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Review of the genus *Shilovia* Makarchenko (Diptera: Chironomidae: Diamesinae: Boreoheptagyiini) from the mountains of Central Asia, with morphological description and DNA barcoding of known species

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Abstract

Chironomids of the genus *Shilovia* Makarchenko (Diamesinae, Boreoheptagyiini) from the mountains of Central Asia are revised using both morphological characters and molecular data. Illustrated descriptions of the adult male *Shilovia xinhuawangi* **sp. nov.** from Xinjiang Uygur Autonomous Region of China, *S. yakovlevi* **sp. nov.** from East Kazakhstan and redescription of *S. rara* Makarchenko from Tajikistan and Kyrgyzstan are provided. The result of morphological study is congruent with DNA barcoding analyses using COI sequences. The average K2P interspecific nucleotide distances within *S. xinhuawangi* **sp. nov.** and *S. yakovlevi* **sp. nov.** are 0.03% and 0.3% respectively. The nucleotide distances between the two new species and *S. rara* can be considered interspecific. Phylogenetic analysis using Maximum likelihood (ML) and Bayesian inferences (BI) support the placement of *S. xinhuawangi* **sp. nov.** and *S. yakovlevi* **sp. nov.** within the monophyletic genus *Shilovia*.

Key words: Diptera, Chironomidae, Diamesinae, Boreoheptagyiini, *Shilovia,* taxonomy, new species, DNA barcoding, Central Asia

Introduction

In this article we provide a further contribution for the revision of the tribe Boreoheptagyiini (Makarchenko *et al.* 2008, 2018, 2020a–b), in particular regarding the little-known genus *Shilovia* Makarchenko. This genus was established with the description of *S. rara* Makarchenko from Tajikistan by a single male (Makarchenko 1989). Originally, *Shilovia* was included in the Podonominae and this conclusion was accepted for a long time (Sæther *et al.* 2000, Ashe & O'Connor 2009). The examination of additional material in 2016 for the *S. rara* from Kyrgyzstan, including both morphology and DNA barcoding, suggested transferring the genus to the tribe Boreoheptagyiini of subfamily Diamesinae (Makarchenko *et al.* 2017). Later, D.M. Palatov collected two more species of the genus *Shilovia* in other regions of Central Asia – *S. xinhuawangi* **sp. nov.** from Xinjiang Uygur Autonomous Region of China and *S. yakovlevi* **sp. nov.** from East Kazakhstan, which are here described, with a redescription of *S. rara* from Tajikistan and Kyrgyzstan.

In this study, both morphological data and DNA sequences of the mitochondrial *cytochrome c oxidase subunit I* gene (COI) are used for species determination and delimitation. COI-based identification in Diamesinae have con-

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firmed the efficiency of DNA barcoding for species delimitation (Makarchenko *et al.* 2014, 2018, 2020b; Montagna *et al.* 2016). We, therefore, obtained DNA barcode sequences of *S. xinhuawangi* **sp. nov.** and *S. yakovlevi* **sp. nov.**, and compared them with *S. rara* and other chironomids of the tribe Boreoheptagyiini. At last phylogenetic relationships within the tribe Boreoheptagyiini using Maximum likelihood (ML) and Bayesian inferences (BI) analyses were reconstructed.

Materials and methods

The adults and preimaginal stages of chironomids were preserved in 96% ethanol for DNA-analysis and in 70% ethanol for further study of morphology. It should be noted that only adult males were used for the morphological study, whereas in some cases pupae and larvae were used for the DNA barcoding. The results of the morphological study of pupae and larvae will be presented in a separate article.

The material was slide-mounted in polyvinyl lactophenol following the recommendations of Moubayed and Langton (2019). The terminology follows Sæther (1980). The photographs were taken using an Axio Lab.A1 (Karl Zeiss) microscope.

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).

For the sequence reaction 2 adult males of *S. rara*, 3 adult males, 2 pupae, 4 larvae of *S, xinhuawangi* **sp. nov.** and 5 adult males, 2 pupae, 2 larvae of *S. yakovlevi* **sp. nov.** were used.

Total DNA was isolated from the thorax using Qiagen DNeasy Blood and Tissue Kit according to the manufacturer protocol with elution in 100 µl of TE buffer. Partial fragment of mitochondrial *cytochrome c oxidase subunit I* (658bp) was amplified by using Folmer *et al.* (1994) universal primer set LCO1490 and HCO2198. PCR reaction had 10 µL of final volume, containing 5 µL of Go Taq Green Master Mix (Promega Corp, Madison, WI, USA), 0.5 µM of each primer, and 1–2µL of eluted DNA. Thermocycler conditions were as follows: initial denaturation for 3 min at 94°C followed by 35 cycles of denaturing for 30 s at 94°C, annealing for 30 s at 48°C and an extension time of 60 s at 72°C, with a final extension for 5 min at 72°C. PCR products were visualized on an 1.5% agarose gel with Ethidium bromide after electrophoresis. Single bands were purified with Exonuclease I and Thermosensitive Alkaline Phosphatase (ThermoFisher Scientific). Purified PCR products were sequenced directly in both directions using an automated sequencer (ABI 3130xl Genetic Analyzer Sequencer; Applied Biosystems, USA) at department of Cell Biology and Genetics of Far Eastern Federal University.

Forward and reverse sequences were manually assembled and edited using Finch TV and MEGA 7 (Kumar *et al.* 2016). Alignment of the DNA sequences were performed using the MUSCLE algorithm (Edgar 2004). Base frequencies and molecular character statistics were calculated in MEGA 7. Phylogenetic trees were constructed using Maximum Likelihood (ML) and Bayesian inference (BI) approaches. The optimal nucleotide substitution model for BI was selected using PartitionFinder 2.1.1 (Lanfear *et al.* 2012) while for the ML analysis was used GTR+G (Gamma distribution). The best models of nucleotide substitution for first, second and third codon positions of COI was SYM (Zharkikh 1994) plus G, HKY (Hasegawa *et al.* 1985) plus I (a proportion of invariable sites) and HKY+G respectively. ML analysis was performed in RAxML v. 8.2.4 using bootstrapping with 1000 replications (Stamatakis 2006). BI was carried out using Markov Chain Monte Carlo (MCMC) randomization in MrBayes v3.2.7 (Ronquist *et al.* 2012). Four Markov chains (three heated chains, one cold) were run for 5 million generations, with the first 25% of sampled trees 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. Bayesian posterior probabilities and ML bootstrap values were used to evaluate branch support. The obtained sequences have been deposited in GenBank under numbers MW136396 – MW136413.

Descriptions

Genus *Shilovia* Makarchenko

Shilovia Makarchenko, 1989: 205.
Shilovia Makarchenko; Sæther *et al*. 2000: 134, Fig. 144; Ashe & O'Conner 2009: 292.
Type species: Shilovia rara Makarchenko, 1989, by original designation.

Generic diagnosis (emended)

Adult male. Small sized species 1.5–2.5 mm long, with wing length 1.6–2.4 mm.

