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MORPHOLOGICAL REDESCRIPTION AND DNA BARCODING OF *MONODIAMESA KAMORA* MAKARCHENKO ET YAVORSKAYA (DIPTERA: CHIRONOMIDAE) FROM THE AMUR RIVER BASIN (RUSSIAN FAR EAST)

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Summary. Illustrated redescription of adult male as well as the results of DNA barcoding of *Monodiamesa kamora* Makarchenko et Yavorskaya in comparison with known species of the genus *Monodiamesa* Kieffer from the low part of Amur River basin in the Russian Far East are provided. 658 bp fragment of the mitochondrial gene cytochrome oxidase I (COI) was used for distinguishing *M. kamora*, *M. bathyphila* Kieffer and all species of subfamily Prodiamesinae available in GenBank. Interspecific distance between *M. kamora* and *M. bathyphila* ranged from 11.9 to 12.5% (mean 12.1%) and these values are sufficient to maintain the species level. Moreover, two independent groups of *M. bathyphila* were determined. We suppose that it is result of incorrect definition of the species.

Key words: Diptera, Chironomidae, Prodiamesinae, *Monodiamesa*, taxonomy, DNA barcoding, Amur River basin, Russia.

А. Е. Макаrenchенко, О. А. Веляев, Н. М. Яворская. Морфологическое переписание и ДНК баркодирование вида *Monodiamesa kamora* Makarchenko et Yavorskaya (Diptera: Chironomidae) из бассейна реки Амур, Дальний Восток // Дальневосточный энтомолог. 2018. N 350. С. 1-8.

Резюме. По материалу из бассейна Нижнего Амура приведены иллюстрированное переписание и результаты анализа фрагмента гена цитохром-оксидазы субъединицы I (658 пн.) мтДНК вида хирономид *Monodiamesa kamora* Makarchenko et Yavorskaya, проведено сравнение с известными видами рода *Monodiamesa* Kieffer. Генетический анализ показал видовую самостоятельность *M. kamora* и его отличие от *M. bathyphila* Kieffer, а также других представителей подсемейства Prodiamesinae, последовательности которых доступны для COI в генетическом банке (GenBank). Межвидовые дистанции (K2P) *M. kamora* и *M. bathyphila* находятся в пределах 11.9–12.5% (в среднем 12.1%), что достаточно для поддержания видового статуса хирономид. С использованием байесовского дерева установлены две высоко обособленные клады *M. bathyphila*. По-видимому, это связано с ошибочной видовой идентификацией одной из двух форм.

INTRODUCTION

The genus *Monodiamesa* was erected by Kieffer (1922) for males which have bare eyes with parallel-sided dorsomedial extensions, apex of third palpal segment with 2 weak medial sensilla clavata, acrostichals absent, supraalars present or occasionally absent, MCu reaching M at distance basally of RM which is less than own, anal point well developed, with basal microtrichia and setae, inferior volsella very long, flat and lobe-like, superior volsella well developed, median volsella with long, sclerotized spine-like seta, sometimes placed on a tubercle or pedestal, gonostylus simple. At the present time the genus *Monodiamesa* includes 8 species from the Palaearctic region, 4 species from Nearctic and 1 species from Neotropical Region (Ashe & O'Connor, 2009). Four species, *M. bathyphila* (Kieffer), *M. improvisa* Makarchenko, *M. nitida* (Kieffer) and *M. kamora* Makarchenko et Yavorskaya we recorded for the Russian Far East (Makarchenko, 2006; Ashe & O'Connor, 2009), the latter of which has been described only by a single adult male from the Lower Amur River basin. Rich additional imaginal material was collected by N.M. Yavorskaya in the Bolon Nature Reserve of the Lower Amur River in May 2016. 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 *M. kamora* from low part of Amur River basin of the Russian Far East as well as give the results of DNA barcoding in comparison with the closely related species of *Monodiamesa* Kieffer and all other species of subfamily Prodiamesinae available in GenBank.

