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A review of the taxonomy, distribution and population genetic analysis of the *Diamesa cinerella* group (Diptera: Chironomidae: Diamesinae), with a description of *D. soktoshensis* sp. nov. and DNA barcoding of known species

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Abstract

We provide original identification keys for adult males, brief redescriptions of the seven known species, a description of a new species, *D. soktoshensis* **sp. nov.,** and a molecular genetic analysis of species of the *Diamesa cinerella* group.

Population genetic analysis of *Diamesa cinerella* group revealed 109 haplotypes for 183 samples and a high level of haplotype diversity—0.984 ± 0.004. We identified four haplogroups, including *Diamesa tsutsuii* (1), *Diamesa lavillei* (2), *Diamesa. soktoshensis* **sp. nov.** (3) and the remaining species (4). The four haplogroups were significantly different from each other by FST values ($P < 0.01$). We explored the use of the internal transcribed spacers (ITS) region as an alternative nuclear DNA barcode for species delimitation of the *Diamesa cinerella* group. Three haplogroups were identified with no obvious connection to the species or sampling location.

Key words: Diptera, Chironomidae, Diamesinae, *Diamesa cinerella* group, taxonomy, population genetics, mitochondrial DNA, ITS

Introduction

As a result of our ongoing revision of the genus *Diamesa* Meigen, we examined the eight species in the *Diamesa cinerella* group: *D. cinerella* Meigen, *Diamesa hamaticornis* Kieffer, *Diamesa hyperborea* Holmgren, *Diamesa kasymovi* Kownacki *et* Kownacka, *D. lavillei* Serra-Tosio, *D. soktoshensis* **sp. nov.**, *Diamesa tonsa* Haliday and *D. tsutsuii* Tokunaga. We provide an original key of species for identifying adult males, a brief redescriptions of known species, a description of a new species, and a genetic analysis of the group.

Previous studies at the molecular level revealed that *D. cinerella, D. tonsa*, and *D. hamaticornis* have extremely low COI interspecific distances which leads to the absence of monophyly of each species and the inability to resolve boundaries using species delimitation approaches (e.g. ABGD, ASAP, bPTP, GMYC) (Montagna *et al*. 2016, Lencioni *et al.* 2021) although these species have clear morphological differences (see below). Later, molecularly indistinguishable species *D. kasymovi* and *D. hyperborea* (from the GenBank and BOLD Systems), as well as a closely related but monophyletic by Bayesian Inference *D. lavillei* were added to the *D. cinerella* group (Makarchenko *et al.* 2023). The most sensitive methods for species delimitation, mPTP and GMYC, placed *D. lavillei* to a separate molecular operational taxonomic unit (mOTU), while BIN BOLD and ASAP united all species of *D. cinerella* group into a single mOTU. Different combinations of nuclear (18S, 28S, CADI and CADIV) and mitochondrial (COI3p, COII and 16S) loci does not change the results obtained using only DNA barcodes (Lencioni *et al*. 2021, Lencioni *et al*. 2024, Semenchenko *et al*. 2024a). The East Palaearctic *D. tsutsuii* was found to be closely related to the *D. cinerella* group species by multi-locus phylogeny, but detailed DNA barcoding of this species was not provided (Semenchenko *et al.* 2024a). The possible reasons for the high similarity of species within groups *D.*

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cinerella apparently is recent divergence from common ancestor which occurred in the late Miocene according to Semenchenko *et al.* 2024a. Other causes may include mitochondrial introgression (Yannic *et al*. 2010, Montagna *et al*. 2016) or incomplete lineage sorting (Montagna *et al*. 2016).

Possible solutions with molecular indistinguishable species are the usage of the highly variable nuclear loci and/or the application of population genetic approaches, analysis of species structures, construction of haplotype networks, and identification of haplogroups with statistical analysis. The use of haplotype networks (Lencioni *et al*. 2021) by COI sequences also did not yield positive results, although limited to three species *D. cinerella, D. tonsa*, and *D. hamaticornis* from Europe, Tien Shan, and Pamir (Kyrgyzstan). In this study, we used all available sequences of the *D. cinerella* group determined to species level from GenBank and BOLD systems. We also deposited many new sequences in GenBank, many of which are from localities far from Europe and include the poorly represented *D. tsutsuii* from the Russian Far East (see Supplementary Table S1).

We attempted to delimit species of *D. cinerella* group using internal transcribed spacers (including complete ITS1, 5.8S ribosomal RNA, ITS2 and partial 28S ribosomal RNA, hereafter ITS), used as an alternative species-delimiting locus and population variability of Culicomorpha (Bower *et al*. 2008, LaRue *et al*. 2009, Paredes-Esquivel *et al*. 2009, Dvorak *et al.* 2024 etc.). DNA barcoding of *Diamesa* larvae from Alpine streams of Austria, including the *D. cinerella* group using concatenated COI and ITS sequences showed hope for success, revealed a resolved tree, but with low nodal support (Dvorak *et al.* 2024). However, the authors did not identify the specimens used in this analysis to the species level and only identified larvae as *D. cinerella*/*tonsa* or *Diamesa* sp.

Materials and methods

Chironomid adults were collected in Russia: Perm Territory (2012), Magadan Region (2014, 2019), Sakhalin Region (2014), Khabarovsk Territory (2019), Dagestan (2021), Khakassia (2022), Karachay-Cherkess (2023), Kabardino-Balkarian (2023). Outside of Russia, samples were collected in Iran (2019, 2021), Mongolia (2019) and France (2020–2021). For detailed information on species, locations and geographic coordinates, see the Supplementary Table S1.

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 polyvinyl lactophenol following the recommendations of Moubayed & Langton (2019). The morphological terminology and abbreviations used below generally follow Sæther (1980) but for some structures of the hypopygium, the terminology of Hansen & Cook (1976) is used.

The photographs were taken using an Axio Lab.A1 (Carl 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.

Holotype and paratypes of the new species, as well as all 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 was extracted from the thorax of adult Chironomidae using a Blood and Tissue Kit (Qiagen, Hilden, Germany) or Invitrogen PureLink Genomic DNA Mini Kit (Invitrogen corp, Carlsbad, CA, USA) and the resultant DNA was eluted in 80–100 µl. A fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994) while ITS locus was amplified using Chir_ITS_Fw (5'-GAAGTAAAAGTCGTAACAAGGT-3') and Chir_28SD1_In_Rw (5'-CAAGCAACATGACTCCTA-3') of our own production. Amplification products were purified by exonuclease I (ExoI) and alkaline phosphatase (FastAP) (Thermo Fisher Scientific Inc., USA) and bidirectionally sequenced by ABI 3130xl or 3500 sequencers (Applied Biosystems) using reagents BigDye terminator v3.1 cycle kit. More details about PCR regime and sequence can be found in Makarchenko *et al.* (2022b, 2022c, 2023). PCR regime of ITS locus corresponds to COI except for the annealing temperature of 55°C and the elongation duration of 90 seconds.

In addition to our own data, we used all available COI DNA barcodes of *Diamesa cinerella* group from GenBank and BOLD systems satisfying the following criteria: the samples are identified to the species level, geographic coordinates are provided, and the sequence length is more than 530 bp. Median joining network with epsilon = 0

(Bandelt *et al.* 1999) and geographical distributions of species were created in the PopART 1.7 program (Leigh & Bryant 2015) with subsequent revision in Adobe Illustrator 25.2.3. In addition, ITS sequences of the *D. cinerella* group from Dvorak *et al*. (2024) were incorporated into our dataset to reconstruct a median-joining network. The obtained sequences have been deposited in GenBank under numbers PV693831–PV693860 for COI (see details in Supplementary Table S1) and PV706923–PV706932 for ITS.

Taxonomy

Diagnosis of *Diamesa cinerella* group

The adult males of this group are characterized by the following features: Antenna with eight or thirteen flagellomeres and reduced setae of plume from very short and very sparse to long but with a reduced number; AR 0.35–0.70; eyes hairy, reniform; preocular setae few; wing with rounded-angular anal lobe; hypopygium usually with a curved gonostylus which in its basal half is expanded, distal portion rather narrower and slender, apex with short megaseta and tooth; transverse sternapodeme usually rectangular or trapezoidal; anal point is short (*ca* 80 µm) to medium (124–192 µm) in length, narrow, with a small preapical dorsal keel near the apex; inferior volsella of gonocoxite in the form of a long blade, often tapering towards the top and covered with numerous short setae or with two branches, from which the longer branch bears a tuft of longer setae at the tip. Other characteristics of this group's species are given in Table 1.

Along with description of *D. soktoshensis* **sp. nov.**, we found it appropriate to provide brief redescriptions of adult males for the known species of the *D. cinerella* group based on our material. For *D. tsutsuii*, only a brief diagnosis is given, since a separate article has recently been devoted to this species (Makarchenko 2023).

Key to species of *Diamesa cinerella* group for adult males

1.	Antenna with 8 flagellomeres.	2
-	Antenna with 13 flagellomeres.	3
2.	Basimedial setae of gonocoxite long and numerous	*D. kasymovi* Kownacki *et* Kownacka
-	Gonocoxite without basimedial setae.	*D. tsutsuii* Tokunaga
3.	Antenna plume's setae104–108 µm long and sparse (2–4 setae on flagellomere) (Figs 3–4)	4
-	Antennal plume's setae, 200–689 µm long, little reduced (3–6 setae on flagellomere) (Figs. 1–2, 5)	5
4.	Gonocoxite with 20–26 basimedial setae, 80–100 µm long (Fig. 22). Gonostylus wider basally and gradually narrowing towards apex (Figs 19–20)	*D. lavillei* Serra-Tosio
-	Gonocoxite with 4–7 basimedial setae, 40–44 µm long (Fig. 25). Gonostylus with inflated basal part and narrowing distal (Fig. 26).	*D. hyperborea* Holmgren
5.	Inferior volsella with two branches, the longer branch bearing a tuft of longer setae at the tip (Figs 31–32).	*D. tonsa* Haliday
-	Inferior volsella simple.	6
6.	Inferior volsella in the form of a wide lobe covered with short setae (Figs 15–17). Gonocoxite with 6–8 basimedial setae, 68–100 µm long (Fig. 18).	*D. cinerella* Meigen
-	Inferior volsella not shaped as above. Gonocoxite with more than 13 basimedial setae	7
7.	Total length 3.6–4.2. AR 0.62–0.67. Gonocoxite with 13–17 basimedial setae (Fig. 24)	*D. hamaticornis* Kieffer
-	Total length 3.0–3.5. AR 0.39–0.40. Gonocoxite with 26–30 basimedial setae (Fig. 28)	*D. soktoshensis* **sp. nov**.

Diamesa cinerella **Meigen**

(Figs. 5, 8, 15–18)

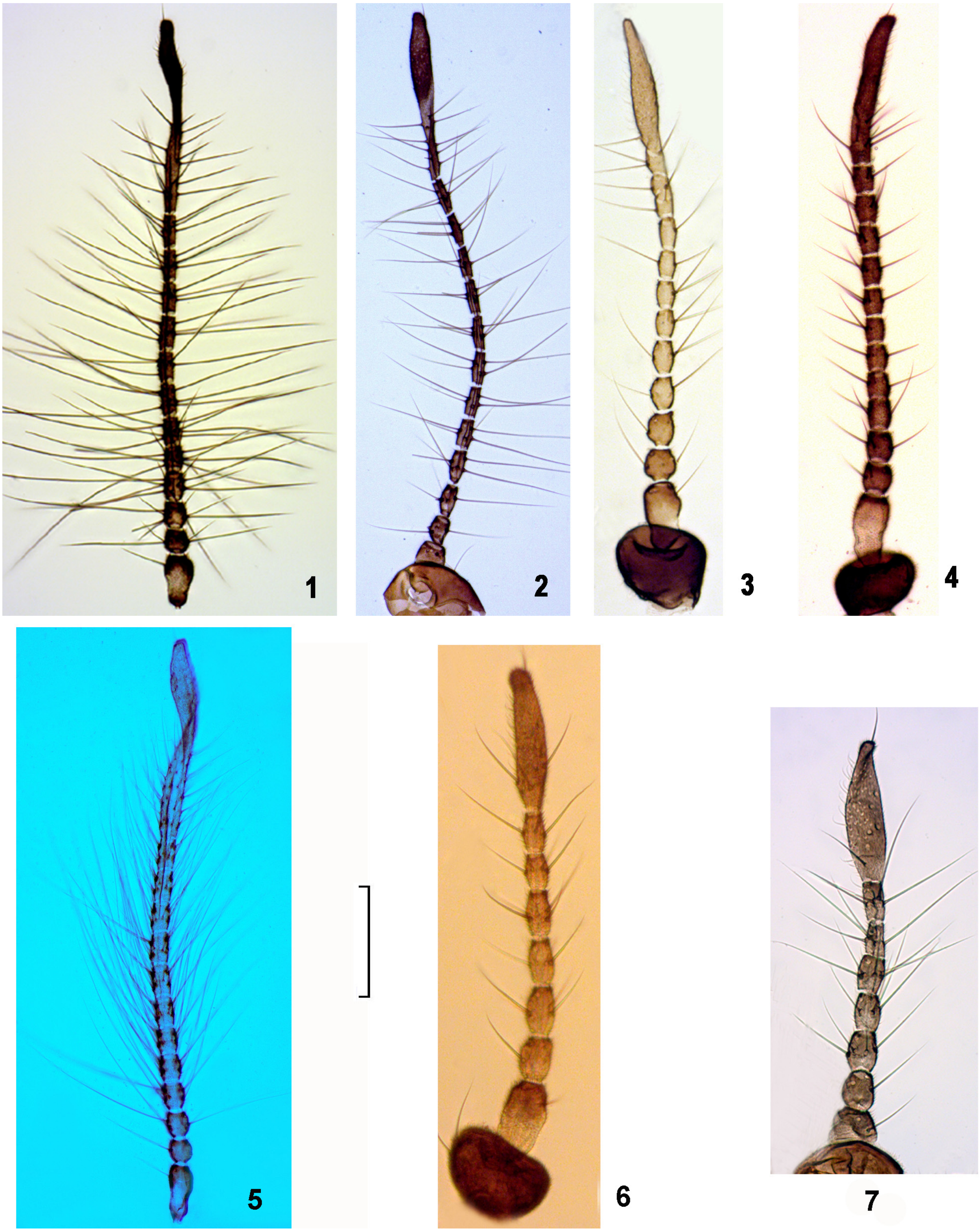
Diamesa cinerella Meigen in Gistl, 1835: 66; Pagast 1947: 481; Serra-Tosio 1971; Willassen & Serra-Tosio 1988: 92; Langton & Visser 2003: 18; Langton & Pinder 2007: 57; Ashe & O'Connor 2009: 273; Rossaro & Lencioni 2015: 73; Montagna *et al.* 2016: 326.

