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Morphological description and DNA barcoding of *Paralimnophyes dolgikh* sp.n. (Diptera, Chironomidae, Orthocladiinae) from the Bolshekhehtsyrsky Nature Reserve (Khabarovsk Territory, Russian Far East)

Морфологическое описание и ДНК-анализ *Paralimnophyes dolgikh* sp.n. (Diptera, Chironomidae, Orthocladiinae) из Болшехехцирского государственного природного заповедника (Хабаровский край, российский Дальний Восток)

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***Key words*:** Diptera, Chironomidae, Orthocladiinae, *Paralimnophyes,* new species, taxonomy, DNA barcoding, Bolshekhehtsyrsky Nature Reserve, Russian Far East.

Ключевые слова: Diptera, Chironomidae, Orthocladiinae, *Paralimnophyes,* новый вид, таксономия, ДНК-анализ, Большехехцирский природный заповедник, российский Дальний Восток.

Abstract. Illustrated description of adult male, as well as DNA barcoding, of *Paralimnophyes dolgikh* **sp.n.** from the Bolshekhehtsyrsky Nature Reserve of the Khabarovsk Territory (Russian Far East) are provided. Genus *Paralimnophyes* Brundin is recorded for the first time for this nature reserve.

Резюме. Приведено иллюстрированное описание, а также ДНК-анализ, нового для науки вида хирономид *Paralimnophyes dolgikh* **sp.n.** из Большехехцирского государственного природного заповедника Хабаровского края российского Дальнего Востока. Род *Paralimnophyes* Brundin впервые приводится для фауны хирономид этого заповедника.

Introduction

The chironomid fauna of the Bolshekhehtsyrsky Nature Reserve numbers more than 65 species of which at least 55 species belongs to the subfamily Orthocladiinae [Yavorskaya, Makarchenko, 2009; Makarchenko et al., 2015]. In May 2015 in this nature reserve for the first time was collected chironomids of the genus *Paralimnophyes* Brundin with the new species *P. dolgikh* sp.n. The morphological description and results of DNA barcoding of this species we present below.

Materials and methods

The terminology follows Sæther [1980]. The material was preserved in 96 % ethanol for DNA-analysis and in 70 % ethanol for further study of morphology and slide-mounting, following the methods by Makarchenko [1985].

Holotype and paratypes of the new species are deposited in the Institute of Biology and Soil Sciences, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia (IBSS FEBRAS).

For molecular analysis were used three males. Reference DNA sequence database, based on partial sequenced from mitochondrial COI gene were obtained by preparing a 50 µl total DNA extract from body of insect using the Invitrogen (Invitrogen corp, Carlsbad, CA, 2007) protocol. The primers for amplification of the 658 bp fragment were LCO1490 (5'-GGTCAACAAATCATAAAGAT ATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'), obtained from Folmer et al.,1994. PCR reaction for this fragment was run in total volume of 10 µl with 5 µl Go Taq Green Master Mix (Promega corp, Madison, WI, USA), 0.5 µl of each primer (100 ng/µl), 3 µl 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, according to the PCR conditions in P.D.N. Hebert [Hebert et al., 2003]. Each PCR fragment was purified using Exonuclease I (ExoI) and Thermosensitive Alkaline Phosphatase (FastAP) (Thermo Fisher Scientific Inc., USA) according to protocols and sequenced on an ABI 3130x (Applied Biosystems) automated sequencer using BigDye terminator v3.1 cycle kit methods. Forward and reverse sequences were aligned and manually edited in MEGA 6.06 [Tamura et al., 2013]. Sequences of *P. dolgikh* sp.n. have been submitted to Gen-Bank by numbers (KT361664–KT361666).

