Zootaxa 5190 (3): 361–392
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5190.3.3
http://zoobank.org/urn:lsid:zoobank.org:pub:AEE72ABB-4358-4BB2-9394-DB5FD8105871

Taxonomy of *Diamesa steinboecki* group (Diptera: Chironomidae: Diamesinae), with description and DNA barcoding of known species. II. Subgroups *davisi, leona* and *loeffleri*

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Abstract

Illustrated redescription of the adult males *Diamesa alpina* Tokunaga from the Russian Far East and North America, *D. amplexivirilia* Hansen from Arctic and the Russian Far East, *D. saetheri* Willassen from Chukotka Region and Kolyma River basin, *D. lupus* Willassen from Alaska, *D. serratosioi* Willassen from Norway and Russian Far East, *D. leoniella* Hansen from Alaska, *D. leona* Roback from Eurasia, *D. japonica* Tokunaga from Japan and Russian Far East, *D. khumbugelida* Saether et Willassen and *D. loeffleri* Reiss from Himalayas are provided.

Morphological data and DNA sequences of the mitochondrial cytochrome c oxidase subunit I gene (COI) were used to delimit of seven species from subgroups *davisi* (*D. alpina, D. amplexivirilia, D. serratosioi*), *leona* (*D. japonica, D. leona*) and *loeffleri* (*D. khumbugelida, D. loeffleri*). Taxonomic remarks with data on geographical distribution of the investigated species are given.

Key words: Diptera, Chironomidae, *Diamesa steinboecki group,* taxonomy, DNA barcoding, Eurasia, North America

Introduction

This article is a continuation of the study at the morphological and molecular genetic level of the *Diamesa steinboecki* group, namely the subgroups *davisi, leona* and *loeffleri*. In the previous work representatives of the *steinboecki* and *longipes* subgroups were considered from the *steinboecki* group, but the species *Diamesa loeffleri* Reiss and *D. khumbugelida* Sæther et Willassen included in the *longipes* subgroup were not presented (Makarchenko *et al.* 2022). This is due to the fact that our study showed relative disagreement in terms of morphological data and barcoding results, in connection with which we found it expedient to separate these two species into a different subgroup, the *loeffleri* subgroup.

Illustrated redescriptions of the adult males *D. alpina* Tokunaga from the Russian Far East and North America, *D. amplexivirilia* Hansen from Arctic and the Russian Far East, *D. saetheri* Willassen from Chukotka Region and Kolyma River basin, *D. lupus* Willassen from Alaska, *D. serratosioi* Willassen from Norway and Russian Far East, *D. leoniella* Hansen from Alaska, *D. leona* Roback from Eurasia, *D. japonica* Tokunaga from Japan and Russian Far East, *D. khumbugelida* Sæther et Willassen and *D. loeffleri* Reiss from Himalayas are provided below.

We used both morphological data and DNA sequences of the mitochondrial cytochrome c oxidase subunit I gene (COI) for species description and delimitation of seven *Diamesa* species from subgroups *davisi* (*D. alpina, D. amplexivirilia, D. serratosioi*), *leona* (*D. japonica, D. leona*) and *loeffleri* (*D. loeffleri, D. khumbugelida*). The use of DNA barcoding for *Diamesa* has proved its worth for species identification and species boundary analysis (Montagna *et al.* 2016, Lencioni *et al.* 2021, Makarchenko *et al.* 2022). Taxonomic remarks with data on the geographical distribution of the investigated species are given.

Accepted by B. Rossaro: 6 Sept. 2022; published: 29 Sept. 2022

Materials and methods

The adults of chironomids were preserved in 96% ethanol for DNA-analysis and in 70% ethanol for further study of morphology. The material was slide-mounted in liquid Fora-Berlese and polyvinyl lactophenol. The terminology follows Sæther (1980).

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

Total genomic DNA was extracted using DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) followed the manufacturer's instructions. We amplified mitochondrial protein coding gene cytochrome *c* oxidase 1 (*CO1*) using the polymerase chain reaction (PCR). For primers, reaction protocols, cycle programs used for amplification and purification of PCR products see Makarchenko *et al.* (2022). Direct sequencing of PCR products was performed using ABI Big Dye Terminator (ver. 3.1, ThermoFisher Scientific) and run on an ABI 3130xl Genetic Analyzer Sequencer (Applied Biosystems, Foster City, CA, USA).

Based on the Kimura-2-Parameter (K2P) model are calculated inter- and intraspecific genetic distances using MEGA7 (Kumar *et al.* 2016). We also used ABGD analysis for species delimitation and Bayesian inference to reconstruct phylogenetic relationships within subgroups *davisi*, *leona* and *loeffleri* of the genus *Diamesa*. Parameter settings for ABGD analysis and MrBayes as well as best models for each codon position of COI gene can also be found in Makarchenko *et al.* (2022). For ABGD analysis we used all available COI sequences of genus *Diamesa* in GenBank and BOLD systems (Approximately 2600 sequences were available at the time of publication).

The obtained sequences have been deposited in GenBank under numbers ON834730–ON834750, ON982481-ON982482

All investigated material 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).

Taxonomy

Diamesa davisi subgroup

The adult males of this subgroup are characterized by the following features. Antenna with eight flagellomeres and reduced setae of plume. Head with separate, weak frontal protrusions. Eyes hairy; frontal setae few or absent. Wings sometimes reduced, with rounded, often small anal lobe. Hypopygium with three-lobed segment IX, lateral lobes (laterosternites) often extending beyond posterior margin of the middle part (tergite IX). Tergite bands usually U or Y-shaped. Transverse sternapodeme usually triangular, often with a spire-shaped apex. Anal point bent downwards, sometimes strongly reduced. Gonocoxite with a knob-like superior volsella covered with microtrichia, sometimes with short setae. Gonostylus with 3–5 apical teeth and megaseta appearing as a wide terminal spine.

Diamesa alpina Tokunaga

(Figs. 1–7)

Diamesa alpina Tokunaga, 1936: 539; Makarchenko 1980: 82–86, 1985: 81–82, 2006: 261; Willassen 1986: 124–125; Makarchenko & Yamamoto 1995: fig. 2; Kobayashi & Endo 2008: 56; Ashe & O'Connor 2009: 269.
Diamesa kurobedistalis Sasa et Okazawa, 1992 :59.
Diamesa kurobenagaia Sasa et Okazawa, 1992 : 61.

Material examined. CANADA: 1 adult male, Jasper-Banff Area, Rocky Mountains, 13.VI.1957, leg. L. Brundin; U.S.A.: 3 adult males, Alaska, Hebert River, about 0.5 mi. downstream of glacier, 20.II.2010, leg. J. Hudson; RUSSIA: 1 adult male, Kamchatka, Avacha River, 11.VI.1970, leg. V. Levanidov; 1 adult male, the same data, except, Korf Bay, unnamed stream near Tilichiki Village, 8.VIII.1972, leg. E. Nikolaeva; 5 adult males, the same data, except, Pravaya Kamchatka River, 7–8.VII.1996, leg. E. Makarchenko; 2 adult males, Chukotka Autonomous

Okrug, Anadyrskyi District, unnamed stream of Velikaya River basin, 1.VIII.1980, leg. E. Makarchenko; 3 adult males, Kurile Islands, Kunashir Island, Sernovodsk Village, Tiurino River, 24.IV.1978, leg. E. Makarchenko; 3 adult males, the same data, except Onekotan Island, Rezvyi Stream, 7.VIII.1996, leg. V. Teslenko; 1 adult male, the same data, except Shiashkatan Island, 12.VIII.1996, leg. V. Teslenko; 3 adult males, Magadan Region, Olskyi District, 137 km of Kolymskaya Road, Ola River, 13.V.2017, 60.412194 N, 151.514564 E, leg. E. Khamenkova; 2 adult males, the same data, except, mouth of Ola River, 03.V.2019, 59.580514 N, 151.272686 E, leg. E. Khamenkova; 3 adult males, Khabarovsk Territory, Solnechnyi District, Gornyi Village, Silinka River, 22.VII.1985, leg. E. Makarchenko.

Description

Adult male (n = 10, except when otherwise stated). Total length 2.6–3.3 mm. Total length/wing length 0.95–1.15.

Coloration. Dark brown to black. Wing greyish, with brownish veins.

Head. Eyes hairy, reniform. Temporal setae including 2–3 frontals and 8–10 verticals. Clypeus with 2–4 setae. Antenna with 8 flagellomeres and reduced plume of setae (Fig. 4); terminal flagellomere with 1–2 subapical setae, 16–28 µm long. Length of 1–8 flagellomeres (µm): 104–120, 34–50, 32–42, 27–38, 29–34, 24–36, 34–48, 104–140; AR 0.33–0.42. Palpomere length (µm): 36–40, 56–72, 88–92, 80–92, 100–136. Palpomere 3 in distal part with sensilla capitata with diameter 16–20 µm. Head width/palpal length 1.27–1.39. Antennal length/palpal length 1.0–1.37.

Thorax. Antepronotum with 8–13 ventrolateral setae. Dorsocentrals 7–11, prealars 2–5, scutellars 24–26.

Wing. Length 2.60–3.60 mm, width 0.76–0.98 mm. Costal extension absent. Anal lobe rounded, sometimes slightly reduced. Squama with 9–21 setae. R and R_1 with 6–20 setae, R_{4+5} with 6–15 setae. RM/MCu 2.4–2.8.

Legs. Spur of front tibia 26–42 µm long. Spurs of mid tibia 38–40 µm and 36–48 µm long. Spurs of hind tibia 50–76 µm and 29–42 µm long. Hind tibial comb with 15–24 setae. Length (µm) and proportions of leg segments for males from Kurile Island, Amur River basin, Chukotka, Kamchatka and North America are as in Table 1. Most long legs and different proportions of leg segments have specimens from Magadan Region and for them the data are given in Table 2.

TABLE 1. Lengths (in µm) and proportions of leg segments of *Diamesa alpina* Tokunaga from North America, Kamchatka, Chukotka, Amur River basin and Kurile Islands, male (n=11)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1513–1800	1445–1720	910–1160	410–510	238–306	98–119	115–136
P_2	1343–1840	1296–1564	607–782	289–344	180–230	82–119	98–136
P_3	1607–1960	1460–1751	968–1156	492–578	246–306	98–119	115–136

continued

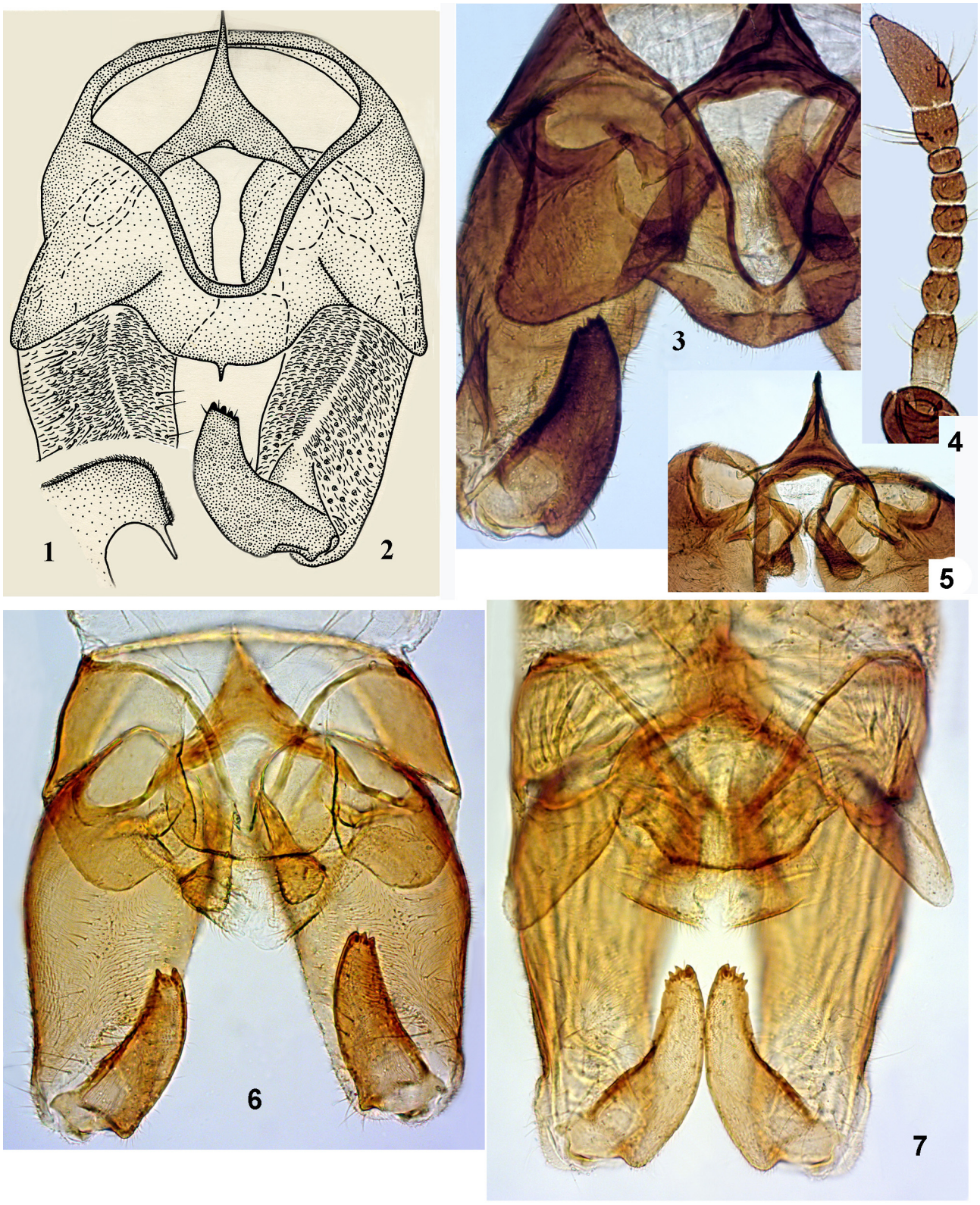
	LR	BV	SV	BR
P_1	0.61–0.67	4.12–5.50	3.04–3.27	0.9–1.2
P_2	0.45–0.50	3.57–5.30	3.90–4.88	1.0–1.1
P_3	0.61–0.70	3.98–4.34	3.02–3.35	1.0–1.2

TABLE 2. Lengths (in µm) and proportions of leg segments of *Diamesa alpina* Tokunaga, male from Magadan Region (Ola River basin) (n=2)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1720–1920	1600–1720	940–1000	435–443	262–279	98–115	131
P_2	1720–1920	1400–1520	600–680	295–312	197–205	82–131	131–148
P_3	1920–2040	1720–1880	1040	495	246–262	98–131	131–148

continued

	LR	BV	SV	BR
P_1	0.58	4.60–4.79	3.53–3.64	1.1–1.4
P_2	0.43–0.45	5.16–5.18	5.06–5.20	1.0–1.1
P_3	0.55–0.60	4.80–4.84	3.50–3.77	1.2–1.4



FIGURES 1–7. Adult male of *Diamesa alpina* Tokunaga from Sakhalin Island **(1–2),** Magadan Region **(3),** Chukotka **(4),** Khakassia **(5),** Alaska **(6)** and Canada **(7). 1,** anal point in lateral view; **2–3, 6–7,** hypopygium in dorsal view; **4,** antenna; **5,** transverse sternapodeme and superior volsellae. **Figs. 1–2** after Makarchenko (1980).

Hypopygium (Figs. 1–3, 5–7). Laterosternites not protruding beyond the posterior margin of tergite IX in East Palaearctic specimens (Figs. 2–3) or slightly (60–68 μm) extending beyond posterior margin of tergite IX in Nearctic adult males (Figs. 6–7). Posterior margin of tergite IX partly straight to slightly rounded, with some weak

setae along, 9–10 μm long. Laterosternites with 9–12 weak setae, 9–15 μm long. Tergite bands broad and distinctly U-shaped (Figs. 2–3, 6–7). Anal point often visible in dorsal view, angled downwards (Fig. 1), 25.2–50.4 μm long; ratio of anal point length to gonostylus length 0.15–0.24. Transverse sternapodeme (TSA) triangular, usually with a spire-shaped apex (Figs. 5–6), 72–160 μm high, 180–224 μm wide at the base; TSA height/TSA width 0.40–0.80. Gonocoxite 360–400 μm long; superior volsella rounded, with microtrichia and sometimes short setae (Fig. 5). Gonostylus 192–208 μm long, weakly curved, gradually narrowing towards distal end, with 3–4 apical teeth of approximately the same size, with strong setae between them and megaseta which in form of wide terminal spine (Figs. 2–3, 6–7), 8–12 μm long; HR 1.75–2.20.

Pupa was described by Tokunaga (1936).

Larva unknown.

