Zootaxa 5357 (1): 144–150
https://www.mapress.com/zt/

Correspondence

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)
https://doi.org/10.11646/zootaxa.5357.1.8
http://zoobank.org/urn:lsid:zoobank.org:pub:85C02912-8244-43C4-81A3-CF0DE416061D

Morphological redescription and DNA barcoding of *Diamesa parancysta* Serra-Tosio (Diptera: Chironomidae: Diamesinae)

EUGENYI A. MAKARCHENKO[1,2 *] & ALEXANDER A. SEMENCHENKO[1,3]
[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] *makarchenko@biosoil.ru; http://orcid.org/0000-0003-2765-8729*
[3] *semenchenko_alexander@mail.ru; https://orcid.org/0000-0001-7207-9529*
[*]*Corresponding author*

Diamesa parancysta Serra-Tosio was described by adult male from Mongolia (Serra-Tosio 1983) and for a long time before its discovery in North Norway (Willassen *et al.* 2005) it was considered as rare East Palaearctic species. As a result of our revision of the genus *Diamesa* Meigen and analysis of DNA barcoding data from the Genbank, it turned out that this species also lives in several regions of Mongolia and Eastern Siberia, Kazakhstan, Altai, China, South Korea, the Russian Far East and Alaska. Also for *D. parancysta* were discovered pupa and fourth instar larva. In this regard we found expedient basing on materials from the different regions to make morphological redescription of the adult male, as well as the original description of the pupa and larva, with DNA barcoding of this species and notes on taxonomy, synonymy and distribution.

Material and methods

The material was preserved in 96% ethanol for DNA-analysis and in 70% ethanol for further study of morphology and slide-mounted in polyvinyl lactophenol. The morphological terminology and abbreviations used below follow Sæther (1980). The photographs were taken using an Axio Lab. A1 (Karl Zeiss) microscope, and then stacked using Helicon Focus software. The final illustrations were post-processed for contrast and brightness using Adobe® Photoshop® software.

After the dissection of hypopygium, wings, legs, head and thorax, total DNA from the abdominal tissues was extracted, using the Qiagen Blood and Tissue Kit (Qiagen, Hilden, Germany). Primers for PCR (Polymerase Chain Reaction) and sequencing was LCO1490 and HCO2198 according to Folmer *et al.* (1994). Amplification products were purified by exonuclease I (ExoI) and alkaline phosphatase (FastAP) (Thermo Fisher Scientific Inc., USA) and bidirectionally sequenced by ABI 3130x sequencer (Applied Biosystems) using reagents BigDye terminator v3.1 cycle kit. More details about DNA extraction, PCR regime and sequence can be found in Makarchenko *et al.* (2022b, 2023).

The obtained sequences were added to a dataset from Barcode of Life Data System (BOLD), containing all published sequences of the *Diamesa* sp. XJC (BIN BOLD:ADL0342) which close to obtained sequences (at Sep 2023). The final dataset also contained morphologically closest species: *D. baicalensis* Tshernovskij, *D. bertrami* Edwards, *D. dactyloidea* Makarchenko, *D. filicauda* Tokunaga, *D. gregsoni* Edwards, *D. insignipes* Kieffer, *D. vernalis* Makarchenko and *D. zernyi* Edwards from GenBank and BOLD.

Phylogenetic analyses were performed using Bayesian inference (BI) in the program MrBayes ver. 3.2.7 (Ronquist *et al*. 2012). The nucleotide substitution models best fitting the COI barcode library for sequences were obtained using the Bayesian Information Criterion (BIC) in PartitionFinder 2.1.1 (Lanfear *et al.* 2012). 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 OR506101–OR506108.

