Identification of an enigmatic lycaenid specimen (Lepidoptera: Lycaenidae) from Sumatra, Indonesia

Идентификация неопознанного экземпляра голубянки (Lepidoptera: Lycaenidae) с Суматры, Индонезия

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Ключевые слова: Lepidoptera, Lycaenidae, неопознанный экземпляр, *Prosotas lutea*, ITS2, последовательности ДНК.

Abstract. With the help of DNA analysis, an enigmatic lycaenid butterfly from Sumatra belonging to the genus *Prosotas* is determined as *Prosotas lutea* (Martin, 1895).

Резюме. С помощью анализа ДНК неопознанная голубянка из Суматры определена как *Prosotas lutea* (Martin, 1895).

Introduction

During an investigation of the entomofauna of Sumatra in 2016, Valentin Tikhonov collected a most unusual lycaenid butterfly (Color plate 7: 1, 2). The specimen is characterized by the complete reduction of its underside pattern, usually consisting of numerous striae, but with the help of genitalic dissection, was determined as belonging to the genus *Prosotas* Druce, 1891 (Color plate 7: 3).

To obtain a reliable determination, we decided to analyse molecular sequences and compared them with ITS2 sequences (Internal Transcribed Spacer 2) known from various *Prosotas* species: *P. lutea* (Martin, 1895) (Color plate 7: 4, 5), *P. gracilis* (Röber, 1886), *P. pia* Toxopeus, 1929 and *P. dubiosa* (Semper, [1879]). We used the ITS2 sequence because of its great variability, providing significant differences at species level.

Material and methods

Material. 1♂, *Prosotas* sp.: Indonesia, Ketambe vill., Sumatra, 28.02.2016 (V. Tikhonov), (Voucher No. ILL253, GenBank accession numbers KX083692); 1♂, *P. lutea:* Myanmar, Hsipaw, Tu River, 1.08.2005 (A. Sokolov), (Voucher No. ILL282, GenBank accession numbers MG551471); 1♂, *P. gracilis*: Indonesia, Ketambe vill., Sumatra, 28.02.2016, (V. Tikhonov), (Voucher No. ILL256, GenBank accession numbers KX083689); 1♂, *P. pia*: Malaysia, Sabah, Borneo, 14.02.2014 (D. Pozhogin), (Voucher No. ILL197, GenBank accession numbers KJ934122); 1♂, *P. dubiosa*: Thailand, Pai, 1.04.2014 (I. Uchevatov), (Voucher No. ILL193, GenBank accession numbers KJ774016).

We amplified DNA 5' section of the nuclear noncoding sequence ITS2 on the Mastercycler gradient (Eppendorf). The following cycling protocols were used: an initial 4 min denaturation at 95 °C and 40 cycles of 30 s denaturation at 95 °C, 30 s annealing at 53 °C and 60 s extension at 72 °C.

We used the following PCR primer pairs: with forward, 5'-GGG CCG GCT GTA TAA AAT CAT A-3' and reverse, 5'-AAA AAT TGA GGC AGA CGC GAT A-3' were used to amplify ITS2 [Stradomsky, 2016].

Amplified fragments were separated using an automated sequencing machine (Applied Biosystems 3500).

The analysis of primary nucleotide sequences was made with the help of the application BioEdit Sequence Alignment Editor, version 7.0.5.3 [Hall, 1999].

ITS2 nucleotide sequences were treated quantitatively using MEGA5 [Tamura et al., 2011] methods Minimum Evolution (ME) and were represented as ME-cladogram.

As additional source for information and calculation of cladograms, we also used sequences from GenBank No. KJ934120, KJ934121, KM586809, KP900999, KX013516.

Results and discussion

Molecular analysis resulted in a cladogram (Color plate 7: 6) showing the enigmatic specimen at the same position and branch where *P. lutea* is located. ITS2 difference is less than 1% between both and thus confirms a determination as *P. lutea*.

Aberrations are not uncommon among lycaenid butterflies and this example shows again that molecular analysis is a powerful tool to determine problematic specimens correctly.

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