Remarks. As noted above, the revision revealed that males from the Eastern Palaearctic are characterized by hypopygium with laterosternites that do not extend beyond the posterior margin of tergite IX, while in males from North America (Alaska and Alberta), the laterosternites extend beyond the posterior margin of tergite IX. Therefore, it is necessary to change the diagnosis of the species given by Willassen (1986) in which he indicated that the laterosternites extend beyond the posterior margin of tergite IX. This error is due to the fact that Willassen analyzed the material only from Alaska and ignored the figure of a male hypopygium from Kamchatka (Makarchenko 1980, Fig. 3A), in which laterosternites do not extend beyond the posterior margin of tergite IX. It is possible that different species inhabit the Eastern Palaearctic and Nearctic, but to confirm this a comparison with the support of DNA barcoding is recommended. Also noteworthy are adult males from the Magadan Region (Ola River basin) possessing the longest legs and leg's indexes differing from other populations, namely LR_1 0.58, BV_1 4.60–4.79, SV_1 3.53–3.64 in specimens from Magadan Region and LR_1 0.62–0.67, BV_1 3.98–4.38, SV_1 3.04–3.27 in specimens from other regions.

Distribution. Known from Japan, Kurile Islands, Kamchatka, Magadan Region, U.S.A. (Alaska) and Canada (Alberta).

Diamesa amplexivirilia Hansen

(Figs. 8–11)

Diamesa amplexivirilia Hansen in Hansen & Cook, 1976: 53; Makarchenko 1980: 86, 1981:108, 1985: 77, 2006: 261, 473, 614; Ashe & O'Connor 2009: 270; Krasheninnikov *et al.* 2020: 591.

Material examined. RUSSIA: 15 adult males, Magadan Region, Tenkinsky District, neighborhood of Sibit-Tyellakh Village, spurs of the peak Vlastny, Olen' Stream (Kolyma River basin), alt. 1300–1400 m a.s.l., 27–30.VII.1977, leg. E. Makarchenko; 10 adult males, Chukotka, Wrangel Island, upper stream of Somnitelnaya River, 22.VII.1978, leg. E. Makarchenko; 33 adult males, the same data, except Krasnyi Flag River, middle stream, 4.VII.1979, leg. E. Makarchenko and M. Makarchenko; 138 adult males, 19 pupae and 65 larvae, the same data, except unnamed stream in upper part of Somnitelnaya River, 14.VII–2.VIII.1979, leg. E. Makarchenko and M. Makarchenko; 1 adult male, Severnaya Zemlya Archipelago, Bolshevik Island, Mikoyan Bay, Chernaya River, 1.IX.2019, 79.20768 N, 102.31090 E, leg. A. Krasheninnikov.

Description

Adult male (n = 16). Total length 2.4–4.2 mm. Total length/wing length 0.92–1.23.

Coloration. Dark brown to black; wing grayish, veins yellowish brown.

Head. Eyes hairy, microtrichia visible along lateral eye margin when head is viewed from front. Temporal setae including 3 frontals and 8 verticals. Clypeus with 2–5 setae. Antenna with 8 flagellomeres and reduced plume of setae, 24–48 μm long; number of setae in flagellomeres 1–7, respectively 2–3 : 2 : 1–2 : 1–2 : 2 : 2 : 4; terminal flagellomere with 3 subapical setae, 12–16 μm long and with 3 setae basally, 32–36 μm long; pedicel with 3 setae. Flagellomeres 1–8 length (μm): 38–76–109, 36–55, 28–50, 24–42, 24–38, 24–38, 24–46, 96–134; AR 0.30–0.46. Palpomeres lengths (in μm): 32–40; 40–80; 72–109; 60–109; 78–143. Palpomere 3 in distal part with sensilla capitata with diameter 16 μm. Antennal length/palpal length 1.18–1.45.

Thorax. Antepronotum with 3–15 ventrolateral setae. Dorsocentrals 9–12, prealars 4–10, scutellars 7–22.

Wing. Length 2.24–3.79 mm, width 0.96–1.25 mm. R and R_1 with 6–26 setae; R_{4+5} with 5–9 setae in distal part. Costa extension 49 μm long. RM/MCu 2.0–4.6. Anal lobe well developed, rounded. Squama with 13–27 setae. VR 0.93.

Legs. Spur of front tibia 31–46 μm long; spurs of mid tibia 20–46 μm long; of hind tibia 44–80 μm and 30–46 μm long. Hind tibial comb with 9–21 setae. Lengths and proportions of leg segments as in Table 3. We did not include in this table the data on the male from the Severnaya Zemlya Archipelago which are given in the article by Krasheninnikov *et al.* (2020).

TABLE 3. Lengths (in μm) and proportions of leg segments of *Diamesa amplexivirilia* Hansen from Vrangel Island and Kolyma River basin, male (n=15)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1530–1924	1343–1872	918–1196	425–572	221–364	102–136	119–170
P_2	1649–2080	1377–1768	646–884	323–391	204–260	102–119	119–156
P_3	1751–2184	1564–2028	935–1222	510–629	272–364	102–136	119–156

continued

	LR	BV	SV	BR
P_1	0.58–0.70	4.03–4.49	3.10–3.49	1.1–1.5
P_2	0.45–0.50	4.66–5.25	4.35–4.83	1.1–1.4
P_3	055–0.65	3.84–4.37	3.29–3.67	1.0–1.4

Hypopygium (Figs. 8–11). Laterosternites very extend beyond posterior margin of tergite IX, evenly sclerotized and with relatively dense 5–9 setae, 16–20 μm long. Posterior margin of tergite IX gently rounded, with 4–5 setae (from one side), 8–12 μm long. Tergite bands weak, widely U-shaped (semi-circular) (Figs. 8–9). Anal point visible in dorsal view, relatively well developed and blunt-tipped, angled downwards, without microtrichia (Fig.10), 52–143 μm long; ratio of anal point length to gonostylus length 0.36–0.64. Transverse sternapodeme 68 μm high, triangular, with rounded apex (Figs. 8–9). Gonocoxite 360–400 μm long; superior volsella rounded, fairly well developed, slightly produced disto-medially, with numerous microtrichia. Gonostylus very strongly curved, broadest in basal 0.4–0.5, then abruptly narrowing, with 3 apical teeth of approximately the same size, with strong 3-4 setae between them and megaseta which in form of wide terminal spine (Fig. 11), 8–12 μm long; HR 1.60-2.27.

Pupa and ***larva*** are described by Makarchenko (1981).

Remarks. According to their main features, specimens from North America and Eurasia are close together, only slightly different is the male from Severnaya Zemlya, with total length 2.4 mm, wing length 2.24 mm, length of the anal point 52 μm, while males from North America, Wrangel Island and Kolyma River basin are 2.8–4.2 mm long, with wing length 2.52–3.79, anal point length 84–143 μm. We noted earlier that this species is closely related to *D. alpina* Tokunaga and *D. davisi* Edwards (Makarchenko 1980) as well as to *D. saetheri* Willassen and *D. serratosioi* Willassen (Makarchenko 1985). The barcoding data (see below) confirm a close similarity of *D. amplexivirilia* with the last two species.

Distribution. Holarctic arcto-alpine species. Known from Canada: Alberta, British Columbia, Northwest Territories, U.S.A.: Montana, Washington, Alaska (Hansen & Cook 1976; Namayandeh 2022) and Russia: Severnaya Zemlya Archipelago (Krasheninnikov *et al.* 2020), upper streams of Kolyma River, Vrangel Island.

Diamesa saetheri Willassen

(Figs. 12–15)

Diamesa saetheri Willassen, 1986: 120; Ashe & O'Connor 2009: 283.

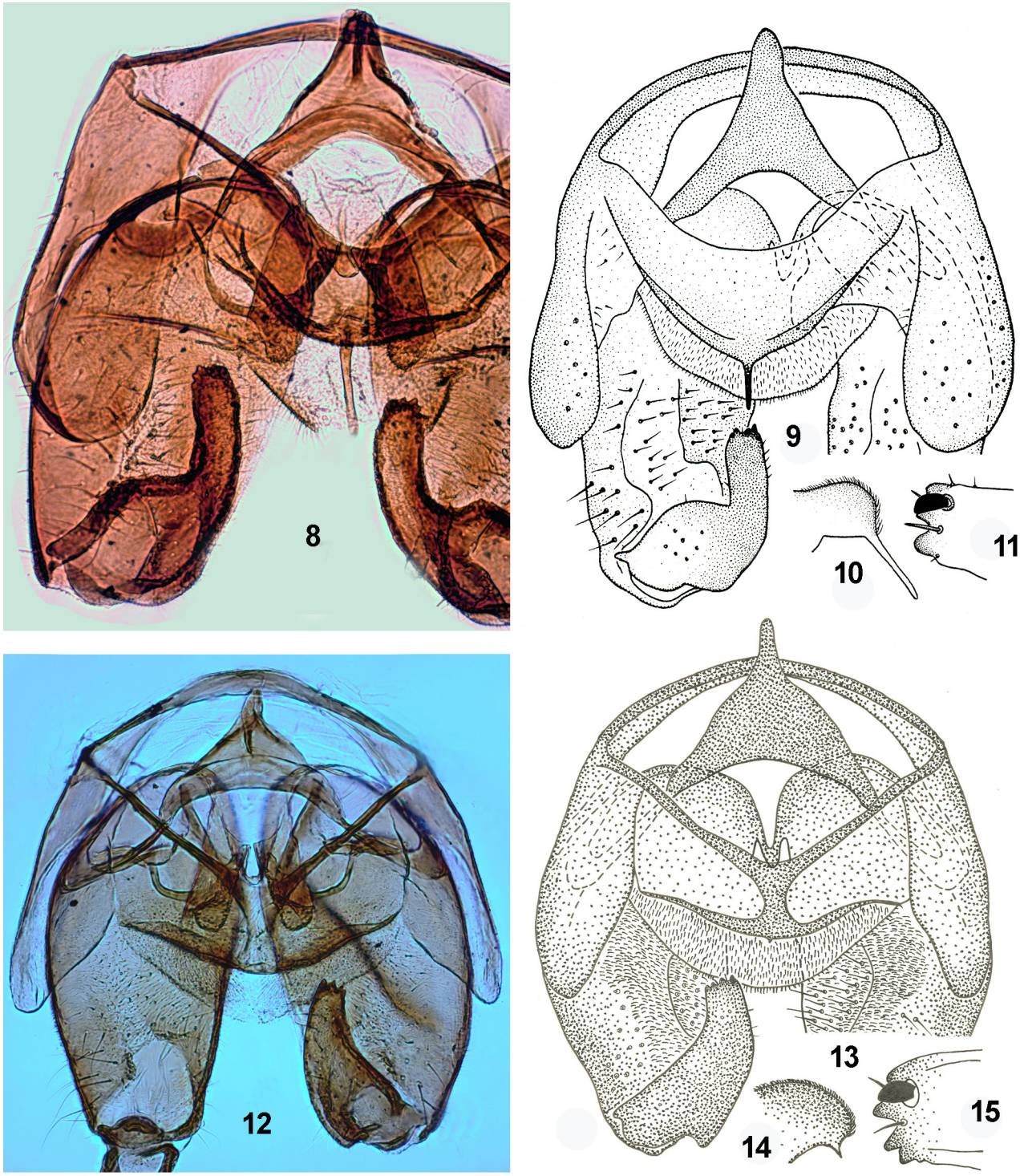
Diamesa davisi auct., nec Edwards 1933. Pagast 1947: 477, 525, Figs. 37, 79; Sæther 1968: 441–444; Makarchenko 1980: 80, Figs. 1–2.

Material examined. RUSSIA: 5 adult males, Chukotka Peninsula, The Gilmimliveem River in the area of thermal mineral springs of the Ioni Lake basin., 4.VIII.1973, leg. I. Levanidova; 5 adult males, the same data, except for the surroundings of the Uelen Village, Nevidimka Stream, 12.VIII.1973, leg. I. Levanidova; 5 adult males, Chukotka, Wrangel Island, Somnitelnaya River, 23.VII.1978, leg. E. Makarchenko; 5 adult males, Magadan Region, Tenkinsky District, neighborhood of Sibit-Tyellakh Village, spurs of the peak Vlastny, Olen' Stream (Kolyma River basin), alt. 1300–1400 m a.s.l., 12–17.VI.1978, leg. E. Makarchenko.

Description

Adult male (n = 20). Total length 2.60–3.25 mm. Total length/wing length 1.04–1.49.

Coloration. Dark brown to black; wing grayish, veins yellowish brown.



FIGURES 8–15. Adult males of *Diamesa amplexivirilia* Hansen from Chukotka Region **(8–11)** and *Diamesa saetheri* Willassen from Chukotka Region **(12–15). 8–9, 12–13,** hypopygium in dorsal view; **10, 14,** anal point in lateral view; **11, 15,** apical part of gonostylus. **Figs. 9–11, 13–15** after Makarchenko (1980).

Head. Eyes hairy. Temporal setae including 0–1 orbitals, 9–15 verticals and 2–6 postorbitals. Clypeus with 4–8 setae. Antenna with 8 flagellomeres and reduced plume of setae 20–50 µm long. Flagellomeres 1–8 length (µm): 88–126, 34–50, 29–46, 29–36, 25–38, 25–34, 34–42, 88–126; terminal flagellomere with 1–2 subapical setae, 12–20 µm long and with 3–4 setae basally, 36–40 µm long; AR 0.28–0.38. Palpomeres lengths (in µm): 32–40; 46–76;

80–109; 71–101; 113–143. Palpomere 3 in distal part with sensilla capitata with diameter 14–16 μm. Antennal length/palpal length 1.12–1.46.

Thorax. Antepronotum with 8–11 ventrolateral setae. Dorsocentrals 7–12, prealars 2–6, scutellars 11–24.

Wing. Length 2.34–2.91 mm, width 0.72–0.93 mm. R and R_1 with 6–16 setae; R_{4+5} with 2–7 setae in distal part. RM/MCu 2.0–3.2. Anal lobe rounded, often reduced. Squama with 19–21 setae.

Legs. Spur of front tibia 20–38 μm long; spurs of mid tibia 29–50 μm and 27–50 μm long; of hind tibia 20–63 μm and 51–126 μm long. Hind tibial comb with 14–21 setae. Lengths and proportions of leg segments as in Table 4.

TABLE 4. Lengths (in μm) and proportions of leg segments of *Diamesa saetheri* Willassen, male (n=20)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1292–1716	1122–1649	680–1003	272–425	170–238	85–119	102–136
P_2	12581716	1003–1598	425–731	187–323	136–204	85–119	102–136
P_3	1377–1872	1224–1664	714–1071	374–544	221–323	94–136	119–153

continued

	LR	BV	SV	BR
P_1	0.52–0.64	4.21–5.12	3.05–4.01	0.7–1.5
P_2	0.38–0.50	5.05–5.54	4.26–5.82	0.7–1.6
P_3	053–0.64	4.00–4.79	3.25–4.12	0.7–1.6

Hypopygium (Figs. 12–15). Laterosternites well extended beyond posterior margin of tergite IX. Tergite IX with weak setae along gently rounded posterior margin; tergite bands Y-shaped (Figs. 12–13); anal point reduced, not visible in dorsal view, angled downwards, without microtrichia (Fig.14), 11–25 μm long; ratio of anal point length to gonostylus length 0.07–0.12. Gonocoxite massive, inner side densely covered with microtrichia and short setae, superior volsella with microtrichia and some short setae. Gonostylus weakly curved with 3–5 apical teeth and megaseta which in form of wide terminal spine (Fig. 15). HR 1.77–2.50.

Pupa and ***larva*** were described by Sæther (1968).

TABLE 5. Comparison of some features of *Diamesa saetheri* Willassen males from different regions of the Palaearctic

Character	Chukotka Peninsula, Gilmimliveem River, n=5	Chukotka Peninsula, Uelen Village, n=5	Wrangel Island, Somnitelnaya River, n=5	Kolyma River basin, Olen' Stream, n=5	Norway, Sweden, Sæther 1968, Willassen 1986, Pagast 1947, n=4
Total length, mm	2.60–3.25	2.50–2.75	3.20–3.60	3.10–3.70	2.5–3.08
Wing length, mm	2.49–2.75	2.34–2.39	2.44–2.70	2.34–2.91	2.47–2.59
TL/WL	1.04–1.16	1.05–1.17	1.29–1.43	1.21–1.49	1.19–1.24
AR	0.28–0.34	0.31–0.34	0.28–0.34	0.32–0.38	0.28–0.38
LR_1	0.55–0.64	0.59–0.60	0.55–0.62	0.52–0.54	0.59–0.62
BV_1	4.21–4.84	4.63–4.66	4.48–4.80	4.70–5.12	4.70–5.01
SV_1	3.05–3.48	3.41–3.58	3.20–3.63	3.79–4.0	3.49–3.55
Anal point length, μm	11–17	13–17	16–25	17–21	-
Anal point length / gonostylus length	0.07–0.09	0.07–0.08	0.08–0.12	0.07–0.09	-
HR	2.0–2.5	1.77–2.1	1.88–2.0	2.1–2.3	1.86–2.15

Remarks. Before the revision of the *Diamesa davisi* group by Willassen (1986) we identified this species as *D. davisi* Edwards. *D. saetheri* and *D. davisi* are very close related morphologically and possibly belong to the same species. To clarify the relationship of these two species, it is necessary in the future to obtain molecular genetic data for *D. davisi* from the type locality. Before this we adhere to Willassen's position on taxonomy of these species.

Males from Wrangel Island and the Kolyma River basin are slightly larger than those from other regions, but the leg indices and other data are similar for all populations (Table 5). The most interesting specimens are from the highlands of the Kolyma River basin. In two out of five males the wings were noticeably reduced. The ratio of total length to wing length is 1.32–1.49, anal lobe reduced, and wing veins are indistinct. A similar reduction was noted earlier for *D. steinboecki* Goetghebuer (Serra-Tosio 1974) and *D. serratosioi* Willassen (Willassen 1986).