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

 Accepted by B. Rossaro: 12 Sept. 2023; published: 17 Oct. 2023

Description

Diamesa parancysta Serra-Tosio

(Figs 1–17)

Diamesa parancysta Serra-Tosio, 1983: 15; Willassen *et al*. 2005: 71; Ashe & O'Connor 2009: 282; Krasheninnirkov 2009: 66, 2011: 248; Kang *et al.* 2017: 120; Makarchenko *et al.* 2022a: 80.
Diamesa corrupta Makarchenko, 1988: 54.
Diamesa sp. Ge, Xia, Wang, Zhang, Ma & Zhou, 2021: 6. Syn. nov.
Diamesa sp. XJC Webb, Cole & Simmons, 2022: 138. Syn. nov.

Material examined. ***Russia:*** 1 adult male, Irkutsk region, Upper Angara River, Padun village, 19.IV.1930, leg. Chekanovsky; 2 adult males, Krasnoyarsk Territory, Ermakovsky District, Kulumys Ridge, Lake Oiskoe, alt. 1400 m a.s.l., 23.VII.2014, leg. E. Borisova; 1 adult male, Republic of Khakassia, Abakan City, valley of the Abakan River, poplar forest, ultraviolet lamp, alt. 245 m a.s.l., 2–3.IV.2020, N 53.71375, E 91.505444, leg. S. Dragan, 2 adult males the same data, except 6.IV.2020, leg. S. Dragan; 2 adult males, Perm Territory, Suksunsky District, Koshelevo Village, mouth of Suksunchik River, alt. 138 m a.s.l., 27.III.2014, N 57.156083, E 57.421111, leg. A. Krasheninnikov; 2 adult males, Magadan Region, Olskyi District, 137 km of Kolymskaya Road, Ola River, 1.V.2015, N 60.412194, E 151.514564, leg. E. Khamenkova. ***Kazakhstan:*** 2 adult males, 2 pupae (males), 2 larvae, East Kazakhstan region, Katon-Karagai District, Sarymsaqty Mountains (Kazakh Mountain Altai), Arasan River, about 0.7 km below of Bolshoe Rakhmanovskoye Lake, alt. 1790–1809 m a.s.l., 5–6.VII.2018, N 49.535983, E 86.500633, leg. D. Palatov. ***Mongolia:*** 1 adult male, Govi-Altay Aymag, Uliastein Gol River at the confluence of Baga Gol River, light trap, alt.1989 m a.s.l., 22.IX.2019, N 45.4846, E 94.277067, leg. D. Palatov.

Adult male (n = 6). Total length 4.5–5.2 mm. Total length/wing length 1.06–1.2.

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

Head. Eyes hairy, reniform. Temporal setae including 8–10 preoculars, 7–14 verticals, 9–15 postorbitals. Clypeus with 12–16 setae. Antenna with 13 flagellomeres and developed plume of seta; terminal flagellomere with 1 subapical setae, 24–50 µm long; AR 1.69–2.46. Palpomere length (µm): 44–77, 88–134, 116–202, 108–190, 172–279. Palpomere 3 in distal part with sensilla capitata with diameter 14–16 µm. Head width/palpal length 1.24–1.45.

Thorax. Antepronotum with 5–15 ventrolateral setae. Dorsocentrals 7–14, prealars 7–13. Scutellum with *ca* 33–50 setae.

Wing. Length 4.2–4.9 mm, width 1.2 mm. Anal lobe rounded, slightly protrudring. Squama with 36–53 setae in 1–2 rows. R and R_1 with 23–28 setae, R_{4+5} with 4–14 setae. RM/MCu 2.67. Costa extension 114 µm long.