Diamesa waltlii Meigen, 1838: 13.

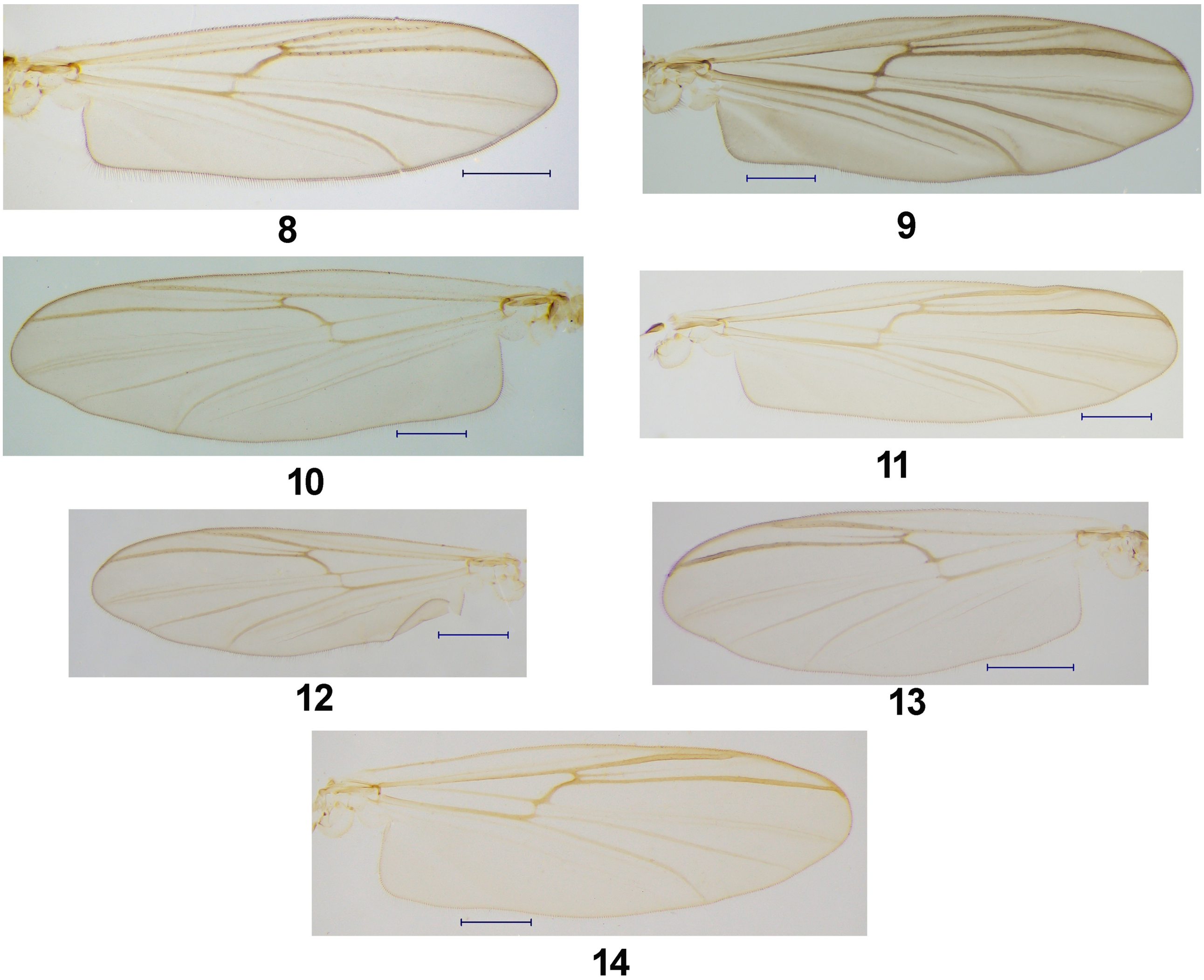
TABLE 1. Comparison of some morphological characters of *Diamesa cinerella* group (males) (our data).

Characters	*D. kasymovi* (n = 4)	*D. tsutsuii* (n = 7)	*D. lavillei* (n = 4)	*D. hyperborea* (n = 1)	*D.hamaticornis* (n = 3)	*D. tonsa* (n = 4)	*D. soktoshensis* (n = 2)	*D. cinerella* (n = 6)
Total length, mm	3.1–3.3	4.3–6.2	3.8–4,0	4.2	3.7–3.9	3.4–4.2	3.0–3.5	3.3–4.6
TL/WL	1.1	1.33–1.35	0.98–1.04	1.1	1.02–1.22	1.10–1.26	0.93–1.15	0.93–1.15
Number of antennal flagellomeres	8	8	13	13	13	13	13	13
AR	0.37–0.47	0.40–0.50	0.33–0.39	0.36	0.50–0.67	0.56–0.69	0.37–0.40	0.49–0.70
Antennal plume	Reduced, 1–3 setae on flagellomere, maximal length 120 µm	Reduced, 2–4 setae on flagellomere, maximal length 82 µm	Reduced, 2–3 setae on flagellomere, maximal length 104 µm	Reduced, 1–4 setae on flagellomere, maximal length 108 µm	Little reduced, 4–6 setae on flagellomere, maximal length of setae 426 µm	Little reduced, 4–6 setae on flagellomere, maximal length of setae 689µm	Little reduced, 3–5 setae on flagellomere, maximal length of setae 200 µm	Little reduced, 4–6 setae on flagellomere, maximal length of setae 410 µm
Wing length, mm	2.8–3.0	3.2–4.0	3.6–4.0	3.76	3.3–3.64	3.1–3.6	3.0–3.2	3.7–4.0
Dc	9	10–13	14–20	17	9–15	10–12	9–11	13–15
Pa	5–7	6–10	6–11	12	6–10	5–8	5–7	8–11
Sc	20–26	20–42	36–50	20	15–38		*ca* 30	31–32
LR_1	0.62–0.67	0.54–0.62	0.65	0.64	0.58–0.59	0.62–0.68	0.64–0.67	0.64–0.67
BV_1	4.9–4.17	3.48–4.04	3.62–3.63	3.76	3.51–3.86	3.67–3.97	3.69–3.70	3.82–3.88
SV_1	2.82–2.97	3.00–3.48	2.97–3.03	3.03	3.13–3.25	2/67–2.93	2.80–2.92	2.80–2.83
Number of Tergite IX setae	20–22	12–24	22–23	18	11–15	17–21	16–19	14–15
Anal point, µm	124–148	124–164	160	192	176–180	120–152	80–88	184
Basimedial setae of gonocoxite, number/ length, µm	22–25/160–192	absent	20–26/108–176	4–5/40–44	12–17/112–196	4–5/64–68	26–28/188–200	6–8/68–100
IVo length, µm	140	*ca* 260	252	316	128–140	168–172	200	188
TSA length/TSA wide, µm	140–160/28–36	204–280/ 44–52	200/20	184/52	180–240/52–60	120–140/40–52	124/52	164/72
HR	1.26–1.41	1.40–1.43	1.16	1.27	1.13	1.40–1.56	1.53	1.43

Material examined. 4 adult males, FRANCE: Machais Nature Reserve, Vosges, Valche glacial stream, collected by Malaise trap, 19.V.2021, leg. J. Claude and A. Badre. 2 adult males SWITZERLAND: Grisons, Macun, Lake Immez, Rive droite, 30.7–15.VIII.2014, leg., B. Lods-Crozet; 1 adult male, the same place except, Valais, Muttbach, 25.VIII.1998, leg. B. Lods-Crozet; 1 adult male, the same place except, Grisons, Macun, Lake Grond, 30.VII–15.VIII.2014, leg. S. Knispel.



FIGURES 1–7. Adult male antennae of *Diamesa hamaticornis* Kieffer **(1)**, *D. soktoshensis* **sp. nov. (2)**, *D. hyperborea* Holmgren **(3)**, *D. lavillei* Serra-Tosio **(4)**, *D. cinerella* Meigen **(5)**, *D. tsutsuii* Tokunaga **(6)** and *D. kasymovi* Kownacki *et* Kownacka **(7)**. Scale bar—50 µm.



FIGURES 8–14. Wings of *Diamesa cinerella* Meigen **(8)**, *D. lavillei* Serra-Tosio **(9)**, *D. hamaticornis* Kieffer **(10)**, *D. hyperborea* Holmgren **(11)**, *D. soktoshensis* **sp. nov. (12)**, *D. kasymovi* Kownacki *et* Kownacka **(13)** and *D. tsutsuii* Tokunaga **(14).** Scale bars—500 µm;

Description

Adult male (n = 6, except when otherwise stated). Total length 3.3–4.6, mm. Total length/wing length 0.93–1.15.

Coloration. Dark brown to brown. Legs brown. Wings grayish to gray, venation brownish.

Head. Eyes hairy, reniform. Temporal setae including 5–9 preoculars, 9–16 verticals. Clypeus with 6–14 setae. Antenna with 13 flagellomeres and little reduced plume of setae (Fig. 5), flagellomere with 4–6 setae, maximal length of which 410 µm; terminal flagellomere with 1 subapical setae, 36–48 µm long; scopus with 2 setae 48–52 µm long. Length of flagellomeres 1–13 (in µm) (n = 1): 100, 44, 48, 48, 48, 48, 52, 56, 56, 52,56, 53, 332; AR 0.49–0.70. Palpomere length (in µm): 44–56, 80–116, 104–156, 120–164, 180–232. Palpomere 3 in distal part with a sensilla capitata with diameter 16–20 µm. Head width/palpal length 1.04–1.29. Antennal length/palpal length 1.37–1.88.

Thorax. Antepronotum with 5–9 ventrolateral setae. Dorsocentrals 13–15, prealars 8–11. Scutellum with 31–32 setae.

Wing (Fig. 8). Length 3.7–4.0 mm, width 1.2–1.3 mm. Anal lobe rounded-angular. Squama with 37–39 setae, 112–140 µm long. $R + R_1$ with 37–39 setae, R_{4+5} with 19–22 setae. RM/MCu 3.0–3.2. Costa extension 80 µm long.