Distribution. Known from Norway and Russian Far East (Chukotka Peninsula, Wrangel Island and Kolyma River basin).

Diamesa lupus Willassen

(Figs. 16–18)

Diamesa lupus Willassen, 1986: 127; Ashe & O'Connor 2009: 281.

Material examined. U.S.A.: Paratype, adult male, Alaska, Glacier Bay, Wolf Creek, 12.VIII.1979, leg. A.M. Milner, Z. M. Bergen Type N 83; 1 adult male, Alaska, Juneau, Hebert River, about 0.5 mi. downstream of glacier, 20.II.2010, 58.532 N, 134.692 E, leg. J. Hudson; 2 adult males, the same data, except 28.X.2012, leg. J. Hudson; 1 adult male, Michigan, Washtenaw Co., Huron River, Hudson Mills Metro Park, 23.III.2013, leg. P. Hudson.

Description

Adult male (n = 4, except when otherwise stated).

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

Head (Fig. 18). Eyes hairy, reniform. Temporal setae including 1–2 frontals, 6 verticals and 9 postorbitals. Clypeus with 2–4 setae. Antenna with 8 flagellomeres and reduced plume of setae; terminal flagellomere with 3–4 basal setae, 60–64 µm long and 2 subapical setae, 16–20 µm long. Length of 1–8 flagellomeres (µm): 84–92, 28–48, 32–38, 28–32, 30–32, 24–32, 40–48, 104–116; AR 0.35–0.39. Palpomere length (µm): 28–40, 48–60, 88–96, 80–88, 96–128. Palpomere 3 in distal part with sensilla capitata with diameter 16–20 µm. Head width/palpal length 1.15–1.21. Antennal length/palpal length 1.04–1.14.

Thorax . Antepronotum with 7–10 ventrolateral setae. Dorsocentrals 5–9, prealars 2–3, scutellars 10–19, epimeron II with 0–1 seta.

Wing. Length 2.65–2.76 mm, width 0.90–0.92 mm. Costal extension absent. Anal lobe rounded, sometimes slightly reduced. Squama with 13–19 setae. R and R_1 with 10–13 setae, R_{4+5} with 4–6. R_{2+3} visible only in basal part. RM/MCu 2.4–2.6.

Legs. Spur of front tibia 32–36 µm long. Spurs of mid tibia 36–40 µm long. Spurs of hind tibia 60–72 µm and 36 µm long. Hind tibial comb with 17–21 setae. Length (µm) and proportions of leg segments as in Table 6.

TABLE 6. Lengths (in µm) and proportions of leg segments of *Diamesa lupus* Willassen (n=3)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1279–1312	1246–1263	754–804	425–572	394–476	98	115–131
P_2	1361	1148–1180	541–607	323–391	262–312	98	115
P_3	1460–1476	1328–1345	820–902	510–629	443–508	98–115	115–131

continued

	LR	BV	SV	BR
P_1	0.60–0.64	4.03–4.10	3.18–3.37	1.0–1.4
P_2	0.47–0.51	4.08–4.71	4.19–4.64	1.1–1.4
P_3	0.62–0.67	3.71–4.17	3.11–3.42	1.2–1.4

Hypopygium (Figs. 16–17). Laterosternites very extended beyond posterior margin of tergite IX, divided into two parts by curved apodeme, ventro-caudal part more weakly sclerotized than the rest of hypopygium, with weak setae and microtrichia. Tergite IX with posterior margin from gently rounded to partly straight, tergite bands Y-shaped, anal point reduced. Transverse sternapodeme (TSA) triangular, usually with a spire-shaped apex (Figs.

16–17), 100–112 μm high, 180–188 μm wide at the base; TSA height/TSA width 0.56–0.60. Gonocoxite 280–292 μm long; superior volsella with dense microtrichia, without setae. Gonostylus 180–200 μm long, curved, narrowed about 0.5 from base, with 3–5 apical teeth and megaseta which in form of wide terminal spine, 8–10 μm long; HR 1.46–1.62.

Pupa *and* ***larva*** *unknown.*

Distribution. Rare Nearctic species, known only from U.S.A. (Alaska ans Michigan) and Canada (Alberta) (Ashe & O'Connor 2009).

Diamesa davisi Edwards

Diamesa davisi Edwards, 1933: 614; Goetghebuer 1939: Hansen & Cook 1976: 81; Willassen 1986: 112; Ashe & O'Connor 2009: 274.

Remarks. We do not have additional material to study morphology and to perform DNA barcoding of this species, so we accept Willassen (1986) conclusions.

Distribution. Nearctic species, known from Canada, Greenland and U.S.A. (Ashe & O'Connor 2009).

Diamesa serratosioi Willassen
(Figs. 19–22)

Diamesa serratosioi Willassen, 1986: 116; Ashe & O'Connor 2009: 284; Makarchenko *et al.* 2022: 80.

Material examined. NORWAY: Paratype adult male, Z. M. Bergen Type N 41, slide NE 61; Ekse, Hoi, Vaksdal, 24–30.VI.1976, leg. T. Andersen. RUSSIA: 3 adult males, Republic of Khakassia, Tashtypsky District, unnamed stream in the basin of the Bolshoy On River, ultraviolet lamp, 51°42'58.2"N 89°51'25.8"E, altitude 1953 m a.s.l., 8–9.VIII.2020, leg. V. Dragan; 2 adult males, Chukotka Autonomous Okrug, Anadyrskyi District, unnamed stream of Velikaya River basin, 1.VIII.1980, leg. E. Makarchenko; 2 adult males, the same data except Chukotsky District, Chegitun River, 23.VII.1981, leg. E. Makarchenko; 2 adult males, Khabarovsk Territory, Solnechnyi District, Gornyi Village, Silinka River (Amur River basin), 26.VII.1983, leg. E. Makarchenko; 6 adult males, Kamchatka, unnamed stream, about 30 km from Esso Village, light trap, 9.VII.1996, leg. E. Makarchenko; 2 adult males, Magadan Region, Olskyi District, 137 km of Kolymskaya Road, Ola River, 1.V.2016, 60.412194 N, 151.514564 E, leg. E. Khamenkova.

Description

Adult male (n = 6, except when otherwise stated). Total length 2.3–3.0 mm. Total length/wing length 0.82–1.0.

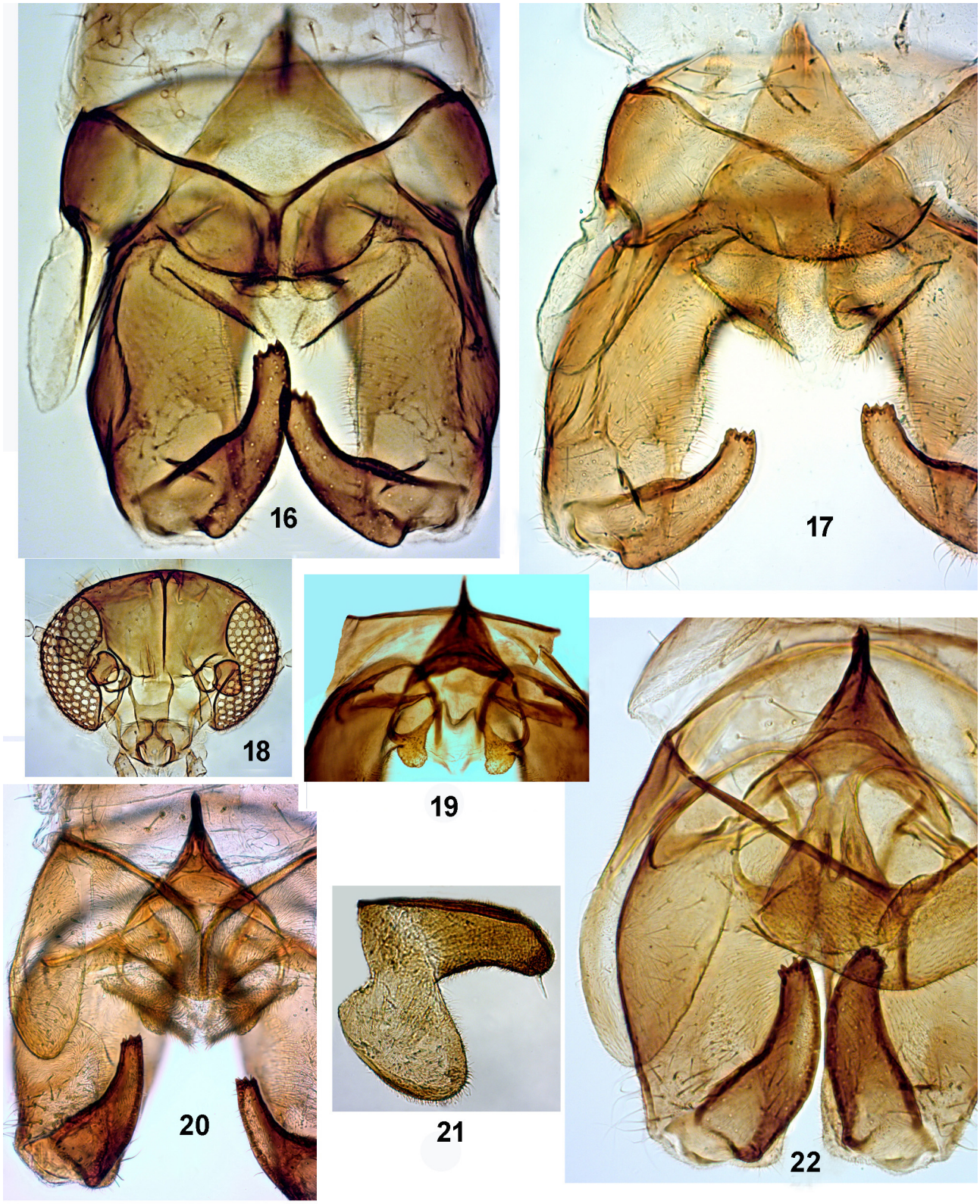
Coloration. Dark brown to brown. Legs brown, sometimes basal 1/3 of femur brownish-yellow. Wings grayish to gray, venation brownish.

Head. Eyes hairy, reniform. Temporal setae including 3–4 frontals, 5–6 verticals. Clypeus with 2–7 setae. Antenna with 8 flagellomeres and reduced plume of setae; terminal flagellomere with 2 subapical setae, 24–32 μm long. Length of 1–8 flagellomeres (μm): 68–104, 36–48, 36–40, 28–32, 28–32, 24–28, 28–36, 68–116; AR 0.25–0.42. Palpomere length (μm): 28–48, 40–48, 76–116, 64–100, 108–156. Palpomere 3 in distal part with sensilla capitata with diameter 12–16 μm. Head width/palpal length 1.05–1.22. Antennal length/palpal length 0.90–1.08.

Thorax. Antepronotum with 6–12 ventrolateral setae. Dorsocentrals 7–12, prealars 3–5, scutellars 22–35.

Wing. Length 2.44–3.0 mm, width 0.72–1.1 mm. Anal lobe rounded angular. Squama with 15–25 setae. R and R_1 with 17–22 setae, R_{4+5} with 4–7 setae. RM/MCu 2.3–2.5.

Legs. Spur of front tibia 24–36 μm long. Spurs of mid tibia 36–44 μm and 32–48 μm long. Spurs of hind tibia 64–84 μm and 32–44 μm long. Hind tibial comb with 16–18 setae. Length (μm) and proportions of leg segments as in Table 7.



FIGURES 16–22. Adult males of *Diamesa lupus* Willassen from Alaska **(16–18)** and *Diamesa serratosioi* Willassen from Khakassia **(19, 21),** Magadan Region **(20)** and Norway **(22). 16, 22,** hypopygium of paratypes in dorsal view; **17, 20,** hypopygium in dorsal view; **18,** head, **19,** transverse sternapodeme, aedeagal lobes and superior volsellae; **21,** segment IX in lateral view.

TABLE 7. Lengths (in µm) and proportions of leg segments of *Diamesa serratosioi* Willassen (n=6)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1312–1624	1230–1689	738–1082	328–492	180–287	82–115	98–131
P_2	1296–1591	1082–1509	508–689	213–312	131–213	82–115	98–131
P_3	1427–1706	1296–1738	787–1099	410–549	197–295	82–115	115–131

continued

	LR	BV	SV	BR
P_1	0.59–0.66	4.18–4.77	2.98–3.44	1.1–1.2
P_2	0.44–0.47	4.80–5.51	4.45–4.75	1.0–1.2
P_3	0.57–0.64	3.99–4.40	3.07–3.47	1.1–1.2

Hypopygium (Figs. 19–22). Laterosternite IX extending beyond posterior margin of tergite IX by 56–80 µm, with weak 18–20 setae, 40–52 µm long (Figs. 20, 22). Tergite IX with 16–18 setae, 20–32 µm long and with pale hyaline anal point, 26–28 µm long, which is not visible from above, in lateral view pointing down (Fig. 21); posterior margin of tergite IX rounded; tergite bands Y-type (Figs. 20, 22). Transverse sternapodeme (TSA) triangular, peaked, 88–140 µm high, 160–196 µm wide at the base; TSA height/TSA width 0.52–0.83. Gonocoxite 312–400 µm long, superior volsella covered with microtrichia and sometimes short setae (Fig. 19). Gonostylus 172–204 µm long, broad at base, more or less strongly curved, with megaseta which in form of wide terminal spine, 4–5 µm long, 3–5 apical teeth and 2 subterminal setae. HR 1.76–2.15.

Pupa and ***larva*** unknown.

Remarks. *D. serratosioi* from Norway and Sweden was previously described as *D. davisi* Edwards by Serra-Tosio (1971). We recoded this species from Russia for Khakassia Republic (Makarchenko *et al.* 2022). This is the first information about the location of *D. serratosioi* in the Russian Far East.

Distribution. Known from Finland, Norway, Sweden (Ashe & O'Connor 2009), Khakassia and Russian Far East (Chukotka and Magadan Region, Amur River basin of Khabarovsk Territory).

Diamesa sonorae Willassen

Diamesa sonorae Willassen, 1986: 125; Ashe & O'Connor 2009: 284.

Remarks. The species has been described and known so far only by one male and two females from North America (California), is close related to *D. alpina* and requires additional revision, including the use of barcoding.

Distribution. Nearctic species, known only from Sonora Pass in California (Ashe & O'Connor 2009).

Diamesa tokunagai Makarchenko et Yamamoto

Diamesa tokunagai Makarchenko et Yamamoto, 1995: 298; Ashe & O'Connor 2009: 286.

Remarks. The species has been described and known so far only by one male from Japan, is close related to *D. alpina* and requires additional revision, possibly supported by DNA barcoding.

Distribution. Known only from Honshu Island of Japan (Ashe & O'Connor 2009).

Diamesa leona subgroup

Adult males of this subgroup have large and heavily chitinized, often bent over the dorsal side hypopygium; tergite IX with delicate pubescence of microtrichia directed anteriorly, with long and wedge-shaped or short anal point, angled downwards and covered in basal part with numerous microtrichia forward and laterally directed. Gonocoxite long, broad, with numerous short, anteriorly-directed setae and with slight broad ridge antero-dorsally; inferior

volsella with rare exceptions absent; superior volsella in form of angular tubercle or collar. Gonostylus slightly curved, with numerous, proximally directed strong setae, in apical part with 1 megaseta and sometimes 1 tooth. Transverse sternapodeme triangular, with a spire-shaped apex.

Diamesa japonica Tokunaga
(Figs. 23–27)

Diamesa japonica Tokunaga, 1936: 542; Makarchenko 1981: 110, 1985: 76, 2006: 261, 2021: 440; Ashe & O'Connor 2009: 278.
Diamesa kurobemijikaia Sasa et Okazawa, 1992: 60.

Material examined. JAPAN: 2 adult males, Honshu, Tochigi Prefecture, Okunikko, Nikko City, Nikko National Park, Toyamazawa River, alt. 1450 m a.s.l., 21.III.1991, leg. R. Ueno. RUSSIA: 23 adult males, Kurile Islands, Kunashir Island, Sernovodsk Village, Tiurino River, 20.IV.1978, leg. E. Makarchenko.

Description

Adult male (n = 14). Total length 3.1–4.2 mm. Wing length 2.76–3.17 mm. Total length/wing length 0.90–1.40.

Coloration. Head, thorax, legs and hypopygium dark brown; antenna and palpomeres brown; abdomen light brown to dark brown; wing greyish, veins brownish.

Head. Eyes hairy. Temporal setae including 4–12 frontals, 6–15 verticals and 4–8 postorbitals. Clypeus with 2–8 setae. Antenna with 8 flagellomeres and reduced plume of setae (Fig. 23); number and length of these setae on 1–7 flagellomeres respectively: 2–3 (28–40 μm), 1–2 (28–36 μm), 1–2 (26–40 μm), 0, 0, 0, 0; terminal flagellomere in basal part with 5–6 setae, 60–80 μm long and with 2 subapical setae, 40–52 μm long. Flagellomeres 1–8 length (μm): 71–88, 34–38, 25–34, 25–30, 25–34, 25–34, 32–38, 193–218; AR 0.68–0.81. Antennal length/palp length 1.05–1.38. Palpomeres lengths (in μm): 32–40; 52–63; 88–105; 76–105; 105–147. Palpomere 3 in distal part with sensilla capitata with diameter 20–24 μm. Palpomeres 1–5 length/head width 0.80–1.38.

