Zootaxa 5159 (3): 445–450
https://www.mapress.com/zt/

Correspondence

https://doi.org/10.11646/zootaxa.5159.3.9

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

http://zoobank.org/urn:lsid:zoobank.org:pub:4401581D-CF16-4508-84EE-5DEA395409BC

Redescription of the Caucasian endemic *Diamesa caucasica* Kownacki et Kownacka (Diptera: Chironomidae: Diamesinae)

EUGENYI A. MAKARCHENKO[1*], ALEXANDER A. SEMENCHENKO[1,3] & DMITRY M. PALATOV[2]
[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] *A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Leninskij prosp. 33, 119071 Moscow, Russia*
triops@yandex.ru; https://orcid.org/0000-0002-8826-9316
[3] *semenchenko_alexander@mail.ru; https://orcid.org/0000-0001-7207-9529*
[*] *Corresponding author. makarchenko@biosoil.ru; http://orcid.org/0000-0003-2765-8729*

Diamesa caucasica Kownacki et Kownacka was described from the watercourses of the Terek River basin flowing down from the high mountain glaciers of the Caucasus (alt. 2000–2700 m a.s.l.), namely from the Chkheri River (Ortsveri Glacier), the Suatisi River (Savitisi Glacier) and the Mnaisidon River (Mna Glacier) in Georgia (Kownacki & Kownacka 1973). So far, no other finds of this species have been recorded in the Caucasus and other mountainous regions of Europe. Apparently, *D. caucasica* is endemic to the Caucasus and its distribution probably does not go beyond the glacial streams of the Terek River basin. This assumption is confirmed by the finds of adult males of this species made by Dmitry Palatov in 2018–2022 in the rivers at an altitude 1402–1980 m a.s.l., originating in the Mostocete, Tanantsete, Skazsky and Tseysky glaciers of North Ossetia and which also belong to the Terek River basin.

We found it expedient in this report to make a morphological redescription of the adult male of this rare species as well as DNA barcoding based on material collected in the glacial streams of North Ossetia.

Material and methods

The adults of chironomids were preserved in 96% ethanol for DNA-analysis and in 70% ethanol for further study of morphology. All material was collected by Dmitry Palatov.

The material was mounted in the polyvinyl lactophenol. The morphological terminology and abbreviations used below generally follow Sæther (1980). The photographs were taken using an Axio Lab.A1 (Karl 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.

Total genomic DNA was isolated from nine specimens of *D. caucasica* using a DNeasy Tissue Kit (Qiagen, Hilden, Germany) and the resultant DNA was eluted in 100 µl. Partial fragment of cytochrome *c* oxidase subunit I (COI-5P: 650 bp) were amplified using LCO1490 and HCO2198 primers (Folmer *et al.* 1994). PCR was performed in 10 µl reaction volume containing 1 µl of template DNA, 10 pmol of each specific primer, 3 µl nuclease-free water and 5 µl Go Taq Green Master Mix (Promega corp, Madison, WI, USA). The thermal conditions for amplifying both genes were set as an initial denaturation of 1 min at 95° C, followed by 35 cycles of 30 sec at 95° C, 30 sec at 48°C, 1 min at 72°C for denaturation, annealing and extension, with a final extension at 72° C for 5 min. The amplicons were purified using Exonuclease I (ExoI) and Thermosensitive Alkaline Phosphatase (FastAP) (Thermo Fisher Scientific Inc., USA) and sequenced in both directions using BigDye terminator v3.1 cycle kit and ABI 3130x sequencer (Applied Biosystems).

Intra and inter specific genetic divergence values were calculated separately for each gene using p-distances implemented in MEGA7 software (Kumar *et al.* 2016). 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 gene 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 were K80+I (Kimura 1980), F81+I (Felsenstein 1981) and GTR+G (Tavaré 1986) respectively. A Bayesian Inferences (BI) analysis was performed with MrBayes v.3.2.7 (Ronquist *et al.* 2012) under the following conditions: 10 million generations with sampling every 500 generations, four chains and a burn-in of 25% trees. Trace files were visually inspected in Tracer v. 1.7 (Rambaut *et al.* 2018).

