

## Two new chironomid species of the genus *Pseudokiefferiella* Zavřel (Diptera: Chironomidae: Diamesinae) from the Amur River basin of Russia

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### Abstract

The adult male, pupa, larva with DNA barcoding of *Ps. matafonovi* **sp. nov.** and the adult male of *Ps. silinka* **sp. nov.** from Amur River basin of Russia are described and illustrated. *Ps. matafonovi* **sp. nov.** is genetically distant from other *Pseudokiefferiella* showing uncorrected p-distances of >6.8 %. The results of species delimitation show that genus *Pseudokiefferiella* includes 10 (mPTP), 13 (ASAP, GMYC) or 14 (BOLD) distinct molecular taxonomic units (mOTUs) that requires a revision of this genus using both morphological and molecular approaches.

**Key words:** Diptera, *Ps. matafonovi* **sp. nov.**, *Ps. silinka* **sp. nov.**, *Pseudokiefferiella*, Chironomidae, Diamesinae

### Introduction

The genus *Pseudokiefferiella* was established by Zavřel (1941) and before the start of molecular genetic study it was considered as monotypic, that is, with one species of *Ps. parva* (Edwards) in Holarctic region (Ashe & O'Connor 2009). According to the published data of Stur and Ekrem (2020), as well as the results of our research and data of GenBank, there are at least 6 species in the genus *Pseudokiefferiella* that differ in DNA barcoding (see below Fig. 26) but poorly differ morphologically. The main problem in the taxonomy of this genus is the lack of DNA barcoding data for the *Ps. parva* from the type locality which is Scotland (Edwards 1932) what makes it impossible to accurately determine who is who. Before to obtaining these information we decided not revise all the species of *Pseudokiefferiella* but found it possible to make description of two new species from the Amur River basin of the Eastern Siberia and Russian Far East. Below we present a descriptions of the adult male, pupa, larva with DNA barcoding of *Ps. matafonovi* **sp. nov.** and of the adult male of *Ps. silinka* **sp. nov.**

### Materials and methods

The larvae, pupae and adult males of *Ps. matafonovi* **sp. nov.** and male adults of *Ps. silinka* **sp. nov.** were preserved in 70% ethanol for further study of morphology and larvae of *Ps. matafonovi* **sp. nov.** 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 (Karl Zeiss) microscope with an AxioCam ERc5s digital camera, and then stacked using Helicon Focus software. The final illustrations were post-processed for contrast and brightness using Adobe® Photoshop® software.

Holotypes and paratypes of the new species, as well as all other 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).

The DNA extraction, thermal regime and primers for amplification, and sequencing conditions of cytochrome c oxidase subunit I gene was done following the protocol similar to that employed in earlier studies (Makarchenko *et al.* 2022, 2023).

The six new COI sequences were added to a dataset from Barcode of Life Data System (BOLD), containing all published sequences of the *Pseudokiefferiella* (at Aug 2023). We then removed most of the sequences from the two highly sampled Barcode Index Numbers (BIN) (BOLD:AAL9436, BOLD:ACI9181) and got the final dataset contained 48 sequences. Inter- and intraspecific genetic distances were calculated based on the observed p-distances using MEGA7 (Kumar *et al.* 2016). Species delimitation was provided using distance-based (ASAP) and tree-based approaches (mPTP and GMYC). Parameter settings for species delimitation including the reconstruction of the input ultrametric tree can be found in Makarchenko *et al.* (2023).

The nucleotide substitution models best fitting the COI barcode library for sequences were obtained using the Bayesian Information Criterion (BIC) in PartitionFinder 2.1.1 (Lanfear *et al.* 2012). The best models for the first, second and third codon position of COI was SYM+I (Zharkikh 1994), F81 (Felsenstein 1981) and GTR+G (Tavaré 1986) respectively. Phylogenetic analyses were performed using Bayesian inference (BI) in the program MrBayes ver. 3.2.7 (Ronquist *et al.* 2012) using the Markov Chain Monte Carlo algorithm (MCMC), with 5 million generations and a burn-in of 25% of the initial trees. Strict clock model (brlenspr=clock:uniform) were used to obtain an ultrametric tree. Moreover, trace files of BI analysis were visually inspected in Tracer 1.7 (Rambaut *et al.* 2018) and then the tree is visualized in FigTree v. 1.4.4. The obtained sequences have been deposited in GenBank under numbers OR465296–OR465301.

## Taxonomy

### *Pseudokiefferiella matafonovi* Makarchenko et Semenchenko, sp. nov.

<http://zoobank.org/NomenclaturalActs/69AA60DC-3129-41FA-9A7F-6CE7F1D20E09>

(Figs. 1–18)

**Type material.** Holotype: adult male, **Russia:** Trans-Baikal Territory, environs of the Chita City, Kaydalovka River, upper stream, N 52.039058, E 113.560243, 18.VIII 2003, leg. P. Matafonov. Paratypes: 1 adult male, 1 pupa (male), 4 pupal exuviae, 7 larvae, the same data as holotype, 18.VIII 2003, leg. P. Matafonov; 8 larvae, the same data as holotype, except 31.V. 2022, N 52.039988, E 113.562473, leg. P. Matafonov; 1 larva, the same data as holotype, except Karpovskiy Stream, 2.VII.2004, leg. O. Klishko.

**Derivatio nominis.** The species is named in honour of the Russian hydrobiologist Dr. Pyotr Viktorovich Matafonov (Institute of Natural Resources, Ecology and Cryology of the Siberian Branch of the Russian Academy of Sciences) who collected material in type locality.

### Description

**Adult male** (n = 2, except when otherwise stated). Total length 3.6–3.9 mm. Total length/wing length 1.06–1.16.

Coloration. Brown, dark brown. Head, thorax and abdomen with hypopygium brown or dark brown. Legs yellowish brown. Wings greyish.