Antenna. Scape bare. Flagellum with 5–6 flagellomeres and reduced plume of short setae, which on flagellomeres 1–4 not more than 12; flagellomeres 1–4 and terminal flagellomere with sensilla chaetica; terminal flagellomere with 1–3 subapical setae. Antennal ratio 0.32–0.44.

Head. Eyes bare or pubescent, not extended dorsomedially. Temporal setae numerous, consisting of inner and outer verticals, frontals, orbitals and postorbitals. Vertex without of frontal tubercles. Tentorium narrow and long; microtrichia absent. Palp with 5 palpomeres; palpomere 3 without sensilla capitata.

Thorax. Antepronotal lobes in contact to widely separated medially; antepronotals present in ventrolateral part. Scutum broad, low, not extended over antepronotal notch, and not covered with macrotrichiae. Acrostichals short, in 1–3 rows, beginning near antepronotum, extending to near scutellum; dorsocentrals short, in 2–4 rows, in anterior part of mesonotum reach or not reach of preanal setae, and in posterior part they are converge or not converge with acrostichal setae; prealars middle size, in 2–3 rows; scutellum covered with numerous setae; some setae on MAII and also supraalars and preepisternals present. Postnotum with or without setae.

Wing. Gray or dark grey. Membrane without setae, covered with good visible microtrichiae. Anal lobe slightly reduced, angular or rounded angular. Costa slightly produced of R_{4+5} apex; R_{2+3} absent; FCu proximal to RM. MCu flows into FCu or into M_{3+4} slightly distal to FCu. R, R_1 and R_{4+5} with setae; Cu and An with or without setae. Squama with setae.

Legs. Tibiae with or without light coloured rings; spurs comparatively short. Fore, mid and hind legs with pseudospurs on tarsomere 1; tarsomere 4 cylindrical, shorter than tarsomere 5. Claws pointed apically; pulvilli as small spinules. LR_1 0.54–0.68, BV_1 3.48-4.21, SV_1 3.04–3.89.

Hypopygium. Tergite IX wide, with concave or almost straight posterior edge, without anal point, covered with numerous very short setae. Sternapodeme long and narrow or wide basally and narrow distally; aedeagal lobe weakly sclerotized, apical part hooked or fingerlike. Gonocoxite with long apical projection ("heel") which densely covered with long and thin setae; this "heel" posterior to gonostylus 0.35–0.63 times as long as gonostylus; inferior volsella in form of tubercle, densely covered with macrotrichiae and setae; basal lobe of gonocoxite poorly developed or reduced. Gonostylus simple, almost straight or curved in middle or distal part, in distal half covered with very short setae, on outer edge with or without rounded extension, apically with a tooth and short megaseta.

Comments

After analyzing all the data on adults and DNA barcoding we placed the genus *Shilovia* in the tribe Boreoheptagyiini, so far represented by the genera *Boreoheptagyia* Brundin, *Kaluginia* Makarchenko and *Palatovia* Makarchenko *et* Semenchenko. Main differences between these genera are given in the key below. However, it should be noted that this key is preliminary, since the final revision of the genus *Boreoheptagyia* has not yet been carried out, where it is possible that some species, at least *B. ortladamellica* Rossaro from Italian Alps and *B. unica* Makarchenko from Japan, can be separated into independent genera (Makarchenko 1994; Rossaro 2017).

Key to genera of the tribe Boreoheptagyiini Brundin

Adult males

1. Antenna with 5–13 flagellomeres, covered with long or short setae and with reduced plume. Acrostichals long, beginning near antepronotum and extending to near scutellum; dorsocentrals long, in 1–3 rows or in 2 groups, 1 near scutellum and 1 on humeral area, or with 1 group dorsocentrals reduced to 2–3 setae in anterior position, near to humeral area, in a large white spot. Aedeagal lobe membraneous, sometimes with spines or setae. Gonocoxite simple with flat median field or with strong basal lobe; stemapodeme narrow, rectangular or triangular. Gonostylus with apical megaseta and megasetae sometimes present on inner margin … . . . . . *Boreoheptagyia* Brundin
– Antenna with 5–7 flagellomeres, covered only with short setae and with reduced plume. Acrostichals and dorsocentrals short, arrangement of these setae on the mesonotum is different. Gonocoxite with or without basal lobe; sternapodeme different shape. Aedeagal lobe weakly or strong sclerotized, another shape and with different structure. Gonostylus different shape and structure … 2

2. Scape of antenna with some setae. Antenna with 7 flagellomeres. Acrostichals beginning near antepronotum and reaching middle of mesonotum. Anal-lateral corners of tergite IX mostly elongated back in form of narrow lobes; anal point present and like small rounded protuberance. Gonostylus scoop-shaped; inner lobe along the margin with 5–9 megasetae and one subterminal tooth; outer lobe widely triangular, inner margin of which with strong setae, outer half with thinner and longer setae. Sternapodeme long, almost trapezoidal, with rounded apex (Makarchenko *et al.* 2020a, Figs. 1–9) . . *Kaluginia* Makarchenko

– Scape of antenna bare. Antenna with 5–6 flagellomeres. Acrostichals beginning near antepronotum, extending to near scutellum; dorsocentrals situated only in anterior part of mesonotum in two groups or in 2–4 rows, in anterior part of mesonotum reaching or not reaching of preanal setae, and in posterior part they are converge or not converge with acrostichal setae. Tergite IX different shape, without anal point. Gonostylus and sternapodeme different shape and structure … 3

3. Antenna with 5 flagellomeres. Dorsocentrals situated only in anterior part of mesonotum in two groups: setae of first group anteriorly of humeral pit or just posterior to antepronotum and setae of second group in humeral area, posteriorly of humeral pit. Postnotum densely covered with aculeiform macrotrichia. Gonocoxite with median volsella, which in basal part bears short lobe. Aedeagal lobe strongly sclerotized with a complex structure, that is, as if several details be glued together in the dorso-ventral direction, with rounded lobe in inner side and with nose or beak shaped apex. Gonostylus with wide basal part, gradually tapering to a rounded apex, with several megasetae in subapical part (Makarchenko *et al.* 2020b, Figs. 1–14) … . . . . . *Palatovia* Makarchenko *et* Semenchenko

– Antenna with 5–6 flagellomeres. Dorsocentrals in 2–4 rows, in anterior part of mesonotum reaching or not reaching of preanal setae, and in posterior part they are converge or not converge with acrostichal setae. Postnotum without aculeiform macrotrichia but sometimes with setae (Fig. 13). Gonocoxite without median volsella, with long apical projection ("heel") which densely covered with long and thin setae (Figs. 14, 19–20); this "heel" posterior to gonostylus 0.35–0.63 times as long as gonostylus. Aedeagal lobe weakly sclerotized, apical part hooked or fingelike (Figs. 25–27). Gonostylus simple, almost straight or curved in middle or in distal part, apically with tooth and short megaseta (Figs. 21–23) . . . . . *Shilovia* Makarchenko

Descriptions

Shilovia rara Makarchenko

(Figs. 1–9, 21, 24–25)

Shilovia rara Makarchenko, 1989: 140; Sæther *et al.* 2000: 134, Ashe & O'Connor 2009: 106.

