



Taxonomy of some Boreoheptagiini Brundin (Diptera: Chironomidae: Diamesinae) from the mountains of Central Asia and the Middle East, with description and DNA barcoding of new taxa

EUGENYI A. MAKARCHENKO^{1,4}, ALEXANDER A. SEMENCHENKO² & DMITRY M. PALATOV³

¹Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, 100 let Vladivostoku 159, 690022 Vladivostok, Russia,

^{1,4}✉ makarchenko@biosoil.ru; <http://orcid.org/0000-0003-2765-8729>

²Laboratory of Ecology and Evolutionary Biology of Aquatic Organisms, Far Eastern Federal University, Suhanova St. 8, 690950 Vladivostok, Russia.

²✉ semenchenko_alexander@mail.ru; <https://orcid.org/0000-0001-7207-9529>

³A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Leninskij prosp. 33, 119071 Moscow, Russia,

³✉ triops@yandex.ru; <https://orcid.org/0000-0002-8826-9316>

⁴Corresponding author

Abstract

Chironomids of the tribe Boreoheptagiini from the mountains of Iran, China and East Kazakhstan are revised using both morphological characters and partial DNA sequences of the mitochondrial cytochrome c oxidase subunit I gene. Using adult males, *Palatovia lorestanica* **gen. nov. sp. nov.**, as well as *Boreoheptagyia iranica* **sp. nov.** both from Iran (Lorestan Province, Zagros Mountains), *B. joeli* **sp. nov.** from China (Tien Shan Mountains), and *B. sarymsactyensis* **sp. nov.** from Eastern Kazakhstan (Kazakh Mountain Altai), are described and figured. A brief redescription of the rare species *B. brevitarsis* (Tokunaga) from Iran (Mazandaran Province), previously known only from Japan, is also given. The DNA barcoding analysis shows well-supported genetic divergence between all studied taxa (four species of the genus *Boreoheptagyia* and one of *Palatovia*). Combining DNA barcodes obtained in this study with available sequences in GenBank, the phylogenetic relationships of the tribe Boreoheptagiini are reconstructed. In the resulting Bayesian and maximum likelihood (ML) tree the polyphyly of the genus *Boreoheptagyia* is recognized. *B. iranica* is placed as a sister species to *P. lorestanica*, despite the lack of confirmation of their morphological similarity.

Key words: Diptera, Chironomidae, Boreoheptagiini, taxonomy, DNA barcoding, phylogeny, Central Asia and Iran.

Introduction

The tribe Boreoheptagiini, with the single genus *Boreoheptagyia*, was erected by Brundin (1966) for the Holarctic species of the genus *Heptagyia* Philippi. *Boreoheptagyia* presently includes 26 species, with the only *B. lurida* (Garrett) from the Nearctic region (Oliver *et al.* 1990; Ashe & O'Connor 2009) and the majority of species recorded from the Palaearctic region (Serra-Tosio 1983, 1989; Makarchenko *et al.* 2008; Ashe & O'Connor 2009; Rossaro 2017). Many mountainous regions of Central Asia and the Middle East remain, however, poorly studied, and representatives of the tribe Boreoheptagiini were not known for Iran's chironomid fauna (Aydin & Samin 2020).

In the process of revising the known species of the genus *Boreoheptagyia* Brundin, which we started several years ago, as well as analyzing a number of closely related genera of Diamesinae, we concluded that the tribe Boreoheptagiini should be expanded to include at least four genera, namely *Boreoheptagyia*, *Kaluginia* Makarchenko, 1987, *Shilovia* Makarchenko, 1989, and *Palatovia* **gen. nov.** (Makarchenko *et al.* 2017, 2018, 2020).

This article is devoted to the description of the adult males of *Palatovia* **gen. nov.** with its single species *P. lorestanica* **sp. nov.** from Iran (Lorestan Province, Zagros Mountains), as well as descriptions of *Boreoheptagyia iranica* **sp. nov.** from Iran (Lorestan Province, Zagros Mountains), *B. joeli* **sp. nov.** from China (Tien Shan Mountains), and *B. sarymsactyensis* **sp. nov.** from Eastern Kazakhstan (Kazakh Mountain Altai), plus a brief redescription of the rare species *B. brevitarsis* (Tokunaga) from Iran (Mazandaran Province), previously known only from Japan.

In this study, we utilize both morphological data and DNA sequences of the mitochondrial cytochrome c oxidase subunit I gene (COI) to determine species delimitation, and we reconstruct phylogenetic relationships from all available molecular data of the tribe Boreoheptagiini. Standardized mitochondrial fragments from COI (DNA barcodes) have been widely used for the subfamily Diamesinae (Carew *et al.* 2005; Montagna *et al.* 2016) as well as for the tribe Boreoheptagiini (Makarchenko *et al.*, 2018, 2020).

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. 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).

Total DNA was extracted from thorax using Qiagen DNeasy Blood and Tissue Kit following manufacturer's protocol with elution in 100 µl of TE buffer. The cytochrome c oxidase I (COI) barcoding fragment was amplified using Folmer *et al.*'s (1994) 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. PCR amplification was carried out on a ProFlex PCR System (Applied Biosystems, Foster City, USA) using the following conditions: a 3 min denaturing step at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 50°C, and 60 s at 72°C, and a subsequent 10-min final extension 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). The amplified products were purified with Exonuclease I and Thermo-sensitive Alkaline Phosphatase, ThermoFisher Scientific, Waltham, MA, USA, and sequenced for both directions. Sequencing reaction was performed using BigDye® Terminator v3.1 Cycle Sequencing Kits and run on an ABI 3130xl Genetic Analyzer Sequencer (Applied Biosystems, Foster City, CA, USA). Forward and reverse sequences were manually assembled and edited using Finch TV and MEGA 7 (Kumar *et al.*, 2016).

Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI) analyses. 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. The best fit models for three codon positions was SYM+G (Zharkikh 1994), HKY+I (Hasegawa *et al.*, 1985) and HKY+G respectively. The ML tree was constructed RAxML v. 8.2.4 using bootstrapping with 1000 replications (Stamatakis 2006). We performed Bayesian analyses with v. 3.2.7 (Ronquist & Huelsenbeck 2003). The four independent chains (one cold and three heated) were run for 5 million generations and sampled every 100 generations. The samples prior to reaching stationary (1,000 trees) were discarded as burn-in and the remaining trees were then used to generate a final tree. 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 obtained sequences have been deposited in GenBank under numbers MT240752 - MT240776.

Results

Genus *Palatovia* Makarchenko *et* Semenenko, gen. nov.

<http://zoobank.org/NomenclaturalActs/D0EF36FA-7C56-430A-BE8B-F95EEF135747>

Type species: *Palatovia lorestanica* Makarchenko *et* Semenenko, sp. nov., by present designation.

Derivatio nominis. The genus is named in honour of Dr. Dmitry M. Palatov from A.N. Severtsov Institute of Ecol-

ogy and Evolution of the Russian Academy of Sciences (Moscow), who collected type material of a new genus and species in Iran mountains as well as other very interesting Diamesinae material in mountains of Caucasus, Central Asia and the Middle East.

Description

Adult male. Small species up to 2.5 mm long, with wing length up to 1.8 mm.

Antenna. Scape and pedicel bare. Flagellum with 5 flagellomeres and reduced plume of short setae, which on flagellomeres 1–4 not more than 7 (Fig. 10); flagellomeres 1–4 and terminal flagellomere with sensilla chaetica; terminal flagellomere with 1–3 subapical setae. Antennal ratio less than 0.50.

Head. Eye bare, not extended dorsomedially. Temporal setae numerous, consisting of inner and outer verticals, frontals, orbitals and postorbitals but some groups of setae are very difficult to be separated. Vertex without of frontal tubercles. Tentorium more swollen in basal half than in distal part; microtrichia absent. Cornua of cibarial pump pointed. Palp with 5 palpomeres; palpomere 3 without sensilla capitata.

Thorax. Anteprenotal lobes in contact to widely separated medially; anteprenotals present in ventrolateral part and situated in basal 2/3 of lobe (Fig. 1). Scutum broad, low, not extended over anteprenotal notch, and covered with macrotrichia. Acrostichals short, in one row, beginning near anteprenotum, extending and sometimes diverging to near scutellum; dorsocentrals short and sparse, situated in two groups only in anterior part of mesonotum: setae of first group in humeral area, posteriorly of humeral pit and setae of second group anteriorly of humeral pit or just posterior to anteprenotum (Fig. 2); prealars middle size, in two groups; scutellum covered with numerous setae; some supraalars and preepisternals present. Postnotum densely covered with aculeiform macrotrichia.