Head. Eyes pubescent, slightly elongate dorsomedially. Temporal setae including 13–19 verticals and 12–13 postorbitals. Clypeus with 2–5 setae. Antenna with 13 flagellomeres and well developed plume of setae; maximal length of these setae on flagellomeres 540 µm; terminal flagellomere with 1 subapical seta, 60–64 µm long; pedicel with 2 setae, 60–72 µm long. AR 1.35–1.57. Palpomere length (µm) (n=1): 44, 84, 156, 120, 156. Palpomere 3 in distal part with sensilla capitata with diameter 8 µm. Head width/palpal length 1.0.

Thorax. Anteprenotum with 7 ventrolateral setae. Dorsocentrals 12–13, prealars 5–6. Scutellum with 9–18 setae in 1 row.

Wing. Length 3.3–3.4 mm, width 0.92 mm. Anal lobe developed and rounded. Squama with 30–46 setae, 75–100 µm long. R and R<sub>1</sub> with 18–20 setae, R<sub>4+5</sub> with 4–5 setae in distal part. RM/MCu 2.3–2.5.

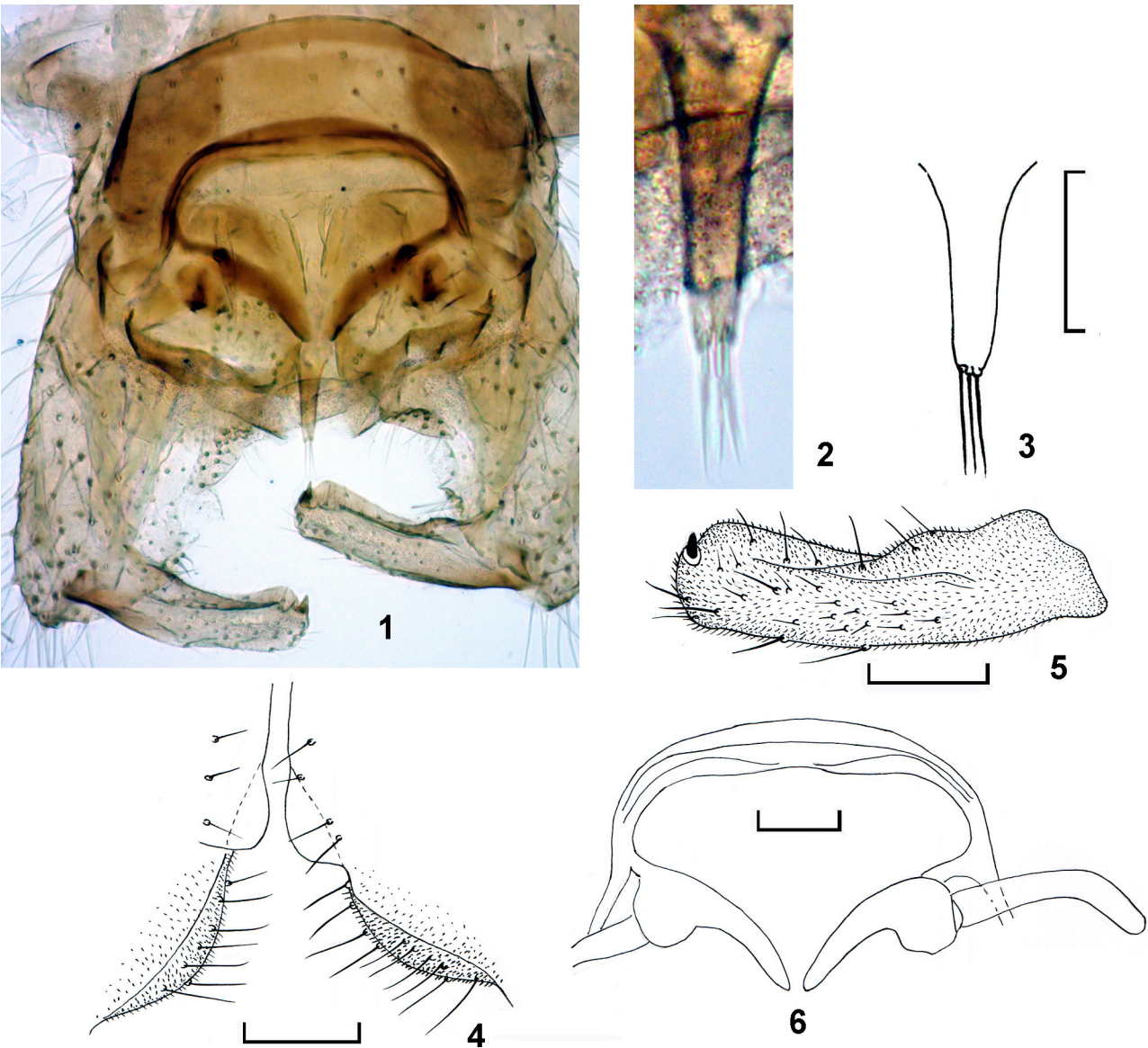
Legs. Spur of front tibia 76–84 µm long. Spurs of mid tibia 60–64 µm and 64 µm long. Spurs of hind tibia 84–96 µm and 60–64 µm long. Hind tibial comb with 16 setae. Length (µm) and proportions of leg segments are as in Table 1.

**TABLE 1.** Lengths (in  $\mu\text{m}$ ) and proportions of leg segments of *Pseudokiefferiella matafonovi* **sp. nov.**, male (n=2).

