Zootaxa 4000 (2): 287–293
www.mapress.com/zootaxa/

Correspondence

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.4000.2.7
http://zoobank.org/urn:lsid:zoobank.org:pub:750409C6-148B-4032-81F3-F78A4F440B19

Morphological description and DNA barcoding of *Hydrobaenus majus* sp. nov. (Diptera: Chironomidae: Orthocladiinae) from the Russian Far East

EUGENYI A. MAKARCHENKO[1,3], MARINA A. MAKARCHENKO[1] & ALEXANDER A. SEMENCHENKO[2]

[1]*Institute of Biology and Soil Science, Far East Branch of the Russian Academy of Sciences, 100 let Vladivostoku 159, 690022 Vladiv ostok, Russia. E-mail: makarchenko@biosoil.ru*

[2]*Far Eastern Federal University, Suhanova St. 8, 690950 Vladivostok, Russia. semenchenko_alexander@mail.ru*

[3]*Corresponding author*

Abstract. Illustrated descriptions of adult male, pupa and fourth instar larva, as well as DNA barcoding, of *Hydrobaenus majus* **sp. nov.** in comparison with the close related species *H. sikhotealinensis* Makarchenko et Makarchenko from the Russian Far East are provided. The species-specificity of *H. majus* **sp. nov.** COI sequences is analyzed and the sequences are presented as diagnostic characters—molecular markers of *H. majus* and *H. sikhotealinensis*.

Key words: Diptera, Chironomidae, *Hydrobaenus,* new species, taxonomy, DNA barcoding, Russian Far East

Introduction

In the spring of 2013–2015 we collected adult males of *Hydrobaenus* Fries species in the Southern Primorye and the Amur River basin of the Russian Far East, which are close related to *H. sikhotealinensis* Makarchenko et Makarchenko and not readily separable from the latter. However, after analyzing the pupae and larvae of this species, as well as DNA barcoding, it was found that it is a new species, *Hydrobaenus majus* **sp. nov**. The morphological description and results of DNA barcoding of this species in comparison with the same of *H. sikhotealinensis* are presented below.

Material and methods

Larva, pupa and adults of *H. majus* were associated by rearing larvae individually to the mature pupae and from the pupae to adults. For morphological comparison, the analysed adult males of *H. majus* and *H. sikhotealinensis* were the same used for DNA barcoding.

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).

Total genomic DNA was extracted from the samples using the Invitrogen (Invitrogen corp, Carlsbad, CA, 2007) protocol. The DNA precipitate was resuspended in TE buffer then stored in -20ºC. The polymerase chain reaction (PCR) was used to amplify fragment of COI gene (mtDNA). The primers for amplification of the COI fragment were LCO1490 (5'-GGTCAACAAATCATAAAGAT ATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'), according to 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 *et al.* 2003. Each fragment was purified using ethanol precipitated, air-dried and cycle 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. The taxon tree was generated using neighbour joining analysis and 1000 bootstrap replicates on Maximum Composite

Accepted by B. Rossaro: 20 Jul. 2015; published: 14 Aug. 2015