Thorax. Antepronotum with 7–12 ventrolateral setae. Dorsocentrals 6–10, prealars 5–8, scutellars 11–24.

Wing. R and R_1 with 15–25 setae; R_{4+5} with 8–12 setae. Costa extension *ca* 48 μm long. RM length/MCu length 2.0–3.6. Anal lobe well developed, rounded. Squama with 18–25 setae 28–52 μm long. VR 0.96–1.0.

Legs. Spur of front tibia 34–46 μm long; spurs of mid tibia 40–55 μm long; of hind tibia 50–71 μm and 32–50 μm long. Hind tibial comb with 20–22 setae. Lengths and proportions of leg segments as in Table 8.

TABLE 8. Lengths (in μm) and proportions of leg segments of *Diamesa japonica* Tokunaga, male (n=14)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1496–1768	1513–1720	918–1105	425–527	255–306	102–136	131–153
P_2	1496–1768	1292–1479	476–544	238–289	170–204	85–119	119–153
P_3	1615–1940	1530–1768	850–1054	442–527	221–255	98–119	131–153

continued

	LR	BV	SV	BR
P_1	0.57–0.64	4.05–4.42	3.15–3.55	0.9–1.3
P_2	0.32–0,39	4.91–5.59	5.41–6.96	0.8–1.3
P_3	053–0.60	4.31–4.99	3.98–4.84	0.8–1.2

Hypopygium (Figs. 24–27). Tergite IX with 7–11 setae (from one side), 20–40 μm long and wedge-shaped anal point 63–136 μm long, angled downwards (Fig. 25) and covered in basal 2/3 with numerous microtrichia forward and laterally directed (Figs. 24–25). Laterosternite IX with 9–11 setae, 28–32 μm long. Transverse sternapodeme (TSA) triangular, with a spire-shaped apex (Fig. 27), 124–128 μm high, 190–196 μm wide at the base; TSA height/TSA width 0.65–0.70. Gonocoxite 350–492 μm long; inferior volsella absent; superior volsella in form of angular tubercle. Gonostylus slightly curved, 220–236 μm long, with numerous, proximally directed strong setae (Figs. 26–27), 44–52 μm long; megaseta 6–8 μm long. HR 1.92–2.23.

Pupa was described by Tokunaga (1936).

Larva unknown.

Distribution. East Palaearctic island species. Known from Japan and Kunashir Island (Kurile Islands).

Diamesa leoniella Hansen

(Fig. 28)

Diamesa leoniella Hansen in Hansen & Cook 1976: 111; Makarchenko 2021: 439.
Diamesa japonica Tokunaga; Makarchenko 1981: 110, misidentification.

Material examined. U.S.A.: 1 adult male, Alaska, Juneau City, Mendenhall Lake, N 58.4217 E 134.5388, 26.III.2013, leg. K. Frangos and P. Hudson; 1 adult male, the same data except Nugget Falls, N 58.4270 E 134.5373, 17.VII.2013, leg. P. Hudson.

Description

Adult male (n = 2). Total length 4.7 mm (n = 1). Wing length 3.44 mm (n = 1). Total length/wing length 1.37.

Coloration. Head, thorax, legs and hypopygium dark brown; antenna brown; palpomeres light brown; abdomen light brown to brown; wing greyish, veins yellowish brown.

Head. Eyes hairy. Frontal tubercles 16–44 μm long, covered with microtrichia. Temporal setae including about 8–12 frontals, 13–15 verticals, 5 postorbitals. Clypeus with 5–6 setae. Antenna with 8 flagellomeres and reduced plume of setae; flagellomeres 1–7 with 2–3 setae, 24–40 μm long; terminal flagellomere in basal part with 4–5 setae, 60–72 μm long and with 2 subapical setae, 16–20 μm long. Flagellomeres 1–8 length (μm): 88–104, 36–40, 40, 32, 32, 26–32, 32–44, 164–248; AR 0.41–0.78. Antennal length/palp length 1.09–1.21. Palpomeres lengths (in μm): 32–40; 56–64; 108; 84–100; 136–156. Palpomere 3 in distal part with sensilla capitata with diameter 20 μm. Palpomeres 1–5 length/head width 0.92.

Thorax. Antepronotum with 9–11 ventrolateral setae. Dorsocentrals 5–7, prealars 7, scutellars 9–11.

Wing. R and R_1 with 25–34 setae; R_{4+5} with 18–19 setae. Costa extension 52–60 μm long. RM/MCu 2.8–3.5. Anal lobe slightly reduced, angularly. Squama with 14–17 setae, 52–68 μm long. VR 0.96–1.0.

Legs. Spur of front tibia 40–44 μm long; spurs of mid tibia 44 μm long; of hind tibia 74–80 μm and 44–48 μm long. Hind tibial comb with 15–16 setae. Lengths and proportions of leg segments as in Table 9.

TABLE 9. Lengths (in μm) and proportions of leg segments of *Diamesa leoniella* Hansen, male (n=2)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1607–2280	1591–2120	1082–1400	541–720	295–440	107–140	148–180
P_2	1706–2160	1394–1760	763–960	377–440	230–360	98–140	148–180
P_3	1738–2400	1624–2160	1033–1440	607–800	312–400	98–160	148–200

continued

	LR	BV	SV	BR
P_1	0.66–0.68	3.92	2.96–3.14	0.9–1.1
P_2	0.55	4.36–4.53	4.06–4.08	0.9–1.0
P_3	0.64–0.67	3.77–3.85	3.17–3.25	1.0–1.1

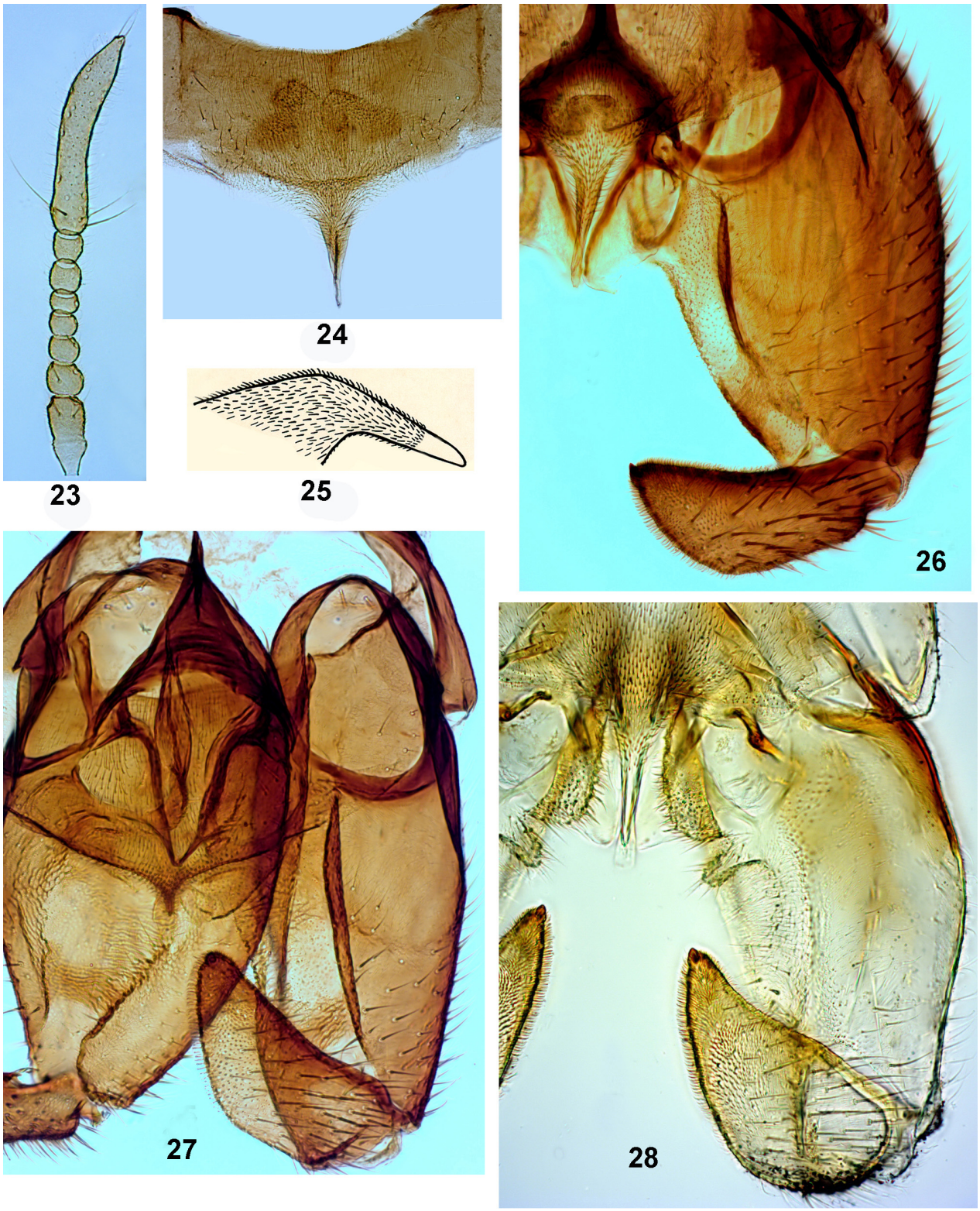
Hypopygium (Fig. 28). Tergite IX with 11–13 setae (from one side), 16–24 μm long and wedge-shaped anal point 100–140 μm long, covered in basal half with numerous microtrichia forward and laterally directed. Laterosternite IX with 10–11 short setae. Transverse sternapodeme triangular, 128 μm long. Gonocoxite 400–496 μm long. Inferior volsella finger-like, 40–48 μm long and 24 μm width, covered with short setae and microtrichia. Superior volsella in form of collar, covered with microtrichia and short setae.Gonostylus slightly curved, 212–246 μm long, with numerous, proximally directed strong setae, 48–64 μm long; megaseta 6–8 μm long. HR 1.65–1.93.

Pupa and ***larva*** unknown.

Remarks. Male of *D. leoniella* is very closely related to *D. japonica* and many years ago senior author misidentified this species as *D. japonica* (Makarchenko 1981). After obtaining comparative material from North America, it turned out that these two species can be well distinguished by the structure of the hypopygium and some

other features (Makarchenko 2021). So, the male of *D. leoniella* has a finger-shaped inferior volsella and a superior volsella in the form of a collar, while *D. japonica* without inferior volsella and superior volsella in form of angular tubercle.

Distribution. Nearctic species. Known from U.S.A.—California, Montana, Utah, Washington, Wyoming (Hansen & Cook 1976) and Alaska (recorded for the first time).



FIGURES 23–28. Adult males of *Diamesa japonica* Tokunaga from Honshu, Japan **(23, 27),** Hokkaido, Japan **(24)** and Kunashir Island **(25–26),** *Diamesa leoniella* Hansen from Alaska **(28). 23,** flagellum of antenna; **24,** tergite IX (bent out on slide); **25,** anal point in lateral view; **26–28,** hypopygium in dorsal view.

***Diamesa leona* Roback**
(Figs. 29–46)

Diamesa leona Roback, 1957: 7, 1959: 2; Hansen & Cook 1976 : 106; Makarchenko 1981: 103, 1985: 73, 2006: 262; Herrmann *et al.* 1987: 311; Linevich & Makarchenko 1989: 30; Ashe & O'Connor 2009: 280.
Diamesa pieta Roback, 1957: 8.
Diamesa caena Roback, 1957: 9.
Diamesa breviala Tokunaga, 1964: 39.
Diamesa renegata Makarchenko, 1977: 1732.
Diamesa mongolica Serra-Tosio, 1983: 11.
Diamesa starmachi Kownacki et Kownacka, 1970: 777; Spies & Sæther 2004: 19 (name correction); Ashe & O'Connor 2009: 285; Giłka *et al.* 2013: 202; Rossaro & Lencioni 2015: 70. **Syn. nov.**

Material examined. RUSSIA: 5 adult males, 3 females, Primorye Territory, Khasansk District, Kedrovaya Pad Nature Reserve, Kedrovaya River, 12.II.1977, leg. E. Nikolayeva; 1 adult male, the same data except 10.II.1979, leg. Yu. Shibnev; 1 mature pupa, the same data except 8.II.1980, leg. E. Makarchenko; 2 adult males, the same data except 19.III.2016, leg. E. Makarchenko; 2 adult females, the same data except 23.II.2019, leg. E. Makarchenko; 3 adult males, the same data except Ussuryiskyi District, Ussuryisky Nature Reserve, 1.XII.1972, leg. I. Chereshnev, L. Budnikova; 1 adult male, the same data except Chuguevskyi District, Elovyi Stream, 31.V.1977, leg. T. Vshivkova; 1 adult male, the same data except Partizanskyi District, Tigrovaya River, 10.V.2020, leg. E. Gorovaya; 4 adult males, the same data except Khabarovsk Territory, Nanaisky District, Anyuisky National Park, Pihtsa River (tributary of Gassi Lake), Amur River basin, N 48.47.804, E 136.47.027, 22–24.V. 2019, leg. N. Yavorskaya; 1 adult male, the same data except Solnechnyi District, Gornyi Village, Silinka River (Amur River basin), 26.VII.1983, leg. E. Makarchenko; 1 adult male, 1 female, the same data except Magadan Region, Tenkinskyi District, not far from Sibit-Tyellakh Village, Ozernyi Stream (Kolyma River basin), 3.VI.1978, S. Kocharina; 2 adult males, the same data except Khasynskyi District, Khasyn River, (Arman River basin) not far from Khasyn Village, 30.VI.2017, leg. I. Zasypkina; 2 adult females, the same data except Olskyi District, Ola River (upper stream), 1.V.2016, leg. E. Khamenkova; 1 adult male, 1 female, the same data except 3.V.2019, leg. E. Khamenkova; 3 adult males, the same data except Kamchatka Territory, Avacha River, 14.VI.1970, leg. V. Levanidov; 4 adult males, the same data except Sakhalin Island, Forest Park of Yuzhno-Sakhalinsk City, Rogatka River, 15.V.1984, leg. E. Makarchenko; 2 adult males, the same data except Krasnoselskaya River near Novoalekseevka Village, 2.VI.1984, leg. E. Makarchenko; 10 adult males, the same data except Chukotka Peninsula, Yoni Lake basin, Gilmimliveem River, 4.VIII.1973, leg. I. Chereshnev; 2 adult males, the same data except Chegitun River, middle stream, 4.VIII.1981, leg. Makarchenko. KAZAKHSTAN: 2 adult males, East Kazakhstan region, Katon-Karagai District, Sarymsaqty Mountains (Kazakh Mountain Altai), Arasan River, about 0.7 km below of Bolshoe Rakhmanovskoye Lake, altitude 1790 m above sea level, 5.VII.2018, 49.535983 N, 86.500633 E, leg. D. Palatov.

Comments. The species *D. leona, D. pieta* and *D. caena* were described from North America by Roback (1957) from adult males collected in the same area but at different times. The first two species differed from each other mainly in color and *D. caena* in reduced wings and the "absence" of the anal point. Hansen (Hansen & Cook1976) comparing the type material of these species, came to the conclusion that *D. pieta* and *D. caena* should be junior synonyms of *D. leona* since all specimens have an anal point and wing reduction is not a diagnostic feature (Hansen & Cook 1976). *D. breviala* Tokunaga from Japan (Tokunaga 1964) and *D. renegata* Makarchenko from the Russian Far East (Makarchenko 1977) with reduced wings also were described as adults males. As a result of studying additional material from various regions of the Far East, we came to the conclusion that these both species are identical to the North American *D. leona*, since have similar hypopygium and occur in populations with both brachypterous and macropterous specimens (Makarchenko 1981). We subscribe to the opinion of Serra-Tosio (1974) and Hansen & Cook (1976) that the brachypterism of the genus *Diamesa* is not a basis for taxonomic identification. In the meanwhile it was suggested that *D. starmachi* Kownacki et Kownacka from the Polish Tatras (Kownacki & Kownacka 1970; Giłka *et al* 2013) may be also a junior synonym of *D. leona,* since it has a similar structures of the hypopygium. But it became possible to confirm this only now, after obtaining the molecular genetic data of these two species. DNA-based methods significantly complement traditional taxonomic approaches in that they facilitate to differentiate closely related species or reveal the presence of distinct taxa that are morphologically indistinguishable. The results of DNA barcoding made it possible to confirm conspecificity of *D. starmachi* from Norway (HQ941609) and *D. leona* from Kazakhstan and Russia (ON834735–ON834742). Phylogenetic analysis

has shown that the identity of *D. starmachi* can no more be supported and it is here stated that *D. starmachi* is a junior synonym of *D. leona*.

Since it's hard to make a general description for the brachypterous and macropterous forms of males from different regions of the Holarctic and to combine all the data available in the literature, we decided to present the main features of these forms in the Table 10 and give a description of hypopygium only.