Legs. Spur of front tibia 76–89 µm long. Spurs of mid tibia 60–88 and 52–73 µm long. Spurs of hind tibia 92–112 and 56–77 µm long. Hind tibial comb with 18– 22 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 parancysta serra-tosio, male (n=6)

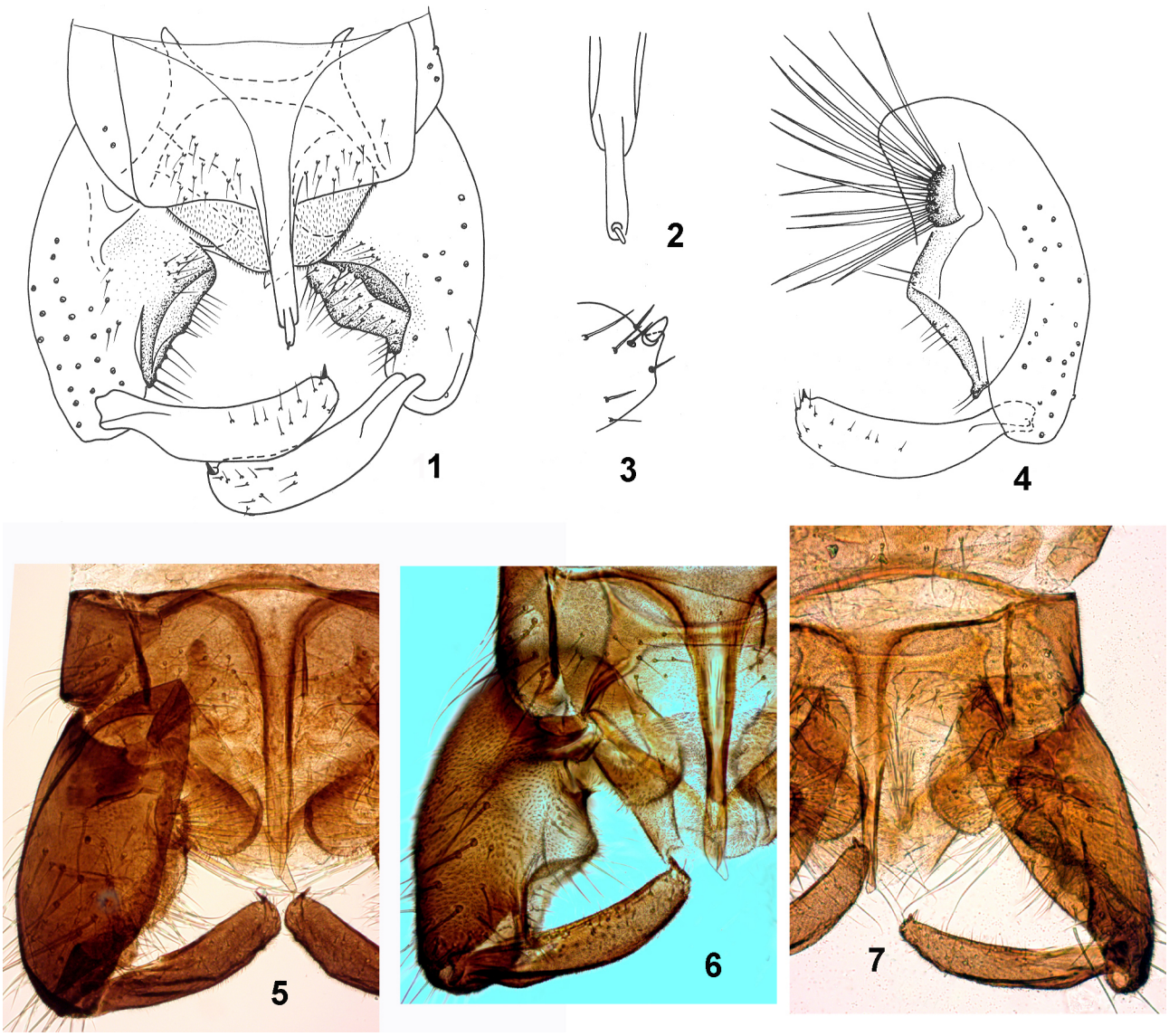
	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
P_1	1066–1440	1312–1853	1050–1296	492–607	295–344	115–148	131–164	0.70–0.80	3.30–3.98	2.26–2.64	3.1–4.2
P_2	1250–1680	1279–1760	689–880	344–492	197–312	98–164	131–164	0.49–0.54	3.73–3.96	3.67–4.15	2.5–2.8
P_3	1345–1840	1624–2120	1050–1400	508–705	262–394	115–164	148–180	0.64-0.70	3.58–4.03	2.83–2.92	3.6–5.4

