

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

Обзор фауны и таксономии хирономид подсемейства
Diamesinae (Diptera, Chironomidae) республики Хакасия
(России), с морфологическим описанием
и ДНК-анализом обнаруженных видов

Е.А. Макаренко*, А.А. Семенченко*, S.V. Dragan**
Е.А. Макаренко*, А.А. Семенченко*, С.В. Драган**

* Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Prosp. 100-letiya Vladivostoka 159, 690022 Vladivostok Russia. E-mail: makarchenko@biosoil.ru; semenchenko_alexander@mail.ru.

* Федеральный научный центр биоразнообразия наземной биоты Восточной Азии ДВО РАН, пр. 100-летия Владивостока 159, Владивосток, 690022, Россия.

** N.F. Katanov Khakas State University, Lenina Str. 90, Abakan 655017 Russia. E-mail: dragan_s@mail.ru.

** Хакасский государственный университет им. Н.Ф. Катанова, ул. Ленина 90, Абакан 655017 Россия.

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Ключевые слова: Diptera, Chironomidae, Diamesinae, фауна, таксономия, переписание, ДНК-анализ, Хакасия, Россия.

Abstract. For the Republic of Khakassia and the bordering territory a literary review of the chironomid investigations have been prepared and for the first time data on the fauna and taxonomy of 8 chironomid species from the subfamily Diamesinae are given. Redescriptions and DNA barcoding of 7 species, *Diamesa baicalensis* Tshernovskij, *D. hamaticornis* Kieffer, *D. khumbugelida* Sæther et Willassen, *D. parancysta* Serra-Tosio, *D. serratosioi* Willassen, *Pseudodiamesa* aff. *nivosa* (Goetghebuer) and *Sympothastia takatensis* (Tokunaga) by adult males are presented. A total of 15 DNA sequences (658 bp) of partial mitochondrial cytochrome c oxidase subunit I (COI) gene of 15 samples belonging to 7 species and 3 genera are newly obtained in this study. We also reconstruct phylogenetic tree of *Diamesa* using obtained sequences and close relatives from Genbank and BOLD systems data.

Резюме. Подготовлен литературный обзор изученности хирономид республики Хакасия и прилегающей территории, впервые приведены данные по фауне и систематике 8 видов подсемейства Diamesinae. Для 7 видов, *Diamesa baicalensis* Tshernovskij, *D. hamaticornis* Kieffer, *D. khumbugelida* Sæther et Willassen, *D. parancysta* Serra-Tosio, *D. serratosioi* Willassen, *Pseudodiamesa* aff. *nivosa* (Goetghebuer) и *Sympothastia takatensis* (Tokunaga) даны переписания имаго сапцов с результатами ДНК-анализа. Впервые было получено 15 частичных последовательностей цитохромоксидазы субъединицы I (658 п.н.) для 15 особей, принадлежащих к 7 видам и 3 родам. Также

было реконструировано филогенетическое дерево рода *Diamesa* с использованием полученных сиквенсов и данных из Генного банка и BOLD systems.

Introduction

The Republic of Khakassia is located in the south of Siberia, along the left bank of the Yenisei River, and includes the flat western part of the Minusinsk Depression, the eastern slopes of the Kuznetsk Alatau and the northwestern part of the Western Sayan. The mountains occupy two-thirds of the territory of the republic and belong to the Altai-Sayan highlands [Resursy poverkhnostnykh vod SSSR..., 1973], which is considered to be part of the South Siberian mountain region [Sochava, Timofeev, 1968]. The length of the territory from north to south is 450 km, the maximum width in its middle part is 200 km. The area is 61,900 km². In the north, east and southeast it borders on the Krasnoyarsk Territory, in the south — on the Republic of Tuva, in the southwest — on the Republic of Altai, in the west — on the Kemerovo Region.

The hydrographic network is developed within three hydrological regions: Altai-West-Sayan, Kuznetsk-Alatau and Minusinsk [Resursy poverkhnostnykh vod SSSR..., 1973]. The river network is represented by 6556 rivers, including the Yenisei River (over 295 km),

its left tributary Abakan River, as well as rivers Tom, Chulym, White Iyus and Black Iyus (Ob River basin). On the territory of the republic there are about 500 lakes with a water surface area of more than 10 hectares, including approximately 390 fresh and 110 salt lakes. The total water surface area of all water bodies in Khakassia exceeds 840 km² [Krutikov, 2008]. At present, the hydrological regime of the upper streams of the Yenisei River, is determined by the influence of the Sayano-Shushenskaya, Mainskaya and Krasnoyarskaya hydroelectric power stations.

Special studies in the field of taxonomy and fauna of chironomids of Khakassia previously have not been carried out. There are studies that are devoted to the investigation of the chironomid fauna of the Yenisei River [Lipina, 1929; Greze, 1957a] and the bays of the Krasnoyarsk Reservoir [Skoptsova, 1987], which cover areas of these water bodies within the boundaries of the region. The earliest, including data on chironomid larvae in the zoobenthos of the Yenisei River, is a short communication by Zvereva [1930] who mentions chironomid larvae from the *thummi* group for the section of the Yenisei River from the mouth of the Mana River to the mouth of the Tuba River.

Most of the studies carried out on the territory of Khakassia are based on material that includes larvae of chironomids. Only for the fauna of Shira Lake, the list includes nine species, which are based on the study of chironomid adults [Borisova et al., 2019].

Among the studied water bodies on the territory of Khakassia, the most studied is the Krasnoyarsk reservoir. As a result of the study of zoobenthos in the reservoir [Vershinin, 1975; Skoptsova, 1987; Gold, Shulepina, 2008; Shulepina, 2008; Gold et al., 2008; Kuznetsova, 2011, 2012, 2017], as well as the nutrition of fish living in it [Romanova, 1975], 181 species and larval forms of chironomids were identified. Of the three reservoirs created in the upper part of the Yenisei, the Mainskoye and Sayano-Shushenskoye remain the least studied. If the Mainskoye reservoir has practically not been studied in relation to hydrofauna, then for the Sayano-Shushenskoye reservoir, 17 larval forms of chironomids are known [Dubovskaya, Gold, 1981; Gold et al., 1985; Burneev, Zadelenov, 1989].

The next in terms of the degree of study is the upper part of the Yenisei River. As a result of the study of zoobenthos, 72 species and larval forms of chironomids were recorded before the creation of a cascade of reservoirs [Zvereva, 1930; Greze, 1957a, b; Greze, Sycheva, 1964]. Later, after the filling of the Krasnoyarsk, Mainskoye and Sayano-Shushenskoye reservoirs, the studies covered sections of the Yenisei riverbed with regulated flow [Andrianova, 2018; Andrianova et al., 2019]. Based on the study of larvae for the upper part of the Yenisei River, namely from the Maina village to the Krasnoyarsk city, 78 species and larval forms of chironomids were identified.

The local chironomid faunas in medium-sized rivers such as the Abakan and Chulym have not been sufficiently studied. In the article by Andrianova [2017],

13 forms of chironomids are given for the section of the Chulym River from the Kopyevo village to the Vladimirovka village. The chironomid fauna in the Abakan River has been studied extremely poorly. Only three forms of chironomids are known from this river [Lipina, 1949; Andrianova, 2018; Andrianova et al., 2019]. In the literature, there are single references to representatives of chironomids from small rivers and streams — tributaries of the Abakan River [Gold, 1976; Ugdyzhkova, 2019]. Gold [1976], in an article devoted to the analysis of the quality of the waters of the small rivers Sora and Byurya, mentioned 7 forms of chironomids.

Among the lakes, the most studied are Bele and Shira, in the fauna of which 21 and 20 species and larval forms are known, respectively [Platonova, 1956; Ozyora Khakassii..., 1976; Romanova, 1989; Rogozin et al., 2011; Tolomeev et al., 2018; Andrianova et al., 2015; Borisova et al., 2019]. The remaining reservoirs of Khakassia have been studied unevenly. The total number of chironomid species in the zoobenthos of lakes ranges from 5 to 37, and the published lists include from 1 to 8 of the most widespread forms of chironomids [Maslennikova, Sycheva, 1964; Ozyora Khakassii..., 1976; Skoptsov, 1976; Sharypov, 2011; Andrianova et al., 2015; Kovaleva, 2019].

To date, 250 species and forms of chironomids are known in the fauna of Khakassia, identified mainly on the basis of the determination by chironomid larvae, of which 155 have been identified to the species level, while the rest are listed in the lists up to the species group level and above. Almost all available data on local faunas of chironomids in reservoirs and streams on the territory of Khakassia, despite the long period of study of some of them, require revision and additions.

In this article, we begin the publication of materials on the fauna and taxonomy of the chironomids from Khakassia, namely of the Diamesinae subfamily. Moreover, in our study we used both morphological data and DNA sequences of the mitochondrial *cytochrome c oxidase subunit I* gene (COI) for species determination. COI-based identification in Diamesinae and the genus *Diamesa* in particular have confirmed the efficiency of DNA barcoding for species delimitation [Montagna et al., 2016; Lencioni et al., 2021; Makarchenko et al., 2022].

Materials and methods

The material was collected in the water bodies of the upper part of the Yenisei River basin, in particular of Abakan, Askiz, Bolshoi On rivers and their basins (Fig. 1).

