Zootaxa 5636 (3): 499–510
https://www.mapress.com/zt/

Article

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

https://doi.org/10.11646/zootaxa.5636.3.5
http://zoobank.org/urn:lsid:zoobank.org:pub:1482A0F6-F2D5-44AE-B9B1-6DE3E131E8A6

On the taxonomy and distribution of *Diamesa gregsoni* Edwards, (Diptera: Chironomidae: Diamesinae), with morphological redescription and DNA barcoding of species from the Far East

ALEXANDER A. SEMENCHENKO[1,2] & EUGENYI A. MAKARCHENKO[1*]
[1]*Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, 100 let Vladivostoku 159, 690022 Vladivostok, Russia.*
[2]*Far Eastern Federal University, Institute of the World Ocean, 10 Ajax Bay, Russky Island, 690922 Vladivostok, Russia.*
semenchenko_alexander@mail.ru; https://orcid.org/0000-0001-7207-9529
makarchenko@biosoil.ru; http://orcid.org/0000-0003-2765-8729
[*]*Corresponding author*

Abstract

By analyzing the DNA barcoding data of the *Diamesa gregsoni* Edwards syntype, we carried out a genetic study of this species from the Far East and made a comparison with DNA barcodes of North American populations. A morphological redescription of the adult male and a description of the pupa and larva are given, and the taxonomy and distribution of the species are clarified in this study. The Bayesian tree revealed two well-supported clades of *D. gregsoni* from Nearctic and Palaearctic. The average K2P genetic divergence between these clades was 1.67%, which corresponds to intraspecific differences. Overall intraspecific p-distances within 23 DNA barcodes of *D. gregsoni* were 1.19%. The Automatic Barcode Gap Discovery (ABGD), Assemble Species by Automatic Partitioning (ASAP), and Multi-rate Poisson tree processes (mPTP) approaches for the species delimitation confirmed that Nearctic and Palaearctic DNA barcodes belong to a single molecular taxonomic unit, while general mixed Yule-coalescent (GMYC) delimit the dataset into three different molecular operational taxonomic units (mOTU).

Key words: Diptera, Chironomidae, *Diamesinae, Diamesa gregsoni*, redescription, DNA barcoding, Far East.

Introduction

Taxonomic studies of Diamesinae often encounter difficulties due to the many molecular taxonomic units within species and the lack of clear morphological differences. Examples of such species are *Pseudokiefferiella parva* (Edwards), *Pseudodiamesa branickii* (Nowicki), and *Pseudodiamesa nivosa* (Goetghebuer) (Makarchenko & Semenchenko 2023, Makarchenko *et al.* 2023). Such taxonomic problems are compounded in species with unusually wide ranges including several biogeographic zones and the absence of DNA barcodes throughout their range, collection of only immatures instead of adult males, and unclear morphological features in their primary description. Unraveling this mosaic is impossible without DNA barcoding and/or clarification of the morphological features of specimens from the type locality, ideally from type specimens. *Diamesa gregsoni* Edwards, 1933 is potentially a cryptic species due to its circumpolar range, including the Nearctic (Akpatok Island, Nunavut [type locality]), the Eastern Palaearctic (China, Japan, Russian Far East), and the Western Palaearctic (Norway, Sweden, Novaya Zemlya) (Ashe & O'Connor 2009).

The project Biodiversity Genomics Europe (https://biodiversitygenomics.eu/) has generated a DNA barcode for the syntype of *D. gregsoni* (BOLD Sample ID: BGE_00011_D03), which united unidentified Nearctic larval and adult chironomids under a single barcode index (BOLD:AAL7629).

In recent years, a project on Integrated Ecological Monitoring of Marine and Terrestrial Ecosystems of Kamchatka has been implemented, during which samples of amphibiotic insects, including *D. gregsoni*, were collected. In this study, we analyzed specimens from the Kamchatka Peninsula, chironomids collected in the Khabarovsk Territory, Magadan Region, and Sakhalin Island, as well as DNA barcodes of chironomids collected in Canada and the USA from the

Accepted by A. Namayandeh: 22 Apr. 2025; published: 19 May 2025

Barcode of Life Data System (BOLD) including the syntype specimen. DNA barcoding using a fragment of cytochrome c oxidase I (COI) has been used to successfully delimit species of chironomids, including those in the genus *Diamesa* (e.g., Montagna *et al.* 2016, Lencioni *et al.* 2021, Lencioni *et al.* 2024, Makarchenko *et al.* 2022a, 2022b, 2023).

The main aims of the present study were: (a) to make a morphological redescription of adult male, describe the pupa and larva of *D. gregsoni* from the Far East, and discuss morphological variability; (b) to provide the DNA barcodes of *D. gregsoni* from the Far East and compare them with Nearctic sequences, including the syntype, using three species delimitation approaches; (c) discuss the transfer of *D. gregsoni* from the *D. aberrata* group to the *D. gregsoni* group based on molecular data.

Materials and methods

The adult males, pupae, and larvae of chironomids were collected in Magadan Region, Sakhalin Island, Kamchatka, and Khabarovsk Territories of Russia, as well as on Hokkaido of Japan, preserved in 96% ethanol for DNA analysis and in 70% ethanol for further study of morphology. The material was slide-mounted in polyvinyl lactophenol. The terminology follows Sæther (1980). The photographs were taken using an Axio Lab.A1 (Carl Zeiss) microscope with an AxioCam ERc5s digital camera and then stacked using Helicon Focus software. The final illustrations were post-processed for contrast and brightness using Adobe® Photoshop® software.

The study employs specimens deposited in the Bioresource Collection of the Federal Scientific Centre of East Asia Terrestrial Biodiversity of the Far East Branch of the Russian Academy of Sciences (reg. number 2797657).

Total genomic DNA extraction was performed using the Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions with elution of resultant DNA in 100 µl. The fragment of mitochondrial gene COI was amplified using the universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994). The following polymerase chain reaction (PCR) regime was employed: initial step at 95°C for 30 s, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 48°C for 30 s and elongation at 72°C for 1 min, with a final extension phase of 72°C for 5 min, and ending with a 10°C hold. PCR was performed in a reaction volume of 10 µl using 5 µl Go Taq Green Master Mix (Promega corp, Madison, WI, USA), 0.5 µM of each primer, 3.5 µl nuclease-free water, and 1.0 µl of genomic DNA. PCR products were electrophoresed in 1.5% agarose gels at 120 V for 30 min. Positive products after electrophoresis were purified using Exonuclease I (ExoI) and Thermosensitive Alkaline Phosphatase (FastAP) (Thermo Fisher Scientific Inc., USA) and then sequenced bidirectionally. Purified PCR products served as templates for sequencing reactions using the same primers used in PCR. Sequencing reactions had a total volume of 10 µl and included 10 pmol of each primer and reagents of BigDye terminator v3.1 cycle kit. The PCR products were sequenced on an ABI 3130x sequencer (Applied Biosystems Foster City, CA, USA) located at the Far Eastern Federal University, Department of Cell Biology and Genetics.