Hypopygium (Figs. 1–7). Tergite IX with distinct tergal bands, 9–19 setae, 20–28 µm long from one side and anal point, 240–366 µm long which has terminal dorsal keel and apical setiform sensilla (Figs. 1– 2). Laterosternite IX with 4–10 setae, 44–72 µm long. Transverse sternapodeme straight or sometimes little concave, with strongly projecting anterolateral corners (Figs. 1, 6), 168–223 µm long. Gonocoxite 340–372 µm long, with large inferior volsella, broad and prominent basal plate (Figs. 1, 5–7). Basimedial setae cluster well developed, with 15–23 long yellowish setae, 160–231 µm long (Fig. 4). Gonostylus gently curved and relatively broad, 208–240 µm long and 36–52 µm wide, with megaseta, 12 µm long and apical tooth, 10 µm long (Figs. 3, 5–7). HR=1.44–1.82.

Pupa (n=2). Total length 5.4–5.5 mm. Cephalothorax dark brown, abdomen yellowish brown. Coloration brownish. Exuviae yellow.

Cephalothorax. Frontal apotome with 2 setae 284–288 μm long. Thorax wrinkled, in anterodorsal and lateral parts granulated. Thoracic horn 390–400 μm long, filiform, with small spinules at the top, yellow except for brown basal part. Precorneal setae lengths (μm): Pc_1—110–164, Pc_2—224–228, Pc_3—84–88 (Fig. 8). Antepronotum with 2 median seta, 120–128 μm long and 1 lateral antepronotals, 80 μm long. Mesonotum with 2 dorsocentrals: Dc_1 strong, 140–148 μm long, Dc_2 hair-like, 24–28 μm long.

Abdomen. Tergite I with delicate shagreen only in anterolateral angles. Tergites II–VII with shagreen in anterior third or half, tergite VIII almost all with shagreen. Sternites I–II without shagreen, sternites III–VIII with rare 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 tergites respectively—9 : 8–9 : 9 : 10 : 9 : 8 : 8–9 (Figs. 9–11). Number of posterior transverse row spines of sternites III–VIII respectively—9 : 7–8 : 8 : 7–8 : 7–8 : 89 (Figs. 9–11). Segment I with 1 pair of lateral setae; segments II–VIII with 3 pairs of strong brown lateral setae, 140–148 μm long (L_1–L_3) and 1 pair of hair-like setae, 80–84 μm long (L_4). Segments II –VIII with spine-like process on posterolateral corner. Anal lobe with 3 yellow anal macrosetae, 300–336 μm long, slightly curved in distal part and pointed. Male genital sac little extended beyond anal lobe (Fig. 12).



FIGURES 1–7. Adult male of *Diamesa parancysta* Serra-Tosio from Upper Angara River **(1–4)**, Magadan Region **(5)**, Khakassia **(6)** and Kazakh Mountain Altai **(7)**. **1, 5, 6–7,** hypopygium in dorsal view; **2,** apical part of anal point; **3,** apex of gonostylus; **4,** gonocoxite and gonostylus in ventral view.