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).

Photographs were taken by Dr. A.A. Semchenko at the microscope Olympus BX53 + DeltaPix Invenio-8DII of the Interdepartmental laboratory "Biology of Marine Invertebrates", School of Natural Sciences, Far Eastern Federal University

DNA was extracted from parts of preserved in 96% ethanol specimens using the Qiagen DNeasy Blood & Tissue kit in accordance with the protocols. A fragment of one mitochondrial gene COI was amplified with the primers COIF-ALT (5'-ACAAATCAYAARGAYATYGG-3') and COIR-ALT (5'-TTCAGGRTGNCCRAARAAYCA-3'), which were obtained from Mikkelsen *et al.* (2006). PCR was performed in a total volume of 10 µl, with the reaction mixture containing 5 µl of Go Taq Green Master Mix (Promega), 0.375 µl of each primer (100 ng/µl), 3.75 µl nuclease-free water and 0.5 µl of purified DNA. The PCR thermal regime consisted of initial denaturation at 94 °C (2.5 min), followed by 35 cycles at 95 °C (denaturation, 30 s), 50 °C (annealing, 30 s), 72 °C (extension, 70 s), and a final cycle at 72 °C (10 min). PCR products were visualized on a 1.5% agarose gel and only positive PCR products were purified for cycle sequencing using Exonuclease I (ExoI) and Thermosensitive Alkaline Phosphatase (FastAP) by ThermoFisher Scientific. A BigDye Cycle Sequencing Kit (Applied Biosystems) was used to analyze the PCR products with an ABI 3130xl sequencer.

We used MEGA7 (Kumar *et al.*, 2016) to edit and assemble double stranded sequences. Sequences of COI of four specimens of *Monodiamesa kamora*, one specimen of *M. bathyphila* and all sequences of COI of Prodiamesinae, that available in GenBank (NCBI), was used in phylogenetic analysis (Fig. 6). Based on the Kimura-2-Parameter (K2P) model were calculated inter- and intraspecific genetic distances. The Bayesian inference method was performed using MrBayes ver. 3.2.6.

Material of the *M. kamora* as well as *M. bathyphila* species 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). DNA sequences of four specimens of *Monodiamesa kamora* are available in GenBank (NCBI) by numbers MG189587-MG189590 and *M. bathyphila* available by number MG520095.

MORPHOLOGICAL REDESCRIPTION

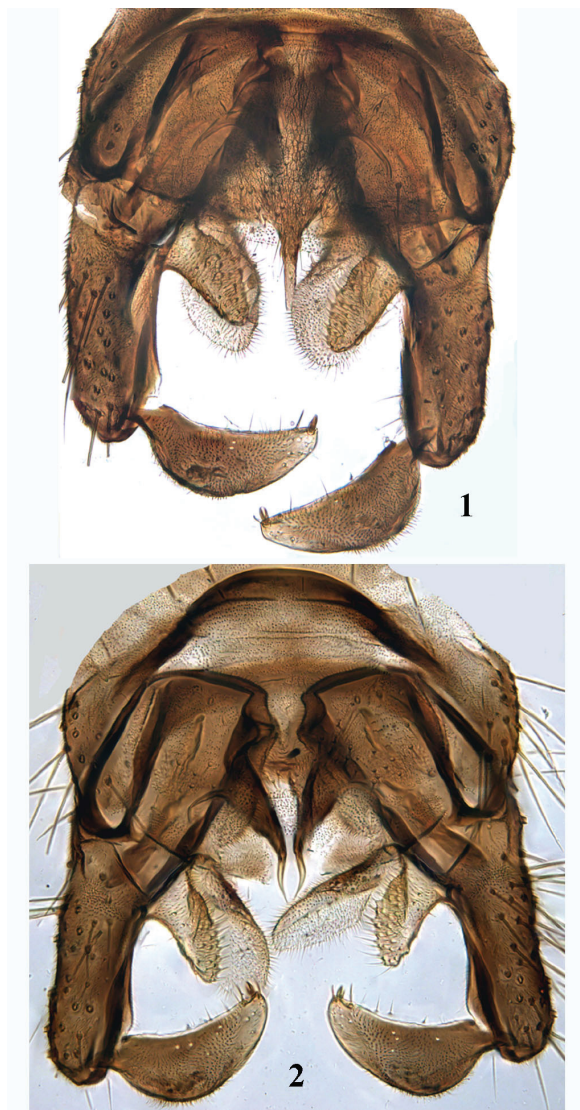
Monodiamesa kamora Makarchenko et Yavorskaya, 2008