Material examined. 1 adult male (holotype), TAJIKISTAN: Varzod District, Gissar Ridge spurs, Varzob River near Kondara Gorge, 1100 m above sea level, 25.III.1987, N 38°48.677', E 68°49.375', leg. L. Zhiltsova; 2 adult males, KYRGYZSTAN: Tien Shan Mountains, Jalal-Abad Region, Chatkal District, Itelgi River, about 20 km above the mouth on the Chatkal River, under a serpentine road to the Chap-Chyma Pass, altitude 2401 m above sea level, 8.VI.2016, N 41°32.822', E 70°46.459', leg. D. Palatov.

Description

Adult male (n = 3, except when otherwise stated). Total length 1.5–2.5 mm. Total length/wing length 0.94–1.11.

Coloration. Brown to dark-brown. Mesonotum brown to brownish yellow, as shown in Fig. 4. Legs spotted: basal 1/3 or half of femur and tibia yellow, distal 2/3 or 1/2 brown; ta_1–ta_5 brown (Fig. 2). Wings grayish to grey. Abdomen dark brown.

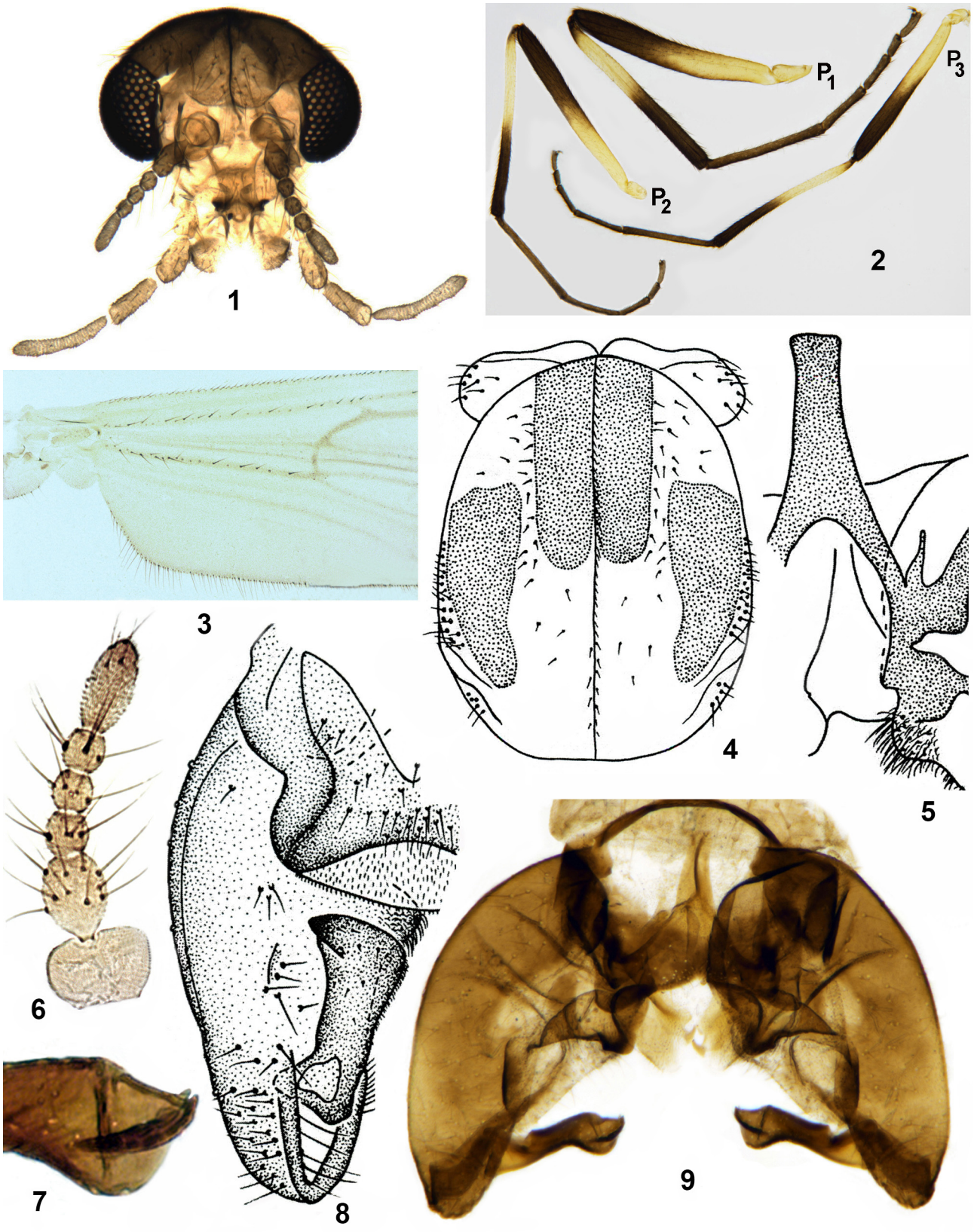
Head. Eyes bare. Temporal setae 37–51, including frontals, verticals, postorbitals which can not be easy separated (Fig. 1). Clypeus with 4–9 setae. Palpomere length (μm): 28–36, 48–52, 68–79, 84–96, 116–148. Head width/palp length 0.98–1.02. Antenna with 5 flagellomeres and reduced plume of setae (Fig. 6); number and length of these setae on 1–4 flagellomeres respectively: 7–10 (44–64 μm), 4–6 (20–72 μm), 4–6 (24–64 μm), 4 (24–68 μm); terminal flagellomere with 2–4 setae, 28–40 μm in subapical and apical area. Flagellomeres 1–5 length (μm): 60, 28–36, 24–30, 24–32, 60–68; AR 0.41–0.44. Antennal length/palp length 0.52–0.63.

Thorax. Antepronotum with 10–12 ventrolateral setae, 44–68 μm long. Acrostichals 19–30, 10–16 μm long, start from anteropronotum, in 1–2 rows. Dorsocentrals 20–22, 16–24 μm long, in 2–3 rows in anterior 1/3, in posterior 2/3 in 1 row and they are not converge with acrostichal setae; prealars 22–30, 40–56 μm long, in 2–3 rows; supraalars 4–6, 32–40 μm long (Fig. 4). Scutellum with 23–53 setae, 64–80 μm long. Postnotum without setae. Preepisternum with 5–15 setae, 32–40 μm long. MAII with 2–5 setae, 30–34 μm long.

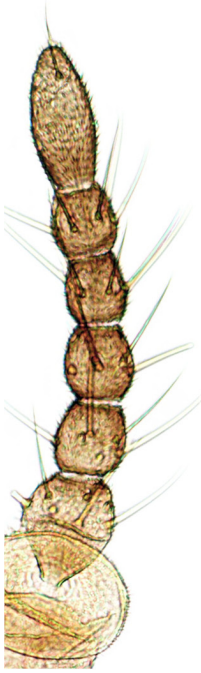
Wing. Length 1.6–2.1 mm, width 0.56–0.60 mm. Costal extension 20–40 μm long. Anal lobe slightly reduced, rounded angular. Squama with 7–8 setae. R and R_1 with 27–38 setae, R_{4+5} with 8–18 setae, R_{2+3} absent, Cu with 6–23 setae (Fig. 3). RM/MCu 2.0–2.2.