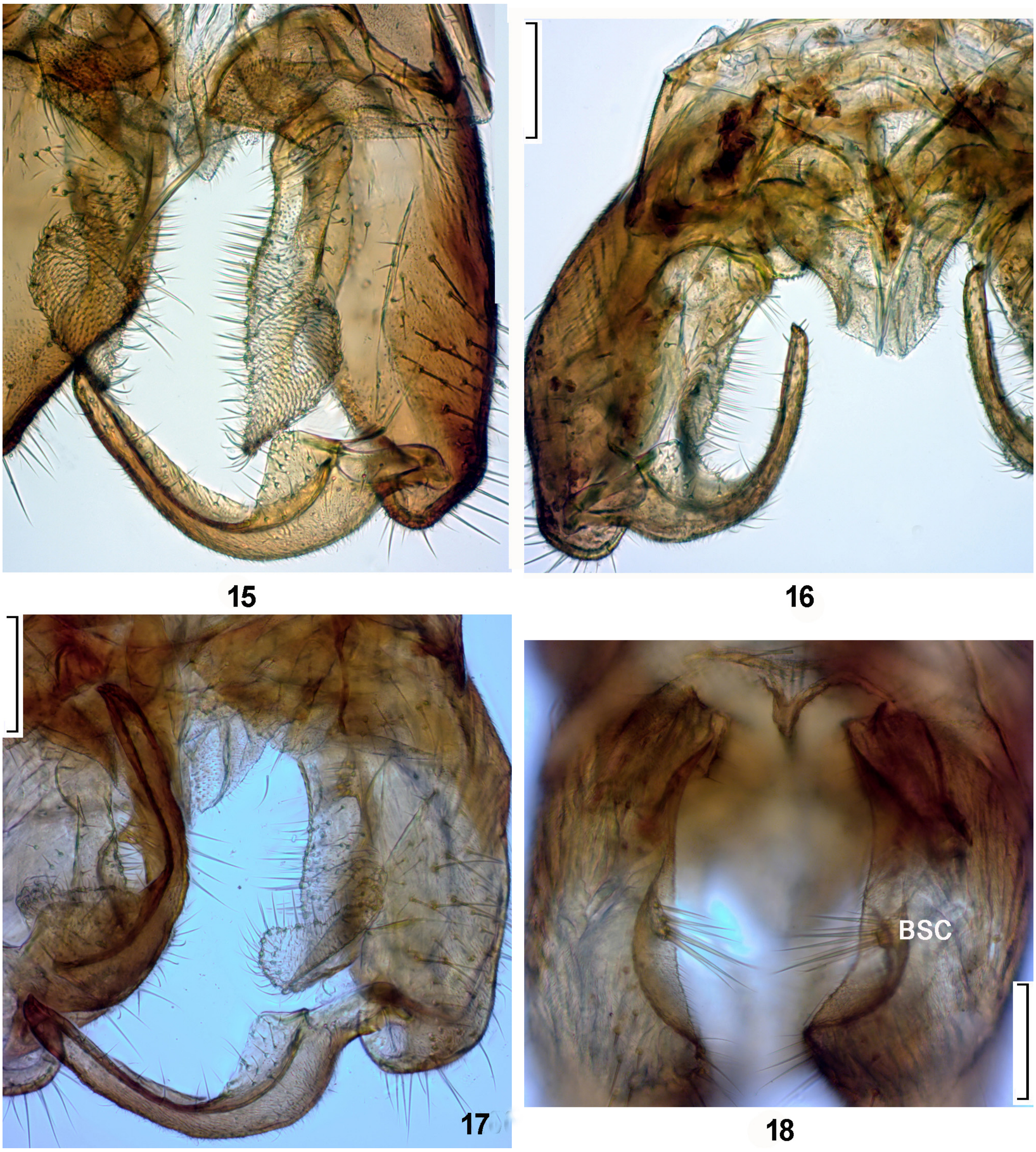
Legs. Spur of front tibia 48–76 µm long. Spurs of mid tibia 60–72 and 52–60 µm long. Spurs of hind tibia 80–92 and 52–60 µm long. Hind tibial comb with 16–20 setae. Length (µm) and proportions of leg segments are as in Table 2.

TABLE 2. Lengths (in µm) and proportions of leg segments of *Diamesa cinerella* Meigen, male (n = 4).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1427–1800	1591–2240	1066–1380	508–705	312–443	115–148	131–164
P_2	1509–1760	1476–1880	640–840	361–459	197–279	115–131	131–148
P_3	1680–1960	2200–2280	1120–1240	607–705	328–394	131	148–164

TABLE 2. (Continued)

	LR	BV	SV	BR
P_1	0.64–0.67	3.82–3.88	2.80–2.83	1.8–2.0
P_2	0.43–0.56	4.31–5.03	3.43–4.66	1.5–1.8
P_3	0.51–0.63	4.06–4.12	2.94–3.46	2.5–2.8



FIGURES 15–18. Adult male of *Diamesa cinerella* Meigen from France **(15)** and Switzerland **(16–18). 15–17,** hypopygium in dorsal view; **18,** hypopygium in ventral view. **BSC** here and further is basimedial setal cluster. Scale bars—50 µm.

Hypopygium (Figs 15–18). Tergite IX with 14–15 setae from one side and anal point, 184 µm long. Laterosternite IX with 9–11 setae. Transverse sternapodeme 164 µm long and 72 µm wide. Gonocoxite 440 µm long; inferior volsella 188 µm long, in the form of a tongue-shaped lobe, covered with short setae. Basimedial setal cluster with 6–8 setae, 68–100 µm long. Gonostylus 308 µm long, curved, widened in basal third and rounded or rounded-triangular along the inner edge; distal two-thirds narrow, with 7–10 short setae along inner edge and with megaseta *ca* 12 µm long and tooth at apex. HR 1.43.

Pupa. Described by Serra-Tosio (1971) and Langton & Visser (2003).

Larva. Described by Schmid (1993) and Rossaro & Lencioni (2015).

Distribution. Widely distributed in the Western Palearctic (Ashe & O'Connor 2009).

Diamesa lavillei **Serra-Tosio**

(Figs 4, 9, 19–22)

Diamesa lavillei Serra-Tosio, 1970: 163; Shilova 1978: 142; Langton & Visser 2003: 30; Ashe & O'Connor 2009: 280; Mohammadi *et al.* 2021: 1234.

Material examined. 1 adult male, RUSSIA: Republic of North Ossetia-Alania, Irafsky District, Caucasus Mountains, Terek River basin, Kumaldon River, 29.XII.2017, alt.1228 m a. s. l., 42.9679 N 43.7867 E, leg. D. Palatov; 2 adult males, the same data, except, Alagirsky District, Caucasus Mountains, Terek River basin, Skazdon River, alt.1943 m a. s. l., 27.VII.2019, 42.7842 N 43.9037 E, leg. D. Palatov;1 adult male, the same data except, Karachay-Cherkess Republic, Zelenchuksky District, Sofia River, Kuban' River basin, 4 km from the tract "Taulu Glade", alt. 1814 m a. s. l., 5.IV.2023, 43.485125 N 41.243800 E, leg. D. Palatov; 1 adult male, the same data, except, Republic of Dagestan, Dokuzparinsky District, Caucasus Mountains, spring sources of the Selda River, alt. 2712 m a. s. l., 20.VIII.2024, 41.251603 N, 47.858658 E, leg. D. Palatov. 1 adult male, the same data, except, Kabardino-Balkarian Republic, left tributary of the Shkhelda River, 2.8 km above the mouth, alt. 2220 m a. s. l., 30.VII.2023, 43.209367 N, 42.649386, leg. D. Palatov; 2 adult males, IRAN: Lorestan Province, Aligudarz County, Zagros Mountains, Dalooni Preserve Park, Dare Daei River, alt. 2244 m a. s. l., 13.VI.2019, 33.186067 N 49.510117 E, leg. D. Palatov.

Description

Adult male (n = 4, except when otherwise stated). Total length 3.8–3.9 mm. Total length/wing length 0.98–1.04.

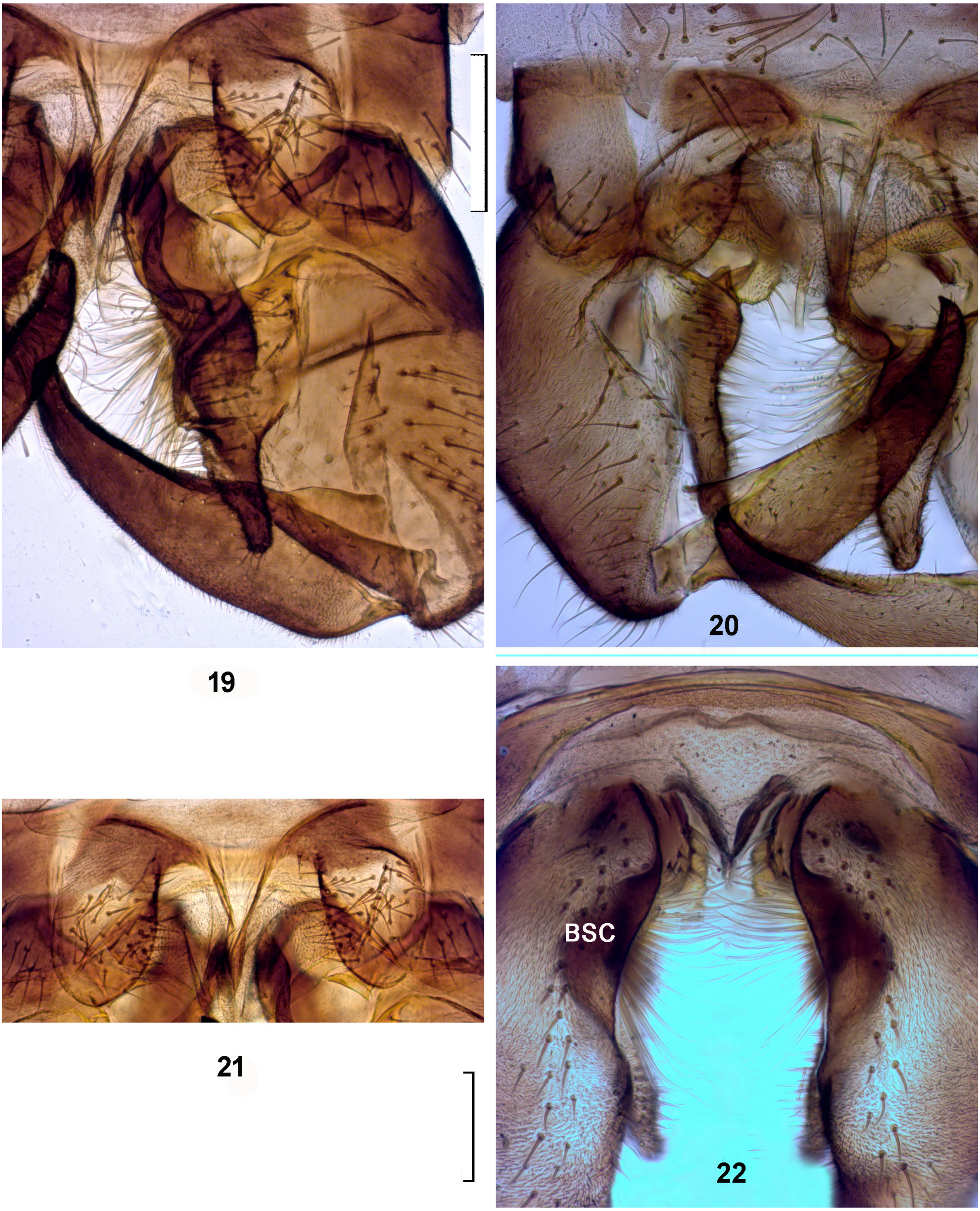
Coloration. Dark brown to black. Wings grey, with brownish veins.

Head. Eyes hairy, reniform. Temporal setae including 5–8 preoculars and 14. Clypeus with 11 setae. Antenna with 13 flagellomeres and reduced plume of setae (Fig. 4); flagellomeres 1–12 with 2–3 setae on flagellomere, 80–104 µm long; terminal flagellomere with 8 setae, 88–104 µm long in basal part and with 1 subapical setae, 36 µm long. Length of flagellomeres 1–13 (in µm) (n = 1): 112, 52, 52, 52, 44, 40, 40, 48, 52, 44, 48, 52, 224; AR 0.33–0.39. Palpomere length (in µm) (n = 1): 44, 108, 148, 144, 192. Palpomere 3 in distal part with sensilla capitata with diameter 20 µm. Head width/palpal length 1.03. Antennal length/palpal length 1.35.

Thorax. Antepronotum with 11–16 ventrolateral setae. Dorsocentrals 14–20, prealars 6–11. Scutellum with 36–50 setae.

Wing (Fig. 9). Length 3.6–4.0 mm, width 1.1 mm. Costal extension 92 µm long. Anal lobe rounded-angular. Squama with 17 setae, 48–92 µm long. $R + R_1$ with 30–38 setae, R_{4+5} with 12–15. RM/MCu 2.0.

Legs. Spur of front tibia 58–60 µm long. Spurs of mid tibia 56–60 µm and 56–64 µm long. Spurs of hind tibia 100 µm and 56–64 µm long. Hind tibial comb with 17–20 setae. Length (µm) and proportions of leg segments are as in Table 3.



FIGURES 19–22. Adult male of *Diamesa lavillei* Serra-Tosio. **19–20,** hypopygium in dorsal view; **21,** tergite IX; **22,** hypopygium in ventral view. Scale bars—50 µm.

