



Morphological description and DNA barcoding of some Diamesinae (Diptera, Chironomidae) from the Severnaya Zemlya Archipelago and the Vaigach Island (Russian Arctic)



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Abstract

Chironomids of the Diamesinae subfamily from the Russian Arctic were studied using both morphological characters and molecular data. Adult males of *Diamesa urvantsevi* sp. nov., *D. amplexivirilia* Hansen, *Arctodiamesa appendiculata* (Lundström) from Severnaya Zemlya Archipelago and *D. arctica* (Boheman), *Pseudokiefferiella* sp. from Vaigach Island were described, redescribed, annotated and figured. A reference 658 bp barcode sequence from a fragment of the mitochondrial gene cytochrome oxidase I (COI) was used as a tool for species delimitation. For *D. arctica* (Boheman) and *Pseudokiefferiella* sp. close DNA barcodes from Norway were performed, which allowed to relate these specimens to the described species. Comparisons with corresponding regions of COI between each described species and close related congeneric species produced K2P genetic distances of 0.11–0.16, values well associated with interspecific variation. Phylogenetic relationships for genera *Arctodiamesa* Makarchenko and *Pseudokiefferiella* Zavřel were reconstructed for the first time.

Key words: Diptera, Chironomidae, *Diamesini*, taxonomy, DNA barcoding, Russian Arctic

Introduction

To date, according to preliminary data 32 chironomid species of the subfamily Diamesinae, belonging to 10 genera for the territory of the Russian Arctic are known (Kieffer 1922, 1923; Lundström 1915; Zasykina *et al.* 1996; Zelentsov & Shilova 1996; Sæther 2004; Zelentsov 2007; Krasheninnikov 2014; Krasheninnikov & Gavrilov 2014; Krasheninnikov & Bogomolova 2017; Makarchenko & Makarchenko 2017). However, the chironomid fauna of most Arctic islands has been studied extremely inadequately. So, only 13 species are known for the Severnaya Zemlya Archipelago of which three species belong to the Diamesinae (Makarchenko 1984; Zelentsov 2007). And for Vaygach Island, only the data of Holmgren (1883) about the discovery of several species of Orthoclaadiinae are available.

This article is devoted to the description, redescription and annotate of the adult males of *Diamesa urvantsevi* sp. nov., *D. amplexivirilia* Hansen, *Arctodiamesa appendiculata* (Lundström) from Severnaya Zemlya Archipelago and *D. arctica* (Boheman), *Pseudokiefferiella* sp. from Vaigach Island.

In this study the mitochondrial cytochrome c oxidase I (COI) gene 650-bp in size was used as the discriminating molecular marker. This marker has been identified as the core of a global bioidentification system at the species level (Hebert *et al.* 2003) and has proved to be useful in the Diamesinae (Makarchenko & Semchenko 2014; Montagna *et al.* 2016a). For two species, *D. arctica* (Boheman) and *Pseudokiefferiella* sp., close DNA barcodes from Norway were performed, which allowed to relate these specimens to the described species. Comparisons with corresponding regions of COI between each described species and close related congeneric species produced K2P genetic distances of 0.11–0.16, values well associated with interspecific variation. Phylogenetic relationships for genera *Arctodiamesa* Makarchenko and *Pseudokiefferiella* Zavřel were reconstructed for the first time using obtained data and available sequences in BOLD systems.

Materials and methods

The adults of chironomids were preserved in 96% ethanol for DNA-analysis and kept cool at 5°C until further processing, and in 70% ethanol for study of morphology. The material was slide-mounted in polyvinyl lactophenol and in sandarac medium without chloral hydrate (Krasheninnikov 2011). 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) and notes on the nomenclature of male genitalia of the genus *Diamesa* (Montagna *et al.* 2016b) are used. The photographs were taken using an Axio Lab. A1 (Karl Zeiss) microscope.

Holotype of the new species is deposited at the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia (FSCEATB FEB RAS). Paratypes of the new species as well as all other material is stored at the Institute of Biological Problems of the North, Far East Branch of the Russian Academy of Sciences, Magadan, Russia (IBPN FEB RAS, CCK).

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 10–25 µL reaction volume using Go Taq Green Master Mix (Promega Corp, Madison, WI, USA) or custom mix consist of 1.25 U Platinum® Taq High Fidelity DNA Polymerase (Invitrogen), 1×PCR buffer, 2.5 mM MgCl₂ and 1mM dNTPs. We add 1.25 µM of each primer, 3 µL of the respective genomic DNA extract and in some cases added 0.06 µL of BSA (Bovine Serum Albumin solution, concentration 20 mg/mL). PCR and Sanger sequencing used the ‘Folmer primer set’ (LCO1490 + HCO2198) (Folmer *et al.* 1994). The general PCR profile consisted of an initial denaturation step at 94° C for 2 min, followed by 35 cycles at 94° C for 1 min, 45° C for 30 s, and 72° C for 2 min, and a final extension step of 10 min at 72° C. Amplification of PCR products was checked by electrophoresis using a 1.5% agarose gel in TBE buffer stained with Ethidium bromide and visualized on GelDoc XR+ imaging systems (BioRad).

PCR products were purified using ExoSAP-IT (USB Corporation, Cleveland, Ohio, USA) or mix of Exonuclease I and Thermosensitive Alkaline Phosphatase (ThermoFisher Scientific, Waltham, MA, USA). Sequencing reaction was performed using BigDye® Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems, Carlsbad, CA, USA) and run on an ABI 3130xl Genetic Analyzer Sequencer in Far Eastern Federal University, Vladivostok or on an ABI 3730 in DNA lab of the Natural History Museum, University of Oslo.

Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI) analyses. PartitionFinder 2.1.1 (Lanfear *et al.* 2012) was used to select the best-fit partitioning scheme and models for BI separately for each codon position of COI. K80 (Kimura 1980), F81 (Felsenstein 1981) and HKY+G (Hasegawa *et al.* 1985) was the best models of nucleotide substitution for the first, second and third of COI codon position respectively on the reconstructed tree of genus *Arctodiamesa*. For phylogenetic tree of genus *Pseudokiefferiella* the best models were SYM+G (Zharkikh 1994), F81 and HKY+G for three COI codon positions. The ML tree was constructed in RAXML v. 8.2.4 using GTR+G model and bootstrapping with 1000 replications (Stamatakis 2006) as well as Bayesian analyses was performed in MrBayes v. 3.2.7 (Ronquist & Huelsenbeck 2003) using 5 000 000 generations, sampled every 100 generations and the first 1000 trees were discarded as burn-in. Moreover, trace files were visually inspected in Tracer 1.7 (Rambaut *et al.* 2018) and then the consensus tree was visualized in FigTree v. 1.4.4. The neighbor-joining tree of genus *Diamesa* was reconstructing in MEGA 7 (Kumar *et al.* 2016).