Wing. Gray or dark grey. Membrane without setae, covered with well visible microtrichia. 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. Squama fully fringed.

Legs. Tibiae without light coloured rings; spurs comparatively short, usually not longer than width of tibial apex. Fore, mid and hind legs with pseudospurs on tarsomere 1; tarsomere 4 cylindrical, little shorter than tarsomere 5. Claws pointed apically; pulvilli as small spinules.

Hypopygium. Tergite IX almost rectangular or in the form of an inverted trapezoid, without anal point and with numerous middle size pale setae; laterosternite IX with rounded and slightly protruding posterolateral lobes which with some setae. Aedeagal lobe strongly sclerotized with a complex structure, that is, as if several details be glued together in a dorso-ventral direction, with rounded lobe on inner side and with nose or beak shaped apex (Figs. 3–4, 7–8, 12). Gonocoxite with median volsella, which in basal part bears a short lobe (Figs. 3–4, 7–8); basimedial setal cluster and basal plate absent. Transverse sternapodeme narrow and triangular, with rounded apex (Fig. 12). Gonostylus with wide basal part, gradually tapering to a rounded apex, with long setae along the outer and inner edges and several megasetae in subapical part (Figs. 5–6, 14).

Taxonomic notes. *Palatovia* is well separated from other genera of Boreoheptagyini, namely *Boreoheptagyia*, *Kaluginia* and *Shilovia*, by a complex of features in adult males. Antenna with 5 flagellomeres and reduced plume of short setae, AR 0.39–0.49. Anteprenotals present in ventrolateral part and situated in basal 2/3 of lobe (Fig. 1). Acrostichals short, in one row, beginning near anteprenotum, extending and sometimes diverging to near scutellum; dorsocentrals short, situated only in anterior part of mesonotum in two groups: setae of first group anteriorly of humeral pit or just posterior to anteprenotum and setae of second group in humeral area, posteriorly of humeral pit (Fig. 2). Postnotum densely covered with aculeiform macrotrichia. R_{2+3} absent. Tergite IX almost rectangular or in the form of an inverted trapezoid, without anal point; laterosternite IX with rounded and slightly protruding posterolateral (Fig. 4). 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 (Figs. 3–4, 7–8, 12). Gonocoxite with median volsella, which in basal part bears a short lobe, basal plate absent (Figs. 3–4, 7–8). Gonostylus with wide basal part, gradually tapering to a rounded apex, with several megasetae in subapical part (Figs. 5–6, 14).

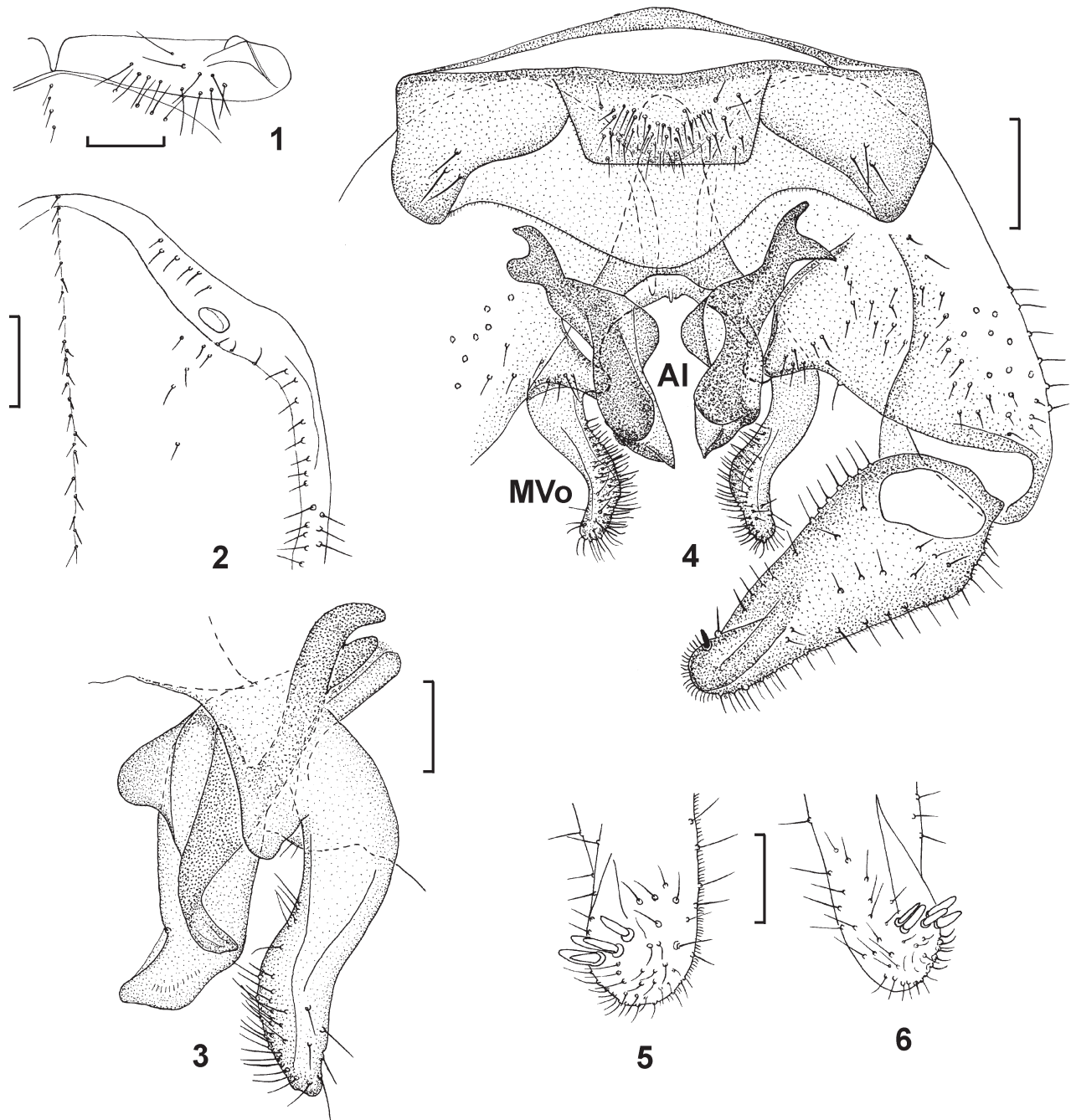
Palatovia lorestanica Makarchenko et Semenchko, sp. nov.

(Figs. 1–14)

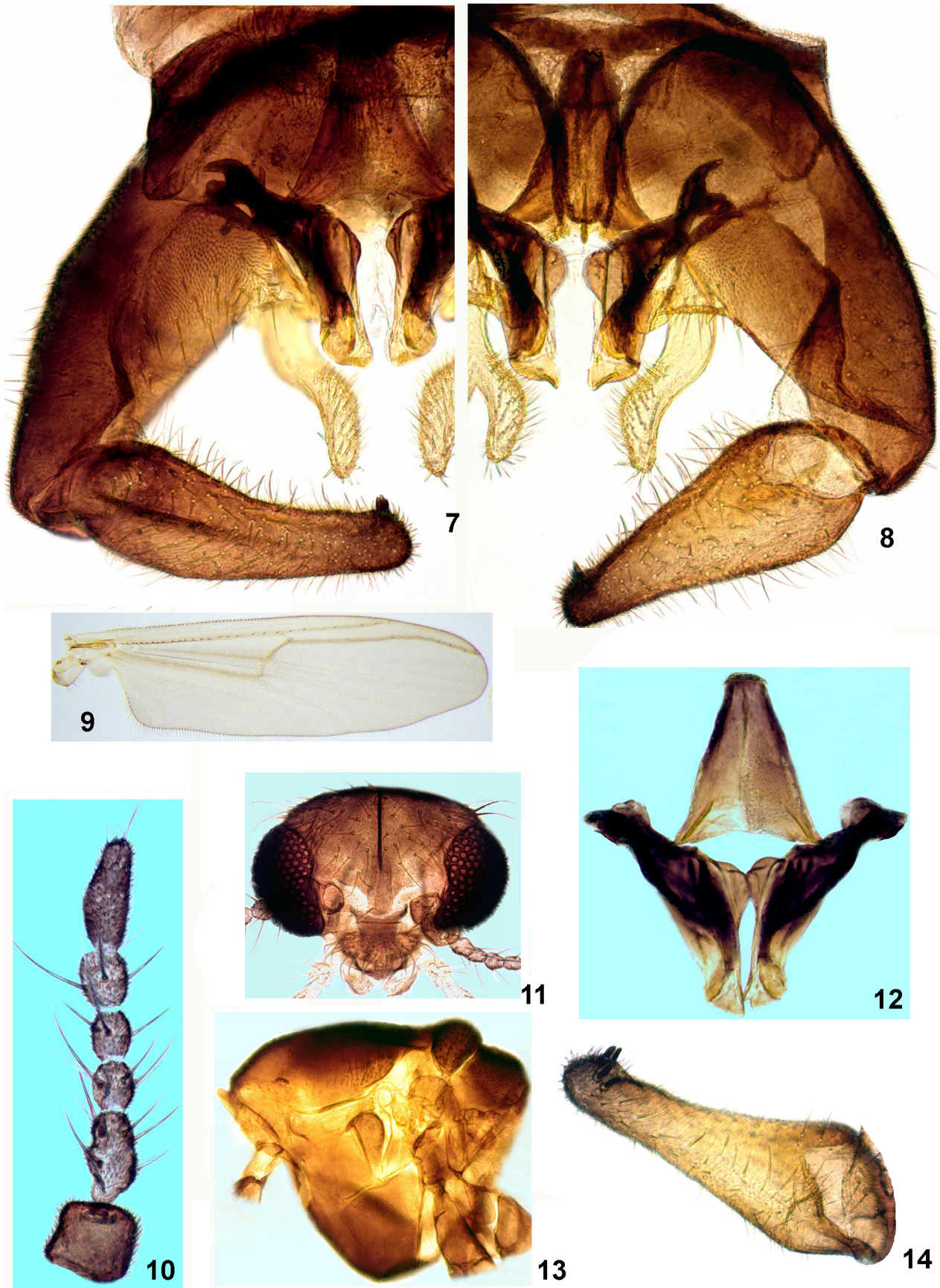
<http://zoobank.org/NomenclaturalActs/3F6E44FF-8662-4D53-A713-C01236023071>

