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Confirmation of a species status and redescription of *Linevitshia yezoensis* Endo, 2007, stat. resurr. (Diptera: Chironomidae: Protanypodinae) from Hokkaido (Japan)

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As a result of the revision of the genus *Linevitshia* Makarchenko in 2014, the Japanese species *L. yezoensis* Endo was reduced to a synonym of *L. prima* Makarchenko from the Russian Far East (Makarchenko & Semenchenko 2014). At that time, DNA barcoding data were known only for *L. prima*, and we did not find sufficient morphological differences between the two species and the differences that did exist were considered to be the result of geographic variation, and DNA barcoding data were only known for *L. prima*. In 2024, Dr. Kazuo Endo collected adult males of *L. yezoensis* from the type locality on Hokkaido and gave us this material for DNA barcoding. The results of the genetic analysis demonstrated that *L. yezoensis* differs from *L. prima* by p-distances of 12.9 %, and it became clear that *L. yezoensis* is a valid species.

Below, we present a redescription of the adult male of *L. yezoensis* with a justification for the restoration of its species status at the morphological and molecular levels.

Materials and methods

The redescription of the male imago of *L. yezoensis* and its comparison with *L. prima* were both carried out on the basis of published data (Makarchenko 1987; Endo *et al.* 2007; Makarchenko & Semenchenko 2014) and based on the review of additional new material which was also used in DNA barcoding.

Adults were preserved in 96% ethanol for study of morphology and DNA barcoding and were slide-mounted in polyvinyl lactophenol. The morphological terminology and abbreviations used below generally follow Sæther (1980). 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.

Genomic DNA was extracted from the whole body of three imago specimens (two females and one male) using ExtractDNA Blood & Cells (Evrogen, Moscow, Russia) following the manufacturer's instructions. We amplified mitochondrial protein coding gene cytochrome c oxidase I using standard primers LCO1490 and HCO2198 (Folmer *et al.* 1994).

The reaction protocols, cycle programs used for amplification, purification of PCR and sequences products followed the process by Makarchenko *et al.* (2022a, 2022b, 2023).

The PCR products were bidirectionally sequenced on an ABI 3500 sequencer (Applied Biosystems). Intra and interspecific genetic divergence values were calculated using p-distances implemented in MEGA7 (Kumar *et al.* 2016) software. We carried out a maximum likelihood tree in IQTREE 2.3.6 (Minh *et al.* 2020) with 1 000 000 bootstrap replicates using ultrafast bootstrapping (Hoang *et al*. 2018) after choosing the optimal models of molecular evolution for each codon of COI with ModelFinder (Kalyaanamoorthy *et al.* 2017) implemented in IQTREE. The obtained sequences have been deposited in GenBank under numbers PV067664–PV067666.

Material is 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).