Fourth instar larva (n = 2). Total length 7.3–7.4 mm. Head capsule 420–460 μm long; dark brown to black, postoccipital margin black and wide; abdomen brownish. S_I short, seta-like; S_{II} and S_{III} simple, hair-like (Fig. 17). Labral lamellae consisting of 5 lobes. Premandible broad, apically with 6 teeth (Fig. 16). Antenna with 5 segments, length of antennal segments (μm): 72, 18, 8, 5, 4. Lauterborn organs small; style reaches base of fourth segment; longest branch of antennal blade reaches the apex of the fourth segment; ring organ *ca* 7 μm diameter located in basal quarter of first segment (Fig. 15); AR 2.0–2.1. Mandible dark brown to black, with apical tooth and 4 inner teeth; apical tooth narrow, twice as long as first inner tooth; seta subdentalis minute; seta interna with 24–26 simple branches (Fig. 14). Mentum with 1 median and 9 pairs of lateral teeth; median tooth about the same size as the first lateral teeth or little wider and together with them slightly lighter than the rest of the lateral teeth; ventromental plate small (Fig. 13). Procercus dark brown, in the form of incompletely sclerotized ring, bearing 4 dark brown strong anal setae, 276–296 μm long and 1 hair-like lateral seta which is on the body, 48–56 μm long. Posterior parapods 656–689 μm long, in 1.3–1.4 times as long as last body segment. Dorsal and ventral pairs of anal tubulus 246 μm long.

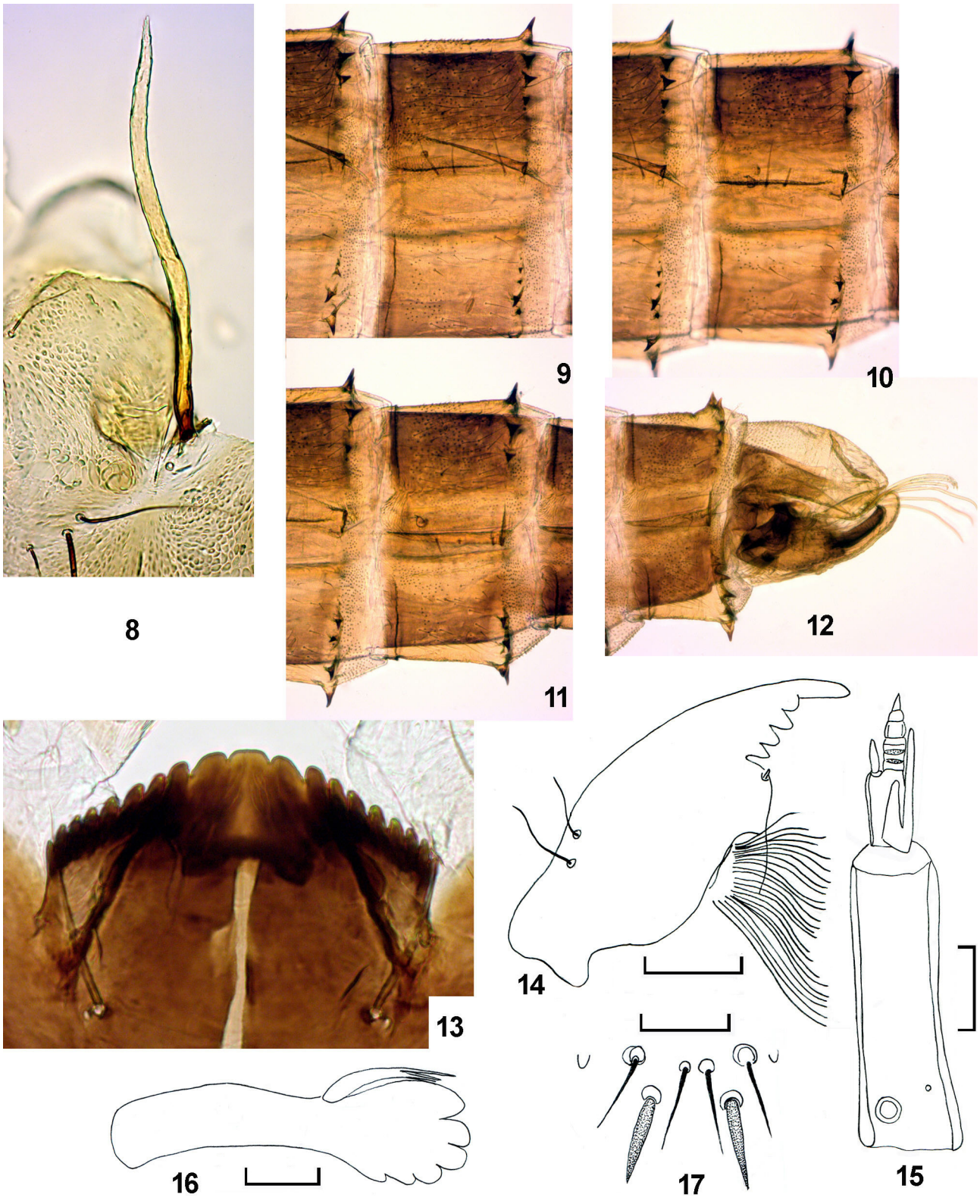
Remarks. Serra-Tosio (1983) brought the adult male of *D. parancysta* closer to the North American *Diamesa nivoriunda* group of species (Hansen & Cook 1976) and, in particular, to *D. ancysta* Roback. Our data on morphology and DNA barcoding indicate a close relationship of this species also to the East Palaearctic species *D. vernalis* and *D. dactyloidea.* The male *D. parancysta* is well separated from the listed species in the structure of the hypopygium, namely in the shape of gonostylus and inferior volsellae, number of basimedial setae. Males of the studied populations fit well into the description of the species. The highest AR (2.12–2.46) are in specimens from the Urals and Khakassia, the lowest are in males from Mongolia (AR 1.49–1.79). The maximum LR value (0.80) is in specimens from the Krasnoyarsk Territory, the minimum (LR 0.66) is in males from the Upper Angara River. Pupa is very similar to *D. tsutsuii* while larva to *D. tsutsuii* and *D. arctica* (Bohemann) but larvae of these species can be good distinguished by mentum. Mentum of *D. parancysta* with 1 median and 9 pairs of lateral teeth; median tooth about the same size as the first lateral teeth or little wider and together with them slightly lighter than the rest of the lateral teeth. Mentum of *D. tsutsuii* with 1 median and 10 pairs of lateral teeth. Mentum of *D. arctica* with 1 median and 9 pairs of lateral teeth, the middle of the mentum is straight, the median tooth and the first 3 pairs of lateral teeth are almost the same size and higher than the lateral ones.

