

## A review of the *Arctodiamesa* Makarchenko, 1983 (Diptera: Chironomidae: Diamesinae), with DNA barcoding of known species

### Обзор рода *Arctodiamesa* Makarchenko, 1983 (Diptera: Chironomidae: Diamesinae), с ДНК-баркодингом известных видов

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**Key words:** Diptera, Chironomidae, Diamesinae, *Arctodiamesa*, review, DNA barcoding.

**Ключевые слова:** Diptera, Chironomidae, *Arctodiamesa*, обзор, ДНК-анализ.

**Abstract.** This article reviews chironomids of the genus *Arctodiamesa* Makarchenko, 1983 from the Arctic regions of Eurasia, as well as from the Russian Far East and Eastern Siberia. Using an integrative method, a new cryptic species, *A. brusnevi* Krasheninnikov, Makarchenko et Semenchenko, **sp.n.**, is described, and the species *A. appendiculata* (Lundström, 1915), *A. amurensis* Makarchenko, 2007, *A. marinae* Makarchenko, 2005 and *A. breviramosa* Makarchenko, 1995 are redescribed. Mean K2P interspecific distances between *A. brusnevi* Krasheninnikov, Makarchenko et Semenchenko, **sp.n.** from Bolshevik Island of and *A. appendiculata* (Lundström) from the northern part of the Russian Far East were 8.87%. Species delimitation (ASAP) confirms that five species of the genus *Arctodiamesa* Makarchenko are valid. A key to the adult males of known species is given.

**Резюме.** Приведён обзор хирономид рода *Arctodiamesa* Makarchenko, 1983 из арктических районов Евразии, а также с российского Дальнего Востока и Восточной Сибири. С использованием интегративного метода описан новый криптический вид *A. brusnevi* Krasheninnikov, Makarchenko et Semenchenko, **sp.n.**, переописаны виды *A. appendiculata* (Lundström, 1915), *A. amurensis* Makarchenko, 2007, *A. marinae* Makarchenko, 2005 и *A. breviramosa* Makarchenko, 1995. Средние межвидовые дистанции, рассчитанные по Кимура-двухпараметрической модели между *A. brusnevi* Krasheninnikov, Makarchenko et Semenchenko, **sp.n.** с острова Большевик архипелага Северная Земля и *A. appendiculata* (Lundström) с северной части российского Дальнего Востока, составили 8,87%. Метод разграничения видов (ASAP) подтвердил валидность пяти видов рода *Arctodiamesa* Makarchenko. Для имаго самцов известных видов рода дана определительная таблица видов.

## Introduction

Based on the materials of the Russian Polar Expedition of 1900–1903, K. Lundström described a new species of chironomid, *Diamesa appendiculata*, from the mouth of the Lena River (Chara-Ullah Mountains)

[Lundström, 1915] by a single adult male and, due to the lack of other finds of this species, remained unchanged in all major reports on Diamesinae [Goetghebuer, 1939; Pagast, 1947]. In 1978, E.A. Makarchenko discovered this species on the Chukchi Peninsula, redescribed its male, described the pupa and larva [Makarchenko, 1978]. From 1978 to 1982, extensive specimens were collected for *D. appendiculata* from various regions of Chukotka and the Kolyma River basin. After studying this material and comparing of *D. appendiculata* with other species and genera of the subfamily Diamesinae, was concluded that this species belongs in its own new genus, *Arctodiamesa* Makarchenko, for which a brief diagnosis was published in 1983 [Makarchenko, 1983]. A year later, a detailed diagnosis of the genus *Arctodiamesa* Makarchenko was published and, taking into account new data, the imago male, pupa and larva of *A. appendiculata* (Lundström, 1915) were redescribed, and new locations of the species were noted [Makarchenko, 1984]. Based on this work, the diagnosis of the genus was included in the collective monographs “Chironomidae of the Holarctic Region” [Oliver, 1986, 1989; Sæther, Andersen, 2013].

Currently the genus *Arctodiamesa* Makarchenko includes four species, of which *A. appendiculata* is distributed on the Arctic coast of Eurasia, in the mountainous regions of the Upper Kolyma River basin and the Irkutskaya Oblast, also apparently in Alaska (as *Diamesa* Alaska sp. 1) [Hansen, Cook, 1976; Tilley 1978; Makarchenko 1985; Ashe, O’Conner, 2009]. *A. breviramosa* Makarchenko is described from males collected in streams near the mouth of the Lena River in the Arctic Region of East Siberia [Makarchenko, 1995] and is recently discovered in the Upper Kolyma River basin. *A. marinae* Makarchenko is known from the Southern Primorye of the Russian Far East [Makarchenko, 2005] and also is recorded from South Korea [Kang et al., 2017].

*A. amurensis* Makarchenko inhabits the Amur River basin [Makarchenko, 2007] and is recently collected in Kamchatka. In addition to the species listed above the immature stages of an unidentified *Arctodiamesa* Makarchenko species were described from the Khabarovskii Krai of the Russian Far East [Makarchenko, 1995].

In this article, we provide an overview of the chironomids of the genus *Arctodiamesa* Makarchenko from the Arctic regions of Eurasia, as well as from the Russian Far East and Eastern Siberia. Using an integrative method, a new cryptic species, *A. brusnevi* Krasheninnikov, Makarchenko et Semenchenko, sp.n., is described, and the species *A. appendiculata* (Lundström), *A. amurensis* Makarchenko, *A. breviramosa* Makarchenko, and *A. marinae* Makarchenko are redescribed. DNA barcoding has been shown to be useful for distinguishing species of the *Arctodiamesa* Makarchenko. A key to the adult males of known species is given.

## Materials and methods

The adult males were preserved in 70 % ethanol for further study of morphology and in 96 % ethanol for DNA barcoding. The material was slide-mounted in polyvinyl lactophenol. The morphological terminology and abbreviations used below generally follow 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 (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.

Holotype and paratypes of the new species, as well as all other material, are deposited in the Bioresource Collection (reg. number 2797657) of the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia (FSCEATB FEB RAS).

Genomic DNA was extracted from thorax and abdomen following the protocol of the Qiagen DNeasy Blood & Tissue Kit. The cytochrome oxidase subunit I (COI) gene was selected for studying. PCR was performed in a 10 µL reaction volume using Go Taq Green Master Mix (Promega Corp, Madison, WI, USA) or 5X ScreenMix-HS DNA polymerase (Evrogen, Moscow, Russia) and primers LCO1490 and HCO2198 [Folmer et al., 1994]. Amplification of PCR products was checked by electrophoresis using a 1.5% agarose gel in TBE buffer stained with Ethidium bromide and visualized on transilluminator. The PCR product was purified using Cleanup St PCR (Evrogen, Moscow, Russia) and sequenced for both directions. Sequencing reaction was performed using BigDye® Terminator v3.1 Cycle Sequencing Kits and run on an ABI 3500 Genetic Analyzer Sequencer (Applied Biosystems, Foster City, CA, USA).

Assemble Species by Automatic Partitioning ASAP [Puillandre et al., 2021] analysis with K2P distances was used to delimit the dataset into molecular taxonomic units.

Phylogenetic analyses were conducted using Bayesian inference (BI) analyses in MrBayes 3.2.7a [Ronquist et al., 2012] using Markov Chain Monte Carlo (MCMC) randomization. Four Markov chains (three heated chains, one cold) were run for 1 million generations, with the first 25 % of sampled trees discarded as burn-in. Strict clock model (brelenspr = clock: uniform) were used to obtain an ultrametric tree. PartitionFinder 2.1.1 [Lanfear et al., 2012] was used to select the best-fit partitioning scheme and models separately for each codon position of the COI gene. The obtained sequences have been deposited in GenBank under numbers PX959799 – PX959807.