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>
P <sub>1</sub>	1115–1148	1509	1082	492–558	303–328	98–115	115–131
P <sub>2</sub>	1214	1378	705	361	312	98	131
P <sub>3</sub>	1378	1525–1607	902–984	492–525	262–279	98–115	148

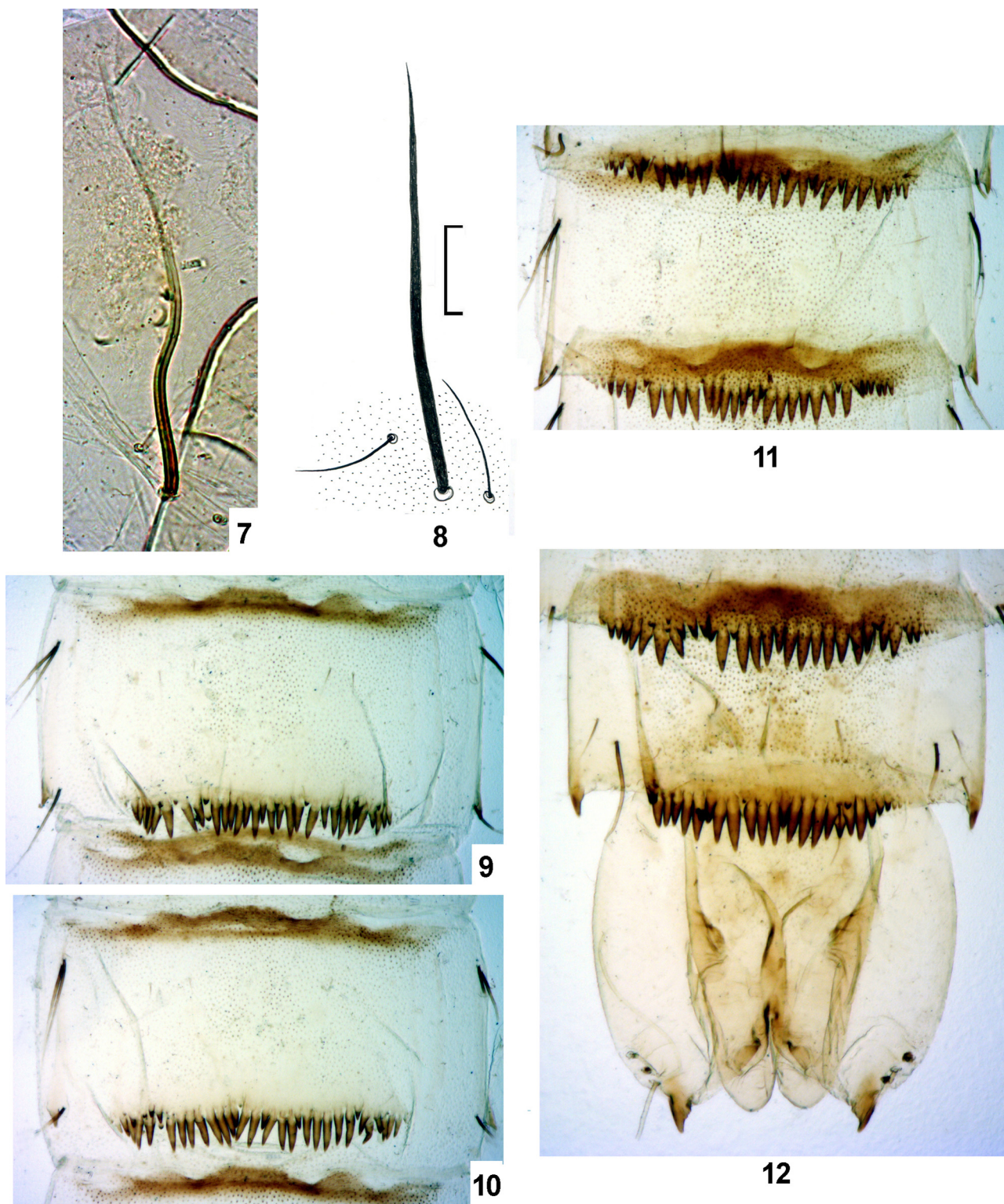
continued.

	LR	BV	SV	BR
P <sub>1</sub>	0.71	3.53–3.56	2.43–2.46	2.5–2.9
P <sub>2</sub>	0.51	3.66	3.68	2.0–2.1
P <sub>3</sub>	0.59–0.61	3.68–3.84	3.03–3.22	2.5–2.8



**FIGURES 1–6.** Adult male of *Pseudokiefferiella matafonovi* **sp. nov.** **1**, hypopygium in dorsal view; **2–3**, anal point; **4**, basal plate of gonocoxite; **5**, gonostylus; **6**, transverse sternapodeme, aedeagal lobes and phallapodeme. Scale bars: 50  $\mu\text{m}$ .





**FIGURES 7–12.** Pupa of *Pseudokiefferiella matafonovi* **sp. nov.** 7–8, precorneals; 9, tergite IV; 10, tergite V; 11, tergites V–VI; 12, tergites VII–VIII and anal segment. Scale bars: 50  $\mu$ m.

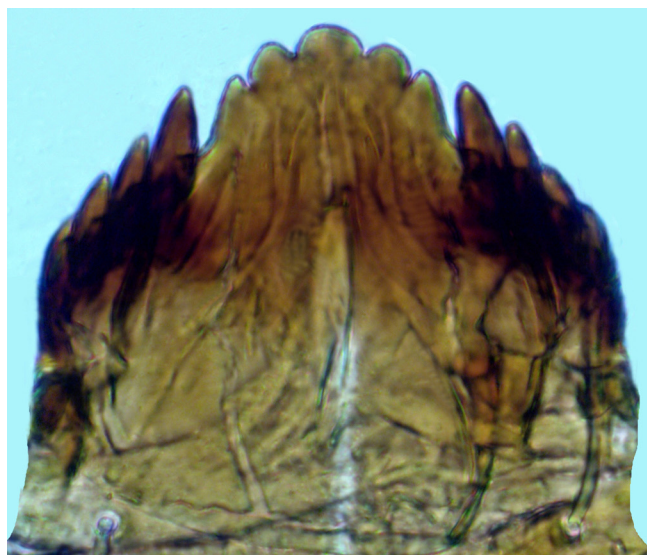
Hypopygium (Figs 1–6). Tergite IX with 17–18 setae from one side, 32–36  $\mu$ m long and with anal point, 80  $\mu$ m long and 16  $\mu$ m wide in base, apically with 3 setae, 24–26  $\mu$ m long (Figs 1–3). Laterosternite IX with 8 setae, 64–76  $\mu$ m long. Transverse sternopodeme consisting of a narrow arch 216–240  $\mu$ m long. Aedeagal lobe 116–132  $\mu$ m long, wide in basal third and narrow finger-shaped in distal two-thirds (Fig. 6); phallapodeme 120  $\mu$ m long. Gonocoxite 308–310  $\mu$ m long, with basal plate in the form of two rounded lobelike projections, lower of which is