Forward and reverse sequences were manually assembled and edited using Finch TV and MEGA 7 (Kumar *et al.*, 2016) and aligned in MEGA7 (Kumar *et al.* 2016) using the CLUSTAL W algorithm (Thompson *et al.* 1994). Based on the K2P, inter- and intraspecific genetic distances are calculated using MEGA7.

To reconstruct phylogenetic tree and delimit species, we obtain a dataset included our own DNA barcodes, syntype (BIN BOLD:AAL7629) from Akpatok Island (Canada) stored in Natural History Museum (London), additional 10 undetermined to species level sequences from Nearctic belonging to the same BIN BOLD, sequence of *D. gregsoni* from Khabarovsk Territory obtained in previous study (Semenchenko *et al.* 2024) as well as DNA barcodes of *D. urvantsevi* Krasheninnikov et Makarchenko and *D. loeffleri* Reiss as outgroups.

Species delimitation for the obtained dataset was provided using distance-based approaches (ABGD, ASAP) and tree-based approaches (mPTP and GMYC). Automatic Barcode Gap Discovery (ABGD) analysis (Puillandre *et al.* 2012) is used on the website (https://bioinfo.mnhn.fr/abi/public/abgd/abgdold.html) with relative gap width (X = 1.0) and intraspecific divergence (P) values between 0.001 and 0.100 with the p-distance model. Assemble Species by Automatic Partitioning (ASAP) analysis was implemented on the website (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html, Puillandre *et al.* 2021) with p-distances. Tree-based approaches Multi-rate Poisson tree processes (mPTP, Kapli *et al.* 2017) and general mixed Yule-coalescent (GMYC, Fujisawa & Barraclough 2013) were run on the web servers (https://mptp.h-its.org/ and https://species.h-its.org/gmyc/) respectively using default parameters. The input ultrametric tree for GMYC was constructed using BEAST (Drummond *et al.* 2012). Settings were as follows: Strict clock, TN93+G nucleotide substitution model (Tamura & Nei 1993), Yule speciation process model (Gernhard 2008) and MCMC chain using 100 million generations.

PartitionFinder 2.1.1 (Lanfear *et al.* 2012) is used to select the best-fit partitioning scheme and models separately for each codon position of COI using the greedy algorithm with linked branch lengths for the corrected Bayesian Information Criterion as the optimality criterion for model selection. The best models for the first, second, and third codon position of COI was K80 (Kimura 1980), F81 (Felsenstein 1981), and HKY+G (Hasegawa *et al*., 1985). Bayesian analyses were performed with MrBayes v3.2.7 (Ronquist *et al*. 2012). For Bayesian analyses, two runs (each with four chains) were started with a setting of 5 000 000 generations and a temperature setting of 0.1, with the first 25% of sampled trees discarded as burn-in. Strict clock model (brlenspr=clock:uniform) was used to obtain an ultrametric tree. Moreover, trace files of BI analysis were visually inspected in Tracer 1.7 (Rambaut *et al.* 2018) and then the tree is visualized in FigTree v. 1.4.4. The obtained sequences have been deposited in GenBank under numbers PV258989–PV258999.

Results of DNA barcoding

We sequenced fragments of the cytochrome oxidase subunit I (658 bp in length) of 11 samples of *D. gregsoni* collected from Kamchatka, Khabarovsk Territories, and Magadan Region. The complete aligned dataset (see Material and Methods) included 23 samples of *D. gregsoni* and 3 outgroups. Among sequences of *D. gregsoni*, 28 synonymous and 2 nonsynonymous mutations of which 21 transitions and 9 transversions were identified. Eighteen mutations were parsimony-informative.