Distribution. Known from Norway, Ural Region, Mongolia, Altai Mountains, Baikal Lake basin, Khakassia, China, South Korea, Alaska (Hayford 2005; Ashe & O'Connor 2009; Krasheninnirkov 2009; Kang *et al.* 2017; Ge *et al.* 2021; Makarchenko *et al.* 2022a; Webb *et al.* 2022).

Results of DNA barcoding

We successfully sequenced 8 barcoding fragments representing *D. parancysta* from Kazakhstan, Mongolia and Russia (Fig. 18). Obtained sequences were conspecific to *Diamesa* sp. XJC from BOLD (BIN BOLD:ADL0342) collected in China: Xinjiang Uygur (XJDQD1074-18 - XJDQD1077-18), USA: Alaska (COLEB087-18) and Russia: Republic of Khakassia (GBMNE58254-22- GBMNE58255-22). The intraspecific p-distances were 0.0–1.5% (0.7% in average). *D. dactyloidea* and *D. vernalis* were the closest species to *D. parancysta*, sequence divergence were 5.1% and 5.4% respectively which is acceptable for an intraspecific level (Montagna *et al.* 2016). Mean distances between *D. parancysta* and the remaining species (Fig. 18) were 6.2–7.1%.

Phylogenetic tree was reconstruct using obtained dataset and *Syndiamesa edwardsi* (Pagast) as outgroup (Fig. 18). The basal polytomic clade was highly supported (Bayesian Posterior Probability, BPP=1) and includes four subclades. *D. parancysta*, *D. dactyloidea* and *D. vernali*s were united in single subclade without support. In its turn clade of *D. parancysta* was high supported (BPP=1).