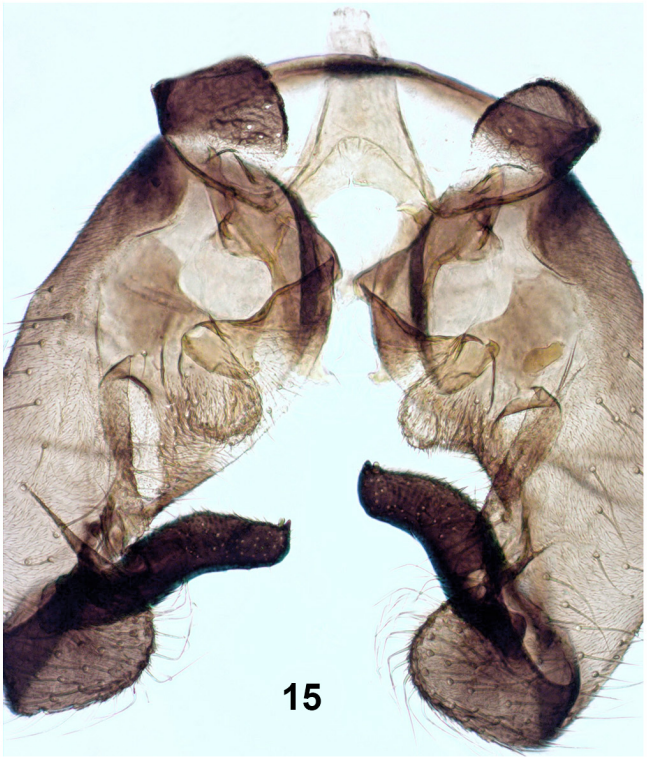
Legs. BR_1 0.9–1.3, BR_2 1.0–1.3, BR_3 1.0–1.1. Spur of front tibia 28–44 μm long. Spurs of mid tibia 32–44 μm

and 32–40 µm long. Spurs of hind tibia 52–63 µm and 40–50 µm long. Hind tibial comb with 6–9 setae. Fore legs with 1–2 pseudospurs, 26–28 µm long on ta_1; middle legs with 2 pseudospurs, 28 µm long on ta_1, hind legs with 2 pseudospurs, 36–40 µm long on ta_1. Length (µm) and proportions of leg segments are as in Table 1.



FIGURES 1–9. *Shilovia rara* Makarchenko, male. **1,** head; **2,** legs; **3,** basal part of wing; **4,** antepronotum and mesonotum, dorsal view; **5,** sternapodeme and inferior volsella; **6,** antenna; **7,** distal part of gonostylus; **8–9,** hypopygium in dorsal view. **1–2, 7, 9,** male from Kyrgyzstan; **3–6, 8,** male from Tajikistan (holotype). P_1– fore leg, P_2 – mid leg, P_3 – hind leg.





FIGURES 10–15. *Shilovia xinhuawangi* **sp. nov.**, male. **10,** basal part of wing; **11,** antenna; **12,** dorsal part of mesonotum; **13,** scutellum and postnotum; **14,** hypopygium in dorsal view; **15,** hypopygium without tergite IX in dorsal view.

Hypopygium (Figs. 5, 7–9, 21, 24–25). Tergite IX with 80–84 setae, 20–32 μm long; posterior edge concave. Laterosternite IX with 3–5 short setae. Sternapodeme long and narrow, 80–82 μm long and 44–48 μm wide in basal part, 26–28 μm wide in subapical part (Fig. 5). Aedeagal lobe weakly sclerotized, apical part hooked (Fig. 25), 100–

118 μm long. Gonocoxite 340–368 μm long, with 44–56 μm long apical projection ("heel") which densely covered with thin setae, 28–40 μm long, and "heel" posterior to gonostylus 0.35–0.44 times as long asgonostylus (Figs. 8–9); inferior volsella in form of tubercle, densely covered with macrotrichiae and setae, 16–24 μm long; basal lobe of gonocoxite as in Fig.24. Gonostylus slightly curved in distal 1/4, 124–128 μm long, in distal half covered with setae, 8–12 μm long, on the outer edge with a rounded extension, apically with a tooth and short megaseta (Figs. 7–9, 21); gonostylus length/gonostylus width 3.1–3.2. HR 2.70–2.72.

Ecology. Adults were collected from stones and bowlders in mountain rivers, located at an altitude of 1100–2401 m, at a flow rate of 0.3–0.6 m/s, with water temperature *ca* 12°C

Distribution. Known from the mountains of Tajikistan (Gissar Range) and Kyrgyzstan (Chatkal District, Tien Shan Mountains) (Figs. 28–29).

TABLE 1. Lengths (in μm) and proportions of leg segments of *Shilovia rara* Makarchenko, male (n=3)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	607-819	590-777	377-525	172-252	115-168	66-84	82-105
P_2	689-945	615-819	312-483	148-231	115-168	66-84	90-105
P_3	722-945	722-882	426-609	213-315	131-210	66-84	82-105

TABLE 1. (continued)

	LR	BV	SV	BR
P_1	0.61-0.68	3.48-3.62	3.04-3.33	0.9-1.3
P_2	0.51-0.60	3.82-3.91	3.65-4.18	1.0-1.3
P_3	0.56-0.69	3.36-3.80	3.0-3.63	1.0-1.3

***Shilovia xinhuawangi* Makarchenko *et* Semenchenko, sp. nov.**
http://zoobank.org/NomenclaturalActs/EFD248D8-FC5F-46E7-938D-A137BAF6071D
(Figs. 10–16, 22, 27)

Type material. Holotype, adult male, CHINA: Xinjiang Uygur Autonomous Region, Tien Shan Mountains, Bogdo-Ula Range, Daong River, altitude 2030 m above sea level, 8.VII.2017, N 43°51.346', E 88°09.633', leg. D. Palatov. Paratypes: 41 adult males, the same data as holotype.

Derivatio nominis. The species is named in honour of the Chinese chironomid taxonomist, Professor of Nankai University Xinhua Wang.

Description

Adult male (n = 6, except when otherwise stated). Total length 2.16–2.36 m. Total length/wing length 0.95–1.07.

Coloration. Brown to dark-brown. Mesonotum dark brown (Fig. 12). Wings grey. Legs: at least basal 3/4 of femur yellowish or yellow, distal 1/4 dark brown; tibia, ta_1–ta_5 dark brown (Fig. 16). Abdomen dark brown.