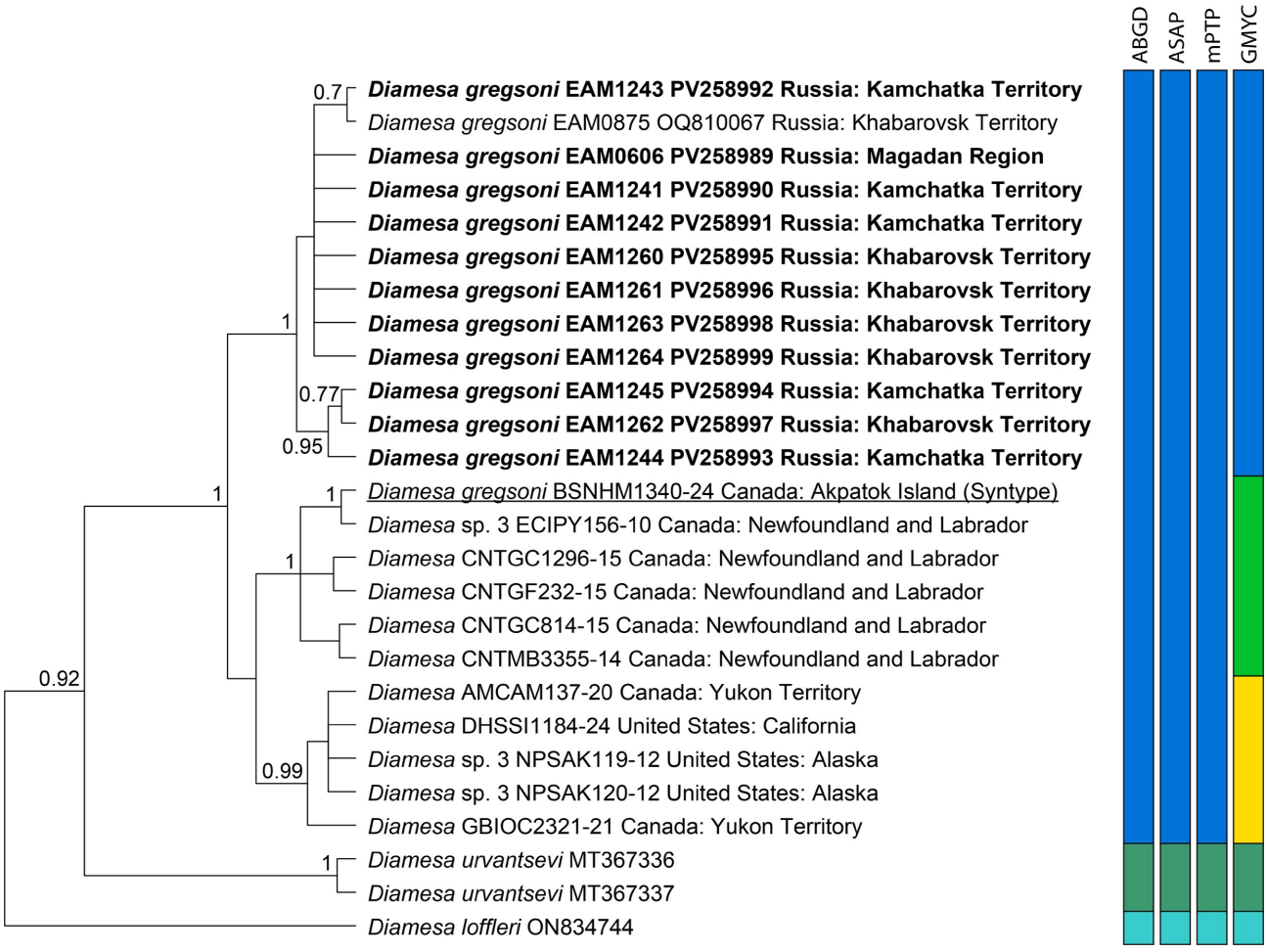

FIGURE 1. Ultrametric Bayesian inference (BI) tree based on the cytochrome c oxidase I (COI) nucleotide sequence data of the *Diamesa gregsoni* Edwards and outgroups *D. urvantsevi* Krasheninnikov et Makarchenko and *D. loffleri* Reiss. Bayesian posterior probabilities (higher than 0.7) are given above tree nodes. The syntype of the *D. grensoni* is underlined. Specimens obtained in this study are in bold.

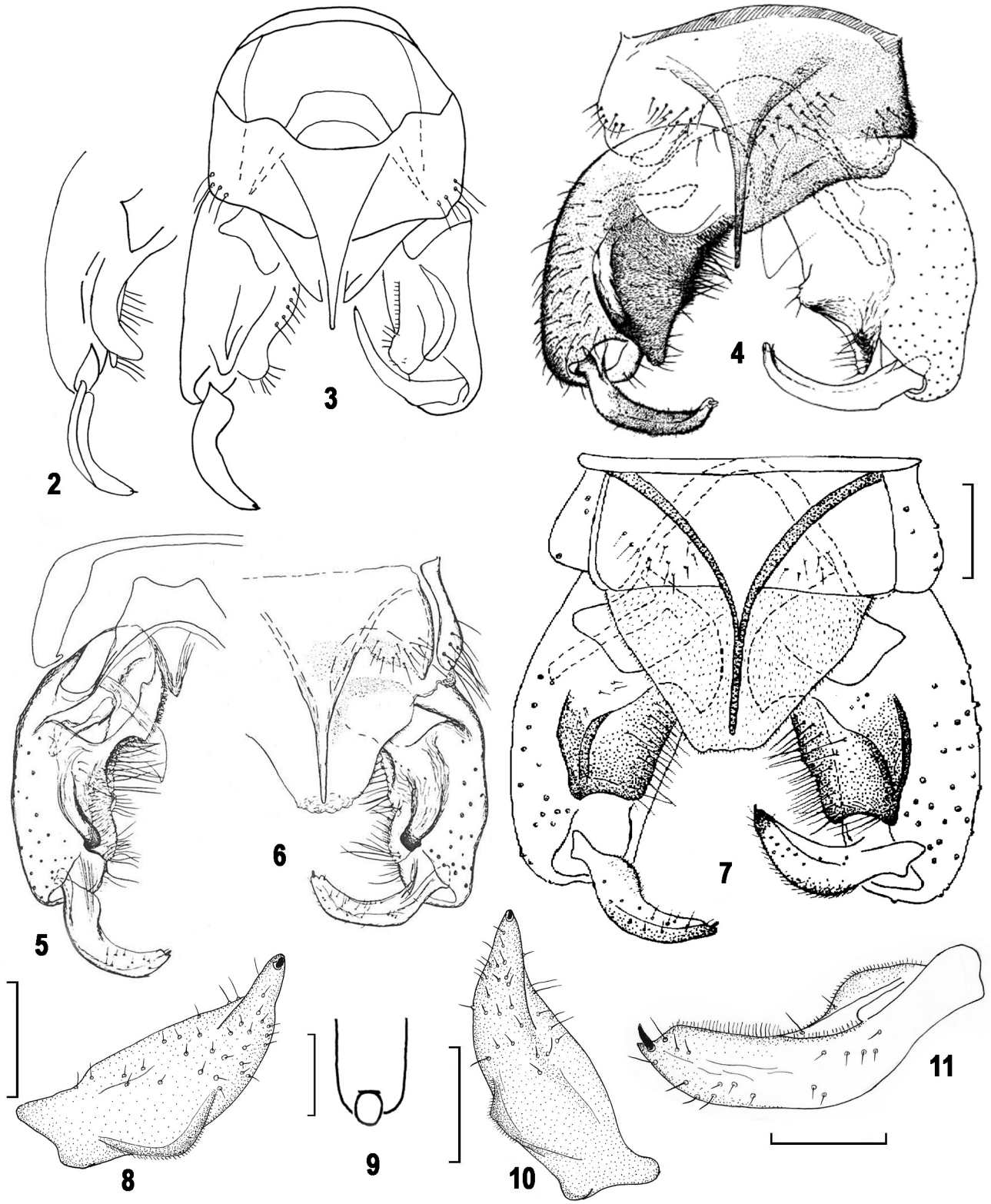

FIGURE 2–11. Adult male hypopygium structures of *Diamesa gregsoni* Edwards. **2,** gonocoxite and gonostylus; **3–4, 6–7,** hypopygium in dorsal view, with tergite IX; **5,** hypopygium in dorsal view, without tergite IX; **8, 10–11,** gonostylus in varies positions; **9,** apex of anal point. **Figs 2–3,** holotype, (after Edwards 1933); **Fig. 4,** from Norway (after Serra-Tosio 1971); **Figs 5–6,** from North America (after Hansen & Cook 1976); **Fig. 7,** from Kamchatka (after Makarchenko 1985).
Scale bars—Figs 7–8, 10–11—50 μm; Fig. 9—20 μm.

The average intraspecific pairwise K2P distance for 23 samples of *D. gregsoni* was 1.19%, while only the values for the Nearctic and Palaearctic chironomids were slightly lower 0.89% and 0.48%, respectively. Genetic divergence between Nearctic and Palaearctic chironomids was 1.67% on average. We identified three fixed mutations in the COI between them. At present, *D. urvantsevi* is molecularly the closest species to *D. gregsoni*, with interspecific distances averaging 4.63%. Another closely related indeterminate species (BOLD:AEB7777) differs from *D. gregsoni* by 6.60%.

