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MORPHOLOGICAL REDESCRIPTION AND DNA BARCODING OF *KALUGINIA LEBETIFORMIS* MAKARCHENKO, 1987 (DIPTERA: CHIRONOMIDAE, DIAMESINAE) FROM SOUTH KOREA

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Summary. Illustrated redescription of adult male as well as the results of DNA barcoding of *Kaluginia lebetifomis* Makarchenko in comparison with known species of the tribe Boreoheptagyiini from South Korea are provided. The species-specificity of *K. lebetiformis* COI sequences is analyzed and the sequences are presented as diagnostic characters – molecular markers. Intergenus K2P distance between three genera and species of the tribe Boreoheptagyiini – *K. lebetiformis*, *Boreoheptagyia* sp. and *Shilovia rara* Makarchenko ranged from 0.105–0.143 (mean 0.124) and these values are sufficient to maintain the genus level. DNA barcodes of *K. lebetiformis* was uploaded to GenBank.

Key words: Diptera, Chironomidae, Diamesinae, *Kaluginia*, taxonomy, DNA barcoding, South Korea.

Е. А. Макарченко, А. А. Семенченко, Х. Кан, Ю. Дж. Бэ. Морфологическое переописание и ДНК баркодинг *Kaluginia lebetiformis* Makarchenko, 1987 (Diptera: Chironomidae, Diamesinae) из Южной Кореи // Дальневосточный энтомолог. 2018. N 367. С. 26-32.

Резюме. По материалу из Южной Кореи приведены иллюстрированное переописание имаго самца и результаты анализа фрагмента гена цитохромоксидазы субъединицы I (658 пн.) мтДНК хирономиды *Kaluginia lebetiformis* Makarchenko из подсемейства Diamesinae. Полученные последовательности для этого вида были депонированы в генном банке. Генетические дистанции (K2P) между родами трёх представителей трибы Boreoheptagyiini – *K. lebetiformis*, *Boreoheptagyia* sp. и *Shilovia rara* Makarchenko находятся в пределах 0,105–0,143 (в среднем 0,124), что достаточно для поддержания родового статуса *Kaluginia* Makarchenko.

INTRODUCTION

The genus *Kaluginia* Makarchenko, 1987 was established with the description of *K. lebetiformis* Makarchenko, 1987 from the south part of Sakhalin Island (Makarchenko, 1987).

Later one male of this species was found in Khasansk District of Primorye Territory (Makarchenko *et al*., 2017). Males of this monotypic genus have antenna with 7 flagellomeres and reduced plume, pedicel with setae; eye bare, sometimes slightly pubescence, not extended dorsomedially; antepronotal lobes narrowly joined medially, lateral antepronotals occupy basal 2/3 of lobe, median antepronotals absent; acrostichals present, beginning near antepronotum and reach middle mesonotum, dorsocentrals erect in 1–2 rows, supraalars present; costa not extending, R_{2+3} reduced and visible only in basal part, FCu distal to MCu, R_{4+5} with setae, squama fringed; legs speckled; anal point like small rounded protuberance, sternapodeme high, almost trapezoidal, gonocoxite with basal lobe and inferior volsella, gonostylus broad and scoop-shaped, with 5–9 megasetae along inner margin.

Some years ago additional imaginal material was collected by Dr. Y.J. Bae and PhD student H. Kang in South Korea. It allows us to make a more detailed description of the male imago, as well as to conduct a DNA analysis of this species. Below we redescribe adult male of *K. lebetiformis* from the South Korea as well as give the results of DNA barcoding in comparison with the closely related members of tribe Boreoheptagyiini, namely *Boreoheptagyia* Brundin and *Shilovia* Makarchenko. The DNA barcode corresponding to the 650-bp fragment of the mitochondrial gene cytochrome c oxidase I (COI) has been identified as the core of a global bio-identification system at the species level (Hebert *et al*., 2003) and has proved to be useful in non-biting midges (Montagna *et al*., 2016; Ekrem *et al*., 2010; Carew *et al*., 2005). K2P genetic distances been used to establish specific independence of the redescribed species.