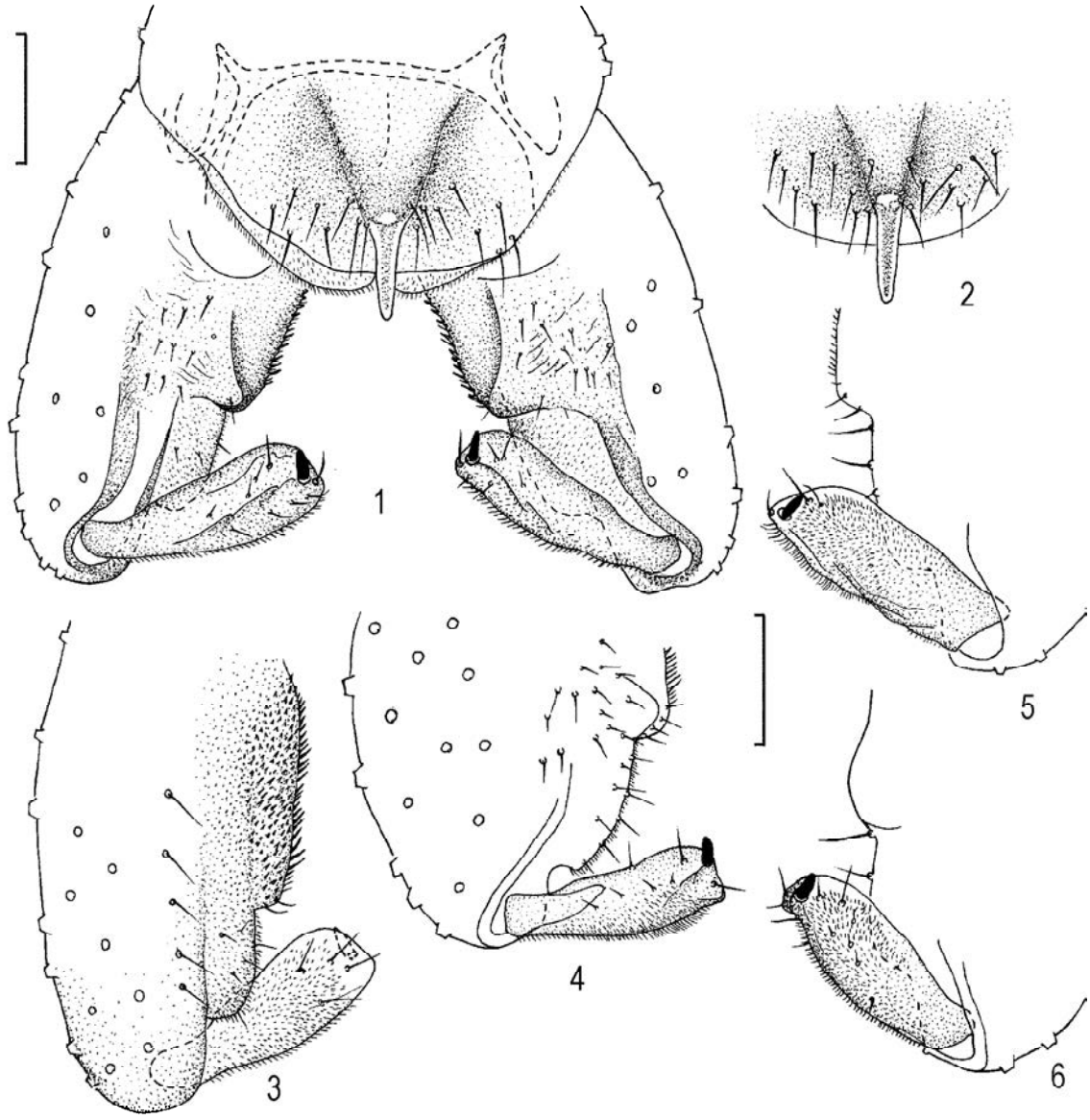
Description

Paralimnophyes dolhikh Makarchenko et Makarchenko, **sp.n.**

Figs. 1–6.