Distance based ABGD analysis yielded 3 molecular operational taxonomic units (mOTU) using a 0.001–0.0077 intraspecific divergence. The results were confirmed by the ASAP with the best asap-score 2.00 (P-val 0.483, threshold distance 0.024) and mPTP analysis (Fig. 1). However, GMYC analysis distinguishes between the Palaearctic and two clades of the Nearctic specimens of *D. gregsoni*.

Trees resulting from the Bayesian analyses yielded two sister clades of *D. gregsoni* from the Nearctic (Canada, USA) and the Palaearctic (Far East, Russia) with a high nodal support (Bayesian posterior probability, BPP = 1) (Fig. 1). Within each clade, two subclades with no clear geographical pattern were identified, and the Nearctic subclades were not supported by Bayesian analyses.

Multilocus analysis (Semenchenko *et al.* 2024) showed that *D. gregsoni* and D. *urvantsevi* belong to the sister clades, which is confirmed only by the COI in this study. However, these species do not share any morphological features with each other; in particular, they have a different structure of the male hypopygium. Nevertheless, to prevent paraphyletic clades, *D. gregsoni* was transferred from the *D. aberrata* group (Lencioni *et al*. 2021, Han *et al*. 2022) to the *D. gregsoni* group, where *D. urvantsevi* was also included (Semenchenko *et al.* 2024). The status of the identified *D. gregsoni* group may be revised in the future with an increase in the sample of sequenced *Diamesa*.

Morphological descriptions

Diamesa gregsoni Edwards

(Figs 7–25)

Diamesa gregsoni Edwards, 1933: 618; Serra-Tosio 1967: 93, 1971: 145; Hansen & Cook 1976: 91; Makarchenko 1985: 93, 2006: 266, 474, 614; Ashe & O'Conner 2009: 276.

Material examined. JAPAN: 1 adult male, Hokkaido, Supporo, Jozenkei, 05.IV.1990, leg. Makarchenko. **RUSSIA:** 4 pharate adult males, extracted from mature pupae, 3 larvae, Magadan Region, Olsky District, Ola River, 127 km, 22.XI.2013, leg. E. Khamenkova; 6 adult males the same data, except 137 km below the bridge, 60.412194 N, 151.514564 E, 29.04–13.V.2017, leg. E. Khamenkova; 5 larvae, Kamchatka Territory, Yelizovsky District, Paratunka River, 52.87888 N 158.20930 E, 18.IX.2022, leg. A. Semenchenko; 5 adult males, 3 pharate adult males extracted from mature pupae, 4 pupae, 6 larvae, Khabarovsk Territory, Solnechny District, the vicinity of the Gorny Village, Levaya Silinka River, 11.V.1984, leg. E. Makarchenko; 1 adult male, the same data, except Nanaisky District, Anyuisky National Park, Pikhtsa River (tributary of Gassi Lake), Amur River basin, 48.796733 N, 136.783783 E, 26.V.2020, leg. Yavoskaya; 5 larvae, the same data, except Tuguro-Chumikansky District, 53.675535 N, 137.040644 E, 10.VIII.2022, leg. N. Yavorskaya; 5 adult males, Sakhalin Island, Noglikskyi District, Chamginsky Pass, Khrebtovyi Stream, 50.758757 N, 143.285754 E, alt. 743 m a.s.l., 28.VII.2003, leg. E. Makarchenko.

Description

Adult male (n =8, except when otherwise stated). Total length 4.3–5.2 (4.7) mm. Wing length 3.64–4.36 (4.17) mm. Total length/wing length 1.03–1.23 (1.14).

Coloration. Dark brown. Head, thorax, abdomen, and hypopygium dark brown. Antenna greyish. Legs brown to dark brown. Wings greyish.

Head. Eyes reniform, bare. Temporal setae including 10–19 preoculars, 14–23 verticals and 17–18 postorbitals. Clypeus with 12–21 setae. Antenna with 13 flagellomeres and well-developed plume; terminal flagellomere with 1 subapical seta, 48–56 μm long; pedicel with 3 setae, 68–108 μm long; AR 1.31–1.71 (1.53). Palpomere length (μm) (n = 6): 48–64, 112–136, 156–188, 164–200, 204–304. Palpomere 3 in distal part with sensilla capitata with diameter 20 μm. Head width/palpal length 0.90–1.27 (n = 6).