Figs 1–5

Monodiamesa kamora Makarchenko et Yavorskaya, 2008: 175–176.

MATERIAL EXAMINED. **Russia:** Khabarovsk Territory: Nikolayevsk District, left bank of Amur River, Kamora River in environs of the city Nikolaevsk-on-Amur,

24.VI 2007, 1♂ (holotype), leg. N. Yavorskaya; Amurskiy District, Bolon Nature Reserve, Simmi River near cordon Cheremshinaya, 25.V 2016, 58♂, leg. N. Yavorskaya; the same river near cordon Kirpu, 25.V 2016, 18♂, leg. N. Yavorskaya; the same river near cordon Vakhtar, 26.V 2016, 6♂, leg. N. Yavorskaya; the same Nature Reserve, Selgon River, 26.V 2016, 24♂, leg. N. Yavorskaya.

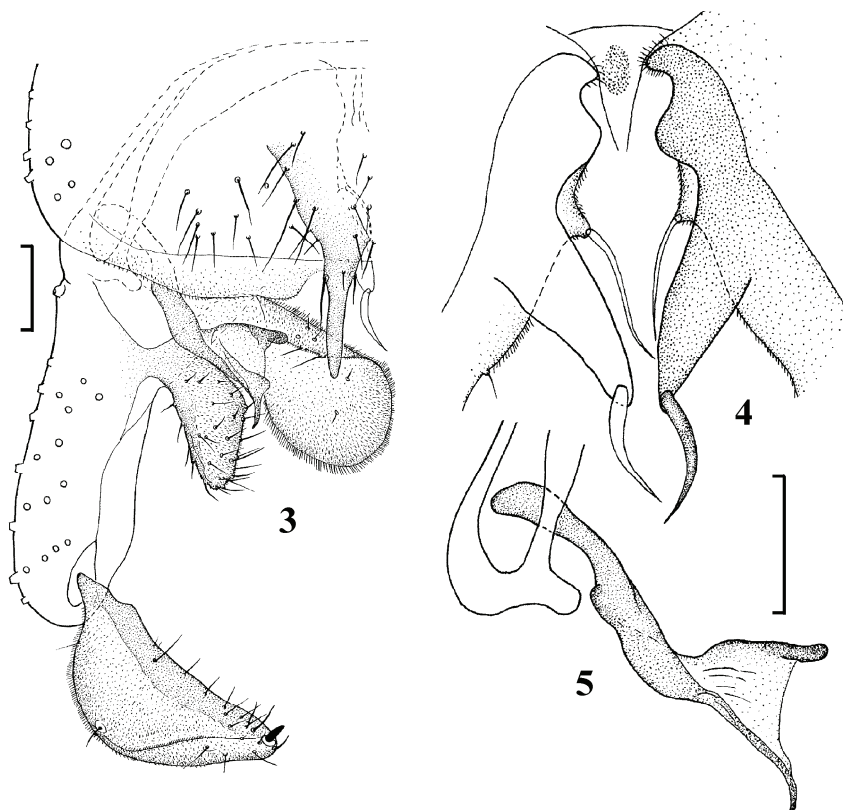


Figs 1–2. Hypopygium of *Monodiamesa kamora* (paratype). 1 – total view, from above; 2 – the same, from below.