Description

Adult male. Hypopygium (Figs. 29–46). Large, usually darker than body segments, heavily chitinized and curved dorsally. Tergite IX with delicate pubescence of microtrichia directed anteriorly; anal point in brachypterous forms 42–80 μm long and 55–108 μm long in macropterous forms, directed downwards at an angle (Fig. 35), almost invisible from above; on straightened tergite IX, viewed from above, anal point often with cut top (Figs. 32–34, 42–42, 45) (more often in brachypterous forms) or rounded top (Figs. 34, 46), sometimes with sharp top and completely covered with microtrichia (Fig. 40), while in most cases the subapical part of the anal point without microtrichia; gonocoxite long, broad, with numerous short, anteriorly-directed setae (Figs. 29, 41–44, 46); gonostylus strong, slightly curved, with numerous short, proximally directed setae; medial surface of gonostylus with fine "pile" of microtrichia, with large terminal spine at end, subterminal setae absent (Figs. 30, 34, 36–39, 41–44, 46). Transverse sternapodeme very strong, triangular, produced to a point antero-medially (Figs. 39, 42–44). Anal point length/ gonostylus length 0.13–0.37). HR 1.50–2.47.

In the process of studying the material from Sakhalin Island several abnormal males were found that lacked the anal point and the posterior margin of tergite IX was concave in the middle (Fig. 31).

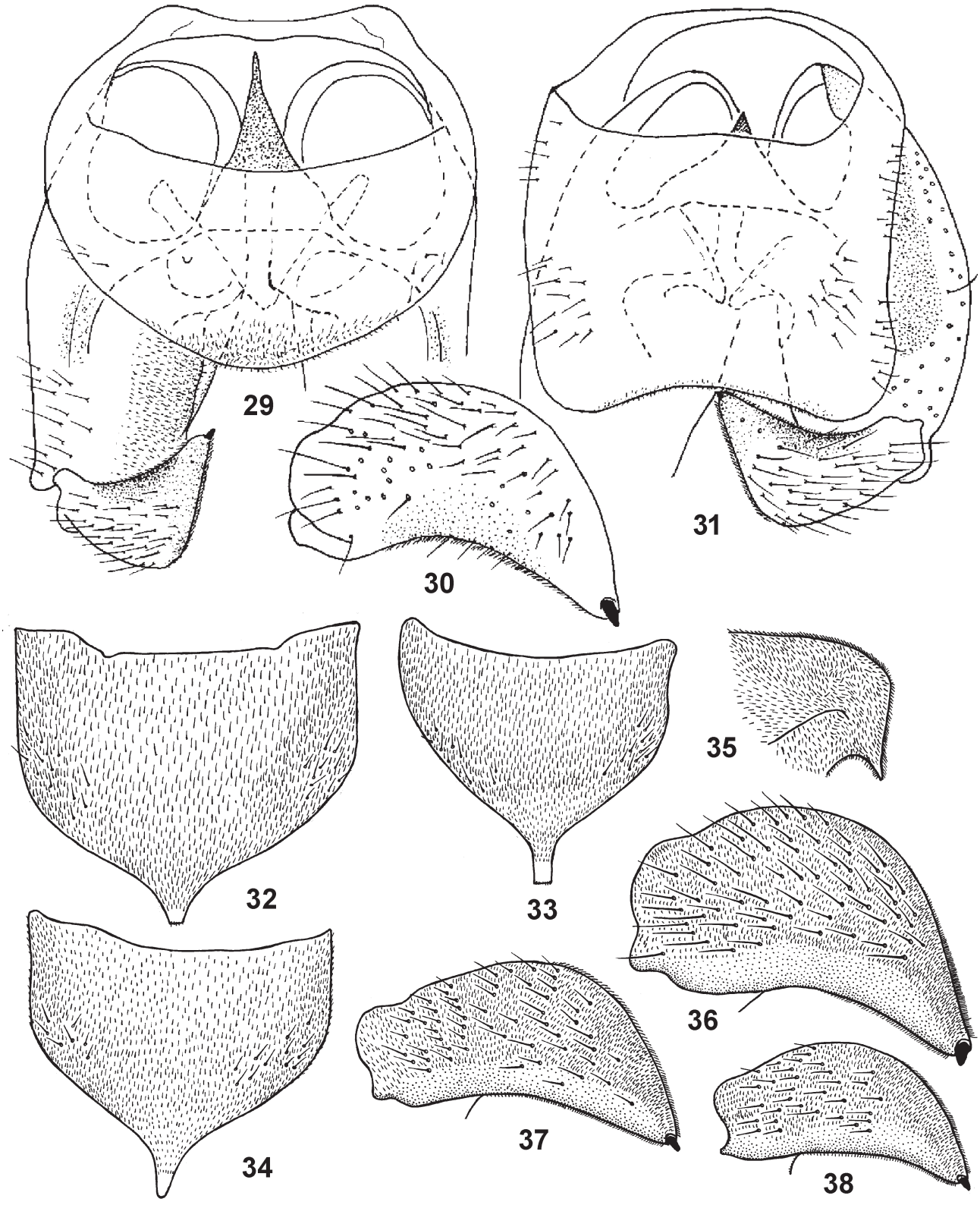
Pupa (as *D. starmachi*) was described by Kownacki & Kownacka (1970) and Makarchenko (1981).

Larva was described by Rossaro & Lencioni (2015).

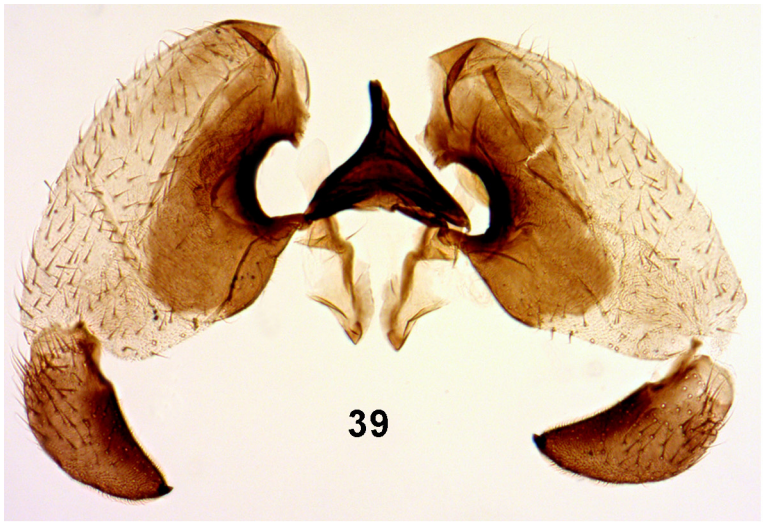
Biology. The biology of *D. leona* is very interesting because adult males and females can lead an active life under extreme conditions, namely low air temperatures in winter time. Quite a lot has been published about this for specimens living in North America (Hansen & Cook 1976, Herrmann *et al.* 1987) and the Polish Tatras (Giłka *et al.* 2013). But there is very little information about this from the Russian Far East. Therefore, below we present some data on the biology of *D. leona* from this region of the Palaearctic.

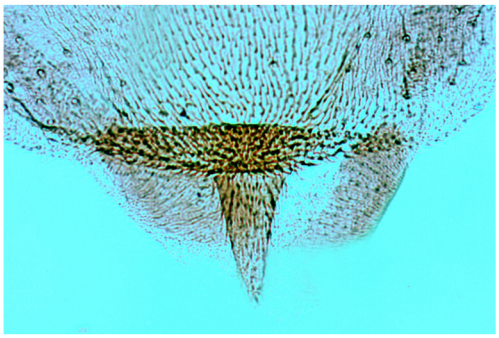
In the south of the Russian Far East the species is apparently bivoltine, in the Northeast it is univoltine. Males and females of the first generation in Primorye Territory are brachypterous; they hatch from pupae from early December to early March at a water temperature in the river from 0.1 to 1.5ºC., and air temperature from –21ºC to +5ºC. The maximum emergence of adults occurs in mid February. Once three brachypterous males were collected in the Ussuriysky Nature Reserve in the snow on December 1, 1972 at an air temperature of—21ºC. Adult insects of the first generation are more like spiders than chironomids. Due to the strong development of the legs, they move well on the ice and are often found on the snow. Males and females of the second generation are macropterous; their emergence occurs in the same area from the end of May to the first half of June. We also encountered brachypterous specimens in the mountains of the Upper Kolyma River basin in early June and in South Sakhalin in the first half of May. Macropterous specimens in South Sakhalin were collected from early May to early June at a water temperature in the rivers from 4 to 17ºC. On the Chukotka Peninsula the emergence of adults was observed from the second half of July to mid August, in Kamchatka—in mid June. In the Chukotka Peninsula and Kamchatka males and females of *D. leona* were collected only as macropterous. But despite well developed wings they do not fly and like brachypterous form sit mainly under damp stones along the banks of watercourses. This is where mating takes place. The inability to fly in macropterous specimens is apparently associated with the underdevelopment of the flight muscles. Larvae and pupae live in foothill and mountain streams on stones with a fast current. On Sakhalin Island the largest number of pupae and larvae was observed among algae *Hydrurus foetidus* Kirchn. *D. leona* reaches its highest altitude (about 800 m) in the Upper Kolyma River basin.

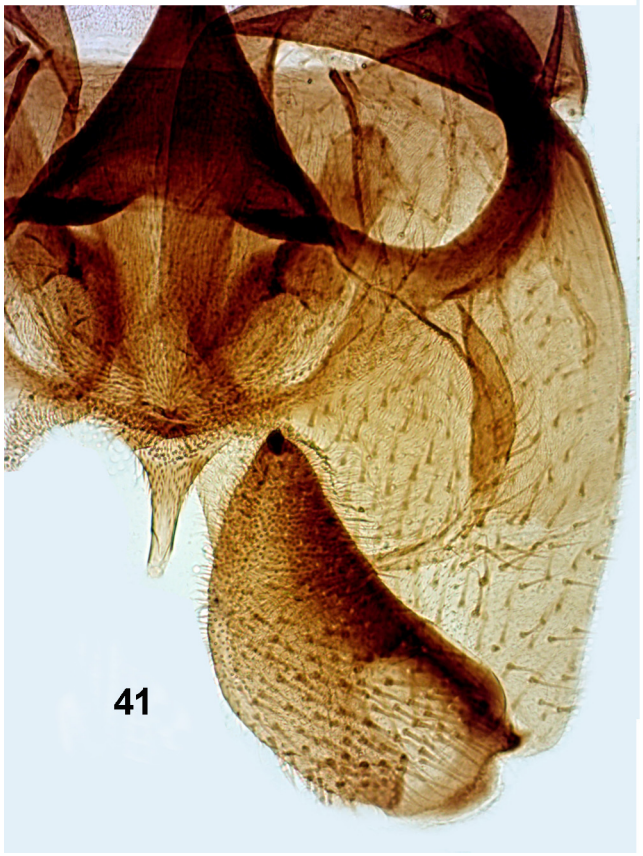
Distribution. Widespread arcto-alpine Holarctic species. Known in North America from Canada (New Brunswick and Quebec) and U.S.A. (Colorado, Idaho, Vichigan, Minnesota, Montana, Nevada, New Mexico, Utah, Washington and Wisconsin). In the Palaearctic recorded from China, Japan, Mongolia, Poland, Austria, Germany, Italy, Luxemburg, Slovakia, East Siberia, Russian Far East (Ashe & O'Connor 2009) and Norway.

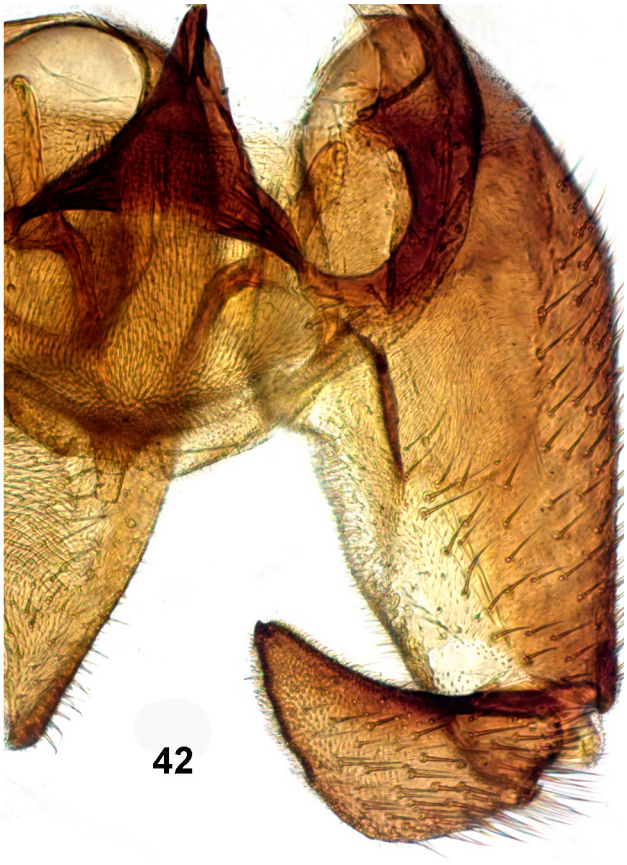


FIGURES 29–38. Adult male of *Diamesa leona* Roback from Sakhalin Island (macropterous form) **(29–31),** Primorye Territory (brachypterous form) **(32, 35–36),** Chukotka (macropterous form) **(33, 38)** and Kamchatka (macropterous form) **(34, 37)**. **29,** typical normal hypopygium in dorsal view; **31,** abnormal hypopygium in dorsal view; **30, 36, 37–38,** gonostylus; **32–34,** tergite IX (bent out on slide); **35,** anal point in lateral view. Figs. 32–38 after Makarchenko (1981).

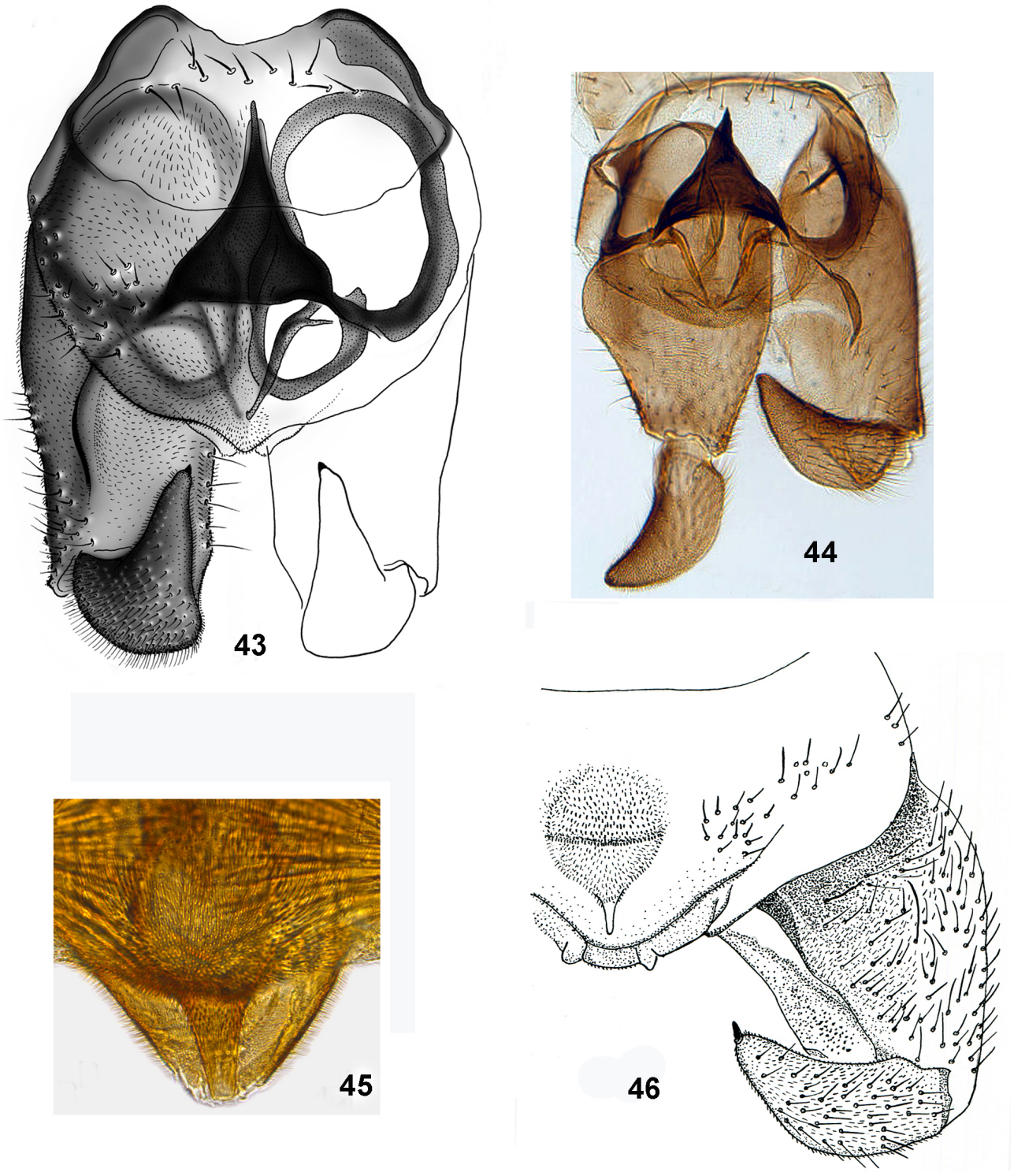








FIGURES 39–42. Adult males of *Diamesa leona* Roback (macropterous form) from Chukotka **(39–40),** Baikal Region **(41)** and Kazakhstan **(42). 39,** hypopygium without tergite IX in dorsal view; **40,** part of tergite IX with anal point (bent out on slide); **41–42,** hypopygium in dorsal view.