FIGURES 8–17. Pupa **(8–12)** and larva **(13–17)** of *Diamesa parancysta* Serra-Tosio. **8,** thoracic horn and precorneals; **9,** segments IV–V, lateral view; **10,** segments VI–VII, lateral view; **11,** segments VII–VIII, lateral view; **12,** segment VIII and anal segment, lateral view; **13,** mentum; **14,** mandible; **15,** antenna; **16,** premandible; **17**, S-setae of labrum. Scale bars: Fig. 14—50 µm; Figs. 15–17—20 µm.

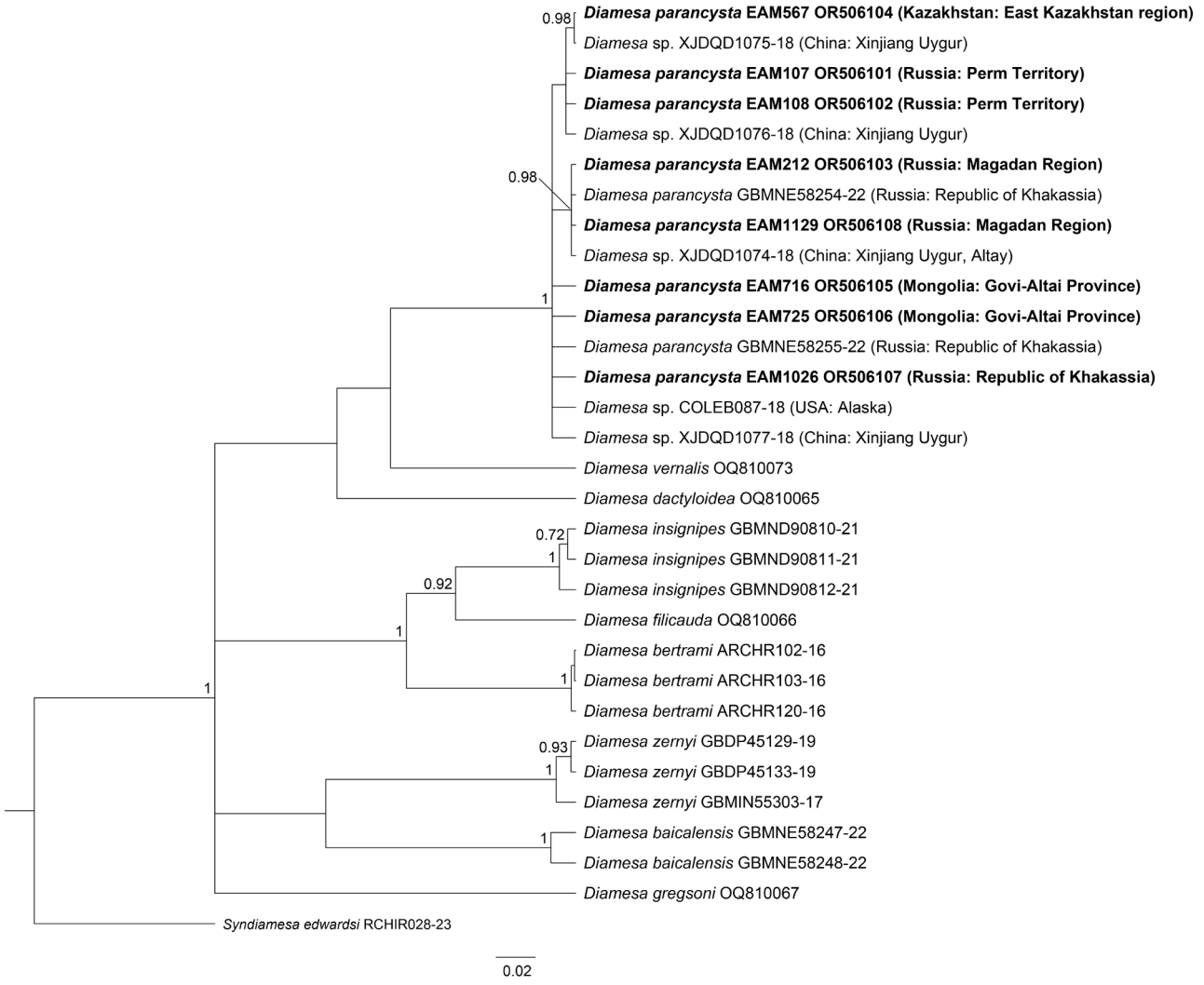


FIGURE 18. Ultrametric Bayesian inference (BI) tree based on the cytochrome c oxidase I (COI) nucleotide sequence data of *Diamesa parancysta* Serra-Tosio and morphologically closest species with *Syndiamesa edwardsi* (Pagast) as outgroup. Bayesian posterior probabilities (higher than 0.7) are given above tree nodes. Specimens obtained in this study are in bold.

Acknowledgements

We are grateful to Drs. E.V. Borisova, E.V. Khamenkova, S.V. Dragan, A.B. Krasheninnikov and D.M. Palatov for making material available to us.

The research was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation (theme No. 121031000147-6).

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.

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, 294–299.

Ge, Y., Xia, C., Wang, J., Zhang, X., Ma, X. & Zhou, Q. (2021) The efficacy of DNA barcoding in the classification, genetic differentiation, and biodiversity assessment of benthic macroinvertebrates. *Ecology and Evolution*, 11, 5669–5681. https://doi.org/10.1002/ece3.7470

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.
Hayford, B. (2005) New records of Chironomidae (Insecta: Diptera) from Mongolis with review of distribution and biogeography of Mongolian Chironomidae. *Journal of the Kansas Entomological Society*, 78 (2), 192–200. https://doi.org/10.2317/0406.24.1
Kang, H.J., Orel, O.V., Makarchenko, E.A. & Bae, Y.J. (2017) Checklist of the Chironomidae (Diptera) recorded from the Korean Indigenous Species Survey of the National Institute of Biological Resources (2014–2016). *Entomological Research Bulletin*, 33 (2), 118–123.