Accepted by B. Rossaro: 12 Jun. 2022; published: 30 Jun. 2022

For the phylogenetic analysis, we used our sequences, as well as sequences from BOLD system. We use all available sequences of *Diamesa latitarsis* group, which include three BIN BOLD numbers of *D. goetghebueri* Pagast, and single BIN BOLD number for *D. latitarsis* (Goetghebuer), *D. lindrothi* Goetghebuer and *D. modesta* Serra-Tosio. Each BIN BOLD number were uniting in triangle on a reconstructed tree. Also, we used *Pseudokiefferiella parva* (Edwards) as outgroup. Obtained sequences have been deposited in GenBank (ON691466–ON691474).

All material is deposited in the collection of the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok (FSCEATB FEB RAS).

Taxonomy

Diamesa caucasica Kownacki et Kownacka

(Figs. 1–6)

Diamesa caucasica Kownacki et Kownacka, 1973: 131; Ashe & O'Connor 2009: 272.

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

Description

Adult male (n = 6, except when otherwise stated). Total length 2.9–3.5 mm. Wing length 3.4–3.6 mm. Total length/wing length 0.83–0.92.

Coloration. Head, thorax and abdomen dark brown; legs brown to light brown; wings greyish, with brownish venation.

Head. Eyes bare and not extended dorsomedially. Temporal setae including 4–6 preoculars, 12–15 verticals and 11–12 postorbitals. Clypeus with 7–10 setae. Antenna with 13 flagellomeres and developed plume; terminal flagellomere without subapical setae; AR 1.0–1.22. Palpomere lengths (in µm) (n=2): 44–48; 80–84; 128–140; 144–152; 176–196. Palpomere 3 distally with sensilla capitata (diameter 16 µm). Palpomeres 1–5 length/head width 0.94–1.0.

Thorax. Antepronotum with 4–7 lateral setae. Acrostichals absent, dorsocentrals 7–12, prealars 4–6, scutellars 19–29.

Wing. Length 3.4–3.6 mm; width 1.0–1.08 mm. R and R_1 with 20–25 setae, R_{4+5} with 2–3 setae. Costa extension 115–131 µm long. RM length/MCu length 3.0–3.6. Anal lobe developed, outline rounded. Squama with 21–26 setae, in 1–2 rows. VR 0.90.