Material. Holotype: adult male, **Russian Far East: *Khabarovsk Territory***, Bolshekhehtsyrsky Nature Reserve, Golovina Stream, Ussuri River basin, N 48°11'131", E 134°41'039", 13.V.2015, leg. N. Yavorskaya. Paratypes: 5 adult males, the same data as holotype, 13.V.2015, leg. N. Yavorskaya.

Etymology. The species is named in honor of Aleksander Mikhailovitch Dolgikh, scientific researcher of the Bolshekhehtsyrsky Nature Reserve, who helped during some years in collecting of aquatic insects.



Figs 1–6. Adult male of *Paralimnophyes dolgikh* sp.n. 1 — hypopygium in dorsal view; 2 — posterior part of tergite IX; 3 — gonocoxite and gonostylus in ventral view; 4 — part of gonocoxite and gonostylus in dorsal view; 5–6 — gonostylus. Scale bars — 50 μm.

Рис. 1–6. Имаго самец *Paralimnophyes dolgikh* sp.n. 1 — гипопигий, вид сверху; 2 — задний край тергита IX; 3 — гонококсит и гоностиль, вид снизу; 4 — часть гонококсита и гоностиль, вид сверху; 5–6 — гоностиль. Масштабная линейка — 50 мкм.

Table 1. Length (μm) and proportions of leg segments of *Paralimnophyes dolgikh* sp.n., male (n = 3)
Таблица 1. Длина члеников ног (мкм) и их индексы самца *Paralimnophyes dolgikh* sp.n. (n = 3)

P	f	t	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV
P_1	608–688	832–928	448–496	288–320	184–224	112	96	0.53–0.54	2.70–2.87	3.21–3.28
P_2	704–768	768–864	336–400	192–224	144–160	96	88–96	0.44–0.47	3.37–3.70	4.08–4.38
P_3	688–752	848–944	456–512	240–256	200–224	104–112	96	0.54	3.09–3.21	3.31–3.37

Description

Male imago (n = 4). Total length 2.7–2.9 mm. Wing length 1.8–1.92 mm. Total length/wing length 1.46–1.51.

Head. Eyes bare, without dorsomedian prolongations. Temporal setae 8–11, including 2 verticals and 2–6 postorbitals. Clypeus with 10–16 setae. Antenna with 13 flagellomeres and well developed plume; apex of 13th flagellomere with pointed apex and subapical seta. AR 0.97–1.03. Length of 5 palpomeres (in μm): 24–32, 48–56, 84–88, 96–100, 136–144.

Thorax. Dark brown. Antepronotal lobes separated in anterior middle part, with 1–3 lateral setae. Acrostichals 8–10, short and scalpellate, restricted to mid-thorax, in 1–2 rows; dorsocentrals 11–16; prealars 3–4, supraalars 1; scutellum with 8–9 setae in one row,

Wing. Greyish and speckled. R with 4–6 setae, R_1 and R_{4+5} without setae. R_{4+5} ending little distal of apex M_{3+4}. Costa extension 80–96 μm. Anal lobe well developed, rectangular-rounded. Squama with 7–10 setae.

Legs. BR_1 2.0–2.8, BR_2 2.3–2.8, BR_3 2.5–3.2. Spur of fore tibia 48–60 μm long. Spurs of mid tibia 16–20 μm long. Spurs of hind tibia 40–48 μm and 16–20 μm long. Hind tibial comb with 9–11 setae. Pseudospurs absent. Mid leg with 4 sensilla chaetica in apical half; hind leg with 1–5 sensilla chaetica on ta_1; one male without sensilla chaetica on ta_1 of mid and hind legs. Pulvilli as groups of short setae. Lengths and proportions of legs as in Table 1.