TABLE 3. Lengths (in µm) and proportions of leg segments of *Diamesa lavillei* Serra-Tosio, male (n = 4).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1840–1960	1920–2080	1240–1360	623–672	426–443	148–164	180–213
P_2	1840–1960	1720–1880	800–880	410–459	279–295	131–148	127–164
P_3	2000–2200	2040–2080	1280–1440	738–836	426–459	148–164	197–213

TABLE 3. (Continued)

	LR	BV	SV	BR
P_1	0.65	3.62–3.63	2.97–3.03	1.0–1.1
P_2	0.47	4.36	4.36–4.45	1.1–1.2
P_3	0.62–0.71	3.40–3.55	2.94–3.19	1.1–1.2

Hypopygium (Figs 19–22). Tergite IX with 22–23 setae, 16–24 µm long and with narrow (4-anal point, 160 µm long. Laterosternite IX with 10–11 setae. Transverse sternapodeme 200 µm length and 20 µm width. Gonocoxite 400 µm long; inferior volsella 252 µm long, widened in basal quarter and narrowed finger-shaped in remaining part, inner edge is covered with short and medium-length setae. Basimedial setal cluster with 20–26 setae, 108–176 µm long. Gonostylus 344 µm long, slightly curved, basally widened, then gradually narrows towards the top, with short megaseta and tooth at apex. HR 1.16.

Pupa. Described by Serra-Tosio (1970, 1971) and Langton & Visser (2003).

Larva. Described by Mohammadi *et al.* (2021)

Distribution. High mountain Palaearctic species. Known from France, Georgia, Spain, Turkey, Iran (Ashe & O'Connor 2009, Mohammadi *et al.* 2021) and Russia (Caucasus Mountains of Dagestan, Kabardino-Balkarian Republic, Republic of North Ossetia–Alania and Karachay-Cherkess Republic).

Diamesa hamaticornis **Kieffer**

(Figs 1, 10, 23–24)

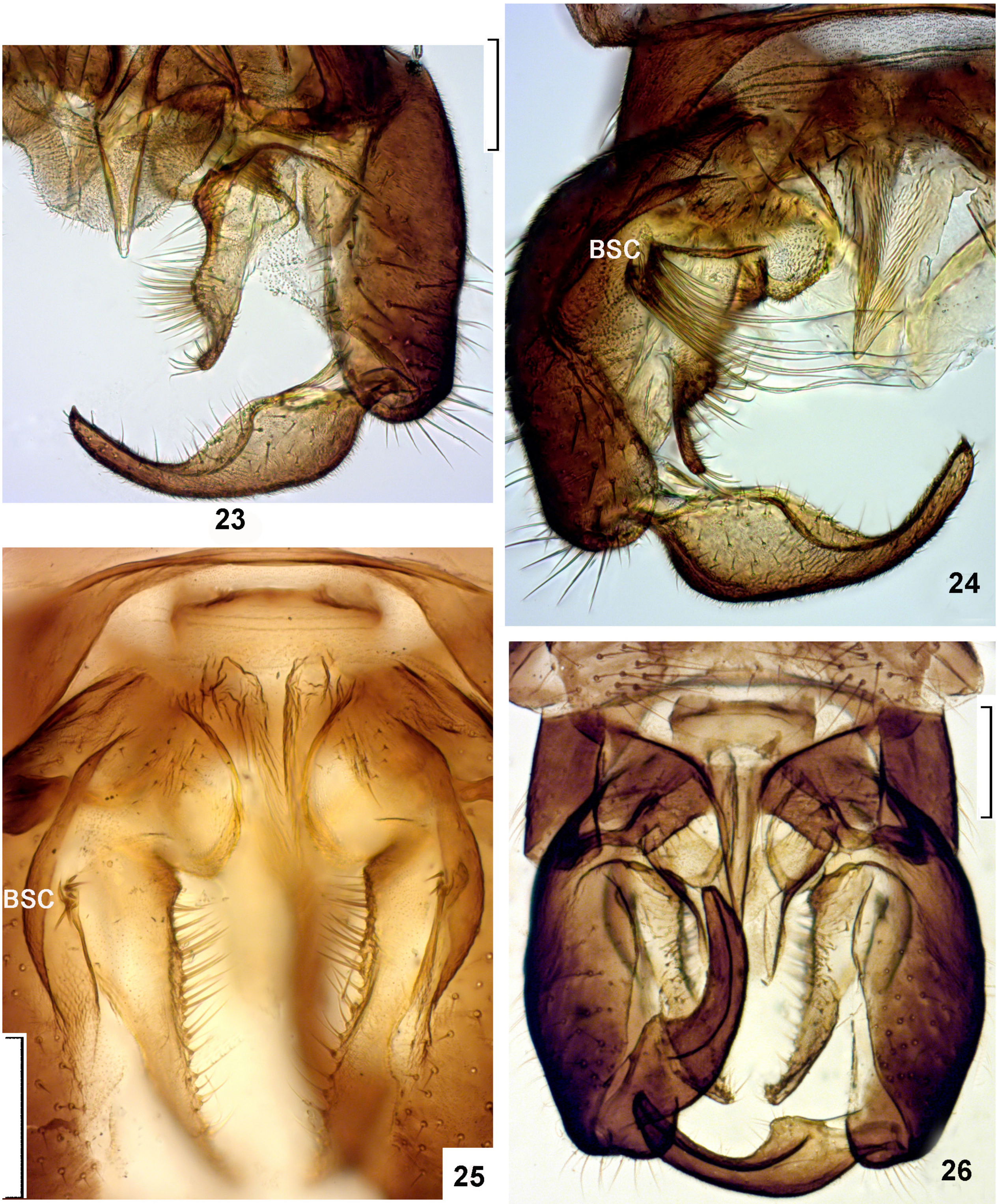
Diamesa hamaticornis Kieffer, 1924: 56; Pagast 1947: 485; Wülker 1959: 350; Serra-Tosio 1971: 236; Schmid 1993: 37; Langton & Visser 2003: 23; Ashe & O'Connor 2009: 276; Makarchenko *et al.* 2022a: 78.

Material examined. 3 adult males, RUSSIA: Republic of North Ossetia-Alania, Irafsky District, Caucasus Mountains, Terek River basin, Karaugomidon River, alt. 1476 m a. s. l., 04.I.2018, 42.8942 N 43.6973, leg. D. Palatov; 2 adult males, the same data, except Alagirsky District, Caucasus Mountains, Terek River basin, Skazdon River, alt. 1943 m a. s. l., 27.VII.2019, 42.7842 N 43.9037 E, leg. D. Palatov; 2 adult males, Magadan Region, Olsky District, mouth of Ola River, 03.V.2019, 59.580514 N, 151.272686 E, leg. E. Khamenkova; 1 adult males, Republic of Khakassia, Abakan City, valley of the Abakan River, poplar forest, alt. 245 m a. s. l., 06.IV.2020, 53.713750 N, 91.522111 E, leg. S. Dragan; 2 adult males, the same data, except Republic of Khakassia, Askizsky District, Askiz River, from surface of snow, 05.XI.2020, 53.339417 N, 89.888500 E, leg. S. Dragan; 1 adult male, KYRGYZSTAN: Talas Region, Chychkan Kiver Valley, Chychkan River, approximately 10–15 km below the pass from the Suusamyr Malley, alt. 2454 m a. s. l., 6.II.2016, 42.19895 N, 72.989667 E, leg. D. Palatov; 2 adult males, MONGOLIA: Govi-Altay Aymag, Uliastein Gol River at the confluence of Baga Gol River, alt.1989 m a. s. l., 22.IX.2019, 45.484600 N 94.277067 E, leg. D. Palatov.

Description

Adult male (n = 3, except when otherwise stated). Total length 3.7–3.9 mm. Total length/wing length 1.02–1.22.

Coloration. Dark brown to brown. Legs brown. Wings grayish to gray, venation brownish.



FIGURES 23–26. Adult male of *Diamesa hamaticornis* Kieffer **(23–24)**, and *D. hyperborea* Holmgren **(25–26)**. **23, 26,** hypopygium in dorsal view; **24–25,** hypopygium in ventral view. Scale bars—50 µm.

Head. Eyes hairy, reniform. Temporal setae including 4 preoculars, 11 verticals. Clypeus with 8 setae. Antenna with 13 flagellomeres (Fig. 1) and little reduced plume of setae, 131–560 µm long; terminal flagellomere with 1–2 subapical setae, 36–52 µm long; AR 0.50–0.67. Palpomere length (in µm): 48–52, 88–96, 120–140, 124–140, 180–216. Palpomere 3 in distal part with sensilla capitata with diameter 12–16 µm. Head width/palpal length 0.99–1.08.

Thorax. Antepronotum with 10–11 ventrolateral setae. Dorsocentrals 9–15, prealars 6–10. Scutellum with 15–38 setae.

Wing. Length 3.2–3.64 mm, width 1.0–1.12 mm. Anal lobe rounded to rounded-angular. Squama with 22–30 setae, 72–88 µm long. R + R_1 with 22–27 setae, R_{4+5} with 10–11. RM/MCu 2.7. Costa extension 98 µm long.

Legs. Spur of front tibia 54 µm long. Spurs of mid tibia 48 µm long. Spurs of hind tibia 84 and 56 µm long. Hind tibial comb with 18 setae. Length (µm) and proportions of leg segments are as in Table 4.

TABLE 4. Lengths (in µm) and proportions of leg segments of *Diamesa hamaticornis* Kieffer, male (n = 3).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1560–1840	1720–2160	1020–1260	541–623	369–426	115–164	164
P_2	1560–1860	1520–1780	680–780	374–459	221–295	115–131	131–148
P_3	1720–2020	1620–2160	1140–1320	599–754	328–402	115–164	156–164

TABLE 4. (Continued)

	LR	BV	SV	BR
P_1	0.58–0.59	3.51–3.86	3.13–3.25	1.1–1.2
P_2	0.44-0.45	4.28–4.50	4.59–4.67	1.2–1.3
P_3	0.61–0.70	3.59–3.71	3.03–3.17	1.2–1.3

Hypopygium (Figs 23–24). Tergite IX with 11–15 setae from one side and anal point, 176–180 µm long. Laterosternite IX with 8–13 setae. Transverse sternapodeme 180–240 µm long and 52–60 µm wide. Gonocoxite 360 µm long; inferior volsella 128–140 µm long, wide in basal 3/4, narrow and finger-shaped in short distal quarter, with long setae along margin (Fig. 23). Basimedial setal cluster with 12–17 setae, 112–196 µm long (Fig. 24). Gonostylus 320 µm long, widened in basal half and narrowed in the distal, a long inward margin with pale short setae, apically with megaseta, 8 µm long. HR= 1.13.

Pupa. Described by Serra-Tosio (1971) and Langton & Visser (2003).

Larva. Described by Schmid (1993).

Distribution. Palaearctic species. Widely distributed in the Western Palaearctic (Ashe & O'Connor, 2009) and known also in East Siberia (Makarchenko *et al.* 2022a) and Magadan Region of the Russian Far East.

Diamesa hyperborea Holmgren

(Figs 3, 11, 25–26)

Diamesa hyperborea Holmgren, 1869: 48; Oliver 1962: 6; Halvorsen *et al.* 1982: 117; Langton & Visser 2003: 24; Ashe & O'Connor 2009: 277; Stur & Ekrem 2020: 18.
Adiamesa ursus Kieffer, 1918: 104; Kieffer in Kieffer & Thienemann 1919: 42.
Diamesa ursus (Kieffer, 1918) Sæther 1968: 458; Serra-Tosio 1971: 227.

Material examined. 1 adult male, NORWAY: Finse, near Blåisen. Flying over small rocky stream, 13.VIII.1980, leg. E. Willassen.

Description

Adult male (n = 1). Total length 4.2 mm. Total length/wing length 1.1.

Coloration. Dark brown to brown. Legs brown. Wings grayish, venation brownish.

Head. Eyes hairy, reniform. Temporal setae including 3–4 preoculars, 14 verticals, 6–8 postorbitals. Clypeus with 14 setae. Antenna with 13 flagellomeres and reduced plume of setae (Fig. 3); flagellomeres with 1–4 setae, maximal length of which 108 µm; terminal flagellomere with 1 seta, 92 µm long in basal part and with 1 subapical setae, 48 µm long. Length of 1–13 flagellomeres (µm): 100, 48, 52, 48, 48, 44, 40, 44, 48, 40, 60, 36, 192; AR 0.36.

Palpomere length (µm): 68, 108, 164, 132, 168. Palpomere 3 in distal part with sensilla capitata with diameter 24 µm. Head width/palpal length 1.05. Antennal length/palpal length 1.25.

Thorax. Antepronotum with 8 ventrolateral setae. Dorsocentrals 17, prealars 12. Scutellum with 20 setae.

Wing (Fig. 11). Length 3.76 mm, width 1.04 mm. Anal lobe rounded-angular. Squama with 43 setae, 44–80 µm long, in 2 rows. R and R_1 with 33 setae, R_{4+5} with 20 setae. RM/MCu 2.6–3.0.

Legs. Spur of front tibia 60 µm long. Spurs of mid tibia 64 and 56 µm long. Spurs of hind tibia 100 and 56 µm long. Hind tibial comb with 17–18 setae. Length (µm) and proportions of leg segments are as in Table 5.