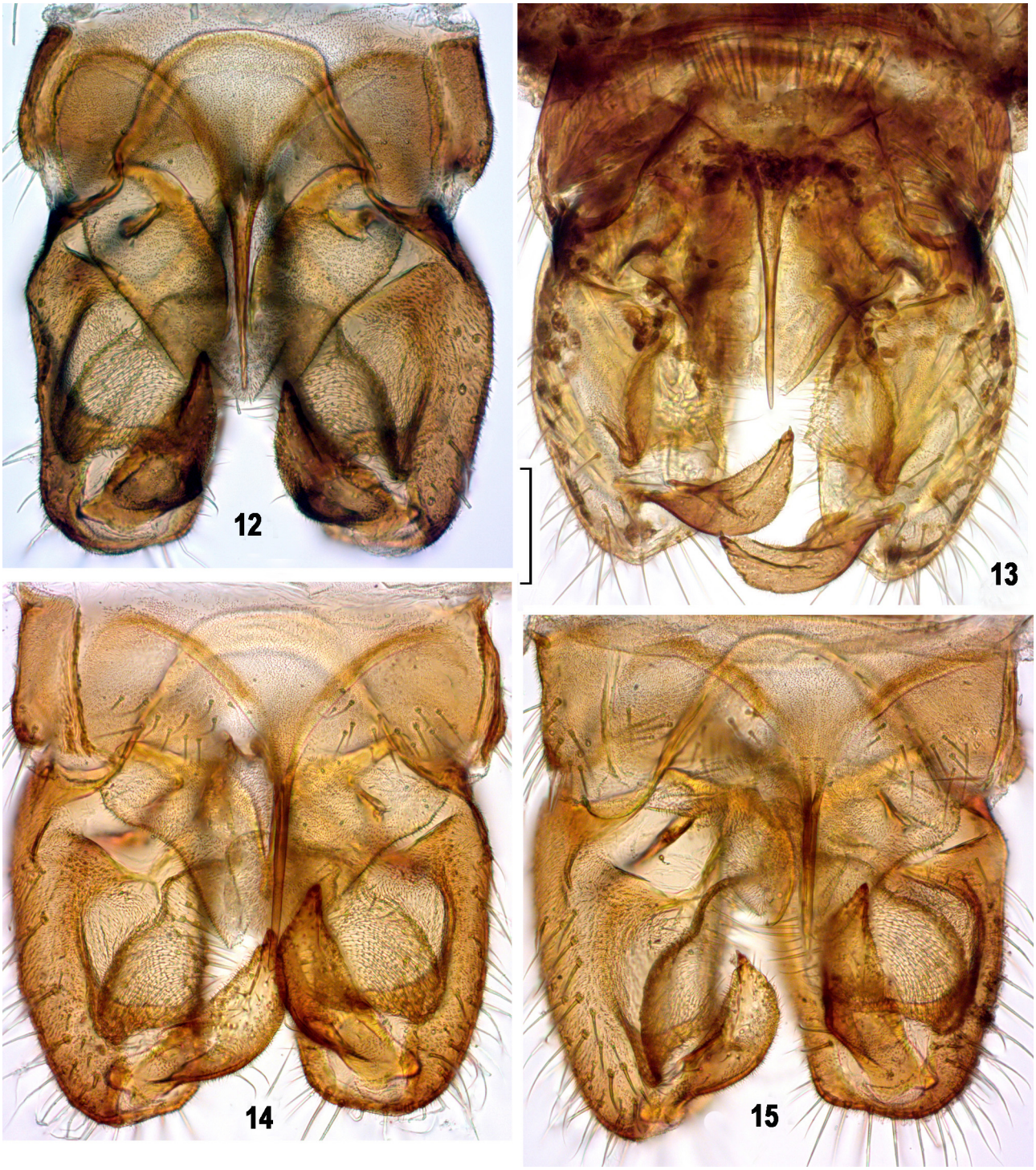

FIGURE 12–15. Adult male hypopygium of *Diamesa gregsoni* Edwards in dorsal view from Ola River (Magadan Region of Russia) **(12)**, Hokkaido (Japan) **(13)**, Sakhalin Island (Russia) **(14)** and Amur River basin (Russia) **(15)**. Scale bar—50 µm.

Thorax. Antepronotum with 5–11 (9) ventrolateral setae. Dorsocentrals 10–15 (13), prealars 7–17 (14). Scutellum with 23–36 setae (n=4).

Wing. Width 1.12–1.40 (1,24) mm. Costal extension 115–131 (118) µm long. Anal lobe outline rounded. Squama with 44–61 (54) setae. R and R_1 with 21–38 setae, R_{4+5} with 3–15 setae. RM/MCu 2.5.

Legs. Spur of front tibia 80–92 µm long. Spurs of mid tibia 52–60 µm and 52–76 µm long. Spurs of hind tibia 60–68 µm and 88–104 µm long. Hind tibial comb with 21 setae. Length (µm) and proportions of leg segments are as in Table 1.

TABLE 1. Lengths (in μm) and proportions of leg segments of *Diamesa gregsoni* Edwards, male (n = 8)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
P_1	1280–1600	1600–1880	1080–1320	508–689	344–426	115–164	148–164	0.67–0.73	3.12–3.74	2.42–2.84	1.8–2.0
P_2	1560–1720	1480–1720	760–840	394–476	230–312	115–148	148–164	0.44–0.51	3.90–4.41	4.0–4.53	2.0–2.4
P_3	1600–2000	1800–2080	1120–1720	558–705	344–394	131–148	148–164	0.62–0.83	3.70–3.95	2.37–3.13	2.0–2.4

Hypopygium (Figs 7–16). Tergite IX with 8–22 setae on one side; anal point narrow and long, slender distally but broadening basally, 136–184 μm long and 8–9 μm wide distally, with minute apical rounded peg (Fig. 9). Laterosternite IX with 5–11 setae. Transverse sternapodeme (TSA) trapezoidal or rounded *ca* 280 μm long. Phallapodeme 156 μm long. Gonocoxite 276–344 μm long; large inferior volsellae covered with long setae (Figs 7, 12–16). Gonostylus 148–192 μm long, slightly curved when pressed against the gonocoxite, with a slightly convex outer edge and an almost straight inner edge; in some males, a slight protrusion is noticeable along the inner edge in the basal half (Figs 8, 10). When bent back, the gonostylus is expanded basally (Figs 11, 16), its surface with short setae; apex with subterminal peg and short macroseta, *ca* 8 μm long. HR 1.5–2.1.

Pupa (n=4) brownish, exuviae brownish yellow to yellow. Total length 5.2–6.4 mm.

Cephalothorax. Frontal tubercles reduced, frontal apotome with 2 setae 280–308 μm long. Thorax scaly in anterior part and wrinkled in posterior. Thoracic horn filiform, 388–528 μm long, brown at base, yellowish distally, approximately same width (16–20 μm) up to the middle, then gradually narrows and at top with some small teeth. Two dark brown precorneal setae anterior to thoracic horn: Pc_1 330–355 μm, Pc_2 236–285 μm long (Fig. 17). Antepronotum with 2 median setae 132–164 μm long and 1 lateral antepronotal 64–88 μm long. Mesonotum with 2 dorsocentrals: Dc_1 strong, 300–336 μm long, Dc_2 hair-like, 28–32 μm long.

Abdomen. Tergite I without shagreen or teeth. Tergites II–VII with shagreen in anterior third or half, tergite VIII almost covered with shagreen. Sternites I–II without shagreen, sternites III–VIII with sparse shagreen and IX without shagreen. Tergite I and sternites I–II without posterior transverse row of spines. Tergites II—VIII with posterior transverse row spines, number of these spines on these tergites: 7–12, 7–12, 8–11, 7–9, 6–8, 7–10, 5–9 (Figs 18–19). Number of posterior transverse row spines of sternites III–VIII: 7–9, 9–11, 8–10, 8–13, 8–14, 8–15 (Fig. 20). The total number of spines of the anal rows of tergites 55–57 and sternites 69–73. Segment I with 2 pairs of lateral setae, 48–96 μm long; segments III–VII with 3 pairs of strong lateral setae, 152–208 μm long (L_1–L_3) and 1 pair of hair-like setae, 24–100 μm long (L_4). Segment VIII with 3 pairs of lateral setae 80–216 μm long. Segments II –VIII with spine-like process on posterolateral corners. Anal lobe with 3 yellow anal macrosetae, 292–312 μm long, slightly curved in distal part and pointed. Male genital sac not extended or slightly extended beyond anal lobe (Fig. 21).