FIGURES 43–46. Adult males of *Diamesa leona* Roback (= *D. starmachi* Kownacki et Kownacka) from Tatra Mountains **(43, 45–46)** and Norway **(44). 43–44, 46,** hypopygium in dorsal view; **45,** part of tergite IX with anal point (bent out on slide). **Figs. 43, 45,** after Gilka *et al.* 2013; **Fig. 44,** after Mærk Aspaas, NTNU Vitenskapsmuseet (from BOLD); **Fig. 46,** after Kownacki & Kownacka 1970.

TABLE 10. Comparison of some morphological characters of the brachypterous and macropterous forms of *Diamesa leona* males from some regions of Palaearctic

Character	Primorye Territory, Russia, n=6	Magadan Region, Russia, n=3	Tatra Mountains, Poland Gilka *et al*. 2013; Kownacki & Kownacka 1970 (in parenthes)	Primorye and Khabarovsk Territories, Sakhalin, Russia, n=8	Kamchatka, Chukotka, n=14	Tatra Mountains, Poland Gilka *et al*. 2013	Kazakhstan, n=4
	Brachypterous			Macropterous			
Total length, mm	3.2–4.6	4.0–5.5	3.2–5.0	3.1–5.45	3.4–4.5	3.2–3.4	3.6–3.8
Total length/wing length	3.43–10.29	10.2–13.1	4.0–6.2	0.88–1.38	1.09–1.42	1.1–1.2	0.90–0.96
AR	0.47–0.63	0.45–0.53	0.40–0.54 (0.68)	0.49–0.57	0.45–0.65	0.45–0.57	0.56–0.57
Antenna length/palp length	1.02–1.23	0.87–1.02	-	1.05–1.1	1.1	-	-
Wing length, mm	0.32–0.34	0.32–0.42	0.79–0.99	3.28–3.95	2.76–4.11	3.17–3.20	2.68–4.0
Clypeal setae	4–8	8–10	-	6–10	4–6	-	6–12
Dorsocentrals	6–10	7–9	7–12	10–18	7–13	15–17	14–16
Prealars	3–5	2–4	3–4	4–8	4–8	4–5	6
LR_1	0.38–0.50	0.39–0.41	0.43–0.49 (0.54)	0.53–0.67	0.58–0.63	0.61–0.62	0.60–0.66
BV_1	6.11–6.76	4.71–5.68	-	3.97–4.77	4.10–4.69	-	4.43–.47
SV_1	4.83–5.38	4.95–5.24	-	3.14–3.42	3.1–3.39	-	3.0–3.29
Anal point length, µm	63–80	42–48	-	56–108	55–76	-	64–108
Anal point length/ gonostylus length	0.16–0.23	0.13	-	0.25–0.37	0.22–0.29	-	0.30–0.36
HR	1.95–2.40	2.2–2.09	-	2.11–2.35	1.5–2.47	-	1.93–2.06

Diamesa loeffleri subgroup

Adult males of this subgroup have antenna with 13 flagellomeres and completely or partly reduced setae of plume. Hypopygium with long anal point, slightly tapering to subapical, with strong anteriorly directed microtrichia in basal half and on tergite IX. Inferior volsella in the form of angular naked projection on the dorsal side of the gonocoxite or like small lobe covered with micro- and macrotrichia and adjacent to a protuberance with some long setae. Superior volsella slightly reduced, in form of rounded angular tubercle or collar. Gonostylus slightly curved, tapering to apex, with short megaseta. Transverse sternapodeme triangular or trapezoidal.

Diamesa khumbugelida Sæther et Willassen
(Figs. 47–60)

Diamesa khumbugelida Sæther et Willassen, 1987: 201; Makarchenko 1989: 84, 2009: 429; Ashe, O'Connor 2009: 279.

Material examined. NEPAL: 1 adult male, Langtang Region, brook near Khyimjung Glacier, alt. 4150 m a.s.l., 4.VIII.1999, leg. R. Endo. CHINA: 4 adult males, Xinjiang Uygur Autonomous Region, Tien Shan Mountains, Dabancheng District, Urumqi City District, Sangecha River, 43°40.583'N, 88°17.006'E, alt. 2356 m a.s.l., 13.VII.2017, leg. D. Palatov. RUSSIA: 8 adult males, Irkutsk Region, Baikal State Biosphere Reserve, Khamar-Daban Ridge, Osinovka char, 1400–1600 m a.s.l., 20.VII.2009, leg.Yu. Sundukov; 1 adult male, Republic of Khakassia, Tashtypsky District, unnamed stream in the basin of the Bolshoy On River, ultraviolet lamp, 51.716167 N, 89.857167 E, alt.1953 m a.s.l., 08–09.VIII.2020, leg. S. Dragan. KAZAKHSTAN: 2 adult males, East Kazakhstan region, Katon-Karagai District, Sarymsaqty Mountains (Kazakh Mountain Altai), Eastern border of Katon-Karagai village, Sarymsaqty River Channel, altitude 1113 m a.s.l., 01.VII.2018, 49.166283 N, 85.633583 E, leg. D. Palatov. TAJIKISTAN: 4 adult males, spurs of the Gissar Range, Khorangon Gorge, Horangon River, 11 km from the Dushanbe City, 29.II.1987, leg. L. Zhiltsova; 5 adult males, the same data except Gissar Range, Ramit Nature Reserve, Sardan-Miyona River, alt. 1200-1300 m a.s.l., 4–5.III.1987, leg. L. Zhiltsova; 5adult males, the same data except Varzob River, alt. about 1100 m a.s.l., 11–14.III.1987, leg. L. Zhiltsova; 2 adult males, the same data except Takob River, alt. about 1200 m a.s.l., 14.III.1987, leg. L. Zhiltsova.

Comments. Analysis of our imaginal material showed that the *D. khumbugelida* male from the type locality in Nepal differs from males of other populations. Therefore, we decided to provide separate descriptions for these males. It is possible that in the future, after obtaining barcoding data for this species from Nepal, it will turn out that these are different species. See more detail in the Remarks.

Description of adult male from Nepal

Adult male (n=1). Total length 3.28 mm. Total length/wing length 1.04.

Coloration. Dark brown. Wings grayish, with brown venation..

Head (Fig. 48). Eyes hairy, reniform. Temporal setae including 3 frontals, 5 orbitals, 7 verticals. Clypeus with 2 setae. Antenna with 13 flagellomeres and reduced plume of setae (Fig. 47); number and length of these setae on 1–12 flagellomeres respectively: 3 (32–60 μm), 2 (40–48 μm), 1 (48 μm), 1 (56 μm), 1 (48 μm), 1 (60 μm), 0, 1 (52 μm), 0, 1 (48 μm), 1 (48 μm), 2 (48–56 μm); terminal flagellomere with 4 setae, 72–100 μm long in basal part and with 2 subapical setae, 28–36 μm long. Length of 1–13 flagellomeres (μm): 96, 52, 48, 40, 40, 36, 32,32, 32, 24, 28, 28, 216; AR 0.44. Palpomeres 1–5 length (μm): 32, 88, 136, 92, 104. Palpomere 3 in distal part with sensilla capitata with diameter 16–20 μm. Head width/palpal length 0.98. Antennal length/palpal length 1.56.

Thorax. Antepronotum with 7 ventrolateral setae. Dorsocentrals 7–8, prealars 4, scutellars 7.

Wing. Length 3.16 mm, width 1.04 mm. Anal lobe rounded. Squama with 11 setae. R and R_1 with 10–11 setae, R_{4+5} with 2 setae in apical part. RM/MCu 3.2.

Legs. Spur of front tibia 56 μm long. Spurs of mid tibia 40–44 μm long. Spurs of hind tibia 68 μm and 40 μm long. Hind tibial comb with 16 setae. Length (μm) and proportions of leg segments are as in Table 11.

TABLE 11. Lengths (in μm) and proportions of leg segments of *Diamesa khumbugelida* Sæther et Willassen from Nepal, male (n=1)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
P_1	1574	1427	968	508	328	82	131	0.68	3.78	3.10	1.1
P_2	1720	1280	680	344	213	82	131	0.53	4.78	4.41	1.0
P_3	1760	1560	1000	525	279	82	131	0.64	4.25	3.32	1.1

Hypopygium (Figs. 49–52). Tergite IX with 7 weak setae, laterosternite IX with 6 setae. Anal point 228 μm long; 20 μm wide at base;14 μm wide at apex; with strong anteriorly directed microtrichia in basal half and on tergite IX (Fig. 51). Phallapodeme 104 μm long. Transverse sternapodeme (TSA) narrow triangular (Fig. 49), 72 μm high, 140 μm wide at the base; TSA height/TSA width 0.51. Gonocoxite 324 μm long; inferior volsella in the form of angular naked projection on the dorsal side of the gonocoxite (Fig. 52). Superior volsella in form of angular tubercle (Fig. 50). Gonostylus slightly curved, tapering to apex, 204 μm long; megaseta 8–10, 14 pm long. HR 1.59.

Description of adult males from Russia, Kazakhstan, China and Tadjikistan

Adult male (n=8, except when otherwise stated). Total length 2.9–4.4 mm. Total length/wing length 0.91–2.7.

Coloration. Dark brown. Wings grayish, with brownish venation.

Head. Eyes hairy, reniform. Temporal setae including 4–5 preoculars, 7–12 verticals, 8–11 postorbitals. Clypeus with 4–8 setae. Antenna with 13 flagellomeres and partly reduced plume of setae (Fig. 55); maximal length of plume setae 410 μm; terminal flagellomere with 2 subapical setae, 28–40 μm long; AR 0.58–0.70. Palpomeres 1–5 length (μm): 48–56, 82–124, 135–164, 119–152, 172–200. Palpomere 3 in distal part with sensilla capitata with diameter 20–24 μm. Head width/palpal length 1.0–1.16.

Thorax. Antepronotum with 2–13 ventrolateral setae. Dorsocentrals 7–12, prealars 4–9, scutellars 16–31.

Wing. Length 3.2–3.9 mm, width 0.86–1.2 mm. Anal lobe rounded. Squama with 21–38 setae. R and R_1 with 10–27 setae, R_{4+5} with 3–4 setae in apical part. RM/MCu 2.7–3.2.

Legs. Spur of front tibia 60–72 μm long. Spurs of mid tibia 40–56 μm and 44–56 μm long. Spurs of hind tibia 64–96 μm and 44–52 μm long. Hind tibial comb with 17–19 setae. Length (μm) and proportions of leg segments are as in Table 12.

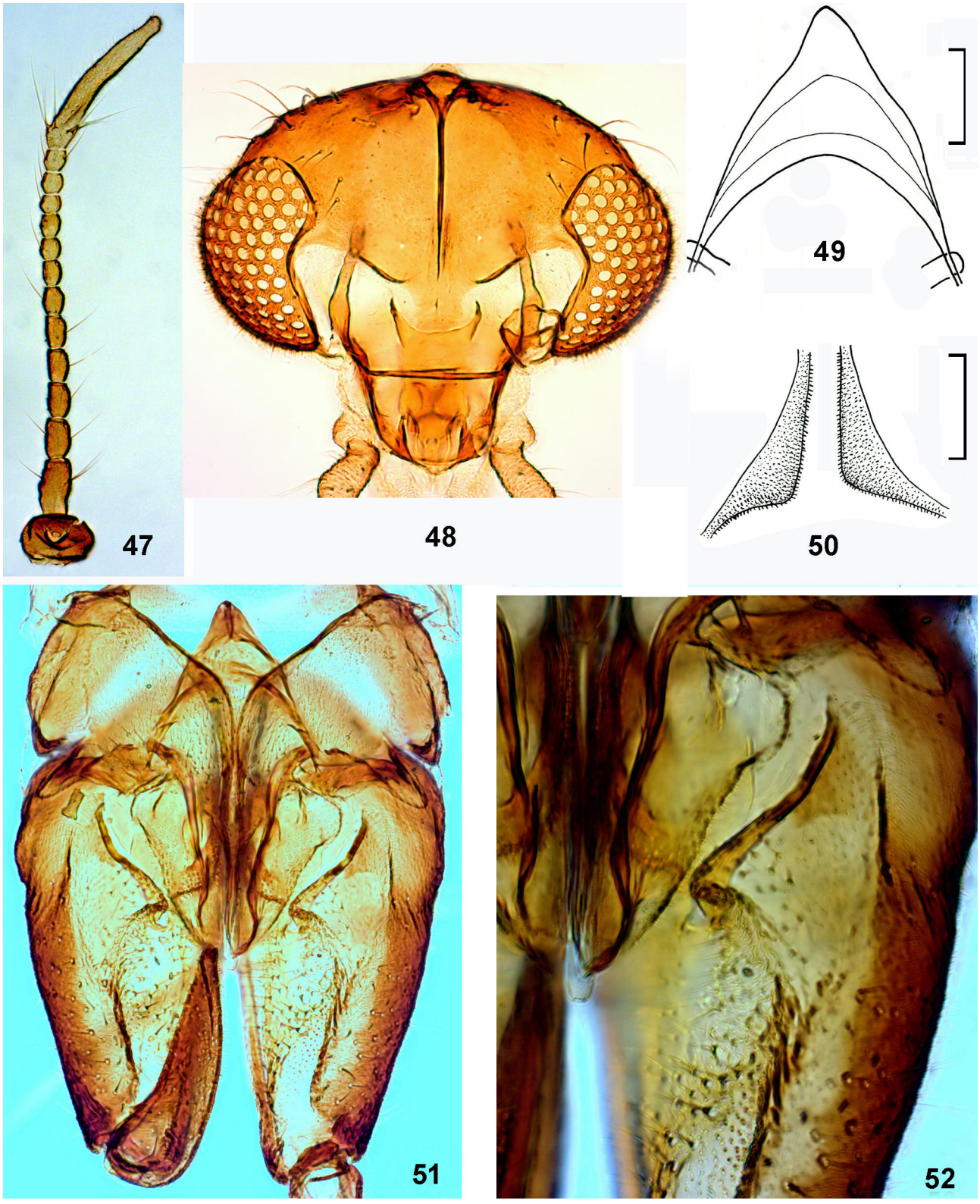
TABLE 12. Lengths (in μm) and proportions of leg segments of *Diamesa khumbugelida* Sæther et Willassen from Russia, Kazakhstan, China and Tajikistan, male (n=8)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1263–1880	1509–2320	951–1160	492–640	294–312	115–148	131–164
P_2	1443–2000	1345–1720	640–820	361–410	230–262	115–131	131–148
P_3	1574–2200	1607–2040	984–1320	558–689	279–410	148	131–164

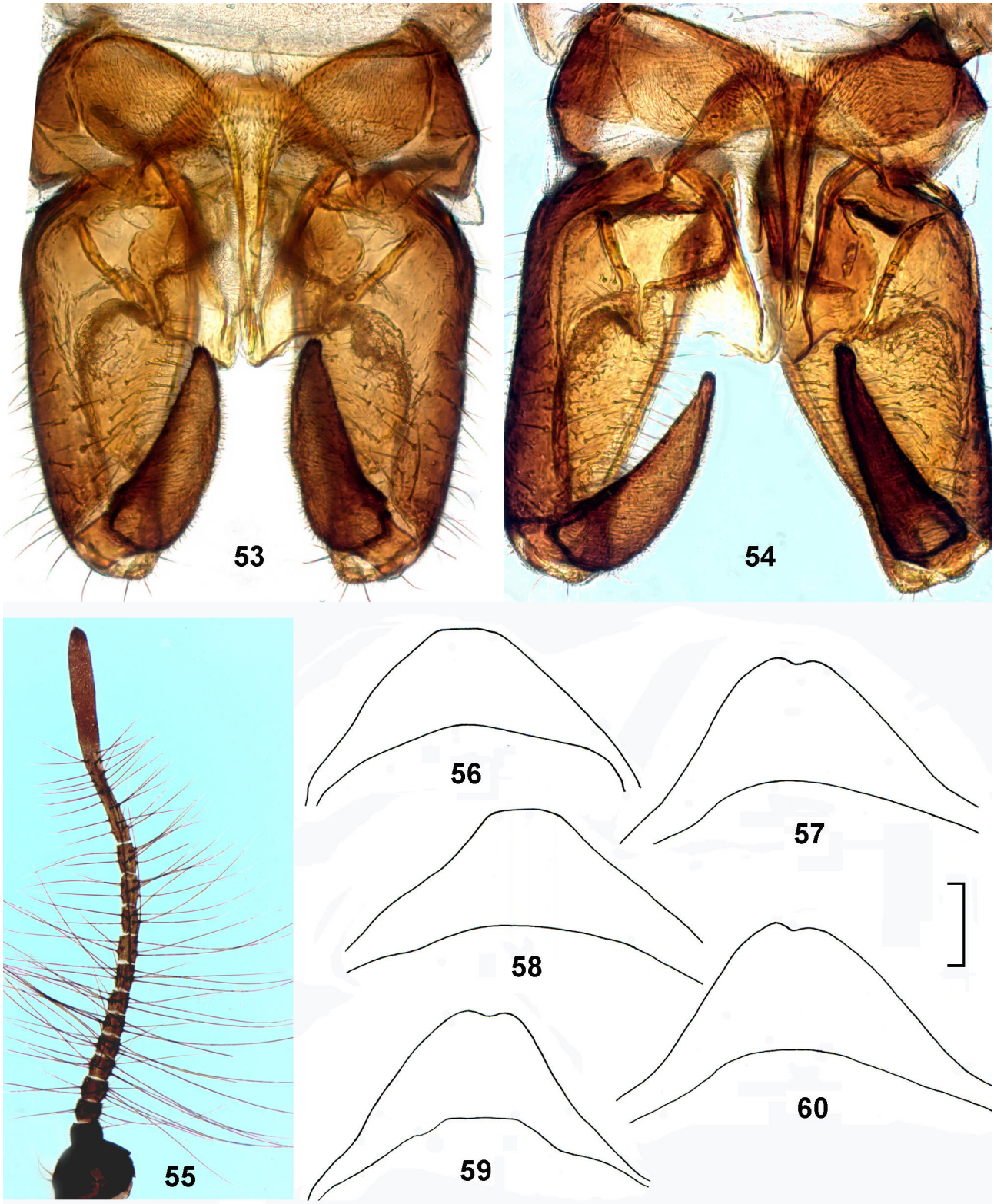
continued

	LR	BV	SV	BR
P_1	0.60–0.69	3.52–3.92	2.85–3.62	1.8–2.0
P_2	0.40–0.51	4.10–5.22	4.04–4.88	1.1–1.4
P_3	0.58–0.66	3.82–4.24	2.97–4.0	1.2–1.7

Hypopygium (Figs. 53–54, 56–60). Tergite IX with 6–15 weak setae, 24–28 μm long; laterosternite IX with 4–8 setae. Anal point 152–240 μm long; 36–48 μm wide at base;16–20 μm wide at apex; with strong anteriorly directed microtrichia in basal half and on tergite IX (Figs. 53–54). Phallapodeme 100–112 μm long. Transverse sternapodeme (TSA) wide trapezoidal (Figs. 56–60), 56–72 μm high, 196–240 μm wide; TSA height/TSA width 0.25–0.33. Gonocoxite 324–492 μm long; Inferior volsella in the form of angular naked projection on the dorsal side of the gonocoxite. Superior volsella in form of rounded angular tubercle. Gonostylus 212–287 μm long, slightly curved, tapering to apex; megaseta 10–12 μm long; HR 1.71–2.14.