Type material. Holotype, adult male, IRAN: Lorestan Province, Zagros Mountains, Tenge-Kholeven River upper of Cham Chit Village, alt. 1338 m above sea level, N 33°22.737', E 48°58.414', 29.VI.2019, leg. D. Palatov. Paratypes: 24 males, the same data as holotype; 5 males, the same data as holotype, excepted Dez (Sezar) River, 1 km to North of Sepid Dasht Town, alt. 1022 m above sea level, N 33°13.567', E 48°53.140' 13.VI.2019, leg. D. Palatov.

Derivatio nominis. The species is named as *lorestanica* after the type locality in mountains of Lorestan Province of Iran.



FIGURES 1–6. *Palatovia lorestanica* sp. nov., male. 1, lobe of pronotum (straightened); 2, anterior part of mesonotum in dorsal view; 3, median volsella and aedeagal lobe in ventral view; 4, hypopygium in dorsal view; 5–6, subapical part of gonostylus. MVo—median volsella; Al—aedeagal lobe. Scale bars: Figs. 1, 4—100 μ m; Fig. 2—200 μ m; Figs. 3, 5–6—50 μ m.



FIGURES 7–14. *Palatovia lorestanica* **sp. nov.**, male. 7, hypopygium in dorsal view; 8, hypopygium in ventral view; 9, wing; 10, antenna; 11, head; 12, transverse sternapodema and aedeagal lobes; 13, thorax in lateral view; 14, gonostylus in ventral view.

Description

Adult male (n = 6, except when otherwise stated). Total length 2.2–2.6 mm. Total length/wing length 1.0–1.8.

Coloration. Dark brown. Anteprepronotum brown or brownish-yellow. Mesonotum mostly dark brown, in some places brown of light brown (Fig. 13). Wings grey to dark grey.

Head. Eyes bare and reniform. Temporal setae 22–26, including frontals, verticals, postorbitals which can not be easily separated (Fig. 11). Clypeus with 16–29 setae. Palpomere length (μm): 28–44, 52–68, 100–124, 80–88, 100–144. Head width/palp length 1.08–1.25. Antenna with 5 flagellomeres and reduced plume of setae 60–80 μm long (Fig. 10); number of setae in flagellomeres 1–4, respectively 6–7 : 4–6 : 3–4 : 3–4. Flagellomeres 1–5 length (μm): 64–76, 24–36, 30–36, 32–38, 68–86; terminal flagellomere with 1–3 subapical setae, 40–64 μm long. AR 0.39–0.49. Antennal length/palp length 0.59–0.64.

Thorax. Anteprepronotum with 11–18 ventrolateral setae, 44–84 μm long, which situated in basal 2/3 of lobe of lobe (Fig. 1). Acrostichals 20–39, 20–68 μm long. Dorsocentrals in two groups: 8–12 setae, 12–40 μm long, before or in humeral area, and 5 setae, 48–80 μm long in posterior part after humeral area (Fig. 2); prealars in two groups: 9–11 setae, 16–44 μm long in 1–2 rows in anterior part and 16–22 setae, 48–52 μm long, in 2–3 rows in posterior part (Fig. 13). Supraalars 4–5, 8–28 μm long. Scutellum with about 60 setae, 80–88 μm long. Preepisternum with 8 setae, 28–32 μm long.

Wing. Length 2.3–2.6 mm, width 0.64–0.72 mm. Costal extension 20–60 μm long. Anal lobe slightly reduced. angular or rounded angular (Fig. 9). Squama with 7–10 setae. R and R₁ with 9–29 setae, R₄₊₅ with 1–7 setae in distal part, R₂₊₃ absent. RM/MCu 2.0.

Legs. BR₁ 0.9–1.8, BR₂ 1.1–2.7, BR₃ 1.3–1.7. Spur of front tibia 44–48 μm long. Spurs of mid tibia 40–44 μm and 44–48 μm long. Spurs of hind tibia 53–54 μm and 44–48 μm long. Hind tibial comb with 6–8 setae. Fore legs with 2 pseudospurs, 26–28 μm long on ta₁, middle legs with 2–5 pseudospurs, 28 μm long on ta₁, hind legs with 5–9 pseudospurs, 28 μm long on ta₁. Length (μm) and proportions of leg segments are as in Table 1.

TABLE 1. Lengths (in μm) and proportions of leg segments of *Palatovia lorestanica* sp. nov., male (n=5)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
P ₁	1033-1164	1000-1132	656-738	279-328	180-197	115-131	148-164	0.63-0.66	3.65-3.86	3.07-3.20	0.9-1.8
P ₂	1181-1328	1017-1197	607-656	238-279	164-180	66-131	131-148	0.53-0.60	3.81-4.69	3.62-3.82	1.0-1.7
P ₃	1246-1492	1164-1312	722-820	361-426	197-221	131-148	148-156	0.59-0.63	3.59-3.91	3.34-3.57	1.2-1.6

Hypopygium (Figs. 3–8, 12, 14). Tergite IX with 52–54 pale yellow setae, 45–60 μm long. Laterosternite IX with 3–8 setae, 40–44 μm long. Transverse sternapodeme length 172–180 μm and 100–124 μm wide in basal part, 48–60 μm wide in subapical part. Aedeagal lobe strongly sclerotized, 220–236 μm long; rounded lobe in inner side 60–64 μm long and 24–32 μm height. Gonocoxite 476 μm long; median volsella yellow, slightly curved, 148–212 μm long and 32–36 μm wide, with basal lobe 36–60 μm long and covered in inner side and in subapical part with pale setae, 20–32 μm long. Gonostylus 316–328 μm long, covered with setae, 32–60 μm long, in subapical part with 4–5 megasetae, 16–20 μm long. HR 1.45–1.51.

Diagnostic characters. See diagnostic characters of the genus.

Ecology. Adults, pupae and larvae were collected from stones and boulders in mountain rivers, located at an altitude of 1022–1344 m, at a flow rate of 0.4–0.9 m/s (Fig. 47).

Boreoheptagyia joeli Makarchenko, sp. nov.