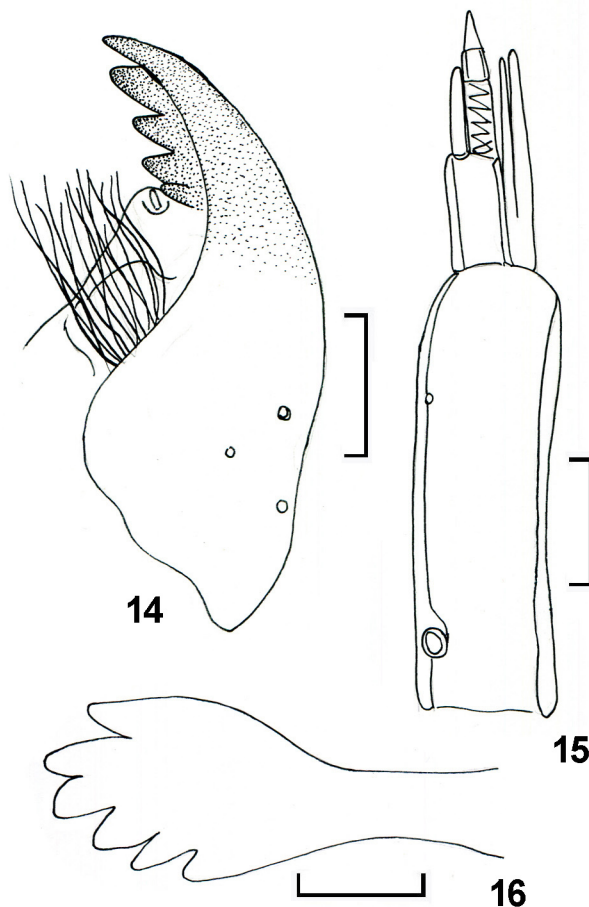
covered with microtrichia and numerous long setae, upper without microtrichia and setae (Fig. 4). Gonostylus massive, 168–176  $\mu\text{m}$  long and 56–60  $\mu\text{m}$  wide, with long crista dorsalis; apex with short macroseta, 16  $\mu\text{m}$  long (Figs 1, 5); HR 1.75–1.76.

**Pupa** (n=4). Total length *ca* 6 mm. Abdomen length 4.28–5.44 mm. Coloration brownish. Exuviae white and transparent, sometimes yellowish.

**Cephalothorax.** Frontal apotoma with 2 setae 152–160  $\mu\text{m}$  long. Thorax in anterodorsal part smooth or slightly wrinkled. Thoracic horn absent. Precorneal setae lengths ( $\mu\text{m}$ ):  $\text{Pc}_1$  – 48–60,  $\text{Pc}_2$  – 180–256,  $\text{Pc}_3$  – 56–104.  $\text{Pc}_1$  and  $\text{Pc}_3$  weak and pale,  $\text{Pc}_2$  strong and brown (Figs. 7–8). Anteprenotum with 1 median seta, 180–236  $\mu\text{m}$  long and 1 lateral anteprenotals, 64–152  $\mu\text{m}$  long. Dorsocentrals absent.



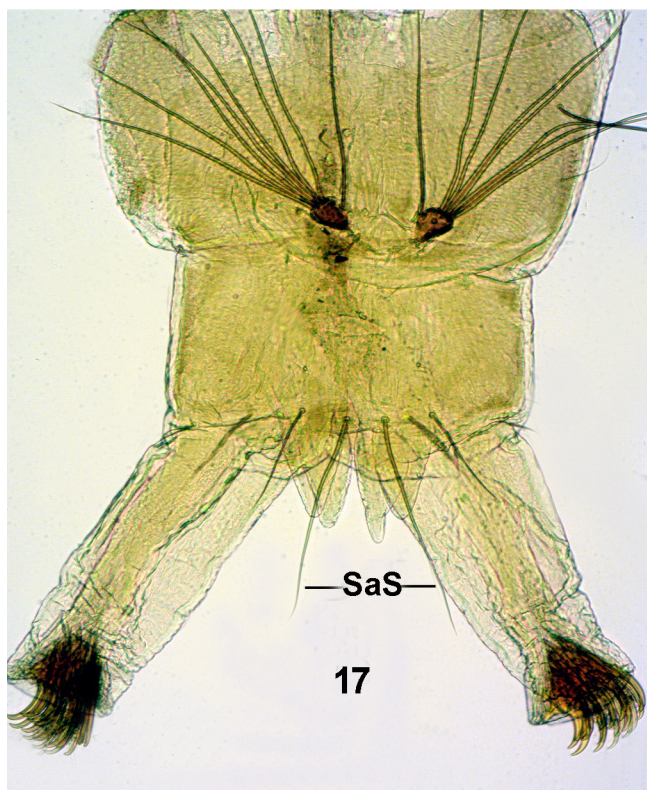
13



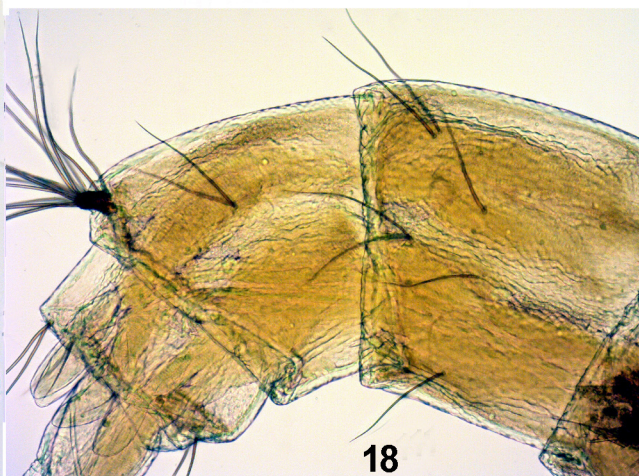
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17