The obtained sequences of described species have been deposited in GenBank under numbers: *Diamesa arctica* (Boheman)—MK592905 and MK592907, *D. urvantsevi* **sp. nov.**—MT367336–MT367337, *D. amplexivirilia* Han-

sen—MT367335, *Arctodiamesa appendiculata* (Lundström) (MT367334) and *Pseudokiefferiella* sp.—MT367333. To obtain a representative tree of the genus *Arctodiamesa* Makarchenko we deposited in GenBank two additional species from Far East—*Arctodiamesa marinae* Makarchenko (MT452263–MT452267) and *A. breviramosa* Makarchenko (MT452268).

Descriptions

Diamesa urvantsevi Krasheninnikov et Makarchenko, sp. nov.

<http://zoobank.org/NomenclaturalActs/2106E8A2-D575-4435-981C-8D4B844676C9>

(Figs. 1–4, 8–10)

Type material. Holotype, adult male, RUSSIA: Severnaya Zemlya Archipelago, Bolshevik Island, *Mikoyan* Bay, Chernaya River, N 79.20768 E 102.3109, 01.IX.2019, leg. A. Krasheninnikov. Paratypes: 7 adult males, the same data as holotype.

Derivatio nominis. The species is named in honor of Nikolay Nikolayevich Urvantsev (1893–1985). He was a Soviet geologist and explorer who together with Georgy Ushakov investigated the Severnaya Zemlya Archipelago and made it first map.

Adult male ($n = 3$). Total length 3.5–3.8 mm. Wing length 3.30–3.84 mm. Total length/wing length 0.98–1.05. Wing length/length of profemur 2.41–2.50.

Colouration. Head, thorax, legs and hypopygium dark brown; antenna brown; palpomeres light brown; abdomen light brown to brown; wing veins yellowish brown.

Head. Eyes hairy, i.e., length of eye microtrichia about 1.5 or more times the height of ommatidial lenses and visible along lateral eye margin when head is viewed from front (after: Hansen & Cook 1976). Temporal setae 27–32, including about 9–18 verticals, 7–8 preoculars, 6 postorbitals. Clypeus with 17–21 setae. Antenna with 13 flagellomeres and well-developed plume; terminal flagellomere 132–140 μm long, with rounded apex and 2–3 subapical setae 20–76 μm long. AR 1.06–1.19. Palpomeres lengths (in μm): 40–44; 84–96; 120–148; 120–132; 148–168. Palpomere 3 in distal part with sensilla capitata (sunken organ) with diameter 16–24 μm . Palpomeres 1–5 length/head width 0.79–0.92.

Thorax. Anteprepronotum with 6–8 ventrolateral setae. Dorsocentrals 5–9, prealars 5–10, scutellars 17–23.

Wing. R with 2–3 setae in basal 1/3, R_1 with 7–10 setae; R_{4+5} with 5–8 setae in distal 1/3. Costa extension 49–66 μm long. RM length/MCu length 3.0–3.6. Brachiolium with 1–2 setae. Anal lobe well developed, angularly rounded. Squama with 29–36 setae 84–144 μm long. VR 0.88–0.94.

Legs. Spur of fore tibia 60–64 μm long; spurs of mid tibia 40–44 μm and 44–52 μm long; of hind tibia 72 μm and 48–52 μm long. Hind tibial comb with 16–18 setae. Lengths and proportions of leg segments as in Table 1.

TABLE 1. Lengths (in μm) and proportions of leg segments of *Diamesa urvantsevi* sp. nov., male ($n=3$)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅
P ₁	1345–1558	1394–1607	771–1000	402–492	246–410	115	148–164
P ₂	1443–1689	1263–1525	541–722	295–344	180–213	115	131–148
P ₃	1656–1837	1378–1738	689–951	310–476	254–312	115–131	148

TABLE 1. (Continued)

	LR	BV	SV	BR
P ₁	0.54–0.62	3.19–3.85	3.17–3.57	1.9–2.7
P ₂	0.41–0.47	4.50–4.80	4.41–5.28	1.4–2.8
P ₃	0.44–0.69	3.96–4.44	3.38–4.66	2.5–2.7

Hypopygium (Figs. 1–4, 8–10). Tergite IX with 14–18 setae (from one side), 16–32 μm long and with very short hyaline “anal point” 12.0–16.4 μm long, which is often missing or not visible, as it tucks under tergite IX (Figs. 3–4, 9). Laterosternite IX with 13–16 setae 28–40 μm long. Phallapodeme 188–224 μm long. Transverse sternapodeme

triangular 80–180 µm long. Gonocoxite 408–476 µm long. Medial field well developed, wide and flat, covered with setae 40–80 µm long; distal end often free. Basimedial setal cluster absent. Gonostylus dark brown to black, darker than gonocoxite, 296–328 µm long, massive, parallel-sided, only slightly curved in basal part, densely covered with microtrichiae and short setae; megaseta 8–9 µm long. HR 1.32–1.45.

Pupa and larva unknown.

Diagnostic characters. The new species is distinguished from all known members of the genus *Diamesa* by the following features. Eyes hairy; antenna with 13 flagellomeres and well-developed plume; AR 1.06–1.19. Dorsocentrals 5–9, prealars 5–10, scutellars 17–23. R with 2–3 setae only in basal 1/3; costa extension 49–66 µm long; anal lobe well developed. LR₁ 0.54–0.62. Tergite IX with very short hyaline “anal point” 12.0–16.4 µm long, which is often not visible or missing; basimedial setal cluster absent; gonostylus much darker than gonocoxite, massive, parallel-sided, only slightly curved in basal part, densely covered with microtrichia and short setae.