Krasheninnikov, A.B. (2009) The chironomids of the genus *Diamesa* (Diptera, Chironomidae) of the mountain rivers of the Middle Urals. *In*: *Animal world of mountain territories*. KMK Publisher, Moskow, pp. 65–67. [in Russian]
Krasheninnikov, A.B. (2011) New data on chironomids (Diptera, Chironomidae) of the Middle Urals. *Vladimir Ya. Levanidov's Biennial Memorial Meetings*, 5, 247–264. [in Russian]
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. (1988) Two new species of *Diamesa* Mg. (Diptera, Chironomidae) from the Eastern Palaearctic. *In*: *Newsletter The Biology of Inland Waters. 79.* Nauka Publisher, Leningrad, pp. 52–56. [in Russian]
Makarchenko, E.A., Semenchenko, A.A. & Dragan, S.V. (2022a) Review on the fauna and taxonomy of Diamesinae subfamily (Diptera, Chironomidae) from the Republic of Khakassia (Russia), with morphological description and DNA barcoding of the discovered species. *Euroasian Entomological Journal*, 21 (Supplement 1), 73–89. https://doi.org/10.15298/euroasentj.21.Spl.1.08
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. & 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
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.
Serra-Tosio, B. (1983) Nouveaux Diamesinae de la Paléarctide méridionale et orientale (Diptera: Chironomidae). *Spixiana*, 6 (1), 1–26.
Webb, J.M., Cole, M.B. & Simmons, T. (2022) DNA barcoding takes bioassessment further: new distribution records for aquatic macroinvertebrates from Alaskan National Parks. *Proceedings of the Entomological Society of Washington*, 124 (1), 131–149. https://doi.org/10.4289/0013-8797.124.1.131
Willassen, E., Hanssen, O. & Koksvik, J.I. (2005) *Diamesa parancysta* Serra-Tosio: an East-Palearctic midge new to Europe (Diptera: Chironomidae: Diamesinae). *The Norwegian Journal of Entomology*, 52, 69–73.