Hypopygium (Figs. 1–6). Anal point hyaline, 32–40 μm long, bare, subparallel-sided, located at the center of tergite IX on convexity; around anal point 16–21 setae (Figs. 1–2) .Laterosternite IX with 3–4 setae on each side. Transverse sternapodeme slightly convex, 112–124 μm long, with high oral projections (Fig. 1). Virga absent. Gonocoxite 228–244 μm long; inferior volsella consists of two lobes, of which anterior lobe (L_1 after Brundin, 1956) rectangular-rounded (Figs 1, 4), ventrally covered by spine-like setae (Fig. 3); posterior lobe (L_2 after Brundin, 1956) more narrow, covered by microtrichiae and some short setae. Gonostylus 92–96 μm long, more narrow at the base and gradually expanded to the top, without crista dorsalis; megaseta 12 μm long (Figs 4–6). HR 2.37–2.65.

Pupa and larva unknown.

Diagnosis. Total length 2.7–2.9 mm. Wing length 1.8–1.92 mm. AR 0.97–1.03. Acrostichals short and scalpellate, restricted to mid-thorax, in 1–2 rows. LR_1 0.53–0.54. Pulvilli as groups of short setae. Anal point hyaline, bare, subparallel-sided, located at the center of tergite IX on convexity; around anal point 16–21 setae. Transverse sternapodeme slightly convex, with high oral projections. Inferior volsella consists of two lobes, of which anterior lobe (L_1) rectangular-rounded, ventrally covered by spine-like setae; posterior lobe (L_2). more narrow, covered by microtrichiae and some short setae. Gonostylus more narrow at the base and gradually expanded to the top, without crista dorsalis, in subapical part without microtrichiae.

Диагноз. Длина тела 2,7–2,9 мм. Длина крыла 1,8–1,92 мм. AR 0,97–1,03. Акростихальные щетинки короткие и Г-образные, расположены в 1–2 ряда в середине среднеспинки. LR_1 0,53–0,54. Пульвиллы в виде группы коротких щетинок. Анальный отросток гипопигия бледный и голый, почти параллельно-сторонний, расположен в середине тергита IX на выпуклости; вокруг анального отростка 16–21 щетинка. Поперечная стернаподема немного выпуклая, с высокими оральными выступами. Нижний придаток гонококсита состоит из двух частей, передняя из которых (L_1 по: Brundin, 1956) более крупная, прямоугольно-округлой формы, вентрально покрыта шиповидными щетинками; задняя часть (L2 по: Brundin, 1956) более узкая, покрыта микротрихиями и короткими щетинками. Гоностиль более узкий в базальной части, к вершине расширяется, без кристы, только с терминальным шипом, субапикально без микротрихий

Remarks. The genus *Paralimnophyes* was established by Brundin [1956] for *Camptocladius hydrophilus* Goetghebuer, 1921 (=*Camptocladius longiseta* Thienemann, 1919). At the present time the genus includes six species, two of which are distributed in Australia, one is occurred in the northern part the Holarctic region, one is found in many parts of the Palaearctic region, one is recorded in Oriental China and one indeterminate species is registered from Mexico [Wang, Sæther, 2002; Sæther, 2004; Makarchenko, Makarchenko, 2008; Ashe, O'Connor, 2012]. Two species, *P. trilineatus* (Lundstrom, 1915) and *P. longiseta* (Thienemann, 1919) were known for Russia [Makarchenko, Makarchenko, 2008; Ashe, O'Connor, 2012]. Male of *P. dolgikh* sp.n. is close related to the same of *P. trilineatus* and *P. longiseta* but good separated from both species by shape of gonostylus and posterior lobe (L_2) of inferior volsella. New species has more long and more narrow posterior lobe (L_2) of inferior volsella and gonostylus with more narrow basal part, without crista dorsalis and with microtrichiae in subapical part.

Results of DNA barcoding

The final alignment of the COI gene yielded 658 bp for 3 individuals of *P. dolgikh* sp.n. The nucleotide composition of the studied sequences of *P. dolgikh* sp.n. COI gene fragments deviated from an equilibrium one, comprising 26.4 % of A, 38.0 % of T, 17.8 % of C, and 17.8 % of G. All three individuals relate to one haplotype.