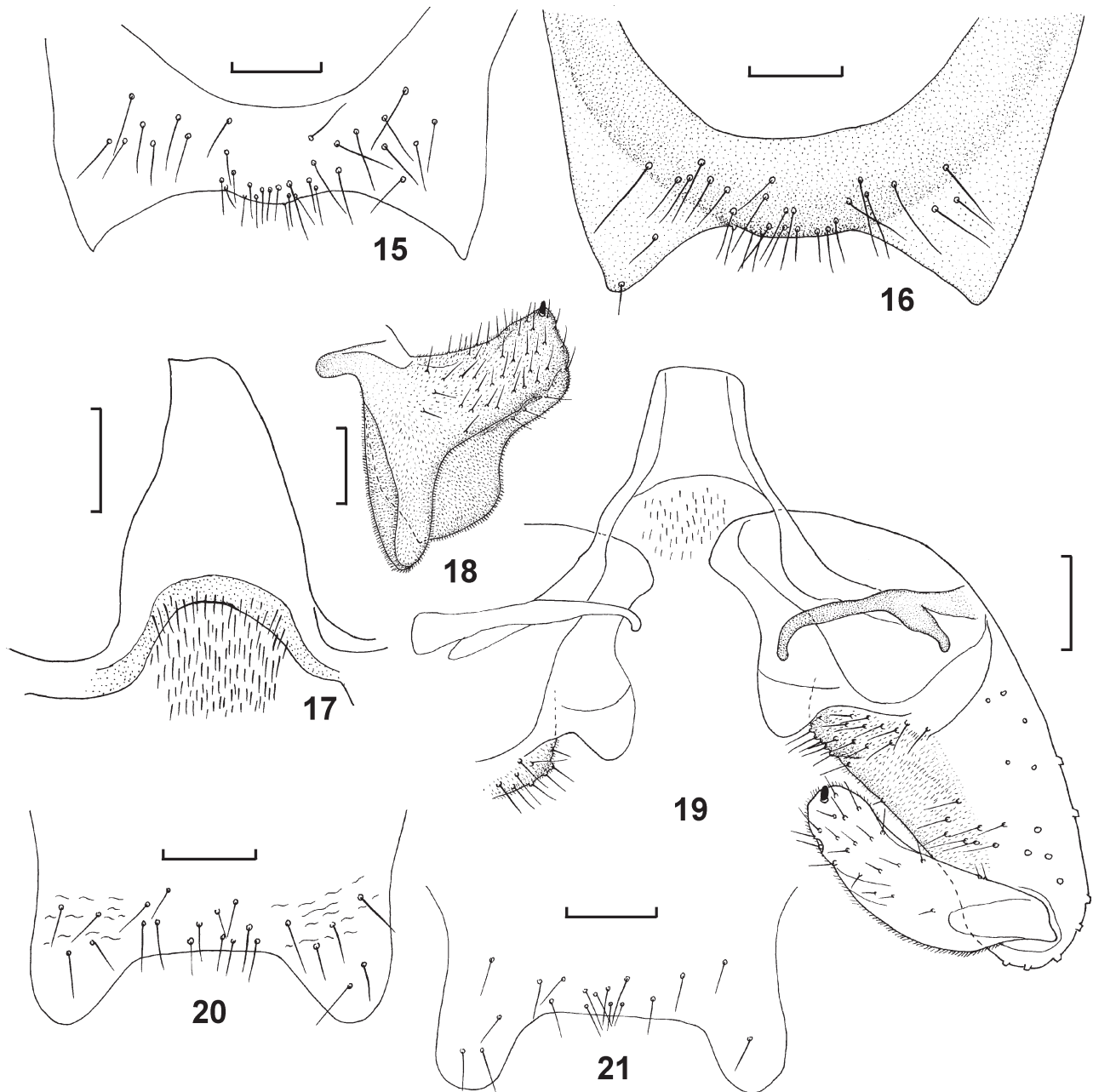
(Figs. 15–18, 22–26)

<http://zoobank.org/NomenclaturalActs/ADC321EA-4997-4F69-B13D-D3D5558FE79B>

Type material. Holotype, adult male, CHINA: Xinjiang Uygur Autonomous Region, Tien Shan Mountains, Bogdo-Ula Range, the headwaters of the Zienzan River at the confluence of the Malu River, altitude 2443 m above sea level, 9.VII.2017, N 43°49.738', E 88°10.351', leg. D. Palatov. Paratypes, 8 adult males, the same data as holo-

type except for 9–10.VII.2017; 1 adult male, the same data as holotype except for Karamsak River, 9.VII.2017, N 43°50.367', E 88°10.203', leg. D. Palatov; 1 pupa, 1 larva, the same data as holotype except for Urumqi City District, unnamed stream of Sangecha River near Malu River, altitude 3001 m above sea level, 13.VII.2017, N 43°43.176', E 88°16.547', leg. D. Palatov.

Derivatio nominis. The species is named in honour of the French chironomid taxonomist Joel Moubayed-Breil.



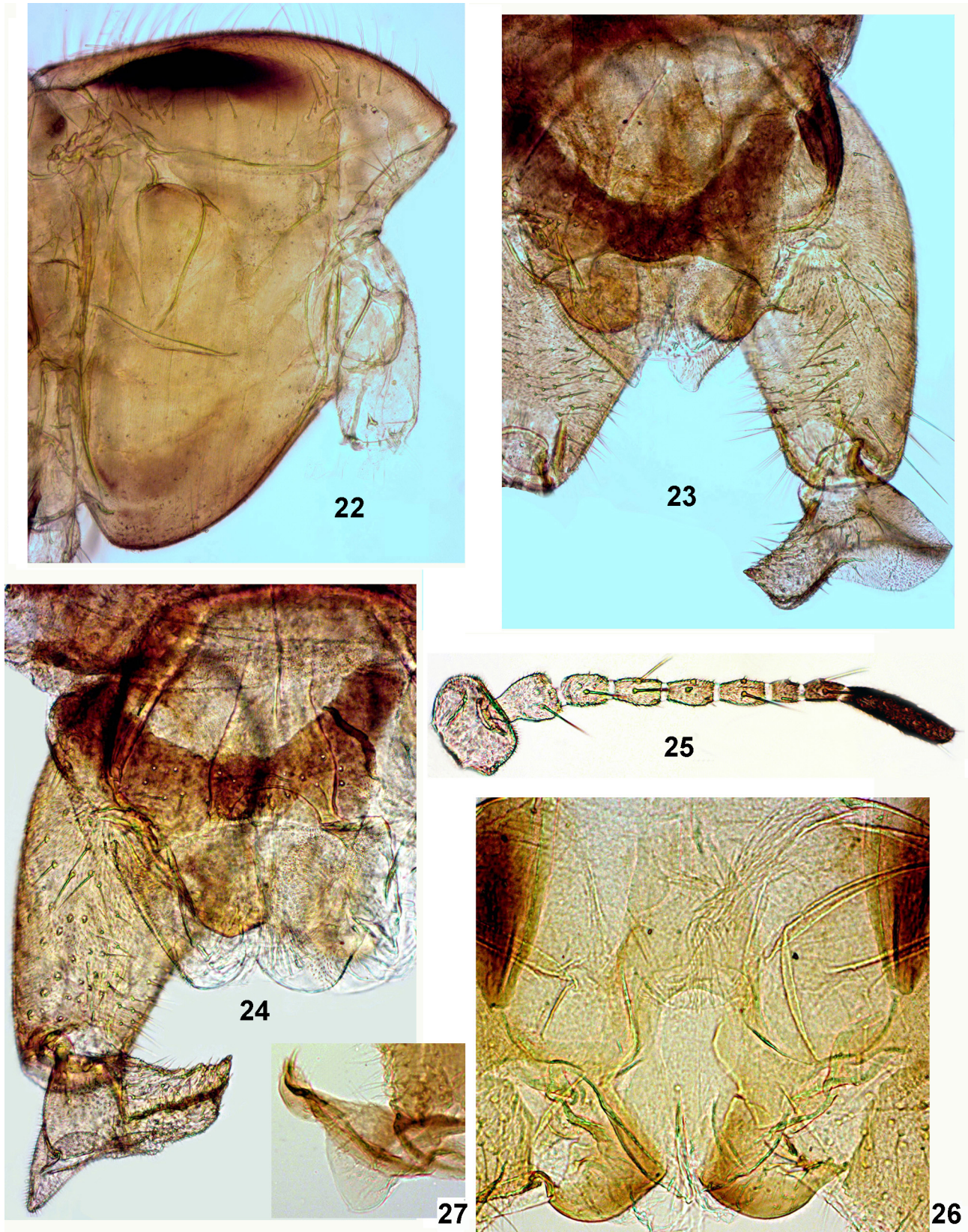
FIGURES 15–21. *Boreoheptagyia joeli* sp. nov. (15–18) and *B. iranica* sp. nov. (19–21), males. 15–16, 20–21, tergite IX; 17, transverse sternapodeme with group of spinules; 18, gonostylus; 19, hypopygium in dorsal view, without tergite IX. Scale bars 50 μ m.

Description

Adult male (n = 4, except when otherwise stated). Total length 2.4–2.9 m. Total length/wing length 0.84–1.0.