Fourth instar larva (n=4). Total length 6.5–8.5 mm. Head capsule uniformly dark brown to black., 656–672 μm long and 380–492 μm wide. S_I and S_{II} short and simple, S_{III} bifurcate, hair-like. Labral lamellae consisting of 5 lobes. Premandible broad, apically with 6–7 teeth (Fig. 24). Antenna with 5 segments; Lauterborn organs small; style reaches base of fourth segment; longest branch of antennal blade reaches the apex of the fourth segment; large ring organ located in basal quarter of first segment (Fig. 22); AR 1.55–1.80, (1.68). Mandible dark brown to black, with apical tooth and 4 inner teeth; apical tooth slightly longer than the first inner tooth; seta subdentalis minute; seta interna with 24–26 simple branches (Fig. 25). Mentum with 1 median and 9–10 pairs of lateral teeth; median tooth twice as wide as the first lateral tooth and same height as it; ventromental plate small (Fig. 23). Procercus dark brown, in the form of incompletely sclerotized ring, bearing 4 dark brown strong anal setae, 268–308 μm long and 1 hair-like lateral seta which is on the body, 56–60 μm long. Posterior parapod is 2 times as long as the last body segment. Dorsal and ventral pairs of anal tubules 160–212 μm long, dorsal pair is slightly thicker.

Remarks. Unfortunately, a detailed comparative morphological analysis of the adult males of known populations of *D. gregsoni* cannot be carried out due to insufficient data for individuals from Canada and Norway, but nevertheless, it can be stated that the males are similar in some basic features (Table 2) and in the structure of the hypopygium. However, it should be noted that there is a difference in the shape of the gonostylus of males shown in the original description (Edwards 1933) from Akpatok Island (Figs 2–3), New Brunswick (Hansen & Cook 1976) (Figs 5–6), Norway (Sierra- Tosio 1971) (Fig. 4), and the Far East (Figs 7–16). This may be due to the position of the gonostylus relative to the gonocoxite, as well as geographic variability, but the protrusion along the outer edge in the basal third of the gonostylus (Figs 8, 10) is present only in males from the Far East. In our opinion, a revision of *D. gregsoni* adult males from North America and other areas is necessary in the future to determine the degree of variability in the form of the gonostylus.

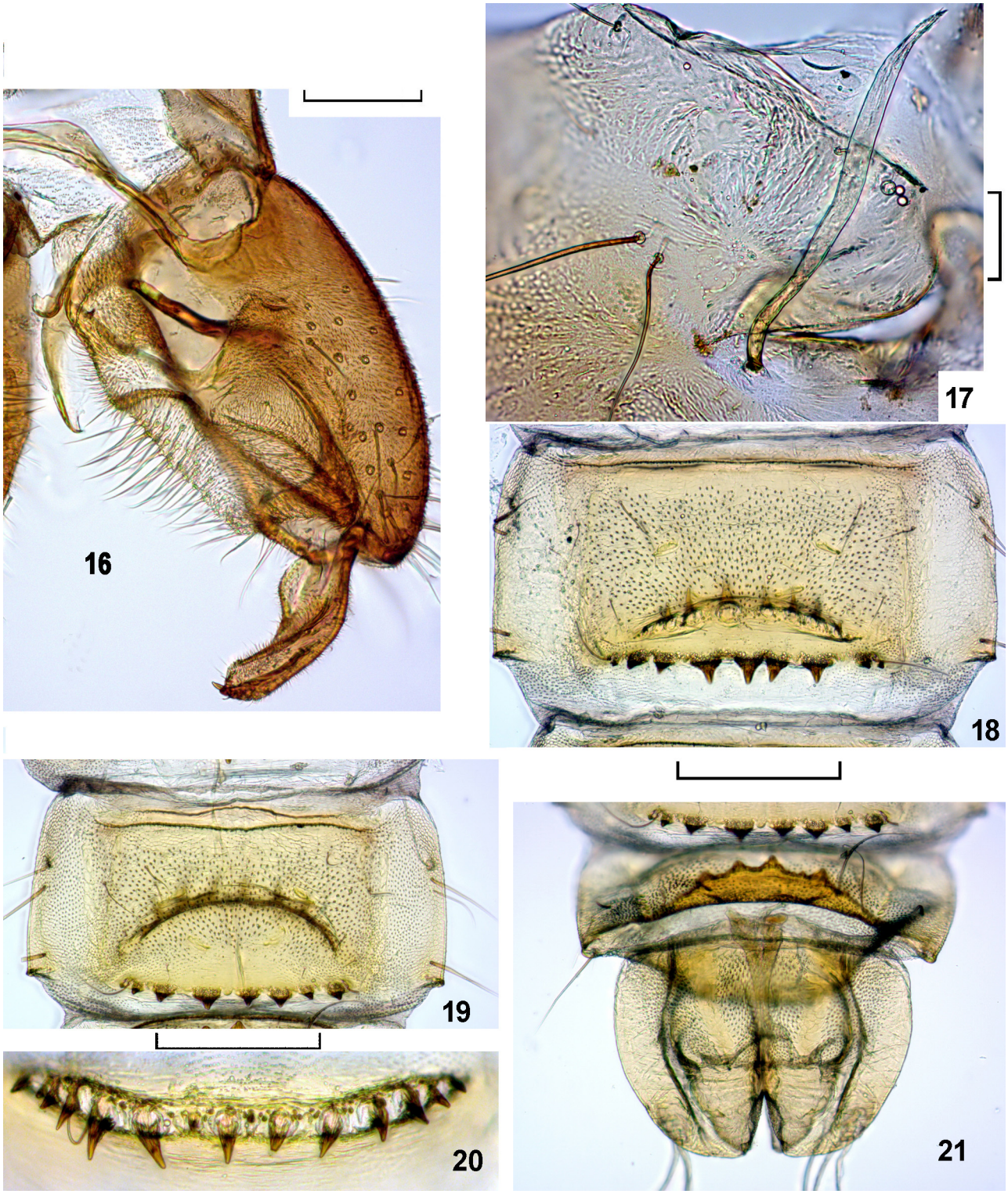

FIGURE 16–21. Adult male **(16)** and pupa **(17–21)** of *Diamesa gregsoni* Edwards. **16,** part of hypopygiuim without tergite IX; **17,** thoracic horn and precorneals; **18,** tergite IV; **19,** tergite VIII; **20,** posterior transverse row spines of sternite V; **21,** anal segment.

Scale bars—Figs 16–17—50 µm; Figs 18–21—200 µm.

TABLE 2. Comparison of some characters in adult males of *Diamesa gregsoni* Edwards from Canada, Norway, and the Far East.