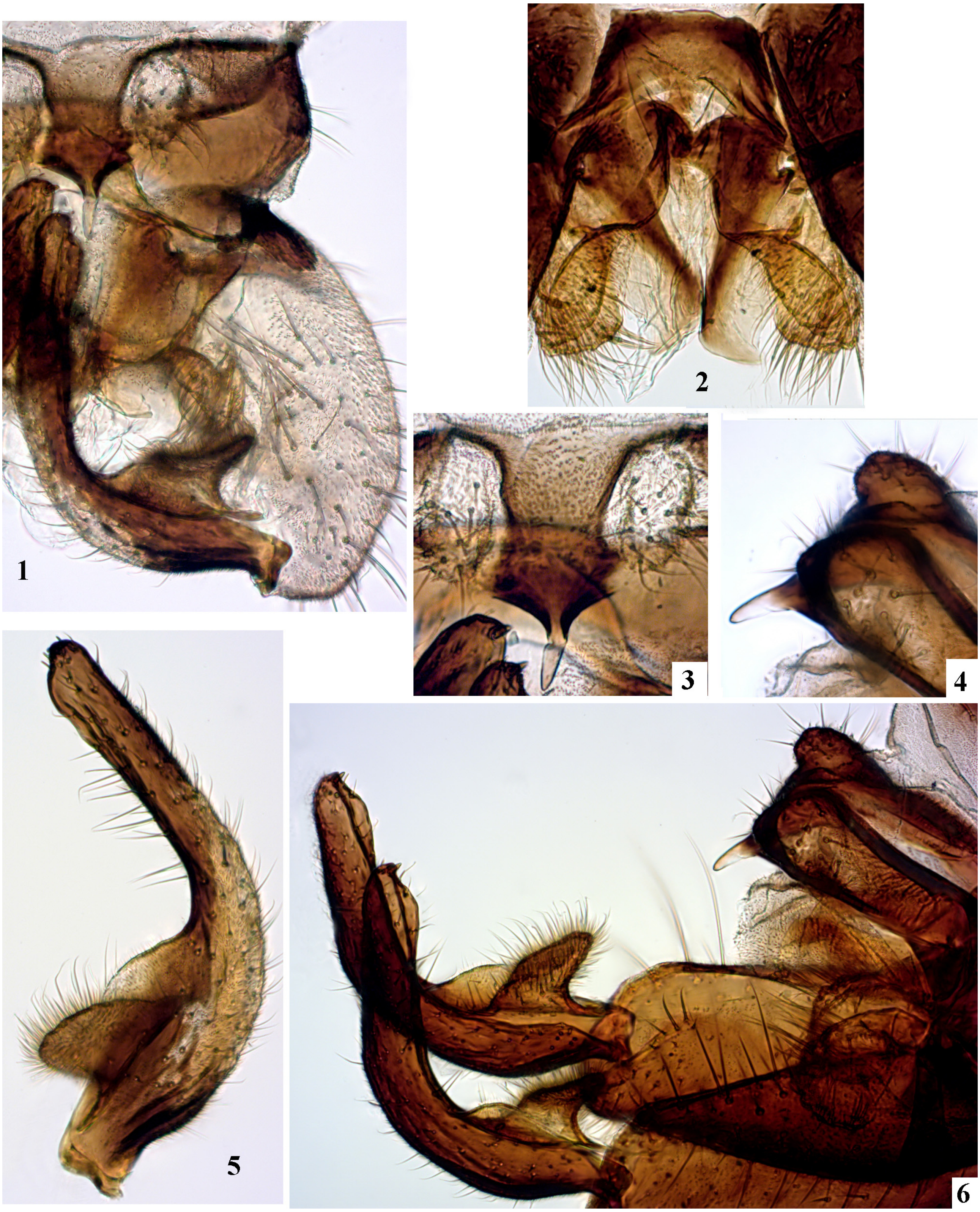
Legs. Spur of fore tibia 64–72 µm long; spurs of mid tibia 60–64 µm and 52–60 µm; of hind tibia 44–52 µm and 84–88 µm long. Hind tibial comb with 14–15 setae. Length (µm) and proportions of leg segments are as in Table 1.

TABLE 1. Lengths (in µm) and proportions of leg segments of Diamesa caucasica Kownacki et Kownacka, male (n=2)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1246–1378	1558–1607	1066	525–558	328–344	115–123	131–148
P_2	1378–1525	1361–1369	672	377	213	98–115	115–131
P_3	1542–1722	1689–1706	1115–1164	574–590	312–328	115	131–148

continued

	LR	BV	SV	BR
P_1	0.66–0.68	3.45–3.52	2,63–2.80	2.8
P_2	0.49	4.08–4.44	4.08–4.31	2.5
P_3	0.66–0.68	3.73–4.00	2.90–2.95	2.4



FIGURES 1–6. Adult male of *Diamesa caucasica* Kownacki et Kownacka. **1,** hypopygium in dorsal view; **2,** transverse sternapodeme, aedeagal lobes and superior volsellae; **3,** tergite IX; **4,** anal point in lateral view; **5,** gonostylus; **6,** hypopygium in lateral view.

FIGURES 7–10. Localities of *Diamesa caucasica* Kownacki et Kownacka. **7,** Greater Caucasus, Urukh River, about 12 km from Mastocete Glacier; **8,** Greater Caucasus, Tanandon River, about 8 km from Tanancete Glacier; **9,** Greater Caucasus, Skazdon River, about 3.5 km from Skazsky Glacier; **10,** Tseydon River, about 7.5 km from Tseysky Glacier (Photos by D.M. Palatov).

Hypopygium (Figs. 1–6). Tergite IX with 14–19 setae (32–56 µm long) from one side of anal point and located on protuberance (Figs. 1, 3, 6). Anal point short (40–48 µm long) and naked, in lateral view slightly curved upwards (Figs. 3–4). Laterosternite IX with 5–7 setae, 40–48 µm long.Transverse sternopodeme (TSA) trapezoidal (Fig. 2), 56 µm high, 100 µm wide; TSA height/TSA width 0.56. Aedeagal lobe as in Fig. 2, 140 µm long, weakly chitinized; phallapodeme sclerotized, 140–144 µm long. Gonocoxite 340–420 µm long; superior volsellae double, covered with setae, 28–36 µm long (Fig. 2). Gonostylus 376–412 µm long, in basal third or half widened along inner margin and with wedge-shaped appendage directed at angle backwards, densely covered with long and fine setae; distal half or two thirds of gonostylus narrow and of the same width, covered with fine setae 28–40 µm long and ending in short megaseta, 8 µm long (Fig. 5–6). HR 0.90–1.02.