Abakan is the middle river, the left tributary of the Yenisei River. The length of the river is 514 km [Resursy poverkhnostnykh vod SSSR..., 1973]. The sources of Abakan (along the Bolshoi Abakan River) are located in the nameless ranges of the junction zone of the Western Sayan with the ranges of the Altai Mountains. The sources are located at altitudes of 2150–2400 m a.s.l. [Gosudarstvennyi doklad..., 2020]. The mouth of the river is located in the Abakan, at an altitude of 244 m a.s.l. The

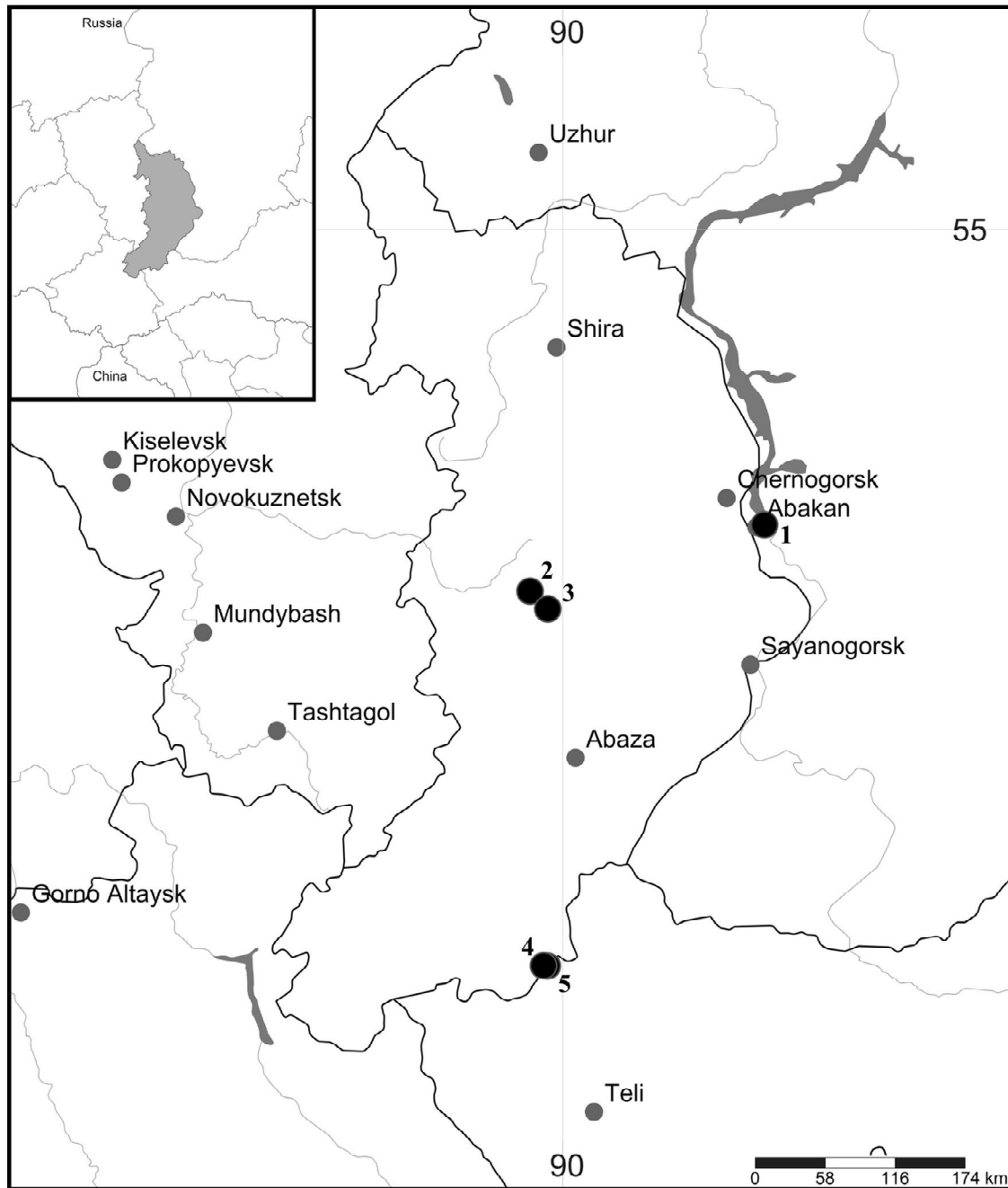


Fig. 1. Map of the Khakassia Republic with places of material collection. 1 — Abakan City, valley of Abakan River; 2 — Askizsky District, Askiz River; 3 — Askizsky District, «Zimnyy» Stream; 4 — Tashtypsky District, unnamed stream in the basin of the Bolshoi On River; 5 — Tashtypsky District, unnamed stream — right tributary of Bolshoi On River.

Рис. 1. Картограмма республики Хакасия с местами сбора материала. 1 — г. Абакан, долина р. Абакан; 2 — Аскизский район, р. Аскиз; 3 — Аскизский район, руч. «Зимний»; 4 — Таштыпский район, безымянный ручей в бассейне р. Большой Он; 5 — Таштыпский район, безымянный ручей — правый приток р. Большой Он.

average catchment height is 1045 m a.s.l. The main tributaries of the river are the river. Ona, Tashtyp, Askiz, Uybat [Resursy poverkhnostnykh vod SSSR..., 1973].

Askiz is a small river, the left tributary of the Abakan River. The length of the river is 124 km. The source of the river is located on the slopes of Karlygan (Abakan Range), at an altitude of about 1200 m a.s.l. [Malyshev,

2007]. The river system includes more than 20 tributaries. The river valley is trough-shaped, asymmetrical with convex slopes. The width of the valley is 1.0–2.5 km [Mazhugina, 1998].

Bolshoi On is a small river, the right tributary of the Ona River. The length of the river is 51 km. The source of the river is on the slopes of the ridge. Sailyg-Khem-

Taiga (Western Sayan), at an altitude of 2200 m a.s.l. The mouth of the river is at an altitude of 1150 m a.s.l. in the vicinity Bolshoi On village (Tashtyp District). The river system includes about 60 tributaries [Resursy pov-erkhnostnykh vod SSSR..., 1973].

The adults of chironomids were collected in traps with ultraviolet lamps of different power — BLB-T5 / 4W and FLU10 T8 G13 / 18W. The trap exposure ranged from 2 to 13 hours, depending on weather conditions. In addition to this method, part of the material was collected with a net by mowing in the floodplain of watercourses and the coastal part of water bodies. Adults of some species were collected directly from snow. Chironomids were preserved in 96 % ethanol. All material was collected by S.V. Dragan. Adult males were slide-mounted in polyvinyl lactophenol. The terminology follows Sæther [1980]. For some structures of the hypopygium, however, the terminology of Hansen & Cook [1976] and Oliver [1989] is used.

The photographs were taken using an Axio Lab.A1 (Karl Zeiss) microscope with an AxioCam ERC5s digital camera and an Olympus SZX16 stereomicroscope with an Olympus DP74 digital camera, and then stacked using Helicon Focus software. The final illustrations were post-processed for contrast and brightness using Adobe® Photoshop® software.

Total DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The *COI* fragment was amplified using LCO1490 (5'-GGTCAA-CAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') [Folmer et al. 1994]. The 10 µl PCR reaction mix contained 3 µl of ultrapure water, 1 µl of DNA template, 0.5 µl of each primer (10 µM), and 5 µl of Go Taq Green Master Mix (Promega Corp, Madison, WI, USA). Thermocycler conditions were as follows: initial denaturation for 3 min at 94 °C followed by 35 cycles of denaturing for 30 s at 94 °C, annealing for 30 s at 48 °C and an extension time of 60 s at 72 °C, with a final extension for 5 min at 72 °C. PCR products were visualized on an 1.5 % agarose gel with Ethidium bromide after electrophoresis. Single bands were purified with Exonuclease I and Thermosensitive Alkaline Phosphatase (ThermoFisher Scientific). Purified PCR products were sequenced directly in both directions using an automated sequencer (ABI 3130xl Genetic Analyzer Sequencer; Applied Biosystems, USA) at department of Cell Biology and Genetics of Far Eastern Federal University.

Sequences were edited and assembled in MEGA 7.0 [Kumar et al. 2016]. Also MEGA 7.0 used to calculate interspecific genetic p-distances. A Bayesian Inference (BI) was performed to reconstruct phylogenetic tree of several *Diamesa* species using MrBayes v.3.2.7 [Ronquist et al., 2012]. The SYM+G [Zharkikh, 1994], F81+I [Felsenstein, 1981] and HKY+G [Hasegawa et al., 1985] models were defined in PartitionFinder 2.1.1 [Lanfear et al. 2012] and used in BI analysis. Four independent runs were conducted with four Monte Carlo Markov Chains (MCMC) as 5,000,000 generations of which 25 % were

discarded as burn-in. For the phylogenetic analysis, we used our sequences, as well as one conspecific sample for each *Diamesa* species from the BOLD system. Also, we used *COI* sequences of *Diamesa dragani* from Khakassia [Makarchenko et al., 2022] and *Pseudokiefferiella parva* as out-group. Tree was displayed and rendered with FigTree v1.4.0 [Rambaut 2012]. Sequences were also deposited in GenBank under accession numbers (ON392048–ON392062).

All material are 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).

Descriptions

Diamesa baicalensis Tshernovskij

Figs 2–5.

Diamesa baicalensis Tshernovskij, 1949: 103; Linevich, 1953: 160, 1963: 70; Pankratova, 1970: 88; Makarchenko, 1987: 202; Linevich, Makarchenko, 1989: 32; Linevich et al., 2002: 56.

Material. 5 adult males, Republic of Khakassia, Abakan City, valley of the Abakan River, poplar forest, 53°42'49.5" N, 91°30'19.6" E, alt. 245 m a.s.l., 2–3.IV.2020 (Fig. 29).