MATERIAL AND METHODS

The material was preserved in 96% ethanol for DNA-analysis and in 70% ethanol for further study of morphology, and slide-mounted following the methods by Makarchenko (1985). The terminology follows Sæther (1980). All material is deposited in the Laboratory of Freshwater Hydrobiology of the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok.

Genomic DNA was extracted with using the Invitrogen PureLink Genomic DNA Mini Kit in compliance with the manufacturer's protocols. Approximately 650 base pairs of the COI were amplified from diluted genomic DNA by polymerase chain reaction (PCR) in a total volume of 10 µl with 5 µl of Go Taq Green Master Mix (Promega Corp, Madison, WI, USA), 0.5 µl of each primer (100 ng/µl), 3 µl of nuclease-free water and 1 µl of total DNA. The PCR thermal regime consisted of one cycle of 1 min at 94 °C; five cycles of 1 min at 94 °C, 1.5 min at 45 °C and 1.5 min at 72 °C; 35 cycles of 1 min at 94 °C, 1.5 min at 50 °C and 1 min at 72 °C; and a final cycle of 5 min at 72 °C. The primers COIF-ALT (5'-ACAAA TCAYAARGAYATYGG-3') and COIR-ALT (5'-TTCAGGRTGNCCRAARAAY CA-3'), which were obtained from Mikkelsen et al. (2006), were used. All PCR products were verified using electrophoresis on a 1,5% TBE agarose by visualizing on GelDoc XR+ imaging systems (BioRad), only positive PCR products were purified for cycle sequencing using Exonuclease I (ExoI) and Thermosensitive Alkaline Phosphatase (FastAP) by ThermoFisher Scientific. The PCR products were bidirectionally sequenced using the BigDye Terminator v3.1 cycle kit and run on an ABI 3130xl DNA analyzer (Applied Biosystems). Sequences were aligned and manually edited in MEGA 7 (Kumar *et al*., 2016). Based on the Kimura-2-Parameter (K2P) model were calculated interspecific and intragenus genetic distances using MEGA 7. Two COI sequences of *K. lebetiformis* have been deposited in GenBank (MH547037–MH547038).

MORPHOLOGICAL REDESCRIPTION

Kaluginia lebetiformis **Makarchenko, 1987**
Figs 1–10

Kaluginia lebetiformis Makarchenko, 1987: 786, 2006: 266; Oliver, 1989: 134; Ashe & Connor, 2009: 291.

MATERIAL EXAMINED. **South Korea**: 2♂, Gyeongi-do, Gapyeong-gun, Gapyeong-cheon, Bukhan River, Han River basin, 15. IV 2016 (Light trap), 37°58' N, 36.4" 127°26' 35.5" E, leg. Y. Bae; 1♂, the same data except 22.V 2014 (Light trap), leg. H. Kang. **Russia**: South Sakhalin, 1♂ (holotype), Belaya River, Sokol Village, Dolinsk District, 29.VI 1985, leg. S. Bestalannaya, E. Makarchenko.

REDESCRIPTION. *Male imago* (n=3). Total length 2.3–2.6 mm. Total length/wing length 1.03–1.10. Total coloration brown to dark-brown; antepronotum light yellow; methonotum yellowish, with brown stripes; legs spotted: basal 2/3 of femur yellowish, distal 1/3 brown; tibia in basal and apical parts brown and yellowish in middle part; basal 2/3 of ta_1 yellowish and distal 1/3 brown; ta_2–ta_5 brown (Fig. 1).