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

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
P_1	2000	2120	1360	656	459	164	180	0.64	3.76	3.03	1.2
P_2	2080	1960	840	459	312	164	180	0.43	4.88	4.81	1.3
P_3	2240	2240	1400	738	410	164	180	0.63	3.94	3.20	1.3

Hypopygium (Figs 25–26). Tergite IX with 18 setae from one side and anal point, 192 µm long. Laterosternite IX with 12–13 setae. Transverse sternapodeme 184 µm long and 52 µm width. Gonocoxite 426 µm long; inferior volsella 316 µm long, wider at the base, gradually narrowing towards apex, with short setae along inner edge. Basimedial setal cluster with 4–5 setae, 40–44 µm long (Fig. 25). Gonostylus 336 µm long, slightly curved, in basal third it is widened, along the inner edge with a small angular protrusion, distal thirds are narrow (Fig. 26). HR 1.27.

Pupa. Described by Serra-Tosio 1971 and Langton & Visser 2003.

Larva. Described by Stur & Ekrem 2020.

Distribution. Palaearctic species, known from Bear Island, Faroe Island, Finland, Iceland and Norway (Ashe & O'Connor 2009, Stur & Ekrem 2020).

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

http://zoobank.org/NomenclaturalActs/C1741C34-32D5-417E-B459-31D7C030CCFC

(Figs 2, 12, 27–28)

Type material. Holotype, adult male, TAJIKISTAN: Gorno-Badakhshan Autonomous Region, Roshtkalinskiy District, Soktosh River, about 2.3 km to West from Deruzh Stronghold, alt. 3343 m a. s. l., 02.VII.2016, 37.3786 N 72.3493 E, leg. D. Palatov. Paratypes: 3 adult males, the same data as holotype.

Derivatio nominis. The species is named as *soktoshensis* after the type locality in the Soktosh River in Pamir.

Description

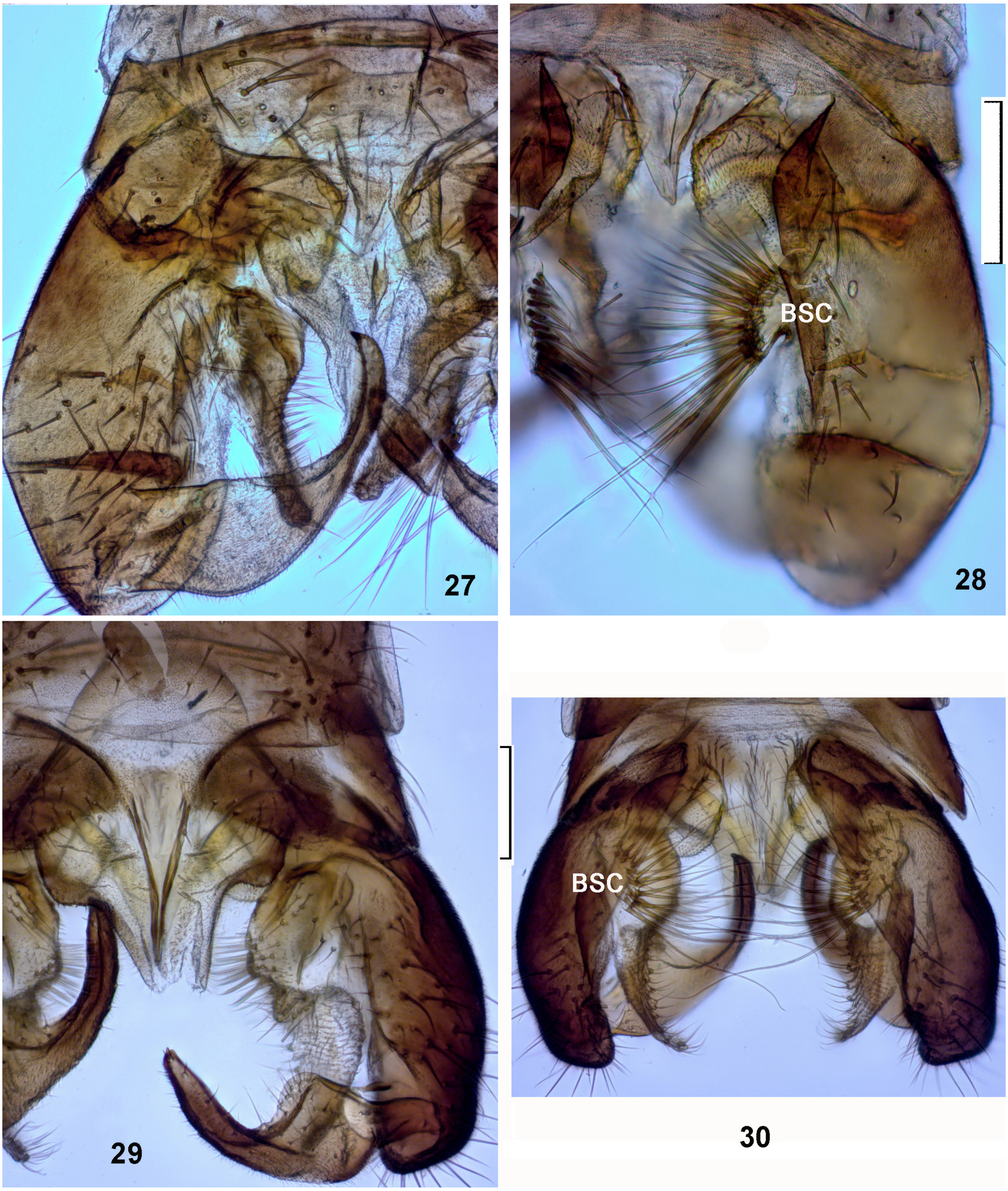
Adult male (n = 2, except when otherwise stated). Total length 3.0–3.5 mm. Total length/wing length 0.93–1.15.

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

Head. Eyes hairy, reniform. Temporal setae including 5 frontals, 11 verticals. Clypeus with 10 setae. Antenna with 13 flagellomeres and reduced plume of setae (Fig. 2); number and length of these setae on 1–12 flagellomeres respectively: 1 (68 µm), 3 (112 µm), 3 (128–164 µm), 5 (200 µm), 5 (96–200 µm), 5 (84–96 µm), 5 (90–200 µm), 5 (120–160 µm), 5 (100–200 µm), 5 (160–200 µm), 5 (96–164 µm), 5 (100–200 µm); terminal flagellomere with 9 setae, 88–112 µm long in basal part and with 1 subapical setae, 32–40 µm long. Length of 1–13 flagellomeres (µm): 84–92, 48, 48–52, 52–60, 52, 52, 52–56, 52–60, 48, 52–56, 52–56, 52–56, 236–272; AR 0.37–0.40. Palpomere length (µm): 44, 92, 148, 156, 208. Palpomere 3 in distal part with sensilla capitata with diameter 20 µm. Head width/palpal length 1.04. Antennal length/palpal length 1.38.

Thorax. Antepronotum with 4–6 ventrolateral setae, 44–52 µm long. Dorsocentrals 9–11, 56–72 µm long; prealars 5–7, 48–60 µm long. Scutellum with *ca* 30 setae.

Wing (Fig. 12). Length 3.0–3.2 mm, width 0.92–0.96 mm. Costal extension 49–66 µm long. Anal lobe rounded-angular. Squama with 20–23 setae, 104–124 µm long. R and R_1 with 33 setae, R_{4+5} with 13 setae. RM/MCu 2.6.



FIGURES 27–30. Adult male of *Diamesa soktoshensis* **sp. nov. (27-28)** and *D. kasymovi* Kownacki et Kownacka **(29–30)**. **27, 29,** hypopygium in dorsal view; **28, 30,** hypopygium in ventral view. Scale bars—50 µm.

Legs. Spur of front tibia 60–68 µm long. Spurs of mid tibia 52–56 µm and 40–48 µm long. Spurs of hind tibia 84–88 µm and 48 µm long. Hind tibial comb with 17–19 setae. Length (µm) and proportions of leg segments are as in Table 6.

TABLE 6. Lengths (in µm) and proportions of leg segments of *Diamesa soktoshensis* **sp. nov.**, male (n = 2).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1460–1509	1656–1706	1066–1148	525–541	328–344	131	148–164
P_2	1460–1525	1443–1542	640–722	312–344	230–246	115–131	144–148
P_3	1640–1720	1760–1840	1120–1160	574–623	328–344	131	164

TABLE 6. (Continued)

	LR	BV	SV	BR
P_1	0.64–0.67	3.69–3.70	2.80–2.92	1.1–1.2
P_2	0.44–0.47	4.28–4.40	4.25–4.54	1.2–1.3
P_3	0.63–0.64	3.74–3.78	3.04–3.07	1.2–1.3

Hypopygium (Figs 27–28). Tergite IX with 16–19 setae, 80–100 µm long and with narrow naked anal point, 80–88 µm long. Laterosternite IX with 8–10 setae, 88–100 µm long. Transverse sternapodeme 124 µm long, 52 µm wide. Gonocoxite 476 µm long; inferior volsellae 200 µm long, expanded basally and finger-shaped distally, with numerous medium-length setae along the inner edge; basimedial setal cluster with 26–28 setae, 188–200 µm long. Gonostylus 312 µm long, slightly curved, widened in basal two-thirds and narrow in distal third, megaseta 8 µm long, next to it there is tooth. HR 1.53.

Pupa and ***larva*** unknown.

Diagnosis. Total length 3.0–3.5 mm, wing length 3.0–3.2 mm. Eyes hairy, reniform. Clypeus with 10 setae. Antenna with 13 flagellomeres and reduced plume of setae (Fig. 2); AR 0.37–0.40. Head width/palpal length 1.04. Antennal length/palpal length 1.38. Anal lobe rounded-angular. Dorsocentrals of mesonotum 9–11, prealars 5–7, scutellars *ca* 30. Squama with 20–23 setae. Hind tibial comb with 17–19 setae. Tergite IX with 16–19 setae, 80–100 µm long and with narrow naked anal point, 80–88 µm long. Transverse sternapodeme 124 µm long, 52 µm wide. Inferior volsella 200 µm long, expanded basally and finger-shaped distally, with numerous medium-length setae along the inner edge; basimedial setal cluster with 26–28 setae, 188–200 µm long. Gonostylus slightly curved, widened in basal two-thirds and narrow in distal third, megaseta 8 µm long, next to it there is tooth. HR 1.53.

Ecology. Adult males were collected from the Soktosh River, which flows through a shallow, semi-desert mountainous valley at an altitude of 3343 m above sea level. The width of the river is 3.5–4 m, the substrate consists of stones, boulders, with scattered pebbles and sand. At the time of collection in July, the flow rate of the river was 0.3–1 m/s, and the water temperature 7°C.

Distribution. Known only from the type locality in the Pamir Mountains (Figs 35–36).

Diamesa kasymovi Kownacki *et* Kownacka

(Figs 7, 13, 29–30)

Diamesa kasymovi Kownacki *et* Kownacka, 1973: 32; Willassen & Serra-Tosio 1988: 94; Langton & Visser 2003: 27; Ashe & O'Connor 2009: 278; Ghaderi *et al.* 2024: 82.

Material examined. 5 adult males, RUSSIA: Republic of Dagestan, Tlyaratinsky District, Caucasus Mountains, Sulak River basin, Dzhurmut River, alt. 1775 m a. s. l., 10.VIII.2021, 41.9727 N 46.5068 E, leg. D. Palatov; 1 adult male, same data except Kabardino-Balkarian Republic, Elbrussky District, The Adyl-Su River, Terek River basin, opposite the Dzhan-Tugan mountain hotel, 06.VIII.2023, 43.217675 N 42.691864 E, leg. D. Palatov.

Description

Adult male (n = 2, except when otherwise stated). Total length 3.1–3.3 mm. Total length/wing length 1.11.

Coloration. Dark brown. Head, thorax, abdomen and legs dark brown. Wings greyish, with brownish veins.

Head. Eyes hairy, reniform. Temporal setae including 4–6 preoculars,8–12 verticals. Clypeus with 8–10 setae. Antenna with 8 flagellomeres and reduced plume of setae (Fig. 7); number and length of these setae on 1–7 flagellomeres respectively: 1 (68 µm), 1 (88 µm), 2 (72–108 µm), 3 (60–96 µm), 2 (120 µm), 3 (80–108 µm), 3 (88–100); terminal flagellomere with 2 setae, 60–64 µm long in basal part and with 1 subapical setae, 36 µm long. Length of 1–8 flagellomeres (µm): 72–76, 40–44, 40–48, 36–40, 36–40, 36, 40, 120–140; AR 0.37–0.47. Palpomere length (µm): 44–48, 76–84, 108–120, 120–124, 128–192. Palpomere 3 in distal part with sensilla capitata with diameter 16 µm. Head width/palpal length 1.11–1.32. Antennal length/palpal length 0.79–0.90.

Thorax. Antepronotum with 12 ventrolateral setae. Dorsocentrals 9, prealars 5–7. Scutellum with 20–26 setae, 172 µm long.

Wing (Fig. 13). Length 2.8–3.0 mm, width 0.92–0.96 mm. Costal extension 49–60 µm long. Anal lobe rounded-angular. Squama with 24–25 setae, 131–361 µm long. R and R_1 with 30–31 setae, R_{4+5} with 17–24. RM/MCu 3.3.