Description. *Adult male* (n = 2, except when otherwise stated). Total length 4.5–4.8 mm. Total length/wing length 1.0–1.2.

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

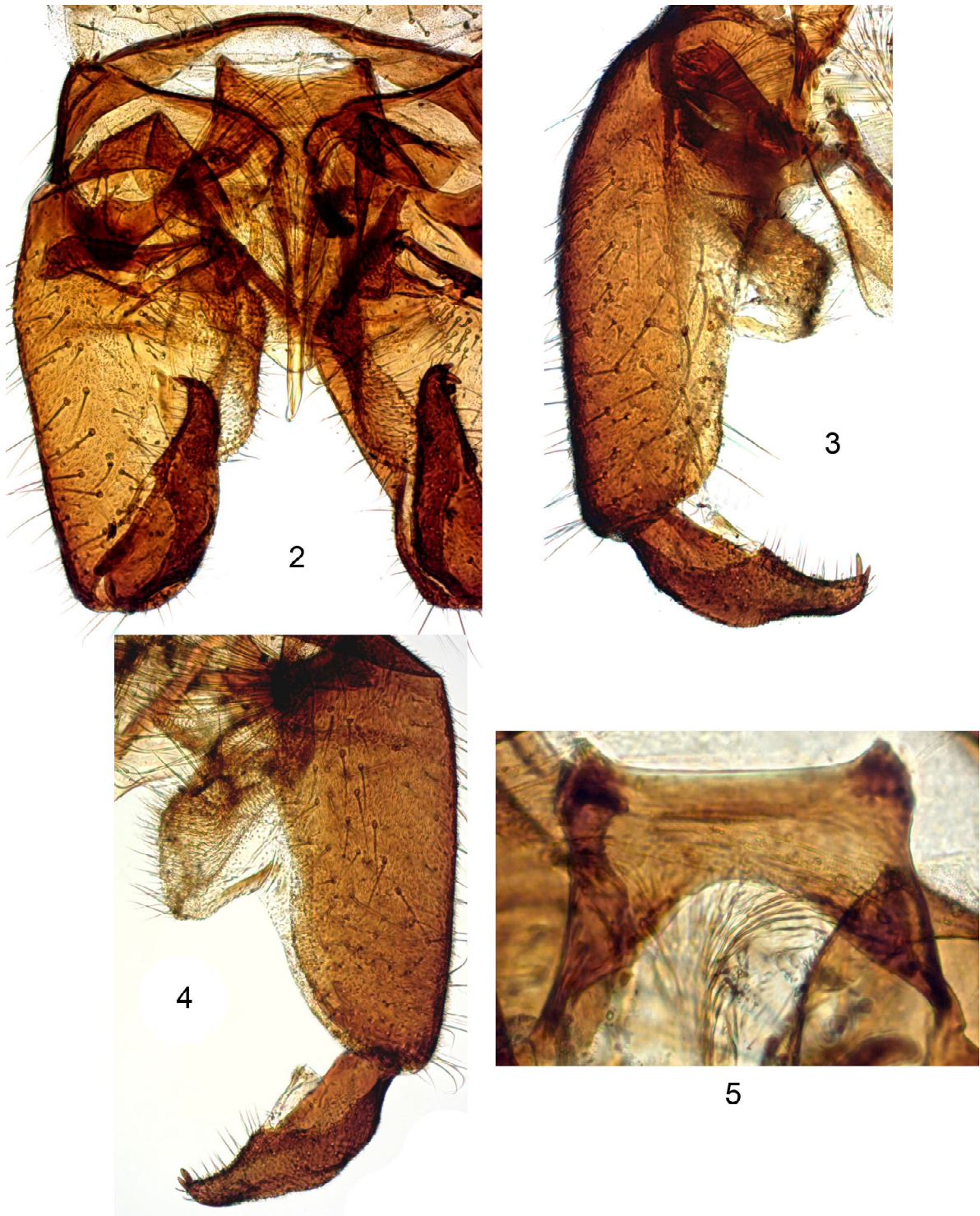
Head. Eyes hairy, reniform. Temporal setae including 5–6 preoculars, 12–15 verticals, 10–14 postorbitals. Clypeus with 5–7 setae. Antenna with 13 flagellomeres and developed plume of setae; terminal flagellomere with 2 setae, 36–40 µm long in subapical and apical area; AR 1.14–1.17. Palpomere length (µm): 52, 48, 116, 148, 156. Palpomere 3 in distal part with sensilla capitata with diameter 16 µm. Head width/palpal length 0.95–1.12.

Thorax. Anteprepronotum with 6–7 ventrolateral setae. Dorsocentrals 12–13, prealars 6–8. Scutellum with 20–30 setae.

Wing. Length 4.0–4.4 mm, width 1.3–1.4 mm. Anal lobe rounded. Squama with 28–46 setae in 1–2 rows. R and R₁ with 25–28 setae, R₄₊₅ with 6–7. RM/MCu 2.5–2.6. Costa extension 44 µm long.

Legs. BR₁ 2.0–2.5, BR₂ 1.3–1.5, BR₃ 1.1–2.0. Spur of front tibia 68–72 µm long. Spurs of mid tibia 60 µm and 60–64 µm long. Spurs of hind tibia 100–120 µm and 60–68 µm long. Hind tibial comb with 18 setae. Length (µm) and proportions of leg segments are as in Table 1.

Hypopygium (Figs 2–5). Tergite IX with 13–19 setae from one side and with long anal point, 280–296 µm long, which in subapical part tapering (Fig. 2). Laterosternite IX with 10–12 setae. Transverse sternapodeme (TSA) 60–68 µm high and 180–236 µm wide, with triangular antero-lateral projections (Fig. 5); high TSA/width TSA 0.29–0.33. Phallopodeme 180 µm long, aedeagal lobe 200 µm long. Gonocoxite 340–400 µm long, with large angular inferior volsella, which in distal part densely covered with short setae; basal part of volsella with group of 10–15 strong setae (Figs 2–3). Basimedial setae cluster with 13–20 long and strong setae radiating fan-like (Fig. 4). Gonostylus 196–236 µm long, tapering to distal end, apically with long megaseta (20–22 µm long) and tooth, 12 µm long (Fig. 2). HR = 1.69–1.73



Figs 2–5. Adult male of *Diamesa baicalensis* Tshernovskij. 2 — hypopygium in dorsal view; 3 — gonocoxite and gonostylus in dorsal view; 4 — the same in ventral view; 5 — transverse sternapodeme.

Рис. 2–5. Имаго самец *Diamesa baicalensis* Tshernovskij. 2 — гипопигий, вид сверху; 3 — гонококсит и гоностиль, вид сверху; 4 — то же, вид снизу; 5 — поперечная стернаподема.

Table 1. Lengths (in μm) and proportions of leg segments of *Diamesa baicalensis* Tshernovskij, male ($n = 2$)
Таблица 1. Длина члеников ног (мкм) и их индексы самца *Diamesa baicalensis* Tshernovskij, ($n=2$)

P	f	t	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV
P ₁	1480	1880–2000	1260–1263	607–640	410	131	148	0.63–0.67	3.52–3.66	2.66–2.76
P ₂	1640–1656	1680–1689	787–800	361–426	246–279	131	148	0.47–0.48	4.33–4.48	4.15–4.25
P ₃	1720–1760	2000	1200–1280	623	344	131–148	148	0.60–0.64	3.90–4.04	2.94–3.10

Pupa was described by Pankratova [1970], Linevich, Makarchenko [1989].

Larva was described by Tshernovskij [1949], Linevich [1953], Pankratova [1970] and Linevich, Makarchenko [1989].

Remarks. *D. baicalensis* is a rare East Palearctic species which was previously known only in the Lake Baikal basin. The find of this species in Khakassia is the first one made outside the type habitat, and also for the first time a DNA barcoding was made for this species.

Distribution. Species is known from Baikal Lake, Angara, Enisey Rivers and Khakassia.

Diamesa dragani Makarchenko et Semenchenko

Diamesa dragani Makarchenko et Semenchenko, 2022 in Makarchenko et al., 2022: 485.

Material. 7 adult males, RUSSIA: Republic of Khakassia, Tashypyskiy District, Sayan Mountains, unnamed stream of Bolshoi On River basin, altitude 2063 m above sea level, 4.X.2020, 51.714667° N, 89.884500° E (Fig. 30).

Remarks. *D. dragani* is described and known only from Khakassia, belonging to *steinboeckii* group and closely related to the *D. maisaraensis* Makarchenko et Semenchenko from Pamir and *D. marinskiyi* Makarchenko et Semenchenko from Tian-Shan, and can be separated from them mostly by smaller size, 7 flagellomeres antenna, X-type of anal tergal bands and by some other features of hypopygium.

Distribution. Known only from the type locality in Sayan Mountains.

Diamesa hamaticornis Kieffer

Figs 6–7.

Diamesa hamaticornis Kieffer, 1924: 56; Pagast, 1947: 485; Wülker, 1959: 350; Serra-Tosio, 1971: 236; Schmid, 1993: 37; Ashe, O'Connor, 2009: 276.

Material. 1 adult male, Republic of Khakassia, Abakan City, valley of the Abakan River, poplar forest, 53°42'49.5" N, 91°30'19.6" E, alt. 245 m a.s.l., 6.IV.2020 (Fig. 29); 2 adult males, Republic of Khakassia, Askizsky District, Askiz River, from surface of snow, 53°20'21.9" N, 89°53'18.6" E, 5.XI.2020 (Fig. 31).

Description. *Adult male* ($n = 1$, except when otherwise stated). Total length 3.7 mm. Total length/wing length 1.02.