It should be noted that only the African species *D. ruvenzoriensis* Freeman (Willassen & Cranston 1986), *D. akhrorovi* Makarchenko et Semenchenko, *D. alibaevae* Makarchenko et Semenchenko from Central Asia (Makarchenko *et al.* 2018) and *D. stenonyx* Serra-Tosio from Nepal (Serra-Tosio 1983) have the similar structure and shape of gonostylus, and such a small “anal point” of tergite IX, as in *D. urvantsevi* **sp. nov.**, does not have any males of known species of the genus *Diamesa*.

Data obtained after DNA barcoding allows us to bring a new species closer to an undescribed species from northeastern Canada (see below).

***Diamesa arctica* (Boheman)**

(Figs. 5–7)

Chironomus arctica Boheman, 1865: 574.

Diamesa arctica (Boheman) Holmgren 1869: 8, 48; Edwards 1924: 173; Goetghebuer 1939: 18; Kureck 1966: 276; Serra-Tosio 1967: 205; Hansen & Cook 1976: 60; Makarchenko 1980: 89, 1985: 87, 2006: 266, 476, 614; Ashe & O'Connor 2009: 270.

Diamesa poultoni Edwards 1922: 197, 213.

Diamesa flavipila Edwards 1922: 214.

Material examined. 2 adult males, RUSSIA: Vaigach Island, Lyamchin Peninsula, stream in the vicinity of Bolshoy Lyamchin Nos Cape, N 69.860028 E 59.133417, 07.VIII.2015, leg. A. Krasheninnikov; 1 adult male, same location except, N 69.857806 E 59.147222, 07.VIII.2015, leg. A. Krasheninnikov.

Adult male (n=3). Total length 5.1–5.6 mm. Wing length 3.4–3.6 mm. Total length/wing length 1.53–1.59.

Coloration. Total color dark brown, wings greyish.

Head. Eyes pubescent. Temporal setae including 18–24 verticals and postorbitals in one group and 4–6 orbitals. Clypeus with 15–18 setae. Antenna with 13 flagellomeres and well-developed plume; terminal flagellomere with 1 subapical seta 47 µm long; AR 1.59. Palpomeres lengths (in µm): 62; 78–109; 140–172; 140–156; 203–234. Palpomere 3 in distal part with sensilla capitata with diameter 18–20 µm.

Thorax. Antepnotum with 7–13 ventrolateral setae. Dorsocentrals 10–20, prealars 11–14. scutellars 34–36 setae.

Wing. R with 11–15 setae, R₁ with 9–14 setae, R₂₊₃ with 1–2 pores, R₄₊₅ with 10–11 setae. Costa extension 78 µm. Anal lobe well developed. Squama with 40–49 setae. VR 0.87–0.91.

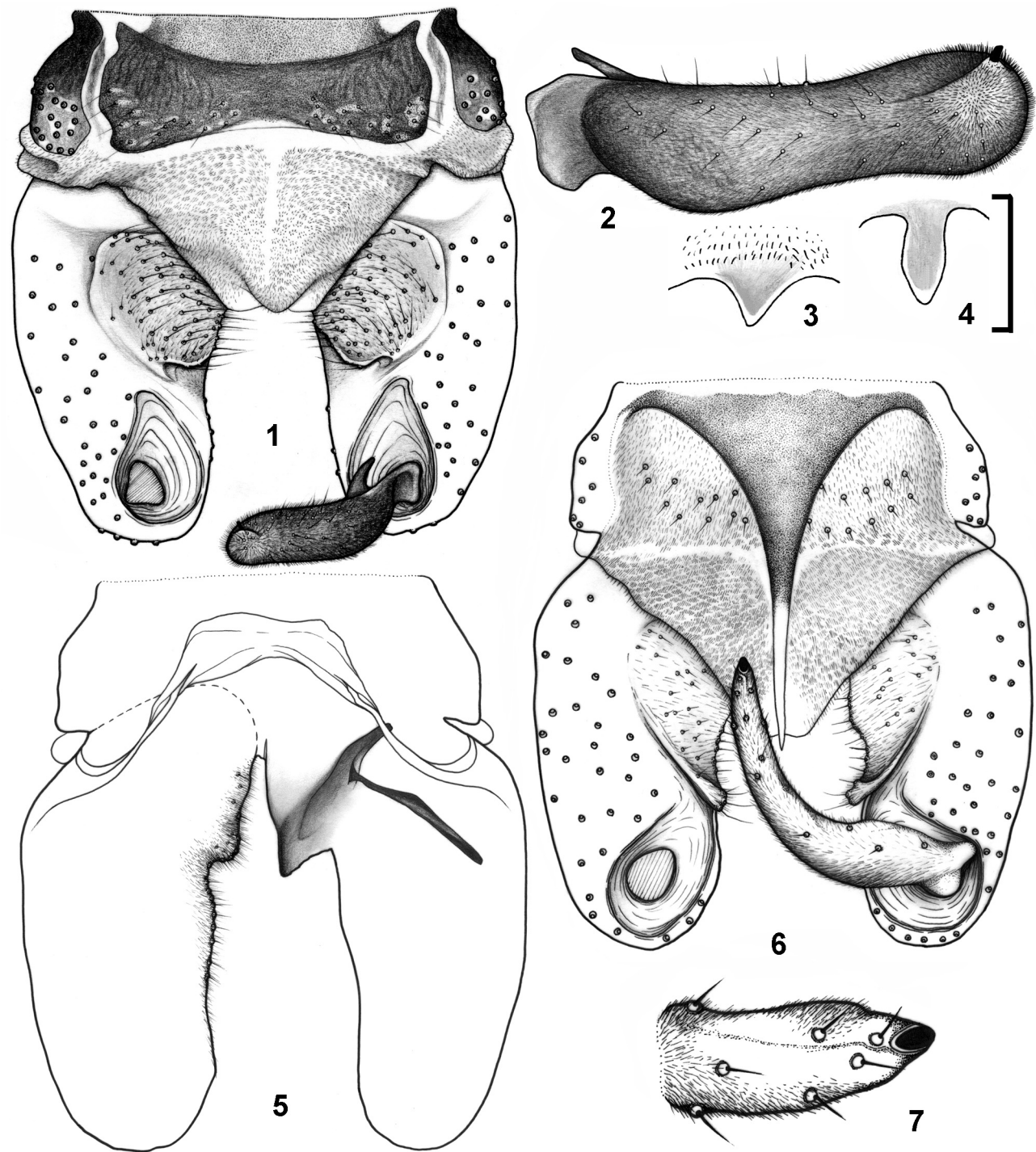
Legs. Spur of fore tibia 93 µm long; both spurs of mid tibia 62 µm long; of hind tibia 93 µm and 78 µm long. Hind tibial comb with 21 setae. Lengths (in µm) and proportions of legs in Table 2.