18

**FIGURES 13–18.** Fourth instar larva of *Pseudokiefferiella matafonovi* sp. nov. 13, mentum; 14, mandible; 15, antenna; 16, distal part of premandible; 17, anal segments in dorsal view; 18, anal segments in lateral view. SaS – supraanal setae. Scale bars: Fig. 4 – 50  $\mu\text{m}$ ; Figs. 15–16 – 20  $\mu\text{m}$ .

Abdomen. Apophyses distinct. Tergite I without shagreen and posterior transverse row of spines. Tergite II with shagreen in anterior third, tergites III–VIII almost completely covered in shagreen. Sternites I and IX without shagreen; II–VIII with median and lateral shagreen. Tergites II–VIII with posterior transverse row of strong, brown or dark brown elongated spines, maximum length 72–80  $\mu\text{m}$  and width 20–24  $\mu\text{m}$ ; sternites without spines. Number of these spines on tergites II–VIII accordingly – 34–40 : 30–33 : 27–34 : 29–32 : 22–33 : 22–35 : 16–25 (Figs 9–12). Segment I with 1 pair of lateral setae. Segments II–VIII with 3 pairs of brown lateral setae 240–288  $\mu\text{m}$  long. The setae extension is shown in Figs 8, 12. Segments II–VIII with spine-like process on posterolateral corner, 28–40  $\mu\text{m}$  long. Anal lobe with 3 anal macrosetae, 300–388  $\mu\text{m}$  long, slightly curved in distal part; apical tubercle pointed, 48–52  $\mu\text{m}$  long. Male genital sac not extended beyond anal lobe (Fig. 12).

**Fourth instar larva** ( $n=6$ ). Coloration greenish. Total length 6.4–7.1 mm. Head capsule 460–514  $\mu\text{m}$  long and 420–429  $\mu\text{m}$  wide; brown or dark brown, postoccipital margin black and narrow.  $S_1$  short, seta-like;  $S_{III}$  simple. Labral lamellae consisting of 2 pectinate lobes. Premandible broad apically with 6 teeth (Fig. 16). Antenna with 5 segments, length of antennal segments ( $\mu\text{m}$ ): 40, 10, 7, 3, 3. Lauterborn organs small, often invisible; style 13  $\mu\text{m}$  long, reaches the middle of the fourth segment; longest branch of antennal blade 35  $\mu\text{m}$  long and reaches the apex of the fourth segment, shorter branch 33  $\mu\text{m}$  long; ring organ 7–8  $\mu\text{m}$  diameter located in basal quarter of first segment. AR 1.60–1.74. Mandible dark brown, with apical tooth and 4 inner teeth; apical tooth same length as first inner tooth but inner tooth slightly wide than apical tooth; seta subdentalis minute; seta interna with 18–20 simple branches (Fig. 14). Mentum with 1 median and 6 pairs of lateral teeth; median tooth not much larger than first lateral tooth and together with two pairs of lateral teeth lighter than other lateral teeth; ventromental plate small (Fig. 13). Abdominal setae dark brown 164–295  $\mu\text{m}$  long; ratio of length of abdominal segment to length of setae 2.22–2.53 (Fig. 18). Procercus dark brown, 48  $\mu\text{m}$  long and 40  $\mu\text{m}$  wide, bearing 7 dark brown anal setae 340–356  $\mu\text{m}$  long and 2 subapical setae from which proximal subapical seta strong and dark brown, 284  $\mu\text{m}$  long, distal seta pale and short. Last abdominal segment with 3 pairs of supraanal setae, from which middle setae most long – 276–280  $\mu\text{m}$  (Fig. 17). Posterior parapods 400  $\mu\text{m}$  long. Dorsal pair of anal tubulus 128  $\mu\text{m}$  long, ventral pair 156  $\mu\text{m}$  long.

**Diagnosis.** Total length of adult male 3.6–3.9 mm. Wing length 3.3–3.4 mm AR 1.35–1.57. Dorsocentrals 12–13, prealars 5–6, scutellars 9–18.  $LR_1$  0.71,  $BV_1$  3.53–3.56,  $SV_1$  2.43–2.46. Anal point apically with 3 setae. Aedeagal lobe wide in basal third and narrow finger-shaped in distal two-thirds. Gonocoxite with basal plate in the form of two rounded lobelike projections, lower of which is covered with microtrichia and numerous long setae, upper without microtrichia and setae. Gonostylus massive, with long crista dorsalis. HR 1.75–1.76. Pupa without thoracic horn.  $Pc_1$  and  $Pc_3$  weak and pale,  $Pc_2$  strong and brown. Tergites II–VIII with posterior transverse row of strong, brown or dark brown elongated spines. Segment I with 1 pair of lateral setae. Segments II–VIII with 3 pairs long brown lateral setae. Segments II–VIII with spine-like process on posterolateral corner. Anal lobe with 3 anal macrosetae, slightly curved in distal part; apical tubercle pointed. Male genital sac not extended beyond anal lobe. Total length of larva 6.4–7.1 mm. Premandible broad, apically with 6 teeth. Antenna with 5 segments, Lauterborn organs small, often invisible; style reaches middle of fourth segment; longest branch of antennal blade reaches the apex of the fourth segment; ring organ located in basal quarter of first segment. AR 1.60–1.74. Mandible dark brown, with apical tooth and 4 inner teeth; apical tooth same length as first inner tooth but inner tooth slightly wide than apical tooth; seta interna with 18–20 simple branches. Mentum with 1 median and 6 pairs of lateral teeth; median tooth with two pairs of lateral teeth lighter than other lateral teeth. Abdominal setae dark brown, ratio of length of abdominal segment to length of setae 2.22–2.53. Procercus dark brown, little longer than wide, bearing 7 strong dark brown anal setae and 2 subapical setae from which proximal subapical seta is strong dark brown.