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

Head. Eyes hairy, reniform. Temporal setae including 4 preoculars, 11 verticals. Clypeus with 8 setae. Antenna

with 13 flagellomeres and developed plume of setae, 131–426 μm long; terminal flagellomere with 2 subapical setae, 36–44 μm long; AR 0.50–0.61. Palpomere length (μm): 48, 88, 120, 124, 180. Palpomere 3 in distal part with sensilla capitata with diameter 12 μm . Head width/palpal length 1.08.

Thorax. Antepronotum with 11 ventrolateral setae. Dorsocentrals 13–15, prealars 10. Scutellum with 38 setae.

Wing. Length 3.64 mm, width 1.12 mm. Anal lobe rounded. Squama with 22 setae in 1 row. R and R₁ with 22 setae, R₄₊₅ with 11. RM/MCu 2.7. Costa extension 98 μm long.

Legs. BR₁ 2.3–2.5, BR₂ 2. 0–2.1, BR₃ 2.5–2.7. 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 2.

Hypopygium (Figs 6–7). Tergite IX with 15 setae from one side and anal point, 176 μm long (Fig. 6). Laterosternite IX with 13 setae. Transverse sternapodeme (TSA) 60 μm high and 240 μm wide; high TSA/width TSA 0.25. Gonocoxite 360 μm long; inferior volsellae 180 μm long, wide in basal 3/4, narrow and finger-shaped in distal quarter, with long setae along margin (Fig. 6). Basimedial setae cluster with 12–14 long setae (Fig. 7). Gonostylus 320 μm long, almost sickle-shaped, tapering in distal half, along the inward margin with pale short bristles, apically with short megaseta, 8 μm long (Figs 6–7). HR = 1.13.

Pupa was described by Serra-Tosio [1971].

Larva was described by Schmid [1993].

Remarks. *D. hamaticornis* is very close morphologically and genetically to *D. cinerella* Meigen, *D. hyperborea* Holmgren and *D. tonsa* (Haliday) (see below) from which it differs poorly. Apparently, an additional revision of this group of species is needed.

Distribution. Widely distributed in the Western Palearctic [Ashe, O'Connor, 2009].

Diamesa khumbugelida Sæther et Willassen

Figs 8–9.

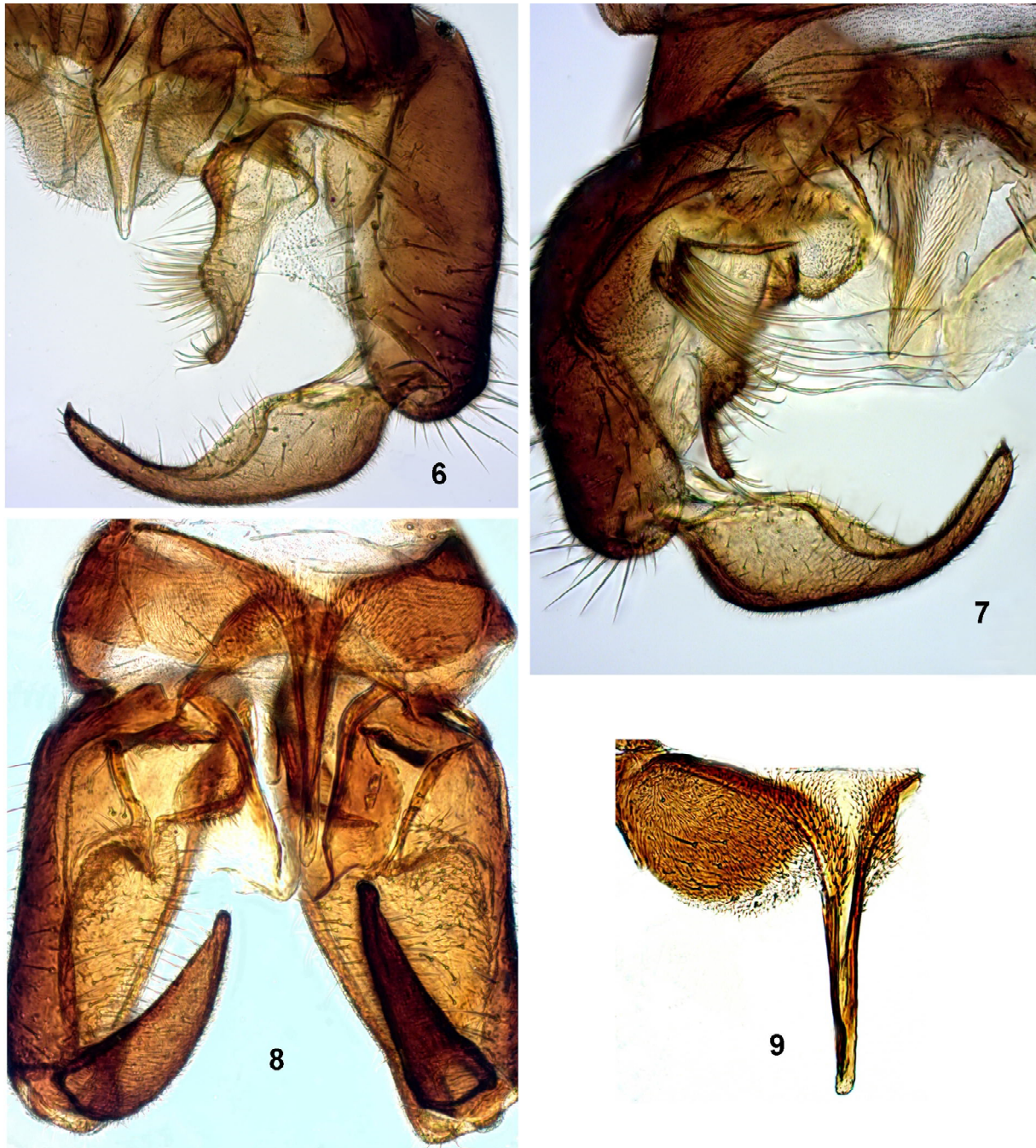
Diamesa khumbugelida Sæther et Willassen, 1987: 201; Makarchenko, 1989: 84, 2009: 429; Ashe, O'Connor, 2009: 279.

Material. 1 adult male, Republic of Khakassia, Tashypyskiy District, unnamed stream in the basin of the Bolshoy On River, ultraviolet lamp, 51°42'58.2" N, 89°51'25.8" E, alt. 1953 m a.s.l., 08–09.VIII.2020.

Description. *Adult male* ($n = 1$). Total length 4.0 mm. Total length/wing length 1.06.

Table 2. Lengths (in μm) and proportions of leg segments of *Diamesa hamaticornis* Kieffer, male ($n = 2$)
Таблица 2. Длина члеников ног (мкм) и их индексы самца *Diamesa hamaticornis* Kieffer, ($n = 2$)

P	f	t	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV
P ₁	1600–1840	1720–2160	1020–1260	541–623	369–426	148–164	164	0.58–0.59	3.51–3.86	3.17–3.25
P ₂	1640–1860	1520–1780	680–780	394–459	221–295	115–131	131–148	0.44–0.45	4.28–4.50	4.65–4.67
P ₃	1800–2020	1620–2160	1140–1320	623–754	344–402	148–164	156–164	0.61–0.70	3.59–3.71	3.0–3.17



Figs 6–9. Adult male of *Diamesa hamaticornis* Kieffer (6–7) and *D. khumbugelida* Sзther et Willassen (8–9). 6, 8 — hypopygium in dorsal view; 7 — hypopygium in ventral view; 9 — part of tergite IX with anal point.

Рис. 6–9. Имаго самец *Diamesa hamaticornis* Кьеффера (6–7) и *D. khumbugelida* Сзтера и Виллассена (8–9). 6, 8 — гипопигий, вид сверху; 7 — гипопигий, вид снизу; 9 — часть тергита IX с анальным отростком.

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

Head. Eyes hairy, reniform. Temporal setae including 5 preoculars, 13 verticals. Clypeus with 4 setae. Antenna with 13 flagellomeres and slightly reduced plume of seta; terminal flagellomere with 2 subapical setae, 32 μm long; AR 0.72. Palpomere length (μm): 44, 116, 176, 284, 128. Palpomere 3 in distal part with sensilla capitata with diameter 24 μm . Head width/palpal length 1.08.

Thorax. Anteprepronotum with 5–6 ventrolateral setae. Dorsocentrals 10–11, prealars 8. Scutellum with 16 setae.

Wing. Length 3.76 mm, width 1.16 mm. Anal lobe unguar. Squama with 27 setae in 1 row. R and R₁ with 36 setae, R₄₊₅ with 3 subapically. RM/MCu 2.6. Costa extension 56–60 μm long.

Legs. Spur of front tibia 60 μm long. Spurs of mid tibia 48 μm long. Spurs of hind tibia 92 and 52 μm long. Hind tibial comb with ca 20 setae. Length (μm) and proportions of leg segments are as in Table 3.

Table 3. Lengths (in μm) and proportions of leg segments of *Diamesa khumbugelida* Sæther et Willassen, male ($n = 1$)
Таблица 3. Длина члеников ног (мкм) и их индексы самца *Diamesa khumbugelida* Sæther et Willassen ($n = 1$)

P	f	t	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV
P ₁	1760	1980	1200	672	443	139	148	0.61	3.52	3.12
P ₂	1880	1760	900	508	312	131	148	0.51	4.13	4.04
P ₃	2040	2000	1360	705	410	131	148	0.68	3.87	2.97

Hypopygium (Figs 8–9). Tergite IX with 11–14 setae from one side and anal point, 296 μm long, with strong anteriorly directed microtrichia in basal half and on tergite IX (Fig. 9). Laterosternite IX with 8 setae. Transverse sternapodeme (TSA) triangular with rounded apex, 68 μm high and 240 μm wide; high TSA/width TSA 0.28. Phallapodeme 230 μm long. Gonocoxite 404 μm long, in basal half dorsally with coracoid protrusion; inferior volsella reduced; superior volsella low. Basimedial setae cluster absent. Gonostylus tapering, 232 μm long, with megaseta, 12 μm long (Fig 8). HR = 2.0.