Coloration. Yellowish-brown to brown or dark brown. Head brown; antennal flagellomeres 1–7 yellowish,

last two subapical flagellomeres brown (Fig. 25). Antepronotum yellow, brownish-yellow. Mesonotum yellow or brownish-yellow, with two dark brown longitudinal stripes dorsolaterally (Fig. 22). Scutellum brownish. Abdomen dark brown. Wings grey. Legs spotted: basal 3/4 of femur yellowish, distal 1/4 dark brown; tibia and ta_1 in basal and apical parts dark brown, in middle part yellowish; ta_2 – ta_5 dark brown.



FIGURES 22–27. *Boreoheptagyia joeli* sp. nov. (22–26) and *B. phoenicia* Moubayed (27), males. 22, thorax in lateral view; 23–24, hypopygium in dorsal view; 25, antenna; 26, middle part of hypopygium without tergite IX; 27, gonostylus.

Head. Eyes bare and reniform. Temporal setae 24–26 (from one side), including frontals, verticals, postorbitals which can not be easily separated. Clypeus with 11–12 setae. Palpomere length (μm): 36–40, 72–80, 116–144, 136–140, 168–172. Head width/palp length 0.95–1.0. Antenna with 8–9 flagellomeres and reduced plume of setae 24–48 μm long; number of these setae on each 2–7 flagellomeres 2 (Fig. 25). Length of flagellomeres (μm) and antennal index for antenna with 9 flagellomeres: 64–68, 28–36, 28–36, 32–38, 32–36, 26–28, 28, 24–28, 80–84; AR 0.27–0.32. Number of setae on flagellomeres 2–8 as: 1, 2, 2, 2, 2, 4–5, 4, 4, 3–4, 3–4, 3–4, 1. Length of flagellomeres (μm) and antennal index for antenna with 8 flagellomeres: 52–56, 32–36, 32–40, 38–40, 38–40, 26–28, 28–32, 84–88; AR 0.33. Terminal flagellomere with 1–2 subapical setae, 20–28 μm long. Antennal length/palp length 0.62–0.66.

Thorax. Anteprepronotum with 5–9 ventrolateral setae. Acrostichals 35–45, 60–112 μm long, start from anteprepronotum, in anterior part in 1 row, in posterior part in 2 rows, sometimes in 3 rows. Dorsocentrals 10–13, 72–96 μm long, in anterior part only (Fig. 22); prealars 16–20, 60–112 μm long; supraalars 1–2, 32–36 μm long. Scutellum with 30–38 setae, 72–152 μm long. Preepisternum without setae.

Wing. Length 2.7–2.9 mm, width 0.80–0.84 mm. Costal extension 48–94 μm long. Anal lobe slightly reduced, angular. Alula with 0–1 seta, 52 μm long. Squama with 26–31 setae, 80–112 μm long. R and R₁ with 23–25 setae, R₄₊₅ with 7–9 setae in distal part, R₂₊₃ absent. RM/MCu 3.0.

Legs. Spur of front tibia 48 μm long. Spurs of mid tibia 44–52 μm and 36–52 μm long. Spurs of hind tibia 44–60 μm and 60–76 μm long. Hind tibial comb with 16 setae. Fore leg without pseudospurs on ta₁, mid leg with 7 pseudospurs, 28 μm long on ta₁, hind legs with 4 pseudospurs, 28 μm long on ta₁. Length (μm) and proportions of leg segments are as in Table 2.

Hypopygium (Figs. 15–18, 23–24, 26). Tergite IX with 33–36 setae, 16–36 μm long, without anal point, posteriorly slightly rounded, anal-lateral corners pointed and elongated back (Figs. 15–16, 23–24). Laterosternite IX with 4–7 setae 24–40 μm long. Transverse sternapodeme tall, with a chopped or rounded apex, 104–148 μm long and 84–92 μm wide in basal part, 36–40 μm wide in subapical part (Fig. 17). Aedeagal lobe weakly sclerotized, hardly visible, with field of small spinules distal of transverse sternapodeme (Figs. 17, 26). Gonocoxite 324–372 μm long; basal lobe widely rounded, with 8–11 setae, 12–16 μm long. Gonostylus bilobate, inner lobe 136–168 μm long, covered with numerous setae, 24–44 μm long and 1 apical megaseta, 8 μm long; outer lobe wide and without setae (Figs. 18, 23–24). HR 2.21.

TABLE 2. Lengths (in μm) and proportions of leg segments of *Boreoheptagyia joeli* sp. nov., male (n=4)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
P ₁	1214-1230	1156-1394	689-820	361-394	197-213	82	115-131	0.59-0.60	4.05-4.20	3.20-3.44	2.1-2.2
P ₂	1214-1361	1214-1394	664-754	189-377	189-213	82	131	0.54-0.55	4.24-4.37	3.65-3.66	1.5-1.7
P ₃	1246-1378	1312-1542	804-935	213-476	213-246	74-82	148	0.61	3.90-4.05	3.12-3.18	1.8-2.0

Diagnostic characters. Adult male of *B. joeli* is most closely related to *B. phoenicia* Moubayed from Lebanon, both with bilobate gonostylus, but with different shape of inner and outer lobes of gonostylus (Figs. 18, 23–24, 27); beside the new species has antenna with 8–9 flagellomeres and reduced plume, preepisternals of mesonotum are absent, acrostichals anteriorly in one row, while antenna of *B. phoenicia* has 12 flagellomeres and relatively well developed plume, all acrostichals in 2 rows and preepisternals are present (Moubayed 1992).

Ecology. Adults, pupae and larvae were collected from stones and boulders in mountain rivers, located at an altitude of 2443–3001 m, at a flow rate of 0.3–1.2 m/s, with water temperatures about 8°C (Fig. 49).

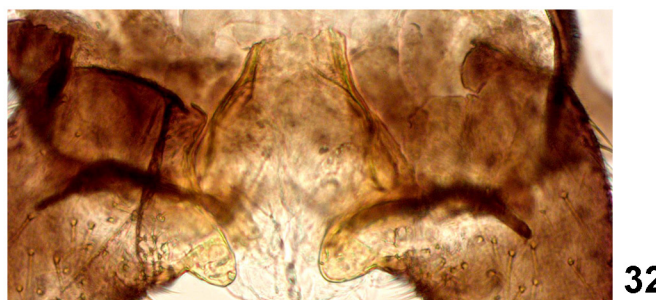
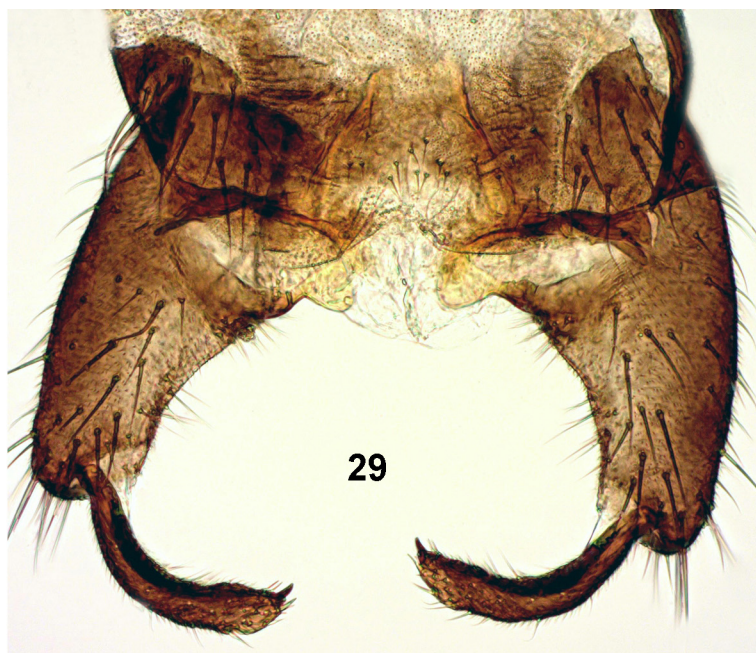
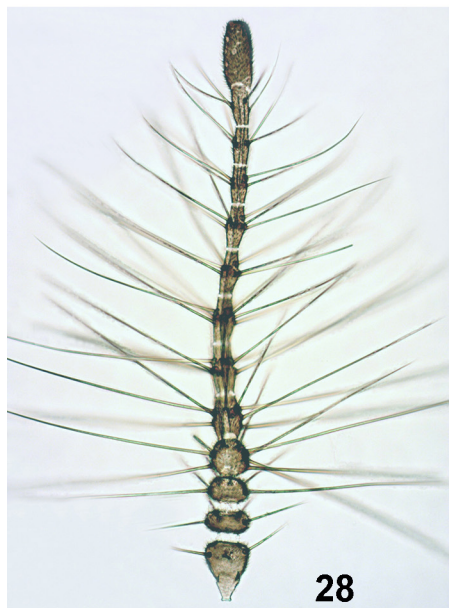
Boreoheptagyia iranica Makarchenko, sp. nov.