Nomenclatural act introduced in the present work is registered in ZooBank (www.zoobank.org) under urn:lsid:zoobank.org:pub:3211339D-859B-4E20-96D0-2BE0FBFE194D

## Taxonomy

### Diptera: Chironomidae: Diamesinae *Arctodiamesa* Makarchenko, 1983

Type species: *Diamesa appendiculata* Lundström, 1915, by original designation.

#### *Arctodiamesa appendiculata* (Lundström, 1915)

Figs 1–11.

*Diamesa appendiculata* Lundström, 1915: 23; Goetghebuer, 1939: 11; Makarchenko, 1978: 56;

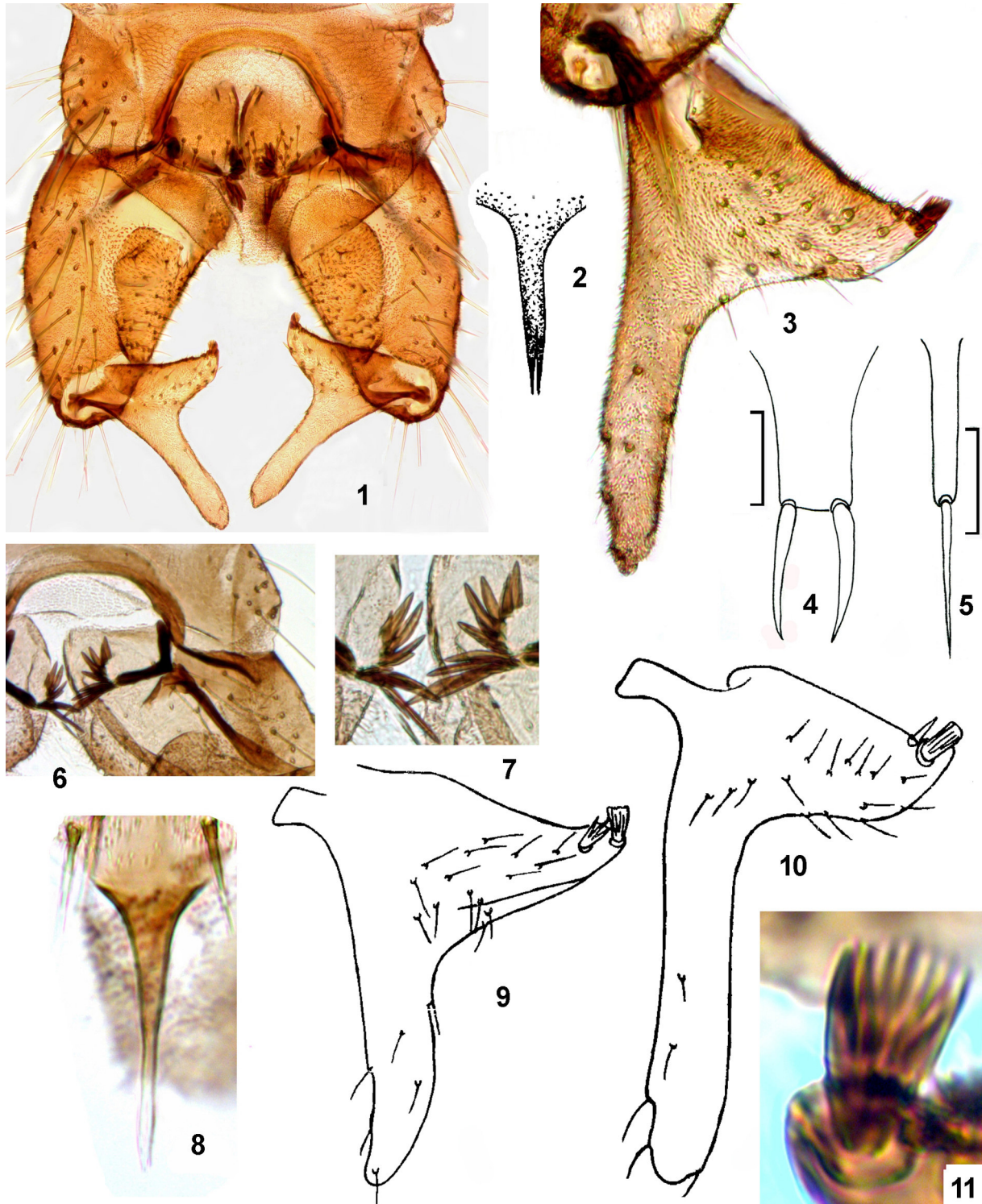
*Arctodiamesa appendiculata* (Lundström, 1915): Makarchenko, 1983: 264, 1984: 96, 1985: 54, 2006: 259, 471, 610; Zelentsov, Shilova, 1996: 55; Sæther et al., 2000: 60; Ashe, O'Connor, 2009: 263.

**Material.** **Russia, Chukotka Autonomous Okrug:** 9 adult ♂♂, 41 pupae, 19 larvae — Iultinsky District, Kresta Bay, nameless stream of the Seutakan Lake basin, 5–30.VII.1976, leg. E.A. Makarchenko; 9 adult ♂♂ — the same data, except Chaunsky district, Chaunskaya Bay, Pucheveyem River, a stream of the northern slope of Neitlin Mount, 13.VII.1978, leg. E.A. Makarchenko; 14 adult ♂♂ — the same data, except Iultinsky District, Wrangel Island, Nasha River, 2.VII.1979, leg. E.A. Makarchenko; 2 adult ♂♂ — the same data, except Somnitelnaya River, 22.VII.1979, leg. E.A. Makarchenko; 3 adult ♂♂ — the same data, except Wrangel Island, Somnitelnaya River, 70.995783° N, 179.539117° E, 18.VII.2015, leg. O.A. Khruleva; 2 adult ♂♂ — the same data, except 70.983333° N, 179.850000° E, 26.VII.2019, leg. O.A. Khruleva; 4 adult ♂♂ — the same data, except middle reaches of the Neozhidannaya River, 71.016667° N, 179.150000° E, 12.VII.2016, leg. O.A. Khruleva; **Magadanskaya Oblast:** 1 adult ♂ — Tenkinsky District, surroundings of the Sibit-Tyellakh village, Olen Stream, Upper Kolyma River basin, 10.VII.1977, leg. E.A. Makarchenko; 4 adult ♂♂ — the same data, except Tenkinsky District, Natalka Mining and Processing Plant, Omchak River, groundwater intake site, 61.68749° N, 147.84925° E, 4.VIII.2024, leg. A.B. Krasheninnikov; **Irkutskaya Oblast:** 1 adult ♂ — Bodaibinskii District, Vitimsky State Nature Reserve, Valley of the Lednikovaya River (tributary of the Levaya Sygykta River), in the area of the pass of the Medvezhy Stream, 56.90419° N, 117.57491° E, 18.VII.2014, h~2000 m a.s.l., leg. I.V. Enushenko.

**Description.** **Adult male** (n = 10). Total length 3.2–4.6 mm, Total length/wing length 1.27–1.45.

**Coloration.** Total color brown to dark brown, wings grayish, antenna brown or dark brown.

**Head.** Eyes hairy. Temporal setae including 2–4 verticals and 8–11 postorbitals. Clypeus with 12–13 setae. Antenna with 13 flagellomeres and well-developed plume; terminal flagellomere with 1–2 subapical setae 29–42 µm long; AR 0.78–1.33. Palpomeres 1–5 lengths (in µm): 40–45 : 63–71; 109–130; 101–109; 130–147; palpomere 3 without sensilla capitata.



Figs 1-11. Details of morphology of adult male of *Arctodiamesa appendiculata* (Lundström). 1 — hypopygium in dorsal view; 2, 4, 5, 8 — anal point; 3, 9, 10 — gonostylus from different regions; 6 — endoskeleton; 7 — apical part of aedeagal lobe; 11 — megaseta. Notes: 3 — from Kolyma River basin, 9 — from Wrangel Island, 10 — from Chaun Bay. Scale bars 25  $\mu\text{m}$ .