FIGURES 47–52. Adult male of *Diamesa khumbugelida* Sæther et Willassen from Nepal. **47,** antenna; **48,** head, **49,** transverse sternapodeme; **50,** superior volsellae; **51,** hypopygium in dorsal view; **52,** inferior volsella of gonocoxite. Scale bars 50 µm.



FIGURES 53–60. Adult males of *Diamesa khumbugelida* Sæther et Willassen from Kazakhstan **(53, 56),** China **(54, 57–58),** Baikal Lake basin **(59)** and Tajikistan **(55, 60). 53–54,** hypopygium in dorsal view; **55,** antenna; **56–60,** transverse sternapodeme. Scale bars 50 µm.

Remarks. As noted above, the male from type locality in Nepalese Himalayas differs in some characters from males of other regions, namely Kazakhstan, Tajikistan, China and Russia. Male of *D. khumbugelida* from type locality has antenna with complete reduction of setae plume (Fig. 47), AR 0.44, clypeals 4–8, squama with 11

setae, scutellars 7, transverse sternapodeme narrow triangular (Fig. 49). Specimens from other areas have antenna with slightly reduced setae of plume (Fig. 55), AR 0.58–0.70, clypeals 4–8, squama with 21–38 setae, transverse sternapodeme wide trapezoidal (Figs. 56–60). To solve the taxonomic problem that has arisen, it is extremely necessary to carry out a molecular genetics study of this species from the Nepal and compare the obtained data with the same of populations from other regions. It is possible that we are dealing with two species.

Diamesa loeffleri Reiss
(Figs. 61–67)

Diamesa loeffleri Reiss, 1968: 56; Ashe & O'Connor 2009: 281; Lin *et al.* 2021: 110.
Diamesa culicoides Heeger; Tokunaga 1966: 275, Figs. 8–14.

Material examined. NEPAL: 1 adult male, Langtang Region, pond near Khyimjung Glacier, alt. 4175 m a.s.l., 4.VIII.1999, leg. R. Endo. INDIA: 1 adult male Uttarakhand, Chamoli District, Rishi Ganga River, right tributary of the Alaknanda River near the Badrinath City, alt. 3128 m a.s.l., 12.V.2018, 30.740733 N, 79.48875 E, leg. D. Palatov; 1 adult male, the same data except Alaknanda River below Badrinath City, 13.V.2018, 30.738128 N, 79.492719 E, leg. D. Palatov.

Description

Adult male (n=3, except when otherwise stated). Total length 3.2–3.8 mm. Total length/wing length 1.0–1.34.

Coloration. Dark brown. Wings grayish, with brownish venation.

Head. Eyes hairy, reniform. Temporal setae including 3–7 preoculars, 7–8 verticals. Clypeus with 6–8 setae. Antenna with 13 flagellomeres and partly reduced plume of setae (Figs. 61–62); maximal length of plume setae 459 µm. Length of 1–13 flagellomeres (n=1) (µm): 68, 36, 36, 40, 36, 36, 40,40, 44, 36, 48, 44, 316; terminal flagellomere with 2 subapical setae, 20–24 µm long; AR 0.63–0.72. Palpomeres 1–5 length (µm): 36–40, 68–88, 116–124, 92–104, 115–164. Palpomere 3 in distal part with sensilla capitata with diameter 16–20 µm. Head width/palpal length 1.04–1.13.

Thorax. Antepronotum with 2–8 ventrolateral setae. Dorsocentrals 5–7, prealars 5–6, scutellars 6–8.

Wing. Length 2.8–3.5 mm, width 0.72–1.04 mm. Anal lobe round angular. Squama with 24–28 setae. R and R_1 with 23–26 setae, R_{4+5} with 4–6 setae in distal part. RM/MCu 2.7–3.0.

Legs. Spur of front tibia 60–98 µm long. Spurs of mid tibia 36–40 µm long. Spurs of hind tibia 68–76 µm and 36–40 µm long. Hind tibial comb with 14–16 setae. Length (µm) and proportions of leg segments are as in Table 13.

TABLE 13. Lengths (in µm) and proportions of leg segments of *Diamesa loeffleri* Reiss, male (n=3)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1263–1410	1246–1607	836–1066	443–525	262–328	98	131
P_2	1246–1394	1099–1345	508–640	295–344	164–213	82–98	131
P_3	1410–1640	1443–1706	820–1033	443–508	280–295	98	131

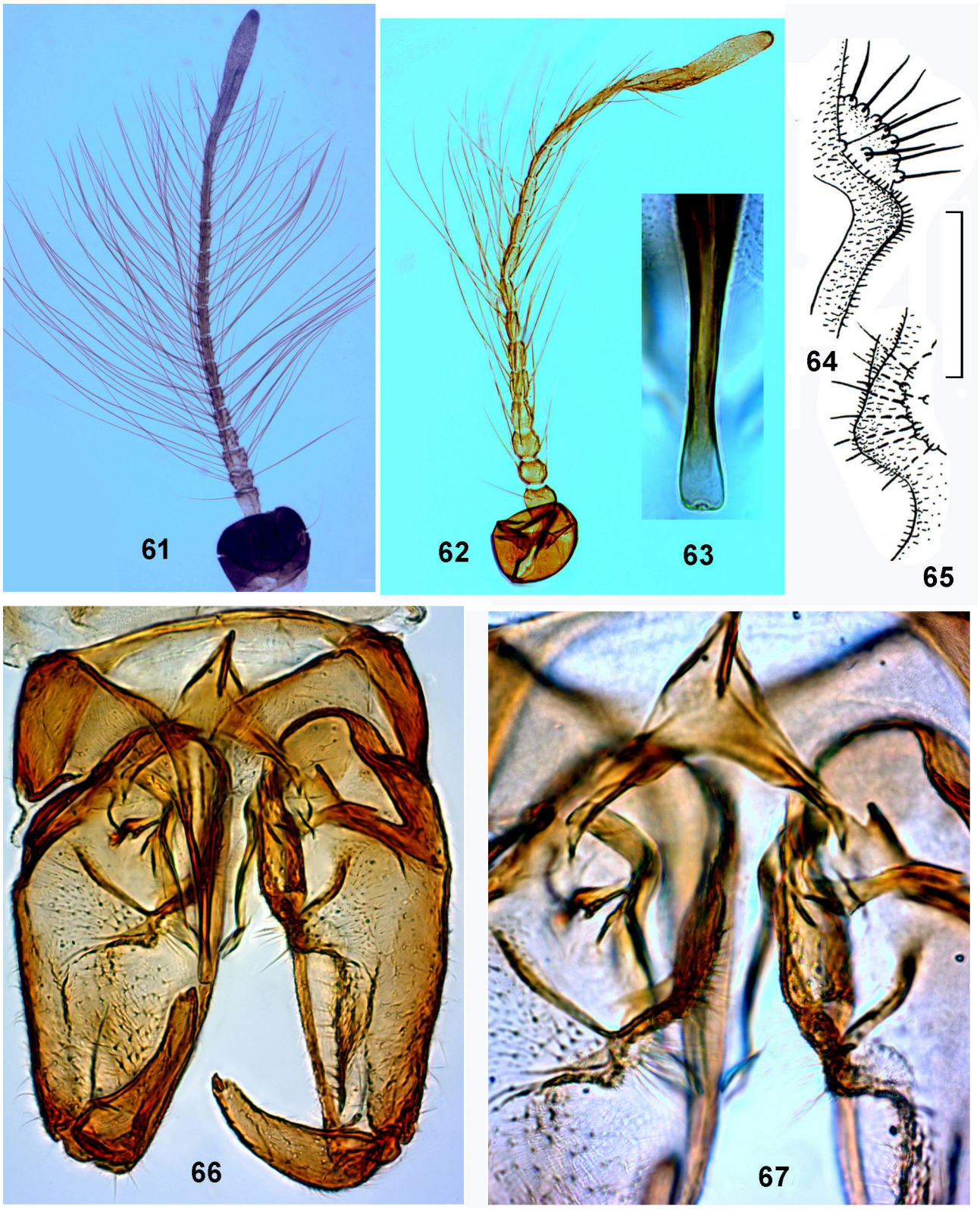
continued

	LR	BV	SV	BR
P_1	0.66–0.67	3.58–3.77	2.83–3.00	1.8–2.0
P_2	0.46–0.48	4.25–4.30	4.30–4.62	1.0–1.2
P_3	0.57–0.61	3.80–4.24	3.24–3.48	1.6–2.0

Hypopygium (Figs. 63–67). Tergite IX with 6–12 weak setae, laterosternite IX with 4–5 setae. Anal point 188–204 µm long, slightly tapering to subapical (Fig. 63), 20–40 µm wide at base;12–16 µm wide at apex; with strong anteriorly directed microtrichia in basal half and on tergite IX. Phallapodeme 92–112 µm long. Transverse sternapodeme (TSA) triangular, 68–92 µm high, 124–144 µm wide at the base; TSA height/TSA width 0.55–0.64. Gonocoxite 296–332 µm long; inferior volsella in the form of small lobe covered with micro- and macrotrichia and adjacent to a protuberance with some long setae (Figs. 64–65, 67). Superior volsella in form of small rounded

angular tubercle. Gonostylus 132–164 µm long, slightly curved, tapering to apex, 204 µm long; megaseta 10–12 µm long. HR 2.02–2.24.

Pupa and ***larva*** unknown.



FIGURES 61–67. Adult males of *Diamesa loeffleri* Reiss from Indian Himalayas **(61)** and Nepal Himalayas **(62–67). 61–62,** antenna; **63,** distal part of anal point; **64–65,** inferior volsellae; **66,** hypopygium in dorsal view; **67,** central part of hypopygium without tergite IX. Scale bar 50 µm.

Remarks. Lin *et al* (2021) redescribed of *D. loeffleri* from Tibet Plateau of China by male which has a common similar structure including the hypopygium but differs of some characters (Table 14). Thus, specimens from China are larger (4.13–4.51 mm) than Himalayan ones, total length/wing length 1.38–1.43, clypeals 30–32, AR 0.70–0.82, acrostichals on the mesonotum 9–11, dorsocentrals 14, anal point of hypopygium length 240–250 μm. For males from our Himalayas material the meanings of these characters are as follows. Total length 3.2–3.8 mm, total length/wing length 1.0–1.34, clypeals 6–8, AR 0.63–0.72, acrostichals on the mesonotum absent, dorsocentrals 5–7, anal point of hypopygium length 188–204 μm. Since the number of compared males of this species from the Himalayas and Tibet Plateau is small, one would assume that this is a geographical variability, but a comparison of the barcoding data showed that these are two different species (see below), that is, the population from China, apparently, should be attributed to a new species.

TABLE 14. Comparison of some features of *Diamesa loeffleri* Reiss males from Himalayas and Tibet Plateau

Character	Nepal Himalayas (Reiss 1968), n=2	Nepal and Indian Himalayas, n=3	Tibet Plateau, China, (Lin *et al.* 2021), n=3
Total length, mm	-	3.2–3.8	4.13–4.51
Wing length, mm	2.3–2.4	2.8–3.5	3.0–3.14
Total lebgth/wing length	-	1.0–1.34	1.38–1.43
Clypeals	*ca* 60	6–8	30–32
Number of setae on squama	23	24–28	21–26
AR	-	0.63–0.72	0.70–0.82
Acrostichals	0	0	9–11
Dorsocentrals	6–9	5–7	14
Prealars	5	5–6	5
LR_1	-	0.66–0.67	0.63–0.67
BV_1	-	3.58–3.77	3.77–4.0
SV_1	-	2.83–3.0	2.88–2.96
Anal point length, μm	-	188–204	240–250
Setae on tergite IX (from one side)	-	6–12	11–12
TSA height, μm	-	68–92	75–95
HR	-	2.02–2.24	2.15–2.28

Results of DNA barcoding

Overall, we have sequenced fragments of the cytochrome oxidase subunit I (658 bp in length) of 23 samples from five *Diamesa* species. To perform the complete data set we, we added COI sequences from GenBank and BOLD systems belonging to *D. loeffleri* (MZ127838), *D. khumbugelida* (ON392058), *D. japonica* (AB704934), *D. starmachi* (HQ941609), *D. amplexivirilia* (MT367335), 13 sequences of *D. serratosioi* and 5 sequences of *D. saetheri* (Fig. 68). The complete data set considered of 47 barcodes with 658 bp in length for each barcode. In total, there were 168 variable sites (25.5%), of which 143 (21,7%) were parsimony informative. Most of substitutions were synonymous transitions. The sequences were heavily AT-biased (65.4%), especially in the third position where 87.2% were A or T.

For the ABGD analysis we combine COI sequences of *Diamesa* from the GenBank, BOLD systems (length > 478 bp) and added the obtained data which gave 2618 sequences. The ABGD analysis, using 0.0264 maximum intraspecific divergence and recursive partition yielded 74 operational taxonomic units (OTUs). Of them 9 OTUs included samples from our data set that consist of 47 barcodes. The list of OTUs was the follows: *D. alpina*, *D. amplexivirilia, D. japonica, D. khumbugelida, D. leona* including *D. starmachi* HQ941609, *D. loeffleri* from India, *D. loeffleri* (MZ127838) from China, *D. saetheri* and *D. serratosioi* (Table 15). Notable that two *D. loeffleri* samples from India were formed different OTUs using 0.007-0.01 maximum intraspecific divergence and recursive partition while the rest 45 samples retained the formed OTUs. *D. starmachi* and *D. leona* formed one OTU using any values of intraspecific divergence in ABGD analysis.