TABLE 2. Lengths (in µm) and proportions of leg segments of *Diamesa arctica* (Boheman) (n=3)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅
P ₁	1313–1350	1575–1688	1050–1125	525–563	300–338	113	113–150
P ₂	1425–1538	1425–1538	713	375–413	225–263	113	150
P ₃	1613–1725	1763–1913	1125	600	338	113	150

TABLE 2. (Continued)

	LR	BV	SV	BR
P ₁	0.62–0.69	3.53–3.79	2.66–2.89	2.5–3.0
P ₂	0.46–0.50	3.96–4.21	4.00–4.31	2.5
P ₃	0.60–0.63	3.75–3.93	3.00–3.20	4.0



FIGURES 1–7. *Diamesa urvantsevi* sp. nov. (1–4) and *D. arctica* (Boheman) (5–7), males. 1, 6, hypopygium in dorsal view; 2, gonostylus; 3–4, “anal point”; 5, inner structures of hypopygium; 6, distal part of gonostylus. Scale bar 20 μ m.

Hypopygium (Figs. 5–7). Tergite IX with 11–15 setae (from one side) and anal point 160–235 μ m long. Lat-erosternite IX with 4–7 setae. Phallapodeme 156 μ m long. Transverse sternapodeme 125 μ m long. Basal plate fairly

developed, obtuse to right-angle distomedially. Gonocoxite 310 µm long; medial field well developed, with numerous microtrichia and setae; distal end of medial field free. Basimedial setal cluster absent. Gonostylus 190 µm long, slender, broadest at about 0.2 its length; megaseta 12 µm long. HR 1.61–1.73.

Comments. Morphological descriptions of the *D. arctica* from different regions of the Arctic are similar (Hansen & Cook 1976; Makarchenko 1985) but the data obtained after comparing the sequences of populations from Svalbard, Greenland, Northern Canada, Ellesmere and Baffin Islands are different (see below) which indicates the heterogeneity of this species. Therefore, in our opinion, an additional comprehensive revision of the populations of this species from different areas of the range is necessary.

Distribution. Holarctic Arctic species, known from Arctic regions of Eurasia and Alaska (Ashe & O'Connor 2009).

Diamesa amplexivirilia Hansen

(Fig. 11)

Diamesa amplexivirilia Hansen in Hansen & Cook, 1976: 53; Makarchenko 1980: 86, 1981:108, 1985: 77, 2006: 261, 473, 614; Ashe & O'Connor 2009: 270.

Material examined. 1 adult male, RUSSIA: Severnaya Zemlya Archipelago, Bolshevik Island, *Mikoyan* Bay, Chernaya River, N 79,20768, E 102,3109. 01.IX.2019, leg. A. Krasheninnikov.

Adult male (n = 1). Total length 2.4 mm. Wing length 2.24 mm. Total length/wing length 1.07. Wing length/length of profemur 2.73.

Colouration. Head, thorax, legs and hypopygium dark brown; abdomen brown to dark brown; wing veins yellowish brown.

Head. Eyes hairy, microtrichia visible along lateral eye margin when head is viewed from front. Temporal setae 11, including 8 verticals and 3 preoculars. Clypeus with 5 setae. Antenna with 8 flagellomeres and reduced plume of setae 24–48 µm long; number of setae in flagellomeres 1–7, respectively 2–3 : 2 : 1–2 : 1–2 : 2 : 2 : 4. Flagellomeres 1–8 length (µm): 76, 36, 28, 24, 24, 24, 96–104; terminal flagellomere with 3 subapical setae, 12–16 µm long and with 3 setae basally, 32–36 µm long; pedicel with 3 setae; AR 0.41–0.44. Antennal length/palpal length 1.18–1.21. Palpomeres lengths (in µm): 32; 40; 72; 60; 78. Palpomere 3 in distal part with sensilla capitata with diameter 16 µm. Palpomeres 1–5 length/head width 0.69.

Thorax. Anteprenotum with 3–4 ventrolateral setae. Dorsocentrals 10, prealars 4, scutellars 7 (in one row).