Рис. 1-11. Детали строения имаго самца *Arctodiamesa appendiculata* (Lundström). 1 — гипопигий, вид сверху; 2, 4, 5, 8 — анальный отросток; 3, 9, 10 — гоностиль самцов из разных регионов; 6 — эндоскелет; 7 — апикальная часть эдегальной лопасти; 11 — мегашетинка гоностыля. Примечание: 3 — из басс. р. Колыма, 9 — из острова Врангеля, 10 — из Чаунской губы. Масштаб: 25 мкм.

Thorax. Anteprepronotum with 8–17 ventrolateral setae. Dorsocentrals 7–17, prealars 5–14, scutellars 17–31.

Wing. Wing length 2.5–3.2 mm. R and R<sub>1</sub> with 12–17 setae, R<sub>4+5</sub> with 1–3 setae in distal part. Anal lobe well developed. Squama with 30–35 setae.

Legs. Spur of fore tibia 54–58 µm long; spurs of mid tibia the same length 41–44 µm; of hind tibia 58 µm and 27 µm long. BR<sub>1-3</sub> 2.8–3.0. Lengths and proportions of leg segments as in Table 1.

Hypopygium (Figs 1–11). Tergite IX with 13–19 setae and anal point 52–66 µm long. Anal point is usually simple (Fig. 8), sometimes forked apically (Fig. 2) or with seta at apex (Fig. 5). Of all material only one apparently aberrant male from Wrangel Island had a rectangular anal point with two setae at the apex, 28 µm long (Fig. 4). Laterosternite IX with 7–12 setae. Transverse sternapodeme 168–212 µm long and 24–32 µm wide; distal part of phallapodeme 140–160 µm long; basimedial part 48–56 µm long. Medial aedeagal lobe strong sclerotized, with large apical spines, 8–44 µm long (Figs 6–7). Usually there are 8–13 such spines, but only one male from the Irkutsk region had ca 20 spines. Gonocoxite 248–320 µm long; medial field broad and flat, covered with short setae. Gonostylus bifurcate (Figs 1, 3, 9–10) length of inner branch to length of outer branch of gonostylus is 1.34–2.03; inner branch with broad and serrated on 5–8 teeth megaseta, 12–15 µm long (Fig. 11); one male had gonostylus with 2 megasetae; next to the megaseta there is simple strong seta, 11–15 µm long, which is sometimes split into 2–3 branches (Fig. 9). HR 1.76–2.43.

*Pupa* and *larva* have described [Makarchenko, 1984, 1985].

**Remarks.** For adult males of *A. appendiculata* from all populations, there is variability in the length of the outer gonostylus branch and several other features, including the values of AR, LR, BV, and SV, as well as the length of legs and the number of spines on the aedeagal lobe. So, for males from the Wrangel Island, Chaun Bay and the Chukchi Peninsula ratio of the length of the inner branch to the length of the outer branch of the gonostylus is respectively 1.34–1.50, 2.03, 1.82. Male from the Irkutskaya Oblast exhibit the greatest differences, with the lowest value of AR (0.78) and the highest number of spines (ca 20) on the aedeagal lobe. It is possible that with future DNA barcoding data, we may discover that we are dealing with a new cryptic species, similar to what happened with chironomids from the population on Bolshevik Island in the Severnaya Zemlya Archipelago. After obtaining genetic data, we have classified these individuals as a new species, *A. brusnevi* sp.n., and will describe it below.

**Distribution.** Holarctic arcto-alpine species, known from the upper stream of Kolyma River, spurs of the Kodar ridge of the Stanovoi Highlands (Irkutskaya Oblast), Arctic regions of East Asia and Alaska [Ashe, O'Connor, 2009; Makarchenko, Orel, 2023]. It is recorded for the Irkutskaya Oblast for the first time, where the species was caught at an altitude of about 2000 m above sea level. Earlier it was noted that the species also lives on Novaya Zemlya [Makarchenko, 1984; Ashe, O'Connor, 2009]. This may be true, but in our opinion, it is

necessary to obtain DNA barcoding data for this population in order to confirm this.

### *Arctodiamesa amurensis* Makarchenko, 2007

Figs 12–20.

*Arctodiamesa amurensis* Makarchenko, 2007: 77; Ashe, O'Connor, 2009: 262.

**Material.** *Russia, Khabarovskii Krai*: 2 adult ♂♂ — Solnechny District, environs of Gorniy Village, Levaya Silinka River, Amur River basin 50.705383° N, 136.369483° E, 28.VII.2006, light trap, leg. E.A. Makarchenko; 2 adult ♂♂ — the same data, except Sonah River, tributary of Amgun' River, Amur River basin, 51.447033° N, 135.255333° E, 19–20.VII.2006, light trap, leg. E.A. Makarchenko; *Kamchatskii Krai*: 1 adult ♂ — Milkovsky District, Kamchatka River basin, Podushka River 1<sup>st</sup>, 54.46284° N, 158.37813° E, 11.VI.2025, leg. A.B. Krashennnikov.

**Description.** *Adult male* (n = 4). Total length 3.9–4.5 mm. Total length/wing length 1.24–1.47. Coloration dark brown. Tibia of all legs with long white spot in middle part.

Head. Eyes hairy. Temporal setae include 2 outer verticals, 5–9 postorbitals and 1 preoculars. Clypeus with 6–10 setae. Antenna with 13 flagellomeres and well developed plume; pedicel with 2 setae. Length of subapical seta of terminal flagellomere 36–40 µm. AR 1.32–1.53. Lengths of palpomeres 1–5 (µm): 40–48: 80–88: 128–192: 128–140: 156–184; palpomere 3 without sensilla capitata. Head width/palp length 1.02–1.22.

Thorax. Anteprepronotum with 7–12 lateral setae. Dorsocentrals 7–13, prealars 7–8. Scutellum with 36–44 setae.

Wing. Length 2.72–3.64 mm. Anal lobe well developed. Squama with 36–65 setae in 1–2 rows. R with 11–14 setae, R<sub>1</sub> with 4–7 setae, R<sub>4+5</sub> with 1–3 setae subapically. Costa projection 120–168 µm. RM/MCu 1.8–2.3.

Legs. BR<sub>1</sub> 2.1–2.5, BR<sub>2</sub> 3.0–4.0, BR<sub>3</sub> 4.3–4.6. Spur of front tibia 72–88 µm. Spurs of middle tibia 40–52 µm and 48–60 µm long, of hind tibia 74–92 µm and 32–48 µm long. Hind tibial comb with 10–11 setae. Pseudospurs absent. LR<sub>1</sub> 0.70–0.81, LR<sub>2</sub> 0.56–0.58, LR<sub>3</sub> 0.58–0.62, BV<sub>1</sub> 3.36–3.53, BV<sub>2</sub> 3.44–4.61, BV<sub>3</sub> 3.39–3.76, SV<sub>1</sub> 2.25–2.48, SV<sub>2</sub> 3.44–3.59, SV<sub>3</sub> 3.12–3.30.

Hypopygium (Figs 12–20). Tergite IX broad, with 26–40 setae and with simple or seta-shaped anal point 36–72 µm long (Figs 12–13, 15); sometimes anal point with apical seta (Fig. 14). Laterosternite IX with 10–17 setae. Medial aedeagal lobe sclerotized, with small spines apically (Fig. 16). Gonocoxite 288–352 µm long, with rounded basal lobe and flat medial field covered by setae. Gonostylus narrow, 164–180 µm long, with small «heel» in subapical outer part (Figs 18–20) and 1–2 narrow and simple megasetae 12–16 µm long; sometimes «heel» invisible (Fig. 17); HR 1.76–1.96.