Pupa and larva unknown.

Distribution. Known from Nepal, Tadjikistan, Baikal Lake basin and Khakassia.

Diamesa parancysta Serra-Tosio

Figs 10–11.

Diamesa parancysta Serra-Tosio, 1983: 15; Willassen et al., 2005: 71; Ashe, O'Connor, 2009: 282; Krashenninikov, 2009: 66.

Diamesa corrupta Makarchenko, 1988: 54.

Material. 1 adult male, Republic of Khakassia, Abakan City, valley of the Abakan River, poplar forest, ultraviolet lamp, 53°42'49.5" N, 91°30'19.6" E, alt. 245 m a.s.l., 2–3.IV.2020; 2 adult males the same data, 6.IV.2020 (Fig. 29).

Description. *Adult male* ($n = 2$). Total length 4.5–5.1 mm. Total length/wing length 1.06–1.07.

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

Head. Eyes hairy, reniform. Temporal setae including 9–10 preoculars, 7–9 verticals. Clypeus with 12–14 setae. Antenna with 13 flagellomeres and developed plume of seta; terminal flagellomere with 1 subapical setae, 36–48 μm long; AR 2.17–2.27. Palpomere length (μm): 52–64, 116–120, 140–164, 132–148, 172–200. Palpomere 3 in distal part with sensilla capitata with diameter 14–16 μm . Head width/palpal length 1.27–1.45.

Thorax. Anteprepronotum with 8–15 ventrolateral setae. Dorsocentrals 11–13, prealars 10. Scutellum with ca 30 setae.

Wing. Length 4.2 mm, width 1.2 mm. Anal lobe rounded, slightly protrude. Squama with 36–53 setae in 1–2 rows. R and R₁ with 23–28 setae, R₄₊₅ with 4–5 setae. RM/MCu 2.67. Costa extension 114 μm long.

Legs. BR₁ 2.8–3.6, BR₂ 1.8–2.5, BR₃ 3.5–4.1. Spur of front tibia 76–88 μm long. Spurs of mid tibia 60–68 and 52–64 μm long. Spurs of hind tibia 92–112 and 56–72 μm long. Hind tibial comb with 18–20 setae. Length (μm) and proportions of leg segments are as in Table 4.

Hypopygium (Figs 10–11). Tergite IX with distinct tergal bands, 9–19 setae from one side and anal point, 272–328

μm long which has terminal dorsal keel and apical setiform sensilla. Laterosternite IX with 8–10 setae. Transverse sternapodeme (TSA) straight or sometimes concave, with strongly projecting anterolateral corners (Fig. 10), 80 μm high and 200 μm wide; high TSA/width TSA 0.40. Gonocoxite 340–372 μm long, with large inferior volsella, broad and prominent basal plate. Basimedial setae cluster well developed, with 16–18 long yellowish setae, 160–200 μm long (Fig. 11). Gonostylus gently curved and relatively broad, 236–240 μm long, with megaseta, 12 μm long and apical tooth, 10 μm long (Fig. 10). HR = 1.44–1.55.

Pupa and larva unknown.

Distribution. Known from Mongolia, Norway, Ural Region, Baikal Lake basin and Khakassia.

Diamesa serratosioi Willassen

Figs 12–14.

Diamesa serratosioi Willassen, 1986: 116; Ashe, O'Connor, 2009: 284.

Material. 3 adult males, Republic of Khakassia, Tashtypsky District, unnamed stream in the basin of the Bolshoy On River, ultraviolet lamp, 51°42'58.2" N, 89°51'25.8" E, altitude 1953 m a.s.l., 8–9.VIII.2020.

Description. *Adult male* ($n = 3$, except when otherwise stated). Total length 2.3–3.0 mm. Total length/wing length 0.82–1.0.

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

Head. Eyes hairy, reniform. Temporal setae including 3–4 preoculars, 5–6 verticals. Clypeus with 2–7 setae. Antenna with 8 flagellomeres and reduced plume of setae; terminal flagellomere with 2 setae, 24–32 μm long in subapical and apical area. Length of 1–8 flagellomeres (μm): 100–104, 40–48, 36–40, 28–30, 32, 24–28, 28–30, 108–116; AR 0.38. Palpomere length (μm): 44–48, 48, 104–116, 84–100, 152–156. Palpomere 3 in distal part with sensilla capitata with diameter 12–16 μm . Head width/palpal length 1.09–1.17. Antennal length/palpal length 0.90–0.95.

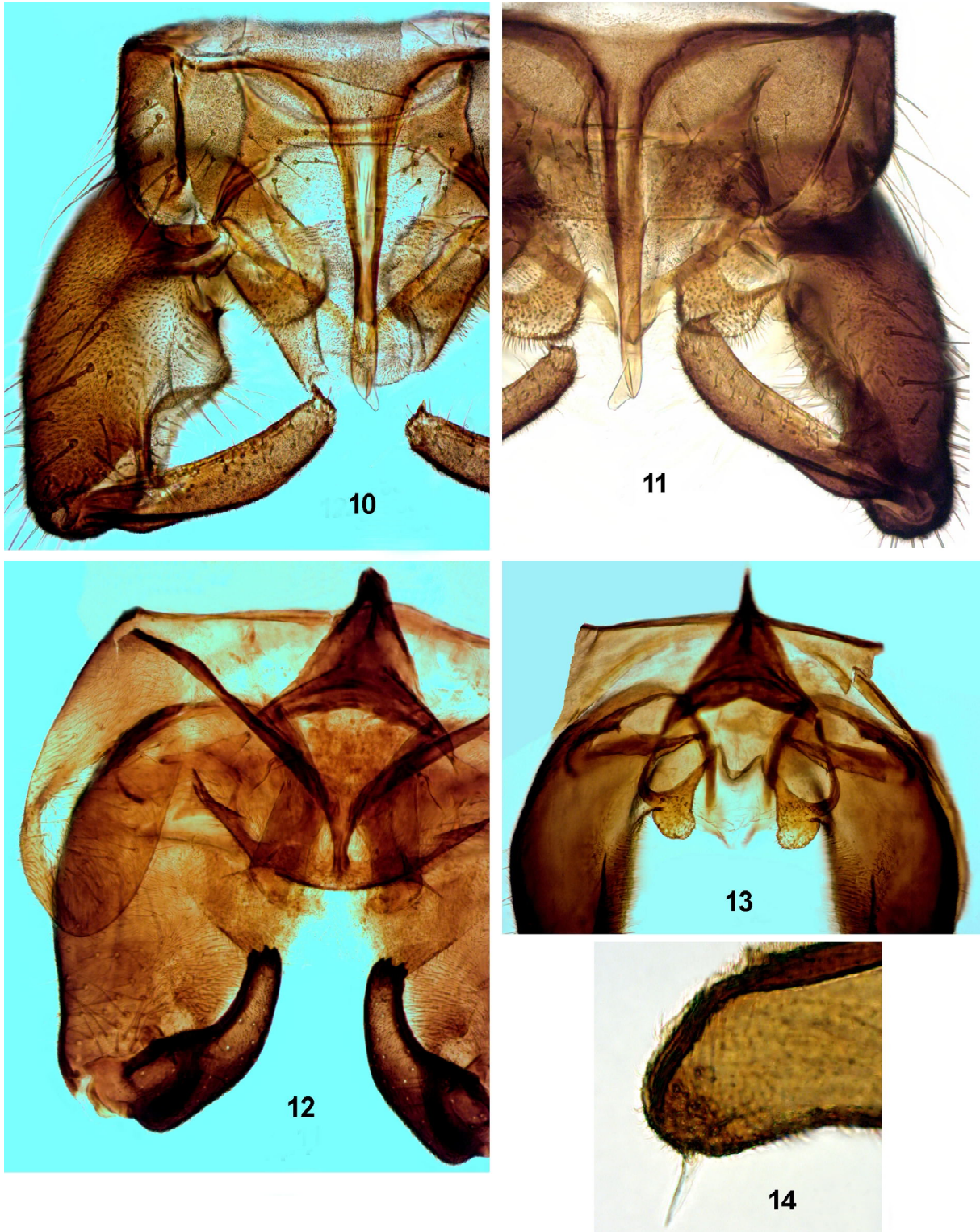
Thorax. Anteprepronotum with 11–12 ventrolateral setae. Dorsocentrals 10–12, prealars 4. Scutellum with 30–35 setae.

Wing. Length 2.8–3.0 mm, width 1.0–1.1 mm. Anal lobe round-angular. Squama with 15–25 setae, 52–88 μm long. R and R₁ with 17–22 setae, R₄₊₅ with 5–7. RM/MCu 2.3–2.5.

Legs. BR₁ 1.1–2.0, BR₂ 1.0–1.3, BR₃ 1.1–2.0. Spur of front tibia 28–36 μm long. Spurs of mid tibia 36 μm and 32–36 μm long. Spurs of hind tibia 64–84 μm and 32–40 μm long. Hind tibial comb with 18 setae. Length (μm) and proportions of leg segments are as in Table 5.