Head. Eyes pubescent. Temporal setae 70–72, including frontals, verticals, postorbitals which can not be easy separated. Clypeus with 11–12 setae. Palpomere length (μm): 28–32, 68–76, 108–116, 108–124, 180–182. Head width/palpal length 0.88–0.95. Antenna with 6 flagellomeres and reduced plume of setae (Fig. 11); number and length of these setae on 1–5 flagellomeres respectively: 5–7 (40–64 μm), 5–6 (48–72 μm), 3–5 (40–68 μm), 5–6 (40–80 μm); terminal flagellomere with 2–3 setae, 24–40 μm long in subapical and apical area. Length of 1–6 flagellomeres (μm): 52–64, 40, 38–44, 32–40, 32–40, 68–84; AR 0.32–0.37. Antennal length/palpal length 0.54–0.62.

Thorax. Antepronotum with 10–15 ventrolateral setae, 40–60 μm long. Acrostichals 29–46, 16–40 μm long, start from anteropronotum, in 1–2 rows. Dorsocentrals 50–70, 25–48 μm long (Fig. 00), in 2–4 rows, in anterior part of mesonotum reach preanal setae, and in posterior part they converge with acrostichal setae (Fig. 12); prealars 35–84, 44–80 μm long, in 2–3 rows; supraalars 4–7, 36–40 μm long. Scutellum with 48–52 setae, 64–76 μm long. Postnotum with 4–17 setae, 40–52 μm long (Fig. 13). Preepisternum with 14–21 setae, 28–48 μm long. MAII with 2–3 setae, 36 μm long.

Wing. Length 2.16–2.36 mm, width 0.58–0.64 mm. Costal extension 60–70 μm long. Anal lobe slightly re-

duced, rounded-angular. Squama with 12–23 setae, 40–68 μm long, in 1–3 rows. R and R_1 with 46–53 setae, in basal ¼ in 2 rows; R_{4+5} with 9–19 setae, R_{2+3} absent, Cu with 5–14 setae, An with 2–16 setae in basal part (Fig. 10). RM/MCu 1.6–2.0.

Legs. Spur of front tibia 36–64 μm long. Spurs of mid tibia 44–60 μm and 48–64 μm long. Spurs of hind tibia 72–84 μm and 44–68 μm long. Hind tibial comb with 11–13 setae. Fore leg with 2 apical pseudospurs on ta_1, 36–40 μm long; mid leg with 2–5 pseudospurs, 32–36 μm long on ta_1, hind legs with 4–6 pseudospurs,32–40 μm long on ta_1. Length (μm) and proportions of leg segments are as in Table 2.

TABLE 2. Lengths (in μm) and proportions of leg segments of *Shilovia xinhuawangi* **sp. nov.**, male (n=6)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	968-1230	918-984	590-607	295-279	164-180	82-98	115-131
P_2	1148-1246	1017-1115	533-558	197-279	164-189	82-90	115-131
P_3	1164-1279	1132-1197	640-672	344-394	197-312	82	115-131

TABLE 2. (continued)

	LR	BV	SV	BR
P_1	0.54-0.66	3.58-4.21	3.11-3.89	0.9-1.1
P_2	0.50-0.53	4.22-4.84	3.25-4.20	1.0-1.1
P_3	0.54-0.58	3.60-4.19	3.50-3.82	1.0-1.1

Hypopygium (Figs. 14–15, 22, 27). Tergite IX with 69–77 setae, 8–20 μm long; posterior edge almost straight (Fig. 14). Laterosternite IX with 4–5 setae, 20–24 μm long. Sternapodeme length 84–120 μm and 68–92 μm wide in basal part, 24–32 μm wide in subapical part. Aedeagal lobe weakly sclerotized, apical part fingerlike or hooked, 120–124 μm long; phallapodeme sclerotized, 60–68 μm long (Figs.15, 27). Gonocoxite 368–412 μm long, with 68–88 μm long apical projection ("heel") which densely covered with thin setae, 48–60 μm long, and "heel" posterior to gonostylus 0.40–0.63 times as long as gonostylus; inferior volsella in form of tubercle, densely covered with macrotrichiae and setae, 8–16 μm long. Gonostylus strongly curved in distal 2/3, 132–176 μm long, in distal half covered with setae, 5–12 μm long, on the outer edge without rounded extension, apically with yellowish-brown megaseta and dark brown or black tooth (Figs. 14–15, 22); gonostylus length/gonostylus width 2.86–3.67. HR 2.06–2.51.

Diagnosis. See the key below.

Ecology. Adults, pupae and larvae were collected from stones and boulders in mountain river, located at an altitude of 2030 m, at a flow rate of 0.3–0.9 m/s, with water temperatures *ca* 8°C.

Distribution. Known only from the type locality – Bogdo-Ula Range of Tien Shan Mountains (Xinjiang Uygur Autonomous Region, China) (Fig. 30).

Shilovia yakovlevi Makarchenko *et* Semenchenko, sp. nov.

http://zoobank.org/NomenclaturalActs/44D857C4-18D5-4E37-8539-B6AB9EE74BC7

(Figs. 17–20, 23, 26)

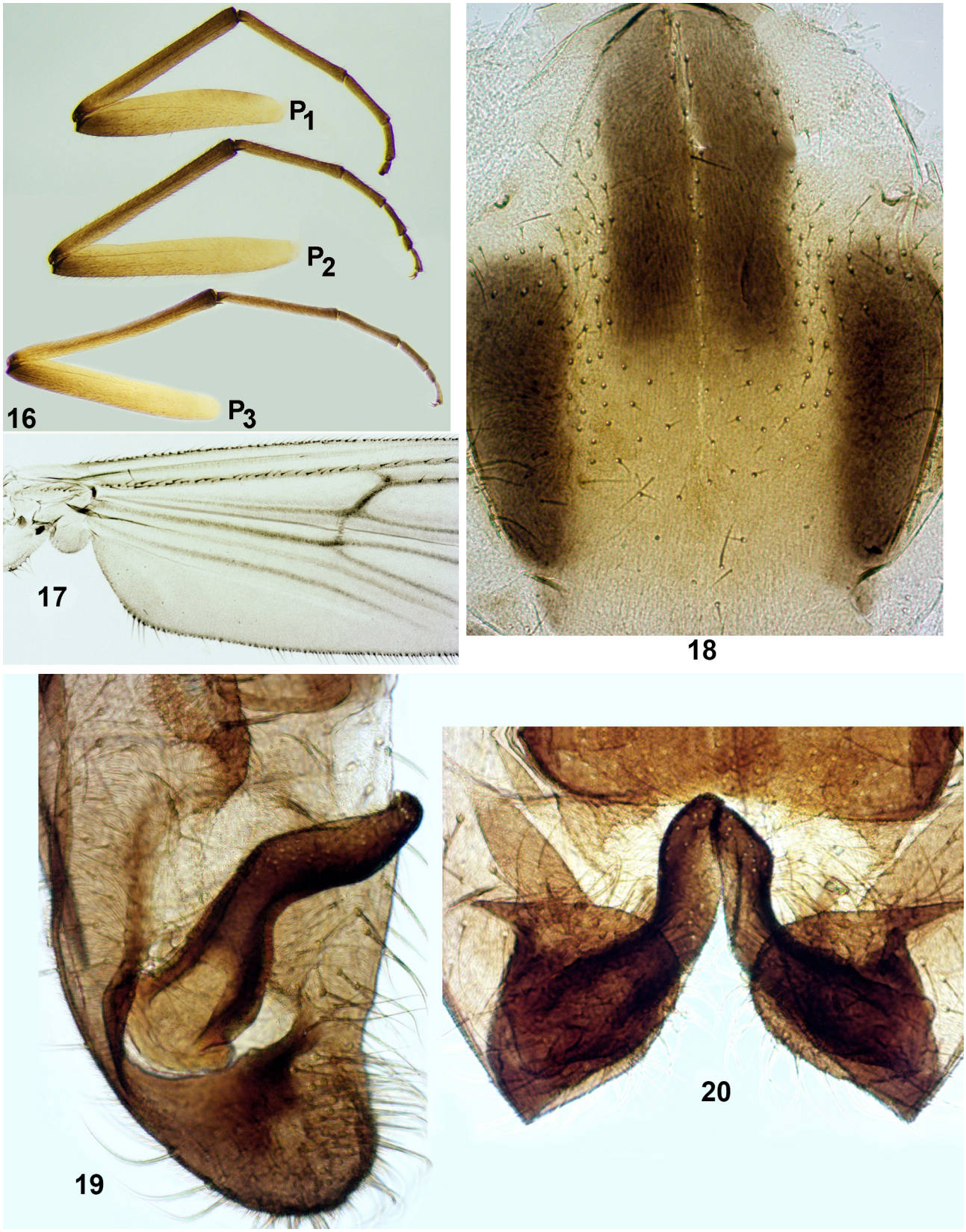
Type material. Holotype, adult male, KAZAKHSTAN: East Kazakhstan Region, Saur-Tarbagatay Mountain system, Zaysan District, Zhemenei River, below the dam in Zaysan Reserve, altitude 648 m above sea level, 13.VII.2018, N 47°27.442', E 84°52.411', leg. D. Palatov. Paratype: 5 adult males, the same data as holotype.