Legs. Spur of front tibia 44–48 µm long. Spurs of mid tibia 40–44 µm and 40–52 µm long. Spurs of hind tibia 76 µm and 44–48 µm long. Hind tibial comb with 15–16 setae. Length (µm) and proportions of leg segments are as in Table 7.

TABLE 7. Lengths (in µm) and proportions of leg segments of *Diamesa kasymovi* Kownacki *et* Kownacka, male (n = 4).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1394–1476	1673–1689	1050–1115	492–508	213–312	98–131	131–148
P_2	1361–1443	1460–1509	640–656	312–328	197–213	98–115	115–131
P_3	1520–1560	1680–1720	1040–1120	541–574	279–295	98–115	131

TABLE 7. (Continued)

	LR	BV	SV	BR
P_1	0.62–0.67	4.00–4.17	2.82–2.97	1.0–1.1
P_2	0.38–0.44	4.41–4.70	4.40–4.50	1.1–1.2
P_3	0.60–0.67	3.93–3.99	3.12–3.15	1.1–1.2

Hypopygium (Figs 29–30). Tergite IX with 20–22 short setae, and anal point, 124–148 µm long. Laterosternite IX with 7–12 setae. Transverse sternopodeme,140–160 µm long, 28–36 µm wide. Gonocoxite 328–356 µm long, with 22–25 basimedial setae, 160–192 µm long; inferior volsella 140 µm long, wider at the base, gradually narrowing towards apex. Gonostylus 252–260 µm long, almost crescent-shaped, in basal half it is widened and rounded, in distal half it is finger-shaped, with short setae along the inner edge, and at the apex with short megaseta and tooth. HR 1.26–1.41.

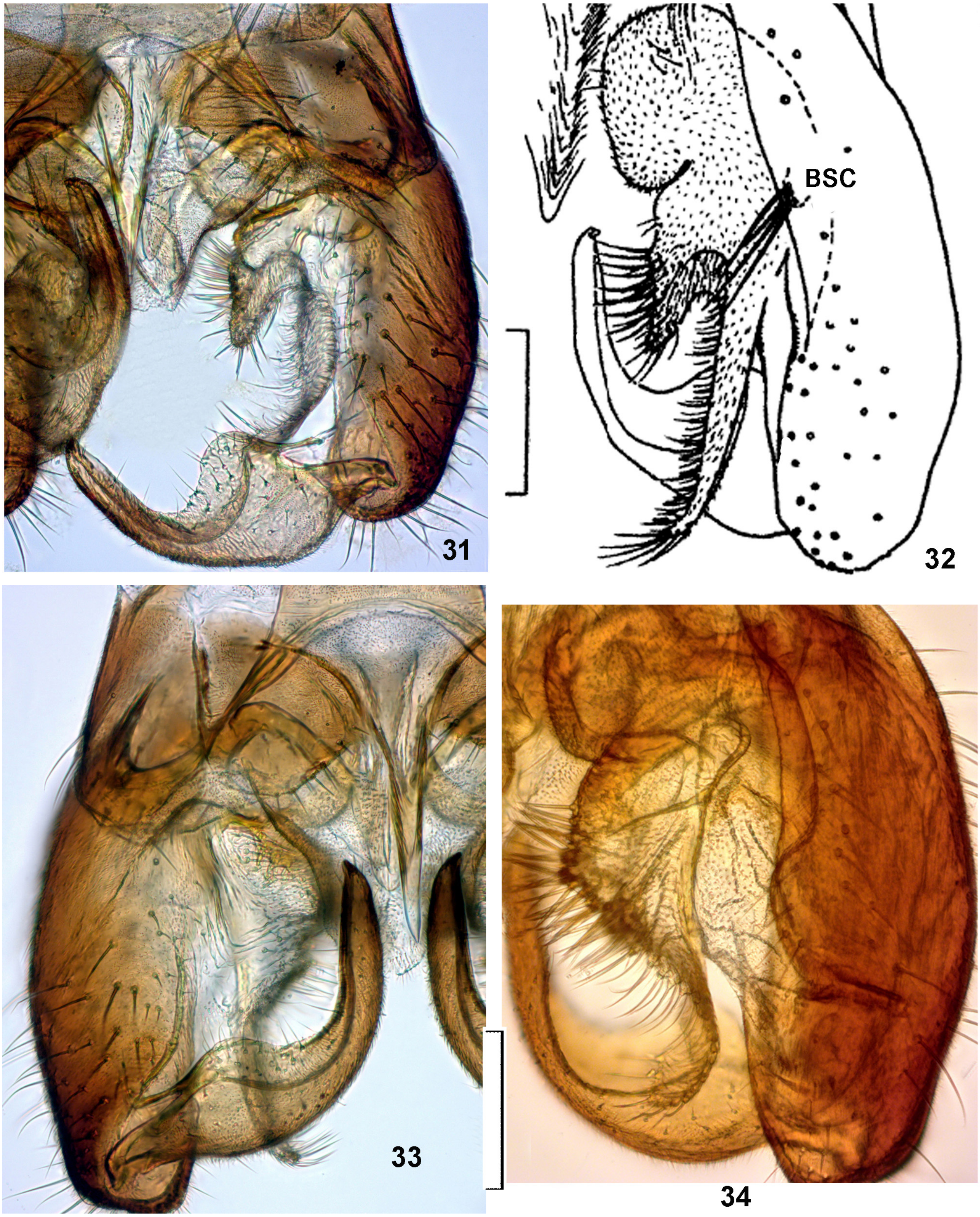
Pupa and ***larva.*** Described by Kownacki & Kownacka (1973).

Distribution. According to Ashe & O'Connor (2009), species is widespread in Austria, Azerbaijan, Belgium, Georgia, Italy, Lebanon, Poland, Slovakia, Switzerland, Turkey but we believe that it is reliably known only from Azerbaijan, Georgia, Lebanon, Iran (Kownacki & Kownacka 1973, Willassen & Serra-Tosio 1988, Ghaderi *et al.* 2024) and from the Caucasus Mountains in Russia (Republic of Dagestan, Kabardino-Balkarian Republic).

Diamesa tonsa Haliday

(Figs 31–32)

Diamesa tonsa Haliday in Walker, 1856: 195; Pagast 1947: 485; Langton & Visser 2003: 50; Langton & Pinder, 2007: 57; Ashe & O'Connor 2009: 286; Krasheninnikov 2009: 65; Rossaro & Lencioni 2015: 74; Montagna *et al.* 2016: 326.
Chironomus pergens Walker, 1856: 187.
Chironomus pertracta Walker, 1856: 188.
Diamesa fissipes Kieffer, 1909: 41.
Diamesa thienemanni Kieffer, 1909: 40; Pagast 1947: 484; Serra-Tosio 1969: 208, 1971: 230.
Diamesa camptoneura Kieffer, 1915: 109; Goetghebuer, 1932: 182, 1939: 12.
Diamesa semireducta Sæther, 1968: 457



FIGURES 31–34. Adult male of *Diamesa tonsa* Haliday **(31–32)** and *D. tsutsuii* Tokunaga **(33–34)**. **31, 33,** hypopygium in dorsal view; **32, 34,** hypopygium in ventral view. **Fig. 32** after Serra-Tosio (1971). Scale bars—50 µm.

Material examined. 4 adult males, RUSSIA: Perm Territory, Suksunsky District, Suksun urban locality, mouth of Suksunchik River, 08.IV.2012, 57.14769 N 57.390170 E, leg. A. Krasheninnikov; 2 adult males, same data except Republic of Dagestan, Tlyaratinsky District, Caucasus Mountains, Sulak River basin, Dzhurmut River, alt. 1775 m

a. s. l., 10.VIII.2021, 41.9727 N 46.5068 E, leg. D. Palatov; 3 adult males, GEORGIA: Samegrelo-Zemo Svaneti, Martvili municipality, Caucasus Mountains, Rioni River basin, Abasha River in the Balda valley, 13.VIII.2016, 42.4847 N 42.3942 E, leg. D. Palatov; 2 adult males, IRAN: Mazandaran Province, Elburs ridge, under Mount Siahseng, above of Devilat Village, alt. 2792 m a. s. l., 15.VI.2019, 36.230917 N, 51.842550 E, leg. D. Palatov; 1 adult male, NORWAY, Ekse, Vaksdal, 10–16.VIII.1976, leg. T. Andersen.

Description

Adult male (n = 4, except when otherwise stated). Total length 3.4–4.2 mm. Total length/wing length 1.10–1.26.

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

Head. Eyes hairy, reniform. Temporal setae including 4–5 preoculars, 7–11 verticals. Clypeus with 8–9 setae. Antenna with 13 flagellomeres and reduced plume of setae, maximal length of which 689 µm; terminal flagellomere with 1 subapical setae, 28–40 µm long. Length of 1–13 flagellomeres (µm) (n=2): 56–84, 32–36, 36–40, 40–44, 44, 44–48, 48, 52–56, 56–60, 56–64, 344–396; AR 0.56–0.69. Palpomere length (µm): 40, 88, 88, 104, 128. Palpomere 3 in distal part with sensilla capitata with diameter 12 µm. Head width/palpal length 1.32.

Thorax. Antepronotum with 7 ventrolateral setae. Dorsocentrals 10–12, prealars 5–8. Scutellum with *ca* 30 setae.

Wing. Length 3.12–3.60 mm, width 0.92–1.4 mm. Anal lobe rounded-angular. Squama with 28–31 setae in 1–2 rows. R and R_1 with 27–32 setae, R_{4+5} with 10–17. RM/MCu 2.7–3.

Legs. Spur of front tibia 56 µm long. Spurs of mid tibia 64 and 60 µm long. Spurs of hind tibia 88 and 60 µm long. Hind tibial comb with 12–14 setae. Length (µm) and proportions of leg segments are as in Table 8.

TABLE 8. Lengths (in µm) and proportions of leg segments of *Diamesa tonsa* Haliday, male (n = 2).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1520–1640	1880–2040	1160–1380	574–656	328–410	115	131–164
P_2	1440–1720	1640–1760	720–840	394–443	230–262	115	115–148
P_3	1640–1880	1880–2160	1160–1240	558–722	312–410	115	131–164

TABLE 8. (Continued)

	LR	BV	SV	BR
P_1	0.62–0.68	3.67–3.97	2.67–2.93	2.0–2.1
P_2	0.44–0.48	4.37–4.80	4.14–4.28	2.8–3.0
P_3	0.57–0.62	3.70–4.19	3.03–3.26	2.1–2.2

Hypopygium (Figs 31–32). Tergite IX with 17–21 setae from one side and anal point, 120–152 µm long. Laterosternite IX with 11–14 setae. Transverse sternapodeme 120–140 µm long and 36–52 µm wide. Gonocoxite 336–392 µm long; inferior volsella with two branches, 57–60 µm long and 168–172 µm long. Basimedial setal cluster with 4–5 setae 64–68 µm long. Gonostylus 249–252 µm long, almost crescent-shaped, with short megaseta and tooth. HR= 1.4–1.6.

Pupa. Described by Langton & Visser (2003).

Larva. Described by Rossaro & Lencioni (2015).

Distribution. Widely distributed in the Western Palaearctic (Ashe & O'Connor 2009).

Diamesa tsutsuii Tokunaga

(Figs 6, 14, 33–34)

Diamesa tsutsuii Tokunaga, 1936: 546; 1964: 22; Makarchenko 1977: 113, 1985: 82; 2006: 261, 477, 614; 2023: 144; Linevich & Makarchenko 1989: 32; Ashe & O'Connor 2009: 287; Makarchenko & Makarchenko 2017: 130.

Diamesa coquilletti Sublette, 1966: 584; Hansen & Cook 1976: 78**.**

Diamesa matuimpedita Sasa, 1989: 149–150.

Material examined. JAPAN: 1 adult male (holotype), Nagano Prefecture, Hosono, from snow, alt. 600 m above sea level, 17.III.1935, leg. K. Tsutsui. 15 adult males, 5 pupae, 11 larvae, RUSSIA: Primorye Territory, Khasansk District, Kedrovaya Pad Nature Reserve, Kedrovaya River, 15–17.II.1975, leg. E. Makarchenko; 1 adult male, Ternei District, Sikhote-Alin Nature Reserve, Spornyi Stream (Serebrianka River basin), leg. O. Zorina; 12 adult males, 6 pupae, 14 larvae, Jewish Autonomous Region, Obluchie District, Bidzhan River, (Amur River basin), from snow, 27.III.2013, 48.640150 N, 131.620283 E, leg. E. Makarchenko; 5 adult males, Khabarovsk Territory, Nanaisky District, Anyuisky National Park, Pihtsa River (tributary of Gassi Lake), Amur River basin, 25.V 2019, leg. N. Yavorskaya; 2 adult males, Magadan Region, Olskyi District, Ola River, 29.IV.2014, leg. E. Khamenkova, 2 adult males, the same data except 3.V.2019, leg. E. Khamenkova; 3 adult males, 3 pupae, Sakhalin Island, Yuzhno-Sakhalinsk City, Bereznjaki River, 17.IV.2015, leg. E. Zhivogliadova.