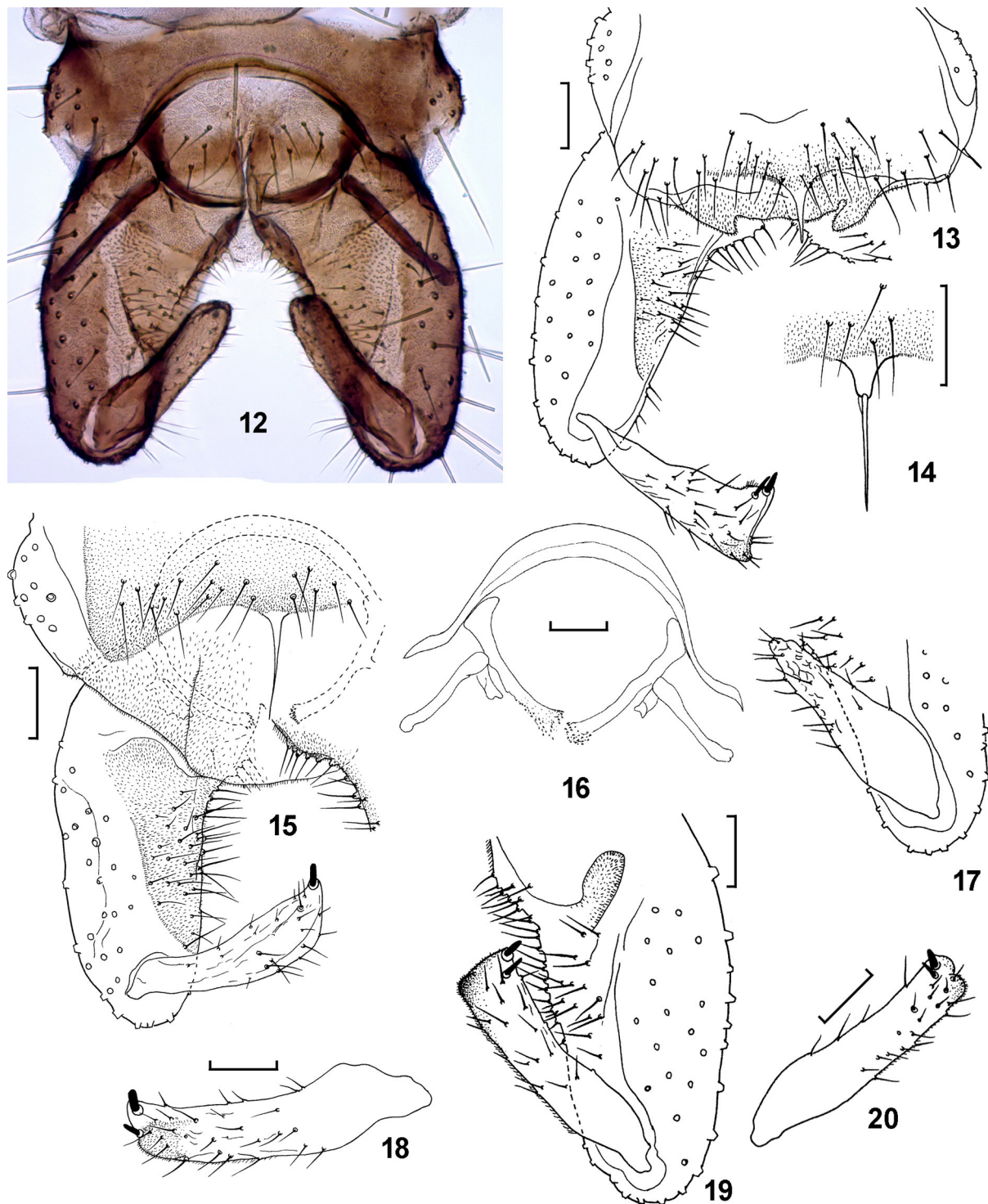
*Pupa* and *larva* unknown.

**Remarks.** The male of *A. amurensis* is separated from other known species of the genus by the shape of the gonostylus and color of tibia. The long and narrow gonostylus with small «heel» in subapical outer part and all legs with long white tibial spots are characteristics.

**Distribution.** *A. amurensis* is known only from type locality in low part of Amur River basin situated in Khabarovskii Krai and from one place in Kamchatka. It is recorded for Kamchatka for the first time. All adult males in Amur River basin

Table 1. Lengths (in µm) and proportions of leg segments of *Arctodiamesa appendiculata* (Lundström), male (n = 5)  
Таблица 1. Длина (в мкм) члеников ног самца и их индексы *Arctodiamesa appendiculata* (Lundström), самец (n = 5)

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV
P <sub>1</sub>	680–901	833–1181	561–816	289–408	187–323	68–102	102–119	0.65–0.76	3.1–3.58	2.39–2.73
P <sub>2</sub>	816–1088	765–1054	459–612	238–340	153–204	68–85	85–115	0.51–0.61	3.63–3.77	3.44–3.98
P <sub>3</sub>	884–1190	952–1292	612–799	340–442	187–255	68–102	102–119	0.56–0.59	3.43–3.96	3.33–3.45



Figs 12–20. Details of morphology of adult male of *Arctodiamesa amurensis* Makarchenko. 12, 13, 15 — hypopygium in dorsal view; 14 — anal point; 16 — endoskeleton; 17, 18, 20 — gonostylus in varies positions; 19 — gonocoxite and gonostylus. Scale bars 50  $\mu\text{m}$ .

Рис. 12–20. Детали строения имаго самца *Arctodiamesa amurensis* Makarchenko. 12, 13, 15 — гипопигий, вид сверху; 14 — анальный отросток; 16 — эндоскелет; 17, 18, 20 — гоностиль в различных положениях; 19 — гонококсит и гоностиль. Масштаб: 50 мкм.

were collected near mountain rivers at a altitude of 300–750 m above sea level.

*Arctodiamesa marinae* Makarchenko, 2005

Figs 21–23.

*Arctodiamesa marinae* Makarchenko, 2005: 60, 2006: 259; Ashe, O'Connor, 2009: 263.

**Material.** Russia, *Primorskii Krai*: 4 adult ♂♂, 4 pupae, 5 larvae — Khasanskiy District, Kedrovaya Pad Nature Reserve, Kedrovaya River, 43.097479° N, 131.556996° E, 2.VI–4.VI.2005, light trap, leg. E.A. Makarchenko. Republic of Korea: 3 adult ♂♂ — Gyeonggi-do, Gapyeonggun, Buk-myeon, Jeockmok-ri, Gapyeong stream, 26.IV.2014, leg. H.J. Kang.

**Description.** Adult male (n = 4, except when otherwise stated). Total length 3.9–4.3 mm. Total length/wing length 1.25–1.31. Coloration brown.

Head. Eyes hairy. Temporal setae including only 14–16 postorbitals, verticals absent. Clypeus with 6 setae. Antenna with 13 flagellomeres and well developed plume; pedicel with 2–3 setae. Length of subapical seta of terminal flagellomere 40–44 µm. AR 1.29. Lengths (µm) of palpomeres 1–5: 44–48: 108–120: 124–160: 124–160: 150–172; second palpomere with sensilla capitata (diameter about 4 µm) in distal part. Head width/palp length 1.34.

Thorax. Anteprepronotum with 11–13 lateral setae. Dorsocentrals 16–21, prealars 13–16. Scutellum with 34–37 setae.

Wing. Length 3.12–3.28 mm. Anal lobe well developed. Squama with 41–43 setae. R with 12–15 setae, R<sub>1</sub> with 2 campaniform sensillae and 8–9 setae, R<sub>4+5</sub> with 2–3 setae subapically. R<sub>2+3</sub> faint with 2 campaniform sensillae in basal part.