Derivatio nominis. The species is named after the lepidopterist Dr. Yakovlev Roman Viktorovich (Altai State University, Barnaul, Russia), who greatly contributed to the study of the entomofauna of the Altai-Sayan and Central Asian regions and, in particular, the Saur mountains.

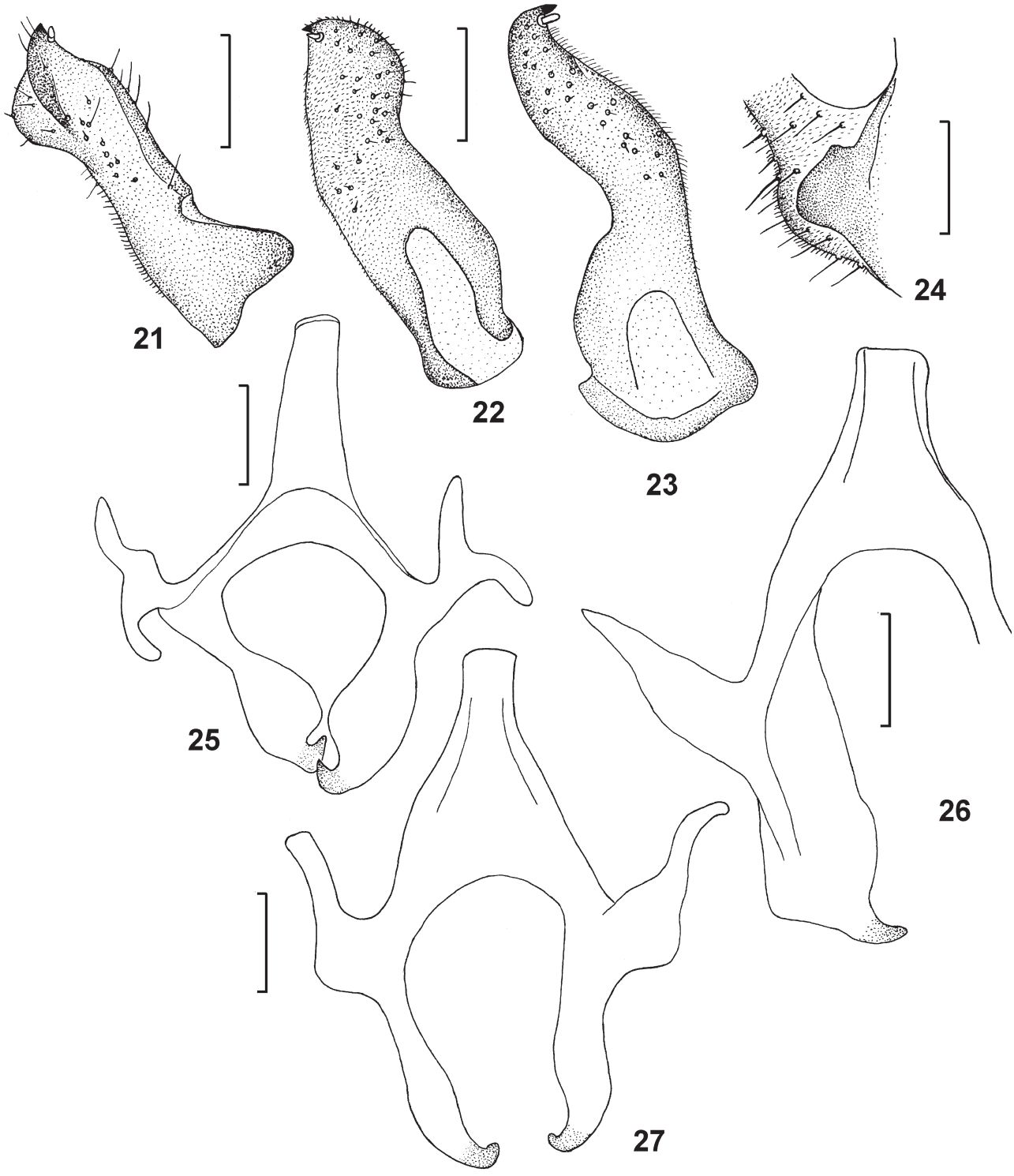
Description

Adult male (n=3, except when otherwise stated). Total length 1.9–2.1 mm. Total length/wing length 0.93–1.06.

Coloration. Brown to dark brown. Mesonotum dark brown in anterior 2/3 and brown to light brown in posterior 1/3; dorsocentrals situated in light stripes (Fig. 18). Scutellun brownish grey. Wings grey. Legs: at least basal 3/4 of femur yellowish or yellow, distal 1/4 dark brown; tibia, ta_1–ta_5 brown to dark brown. Abdomen dark brown.



FIGURES 16–20. *Shilovia xinhuawangi* **sp. nov. (16)** and *S. yakovlevi* **sp. nov. (17–20)**, males. **16,** legs; **17,** basal part of wing; **18,** dorsal part of mesonotum; **19,** gonocoxite and gonostylus; **20,** part of hypopygium in dorsal view. Designations are the same as in Figures 1–9.