Gen-Bank contains another species of the genus *Paralimnophyes*. P-distance between *P. dolgikh* **sp.n.** and *Paralimnophyes* sp. (*KC750464-KC750466*) is

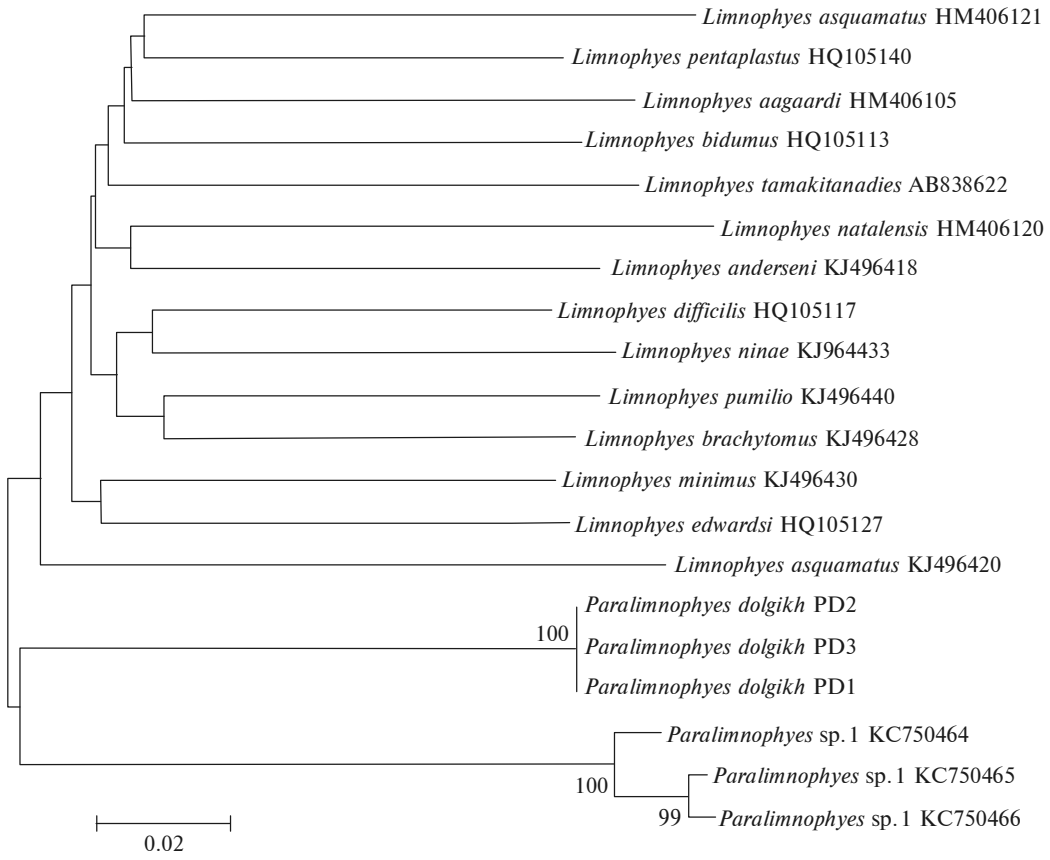


Fig 7. Taxon tree from Neighbour Joining analysis on Maximum Composite Likelihood model using 1000 bootstrap replicates. Bootstrap values >70 are given on branches.

Рис. 7. Филогенетическое дерево, построенное методом ближайшего связывания с использованием модели максимального правдоподобия с бутстреп-поддержкой (1000 репликаций). Бутстреп-поддержка в 70 % и выше дана в соответствующих узлах ветвления.

equal to 16.2 %. The neighbour joining tree of *P. dolgikh* **sp.n.**, *Paralimnophyes* sp. and species of the genus *Limnophyes* from the GenBank is shown in Fig. 7.

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