(Figs. 19–21, 28–32)

<http://zoobank.org/NomenclaturalActs/45D3F40D-35E6-4BDA-B14C-E29C3D1A36CD>

Type material. Holotype, adult male, IRAN: Lorestan Province, Zagros Mountains, Tenge-Kholeven River upper of Cham Chit Village, alt. 1338 m above sea level, N 33°22.737', E 48°58.414', 29.VI.2019, leg. D. Palatov. Paratypes: 3 males, the same data as holotype.

Derivatio nominis. The species is named as *iranica* after the type locality in mountains of Iran.



FIGURES 28–32. *Boreoheptagyia iranica* sp. nov., male. 28, 1–13 flagellomeres of antenna; 29–30, hypopygium in dorsal view; 31–32, middle part of hypopygium without tergite IX.

Description

Adult male (n = 4, except when otherwise stated). Total length 2.2–2.5 m. Total length/wing length 1.16–1.32.

Coloration. Brown to dark brown. Wings grey. Thorax dark brown, mesonotum dorsally in middle part a little lighter longitudinal. Scutellum brownish. Legs brown to dark brown, without spots, only basal 2/3 of femur a little lighter than other leg segments.

Head. Eyes bare and reniform. Temporal setae 14–17 (from one side), including frontals, verticals, postorbitals. Clypeus with 11–16 setae. Palpomere length (μm): 32–40, 56, 100–112, 108–116, 168–196. Head width/palp length 1.08–1.18. Antenna with 13 flagellomeres and reduced plume of setae, 98–295 μm long (Fig. 28). Length of 1–13 flagellomeres (μm): 52–56, 20–24, 24, 36–44, 40–48, 48–52, 44–48, 44–46, 40–44, 36–40, 34–36, 36, 60–76; AR 0.13–0.16. Terminal flagellomere with 0–1 subapical setae, 28–34 μm long. Antennal length/palp length 1.04–1.17.

Thorax. Anteprenotum with 9–10 ventrolateral setae, 72–76 μm long. Acrostichals 21–26, 12–24 μm long, in 1–2 rows, start from anteprenotum and occupy anterior 2/3 of mesonotum. Dorsocentrals 12–15, 72–88 μm long, in one row; prealars 20–21, 20–64 μm long; supraalars 2–3. Scutellum with 33–36 setae, 48–80 μm long. Preepisternum with 8–9 setae, 24–40 μm long.

Wing. Length 1.8–1.9 mm, width 0.56 mm. Costal extension absent. Anal lobe developed, rounded. Alula without setae. Squama with 21–26 setae, 56–72 μm long. R and R_1 with 23–31 setae, R_{4+5} with 0–3 setae in distal part, R_{2+3} absent. RM/MCu 1.2–1.3.

Legs. Spur of front tibia 52 μm long. Two spurs of mid tibia 44–52 μm long. Two spurs of hind tibia 52–68 μm long. Hind tibial comb with 11–12 setae. Fore leg with 2 pseudospurs on ta_1 and ta_2 , mid leg with 4 pseudospurs on ta_1 and 2 pseudospurs on ta_2 , hind legs with 6–7 pseudospurs on ta_1 , 2 pseudospurs on ta_2 and 0–2 pseudospurs on ta_3 . Length (μm) and proportions of leg segments are as in Table 3.

TABLE 3. Lengths (in μm) and proportions of leg segments of *Boreoheptagyia iranica* sp. nov., male (n=2)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
P_1	754-763	836-902	533-541	279	164-180	82	115	0.60-0.64	3.24-3.45	2,98-3.08	1.3-1.5
P_2	738-820	894-910	476	246-279	148-164	82	115	0.52-0.53	3.38-3.63	3.43-3.63	1.5-1.7
P_3	853-886	1066	607-615	344	180	82	115-123	0.57-0.58	3.48-3.55	3.12-3.55	1.5-1.7

Hypopygium (Figs. 19–21, 29–32). Tergite IX with 19–22 setae, 12–27 μm long, without anal point, posterior edge in middle part straight, anal-lateral corners elongated in form of narrow lobes back (Figs. 20–21). Laterosternite IX with 11–13 setae 40–60 μm long. Transverse sternapodeme tall, with a chopped apex, 88–144 μm long and 96–108 μm wide in basal part, 48–56 μm wide in subapical part (Figs. 19, 31–32). Aedeagal lobe weakly sclerotized, 108–120 μm long (Figs. 19, 31–32). Gonocoxite 248–260 μm long; basal lobe rounded or triangular-rounded, 28–36 μm height and 40 μm width, without setae (Figs. 19, 29–32). Small superior rounded volsellae present (Fig. 19). Gonostylus narrow and slightly curved, 140–148 μm long, with 1 apical megaseta, 8–12 μm long (Figs. 29–30). HR 1.75–1.77.

Diagnostic characters. The adult male of *B. iranica* with 13 antennal flagellomeres and reduced of plume setae, the characters typical of the genus, can be separated from known species of *Boreoheptagyia* in a set of characters, namely by thorax and legs colour, some features of mesonotum, wing and hypopygium. Thorax of the new species dark brown, mesonotum dorsally in middle part a little lighter longitudinal. Legs brown to dark brown without spots, only basal 2/3 of femur a little lighter than other leg segments. Mesonotum with acrostichals in 1–2 rows, which start from anteprenotum and occupy anterior 2/3 of mesonotum; dorsocentrals in one row. Wing without costal extension. Tergite IX without anal point, posterior edge in middle part straight, anal-lateral corners elongated in form of narrow lobes back (Figs. 20–21). Gonocoxite with rounded or triangular-rounded basal lobe, which without setae and with small superior volsellae. Gonostylus narrow and slightly curved.

Ecology. Adults were collected from stones and boulders in mountain rivers, located at an altitude of 1344 m, at a flow rate of 0.4–0.9 m/s (Fig. 48).

Boreoheptagyia brevitarsis (Tokunaga)

(Figs. 33–39)

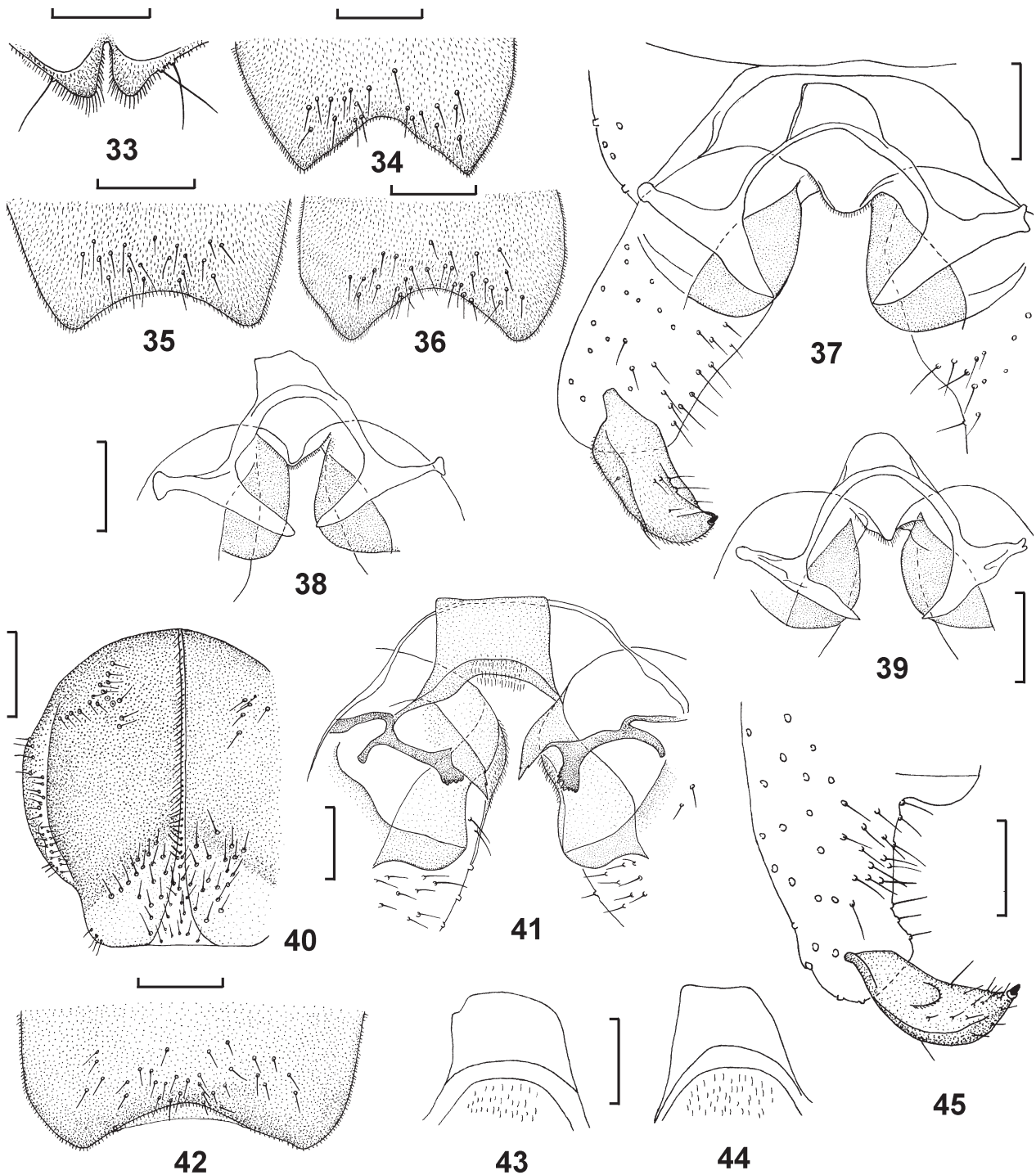
Prodiamesa (*Monodiamesa*) *brevitarsis* Tokunaga, 1936: 528.