Legs. BR<sub>1</sub> 2.7, BR<sub>2</sub> 2.0, BR<sub>3</sub> 4.3. Spur of front tibia 92 µm. Spurs of middle tibia 48 µm and 40 µm long, of hind tibia 76 µm and 44 µm long. Hind tibial comb with 10 setae. Middle ta, with 2 pseudospurs 28 µm long, hind ta, with 2 pseudospurs 36 µm long. LR<sub>1</sub> 0.78, LR<sub>2</sub> 0.57, LR<sub>3</sub> 0.62, BV<sub>1</sub> 3.23, BV<sub>2</sub> 3.92, BV<sub>3</sub> 3.50, SV<sub>1</sub> 2.15, SV<sub>2</sub> 2.38, SV<sub>3</sub> 3.04.

Hypopygium (Figs 21–23). Tergite IX with 30 setae and narrow anal point, 68 µm long (Fig. 21). Laterosternite IX with 12 setae. Transverse sternapodeme 144 µm long. Medial aedeagal lobe sclerotized, with strong spines apically (Fig. 22). Gonocoxite 284–288 µm long, medial field broad and flat, covered with short setae (Fig. 21). Gonostylus 156 µm long, with rounded wide (36–40 µm) lobe in basal half and small «heel» in subapical outer part; megaseta 20 µm long (Fig. 23). HR 1.82–1.85.

*Pupa* and *larva* have been described [Makarchenko, 2005].

**Distribution.** Known from type locality in South Primorye Territory of the Russian Far East and from South Korea.

*Arctodiamesa breviramosa* Makarchenko, 1995

Figs 24–30.

*Arctodiamesa breviramosa* Makarchenko, 1995: 88; Sæther et al., 2000: 60; Ashe, O'Connor, 2009: 263.

**Material.** Russia, Republic of Sakha Yakutia: 3 adult ♂♂ — Bulunsk District, Ust-Lenskiy Nature Reserve, unnamed stream in 7 km from Tiksi Town, Arctic of East Siberia, 20.VI.1990, leg. N.I. Zelentsov; 4

adult ♂♂ — the same data except, Arangastah Str., 25.VI.1990, leg. N.I. Zelentsov; 9 adult ♂♂ — the same place, 4.VII.1990, leg. N.I. Zelentsov; *Magadanskaya Oblast*: 1 adult ♂ — Momsky District, Bolshoy Darpir Lake, Kolyma River basin, 64.093178° N, 148.030117° E, 31.VII.2018, leg. E.V. Khamenkova.

**Description.** Adult male (n = 5). Total length 3.4–4.0 mm. Total length/wing length 1.2–1.4.

Coloration. General color dark brown, antenna brown, wing greyish, wing squama dark grey, legs grayish-brown.

Head. Eyes hairy. Verticals 2–6, postorbitals 11–13, clypeals 7–9. AR 1.1–1.2. Length of last 4 maxillary palp segments (µm): 24 : 36–64 : 44–96 : 86–108 : 92–18.4. Head width/palp length 1.7–2.05.

Thorax. Anteprepronotum with 4–7 ventrolateral setae. Dorsocentrals 9, prealars 8–10, scutellars 28–31.

Wing. Length 2.5–2.7 mm. RM/MCu 2. R and R with 8–15 setae, R with 0–2 setae. Squama with 30–32 setae.

Legs. BR<sup>±5</sup> 2.5–3.0, BR 2.3–3.8, BR 3.7–4.1. Front tibial spur length 54.4 µm, middle tibial spurs 44.8 and 35.2 µm, hind tibial spurs 60.8 and 32 µm long, respectively. Hind tibial comb absent; ta distally nearly cordiform. Length (µm) and proportions of legs as in Table 2.

Hypopygium (Figs 24–30). Tergite IX with 15–20 setae on each side. Anal point with apical seta 32–35.2 µm long (Figs 29–30), sometimes anal point without apical seta (Fig. 28) (only once, among 16 males). Laterosternite IX with 5–9 setae. Transverse sternapodeme 212 µm long and 16 µm wide; phallapodeme 124–128 µm long. Medial aedeagal lobe strong sclerotized, with 6–7 large apical spines, 8–16 µm long (Figs 26–27). Gonocoxite with weak basal plate and flat median field covered short setae. Gonostylus 156 µm long, bifurcate but with short outer branch which is shorter than or equal to inner branch (Figs 24–26); megaseta broad, 16–29 µm long, serrated on 5–6 teeth.

*Pupa* and *larva* unknown.

**Distribution.** Known from Arctic region of East Siberia and of Kolyma River basin. It is recorded for the first time for the Kolyma River basin.

*Arctodiamesa brusnevi* Krasheninnikov, Makarchenko et Semenchenko, sp.n.

Figs 31–36.

urn:lsid:zoobank.org:act:D029785E-C370-4D40-8DF3-E1E-6F3B53123

*Arctodiamesa appendiculata* (Lundström): Krasheninnikov et al., 2020: 594.

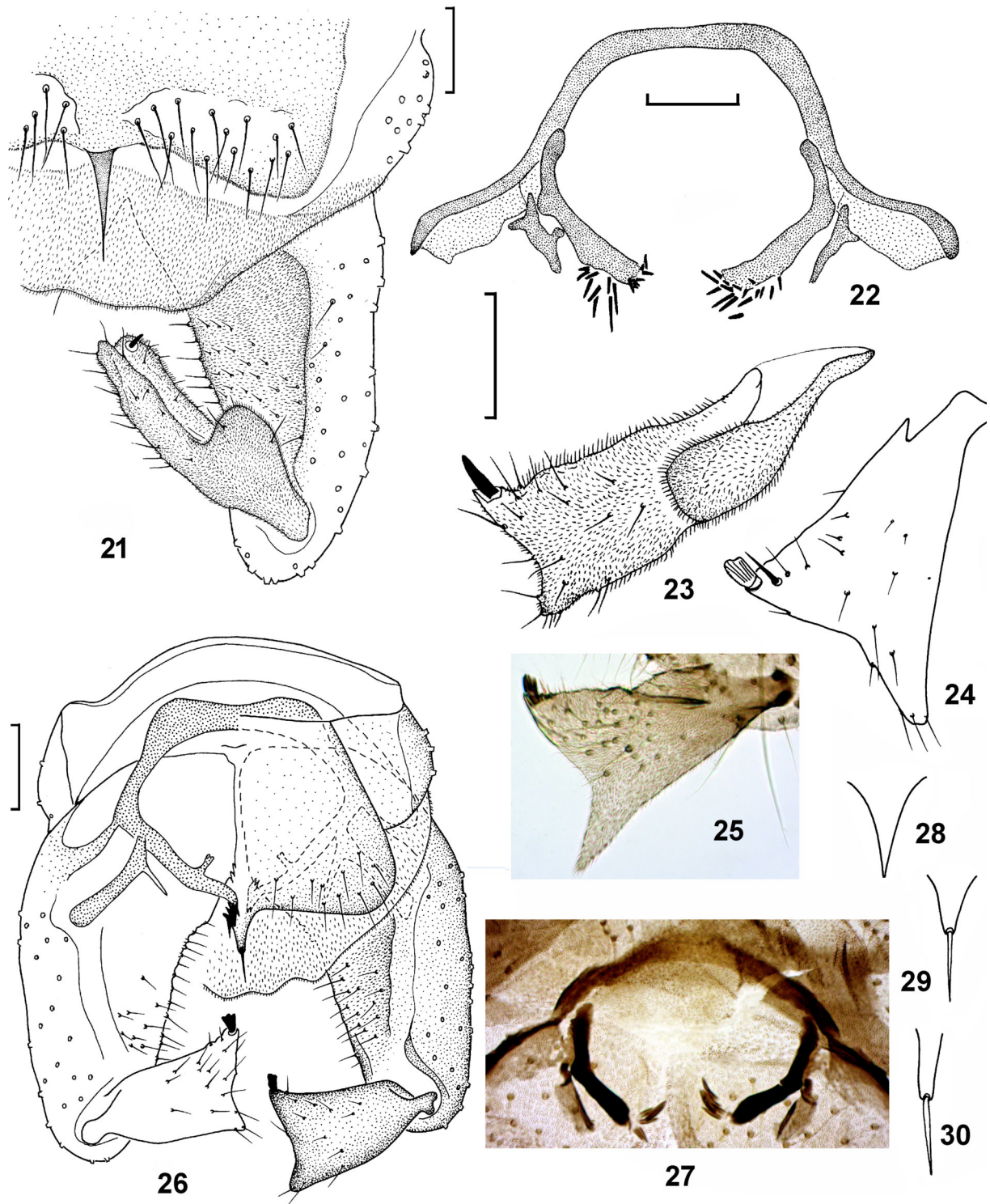
**Material.** Russia, *Severnaya Zemlya Archipelago*: Holotype, adult ♂, Bolshevik Island, estuary of the nameless river, near the base on Baranova Cape, 27.VII.2018, leg A.I. Loginov; paratypes: 1 adult ♂, the same data as holotype; 1 adult ♂, the same data, except Mikoyan Bay, Chernaya River, 79.20768° N, 102.3109° E, 1.IX.2019, leg A.B. Krasheninnikov.