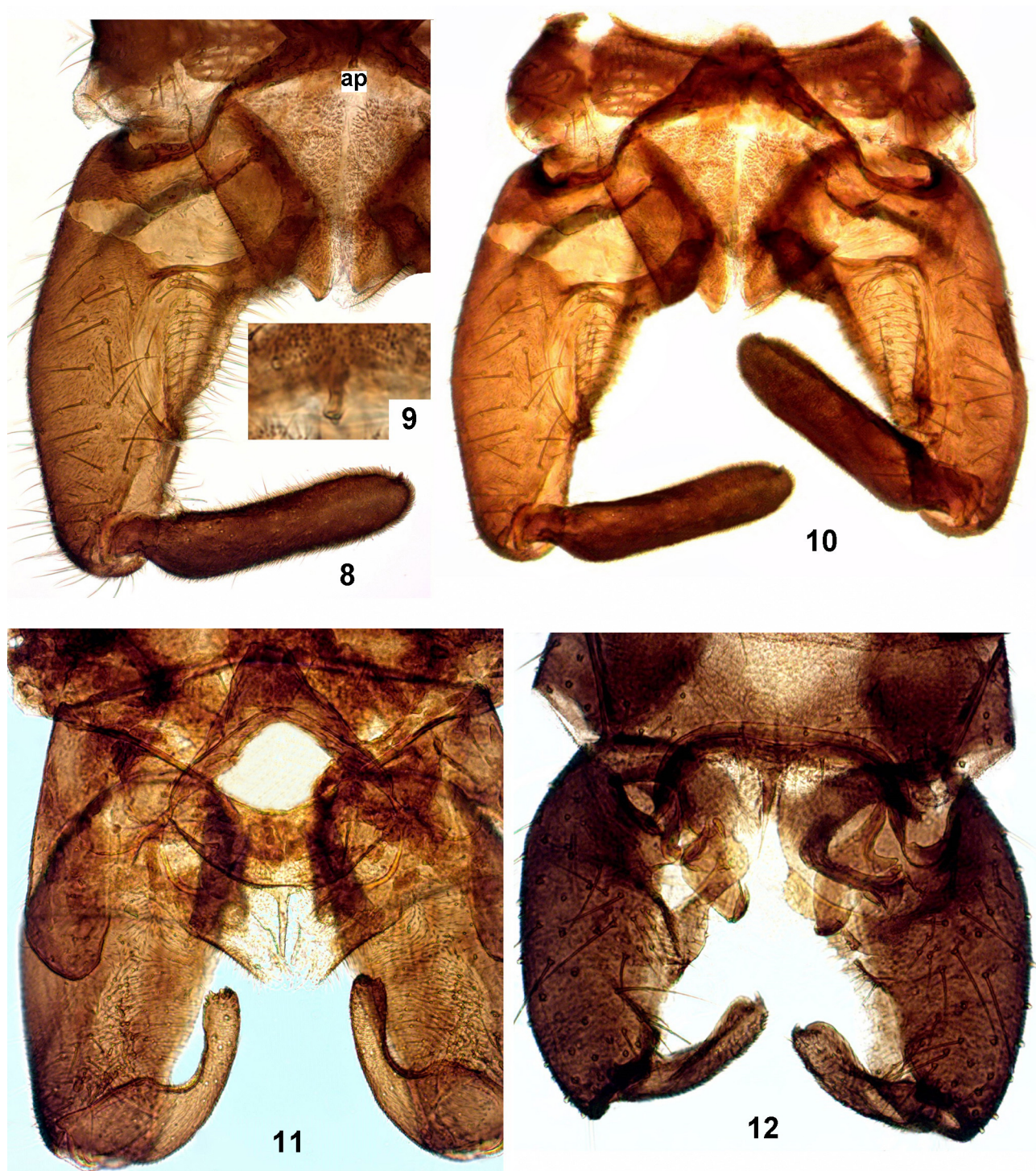
Wing. R with 6 setae, R₁ with 8 setae; R₄₊₅ with 5 setae in distal part. Costa extension 49 µm long. RM length/MCu length 2.33. Brachiolum with 1–2 setae. Anal lobe well developed, rounded. Squama with 14–15 setae. VR 0.93.

Legs. Spur of fore tibia 32 µm long; spurs of mid tibia 32 µm long; of hind tibia 44 µm and 32 µm long. Hind tibial comb with 9 setae. Lengths and proportions of leg segments as in Table 3.

Hypopygium (Fig. 11). Tergite IX with 4–5 setae (from one side), 8–12 µm long and with slender anal point 52 µm long, which directed somewhat to nearly ventrad. Laterosternite IX with dorsolateral region greatly produced along side of gonocoxite and with 5–9 setae, 16–20 µm long. Basal plate fairly well developed, slightly produced disto-medially, with numerous microtrichia ventrally. Medial field scarcely developed, with short setae. Basimedial setal cluster absent. Gonostylus broadest in basal 0.4, then abruptly narrowing, with megaseta and about 3–4 terminal teeth. Transverse sternapodeme 68 µm long, triangular, with rounded apex. HR 1.65.

Comments. The morphological description of the *D. amplexivirilia* from the of Severnaya Zemlya Archipelago is similar to that of males from other regions of the Holarctic. We noted earlier that this species is closely related to *D. alpina* Tokunaga and *D. davisi* Edwards (Makarchenko 1980), as well as to the species *D. saetheri* Willassen and *D. serratosioi* Willassen (Makarchenko 1985). The obtained barcoding data (see below) confirm a close similarity of *D. amplexivirilia* with the last two species.

Distribution. Holarctic arcto-alpine species, known from mountains of Kolyma River upper stream, Arctic regions of Eurasia and Canada (Ashe & O'Connor 2009).



FIGURES 8–12. *Diamesa urvantsevi* sp. nov. (8–10), *D. amplexivirilia* Hansen (11) and *Pseudokiefferiella* sp. (12), males. 8, 10–12, hypopygium in dorsal view; 9, “anal point”. ap—“anal point”.

TABLE 3. Lengths (in μm) and proportions of leg segments of *Diamesa amplexivirilia* Hansen, male (n=1)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
P ₁	820	771	508	230	148	66	98	0.66	3.87	3.13	0.8
P ₂	1000	771	377	180	131	66	98	0.49	4.52	4.70	0.7
P ₃	1066	935	590	312	164	82	98	0.63	3.95	3.39	1.5

***Arctodiamesa appendiculata* (Lundström)**

(Figs. 13–15)

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

Arctodiamesa appendiculata (Lundström) Makarchenko 1983: 264, 1984: 96, 1985: 54, 2006: 259, 471, 610; Ashe & O'Connor 2009: 263.

Material examined. 2 adult males, RUSSIA: Severnaya Zemlya Archipelago, Bolshevik Island, estuary of the nameless river, 27.VII.2018, leg. A. Loginov.

Adult male (n=2). Wing length 3.3–3.4 mm.

Colouration. Total colour brown to dark brown, wings greyish.