**Remarks.** Unfortunately, due to the uninformative description of the adult male holotype of *Ps. parva* (Edwards 1932) and often other conflicting descriptions of adults, pupa and larvae of this species (Pagast 1947; Wülker 1959; Zavřel 1941; Oliver 1959; Pankratova 1970; Schmid 1993 and other), it is impossible to make an objective and reliable comparison of this species with *Ps. matafonovi* **sp. nov.** The most complete and correct description of the imago and pupa of *Ps. parva* is given only in the work of Serra-Tosio (1971), according to which the gonocoxite of the male in dorsolateral position at the base of the gonostylus has a rounded-triangular protrusion, which is absent in the male *Ps. matafonovi* **sp. nov.** Also that the shape and structure of anal point and the aedeagal lobes differ in both species. Pupa of the new species without thoracic horn and tergites II–VIII with posterior transverse row of strong, brown or dark brown elongated spines, segments II–VIII with 3 pairs of lateral setae while pupa of *Ps. parva* with thoracic horn and tergites II–VIII with transverse row of thorn-like spines, segments II–VIII with 4 pairs of lateral setae.



**Ecology.** Larvae and pupae were collected in the upper reaches of the Kaydalovka River, at an altitude of 960–980 m above sea level, among the fouling of small boulders with moss and yellow-green algae *Vausheria geminata* f. *geminata* and *Tribonema* sp., at a depth of about 10 cm at a water temperature of 3.5–4.5 °C.

**Distribution.** Known only from the type locality in upstream of Amur River basin.

***Pseudokiefferiella silinka* Makarchenko et Semenchenko, sp. nov.**

<http://zoobank.org/NomenclaturalActs/D9D8264A-89D7-4D06-8933-D509CCB0A908>

(Figs 19–25)

**Type material.** Holotype: adult male, **Russia:** Khabarovsk Territory, Solnechnyi District, environs of Gornyi Village, Miao Chan Ridge, valley of the Levaya Silinka River, Amur River basin, 27.VII.2006, N 50.710861, E 136.365278, light trap, leg. E. Makarchenko.

**Derivatio nominis.** The species is named as *silinka* after the type locality in Levaya Silinka River of the Amur River basin. The name is a noun in apposition.

**Description**

**Adult male** (n = 2, except when otherwise stated). Total length 2.7–2.8 mm. Total length/wing length 1.16.

Coloration. Grayish brown. Head, thorax and abdomen with hypopygium grayish brown or brown. Legs brownish gray. Wings greyish.

Head. Eyes pubescent, slightly elongate dorsomedially. Temporal setae including 7–8 verticals and 12 postorbitals. Clypeus with 2 setae, 52–68 µm long. Antenna with 13 flagellomeres and well developed plume of setae; maximal length of these setae on flagellomeres 558 µm; terminal flagellomere with 1 subapical seta, 36 µm long; pedicel with 2 setae. AR 0.81. Palpomere length (µm): 32–40, 48–56, 88–108, 92–96, 132–160. Palpomere 3 in distal part with sensilla capitata with diameter 8 µm. Head width/palpal length 1.0.

Thorax. Anteprenotum with 2–4 ventrolateral setae, 32–40 µm long. Dorsocentrals 7–10, 84–88 µm long, prealars 3–4, 72–80 µm long. Scutellum with 7–8 setae in 1 row.

Wing. Length 2.3–2.4 mm, width 0.64 mm. Anal lobe developed and rounded. Squama with 14 setae, 52–68 µm long. R and R<sub>1</sub> with 17 setae, R<sub>4+5</sub> with 5 setae in distal part. RM/MCu 2.5.

Legs. Spur of front tibia 40–60 µm long. Spurs of mid tibia 40–44 µm and 40 µm long. Spurs of hind tibia 56–60 µm and 40–44 µm long. Hind tibial comb with 14–15 setae. Length (µm) and proportions of leg segments are as in Table 2.