REDESCRIPTION. *Male imago* (n=5). Total length 3.9–5.1 mm. Total length/wing length 1.27–1.47. Coloration dark-brown.

Head. Temporal setae including 2–4 verticals and 6–7 postorbitals. Clypeus with 15–16 setae. Palpomere length (μm): 32–52, 84–104, 160–164, 140–152, 204–220. Head width/palpal length 1.–1.31. Antenna with 13 flagellomeres, AR 1.75–2.08.

Thorax. Antepronotum with 4–10 lateral setae. Acrostichals absent, dorsocentrals 18–23 (in 1–2 rows), prealars 8, supraalars 1. Scutellum with 41–55 setae.



Figs 3–5. Hypopygium of *Monodiamesa kamora* (holotype). 3 – total view, from above; 4 – median volsellae and basal setae; 5 – phallapodema and aedeagal lobe. Scale bars = 50 μm.

Wing. Length 3.04–3.16 mm. Anal lobe well developed, roundish and projected forward. Squama with 27–32 setae in 1–2 rows. R and R₁ with 27–33 setae, R₄₊₅ with 2–4 setae in subapical part. Costal projection 80–82 μm long.

Legs. Femur of all legs dark-brown, tibia and tarsus visibly lighter. BR₁ 2.10–2.33; BR₂ 1.27–2.22; BR₃ 1.56–3.0. Spur of front tibia 64–92 μm long. Both spurs of middle tibia 68–72 μm long. Spurs of hind tibia 96–112 μm and 71–84 μm long. Hind tibial comb with 11–13 spine-like setae. Pulvilli spine-like. Middle and hind

legs with 2 pseudospurs in apical part ta_1 and ta_2 32–36 μm long. 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	1058–	1328–	951–	508–	377–	213–	180–	0.67–	2.56–	2.49–
	1148	1460	1033	574	402	221	197	0.72	2.65	2.65
P ₂	1164–	1378–	566–	361–	262–	172–	180–	0.41–	3.12–	4.13–
	1246	1427	623	394	279	180	187	0.45	3.19	4.49
P ₃	1230–	1558–	754–	476–	344–	197	197–	0.48–	2.89–	3.38–
	1263	1640	853	508	361	213	213	0.53	3.0	3.72

Hypopygium (Figs 1–5). Tergite IX with anal point 72–88 μm long and with 46–49 setae, some of that situated in basal part of anal point; laterosternite IX with 12–13 setae. Gonostylus in middle outer part prominent, 152–156 μm long, with megaseta 64 μm long and 1–2 setae nearest 80 μm long. Gonocoxite 356–368 μm long, shape of superior and inferior volsellae as in Figs 1–3. Length of superior volsella 92–104 μm , inferior volsella 124–132 μm . Median volsella setae-like, slightly curved 40–44 μm long and situated on dark-brown pedestal 84–104 μm long. Near of median volsella in antero-ventral part situated seta 52–60 μm long on small tubercle (Fig. 4). Phallapodeme with aedeagal lobe as in Fig. 5.

REMARKS. *M. kamora* belongs to the group of species *bathyphila*. Male imagines of *M. kamora* is close related to *M. alpicola* Brundin and *M. bathyphila* Kieffer but can be separated from these species by shape of gonostylus which in middle outer part prominent. Median volsella spine-like, slightly curved and situated on long pedestal. Near of median volsella in antero-ventral part situated seta on small tubercle. Inferior volsella in distal part wide-roundish and just a little longer of superior volsella.

DISTRIBUTION. Russia (the low part of Amur River basin).