FIGURES 21–27. *Shilovia rara* Makarchenko **(21, 24–25)**, *S. xinhuawangi* **sp. nov. (22, 27)** and *S. yakovlevi* **sp. nov. (23, 26)**, males. **21–23,** gonostylus; **24,** inferior volsella and basal lobe of gonocoxite; **25–27,** sternapodeme, phallapodeme and aedeagal lobe. Scale bars: 50 μm.

Head. Eyes bare. Temporal setae 56–60, including frontals, verticals, postorbitals which can not be easy separated. Clypeus with 12–19 setae. Palpomere length (μm): 24–36, 40–52, 76–92, 88–92, 136–156. Head width/palpal length 0.90–1.11. Antenna with 6 flagellomeres and reduced plume of setae; number of setae on 1–5 flagellomeres respectively: 4–5, 5, 5–6, 5, 5–6; length of setae on 1–5 flagellomeres 48–56 μm; terminal flagellomere with 2–3

setae, 24–28 μm long in subapical and apical area. Length of 1–6 flagellomeres (μm): 48–52, 32, 28–36, 28–38, 32–34, 6–84; AR 0.39–0.44. Antennal length/palpal length 0.61–0.71.

FIGURES 28–31. Type localities of *Shilovia rara* Makarchenko **(28–29)**, *S. xinhuawangi* **sp. nov. (30)**, and *S. yakovlevi* **sp. nov. (31)**. **28,** Varzob River near Kondara Gorge, Varzod District, Tajikistan (photo by M.V. Vinarski); **29,** Itelgi River, Chatkal District, Kyrgyzstan, (photo by E.S. Chertoprud); **30,** Daong River, Xinjiang Uygur Autonomous Region, China (photo by M.V. Vinarski); **31,** Zhemenei River, East Kazakhstan Region, Kazakhstan (photo by M.V. Vinarski).

Thorax. Antepronotum with 6–10 ventrolateral setae, 40–50 μm long. Acrostichals 30–56, 20–28 μm long, start from anteropronotum, in 1–3 rows. Dorsocentrals 46–71,12–24 μm long , in 2–4 rows, in anterior part of mesonotum reach preanal setae, and in posterior part they are not converge with acrostichal setae (Fig. 18); prealars 30–34, 40–64 μm long, in 2–3 rows; supraalars 3, 30–40 μm long. Scutellum with 40–51 setae, 72–80 μm long. Postnotum without setae. Preepisternum with 9–13 setae, 28–40 μm long. MAII with 2–3 setae, *ca* 32 μm long.

Wing. Length 1.76–2.24 mm, width 0.52–0.56 mm. Costal extension 40–48 μm long. Anal lobe slightly reduced, rounded-angular. Squama with 5–15 setae, 44–60 μm long. R and R_1 with 36–50 setae; R_{4+5} with 8–10 setae, R_{2+3} absent, Cu and An without setae (Fig. 17). RM/MCu 1.7–2.0.

Legs. Spur of front tibia 48–52 μm long. Two spurs of mid tibia 56–60 μm long. Spurs of hind tibia 72 μm and 60–64 μm long. Hind tibial comb with 10–11 setae. Fore leg with 2 pseudospurs on ta_1, 28–30 μm long; mid leg with 2 pseudospurs, 24–28 μm long on ta_1; hind legs with 2–5 pseudospurs 28–36 μm long on ta_1. Length (μm) and proportions of leg segments are as in Table 3.

TABLE 3. Lengths (in μm) and proportions of leg segments of *Shilovia yakovlevi* **sp. nov.**, male (n=3)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	738-918	672-902	435-558	213-279	148-180	66-82	98-115
P_2	853-1099	820-984	410-508	197-238	131-180	66-82	98-115
P_3	877-1132	836-1082	492-607	262-344	164-205	66-74	107-115

TABLE 3. (continued)

	LR	BV	SV	BR
P_1	0.60-0.65	3.51-3.71	3.15-3.36	0.9-1.0
P_2	0.50-0.54	4.11-4.44	4.0-4.10	1.0-1.1
P_3	0.55-0.59	3.68-3.86	3.48-3.68	1.0-1.1

Hypopygium (Figs. 19–20, 23, 26). Tergite IX with 49–56 setae, 10–12 μm long; posterior edge almost straight or slightly concave (Fig. 20). Laterosternite IX with 3–6 setae, 10–14 μm long Sternapodeme length 80–84 μm and 36–80 μm wide in basal part, 16–28 μm wide in subapical part. Aedeagal lobe weakly sclerotized, apical part fingerlike, 96–100 μm long; phallapodeme sclerotized, 72–76 μm long (Fig. 26). Gonocoxite 320–380 μm long, with 80–100 μm long apical projection ("heel") which densely covered with thin setae, 35–52 μm long, and "heel" posterior to gonostylus 0.45–0.54 times as long as gonostylus; inferior volsella in form of tubercle, densely covered with macrotrichiae and setae, 16–24 μm long (Fig. 19–20). Gonostylus wide in basal part, in middle part strongly curved, 164–188 μm long, in distal half covered with setae, 6–12 μm long, on the outer edge without rounded extension, apically with yellowish-brown megaseta 8 μm long and dark brown or black tooth (Figs. 19–20, 23); gonostylus length/gonostylus width 5.22–7.20. HR 2.0–2.13.

Diagnosis. See the key below.

Ecology. Adults, pupae and larvae were collected from stones and bowlders in mountain river, located at an altitude of 648 m, at a flow rate of 1.0–1.2 m/s.

Distribution. Known only from the type locality – Saur Mountains of East Kazakhstan (Fig. 31).

Key to the known species of *Shilovia* Makarchenko

Males

1. Antenna with 5 flagellomeres. Legs spotted: basal 1/3 or half of femur and tibia yellow, distal 2/3 or half brown; ta_1–ta_5 brown (Fig. 2). Dorsocentrals in anterior part of mesonotum not reach preanal setae (Fig. 4). Posterior margin of tergite IX concave; gonostylus in subapical part along outer edge with rounded extension; inferior volsella and basal lobe of gonocoxite present; sternapodeme long and narrow (Figs. 5, 7–9, 21, 24–25) … . . . . . *Shilovia rara* Makarchenko

– Antenna with 6 flagellomeres. Legs not spotted: at least basal 3/4 of femur yellowish or yellow, distal 1/4 dark brown; tibia, ta_1–ta_5 dark brown (Fig. 16). Dorsocentrals in anterior part of mesonotum reach preanal setae (Fig. 18). Posterior margin of tergite IX straight or slightly concave; gonostylus another shape, in subapical part along outer edge without rounded extension;

basal lobe of gonocoxite reduced; sternapodeme wide basally and narrow distally (Figs. 14–15, 19–20, 22–23, 26–27) 2

2. Dorsocentrals in posterior part converge with acrostichal setae (Fig. 12). Postnotum with setae (Fig. 13). Wing with setae on Cu and An (Fig. 10). Gonostylus length/gonostylus width 2.86–3.67 . . . . . *Shilovia xinhuawangi* **sp. nov.**