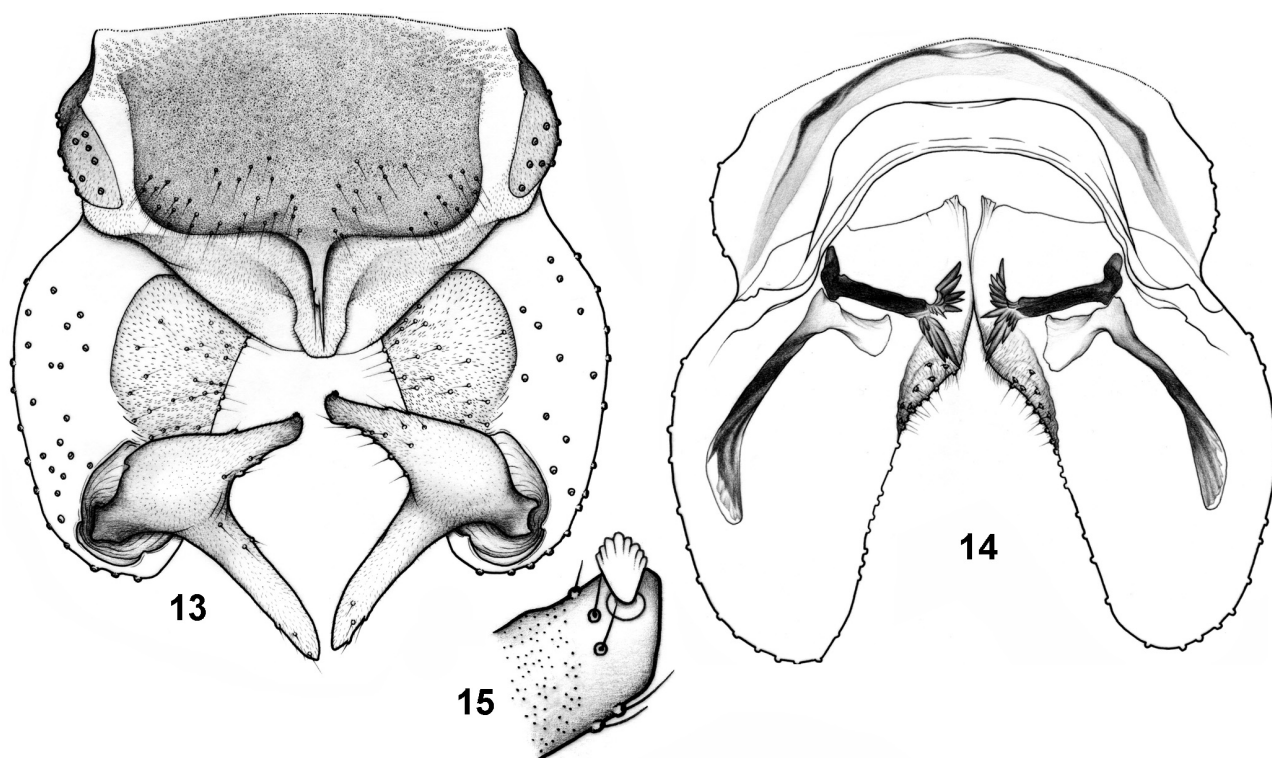
Head. Eyes hairy. Temporal setae including 9 outer verticals and 3–4 postorbitals. Clypeus with 10–11 setae. Antenna with 13 flagellomeres and well-developed plume; terminal flagellomere with 1–2 subapical setae 27–39 μm long; AR 1.2–1.4. Palpomeres lengths (in μm): 47–51; 82–90; 133; 106–110; 149–161;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

Thorax. Antepnotum with 8 ventrolateral setae. Dorsocentrals 7–10, prealars 6–8, scutellars *ca* 20.

Wing. 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 62–78 μm . Squama with 35 setae.

Legs. Spur of fore tibia 67–71 μm long; spurs of mid tibia 55–59 μm and 39–43 μm long; of hind tibia 71–78 μm and 47 μm long. Hind tibial comb with 14 setae. Lengths and proportions of leg segments as in Table 4.

Hypopygium (Figs. 13–15). Laterosternite IX with 5–13 setae. Tergite IX with 42–49 setae. Anal point 51 μm long, with 2 setae 27–35 μm long on apex. Transverse sternapodeme 176–184 μm long; distal part of phallapodeme 165–180 μm long; basimedial part 60 μm long. Medial aedeagal lobe strong sclerotized, with large apical spines. Gonocoxite 353–361 μm long; medial field broad and flat, covered with short setae. Gonostylus bifurcate, 157–176 μm long, outer branch 125–137 μm long, in 1.6–2.1 times longer than inner branch; inner branch with broad and serrated megaseta 10–12 μm long. HR 2.0–2.3.



FIGURES 13–15. *Arctodiamesa appendiculata* (Lundström), male. **13**, hypopygium in dorsal view; **14**, inner structures of hypopygium; **15**, distal part of gonostylus.

Comments. The adult males of *A. appendiculata* from Severnaya Zemlya Archipelago fits into the description of the species from other regions of the Arctic and Subarctic (Makarchenko 1984, 2006). Specimens of all popula-

tions of this species are characterized by a slight variability in the ratio of the length of the inner branch to the length of the outer branch of the gonostylus. So, for males from the Wrangel Island, Chaun Bay, the Chukchi Peninsula and the Severnaya Zemlya Archipelago this ratio is respectively 1.34–1.50, 2.03, 1.82, 1.6–2.1.

Distribution. Holarctic arcto-alpine species, known from the upper stream of Kolyma River, Arctic regions of Eurasia and Alaska (Ashe & O'Connor 2009).

TABLE 4. Lengths (in μm) and proportions of leg segments of *Arctodiamesa appendiculata* (Lundström), male (n=2)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅
P ₁	952–1045	1201–1232	780–827	218–406	218	94	125
P ₂	1108–1201	1030–1076	577–593	250–312	187	94	109
P ₃	1248–1342	1342–1357	749	312–406	218	94	125

TABLE 4. (Continued)

	LR	BV	SV	BR
P ₁	0.65–0.67	3.48–3.96	2.75–2.76	3.5–4.3
P ₂	0.55–0.56	4.09–4.24	3.70–3.84	5.0–5.3
P ₃	0.55–0.56	4.09–4.46	3.46–3.60	3.5–4.6

Pseudokiefferiella sp.

(Fig. 12)

Pseudokiefferiella sp. | Male, available from:

http://v3.boldsystems.org/index.php/Public_RecordView?processid=CHRFI378-11

GenBank accession number: JN266675, available from:

<https://www.ncbi.nlm.nih.gov/nuccore/JN266675>

Material examined. 1 adult male, RUSSIA: Vaigach Island, Lyamchin Peninsula, stream in the vicinity of Bolshoy Lyamchin Nos Cape, N 69.860028 E 59.133417, 07.VIII.2015, leg. A. Krasheninnikov; 1 adult male, the same location except, mouth part of the stream from the walrus rookeries, N 69.856278 E 59.132917, 07.VIII.2015, leg. A. Krasheninnikov; 1 adult male, the same locality except, stream, N 69.855972 E 59.163417, 07.VIII.2015, leg. A. Krasheninnikov; 1 adult male, the same locality except, puddles on the shore of the Okhotnich'ya sea lip, N 69.88425 E 59.357556, 07.VIII.2015, leg. A. Krasheninnikov.