Description

Adult male (n = 7). Total length 4.3–6.2 mm. Total length/wing length 1.33–1.35.

Antenna with 8 flagellomeres and reduced plume of setae (Fig. 1); number and length of these setae on 1–7 flagellomeres respectively (n=2): 2–3 (36–40 µm), 1 (40–42 µm), 3–4 (56–60 µm), 3–4 (48–56 µm), 3–4 (48–56 µm), 2–3 (80–82 µm), 2–3 (80–82 µm); terminal flagellomere with 3–4 setae, 72–80 µm long in basal part and with 1 subapical setae, 20–22 µm long. Length of 1–8 flagellomeres (µm) (n=4): 76–84, 52–56, 60–64, 48–56, 52–56, 42–44, 38–44, 156–164; AR 0.40–0.50. Palpomere length (µm): 46–48, 55–100, 134–140, 143–152, 212–220. Head width/palpal length 1.05–1.06. Antennal length/palpal length 0.80–0.84. Thorax. Antepronotum with 9–11 ventrolateral setae. Dorsocentrals 10–13, prealars 6–10. Scutellum with 20–42 setae. Wing 3.2–4.0 mm long. Anal lobe rounded-angular. LR_1 0.54–0.62, BV_1 3.48–4.04, SV_1 3.0–3.48.

Hypopygium (Figs 33–34). Tergite IX with 12–24 setae and anal point, 124–164 µm long, *ca* 40 µm wide, which slender apically, broadening basally, usually does not go beyond top of proctiger or only slightly goes. Laterosternite IX with 10–11 setae. Transverse sternapodeme trapezoidal, without antero-lateral projections, 204–280 µm long, 44–52 µm wide. Gonocoxite 400–508 µm long; inferior volsella *ca* 260 µm long, basal half widened, narrow distal, with setae along inner margin, which are longest in basal part; basimedial setal cluster absent. Gonostylus 280–360 µm long, curved, widened and rounded in basal third, narrow in distal two-thirds, with megaseta 32–36 µm long. HR 1.40–1.43.

Pupa and ***larva.*** Described by Makarchenko (1985, 2023).

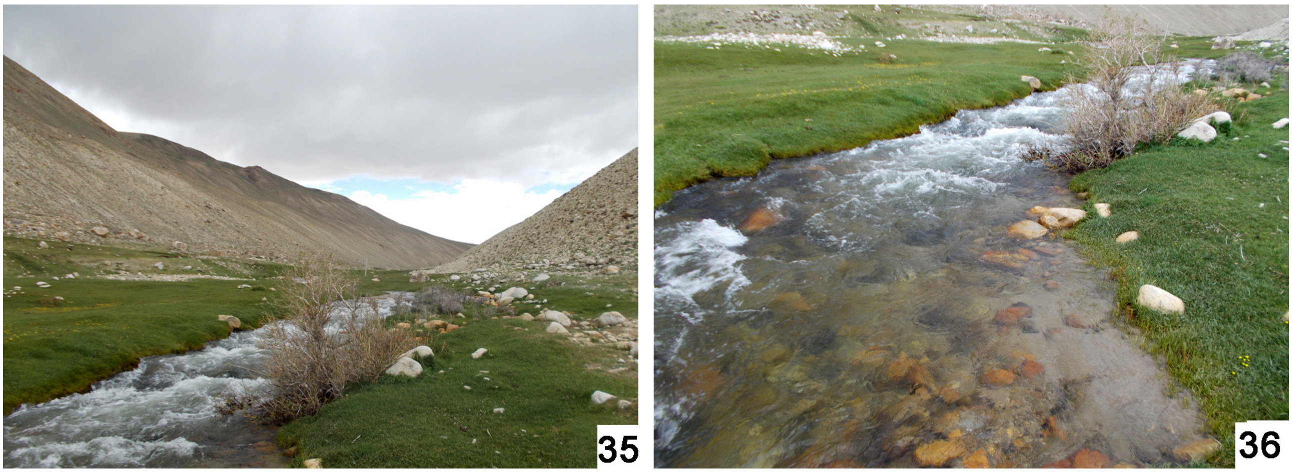
Distribution. East Palaearctic species. The western border of the range is the basin of Baikal Lake.

Molecular results

Overall, we sequenced fragments of the cytochrome oxidase subunit I (609–658 bp in length) for 30 samples which included 6 species: *D. cinerella, D. hamaticornis, D. kasymovi, D. lavillei, D. tonsa*, and *D. tsutsuii*. We also sequenced 10 samples for ITS locus (998–1003 bp in length), 1–3 sample for each of the 6 species.

In the present study, we used the COI DNA barcodes with previously deposed sequences of non-biting midges from *Diamesa cinerella* group in accordance with the criteria described in the Material and Methods section. As of June 2025, 153 sequences have been found, to which we added 30 new ones and obtained a final dataset of 183 sequences (Supplementary Table S1). This dataset added *D. hyperborea*, which was not represented in our data. The number of samples for each species in the dataset is significantly skewed towards the widespread, Western Palaearctic *D. tonsa* (88 samples) and the northern, Western Palaearctic *D. hyperborea* (41 samples). The remaining species are represented by 6–15 samples.

A total of 109 haplotypes for 183 samples were observed for *D. cinerella* group. Haplotype diversity and nucleotide diversity for all datasets were 0.984 ± 0.004 and 0.015 ± 0.008. The total number of polymorphic sites was 77. The values of nucleotide and haplotype diversity and number of nucleotide differences are in Table 9. However, according to the median joining haplotype network (Fig. 37), most species were homogeneous, leading to non-representativeness of species analysis in comparison with the analysis of identified haplogroups.



FIGURES 35–36. Type locality of *Diamesa soktoshensis* **sp. nov.** in Tajikistan, Gorno-Badakhshan Autonomous Region, Roshtkalinskiy District, Soktosh River, about 2.3 km to West from Deruzh Stronghold, alt. 3343 m above sea level, 37.3786 N, 72.3493 E (Photos by D.M. Palatov).

TABLE 9. Main values of COI mtDNA variability for species of *Diamesa cinerella* group. N—number of individuals, S—number of polymorphic sites, h—number of haplotypes, Hd—haplotypic diversity, π—nucleotide diversity, k—average number of nucleotide differences, SE—standard error.

Species	N	S	h	Hd ± SE	π ± SE	k ± SE
D. cinerella	13	26	12	0.987 ± 0.035	0.013 ± 0.007	7.397 ± 3.700
D. hamaticornis	15	22	14	0.991 ± 0.028	0.009 ± 0.005	4.914 ± 2.536
D. hyperborea	36	17	13	0.764 ± 0.070	0.006 ± 0.003	3.919 ± 2.011
D. kasymovi	8	16	7	0.964 ± 0.077	0.010 ± 0.006	5.821 ± 3.114
D. lavillei	8	14	7	0.964 ± 0.077	0.008 ± 0.005	4.893 ± 2.666
D. soktoshensis **sp. nov.**	6	3	3	0.733 ± 0.155	0.002 ± 0.002	1.400 ± 0.990
D. tonsa	89	37	52	0.978 ± 0.006	0.010 ± 0.005	5.013 ± 2.459
D. tsutsuii	8	11	7	0.964 ± 0.077	0.005 ± 0.003	3.107 ± 1.800
All species	183	77	109	0.984 ± 0.004	0.015 ± 0.008	7.828 ± 3.660

Diamesa cinerella group haplotypes were organized in four well-supported haplogroups by haplotype network (Fig. 37):

1) Haplogroup 1 (H1) includes sequences of the *D. tsutsuii* from Eastern Palaearctic
2) Haplogroup 2 (H2) includes sequences of the *D. lavillei* and one sequence of *D. hyperborea* (BOLD Process ID: GBMIN34289-13) from Caucasus and Iran
3) Haplogroup 3 (H3) includes sequences of the *D. soktoshensis* **sp. nov.** from Tadzhikistan
4) Haplogroup 4 (H4) includes the sequences of the remaining species: *D. cinerella, D. hamaticornis, D. hyperborea, D. kasymovi, D. tonsa* from the Western and partly Eastern Palaearctic, the northernmost locality is on Bear Island.

The four different haplogroups are ordered geographically, except for H2 which is sympatric with H4 in the Caucasus and Iran (Fig. 38). Samples of *D. soktoshensis* **sp. nov.** (H3) were collected in Tadzhikistan in which samples from haplogroup 4 were not observed, although they distributed in the bordering Kyrgyzstan (Supplementary Table S1)

Indicators of molecular diversity of haplogroups, as well as the results of neutrality tests are given in Table 10. Haplotype diversity for the H1, H2 and H4 haplogroups was high while H3 includes only 6 sequences belonging to

3 haplotypes which decrease the haplotype and nucleotide diversity rates (Tables 9–10). For the same reason and in contrast to the other haplogroups, neutrality tests show positive values for H3 but without reliable significance (Table 10).

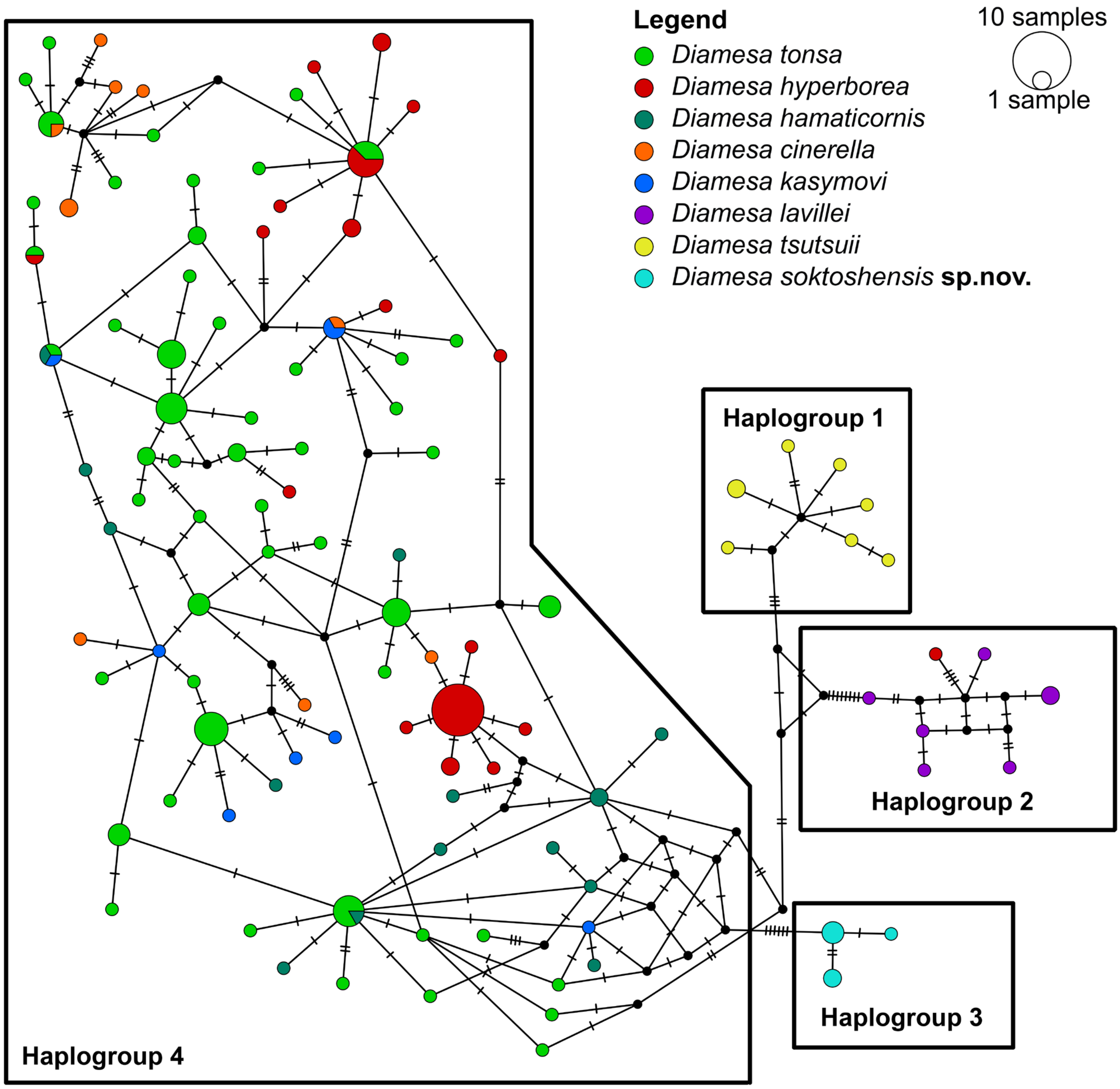


FIGURE 37. A maximum parsimony median-joining (MP) haplotype network of the COI gene from 183 samples of *Diamesa cinerella* group.

TABLE 10. Main values of COI mtDNA variability for observed haplogroups. N—number of individuals, S—number of polymorphic sites, h—number of haplotypes, Hd—haplotypic diversity, π—nucleotide diversity, k—average number of nucleotide differences, SE—standard error. **-$P < 0.01$; ***-$P < 0.001$.

