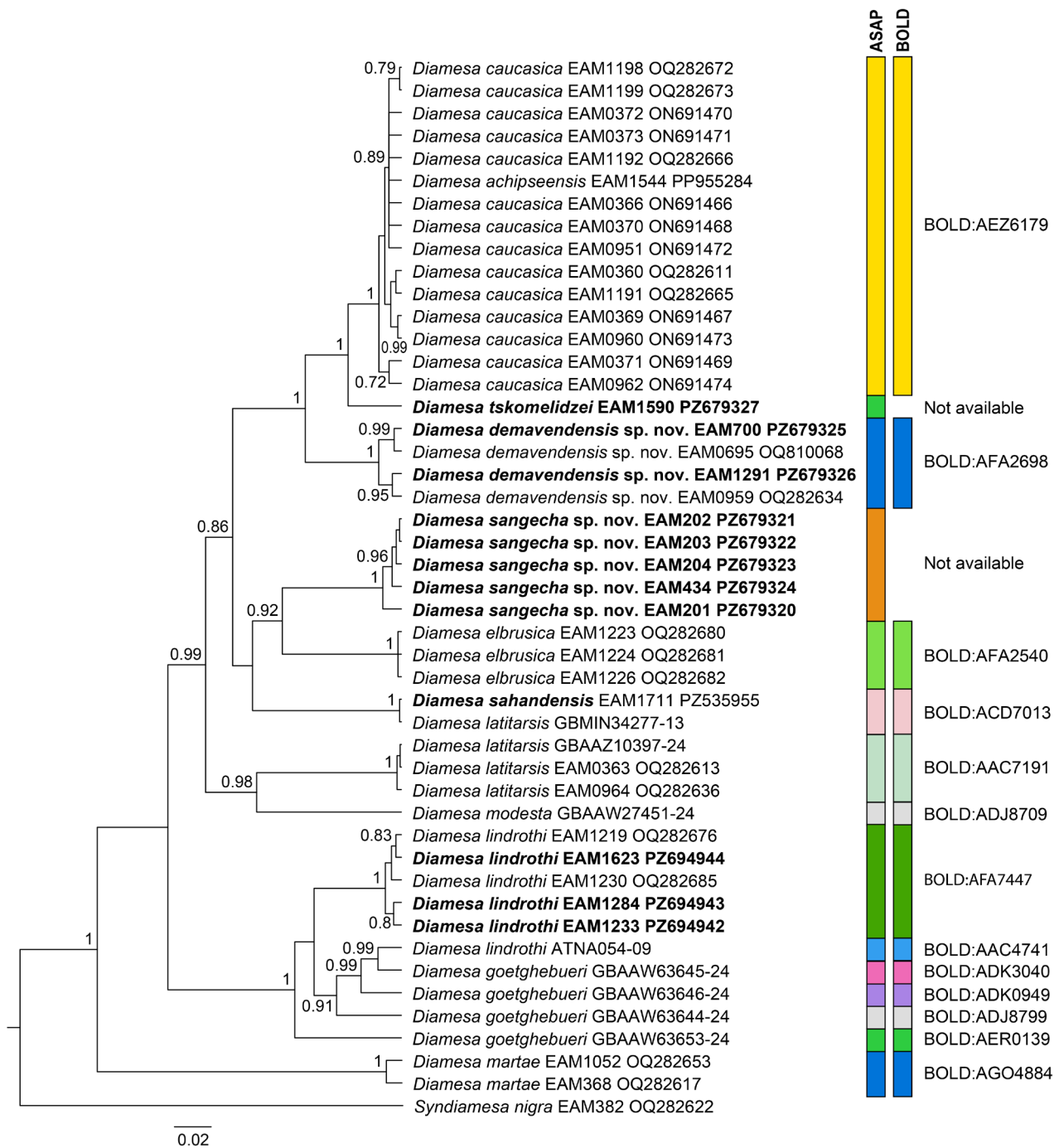


FIGURE 55. Bayesian inference (BI) phylogenetic tree of the *Diamesa latitarsis* species group based on the mitochondrial cytochrome c oxidase subunit I (COI) nucleotide sequence data. *Syndiamesa nigra* Rossaro is utilized as the outgroup. Bayesian posterior probabilities (BPP > 0.70) are indicated at the respective tree nodes. Specimens newly sequenced or re-evaluated in this study are highlighted in bold. The colored vertical bars on the right indicate the results of the molecular species delimitation analyses performed via Assemble Species by Automatic Partitioning (ASAP) and the Barcode Index Number (BIN) system from the Barcode of Life Data Systems (BOLD).

The second resolved clade lacked strong statistical support and encompassed all molecular variants of *D. goetghebuerei* and *D. lindrothi*, aggregating a total of 6 molecular operational taxonomic units (mOTUs). Crucially, despite high Bayesian posterior probabilities (BPP) at the majority of internal nodes, the lineages corresponding to each of these two species were recovered as strictly non-monophyletic. The type localities of *D. goetghebuerei* are restricted to Austria: “Nord-Tirol, Sölden” and “Nord-Tirol, St. Sigmund” (Ashe & O’Connor 2009). This

geographic designation fails to resolve the molecular conundrum, as all four detected *D. goetghebueri* mOTUs were sympatrically collected from Tyrol, Austria (Dvorak *et al.* 2024), along with other scattered Alpine sites. Furthermore, a critical metadata audit revealed that all individuals within these four mOTUs (totaling 27 specimens) were identified solely based on morphologically ambiguous larvae or adult females, rendering definitive species-level identification highly problematic.