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Description

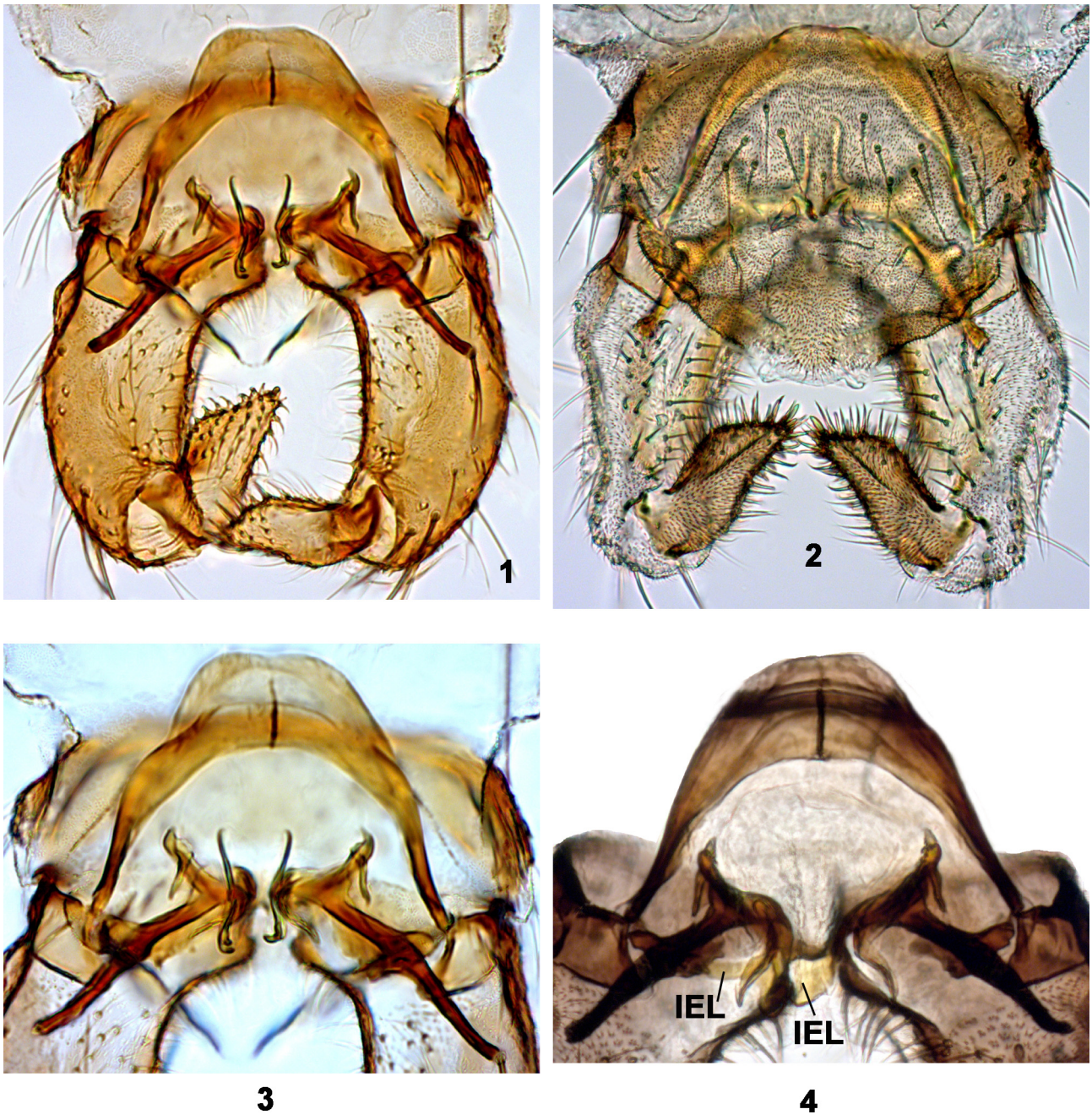
Linevitshia yezoensis Endo, 2007, stat. resurr.

(Figs. 1–4, 7–8)

Linevitshia yezoensis Endo in Endo, Makarchenko & Willassen, 2007: 93, Figs 1–4, 7–13; Ashe & Connor 2009: 292; Makarchenko & Endo 2009: 65.

New material examined. *Japan*: 5 adult males, 2 adult females, Hokkaido, Obihiro City, Taisho, Nuppuku River, N 42.819383, E 143.187231, altitude 102 m a.s.l., 06.X. 2024, leg. K. Endo.

Adult male (n = 14, except when otherwise stated) Total length 3.0–4.14 mm. Total length/wing length 1.30–1.52. Coloration. Body largely brown to dark brown; head and thorax more or less grayish.



FIGURES 1–4. Males of *Linevitshia yezoensis* Endo **(1, 3–4)** and *L. prima* Makarchenko **(2)**. **1–2,** hypopygium in dorsal view; **3–4,** endoskeleton. IEL—inner part of aedeagal lobe.

Head. Eyes reniform with weak microtrichia between ommatidia. Frons with weak protrusions near dorsomesal corner of the eye. Temporal setae including 0–1 weak and short inner verticals and 3–5 stronger postorbitals. Clypeus without setae. Antenna with 13 flagellomeres and well-developed plume; ultimate flagellomere with 2 subapical setae 38–40 μm long, pedicel with 2–3 setae, scape without setae. AR 1.00–1.11. Palpomere length (μm): 40–48, 61–79, 112–141, 127–146, 192–225.

Thorax. Antepronotum with U-shaped notch in frontal view, with 4–9 dorsal and 14–21 lateral setae. Acrostichals 17–33, dorsocentrals 11–16, prealars 6–9, supralars 1–3. Scutellum with 10–16 setae. Posterior anepisternum II with 3–5 setae, epimeron II with 3–8 setae, preepisternum without setae.