Characters	Canada (Akpatok Island, New Brunswick) (n=2) Edwards 1933*; Hansen & Cook 1976	Norway (n = 1) Serra-Tosio 1967, 1971	Far East (n = 8)
Total length, mm	4.6	4–5	4.3–5.2
Wing length, mm	3.3–3.5	3.7	3.64–4.36
AR	1.38–1.40; 1.8*	1.45	1,31–1.71
Antepronotals	12	6	5–11
Dorsocentrals	10–12	12–13	10–15
Prealars	10–15	6–8	10–17
Scutellars	36–42	–	23–36
Squamal setae	45–76	–	44–61
LR_1	0.75–0.76	0.67	0.67–0.73
BV_1	3.45–3.66	3.52	3.12–3.74
SV_1	2.45–2.46	2.83	2.42–2.84
Tergite IX, number of setae	12–14	–	10–22

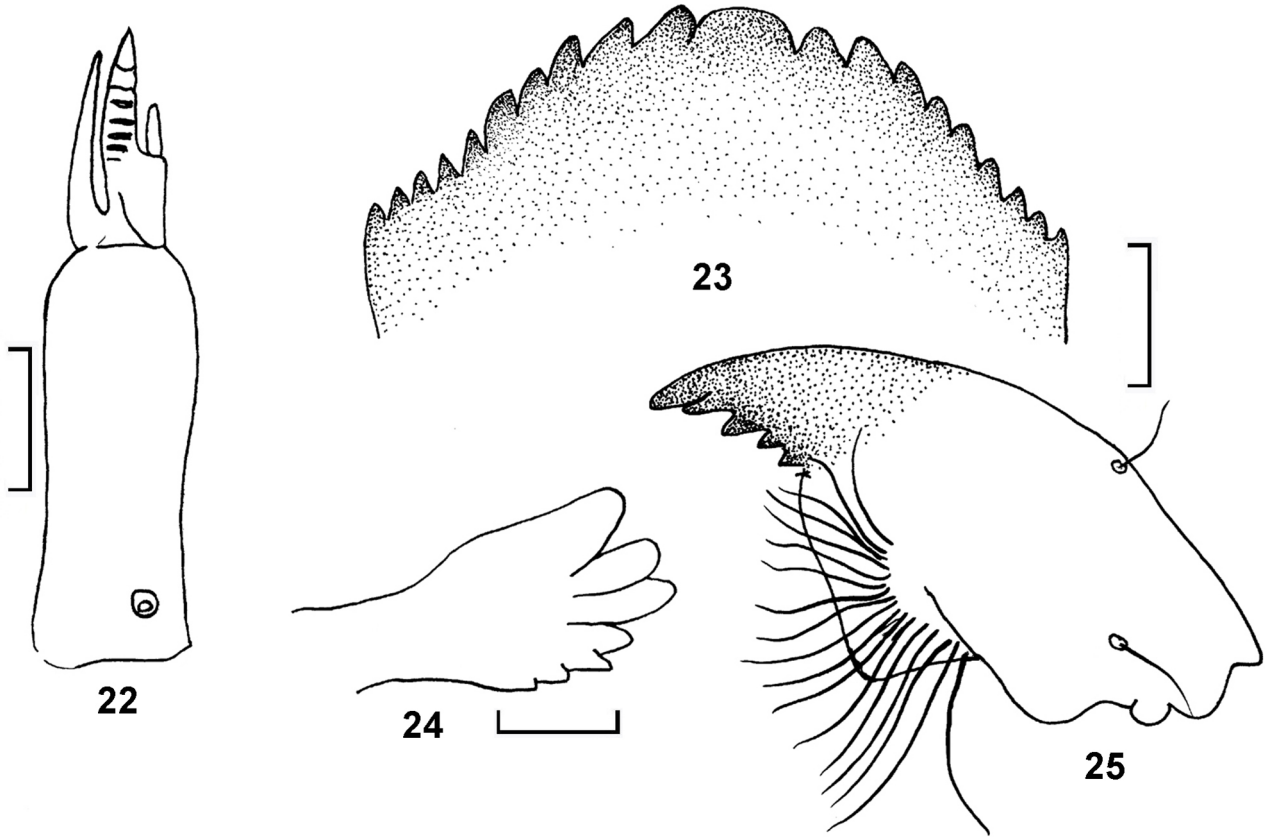

FIGURE 22–25. Larva of fourth instar of *Diamesa gregsoni* Edwards. **22,** antenna; **23,** mentum; **24,** distal part of premandible; **25,** mandible. Scale bars are 20 µm. **Figs 22, 24–25** redrawn from Makarchenko (1985). Scale bars—20 µm.

Biology and ecology. In North America, adults were caught in the first half of April in New Brunswick and in early September on Akpatok Island, Nunavut (Hansen & Cook 1976). In Europe, the species was collected in Norway at an altitude of 1837 m a.s.l. on August 11 (Serra-Tosio 1971). In the Far East, the species is bivoltine. In Kamchatka, the emergence of adults occurs from the first half of June to mid-July and in the first half of October. On the Ola River in the vicinity of Magadan City *D. gregsoni* adults were caught in late April–early May and late November. The earliest collection of males in the vicinity of Magadan City on the Dukcha River was on April 22 on

the snow. In the Amur River basin, adults were collected in late May and July, on Sakhalin, in late July at an altitude of 743 m a.s.l.

Larvae and pupae live in foothill and mountain rivers on cobble and gravel substrates with interstitial sand (Figs 26–29).

Distribution. According to our data *D. gregsoni* currently known from Canada (Akpatok Island in the Hudson Strait, Nunavut, New Brunswick, Newfoundland, Labrador, Yukon Territory), USA (Alaska, California), Norway, Japan (Hokkaido Island) and the Russian Far East—in the vicinity of Magadan, from the Kamchatka Peninsula, Sakhalin Island, the Amur River basin and the northeast of Khabarovsk Territory. Ashe & O'Connor (2009) also pointed out to the occurrence of this species in Sweden, Novaya Zemlya and China. We believe that this information should be clarified.

FIGURE 26–29. Localities of *Diamesa gregsoni* Edwards. **26–27,** Sakhalin Island, Chamginsky Pass, Khrebtovyi Stream, alt. 743 m a.s.l. (Photos by E.A. Makarchenko); **28,** Kamchatka, Paratunka River (photo by A.A. Semenchenko); **29,** Amur River basin, Anyuisky National Park, Pikhtsa River (tributary of Gassi Lake) (photo by N.M. Yavorskaya).

Acknowledgements

We are much grateful to E.V. Khamenkova, N.M. Yavorskaya, N.A. Seliverstov for collecting chironomids on the Russian Far East and making material the available to us.