Group	N	S	h	Hd ± SE	π ± SE	k ± SE	Tajima's D	Fu's Fs
Haplogroup 1	8	11	7	0.964 ± 0.077	0.005 ± 0.003	3.107 ± 1.800	-1.219	-3.671**
Haplogroup 2	8	14	7	0.964 ± 0.077	0.007 ± 0.005	4.893 ± 2.666	-0.555	-2.182
Haplogroup 3	6	3	3	0.733 ± 0.155	0.002 ± 0.002	1.400 ± 0.990	0.338	0.381
Haplogroup 4	161	62	92	0.979 ± 0.005	0.011 ± 0.006	5.625 ± 2.713	-1.474	-25.175***

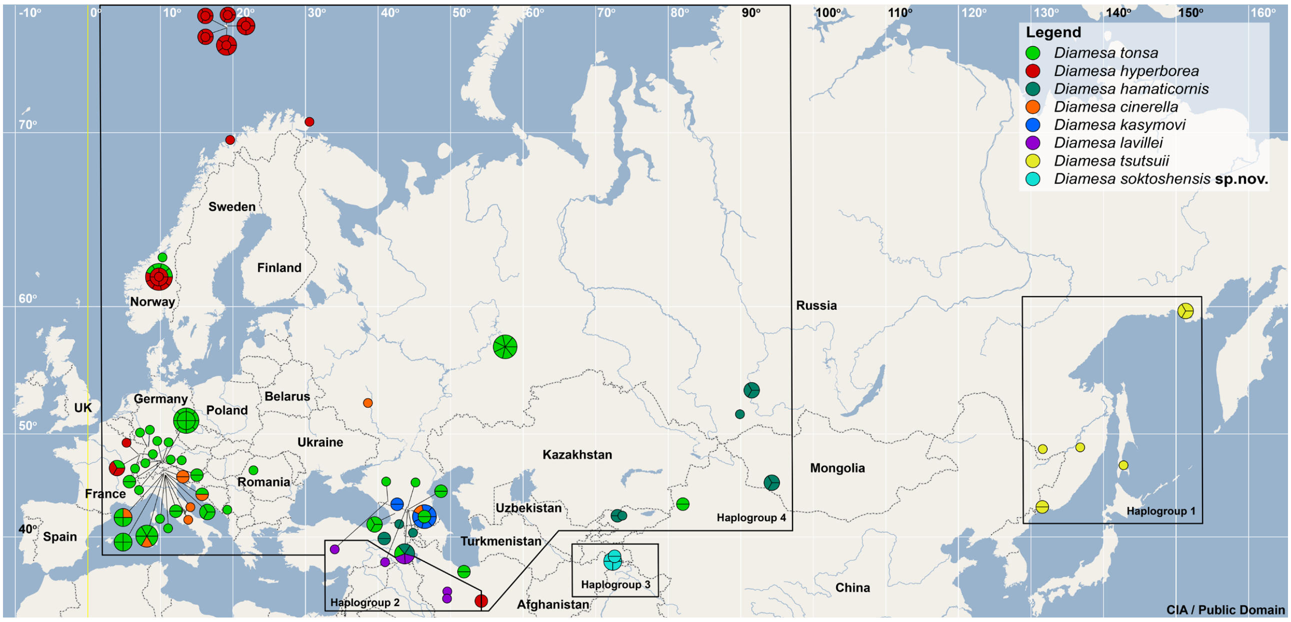


FIGURE 38. Map of *Diamesa cinerella* group localities in the Palaearctic. Indicated haplogroups corresponds to haplotype network (Fig. 37).

The study of haplogroup differentiation showed high significant FST values ($P < 0.05$) between haplogroups and a low level of gene flow (from 0.074 to 0.246) (Table 11). At a threshold value of 0.05, all haplogroups differed significantly in the number of nucleotide substitutions per site between groups, except for the pair H1 and H2. The AMOVA analysis, used to measure the proportion of genetic variation among haplogroups, confirmed significant differentiation of haplogroups ($\Phi st = 0.686$, $P < 0.001$).

TABLE 11. Genetic differentiation indices of the *Diamesa cinerella* group haplogroups: Fst values, average number of nucleotide differences per site between groups (Dxy) with standard error (SE) and flow of migrants (Nm). *-$P < 0.01$, **-$P < 0.001$.

Pairwise comparisons	Fst	Dxy ± SE	Nm
H1–H2	0.217*	0.214 ± 0.005	0.138
H1–H3	0.005**	0.048 ± 0.002	0.074
H1–H4	0.023*	0.002 ± 0.002	0.246
H2–H3	0.004**	0.047 ± 0.003	0.103
H2–H4	0.021*	0.001 ± 0.001	0.215
H3–H4	0.000**	0.004 ± 0.004	0.268

For the ITS sequences, we reconstructed a haplotype network that revealed three haplogroups. (Fig. 39). Haplogroup 1 included *D. cinerella, D. hamaticornis, D. kasymovi, D. lavillei, D. tonsa, D. tsutsuii* from Primorye Territory and five unidentified samples from Austria (*Diamesa cinerella/tonsa*). Haplogroup 2 included the remaining 9 samples from Austria. Haplogroup 3 included one sample of *D. tsutsuii* from the Magadan region (see Supplementary Table S1 for detals). The observed p-distances between clades were 2.44%, 1.78%, 2.83% between clades 1–2, 1–3 and 2–3 respectively. However, FSTst values between observed haplogroups were insignificant (FST > 0.05, -$P < 0.05$) and comparison of the 2 and 3 haplotypes resulted in zero values. Negative results are due to low sampling for each haplotype.

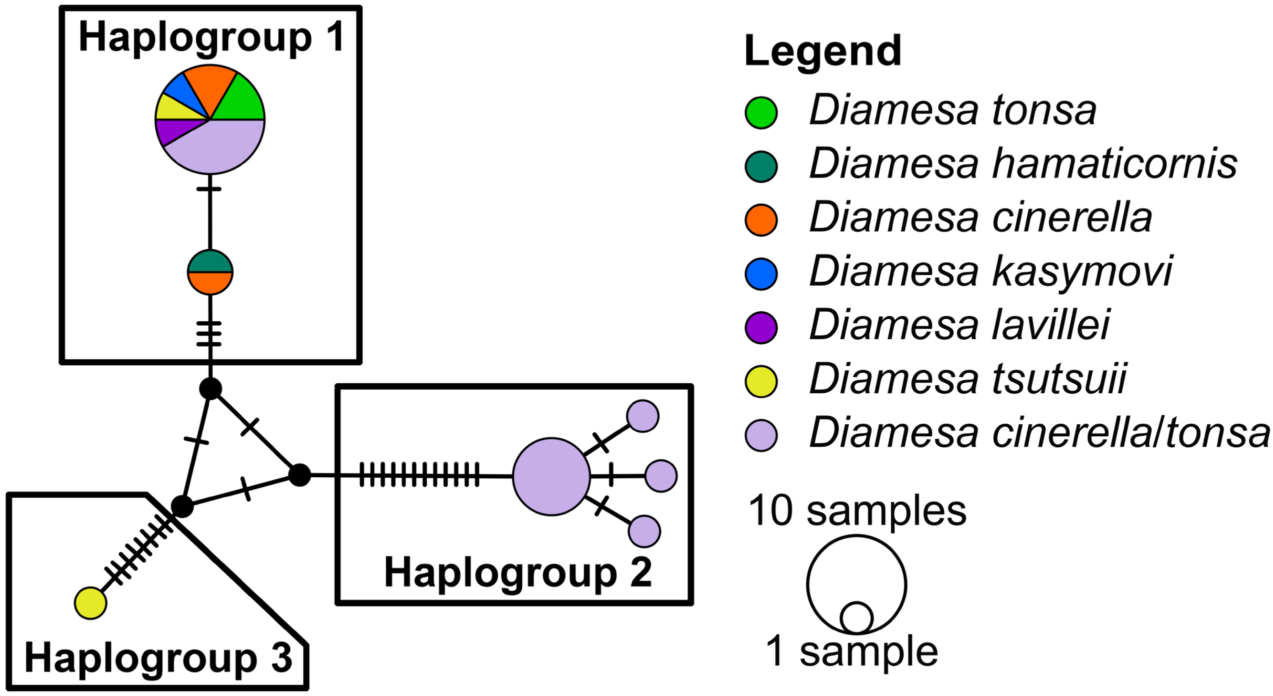


FIGURE 39. A maximum parsimony median-joining (MP) haplotype network of the ITS region from 24 samples of *Diamesa cinerella* group.

Discussion

Sequences of the *Diamesa cinerella* group using COI gene showed high haplotype diversity (0.984 ± 0.004), a large number of polymorphic sites (77), and relatively low nucleotide diversity (0.015 ± 0.008). Tajima and Fu's neutrality tests for the 1 and 4 haplogroups indicate an expansion ($P < 0.01$ and $P < 0.001$ respectively). Moreover, the first 1–3 haplogroups included only 6–8 samples, which is insufficient for reliable statistical results. However, our analysis highlighted a high genetic structure among haplogroups of the *Diamesa cinerella* group with distinctive mtDNA lineages. Each haplogroup occupies its own geographic area except for the *D. lavillei* (haplogroup 2), which shares the Caucasus with the multispecies haplogroup 4 (Fig. 38). Differentiation of haplogroups is confirmed by average number of nucleotide differences per site (0.002–0.214), the FST values (0.004–0.217, $P < 0.01$ or $P < 0.001$) and low gene flow (0.074–0.268) (Table 11). Three of the four identified lineages belong to morphologically distinct species: *D. tsutsuii* (haplogroup 1)*, D. lavillei* (haplogroup 2), and *D. soktoshensis* **sp. nov.** (haplogroup 3). Haplogroup 4 includes the remaining currently known species of the *Diamesa cinerella* group, which is confirmed by previous studies (Montagna *et al.* 2016; Lencioni *et al.* 2021, 2024; Makarchenko *et al.* 2023). The haplotype network did not reveal any clear pattern in either species or geography for haplogroup 4. For example, we expected to observe samples of *D. hyperborea* from Bear Island (big red circle in Fig. 37) at the edge of the haplotype network, however they occupied a middle position, were separated by 6–8 mutational steps from the other haplogroups. In contrast, samples from Germany, Italy, Georgia, Switzerland, and Russia belonging to *D. tonsa* and *D. cinerella* were at the edge of the haplotype network, sharing almost identical haplotypes (upper left group in Fig. 37). Weak geographic subdivision, usually combined with high haplotype diversity and low nucleotide diversity, has many examples among the Culicomorpha (Motoki *et al.* 2019, Semenchenko *et al.* 2024b, Bursali & Simsek 2024, Askari *et al.* 2024, Helleman *et al.* 2025). Geographical barriers in the form of straits between islands (Helleman *et al.* 2025), mountains (Bursali & Simsek, 2024), vast land distances (Semenchenko *et al.* 2024b), and oceans between continents (Motoki *et al.* 2019) are often unable to exert a significant influence on the identification of geographically determined haplogroups. The low flight ability of many groups of chironomids, for example the closely related *Pagastia orientalis* (Semenchenko *et al.* 2024b), also does not significantly contribute to the emergence of geographically isolated populations.

A remarkable situation arose with two larvae identified as *D. hyperborea* collected in Iran (GenBank accession numbers JF764759 and JF764772). The first larva was found among Caucasian and European *D. tonsa*, while the second larva belongs to haplogroup 2 that belonged to *D. lavillei* from the Caucasus and Iran (Figs 37, 38). On this basis, we can confidently conclude that at least the second larva belongs to *D. lavillei*, and not to *D. hyperborea*, whose range is much further north (Serra-Tosio 1971, Ashe & O'Connor 2009, Stur & Ekrem 2020).

Haplotype networks using ITS locus showed even more contradictory results (Fig. 39). Three well separated clades were identified that had no obvious pattern in either species or their geography. Moreover, samples collected in the same locality were found in different clades. Thus, our results suggest that the ITS locus lacks sufficient resolution for species level delimitation within the *D. cinerella* group, potentially due to high intra- and interspecific variation as well as the presence of paralogous copies resulting from incomplete concerted evolution of the ribosomal DNA array (Li & Wilkerson 2007, Mishra *et al*. 2021). Despite the poorly sampled dataset, we conclude that the inconsistency of the ITS locus resolves the conflict within the *D. cinerella* group. However, for example, using the 22 species of the genus *Chironomus*, it was shown that species-specific sequences appear and are fixed in the highly polymorphic transcribed spacers of chironomids, which are suitable for species identification (Gunderina & Katokhin 2020).

In our opinion, the prospects for DNA barcoding of the *Diamesa cinerella* group are associated with the analysis of concatenated nuclear genes after high-throughput sequencing and/or the search for and analysis of genes associated with the reproductive system of chironomids.

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Supplementary Materials. The following supporting information can be downloaded at the DOI landing page of this paper.

Supplementary Table S1