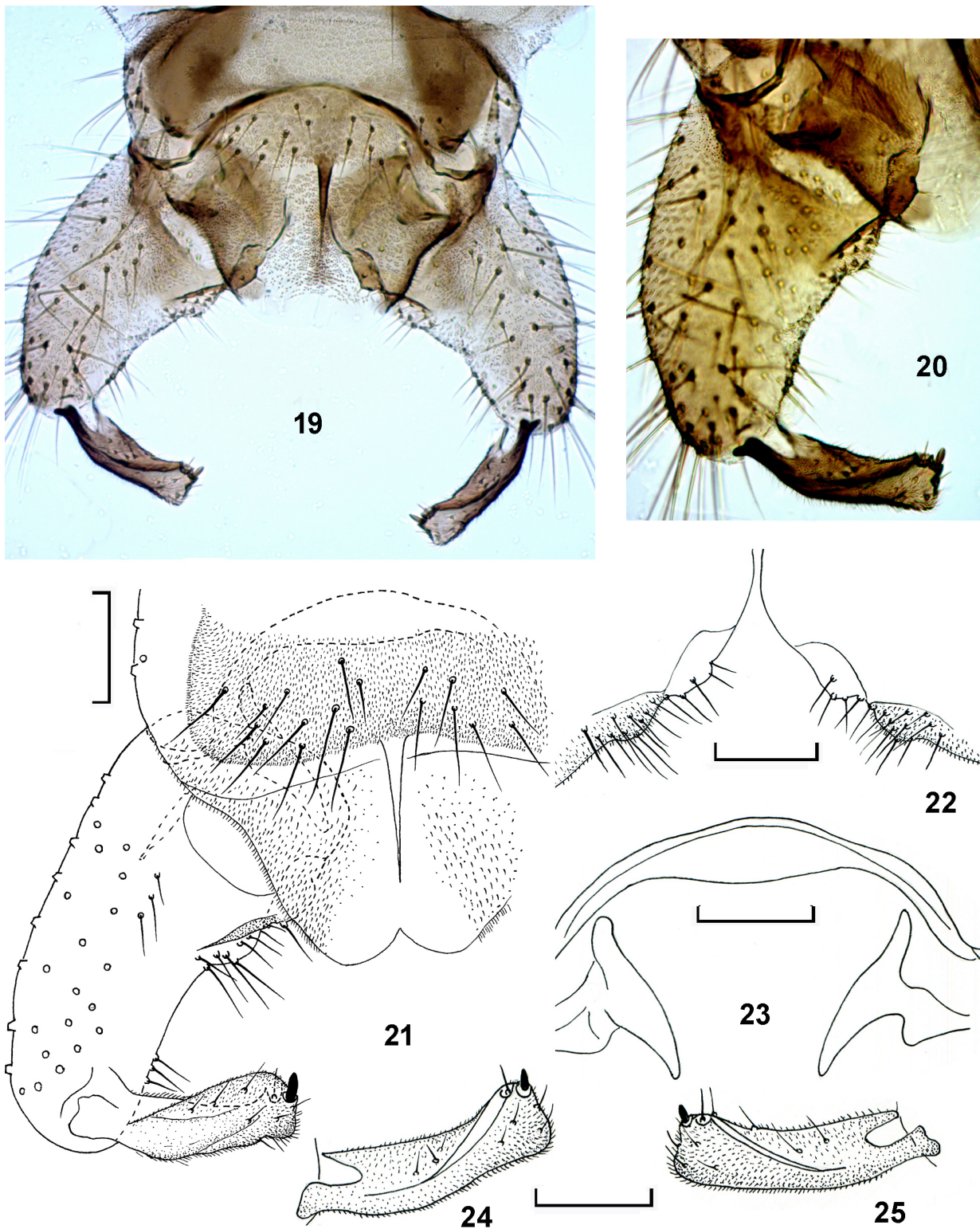
**TABLE 2.** Lengths (in µm) and proportions of leg segments of *Pseudokiefferiella silinka* sp. nov., male (n=1).

	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>	820	1017	672	312	197	98	115	0.66	3.51	2.68	1.88
P <sub>2</sub>	836	935	410	230	164	82	98	0.44	3.80	4.32	1.63
P <sub>3</sub>	1050	1000	623	344	180	82	115	0.62	3.71	3.29	2.14

Hypopygium (Figs 19–25). Tergite IX with 12–14 setae, from one side, 28–40 µm long and with narrow, sharply pointed anal point, 68–80 µm long (Figs 19, 21). Laterosternite IX with 5–8 setae, 60–72 µm long. Transverse sternopodeme consisting of a narrow arch 176 µm long, expanded in the middle (Fig. 23). Aedeagal lobe wedge-shaped, 68 µm long (Fig. 23). Gonocoxite 212 µm long, with basal plate in the form of two rounded or rounded triangular tubercles, the lower of which is covered with microtrichia and numerous long setae, the upper without microtrichia and only with 3–4 setae (Fig. 22). Gonostylus narrow, 100–104 µm long, with long crista dorsalis; apex with short macroseta, 8–12 µm long (Figs 24–25); HR 1.9–2.2.

**Pupa and larva** unknown.

**Diagnosis.** Total length of adult male 2.7–2.8 mm. Wing length 2.3–2.4 mm. AR 0.81. Dorsocentrals 7–10, prealars 3–4, scutellars 7–8. LR<sub>1</sub> 0.66, BV<sub>1</sub> 3.51, SV<sub>1</sub> 2.68. Anal point narrow, sharply pointed. Transverse sternopodeme consisting of a narrow arch expanded in the middle. Aedeagal lobe wedge-shaped. Gonocoxite with basal plate in the form of two rounded or rounded triangular tubercles, lower of which is covered with microtrichia and numerous long setae, upper without microtrichia and only with 3–4 setae. Gonostylus narrow, with long crista dorsalis; apex with short macroseta. HR 1.9–2.2.



**FIGURES 19–25.** Adult male of *Pseudokiefferiella silinka* **sp. nov.** 19, 21, hypopygium in dorsal view; 20, gonocoxite and gonostylus in dorsal view; 22, basal plate of gonocoxite; 23, transverse sternapodeme and aedeagal lobes; 24–25, gonostylus. Scale bars: 50  $\mu$ m.

**Remarks.** The adult male of *Ps. silinka* **sp. nov.** is more closely related to *Ps. parva* but can be well distinguished from it and from *Ps. matafonovi* **sp. nov.** in smaller size (2.7–2.8 mm), AR value (0.81), number of setae on squama (14), narrower gonostylus and by structure of gonocoxite basal plate, which has the form of two rounded or rounded triangular tubercles. In adult males of *Ps. parva* and *Ps. matafonovi* **sp. nov.** are respectively: total lengths