Table 4. Lengths (in μm) and proportions of leg segments of *Diamesa parancysta* Serra-Tosio, male ($n = 3$)
Таблица 4. Длина члеников ног (мкм) и их индексы самца *Diamesa parancysta* Serra-Tosio, ($n = 3$)

P	f	t	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV
P ₁	1279–1440	1620–1673	1160–1164	560–607	328–344	139–148	156–164	0.70–0.72	3.30–3.52	2.54–2.64
P ₂	1525–1640	1525–1760	787–880	459–492	246–312	115–164	148–164	0.50–0.52	3.73–3.96	3.86–3.88
P ₃	1673–1840	1804–2120	1197–1400	664–705	344–394	148–164	148–180	0.66	3.58–3.71	2.83–2.90



Figs 10–14. Adult male of *Diamesa parancysta* Serra-Tosio (10–11) and *D. serratosoi* Willassen (12–14). 10–12 — hypopygium in dorsal view; 13 — basal part of hypopygium without tergite IX in dorsal view; 14 — anal point in lateral view.

Рис. 10–14. Имаго самец *Diamesa parancysta* Serra-Tosio (10–11) и *D. serratosoi* Willassen (12–14). 10–12 — гипопигий, вид сверху; 13 — базальная часть гипопигия без тергита IX, вид сверху; 14 — анальный отросток, вид сбоку.

Table 5. Lengths (in μm) and proportions of leg segments of *Diamesa serratosioi* Willassen, male ($n = 3$)
Таблица 5. Длина члеников ног (мкм) и их индексы самца *Diamesa serratosioi* Willassen ($n = 3$)

P	f	t	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV
P ₁	1525–1574	1558–1681	1033–1082	476–492	279–287	98–115	131	0.64–0.66	4.18–4.23	2.98–3.07
P ₂	1525–1558	1378–1509	640–689	295–312	197–213	98–115	131	0.46	4.80–4.98	4.45–4.54
P ₃	1574–1640	1591–1738	1017–1099	541–549	262–295	82–115	115–131	0.63–0.64	3.99–4.30	3.07–3.11

Hypopygium (Figs 12–14). Laterosternite IX extending beyond posterior margin of tergite IX by 56–80 μm , with weak 18–20 setae, 40–52 μm long (Fig. 12). Tergite IX with 16–18 setae, 20–32 μm long and with pale hyaline anal point, 26–28 μm long, which is not visible from above, in lateral view pointing down (Fig. 14); posterior margin of tergite IX rounded; tergal bands Y-type (Fig. 12). Transverse sternopodeme (TSA) triangular, peaked, 120–140 μm high, 160–168 μm wide at the base; TSA height/TSA width 0.75–0.83. Aedeagal lobe as in Figs. 13, weakly chitinized. Gonocoxite 372–380 μm long, superior volsellae in the form of a small cone covered with microtrichia and short setae (Fig. 13). Gonostylus 200–204 μm long, curved, broad at base, with megaseta, 4–5 μm long, 3 api-cal teeth and 2 subterminal setae. HR 1.82–1.86.

Pupa and larva unknown.

Remarks. *D. serratosioi* is belonging to *davisi* group and males of this species from Norway and Sweden were previously described as *D. davisi* Edwards by Serra-Tosio [1971]. It is the first record of *D. serratosioi* for Russia.

Distribution. Species is known from Finland, Norway, Sweden [Ashe, O'Connor, 2009] and Khakassia.

Pseudodiamesa aff. *nivosa* (Goetghebuer)

Figs 15–20.

Syndiamesa nivosa Goetghebuer, 1928: 126;

Pseudodiamesa nivosa (Goetghebuer); Pagast, 1947: 510; Reiss, 1968: Fig. 6; Serra-Tosio, 1964: Fig. 1A–1C, 1971: 69, 1976: 126; Ashe, O'Connor, 2009: 305.

Material. 8 adult males, Republic of Khakassia, Askizsky District, «Zimnyi» Stream, Askiz River basin, 53°25'07.5" N, 89°45'22.6" E, alt. 646 m a. s. l., 1.VIII.2020 (Fig. 32).

Description. *Adult male* ($n = 3$). Total length 5.9–6.3 mm. Total length/wing length 1.08–1.13.

Coloration. Head, thorax, legs, and abdomen dark brown to black; antennae brown to dark brown; wings greyish.

Head. Eyes bare and extended dorsomedially. Temporal setae including 14–15 preoculars, 6–8 verticals and 7–9 post-orbitals. Clypeus with 34–36 setae. Antenna with 13 flagellomeres and developed plume; pedicel without setae; terminal flagellomere with 1–2 subapical setae 36–40 μm long; AR 3.22–3.82. Palpomere lengths (in μm): 68–72; 172–188; 248–250; 192–228; 252–272. Palpomere 3 distally with sensilla capitata (diameter 16 μm). Palpomeres 1–5 length/head width 0.91–1.0.

Thorax. Antepnotum with 7–8 lateral setae. Acrostichals absent, dorsocentrals 29–39 (in 2–3 rows), prealars 32–34, scutellars ca 58–60.

Wing. Length 5.48–5.56 mm; width 1.32–1.44 mm. Membrane without setae. R and R₁ with 26–29 setae, R₄₊₅ with 1–3 setae on apex. Costa extension 148–150 μm long. RM length/MCu length 2.5. R₂₊₃ visible only in basal 140–180 μm . MCu flows into Cu, distally of fork FCu at 230–246 μm (Fig. 18). Anal lobe developed, outline rounded. Squama with ca 80 setae in 2–3 rows. VR 0.90–0.91.

Legs. BR₁ 5.40–5.46, BR₂ 2.90–3.0, BR₃ 4.58–5.0. Spur of fore tibia 120–132 μm long; spurs of mid tibia 76–84 μm and 76–84 μm ; of hind tibia 120–144 μm and 80–92 μm long. Hind tibial comb with 16–18 setae. Lengths and proportions of leg segments as in Table 6.

Hypopygium (Figs 15–17, 10–20). Tergite IX with 18–26 setae on each side. Anal point wide in basal part, tapering to apex and often distally setiform, 72–100 μm long (Figs 15, 19–20). Laterosternite IX with 10–12 setae. Gonocoxite 388–412 μm long; basal lobe of large size, wedge-rounded, with some long setae at inner part (Figs 16–17). Gonostylus 238–244 μm long, in dorsal view with large lobe on the outer edge (Figs. 15–17); apically with short pale megaseta, 14–16 μm long. Transverse sternopodeme trapezoidal, 272–308 μm wide and 48–64 μm high (Fig. 16). Phallapodeme 184 μm long, with lateral aedeagal lobe 220–260 μm long, which more wide in basal 1/4 and narrow in distal 3/4, elongated in rounded apex (Figs 16–17). HR 1.61–1.63.

Remarks. The *Pseudodiamesa* species which found in Khakassia belongs to *nivosa* group and is most similar to the *Ps. nivosa*, but differences in the shape of the gonostylus, as well as DNA barcoding data, do not allow us to confidently attribute this species to *Ps. nivosa*.

Sympotthastia takatensis (Tokunaga)

Figs 21–28.

Syndiamesa takatensis Tokunaga, 1936: 531; 1937: 49.

Sympotthastia toyamayezae Sasa, 1996: 44.

Sympotthastia khorensis Makarchenko, 1984: 91, 1985: 60.

Sympotthastia takatensis (Tokunaga); Makarchenko, 1994: 51, 2006: 276; Wang, 2000: 634; Kobayashi, Endo, 2008: 58; Ashe, O'Connor, 2009: 331; Liu et al., 2016: 428.