Adult male (n=4). Total length 3.4–4.1 mm. Wing length 2.3–2.7 mm. Total length/wing length 1.25–1.71. Wing length/length of profemur 2.73–3.0.

Colouration. Total colour brown, wings greyish.

Head. Eyes pubescent, slightly elongate dorsomedially. Temporal setae 15–22, including 10–16 verticals and 5–6 postorbitals. Clypeus with 0–2 setae. Antenna with 13 flagellomeres and well-developed plume; terminal flagellomere with 1 subapical seta 30 μm long; AR 1.31–1.54. Palpomeres lengths (in μm): 31; 47; 125–140; 109–125; 94–109. Palpomeres 1–5 length/head width 0.70–0.79.

Thorax. Antepronotum with 3–5 ventrolateral setae. Dorsocentrals 9–10, prealars 5–6, scutellars 7–9.

Wing. R with 6–20 setae, R₁ with 2–4 setae in basal part; R₂₊₃ with 1 pore, R₄₊₅ with 0–1 seta. Costa extension 47–49 μm long. RM length/MCu length 2.70. Brachiolium with 1–2 setae. Anal lobe developed, rounded and slightly protrude. Squama with 24–31 setae 80–104 μm long. VR 0.86–0.98.

Legs. Spur of fore tibia 62–78 μm long; both spurs of mid tibia 47 μm long; of hind tibia 62 μm and 30 μm long. Hind tibial comb with 12 setae. Lengths and proportions of leg segments as in Table 5.

Hypopygium (Fig. 12). Tergite IX with 11–18 setae (from one side) and with anal point 62–78 μm long, which in distal half slender and hair-like. Laterosternite IX with 5–8 setae. Transverse sternapodeme like narrow arch, 170–180 μm long. Gonocoxite 250–280 μm long, with broad basal lobe and lobe-like medial field, covered with 6–9 setae 24–32 μm long. Gonostylus 110–140 μm long, with long and rounded apically crista dorsalis; macroseta 8–9 μm long. HR 2.00–2.28.

Comments. After comparing the male hypopygium and DNA analysis data of the *Pseudokiefferiella* sp. from

Vaigach Island and the *Pseudokiefferiella* sp. from Northern Norway (BOLD ID: CHRFI378, BIN BOLD:AAV2899) we came to the conclusion that this is the same and apparently new to science species. However, we believe that in order to fully confirm this it is necessary to conduct an additional revision of the known species of this genus shown in Figure 17. It is also necessary to determine which of them is really the *Pseudokiefferiella parva* (Edwards).

TABLE 5. Lengths (in μm) and proportions of leg segments of *Pseudokiefferiella* sp. (n=3)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅
P ₁	842–889	1014–1139	764–858	312–374	187–218	94	109
P ₂	858–936	889–1014	359–484	250–281	156–172	94	109–125
P ₃	998–1092	1092–1217	624–702	328–406	172–203	94	109–125

TABLE 5. (Continued)

	LR	BV	SV	BR
P ₁	0.73–0.75	3.29–3.89	2.34–2.50	2.0–2.5
P ₂	0.38–0.49	3.50–3.62	4.00–5.13	2.0–2.5
P ₃	0.53–0.61	3.68–4.02	3.11–3.63	3.5–4.0

Results of DNA barcoding

After assembly and align we obtain 13 new COI sequences (658 bp in size) of chironomids from 3 genera: *Diamesa* Meigen, *Arctodiamesa* Makarchenko and *Pseudokiefferiella* Zavřel. The total sequence divergence within genus *Diamesa* including one sequence of *D. amplexivirilia*, two of *D. arctica* and two of *D. urvantsevi* **sp. nov.** was 0.072 ± 0.007 . In total, we have determined 88 synonymous and one non-synonymous substitutions most of which were transitions. The average intraspecific pairwise distance between three *Diamesa* species was 0.093. Reconstructed neighbor joining phylogenetic tree (not shown), consists of over 2200 *Diamesa* COI barcodes reveal that all three species locate in various clusters. The closest species to *D. amplexivirilia* were *D. saetheri* Willassen, *D. serratosioi* Willassen and *Diamesa* sp. (BOLD: CNAUY568-14), p-distances were 0.090 ± 0.010 , 0.098 ± 0.010 and 0.090 ± 0.012 respectively. Similarity of *D. amplexivirilia*, *D. saetheri* and *D. serratosioi* is also confirmed by morphological data (see above). Obtained sequences of *D. arctica* were close to specimen *D. arctica* from Norway (BOLD ID: ATNA401-10, BIN BOLD:AAD7062). Low genetic distances (0.014) suggest that these specimens are conspecific. The sister clade to obtained specimens were diverged into two clades—*D. arctica* from Svalbard and Greenland (BIN BOLD:AAD7061) and *D. arctica* from Northern Canada, Greenland and nearest Islands (BIN BOLD:AEC0191). Another BIN number, BOLD:AAL5960 from Northern Canada, Greenland, Ellesmere and Baffin Islands includes *D. arctica* which indicates the need for taxonomic revision of this species.

Sequence of *A. appendiculata* from Bolshevik Island was the first molecular data in GenBank for genus *Arctodiamesa*. To carry out comparative analysis within genus we depose in GenBank six sequences belonging to *A. breviramosa* Makarchenko from Magadan region of Russia, and *A. marinae* Makarchenko from South Korea. The average interspecific sequence divergence between three species was 0.124 ± 0.012 . *A. appendiculata* was sister (Bayesian posterior probabilities = 1, ML bootstrap support = 100) to *A. breviramosa* on Bayesian tree (Fig. 16).

The closest specimen from GeneBank and BOLD Systems to *Pseudokiefferiella* sp. obtained from Vaygach Island was *Pseudokiefferiella* sp., BOLD ID: CHRFI378, BIN BOLD:AAV2899 from Northern Norway. Low sequence divergence between specimens (0.002) as well as morphological similarity of male hypopygium and nearness of habitats suggest that these specimens are conspecific. The sister clade (Fig. 17) to *Pseudokiefferiella* sp. was *Pseudokiefferiella* sp. from Greenland and Svalbard Islands (BIN BOLD:ACI9181) on a Bayesian tree (PP = 0.86). Interspecific genetic divergence between two species was 0.035. Another close specimen to *Pseudokiefferiella* sp. was *Pseudokiefferiella* sp. BOLD ID: CNTUB3438, BIN BOLD:ACR4757, collected in Northern Canada, p-distance between them was 0.042. The phylogeny of the remaining *Pseudokiefferiella* species is shown on tree (Fig.17).