In contrast, the two non-monophyletic mOTUs assigned to *D. lindrothi* (BOLD:AAC4741 and BOLD:AFA7447), originating from Norway and the Caucasian mountains of Russia respectively, were securely identified based on adult male morphology. The historical type localities for *D. lindrothi* are strictly insular, designated from Iceland (Ashe & O'Connor 2009). Strikingly, not a single available DNA barcode has been sampled from the remote island of Iceland, although the Norwegian populations remain geographically closest to it. Morphologically, adult males of *D. lindrothi* and *D. goetghebueri* are segregated by an extremely narrow suite of hypopygial characters, specifically the structure of the dorsolateral lobe of the inferior volsella, which is short and finger-like in *D. lindrothi* but large and flat in *D. goetghebueri*. Based on the prominent lack of monophyly and exceptionally low K2P distances (starting from 1.24%), we hypothesize that these taxa are the evolutionary products of a recent Pleistocene radiation and active, ongoing speciation in Europe. This scenario directly aligns with the evolutionary model of Lencioni *et al.* (2021), which suggests that Plio-Pleistocene climatic cycles drove rapid diversification waves among cold-stenothermic Alpine chironomids, resulting in morphologically fluid and poorly segregated *Diamesa* lineages including the *D. lindrothi* and *D. goetghebueri* complex. Furthermore, according to the subsequent chronological data by Lencioni *et al.* (2025), the deeper interspecific gaps exceeding 5.0% K2P observed elsewhere in our wider dataset are well-explained by the remarkably low nucleotide substitution rates estimated for cold-adapted *Diamesa* (0.342% per site/lineage/Myr), maintaining deep divergence under intense purifying selection in extreme environments. Concurrently, the recent nature of this Plio-Pleistocene explosive radiation and subsequent incomplete lineage sorting fully account for the minimal genetic distances observed in these young, emerging evolutionary complexes.

Based on the compelling evidence discussed above, we can hypothesize that the entire unresolved second clade consists exclusively of genetically heterogeneous populations of *D. goetghebueri*, given that a significant portion of this material was sampled directly from its historical type locality in Tyrol. Conversely, the authentic *D. lindrothi* is apparently absent from the currently available global molecular databases, as all sequences provisionally assigned to this taxon originate from mainland regions (Norway and the Caucasus) situated far from its insular type locality in Iceland. Consequently, the observed non-monophyly and critically low interspecific K2P distances within this complex might represent an artifact of systematic larval misidentifications in public repositories rather than genuine evolutionary reticulation.

However, a compelling counterargument arises from authoritative morphological identifications. Imago males of *D. lindrothi* from Norway and the Caucasus were independently verified by leading chironomid experts (E. Stur and E.A. Makarchenko, respectively), yet the K2P distance between their respective lineages (BINs BOLD:AAC4741 and BOLD:AFA7447) reaches 3.71–4.56% (mean 4.15%). These values are strictly borderline between intraspecific polymorphism and interspecific divergence (see above), indicating deep allopatric differentiation but preventing definitive taxonomic segregation. Adhering to these molecular and morphological baselines suggests that the entire clade two, including the ambiguous Alpine larval sequences, might actually represent highly polymorphic allopatric populations of *D. lindrothi*.

Conversely, such a scenario invokes a critical dilemma: to date, verified DNA barcodes for adult males of *D. goetghebueri* are absent from global molecular databases, both from its historical type locality in Tyrol and any other Alpine regions. Ultimately, neither the geographic isolation of the type localities nor these borderline genetic distances resolve the molecular conundrum, merely introducing further ambiguities. Consequently, obtaining authentic topotypic DNA barcodes of *D. lindrothi* directly from Iceland, along with the collection and sequencing of morphologically secured adult males of *D. goetghebueri* from the Austrian Alps, remains a key imperative for a comprehensive future taxonomic revision to definitively resolve this puzzle.

Thus, by pooling all globally available sequences, including a comprehensive matrix of DNA barcodes and multi-locus data for the entire *latitarsis* group, a significant taxonomic conflict has been exposed. The integration of global data reveals ambiguous and highly problematic boundaries between *D. lindrothi* Goetghebuer and *D. goetghebueri*, suggesting a complex evolutionary scenario of recent radiation or cryptic polymorphism that challenges traditional morphological and automated molecular species delimitation.

The third resolved clade received high branch support (BPP = 1.00) and comprised two well-demarcated sister species, *D. latitarsis* and *D. modesta*, with each cluster incorporating a high density of sequences from diverse European and Alpine localities (Table 1). Notably, our analysis demonstrated that the standard single-locus COI Folmer region provides an unambiguous resolution of these lineages. This finding contrasts with the conclusions of Dvorak *et al.* (2024), who suggested that the single COI marker is insufficient for this species pair, but agrees with Lencioni *et al.* (2024), who showed that additional nuclear and mitochondrial loci do not alter the results obtained from COI alone. Our results indicate that under a balanced sampling strategy, the standard COI barcode retains sufficient diagnostic power to reliably segregate *D. latitarsis* and *D. modesta*.

The fourth clade (BPP = 0.86) grouped the Iranian *D. sahandensis*, the Caucasian *D. elbrusica*, and the Central Asian *D. sangecha* **sp. nov.** (Kyrgyzstan/China). Within this cluster, a prominent database misidentification was exposed: a larval specimen originally deposited in BOLD as *D. latitarsis* (Process ID: GBMIN34277-13) was securely recovered deep within the *D. sahandensis* lineage, representing its true biological identity.

The fifth clade accommodated the widely distributed new species *D. demavendensis* **sp. nov.** (Iran/Caucasus), nesting as a sister group to two hyper-close species, *D. tskhomelidzei* and *D. caucasica*. In full agreement with recent taxonomic revisions, the *D. caucasica* cluster completely subsumed the genetically indistinguishable *D. achipseensis* (Makarchenko *et al.* 2024).

Acknowledgments

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