**Description.** Adult male (n=3). Total length 3.9–4.2 mm, Total length/wing length 1.2.

Coloration. Total color brown to dark brown, wings grayish, legs grayish-brown.

Table 2. Lengths (in µm) and proportions of leg segments of *Arctodiamesa breviramosa* Makarchenko, male (n = 3)  
Таблица 2. Длина (в мкм) члеников ног самца и их индексы *Arctodiamesa breviramosa* Makarchenko, самец (n = 3)

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV	BR
P <sub>1</sub>	756–869	945–1115	621–754	311–344	189–295	81–82	95–115	0.66– 0.68	3.28– 3.44	2.63–2.74	2.5–3.0
P <sub>2</sub>	891–935	837–1017	432–541	262–270	162–180	81–82	95–107	0.52– 0.53	3.56– 4.11	3.61–4.0	2.3–3.8
P <sub>3</sub>	986– 1033	1013–1181	554–689	338–394	203–213	81–98	108–115	0.55–058	3.50– 3.58	3.21–3.58	3.7–4.1

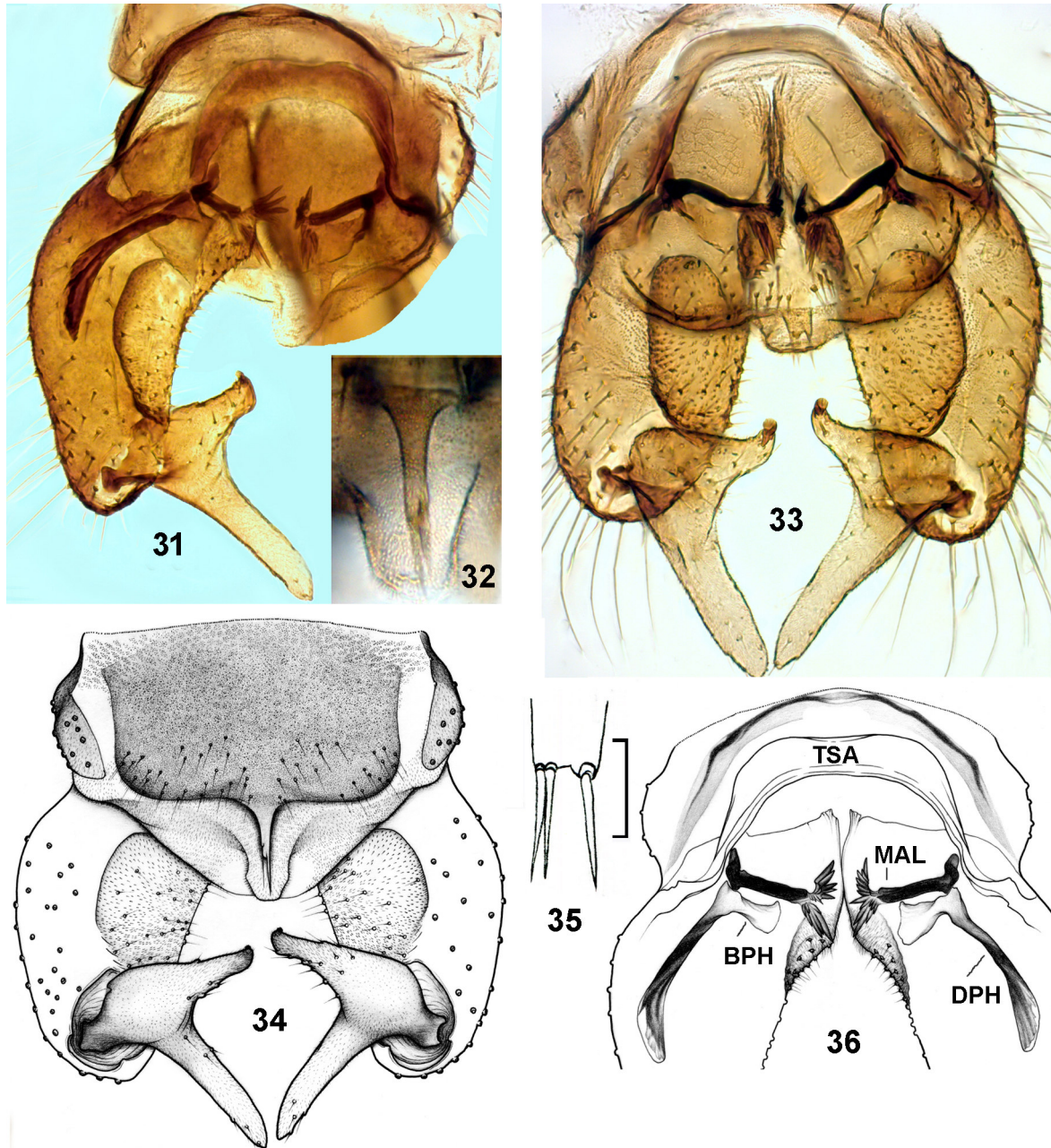


Figs 21–30. Details of morphology of adult males of *Arctodiamesa* sp.: *A. marinae* Makarchenko (21–23) and *A. breviramosa* Makarchenko (24–30). 21, 26 — hypopygium in dorsal view; 22, 27 — endoskeleton; 23–25 — gonostylus; 28–30 — anal point. Scale bars 50  $\mu$ m.

Рис. 21–30. Детали строения имаго самцов двух видов рода *Arctodiamesa* sp.: *A. marinae* Makarchenko (21–23) и *A. breviramosa* Makarchenko (24–30). 21, 26 — гипопигий, вид сверху; 22, 27 — эндоскелет; 23–25 — гоностиль; 28–30 — анальный отросток. Масштаб: 50 мкм.