Pupa described by Kownacki & Kownacka (1973) who recorded some information on larva too.

Ecology. Adult males were collected from snow, stones and bowlders near mountain rivers and springs located at 1402–1943 m a.s.l., with flow rate of 0.2–1.0 m/s, water temperatures 0.8–11°C, and mineralization of 37–65 ppm.

Remarks. *D. caucasica* belongs to the *latitarsis* group, from the representatives of which adult males differ in the structure of the hypopygium, namely, in the shape of the gonostylus and presence of a basal wedge-shaped appendage, as well as in the arrangement of setae of tergite IX on protuberances and in a short naked anal point at angle directed downwards.

In general, adult males from North Ossetia are similar to those of the original description (Kovnacki & Kownacka

1973), but specimens from our material have total length 2.9–3.5 mm, AR 1.0–1.22, dorsocentrals 7–12, while in males from Georgia total length 6 mm, AR 0.96, dorsocentrals 10–12. It should be noted that our redescription has been supplemented with a number of features which were absent in the original description.

Distribution. Known only from glacial streams of Terek River basin in Caucasus (Figs. 7–10).

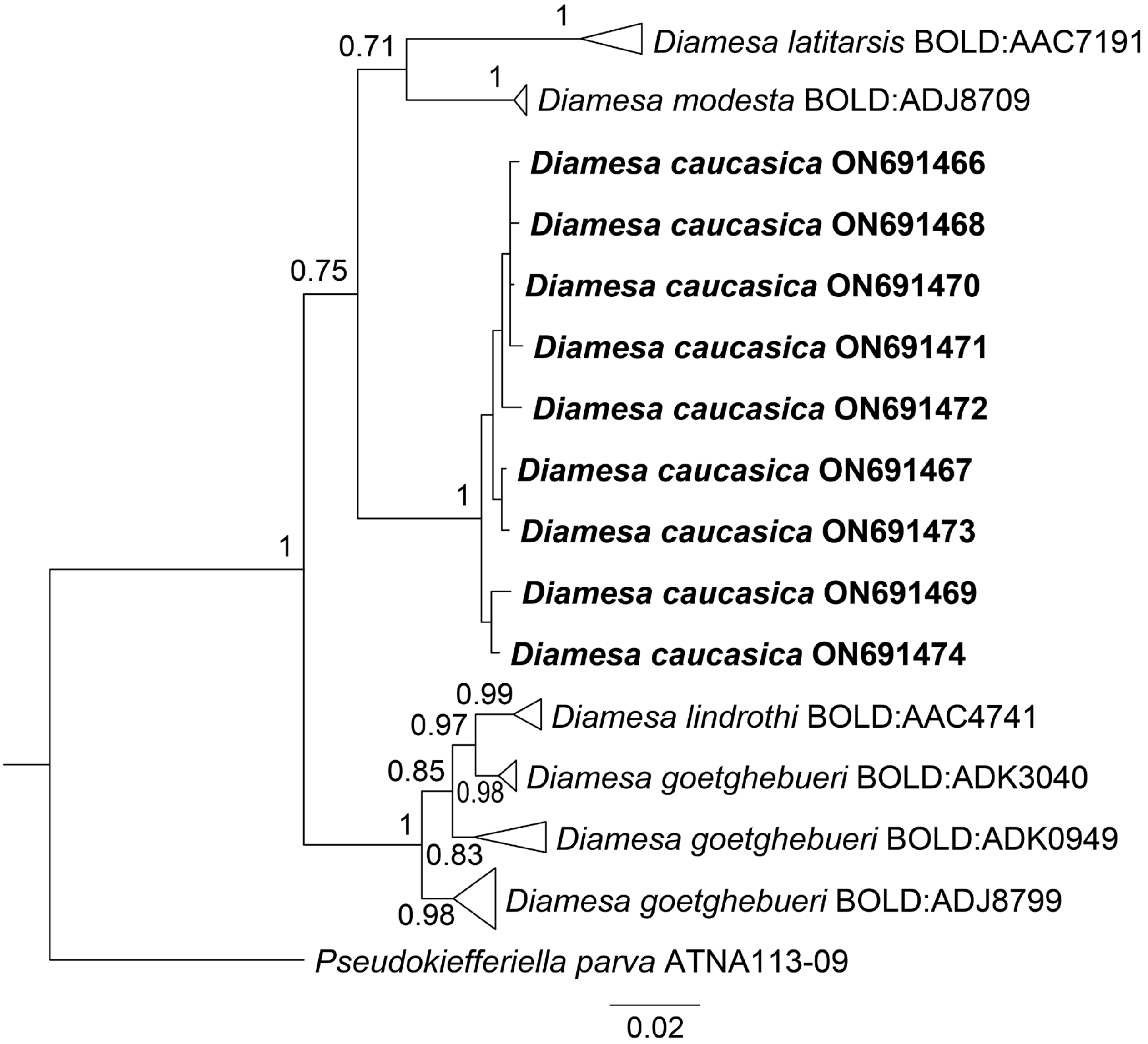


FIGURE 11. Bayesian inference (BI) tree based on the cytochrome c oxidase I (COI) nucleotide sequence data of the *Diamesa latitarsis* group and *Pseudokiefferiella parva* (Edwards) as outgroup. Specimens obtained in this study are in bold.

Results of DNA barcoding

A total of 658 base pairs (COI-5P gene) were observed after aligning sequences of nine specimens of *Diamesa caucasica*. After assembly and alignment, nucleotide frequencies across these sequences were as follows: A=26.4%, T=41.0%, G=17.0%, and C= 15.6%.