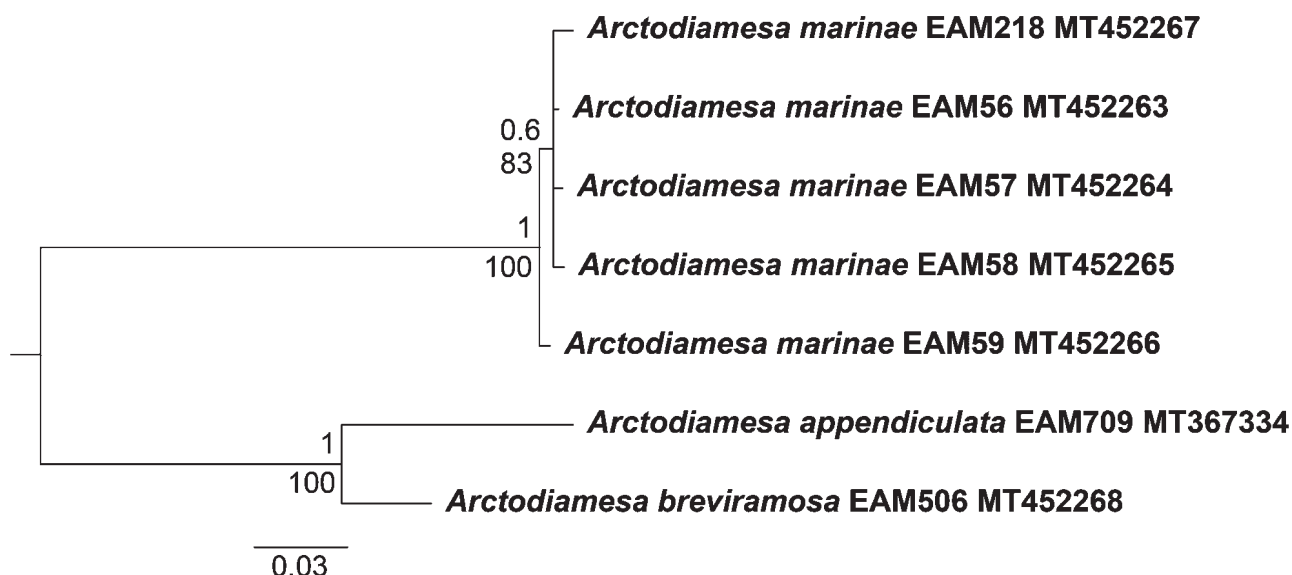


FIGURE 16. Bayesian tree based on mitochondrion COI gene for three species of the genus *Arctodiamesa* Makarchenko. K80, F81 and HKY+G was the best models of nucleotide substitution for the first, second and third of COI codon position respectively. Bayesian posterior probabilities (PP) are given above tree nodes and bootstrap support values found in the ML analysis are shown below nodes. Specimens obtained in this study are in bold.

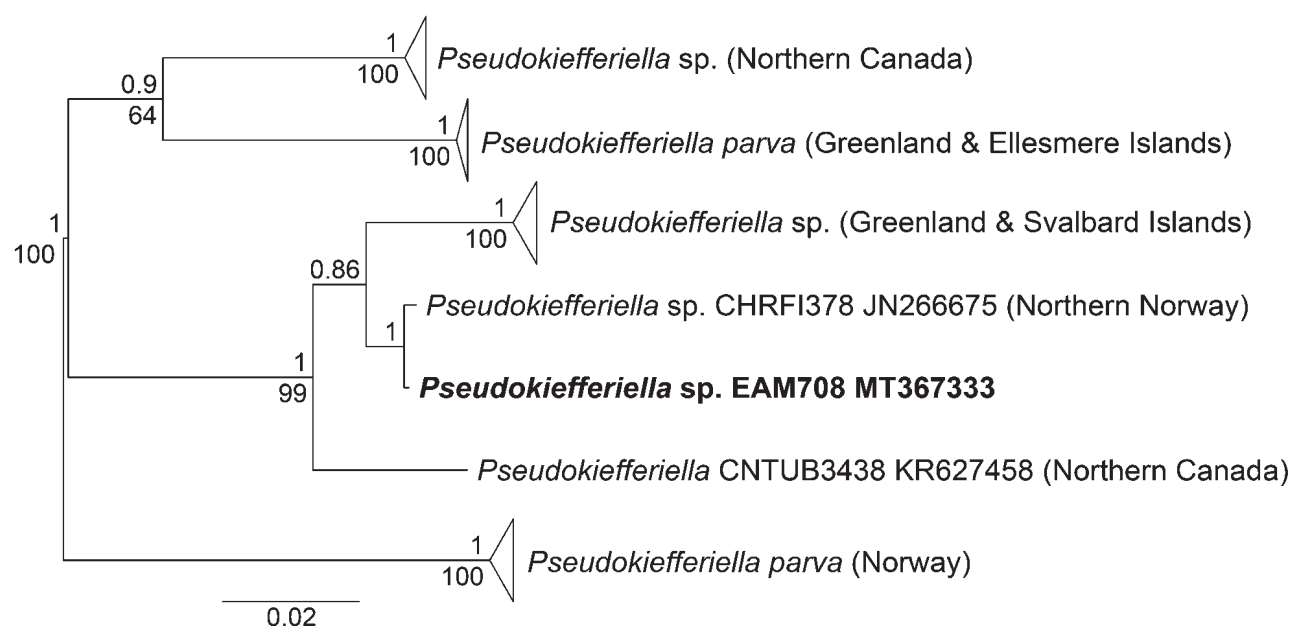


FIGURE 17. Bayesian tree based on mitochondrion COI gene for the genus *Pseudokiefferiella* Zavřel. SYM+G, F81 and HKY+G was the best models of nucleotide substitution for the first, second and third of COI codon position respectively. Bayesian posterior probabilities (PP) are given above tree nodes and bootstrap support values found in the ML analysis are shown below nodes. Specimens obtained in this study are in bold.

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