Heptagyia brevitarsis Tokunaga, 1939: 302; Sasa & Kikuchi 1995: 207.

Boreoheptagyia brevitarsis (Tokunaga); Serra-Tosio 1989: 140; Endo 2002: 12; Makarchenko *et al.* 2008: 6.

Boreoheptagyia brevitarsis (Tokunaga); Makarchenko 1981: 89; 1985: 38; 2006: 259, misidentification.

Boreoheptagyia kurobeia Sasa *et Okazawa*, 1992; Endo 2002: 12.



FIGURES 33–45. *Boreoheptagyia brevitarsis* (Tokunaga) (33–39) and *B. sarymsactyensis* sp. nov., (40–45), males. 33, frontal tubercles of head vertex; 34–36, 42, tergite IX; 37, hypopygium in dorsal view, without tergite IX; 38–39, 41, middle part of hypopygium without tergite IX; 40, scutum in dorsal view; 43–44, transverse sternapodeme; 45, gonocoxite and gonostylus. Scale bars: Figs. 33–39, 41–45—50 µm; Fig. 40—200 µm.

Material examined. Three adult males extracted from the mature pupae, IRAN: Mazandaran Province, the right source of the Gelendrud River is slightly higher than the confluence with the left, altitude 1987 m above sea level, 36°18.276'N, 51°50.087'E. 26.VI.2019, leg. D. Palatov.

Remarks. The male of *B. brevitarsis* was described by Tokunaga (1936) from Japan and redescribed by Makarchenko (2008), with notice that this species is known only from Japan, all other records based on larvae from China and the Russian Far East should be revised.

B. brevitarsis is collected outside the Eastern Palearctic for the first time and therefore we found expedient to adduce here the short description of a male from the mountains of Iran.

In our opinion, the distribution of this species looks rather strange and in the future it is necessary to compare the data of DNA-barcoding of Japanese and Iranian populations to clarify their species unity or difference.

Description

Adult male extracted from mature pupae ($n = 3$, except when otherwise stated). Total length *ca* 2.0 mm. Brown to dark brown. Mesonotum dark brown. Scutellum brownish grey. Abdomen dark brown. Temporal setae of head 25–26 (from one side), including frontals, verticals, postorbitals. Frontal tubercles of head vertex rounded triangular, 24 μm long, with 1–2 lateral setae, 36–60 μm long (Fig. 33). Clypeus with 7–9 setae. Antenna with 13 flagellomeres and reduced plume of setae. Length of 1–13 flagellomeres (μm): 32, 28, 24, 32, 36, 44, 40, 44, 48, 46, 40, 44, 80; AR 0.17. Terminal flagellomere in distal part with 5 setae, 28–48 μm long. Anteprenotum with 7–12 ventrolateral setae, 48–56 μm long. Acrostichals 32–35, 36–52 μm long, start from anteprenotum, in posterior part without connection with dorsocentrals. Dorsocentrals 8–11 in anterior part and 5–8 in posterior part, 44–72 μm long; prealars 19–25, 36–52 μm long; supraalars 4, 40 μm long. Scutellum with *ca* 60 setae. Preepisternum and MAII without setae. Wings are not spread. Anal lobe well developed, rounded. Alula without setae. Squama with 34 setae.

Hypopygium (Figs. 34–39). Tergite IX with concave posterior margin and 20–32 setae, 12–16 μm long (Figs. 34–36). Laterosternite IX with 7 setae, 24–28 μm long. Transverse sternapodeme as in Figs. 37–39. Aedeagal lobe weakly sclerotized, sharply narrow triangular or triangular, without field of small spinules distal of transverse sternapodeme; phallopodeme reduced (Figs. 37–39). Gonocoxite 136–144 μm long; basal lobe rounded, without setae. Gonostylus 72 μm long, slightly curved, ending in megaseta, 4 μm long (Fig. 37). HR 1.89–2.0.

Distribution. Known only from Japan and Iran.

Boreoheptagyia sarymsactyensis Makarchenko, sp. nov.

(Figs. 40–45)

<http://zoobank.org/NomenclaturalActs/27EC911F-0609-40FA-8EE0-5FA037C4FC9A>

Type material. Holotype, adult male extracted from the mature pupa, KAZAKHSTAN: East Kazakhstan region, Katon-Karagai District, Sarymsaqty Mountains (Kazakh Mountain Altai), Eastern border of Katon-Karagai village, Sarymsaqty River Channel, altitude 1113 m above sea level, 01.VII.2018, 49°09.977'N, 85°38.015'E. leg. D. Palatov. 3 males extracted from the pupae, the same data as holotype.

Derivatio nominis. The species is named as *sarymsactyensis* after the type locality in Sarymsaqty River of East Kazakhstan.

Description

Adult male extracted from mature pupae ($n = 4$, except when otherwise stated). Total length *ca* 2.4–2.8 mm.

Coloration. Brown to dark brown. Mesonotum dark brown in anterior 2/3 and brown to light brown in posterior 1/3 (Fig. 40). Scutellum brownish grey. Abdomen dark brown. Legs brown, without spots (?).

Head. Eyes bare and reniform. Temporal setae 44–52 (from one side), including frontals, verticals, postorbitals. Clypeus with 17 setae. Palpomere length (μm) ($n=2$): 36–44, 60–68, 60–92, 13680–104, 120–136. Head width/palp length 1.0–1.11. Antenna with 13 flagellomeres and reduced plume of setae, 180–295 μm long. Length of 1–13 flagellomeres (μm): 44–60, 24, 32, 40–44, 48–52, 48–52, 48–52, 48–52, 44–52, 48–52, 50–52, 52, 92–96; AR 0.17. Terminal flagellomere in distal part with 2–3 setae, 28–40 μm long. Antennal length/palp length 1.40–1.51.

Thorax. Anteprenotum with 12–15 ventrolateral setae, 56–64 μm long. Acrostichals 48–65, 28–32 μm long, start from anteprenotum, in posterior part connect with dorsocentrals (Fig. 40). Dorsocentrals 18–21 in anterior part and 24–26 in posterior part, 32–56 μm long (Fig. 40); prealars 29–45, 44–52 μm long; supraalars 3–5, 44 μm long.

Scutellum with 119–124 setae, 62–68 μm long. Preepisternum with 16–19 setae, 52–60 μm long; MAII without setae.

Wings are not spread. Anal lobe well developed, rounded. Alula without setae. Squama with 40–43 setae, 80–104 μm long.

Legs. Spur of front tibia 44 μm long. Spurs of mid tibia 48–50 μm long. Spurs of hind tibia 60 μm and 52 μm long. Hind tibial comb with 13 setae. Fore leg without pseudospurs on ta_1 , mid leg with 2 pseudospurs, 24–26 μm long on ta_1 , hind legs with 2 pseudospurs, 24–26 μm long on ta_1 . Length (μm) and proportions of leg segments are as in Table 4.