Table 3. Lengths (in  $\mu\text{m}$ ) and proportions of leg segments of *Arctodiamesa brusnevi* sp.n., male ( $n = 2$ )  
 Таблица 3. Длина (в  $\mu\text{м}$ ) члеников ног самца и их индексы *Arctodiamesa brusnevi* sp.n., самец ( $n = 2$ )

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV	BR
P <sub>1</sub>	952–1045	1201–1232	780–827	218–406	218	94	125	0.65–0.67	3.48–3.96	2.75–2.76	3.5–4.3
P <sub>2</sub>	1108–1201	1030–1076	577–593	250–312	187	94	109	0.55–0.56	4.09–4.24	3.70–3.84	5.0–5.3
P <sub>3</sub>	1248–1342	1342–1357	749	312–406	218	94	125	0.55–0.56	4.09–4.46	3.46–3.60	3.5–4.6



Figs 31–36. Details of morphology of Adult male of *Arctodiamesa brusnevi* Krasheninnikov, Makarchenko et Semenchenko, sp.n. 31, 33–34 — hypopygium in dorsal view; 32, 35 — anal point; 36 — endoskeleton and basal lobes. Designations: TSA — transverse sternapodeme, MAL — medial aedeagal lobe, BPH — basimedial part of phallapodeme, DPH — distal part of phallapodeme; 34, 36 — after Krasheninnikov et al. [2020]. Scale bar 25  $\mu\text{m}$ .

Рис. 31–36. Детали строения имаго самца *Arctodiamesa brusnevi* Krasheninnikov, Makarchenko et Semenchenko, sp.n. 31, 33–34 — гипопигий, вид сверху; 32, 35 — анальный отросток; 36 — эндоскелет и базальные лопасти гонококситы. Обозначения: TSA — поперечная стернаподема, MAL — срединная эдегальная лопасть, BPH — базимедиальная часть фаллоподемы, DPH — дистальная часть фаллоподемы; 34, 36 — по Krasheninnikov et al. [2020]. Масштаб: 25  $\mu\text{м}$ .

Head. Eyes hairy. Temporal setae including 9 outer verticals and 3–4 postorbitals. Clypeus with 10–11 setae. Antenna with 13 flagellomeres and well-developed plume; terminal flagellomere with 1–2 subapical setae 27–39  $\mu\text{m}$  long; AR 1.2–1.4. Palpomeres lengths (in  $\mu\text{m}$ ): 47–51; 82–90; 133; 106–110; 149–161; palpomere 3 without sensilla capitata. Head width/palp length 1.04.

Thorax. Antepnotum with 6–8 ventrolateral setae. Dorsocentrals 7–13, prealars 6–14, scutellars ca 20.

Wing. Length 3.3–3.7 mm. R with 8–11 setae,  $R_1$  with 7 setae,  $R_{2+3}$  with 4 pores,  $R_{4+5}$  with 1–2 setae. Anal lobe well developed. Costa extension 62–98  $\mu\text{m}$ . Squama with 35–38 setae.

Legs. BR<sub>1</sub> 3.5–4.3, BR<sub>2</sub> 5.0–5.3, BR<sub>3</sub> 3.5–4.6. Spur of fore tibia 67–71  $\mu\text{m}$  long; spurs of mid tibia 55–59  $\mu\text{m}$  and 39–43  $\mu\text{m}$  long; of hind tibia 71–78  $\mu\text{m}$  and 47  $\mu\text{m}$  long. Hind tibial comb with 14 setae. Lengths and proportions of leg segments as in Table 3.

Hypopygium (Figs 31–36). Tergite IX with 21–28 setae from one side. Anal point of two males is finger-shaped, 68–72  $\mu\text{m}$  long, with 2 setae on apex, 27–35  $\mu\text{m}$  long (Figs 32, 34) and one male with anal point almost square in shape, 52  $\mu\text{m}$  long, with three setae on apex, 20–28  $\mu\text{m}$  long (Figs 33, 35). Laterosternite IX with 5–13 setae. Transverse sternapodeme 176–184  $\mu\text{m}$  long; distal part of phallapodeme 165–180  $\mu\text{m}$  long; basimedial part 60  $\mu\text{m}$  long. Medial aedeagal lobe strong sclerotized, with 7–9 large apical spines, 36–40  $\mu\text{m}$  long (Fig. 36). Gonocoxite 353–361  $\mu\text{m}$  long; medial field broad and flat, covered with short setae (Figs 31, 33). Gonostylus bifurcate, 157–176  $\mu\text{m}$  long, outer branch 125–140  $\mu\text{m}$  long, in 1.6–2.1 times longer than inner branch; inner branch with broad and serrated megaseta, 10–12  $\mu\text{m}$  long. HR 2.0–2.3.

*Pupa and larva unknown.*

**Diagnosis.** Total length 3.9–4.2 mm, Total length/wing length 1.2. Clypeus with 10–11 setae. Antenna with 13 flagellomeres and well-developed plume; AR 1.2–1.4. Head width/palp length 1.04. Dorsocentrals of mesonotum 7–13, prealars 6–14, scutellars ca 20. Wing length 3.3–3.7 mm; squama with 35–38 setae. LR<sub>1</sub> 0.65–0.67, BV<sub>1</sub> 3.48–3.96, SV<sub>1</sub> 2.75–2.76. Two males with finger-shaped anal point and 2 setae on apex, one male with almost square anal point and three setae on apex. Transverse sternapodeme 176–184  $\mu\text{m}$  long; distal part of phallapodeme 165–180  $\mu\text{m}$  long; basimedial part 60  $\mu\text{m}$  long. Medial aedeagal lobe strong sclerotized, with 7–9 large apical spines. Medial field of gonocoxite broad and flat, covered with short setae. Gonostylus bifurcate, outer branch in 1.6–2.1 times longer than inner branch; inner branch with broad and serrated megaseta. HR 2.0–2.3.

**Диагноз.** Длина тела 3,9–4,2 мм, длина тела/длина крыла 1,2 мм. Клипеус с 10–11 щетинками. Антенна с 13 флагелломерами и хорошо развитыми щетинками опухала; AR 1,2–1,4. Ширина головы/длина максиллярного щупика 1,04. Дорсоцентральных щетинок мезонотума 7–13, преалярных 6–14, скутеллярных около 20. Длина крыла 3,3–3,7 мм; чешуйка с 35–38 щетинками. LR<sub>1</sub> 0,65–0,67, BV<sub>1</sub> 3,48–3,96, SV<sub>1</sub> 2,75–2,76. У двух самцов анальный отросток пальцевидный, с 2 щетинками на вершине, у одного самца анальный отросток почти квадратный, с 3 щетинками на вершине. Длина поперечной стернаподемы 176–184 мкм; длина дистальной части фалаподемы 165–180 мкм, базимедиальной части 60 мкм. Срединная эдиагальная лопасть сильно склеротизована, с 7–9 крупными апикальными шипами. Срединное поле гонококсита широкое, покрыто короткими щетинками. Гоностил двуветвистый, наружная ветвь в 1,6–2,1 длиннее внутренней ветви; внутренняя

ветвь с широкой зазубренной апикально мегашетинкой. HR 2,0–2,3.

**Remarks.** In the description of *A. brusnevi* sp.n., we used part of the drawings and data obtained and used by A.B. Krasheninnikov for article in 2020, when describing the population of *A. appendiculata* from Bolshevik Island [Krasheninnikov et al., 2020]. In this regard, we consider it necessary to include A.B. Krasheninnikov as an author of the new species.

*A. brusnevi* sp.n. is a cryptic species isolated from *A. appendiculata* after receiving the results of DNA barcoding and is morphologically little different from the latter. The male of the new species has longer legs, a mostly finger-like anal point of the hypopygium, with two setae apically, and a distal part of the phallapodeme 165–180  $\mu\text{m}$  long. The male of *A. appendiculata* has shorter legs, and the anal point is simple, sometimes bifurcate, or with a single setae apically. The distal part of the phallapodeme 140–160  $\mu\text{m}$  long. It is possible that after obtaining the pupa and larva of *A. brusnevi* sp.n., additional characteristics will appear that will allow us to distinguish the species at the morphological level.

**Distribution.** Known only from type locality in Bolshevik Island of the Severnaya Zemlya Archipelago.

**Etymology.** The species is named in honor and memory of polar explorer Mikhail Ivanovich Brusnev, who collected chironomid material in the Lena River delta area (Chara-Ullach Mountains) in 1902.