Head. Temporal setae (from one side) including 3–4 frontals, 7 orbitals and 7–10 verticals. Clypeus with 17–26 setae. Palpomere length (μm): 28–48, 60–64, 104–120, 132–140, 212–220. Head width/palpal length 0.92–1.0. Antenna with 7 flagellomeres and reduced plume of setae 48–68 μm long (Fig. 2). Flagellomeres length (μm): 52–56, 24, 28, 24–28, 28–32, 28–32, 68–84; terminal flagellomere with 2 subapical setae 60–76 μm long; scape with 3 setae 28–36 μm long. AR 0.34–0.46.

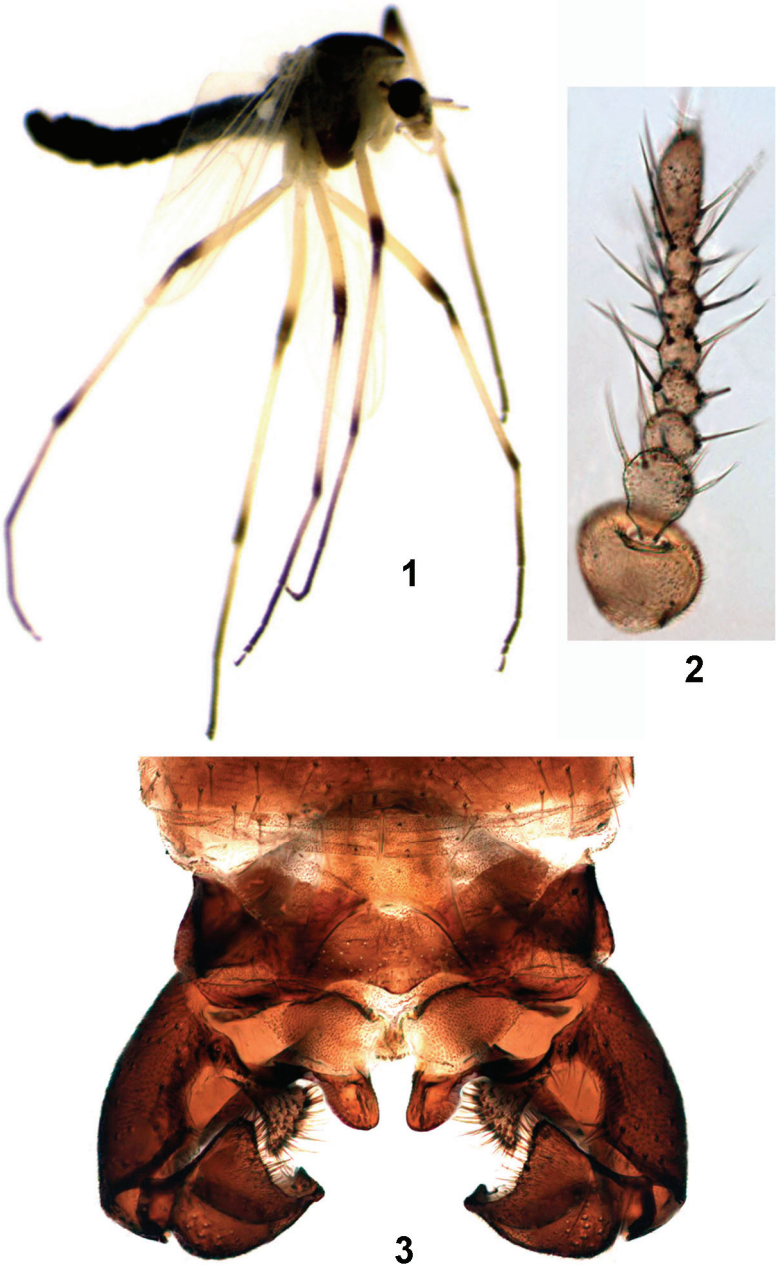
Thorax. Antepronotum with 18–20 lateral setae. Acrostichals 25 (48–56 μm), dorsocentrals 21–27 (72–100 μm), prealars 16, supraalars 1, scutellars *ac* 50.