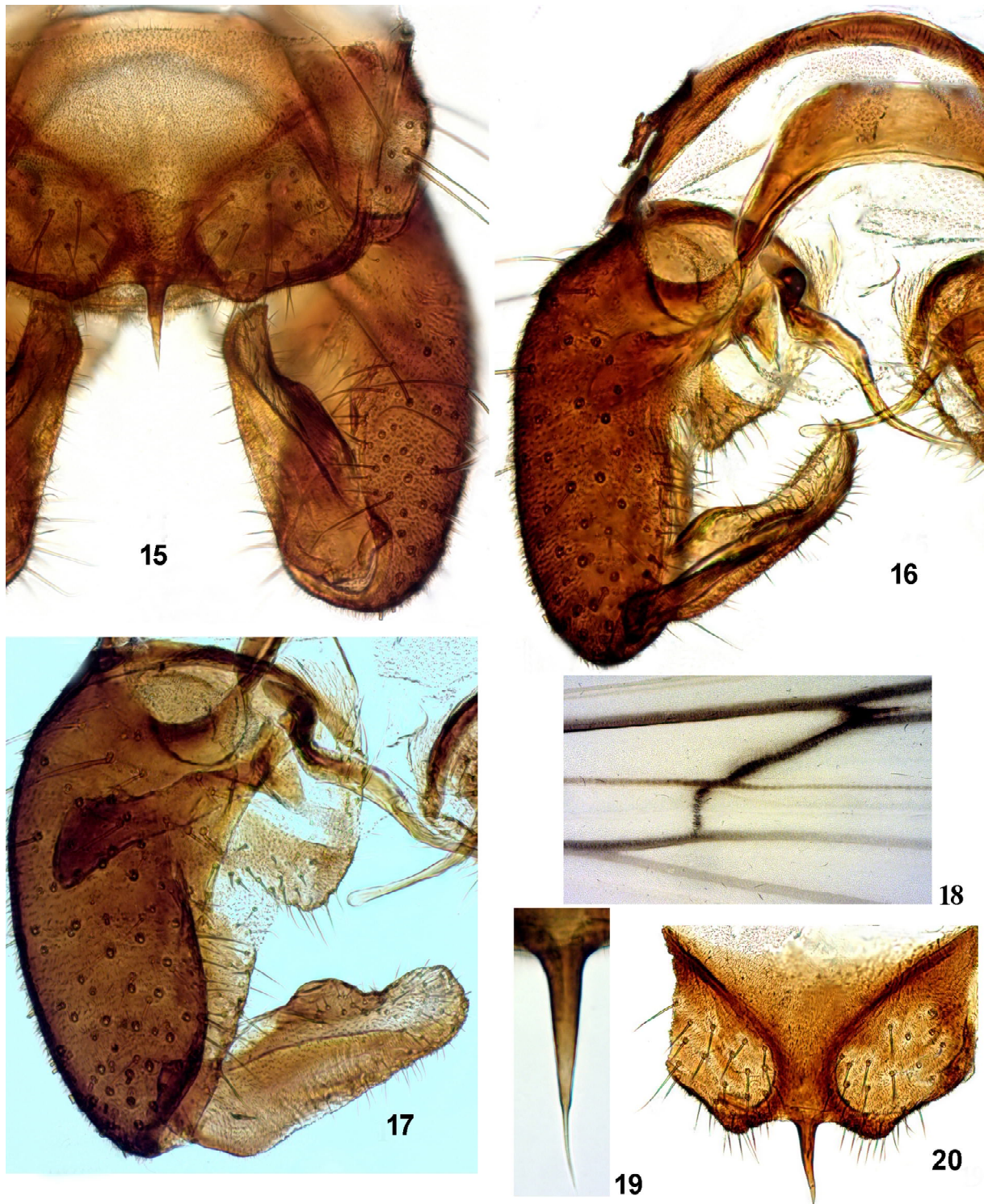
Material. 1 adult male, Republic of Khakassia, Abakan City, valley of the Abakan River, poplar forest, ultraviolet lamp, 53°42'49.5" N, 91°30'19.6" E, alt. 245 m a.s.l., 30.IV.2020; 2 adult males the same data, 10–11.V.2020; 1 adult male, the same data, 8–9.VII.2020 (Fig. 29).

Description. *Adult male* ($n = 3$). Total length 4.5–5.9 mm. Total length/wing length 1.09–1.16.

Coloration. Head, thorax, legs, and abdomen dark brown to black; antennae brown to dark brown; wings grayish, squama grey, venation brown.

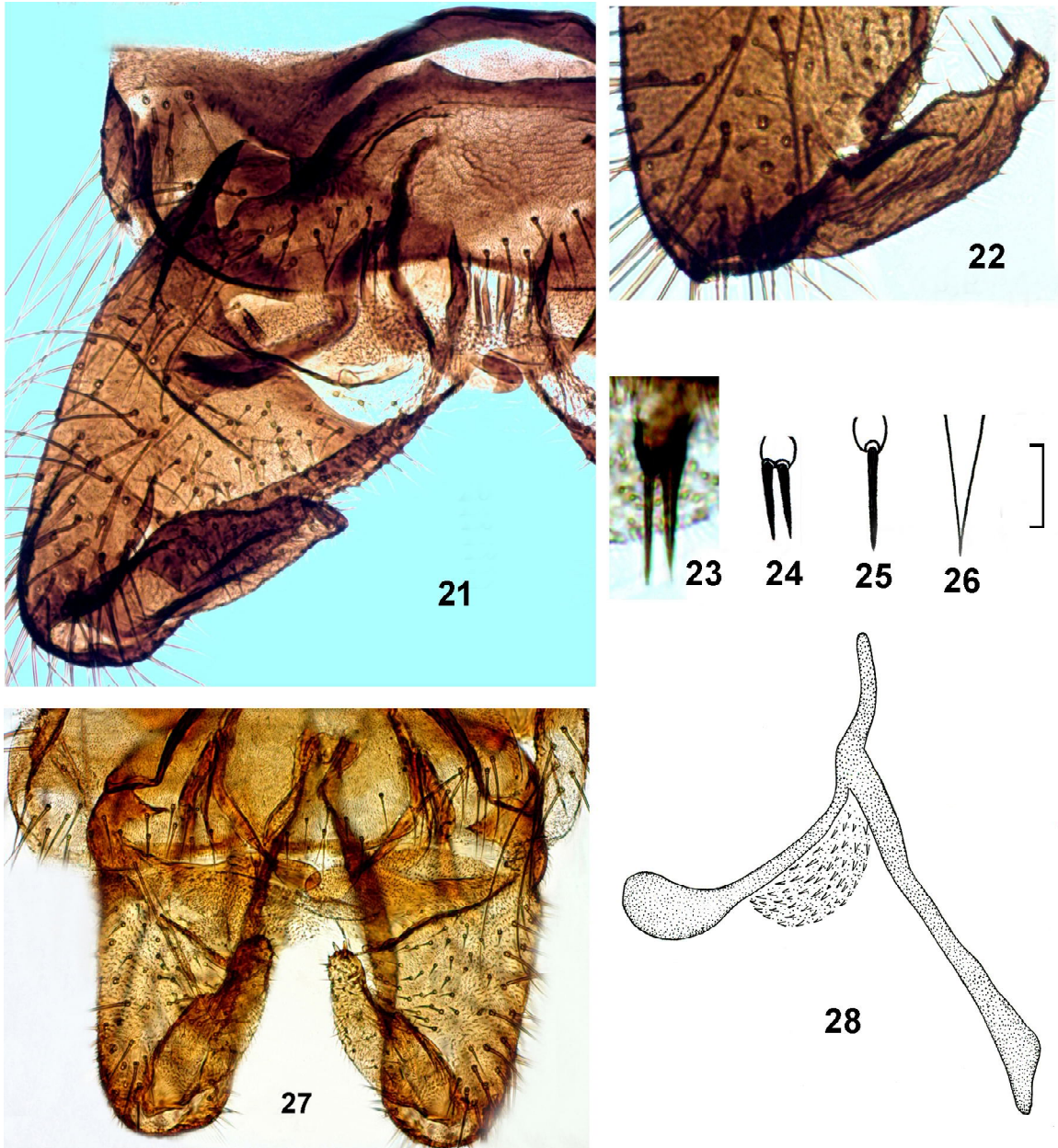
Table 6. Lengths (in μm) and proportions of leg segments of *Pseudodiamesa* aff. *nivosa* (Goetghebuer), male ($n = 3$)
Таблица 6. Длина члеников ног (мкм) и их индексы самца *Pseudodiamesa* aff. *nivosa* (Goetghebuer) ($n = 3$)

P	f	t	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV
P ₁	1800–1820	2000–2080	1320–1440	738–787	476–623	271–287	230–238	0.66–0.69	2.19–2.97	2.69–2.88
P ₂	1960–2040	2100–2160	1040–1120	607–640	394–476	230–246	213–230	0.49–0.52	3.27–3.61	3.71–4.0
P ₃	2320–2400	2760–2800	1300–1520	820–886	459–508	295–312	230–279	0.47–0.54	3.42–3.61	3.42–3.97



Figs 15–20. Adult male of *Pseudodiamesa* aff. *nivosa* (Goetghebuer). 15 — hypopygium in dorsal view; 16–17 — hypopygium without tergite IX in dorsal view; 18 — gonostylus; 19 — anal point; 20 — tergite IX.

Рис. 15–20. Имаго самец *Pseudodiamesa* aff. *nivosa* (Goetghebuer). 15 — гипопигий, вид сверху; 16–17 — гипопигий без тергита IX, вид сверху; 18 — гоностиль; 19 — анальный отросток; 20 — тергит IX.



Figs 21–28. Adult male of *Symptothastia takatensis* (Tokunaga). 21, 27 — hypopygium in dorsal view; 22 — gonostylus; 23–26 — anal point; 27 — lateral endomere and fallapodeme.

Рис. 21–28. Имаго самец *Symptothastia takatensis* (Токунэга). 21, 27 — гипопигий, вид сверху; 22 — гоностиль; 23–26 — анальный отросток; 28 — латеральная эндомера и фаллопоема.

Head. Eyes bare and extended dorsomedially. Temporal setae including 5–7 verticals and 10–12 postorbitals. Clypeus with 11–14 setae. Antenna with 13 flagellomeres and developed plume; terminal flagellomere with 1–2 subapical setae 40–42 μm long; AR 2.76–2.81. Palpomere lengths (in μm): 48; 100; 228; 240; 248. Palpomere 3 distally with sensilla capitata (diameter 12 μm) and with projection apically. Palpomeres 1–5 length/head width 1.85–1.91.

Thorax. Antepnotum with 5–7 lateral setae. Acrostichals absent, dorsocentrals 18–19 (in 2 rows), prealars 20–25 (in 2 groups), scutellars ca 35.

Wing. Length 4.8–5.4 mm; width 1.24–1.40 mm. R and R_1 with 22–23 setae, R_{4+5} without setae. Costa extension

115–148 μm long. RM length/MCu length 2. Anal lobe developed, rounded and protrudes forward. Squama with 60–65 setae in 2–3 rows.

Legs. BR_1 4.1–7.5, BR_2 3.0–6.2, BR_3 4.3–6.5. Spur of fore tibia 116–140 μm long; spurs of mid tibia 68–72 μm and 68–72 μm ; of hind tibia 116–140 μm and 68 μm long. Hind tibial comb with 16–18 setae. Lengths and proportions of leg segments as in Table 7.