RESULTS OF DNA BARCODING

The final alignment of the COI gene yielded 658 bp for *Monodiamesa kamora*. We obtained 658 bp of COI gene. Intraspecific sequence divergence ranged from 0.0 to 0.76% with a mean divergence value of 0.5%, which is within the normal range for chironomids (Montagna *et al.*, 2016). Within the specimens of *M. kamora*, four haplotypes were encountered, which are divergent by eight synonymous transitional substitutions. Interspecific distance between *M. kamora* and two independent groups of *M. bathyphila* (Fig. 6) ranged from 11.9 to 12.5% (mean 12.1%). According to Montagna *et al.* (2016) and Ekrem *et al.* (2010) these values are sufficient to maintain the species level. The Bayesian tree contains all available species of subfamily Prodiamesinae shown in Fig. 6. The monophyly of the species *M. kamora* strongly supported, but *M. bathyphila* formed paraphyletic group contains one specimen from South Korea (Kim *et al.*, 2012) and 4 specimens from Great Britain (Bista *et al.*, 2017). To solve the revealed contradiction, we obtained a sequence of the *M. bathyphila* which was identical to acc. number JN887070 from Korea. We suppose that the chironomids by numbers KY225334, KY225363, KY225355, KY225354 have incorrect definition of the species because were identified only by

larvae which in representatives of this genus are very poorly different and are usually referred to the *bathyphila* group species.

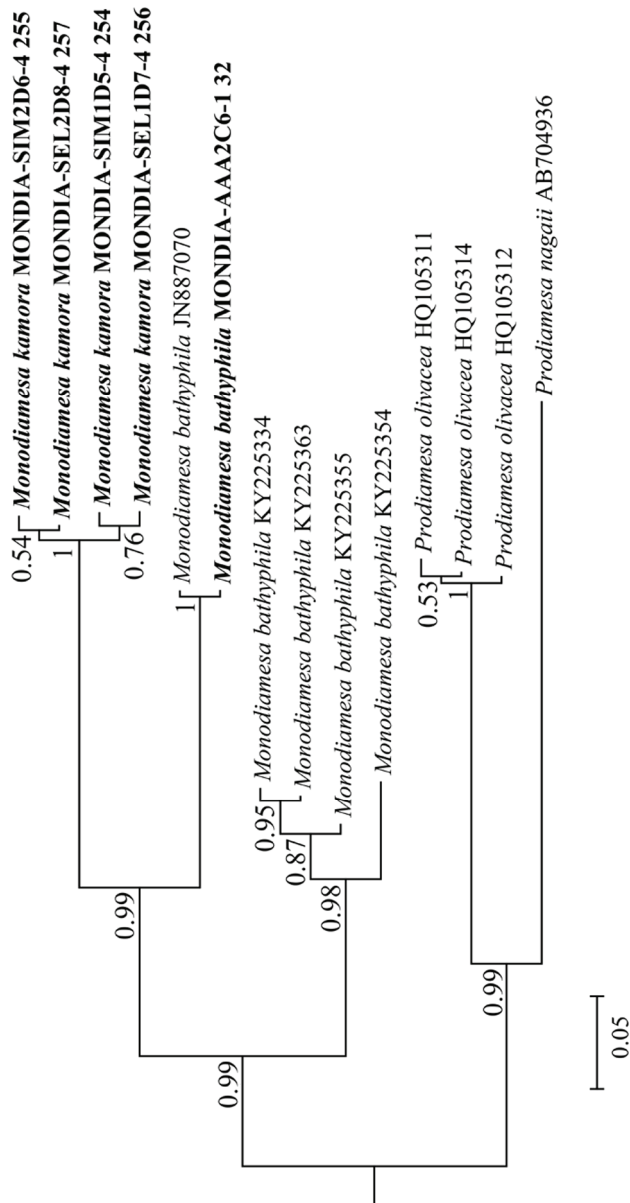


Fig. 6. Bayesian tree of chironomids subfamily Prodiamesinae based on the mitochondrial cytochrome c oxidase I (COI) barcode gene sequences (658 bp). Numbers are Bayesian posterior probability. Specimens obtained in this study are in bold. According to Kobayashi & Endo (2008) the species *Prodiamesa nagaii* Sasa et Kawai, 1985 that is given in tree is synonym of *Prodiamesa levanidovae* Makarchenko, 1982.

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