Wing. Length 2.08–2.52 mm, width 0.68–0.76 mm. Anal lobe well developed; squama with 19–31 setae (64–80 μm) in 1–2 rows. R and R_1 with 55–58 setae (32–36 μm), R_{4+5} with 24–29 setae (28–40 μm) in subapical part. Wing of one male with 3 short setae on M_{3+4}.

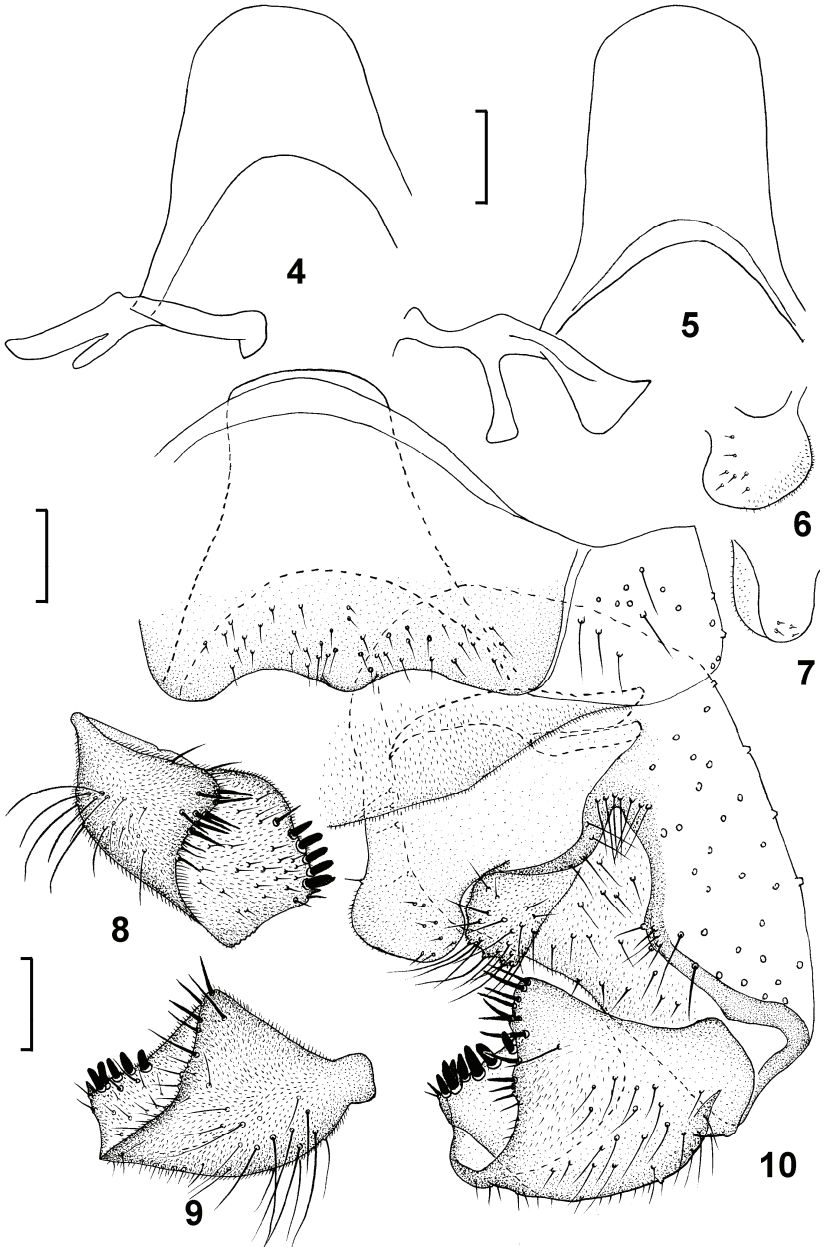
Legs. BR_1 2.0; BR_2 1.8; BR_3 1.9. Spur of front tibia 44 μm long. Spurs of middle tibia 28–52 and 44–53 μm long. Spurs of hind tibia 68–72 μm and 44–52 μm long. Hind tibial comb with 12–14 spine-like setae. Middle legs with 4 pseudospurs 36–40 μm long on ta_1; hind legs with 7 pseudospurs on ta_1. Claw with 4 denticles apically. Length (μm) and proportions of legs segments are as follow:

P	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV
P_1	918–1115	1132–1328	722–886	394–459	197–213	66–74	98–115	0.64–0.67	3.67–3.87	2.76–2.84
P_2	984–1197	1082–1246	574–738	312–394	189–213	115–74	115	0.53–0.59	3.61–4.0	3.31–3.60
P_3	1000–1214	1230–1443	705–886	394–508	213–246	66–74	98–131	0.57–0.61	3.69–3.81	3.0–3.16

Hypopygium (Figs 3–10). Tergite IX with "anal point" which like small rounded protuberance, and with 29–42 setae, 12–20 μm long; laterosternite IX with 15–20 setae (from one side), 44–48 μm long. Transverse sternapodeme high, almost trapezoidal, 128–132 μm long of specimens from South Korea, 92 μm long of specimen from South Sakhalin, and 148–152 μm wide in basal part, 88–100 μm wide in subapical part, with rounded apex (Figs 4–5). Gonocoxite 308–310μm long. Basal lobe of gonocoxite is various shapes and depends on its position; in the inner half with microtrichia, along the outer margin with short setae (Figs 3,



Figs 1–3. Adult male of *Kaluginia lebetiformis* Makarchenko from South Korea. 1 – total view of male, from one side; 2 – antenna; 3 – total view of hypopygium, from above.



Figs 4–10. Hypopygium of *Kaluginia lebetiformis* Makarchenko from South Sakhalin (4) and South Korea (5–10). 4–5 – transverse sternapodeme and aedeagal lobe; 6–7 – basal lobe of gonocoxite; 8–9 – gonostylus; 10 – total view of hypopygium, from above. Scale bars = 50 μm.

6–7, 10); inferior volsella as in Figs 3, 10, covered by setae 40–60 μm long. Gonostylus 156 μm long, scoop-shaped; inner lobe along the margin with 5–9 megasetae 12–16 μm long and one subterminal tooth; outer lobe widely triangular, inner margin of which with strong setae 20–24 μm long, outer half with thinner and longer setae 56–72 μm long (Figs 3, 8–10). HR 1.97.

REMARKS. Additional material from South Korea allowed a more detailed study of the internal structure of the male hypopygium, namely transverse sternapodeme, which in complex with aedeagal lobe, basal lobe of gonocoxite and inferior volsella is typical for Boreoheptagyiini. Males from both populations are close related by all features but specimens from South Korea have more a long transverse sternapodeme (128–132 μm) than specimen from South Sakhalin (92 μm).

DISTRIBUTION. East Palaearctic continental-insularis species known from Sakhalin Island and Primorye Territory in Russia and from South Korea.

RESULTS OF DNA BARCODING

The final alignment of the COI gene yielded 658 bp for 2 samples of *K. lebetiformis* that were 2 haplotypes. The nucleotide composition of the studied sequences of *K. lebetiformis* COI gene fragments deviated from an equilibrium one, comprising 26.4 % of A, 40.0 % of T, 17.0 % of C, and 16.5 % of G. Total intraspecific sequence divergence was 0.0061, which is based on four nucleotide substitutions. All the substitutions were synonymous and observed only in the third codon positions. Three substitutions were transitions and one transversion. Average intergenus K2P distance between *K. lebetiformis* and other genera of the tribe Boreoheptagyiini (Diamesinae) showed the following results: *Boreoheptagyia* sp. (KY640386) – 0.143, *Shilovia rara* Makarchenko (KY640384–KY640385) – 0.124. High differences between the groups can argue genus independence of the *Kaluginia* (Ekrem *et al*., 2007).

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