We would like to acknowledge to Torbjørn Ekrem and Elisabeth Stur from NTNU University Museum, Norway and Benjamin W. Price, Duncan Sivell, Joana P. Cristóvão and Owain Powell from the Natural History Museum, London, UK for providing the COI sequence of the syntype of *Diamesa gregsoni* Edwards through the Horizon Europe funded project Biodiversity Genomics Europe (https://biodiversitygenomics.eu/).

The study was funded by the Ministry of Science and Higher Education of the Russian Federation, project

number FZNS-2024-0037 «Integrated environmental monitoring of coastal marine and terrestrial ecosystems of Kamchatka» and partly of theme No. 124012400285-7.

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.

Drummond, A.J., Suchard, M.A., Xie, D & Rambaut, A. (2012) Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, 29 (8), 1969–1973. https://doi.org/10.1093/molbev/mss075

Edwards, F.W. (1933) Oxford University Expedition to Hudson's Strait, 1931: Diptera Nematocera. With notes on some other species of the genus *Diamesa*. *The Annals and magazine of natural history; zoology, botany, and geology*, Series 10, 12, 611–620. https://doi.org/10.1080/00222933308673727

Felsenstein, J. (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution*, 17, 368–376. https://doi.org/10.1007/BF01734359

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3 (5), 294–299.

Fujisawa, T. & Barraclough, T.G. (2013) Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent approach: A revised method and evaluation on simulated data sets. *Systematic Biology*, 62 (5), 707–724. https://doi.org/10.1093/sysbio/syt033

Gernhard, T. (2008) The conditioned reconstructed process. *The Journal of Theoretical Biology*, 253 (4), 769–778. https://doi.org/10.1016/j.jtbi.2008.04.005

Han, W., Wei, L., Ni, Z. & Tang, H. (2022) New insights on *Diamesa* Meigen (Diptera, Chironomidae, Diamesinae) in the Tibetan Plateau of China. *Euroasian Entomological Journal*, 21, 23–41. https://doi.org/10.15298/euroasentj.21.Spl.1.04

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.

Hasegawa, M., Kishino, H. & Yano, T. (1985) Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, 22, 160–174. https://doi.org/10.1007/bf02101694

Kapli, P., Lutteropp, S., Zhang, J., Kobert, K., Pavlidis, P., Stamatakis, A & Flouri, T. (2017) Multi-rate Poisson tree processes for singlelocus species delimitation under maximum likelihood and Markov chain Monte Carlo. *Bioinformatics*, 33 (11), 1630–1638. https://doi.org/10.1093/bioinformatics/btx025

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16 (2), 111–120. https://doi.org/10.1007/BF01731581

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

Lanfear, R., Calcott, B., Ho, S.Y & Guindon, S. (2012) Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, 29 (6), 1695–1701. https://doi.org/10.1093/molbev/mss020

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

Lencioni, V., Prat, N., Paoli, F., Acosta, R, Rodriguez-Prieto, A & Allegrucci, G. (2024) Revisiting European and Asian Diamesa species (Diptera: Chironomidae: Diamesinae): morphological and molecular insights. *Zoological Journal of the Linnean Society*, 202 (4), zlae136. https://doi.org/10.1093/zoolinnean/zlae136

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. (2006) 3. Subfamily Diamesinae. *In:* Lelej, A. (Ed.), *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., Semenchenko, A.A & Palatov, D.M. (2022a) Taxonomy of *Diamesa steinboecki* group (Diptera: Chironomidae: Diamesinae), with description and DNA barcoding of known species. II. Subgroups *davisi*, *leona* and

loeffleri. *Zootaxa*, 5190 (3), 361–392.
https://doi.org/10.11646/zootaxa.5190.3.3
Makarchenko, E.A., Semenchenko, A.A & Palatov, D.M. (2022b) 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. (2023) Two new chironomid species of the genus *Pseudokiefferiella* Zavřel (Diptera: Chironomidae: Diamesinae) from the Amur River basin of Russia. *Zootaxa*, 5339 (5), 481–491.
https://doi.org/10.11646/zootaxa.5339.5.5
Makarchenko, E.A., Semenchenko, A.A & Palatov, D.M. (2023) Fauna and taxonomy of Diamesinae (Diptera, Chironomidae) from the Caucasus, with a morphological description and DNA barcoding of new taxa and a discussion of diagnostic problems for *Diamesa* Meigen and *Pseudodiamesa* Goetghebuer. *Zootaxa*, 5271 (2), 313–328.
https://doi.org/10.11646/zootaxa.5271.2.6
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
Puillandre, N., Brouillet, S & Achaz, G. (2021) ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources*, 21 (2), 609–620.
https://doi.org/10.1111/1755-0998.13281
Rambaut, A., Drummond, A.J., Xie, D, Baele, G & Suchard, M.A. (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67 (5), 901–904.
https://doi.org/10.1093/sysbio/syy032
Ronquist, F., Teslenko, M., Mark, P.V.D, Ayres, D.L, Darling, A, Höhna, S, Larget, B, Liu, L, Suchard, M.A & Huelsenbeck, J.P. (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology*, 61, 539–542.
https://doi.org/10.1093/sysbio/sys029
Sæther, O.A. (1980) Glossary of chironomid morphology terminology (Diptera: Chironomidae). *Entomologica scandinavica*, Supplement 14, 1–51.
Semenchenko, A.A., Cranston, P.S & Makarchenko, E.A. (2024) A multi-locus phylogeny for the Diamesinae (Chironomidae: Diptera) provides new insights into evolution of an amphitropical clade. *Zoological Journal of the Linnean Society*, 202 (4), zlae035.
https://doi.org/10.1093/zoolinnean/zlae035
Serra-Tosio, B. (1967) Un Chironomide boréal nouveau pour l'Europe: *Diamesa gregsoni* Edwards (Diptera). *Travaux du Laboratoire d'hydrobiologie et de pisciculture de l'Université de Grenoble*, 57–58, 93–96.
Serra–Tosio, B. (1971) *Contribution à l'étude taxonomique, phylogénétique, biogéographique et écologique des Diamesini (Diptera, Chironomidae) d'Europe*. Doct. Thesis, A l'Université Scientifique et Médicale de Grenoble, Grenoble, 7 + 462 + [1] pp., 184 pls. [pp. I–II, 2A–2E + 1–303 + 304–462 + [1], pls. 1–184]
Tamura, K. & Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10 (3), 512–526.
https://doi.org/10.1093/oxfordjournals.molbev.a040023
Thompson, J.D., Higgins, D.G & Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22 (22), 4673–4680.
https://doi.org/10.1093/nar/22.22.4673