– Dorsocentrals in posterior part not converge with acrostichal setae (Fig. 18). Postnotum, Cu and An of wing without setae (Fig. 17). Gonostylus length/gonostylus width 5.22–7.20 . . . . . *Shilovia yakovlevi* **sp. nov.**

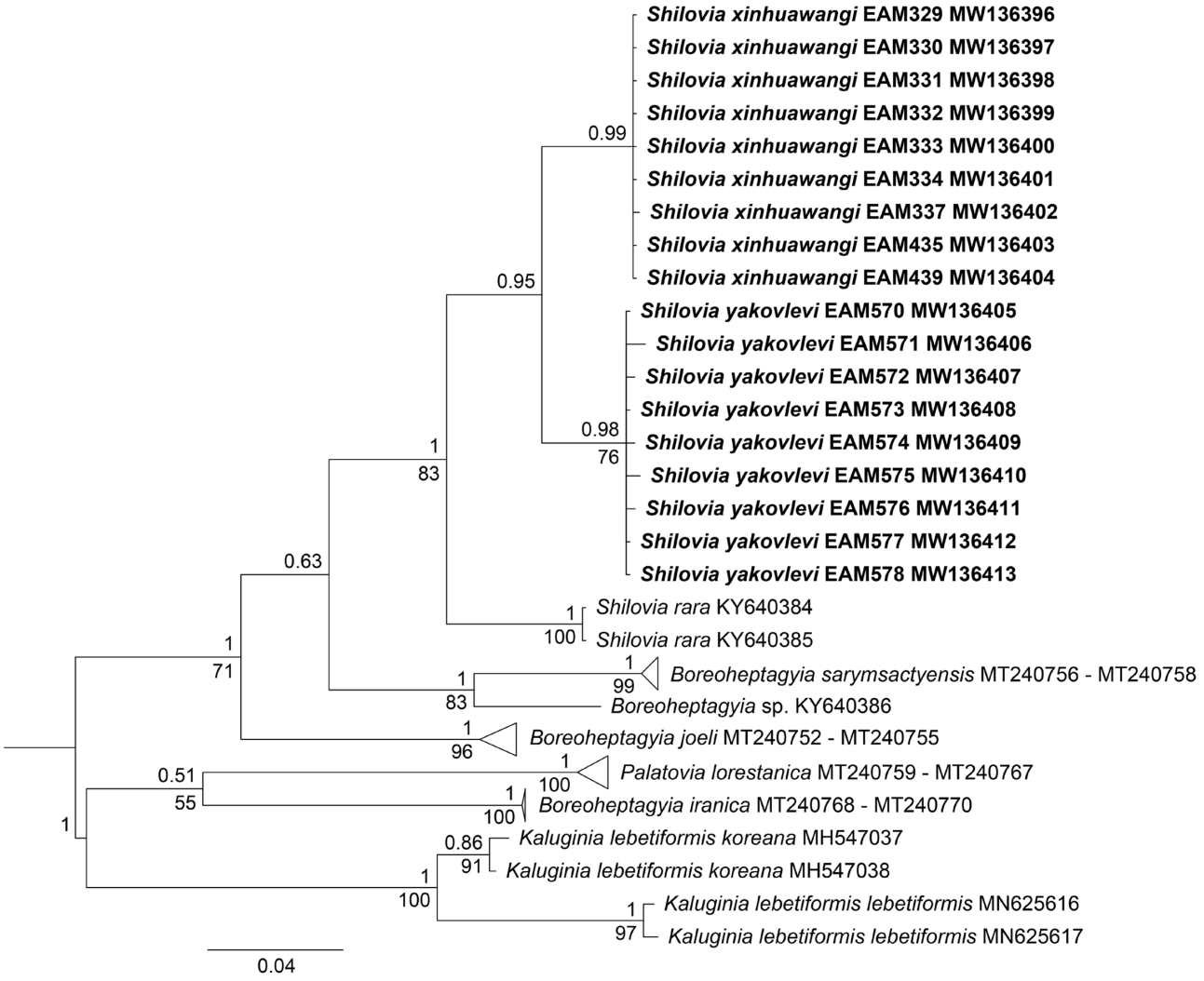


FIGURE 32. Bayesian tree based on mitochondrion COI gene for available members of the tribe Boreoheptagyiini. Bayesian posterior probabilities (PP) are given above tree nodes and bootstrap support values found in the ML analysis are shown below nodes. Specimens obtained in this study are in bold.

Results of DNA barcoding

A total of 9 specimens of each new species were sequenced. The final alignment of the COI barcode region yielded 658 base pairs. The total pairwise K2P distance within *S. xinhuawangi* **sp. nov.** ranged from 0.000% to 0.152% (average 0.03%), which is based on one synonymous nucleotide substitution. The values of the K2P intraspecific distances for the *S. yakovlevi* **sp. nov.** were significantly higher – from 0.000% to 0.920% (average 0.306%), which is based on seven synonymous and two nonsynonymous nucleotide substitutions.

The average interspecific K2P distance (COI) between *S. xinhuawangi* **sp. nov.** and *S. yakovlevi* **sp. nov.** was 4.464%. Each species was different from *S. rara* (KY640384 – KY640385) at 7.573% and 6.289% respectively (Fig. 32). The high differences between three *Shilovia* species were also confirmed by ABGD analysis, which yielded 3 operational taxonomic units (OTU) using a 0.0046–0.0359 intraspecific divergence. We combined the COI sequences of three species of genus *Shilovia* to compute the intergeneric distances within the tribe Boreoheptagyiini. A comparison of the genus *Shilovia* with other genera of the tribe Boreoheptagyiini yielded the following

results: *Kaluginia* (MN625616 – MN625617, MH547037 - MH547038) — 13.30%, *Boreoheptagyia* (MT240768 – MT240770, MT240752 –MT240755, MT240756 – MT240758, KY640386) — 11.32% and *Palatovia* (MT240759 – MT240767) – 14.94%.

We used COI to reconstruct phylogenetics relationships of the tribe Boreoheptagyiini. Bayesian inference and Maximum Likelihood trees had a nearly similar topology, so we present only Bayesian tree with posterior probability (PP) above nodes, including bootstrap support for ML tree under nodes. The phylogenetics tree has two primary clades. First clade was strongly supported (PP = 1.00) and includes genera *Kaluginia, Palatovia* and *Boreoheptagyia iranica* Makarchenko. The second clade (PP = 1.00, ML = 71) formed by remaining *Boreoheptagyia species* and three *Shilovia* species. Thus, phylogenetic analysis revealed polyphyly of the genus *Boreoheptagyia.* Further studies using a multilocus approach will increase the support for identified nodes and allow us to analyze the identified polyphyly (Makarchenko *et al.* 2020b). However, clade *Shilovia* was monophyletic. *S. rara* was the earliest branching lineage (PP = 1.00, ML = 83) after which the two sister clades, namely, a well-supported clade (PP = 0.95) uniting *S. xinhuawangi* **sp. nov.** and *S. yakovlevi* **sp. nov.**

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