Hypopygium (Figs 21–28). Tergite IX with 12–16 setae on each side. anal point variable, often in the form of one or two setae, 20–28 μm long located on the tubercle, 8 μm long (Figs 23–25), but may be of the usual structure, wider basally and tapering to a pointed apex, 44 μm long (Fig. 26). Lateros-



Figs 29–32. Localities of some Diamesinae from Khakassia. 29 — Abakan City, channel of the Abakan River, poplar forest; 30 — unnamed stream of Bolshoi On River basin, altitude 2063 m above sea level; 31 — Askiz River; 32 — “Zimnyi” Stream of Askiz River basin (Photos by S.V. Dragan).

Figs 29–32. Localities of some Diamesinae from Khakassia. 29 — Abakan City, channel of the Abakan River, poplar forest; 30 — unnamed stream of Bolshoi On River basin, altitude 2063 m above sea level; 31 — Askiz River; 32 — “Zimnyi” Stream of Askiz River basin (Photos by S.V. Dragan).

termite IX with 12–14 setae. Aedeagal lobe 160–180 μm long, in distal part shaped like a golf-club (Fig. 28). Phallapodeme 172 μm long. Gonocoxite 356–360 μm long; superior volsella small, rounded, with microtrichia; median volsella spiniferous. Gonostylus 188–200 μm long widest in about middle and narrowing towards apex with megaseta 20–24 μm long and 2 subapical setae, 36–40 μm long (Figs 21–22, 27). Transverse sternapodeme trapezoidal, 240 μm wide and 24–28 μm high. HR 1.85–1.91.

Pupa and larva are described by Liu et al. [2016].

Distribution. East Palearctic species. Was known from China, Korea, Japan and Russian Far East. For East Siberia is recorded for the first time.

Results of DNA barcoding

A total of 15 specimens belonging to *Diamesa* Meigen, *Symphotthastia* Pagast and *Pseudodiamesa* Goetghebuer were sequenced. The final alignment of the COI barcode region yielded 658 base pairs.

We made a comparison of the obtained sequences with data of BOLD systems. The closest sequence to *D. parancysta* were *Diamesa* sp. XJC (BIN

BOLD:ADL0342), the average p-distance between them were 0.38 % which corresponding to species level. Notably that *Diamesa* sp. XJC consist of five specimens in BOLD systems, of which 4 specimens (XJDQD1074-18 - XJDQD1077-18) collected in Xinjiang, China and specimen COLEB087-18 collected in Wrangell—St. Elias National Park and Preserve, Canada. Thus, *D. parancysta* have a large area with minimal intraspecific divergence. Monophyly of *D. parancysta* and 2 specimens of *Diamesa* sp. XJC from China (XJDQD1074-18) and Canada (COLEB087-18) were high supported (Bayesian posterior probability, BPP=1) which confirms their conspecificity (Fig. 33).

D. hamaticornis was closed to a wide number of specimens belonging to *D. cinerella*, *D. hamaticornis*, *D. hyperborea* and *D. tonsa*. The average p-distances between 7 specimens of 4 species were 0.46 %. Close High similarity of *D. cinerella*, *D. hamaticornis* and *D. tonsa* was discussed in Lencioni et al. [2020]. Apparently, *cinerella* group also includes the *D. hyperborea* from continental Norway and Svalbard. Four species were included in the high supported clade (BPP = 1).

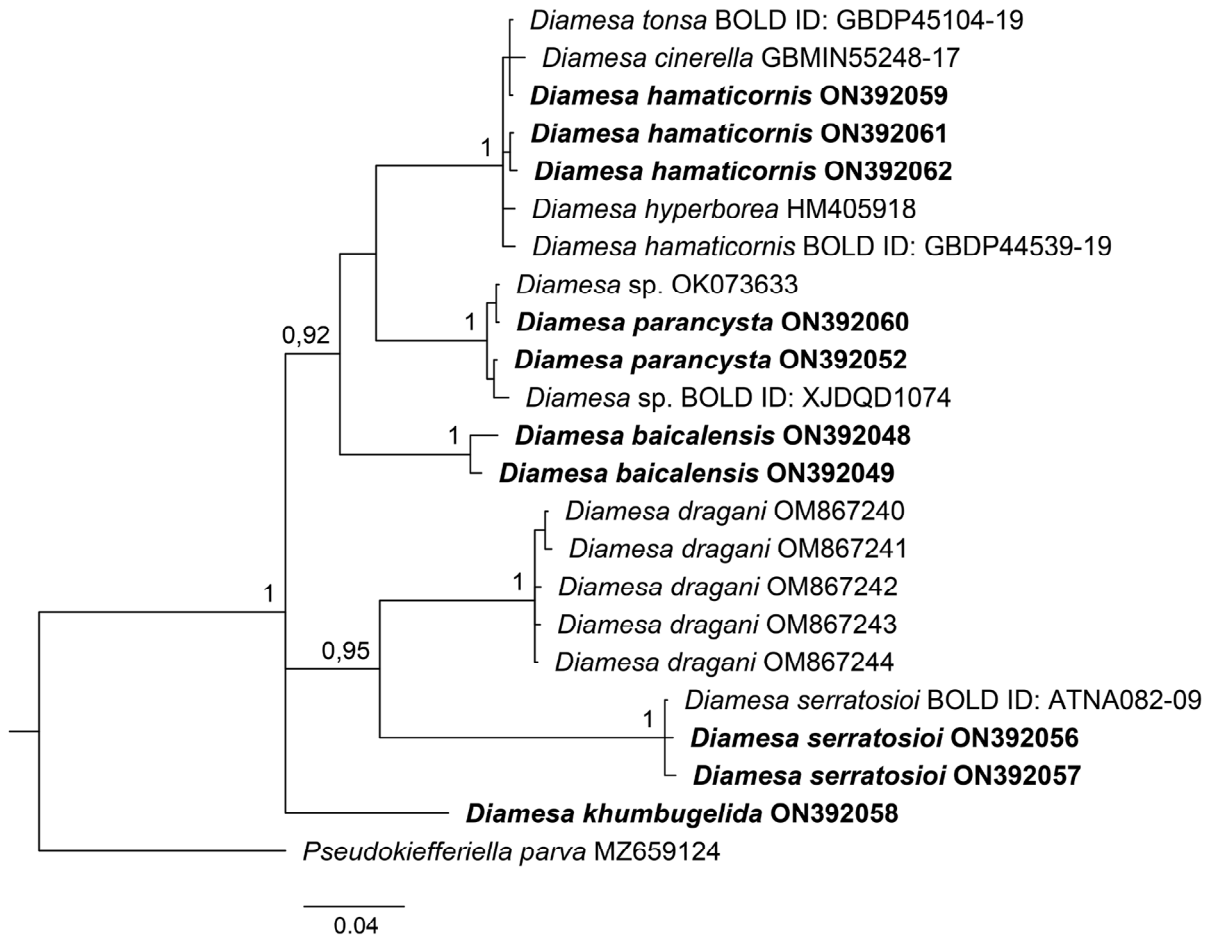


Fig. 33. Bayesian tree on mitochondrion COI gene of genus *Diamesa* using obtained sequences and close sequences from GenBank and BOLD systems. Clade support is indicated by posterior probabilities $e \geq 0.70$ above each branch. Specimens obtained in this study are in bold.

Рис. 33. Байесовское дерево, построенное с использованием гена цитохромоксидазы 1 на основании полученных последовательностей рода *Diamesa*, а также сходных сиквентов из генного банка и системы BOLD. Значения апостериорной вероятности выше 0,70 указаны над узлами ветвления. Секвенированные в настоящей работе особи выделены полужирным шрифтом.

Sequences of *D. baicalensis* and *D. khumbugelida* were unique. The closest available species were *D. zernyi* and *D. tonsa* which differed from the obtained sequences by 6.80 % and 7.65 % respectively.

Sequences of *D. serratosioi* were conspecific to *D. serratosioi* ATNA082-09 (BIN BOLD: AAD4446). The average sequences divergence between sequences were 0.38 %.

The obtained sequences of *S. takatensis* are most similar to the sequences of *S. takatensis*, which are privately available in BOLD. The closest published se-

quence belongs to *S. spinifera* with sequence divergence 8.42 %.

We also add to the Bayesian tree sequences of *D. dragani* (OM867240-OM867244) which was related to the *D. steinboeckii* group and collected from Khakassia [Makarchenko et al., 2022].

The p-distances between *Ps. aff. nivosa* and specimen of *Ps. nivosa* collected from Perm Krai (Russia) and deposited in BOLD systems under number GBMNB14891-20 (BIN BOLD: ADB8603) were 2.51 % which indicates their conspecificity. Another species of *Ps. nivosa*

Table 7. Lengths (in μm) and proportions of leg segments of *Sympotthastia takatensis* (Tokunaga), male ($n = 2$)
Таблица 7. Длина члеников ног (мкм) и их индексы самца *Sympotthastia takatensis* (Tokunaga) ($n = 2$)

P	f	t	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV
P ₁	1443–1640	1738–1920	1263–1300	656–705	443–508	279–410	180–213	0.68–0.73	2.63–2.85	2.51–2.74
P ₂	1525–1760	1804–1860	900–986	556–558	385–402	213	164–197	0.48–0.49	3.19–3.30	3.76–4.02
P ₃	1840–2120	2080–2240	1320–1360	672–722	443–476	246–262	197	0.61–0.63	3.36–3.45	2.97–3.21

(BIN BOLD: AAF6936) uniting specimens from Norway, Finland and Leningrad Oblast of Russia. The average sequence divergence between *Ps.* aff. *nivosa* and *Ps. nivosa* (BIN BOLD: AAF6936) were 6.39 %. These results indicate the need for a taxonomical revision of this species.

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