Hypopygium (Figs. 41–45). Tergite IX with slightly concave posterior margin and 38–40 setae, 15–16 μm long (Fig. 42). Laterosternite IX with 5–7 setae, 20–32 μm long. Transverse sternapodeme from almost rectangular to nearly trapezoidal shape, 48–60 μm long and 60–68 μm wide in basal part (Figs. 41, 43–44). Aedeagal lobe weakly sclerotized, sharply triangular or conical, with field of small spinules distal of transverse sternapodeme; phallapodeme well sclerotized, extended distally and serrated apically (Fig. 41). Gonocoxite 150–156 μm long; basal lobe rounded, without setae. Gonostylus 80 μm long, wrinkled, slightly curved, ending in megaseta, 4 μm long (Fig. 45). HR 1.95.

TABLE 4. Lengths (in μm) and proportions of leg segments of *Boreoheptagyia sarymsactyensis* sp. nov., male (n=2)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
P_1	426–476	649–672	312–344	172	98	49	98	0.50–0.51	3.30–3.58	3.34–3.42	1.4
P_2	656	672	353	189	139	72	98	0.53	3.38	3.76	1.5
P_3	607	804	377	213	148	74	115	0.47	3.26	3.74	2.5

Diagnostic characters. Adult male of *B. sarymsactyensis* is most closely related to *B. sasa* Makarchenko *et al.* Endo from Hokkaido (Japan) and Kolyma River basin of the Northeastern Russia, male of which also has acros-tichals connecting with dorsocentrals in the posterior part of the mesonotum, dorsocentrals in anterior and posterior groups, presence of setae on preepisternum and field of small spinules distal of transverse sternapodeme (Makarchenko *et al.* 2008). But male of the new species has not setae on median anepisternum II, and with numerous (119–124) of scutellars, aedeagal lobe widely triangular or conical, phallapodeme well sclerotized, extended distally and serrated apically (Fig. 41), while *B. sasai* has some setae on median anepisternum II, with 58–62 scutellars, aedeagal lobe long and narrow, phallapodeme very narrow (Makarchenko *et al.* 2008).

Ecology. Pupae were collected from stones and boulders covered with moss in mountain rivers, located at an altitude of 1113 m, at a flow rate of 0.8–1.0 m/sc (Fig. 50).

Results of DNA barcoding

We obtained the partial COI sequences of 25 specimens (658 bp), of which 5 specimens were *Boreoheptagyia brevitarsis*, 4 – *B. iranica*, 4 – *B. joeli*, 3 – *B. sarymsactyensis* and 9 – *Palatovia lorestanica*. None of these protein-coding sequences had premature stop codons or ambiguous nucleotides in translation. The mean nucleotide base compositions were A 27.0%, C 17.5%, G 16.8%, and T 38.7% across all sites for all sequences. The transition/transversion ratio (R) was 7.34. Overall, there were 161 variable sites most of which were interspecific synonymous substitutions. Pairwise intraspecific K2P sequence divergence for *B. brevitarsis*, *B. iranica*, *B. joeli*, *B. sarymsactyensis* and *P. lorestanica* were 0.011, 0.000, 0.008, 0.005 and 0.005 respectively.

Interspecific K2P pairwise distances between 4 *Boreoheptagyia* species ranges from 0.101 to 0.162 (mean 0.128). According to Montagna *et al.* (2016) and Ekrem *et al.* (2007, 2010) these values are sufficient to maintain the species level. Intergeneric distances between *P. lorestanica* and *Boreoheptagyia* species ranges from 0.137 to 0.160 (mean 0.148). However, the COI locus isn't optimal for the correct genus identification (Ekrem *et al.* 2007).

The Bayesian tree is shown in fig. 46. In order to reconstruct a more representative tree, we added to the obtained sequences seven samples belonging to the three genera of the tribe of Boreoheptagyiini – *Kaluginia* (including *K. lebetiformis koreana* Makarchenko and *K. lebetiformis lebetiformis* Makarchenko), *Shilovia rara* Makarchenko and *Boreoheptagyia* sp. (KY640386). The topology of the ML tree (not shown) was essentially identical to that of the Bayesian tree except clade *B. iranica* + *P. lorestanica* was placed sister to the genus *Kaluginia* instead polytomy

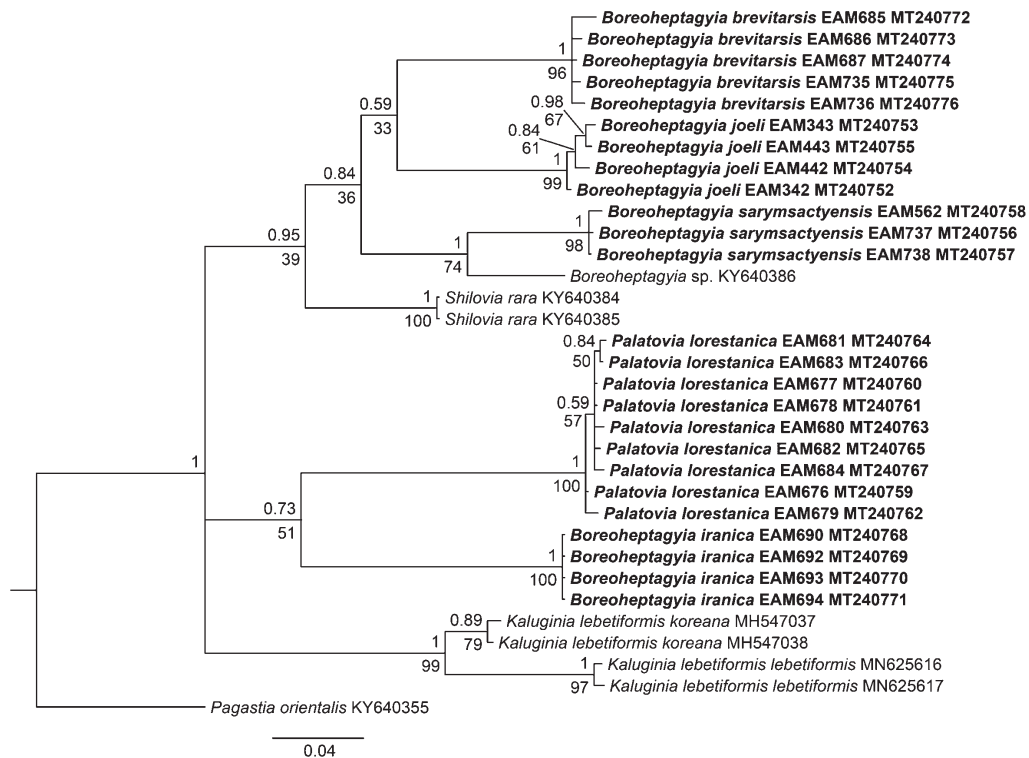


FIGURE 46. Bayesian tree based on mitochondrion COI gene for available members of the tribe Boreoheptagiini. *Pagastia orientalis* (Tshernovskij) (tribe Diamesini) was used as outgroup to root the tree. Bayesian posterior probabilities (PP) are given above tree nodes and bootstrap support values found in the ML analysis are shown below nodes. Specimens obtained in this study are in bold.



47



48



49



50

FIGURES 47–50. Type localities of *Palatovia lorestanica* sp. nov. (47), *Boreoheptagyia iranica* sp. nov. (48), *B. joeli* sp. nov. (49) and *B. sarymsactyensis* sp. nov. (50). 47, Dez (Sesar) River, Lorestan Province, Iran (photo by E.S. Chertoprud); 48, Tenge-Kholeven River, Lorestan Province, Iran (photo by D.M. Palatov); 49, Zienzan River, Xinjiang Uygur Autonomous Region, China (photo by M.V. Vinarski); 50, Sarymsaqty River, East Kazakhstan Region, Kazakhstan (photo by R.N. Krykbaeva).

in the basal node of BI tree. The monophyly of the genera *Palatovia* and *Shilovia* was moderately supported, the Bayesian posterior probability is PP=0.73 and 0.95 respectively; maximum likelihood bootstrap value percents of ML=51 and 39 respectively. *S. rara* was placed as a sister species to all *Boreoheptagyia* species, except *B. iranica*, which was sister to *P. lorestanica*. Since *B. iranica* and *P. lorestanica* do not share a group of morphological characters, the genus *Boreoheptagyia* appears to be polyphyletic. Further studies using a multilocus approach and a wide list of *Boreoheptagyia* species will allow us to analyze the identified polyphyly.

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