#### KEYS TO ADULT MALES OF THE KNOWN SPECIES OF *ARCTODIAMESA* MAKARCHENKO

1. Gonostylus distinctly bifurcate, with wide serrated megaseta (Figs 3, 11)..... 2
  - Gonostylus simple, megaseta narrow and simple (Figs 15, 23) ..... 4
2. Gonostylus bifurcate, outer branch is longer than inner branch. Aedeagal lobe with more than 8 spines apically (Fig. 7) ..... 3
  - Gonostylus bifurcate, outer branch is shorter than or equal to the inner branch (Figs 24–25). Aedeagal lobe with 6–7 spines ..... *A. breviramosa* Makarchenko
3. Anal point usually simple (Fig. 8), sometimes forked apically (Fig. 2) or with seta on apex (Fig. 5). Distal part of phallapodeme 140–160  $\mu\text{m}$  long .....
  - ..... *A. appendiculata* (Lundström)
  - Anal point usually finger-shaped, with 2 setae on apex (Fig. 32). Distal part of phallapodeme 165–180  $\mu\text{m}$  long .....
    - ..... *A. brusnevi* sp.n.
4. Gonostylus narrow, with 2 megasetae and small «heel» in subapical outer part (Figs 18–19) .....
  - ..... *A. amurensis* Makarchenko
  - Gonostylus as on Figs 21–23, with 1 megaseta, wide rounded lobe in basal half and small «heel» in subapical outer part ..... *A. marinae* Makarchenko

Key for pupae and larvae of known species was publishes in 2005 [Makarchenko, 2005]. There are still no data available for the preimaginal stages of *A. amurensis*, *A. breviramosa* and *A. brusnevi* sp.n.

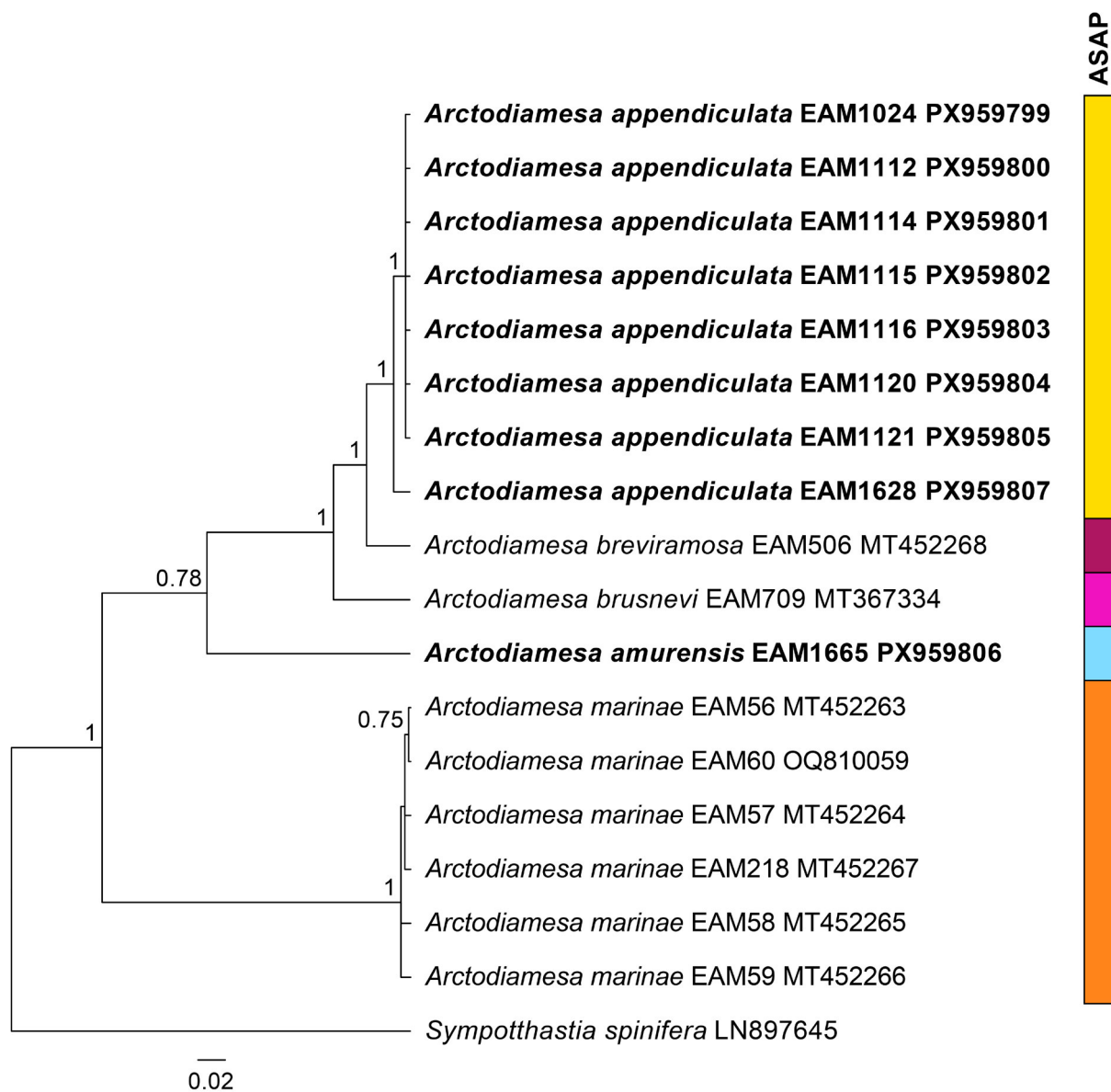


Fig. 37. Ultrametric Bayesian inference (BI) tree based on the cytochrome oxidase I (COI) nucleotide sequence data of the *Arctodiamesa* Makarchenko and *Sympotthastia spinifera* Serra-Tosio as outgroup. Bayesian posterior probabilities (higher than 0.7) are given above the tree nodes. Specimens obtained in this study are in bold.

Рис. 37. Ультраметрическое байесовское дерево, построенное на основе гена цитохром-с-оксидазы I (COI) рода *Arctodiamesa* Makarchenko с использованием *Sympotthastia spinifera* Serra-Tosio в качестве внешней группы. Байесовские апостериорные вероятности (выше 0.7) указаны над узлами дерева. Образцы, полученные в данном исследовании, выделены полужирным шрифтом.

## Results and discussion of COI DNA barcoding

We sequenced fragments of the cytochrome oxidase subunit I (571–670 bp in length) of eight specimens of *A. appendiculata* and one specimen belonged to *A. amurensis* (658 bp in length). Mean K2P intraspecific sequence divergence within *A. appendiculata* were 0.54 %. Moreover, specimen EAM1628 (Fig. 37) from the Omchak River (Magadan Region) differed from seven specimens from Wrangel Island by 2 %.

Sample EAM709 (NCBI accession number MT367334) from Bolshevik Island was erroneously identified as *A. appendiculata* [Krasheninnikov et al., 2020] due to the lack of sequences of Far Eastern samples of *A. appendiculata* at that time. In this study, we found that interspecific distances between Far Eastern specimens and EAM709 averaged 8.87 %, which significantly exceeds the intraspecific threshold for diamesines [Montagna et al., 2016]. Bayesian analyses confirm did not confirm the monophyly between *A. appendiculata* and *A. brusnevi* sp.n. (Fig. 37). The sister

of the first species was *A. breviramosa* (EAM506) from Bolshoy Darpir Lake (Bayesian posterior probability, BPP = 1). *A. amurensis* from Kamchatka was sister to all three species with moderate support (BPP = 0.78). Species delimitation for the obtained dataset confirms the validity of each species of *Arctodiamesa*.

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