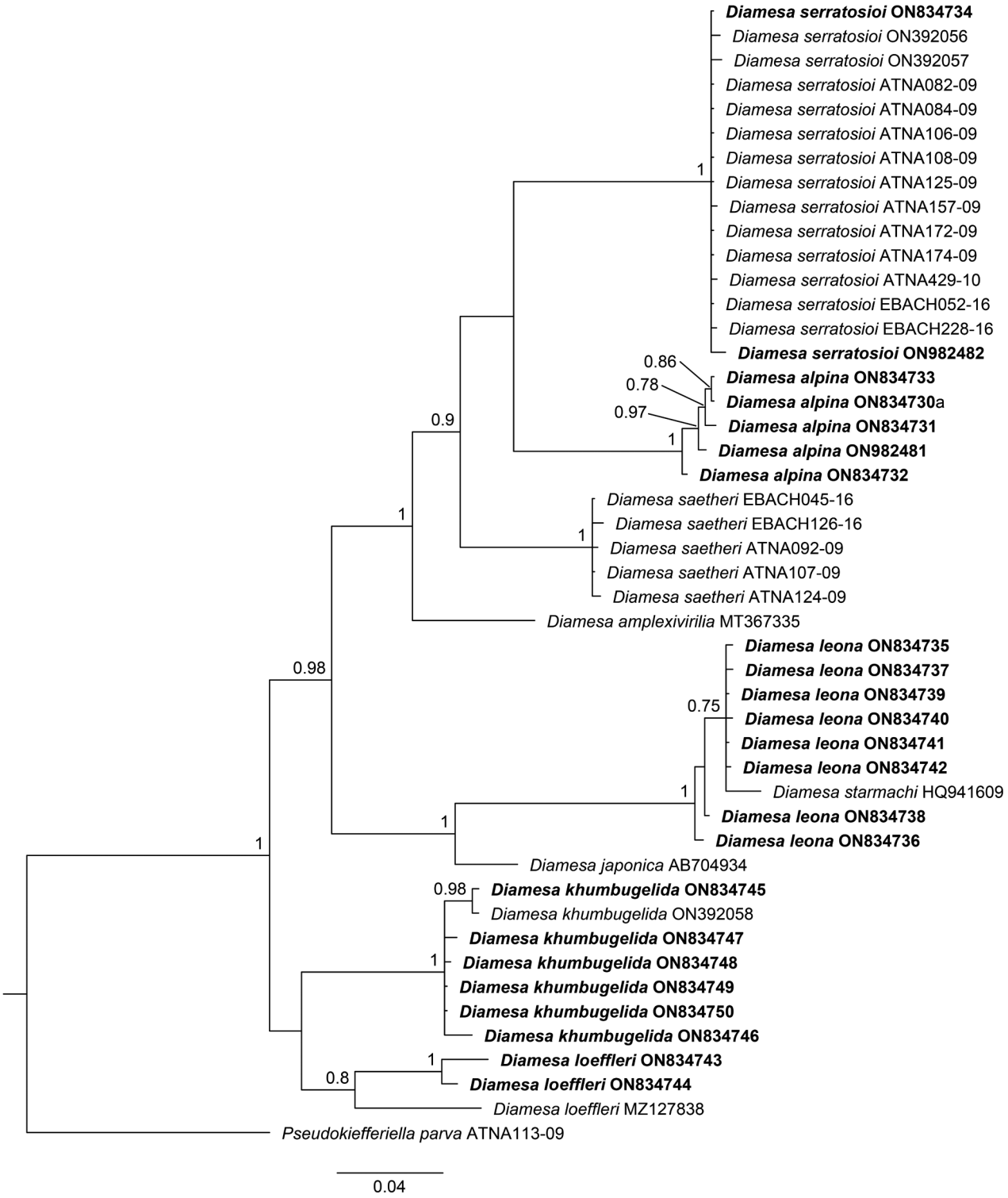


FIGURE 68. Bayesian inference (BI) trees based on the cytochrome c oxidase I (COI) nucleotide sequence data of the genus *Diamesa* Meigen and outgroup *Pseudokiefferiella parva* (Edwards) Bayesian posterior probabilities (higher than 0.7) are given above tree nodes. Specimens obtained in this study are in bold.

The K2P sequence divergence within and between nine *Diamesa* species shown in the Table 15. The grouping of samples into species is based on ABGD analysis with 0.0264 maximum intraspecific divergence and recursive partition. K2P distance between *D. loeffleri* from India and *D. loeffleri* (MZ127838) from China was 7.71% in average which corresponds to species level (Montagna *et al.* 2016). Type habitat of *D. loeffleri* in India therefore, we assume that *D. loeffleri* (MZ127838) from China belongs to a new species. Intraspecific sequence divergence

between two samples of *D. loeffleri* from India was 2.47% despite low distance between collection points (43.0956 N 131.5577 E and 30.740733 N 79.48875 E), which was 488 meters. Interspecific distances between the remaining species were also corresponds to species level (Montagna *et al.* 2016).

TABLE 15. 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	8
1. *D. alpina*	5	0.61								
2. *D. amplexivirilia*	1	-	9.39							
3. *D. japonica*	1	-	11.95	9.39						
4. *D. khumbugelida*	7	1.19	13.07	11.73	11.26					
5. *D. leona* (+*D. starmachi*)	9	0.79	14.12	13.21	8.38	11.66				
6. *D. loffleri*	2	2.47	12.49	10.55	10.11	9.83	12.29			
7. *D. loffleri* (MZ127838)	1	-	13.60	11.73	10.27	8.38	10.70	7.71		
8. *D. saetheri*	5	0.37	9.55	10.57	11.56	12.11	13.98	12.87	12.73	
9. *D. serratosioi*	15	0.24	10.77	11.31	11.93	12.54	14.73	12.39	13.97	10.80

We used COI to reconstruct phylogenetics relationships of *Diamesa* sequences from our data set (Fig. 68). The Bayesian inference phylogeny revealed two well-supported primary clades (Bayesian PP, BPP = 1), one including *D. loffleri* (form India and China) and *D. khumbugelida* and one including the remaining species. Species *D. japonica* was sister to *D. leona* (including *D. starmachi*) (BPP = 1.00). *D. amplexivirilla* was sister to *D. saetheri* + (*D. alpina* + *D. serratosioi*) with high support (PP = 1). Thus, the monophyly of subgroups *davisi* (*D. alpina, D. amplexivirilia, D. saetheri* and *D. serratosioi*) and *leona* (*D. japonica, D. leona*) was highly supported on the Bayesian tree (BPP = 1.00), while the support of subgroup *loeffleri* (*D. loeffleri, D. khumbugelida*) was low (BPP = 0.51).

Acknowledgements

The authors are very thankful to Dr. Endre Willassen (University of Bergen, Norway) who presented to E.A. Makarchenko many years ago microscope slides of some species of *Diamesa* from Bergen University Museum, namely paratypes of *Diamesa serratosioi* Willassen and *D. lupus* Willassen thanks to which we were able to more correctly identify and redescribe these species from the Far East and North America. The same gratitude goes to Dr. Patrick L. Hudson, Emeritus Scientist (Great Lakes Science Center, Michigan, U.S.A.) for the opportunity to study Diamesinae of the Chironomid Reference Collection of USGS Great Lakes Science Center and to Dr. K. Endo for slides of *D. khumbugelida* Sæther et Willassen and *D. loeffleri* Reiss from the type locality of these species in Nepal. We are also very grateful to all the many collectors of material that was used in the preparation of this article for making material available to us and to Dr. V.M. Loktionov (Federal Scientific Center of the East Asia Terrestrial Biodiversity, Vladivostok) for help with preparing of some microphotographs and Dr. W. Giłka (University of Gdańsk, Poland) for sending us some figures of *D. starmachi* which we used in article.

The research was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation (theme No. 121031000147-6). In addition, the work was supported by the Russian Scientific Fund (RSF), project No. 22-24-00162.

References

Ashe, P. & O'Connor, J.P. (2009) *A World Catalogue of Chironomidae (Diptera). Part 1. Buchonomyiinae, Chilenomyiinae, Podonominae, Aphroteniinae, Tanypodinae, Usambaromyiinae, Diamesinae, Prodiamesinae and Telmatogetoninae.* Irish Biogeographical Society & National Museum of Ireland, Dublin, 445 pp.

Edwards, F.W. (1933) Oxford University Expedition to Hudson's Strait, 1931: Diptera Nematocera. With notes on some other

species of the genus *Diamesa*. *Annals and Magazine of Natural History*, Series 10, 12, 611–620.
https://doi.org/10.1080/00222933308673727
Giłka, W., Soszyńska-Maj, A. & Paasivirta, L. (2013) The peculiar winter-active midge *Diamesa starmachi* (Diptera: Chironomidae). *Polish Journal of Entomology*, 82, 201–211.
https://doi.org/10.2478/v10200-012-0035-6
Goetghebuer, M. (1939) Tendipedidae (Chironomidae). Subfamilie Diamesinae. A. Die Imagines. *In*: Lindner, E. (Ed.), *Die Fliegen der Palaearktischen Region*, 3 (13d), pp. 1–28.
Hansen, D.C. & Cook, E.F. (1976) The systematics and morphology of the Nearctic species of *Diamesa* Meigen, 1835 (Diptera: Chironomidae). *Memoirs of the American Entomological Society*, 30, 1–203.
Herrmann, S.J., Sublette, J.E., Sublette, M. (1987) Midwinter emergence of *Diamesa leona* Roback in the Upper Arkansas River, Colorado, with notes on other diamesines (Diptera, Chironomidae). *Entomologica scandinavica*, Supplement 29, 309–322.
Kobayashi, T. & Endo, K. (2008) Synonymic notes on some species of Chironomidae (Diptera) described by Dr. M. Sasa (†). *Zootaxa*, 1712 (1), 49–64.
https://doi.org/10.11646/zootaxa.1712.1.3
Kownacki, A. & Kownacka, M. (1970) *Diamesa starmachii* sp.n. (Diptera, Chironomidae). *Bulletin de l'Académie Polonaise des Sciences*, Cl. II, Série des Sciences Biologiques, 18 (12), 777–780.
Krasheninnikov, A.B., Makarchenko, E.A., Semenchenko, A.A., Gavrilo, M.V. & Vshivkova, K.A. (2020) Morphological description and DNA barcoding of some Diamesinae (Diptera, Chironomidae) from the Severnaya Zemlya Archipelago and the Vaigach Island (Russian Arctic). *Zootaxa*, 4802 (3), 587–600.
https://doi.org/10.11646/zootaxa.4802.3.13
Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33 (7), 1870–1874.
https://doi.org/10.1093/molbev/msw054
Lencioni, V., Rodriguez-Prieto, A. & Allegrucci, G. (2021) Congruence between molecular and morphological systematics of Alpine non-biting midges (Chironomidae, Diamesinae). *Zoologica Scripta*, 50 (4), 455–472.
https://doi.org/10.1111/zsc.12480
Lin, X.L., Chang, T., Yan, C.C., Wang, B. & Liu, W.B. (2021) Redescription of *Diamesa loeffleri* Reiss, 1968 (Diptera, Chironomidae) and new record from China. *Annales Zoologici Fennici*, 58, 109–113.
Linevich, A.A. & Makarchenko, E.A. (1989) New or little known species of subfamily Diamesinae (Diptera, Chironomidae) from Baikal Territory. *In*: *Systematics and ecology of river organisms.* DVNC AN SSSR, Vladivostok, pp. 20–37. [in Russian]
Makarchenko, E.A. (1977) New species of Diamesa (Diptera, Chironomidae) from the South Primorye. *Zoologicheskyi Zhurnal*, 56, 1732–1734. [in Russian]
Makarchenko, E.A. (1980) New or little known species of chironomid of subfamily Diamesinae (Diptera, Chironomidae) from the Soviet Far East. *In*: *Freshwater fauna of the Far East.* DVNC AN SSSR, Vladivostok, pp. 80–94. [in Russian]
Makarchenko, E.A. (1981) Taxonomy and distribution of some chironomids of subfamily Diamesinae (Diptera, Chironomidae) from the Soviet Far East. *In*: *Invertebrate animals in ecosystems of salmon rivers of the Far East.* DVNC AN SSSR Press, Vladivostok, pp. 89–113. [in Russian]
Makarchenko, E.A. (1985) *Chironomids of the Soviet Far East. Subfamilies Podonominae, Diamesinae and Prodiamesinae (Diptera, Chironomidae).* DVNC AN SSSR Press, Vladivostok, 208 pp. [in Russian]
Makarchenko, E.A. (1989) A new and a little known species of the genus *Diamesa* (Diptera, Chironomidae) from Tadjikistan. *Vestnik Zoologii*, 2, 82–85. [in Russian]
Makarchenko, E.A. (2006) Subfamily Diamesinae. *In*: *Key to the Insects of Russian Far East. Vol. 6. Diptera and Siphonaptera. Pt. 4.* Dal'nauka, Vladivostok, pp. 253–276 + 468–480 + 607–621. [in Russian]
Makarchenko, E.A. (2009) A new finding of *Diamesa khumbugelida* Sæther et Willassen (Diptera, Chironomidae, Diamesinae) from Khamar-Daban Range (Baikal Nature Reserve). *Euroasian Entomological Journal*, 8 (4), 429–430.
Makarchenko, E.A. (2021) Confirmation of a species status of *Diamesa leoniella* Hansen, 1976, stat. resurr. (Diptera, Chironomidae) from North America. *Zootaxa*, 4985 (3), 439–442.
https://doi.org/10.11646/zootaxa.4985.3.11
Makarchenko, E.A. & Yamamoto, M. (1995) Chironomids of the Diamesinae (Diptera, Chironomidae) from Japan. V. New and little-known species of *Diamesa* Meigen. *Japanese Journal of Entomology*, 63 (2), 297–301.
Makarchenko, E.A., Semenchenko, A.A. & Palatov, D.M. (2018) New data on taxonomy and systematics of the genus *Diamesa* Meigen (Diptera: Chironomidae: Diamesinae) from Tien Shan and Pamir Mountains, with description of two new species. *Journal of Limnology*, 77 (S1), 50–58.
https://doi.org/10.4081/jlimnol.2018.1783
Makarchenko, E.A., Semenchenko, A.A. & Palatov, D.M. (2022) Taxonomy of *Diamesa steinboecki* group (Diptera: Chironomidae: Diamesinae), with description and DNA barcoding of new species. I. Subgroups *steinboecki* and *longipes*. *Zootaxa*, 5125 (5), 483–512.
https://doi.org/10.11646/zootaxa.5125.5.2
Makarchenko, E.A., Semenchenko, A.A. & Dragan, S.V. (2022) Review on the fauna and taxonomy of Diamesinae subfamily

(Diptera, Chironomidae) from the Republic of Khakassia (Russia), with morphological description and DNA barcoding of the discovered species. *Euroasian Entomological Journal*, 21, Supplement 1, 73–89.
https://doi.org/10.15298/euroasentj.21.Spl.1.08

Montagna, M., Mereghetti, V., Lencioni, V. & Rossaro, B. (2016) Integrated Taxonomy and DNA Barcoding of Alpine Midges (Diptera: Chironomidae). *PLoS ONE*, 11 (3), e0149673.
https://doi.org/10.1371/journal.pone.0149673

Namayandeh, A. (2022) Selected Flies (Chironomidae). *In*: Working Group on General Status of NWT Species. 2021. *NWT Species 2021–2025—General Status Ranks of Wild Species in the Northwest Territories*. Department of Environment and Natural Resources, Government of the Northwest Territories, Yellowknife, Northwest Territories, pp. 166–167 + 174–179.

Pagast, F. (1947) Systematik und Verbreitung der um die Gattung *Diamesa* gruppierten Chironomiden. *Archiv für Hydrobiologie*, 61, 435–596.

Reiss, F. (1968) Neue Chironomiden Arten (Diptera) aus Nepal. *Khumbu Himalaya*, 3 (1), 55–73.

Roback, S.S. (1957) Some Tendipedidae from Utah. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 109, 1–24.

Rossaro, B. & Lencioni, V. (2015) A key to larvae of species belonging to the genus *Diamesa* from Alps and Apennines (Italy). *European Journal of Environmental Sciences*, 5 (1), 62–79.
https://doi.org/10.14712/23361964.2015.79

Sæther, O.A. (1968) Chironomids of the Finse area, Norway, with special reference to their distribution in a glacier brook. *Archiv für Hydrobiologie*, 64, 426–483.

Sæther, O.A. (1980) Glossary of chironomid morphology terminology (Diptera, Chironomidae). *Entomologica scandinavica*, Supplement 14, 1–51.

Sæther, O.A. & Willassen, E. (1987) Four new species of *Diamesa* Meigen, 1835 (Diptera: Chironomidae) from the glaciers of Nepal. *Entomologica scandinavica*, Supplement 29, 189–203.

Sasa, M. & Okazawa, T. (1992) Studies on the chironomid midges (Yusurika) of Kurobe River, *Research Report from Toyama Prefectural Environmental Pollution Research Center*, 1992, 40–91.

Serra-Tosio, B. (1971) s.n. *In*: *Contribution à l'étude taxonomique, phylogénétique, biogéographique et écologique des Diamesini (Diptera, Chironomidae) d'Europe*. *Vols. I & II.* Doctor Thesis, A l'Université Scientifique et Médicale de Grenoble. pp. 2A-2E + 1–303 & pp. 304–462 + [1], pls. 1–184.

Serra-Tosio, B. (1974) La mouche des glaciers *Diamesa steinboecki* Goetgh., insecte de montagne à ailes réduites (Diptera, Chironomidae). *Travaux scientifiques du Parc National de la Vanoise*, 5, 165–189.

Serra-Tosio, B. (1983) Nouveaux Diamesinae de la Paléarctide méridionale et orientale (Diptera, Chironomidae). *Spixiana*, 6 (1), 1–26.

Spies M., Sæther, O.A. (2004) Notes and recommendations on taxonomy and nomenclature of Chironomidae (Diptera). *Zootaxa*, 752 (1), 1–90.
https://doi.org/10.11646/zootaxa.752.1.1

Tokunaga, M. (1936) Chironomidae from Japan (Diptera). VI. Diamesinae. *Philippine Journal of Science*, 59, 525–552.

Tokunaga, M. (1964) A snow midge from Japan. *Akitu*, 11, 39–40.
https://doi.org/10.1093/nq/11-1-39

Tokunaga, M. (1966) Some nematocerous Diptera of the North-East of Afghanistan. *Results of the Kyoto University Scientific Expedition to the Karakoram and Hindukush*, 1955, 273–286.

Willassen, E. (1986) A review of *Diamesa davisi* Edwards and the *davisi* group. *Spixiana*, Supplement 11, 109–137.