Interspecific pairwise p-distances between specimens of *D. caucasica* ranged from 0.15 to 1.06% (mean 0.64%). This divergence is based on 12 synonymous mutations. Interspecific divergence between obtained sequences of *D. caucasica* and species of *D. latitarsis* group from BOLD systems were the follows: *D. goetghebueri* (BIN BOLD: ADK0949)—6.80%, *D. goetghebueri* (BIN BOLD: ADJ8799)—6.90%, *D. goetghebueri* (BIN BOLD: ADK3040)—7.40%, *D. latitarsis* (BIN BOLD: AAC7191)—7.27%, *D. lindrothi* (BIN BOLD: AAC4741)—7.62% and *D. modesta* (BIN BOLD: ADJ8709)—6.47%. The obtained interspecific divergence corresponds to species level (Montagna *et al.* 2016, Makarchenko *et al.* 2022).

The Bayesian inference (BI) phylogeny revealed three clades (Fig. 11). The first well-supported clade (Bayesian posterior probability BPP = 1) was the earliest branching and includes three BIN BOLD numbers of *D. goetghebueri* and *D. lindrothi*. *D. caucasica* (BPP = 1) was placed as sister to the clade uniting *D. latitarsis* and *D. modesta* (BPP = 0.71). Thus, the monophyly of the *D. caucasica* was highly supported.

Acknowledgements

Authors are grateful to A.G. Sabeev (Alaniya National Park) for organizing field work in the Digor gorge.

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

References

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

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.

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

Kownacki, A. & Kownacka, M. (1973) Chironomidae (Diptera) from the Caucasus. II. *Diamesa* Waltl group *latitarsis*. *Bulletin de L'Académie des Polonaise des Sciences. Série des sciences biologiaues*, 21, 131–138

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

Makarchenko, E.A., Semenchenko, A.A. & Palatov, D.M. (2022) Taxonomy of *Diamesa steinboecki* group (Diptera: Chironomidae: Diamesinae), with description and DNA barcoding of new species. I. Subgroups steinboecki and longipes. *Zootaxa*, 5125 (5), 483–512.
https://doi.org/10.11646/zootaxa.5125.5.2

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

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.

Tavaré, S. (1986) Some Probabilistic and Statistical Problems in the Analysis of DNA Sequences. Lectures on Mathematics in the Life Sciences. *American Mathematical Society*, 17, 57–86.