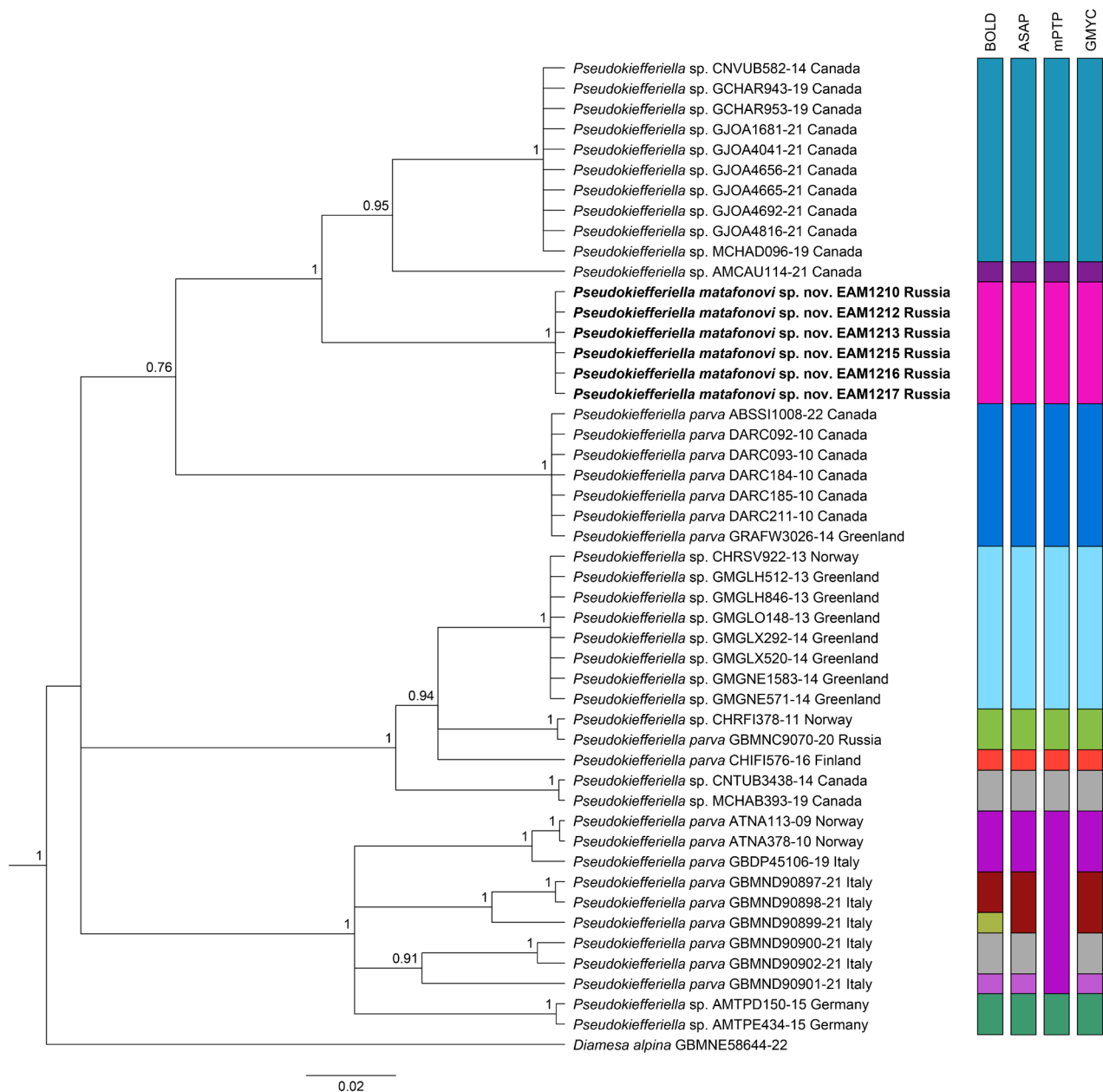


3.1–3.5 mm, 3.6–3.9 mm; AR 0.85–1.0, 1.35–1.87; number of setae on squama 23–30, 30–46. In the same species gonostylus is more massive; gonocoxite has basal plate in the form of two rounded lobelike projections. Gonocoxite of *Ps. silinka* **sp. nov.** as in *Ps. matafonovi* **sp. nov.**, in dorsolateral position at the base of the gonostylus both species are without rounded-triangular protrusion.

**Distribution.** Known only from the type locality in middle stream of Amur River basin.

## Results of DNA barcoding

Overall, we have sequenced fragments of the cytochrome oxidase subunit I (641 bp in length) of six *Ps. matafonovi* **sp. nov.** samples. All 6 sequences belonged to the same haplotype, so the intraspecific distances were zero. Inter-specific (according to BOLD, Fig. 26) distances in our dataset of *Pseudokiefferiella* varied between 2.4–13.8%. *Ps. matafonovi* **sp. nov.** shows the lowest genetic distance to Canadian *Pseudokiefferiella* sp. (BOLD:AAL9436 and BOLD:AEI1803) with 6.8% and 8.0% divergence respectively.



**FIGURE 26.** Ultrametric Bayesian inference (BI) tree based on the cytochrome c oxidase I (COI) nucleotide sequence data of the genus *Pseudokiefferiella* Zavřel and *Diamesa alpina* Tokunaga as outgroup. Bayesian posterior probabilities (higher than 0.7) are given above tree nodes. Specimens obtained in this study are in bold.

Phylogenetic tree was reconstructed using obtained dataset and *Diamesa alpina* Tokunaga as outgroup (Fig. 26). The basal polytomic clade was moderately supported (BPP=0.65) and includes three subclades. The first subclade contains specimens from Germany, Italy and Norway (BIN numbers: BOLD:ACU5487, BOLD:AEB0971, BOLD:AEL6605, BOLD:AEK8333, BOLD:AEZ3104, BOLD:AAH8220). Specimens of the second subclade collected from Canada, Finland, Greenland, Norway and Russia (BIN numbers: BOLD:ACR4757, BOLD:ADF0056, BOLD:AAV2899, BOLD:AAV2899). Finally, the third subclade includes *Ps. matafonovi* **sp. nov.** and three OTUs from Canada and Greenland (BIN numbers: BOLD:AAM6306, BOLD:AEI1803 and BOLD:AAL9436). The most of the nodes of subclades were highly supported (BPP=0.91–1).

The results of four species delimitation approaches confirm the validity of the *Ps. matafonovi* **sp. nov.** and shows that genus *Pseudokiefferiella* includes 10 (mPTP), 13 (ASAP, GMYC) or 14 (BOLD) distinct molecular taxonomic units (mOTUs) that requires a large revision using both morphological and molecular approaches (Fig. 26).

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