Wing. Wing length 2.12–2.6 mm, width 0.56–0.68. Costal extension 60–80 μm long; R_{2+3} weak, but distinct. Anal lobe weakly developed. Membrane without macrotrichia. Brachiolum with 3–5 setae. R with 22–28 setae, R_1 with 5–11 setae, R_{4+5} with 6–11 setae. RM/Mcu 3.2–3.5. Alula with 4–9 setae. Squama with 18–25 setae.

Legs. Spur of front tibia 70–79 μm. Spurs of mid tibia 56–78 and 60–75 μm. Spurs of hind tibia 81–90 and 65–73 μm long. Hind tibial comb with 9–11 setae. Tarsal sensilla chaetica absent. Ta_4 cylindrical; ta_5 slightly curved; pulvilli small; tip of claws serrate, with approximately 5 teeth. Lengths and proportions of leg segments are as in Table 1.

TABLE 1. Lengths (in μm) and proportions of leg segments of *Linevitshia yezoensis* Endo, male (n=14)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1050–1172	1033–1285	476–980	262–461	180–293	118–181	131–145
P_2	984–1159	1031–1182	462–553	259–303	175–199	115–125	125–139
P_3	1230–1381	1354–1504	702–843	394–843	224–312	132–156	131–153

continued

	LR	BV	SV	BR
P_1	0.66–0.76	3.18–3.40	2.51–2.90	3.3–3.5
P_2	0.43–0.48	3.60–3.83	3.94–4.62	3.0–3.4
P_3	0.52–0.56	3.43–3.86	3.42–3.73	3.2–4.0

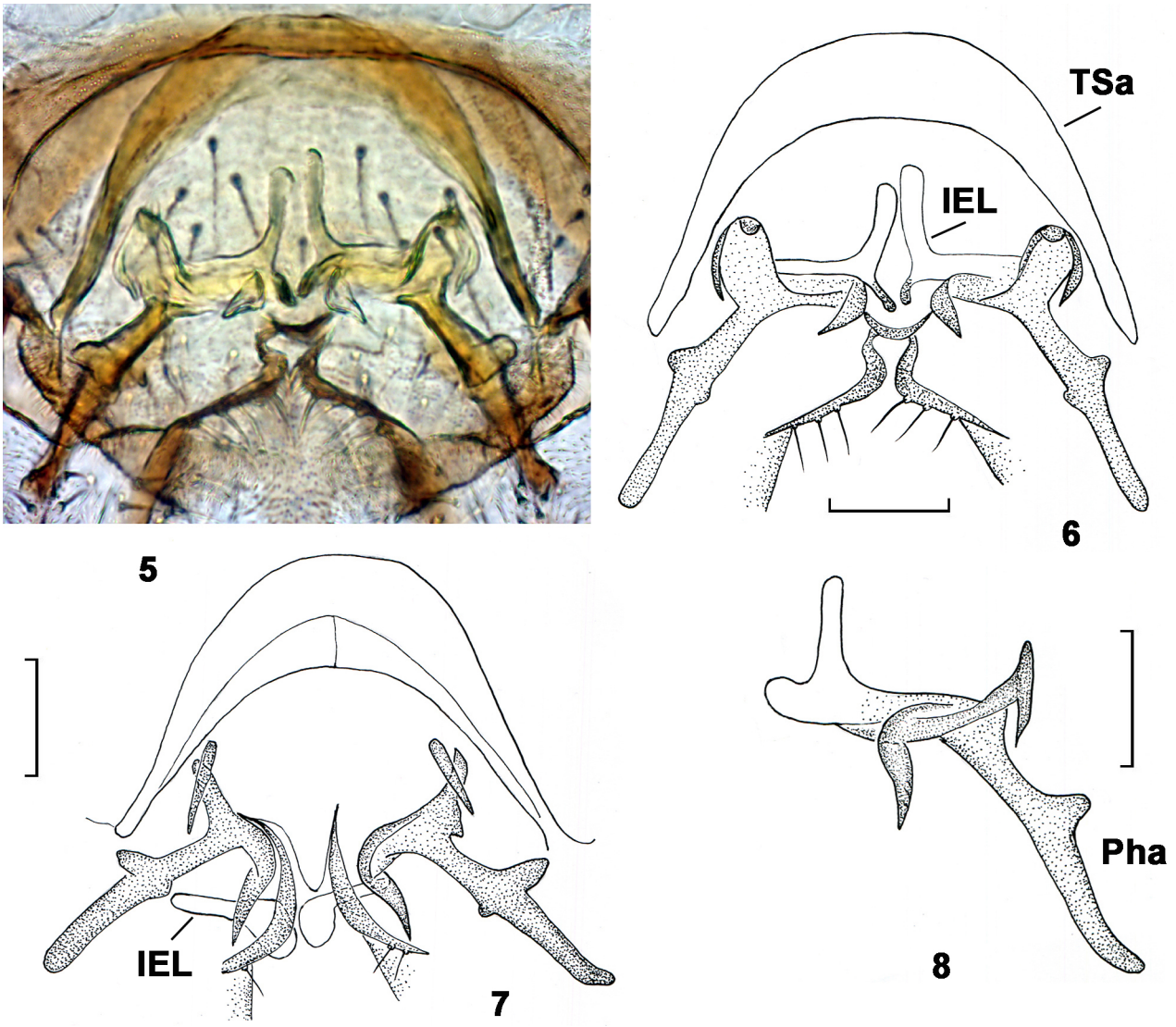
Hypopygium (Figs. 1–4, 7–8). Tergite IX with 9–14 setae. Anal point absent. Laterosternite IX with 8–11 setae. Gonocoxite simple, 160–180 μm long. Transverse sternapodeme broadly arched (Figs. 1, 7), 128–164 μm long and 40–56 μm width; TSA length/TSA width 3.7–4.5. Phallapodeme 96–120 μm long; aedeagal lobe distally with yellowish, weakly chitinized, finger-like inner part, which with large, noticeably protruding "heel" basally (Fig. 8). Gonostylus 88–96 μm long; in distal part with strong setae 12–22 μm long and 1–2 apical megaseta 12–14 μm long (Fig. 1). HR 1.7–2.4.

Pupa of *L. yezoensis* is no different from that of *L. prima* (Endo *et al.* 2007).

Larva unknown.

Remarks. Comparison of adult males of *L. prima* and *L. yezoensis* showed that the values of most morphological characters of these species overlap and do not provide clear differences, although males of the first species are larger, have longer wings and a greater number of acrostichal and dorsocentral setae of the mesonotum, as well as setae of the wing squama (Table 2). The main difference between these species lies in the structure of the hypopygium. Thus, the length of the gonostylus of *L. prima* 112–124 μm, gonocoxite—200–288 μm, length of TSA 180–228 μm, width of TSA 24–46 μm, ratio of TSA length to its width 5.0–5.9, gonostylus with 1–4 megasetae. In *L. yezoensis*, length of gonostylus 88–96 μm, gonocoxite—160–180 μm, length of TSA 128–164 μm, width of TSA 32–56 μm, the ratio of the TSA length to its width 3.0–4.5, gonostylus with 1–2 megasetae (Table 2). However, the more important difference between these two species is in the complex structure of the aedeagal lobe, which is difficult to describe, but nevertheless the internal part of aedeagal lobe in *L. prima* is finger-like, without a "heel" basally (Figs. 4–6), and in *L. yezoensis* it is also finger-like but with a large, noticeably protruding "heel" basally (Figs. 3, 7–8). It should be noted that the inner part of the aedeagal lobe of *L. yezoensis* may sometimes be invisible (Fig. 3) or difficult to distinguish (Fig. 4).

Distribution. Known from type locality in Hokkaido (Japan) and it is possible that this species lives on Kunashir Island (Kurile Islands) and in Oriental China (Sun *et al.* 2019), but this can only be confirmed after DNA barcoding of specimens from these places.



FIGURES 5–8. Males of *Linevitshia prima* Makarchenko **(5–6)** and *L. yezoensis* Endo **(7–8)**. **5–7,** endoskeleton; **8,** phallapodeme and aedeagal lobe. IEL—inner part of aedeagal lobe; TSa—transverse sternapodeme; Pha—phallapodeme. Scale bars are 50 μm.

TABLE 2. Comparison of adult males characters of *Linevitshia yezoensis* Endo and *L. prima* Makarchenko.

Characters	*Linevitshia yezoensis* Endo n=14 (Endo *et al.* 2007and orig.)	*L. prima* Makarchenko n=8 (Makarchenko 1987, 2014 and orig,)
Total length, mm	3.0–4.1	3.8–5.4
Wing length, mm	2.1–2.3	3.2–3.3
TL/WL	1.3–1.5	1.1–1.5
AR	1.0–1.11	1.10–1.25
Antepronotals	4–9 dorsal/14–21 lateral	2–6 dorsal, 8–19 lateral
Acrostichals	17–33	17–19
Dorsocentrals	11–16	17–21
Prealars	6–9	8–10
Su	1–3	3

.....continued on the next page

TABLE 2. (Continued)

Characters	*Linevitshia yezoensis* Endo n=14 (Endo *et al.* 2007and orig.)	*L. prima* Makarchenko n=8 (Makarchenko 1987, 2014 and orig,)
Scutellars	10–16	14–17
PAII setae	3–5	4–6
EII setae	3–8	8
Alula setae	4–9	4–8
Squamal setae	18–25	25–73
LR_1	0.66–0.76	0.68–0.73
BV_1	3.18–3.40	2.74–3.28
SV_1	2.51–2.90	2.53–2.76
Tergite IX, number of setae	9–14	11–18
TSA length, μm	128–164	180–228
TSA width, μm	32–56	24–46
TSA length/ TSA width	3.0–4.5	5.0–5.9
Phallapodeme length, μm	96–120	92–132
Inner part of aedeagal lobe	with "heel"	without "heel"
Gonostylus length, μm	88–96	112–124
Megaseta of gonostylus	1–2	1–4
Gonocoxite, length, μm	160–180	200–288
HR	1.70–2.40	1.79–2.32

Results of DNA barcoding

In this study we obtained 3 DNA barcodes of cytochrome c oxidase subunit I (658 bp) belonging to the described species *L. yezoensis.* Intraspecific sequence divergence within these taxa were 0.2%. The mean intraspecific distances between *L. yezoensis* and *L. prima* were 12.9%. Such values correspond to species level differences for other chironomid subfamilies (e.g. Montagna *et al.* 2016, Makarchenko *et al.* 2022a, 2022b, 2023), but species boundary calculations for the Protanypodinae subfamily are not available.

The maximum likelihood tree (Fig. 9) using *Protanypus morio* Zetterstedt and *P. caudatus* Edwards as outgroups support monophyly (bootstrap support values = 100) of the genus *Linevitshia* as well as *L. yezoensis.*

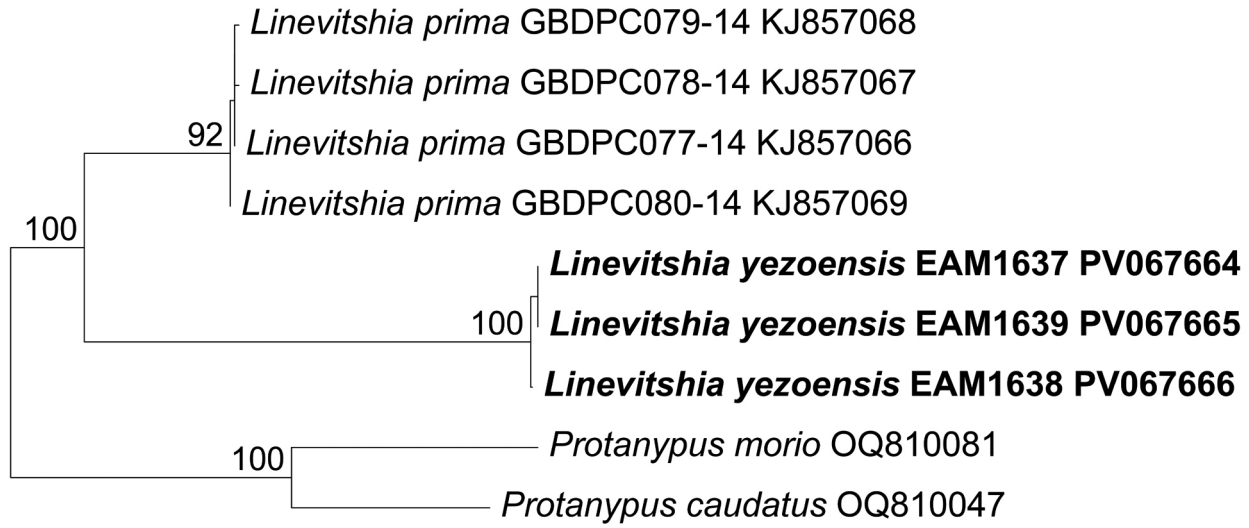


FIGURE 9. Maximum likelihood tree based on the COI nucleotide sequence data of the genus *Linevitshia* Makarchenko with two species of *Protanypus* Kieffer as outgroups. Bootstrap support values (higher than 70%) are given above the tree nodes. The sequences obtained in this study are in BOLD.

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