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Polyommatus fominae Stradomsky, 2005 and *P. szabokyi* Bálint, 1990 (Lepidoptera: Lycaenidae) — species of hybrid origin of *P. icarus* (Rottemburg, 1775) and *P. icadius* (Groum-Grshimaïlo, 1890). Part II

Polyommatus fominae Stradomsky, 2005 и *P. szabokyi* Bálint, 1990 (Lepidoptera: Lycaenidae) — вид гибидного происхождения от *P. icarus* (Rottemburg, 1775) и *P. icadius* (Groum-Grshimaïlo, 1890). Часть II

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КЛЮЧЕВЫЕ СЛОВА: гибрид, фауна, энтомология, Lepidoptera, Lycaenidae, Polyommatinae.

ABSTRACT. Studied specimens of the taxa *fominae* and *szabokyi* possess the gene Ef-1a characteristic of *P. icarus*. Specimens previously examined have a genetic mixture of two species: *fominae* — COI from *P. icadius*, ITS2 and Ef-1a from *P. icarus*; *szabokyi* — COI and ITS2 from *P. icadius*, Ef-1a from *P. icarus*. New data confirm that the taxa *fominae* and *szabokyi* are of hybrid origin with the sharing the parental species *P. icarus* and *P. icadius*.

РЕЗЮМЕ. Изученные экземпляры таксонов *fominae* и *szabokyi* обладают геном Ef-1a, характерным для *P. icarus*. Образцы, исследованные ранее , обладают смешанным набором генов: *fominae* — COI от *P. icadius*, ITS2 и Ef-1a от *P. icarus*; *szabokyi* — COI и ITS2 от *P. icadius*, Ef-1a от *P. icarus*. Новые данные подтверждают, что таксоны *fominae* и *szabokyi* имеют гибридное происхождение от родительских видов *P. icarus* и *P. icadius*.

Introduction

We had previously shown that the taxa *Polyommatus fominae* Stradomsky, 2005 and *P. szabokyi* Bálint, 1990 originated as a result of hybridization of the species *P. icarus* (Rottemburg, 1775) and *P. icadius* (Groum-Grshimaïlo, 1890) [Stradomsky, Yakovlev, 2018]. Hybridization was demonstrated by the presence in specimens of *fominae* and *szabokyi* the mitochondrial gene COI, characteristic of *P. icadius*, and nuclear sequence ITS2, characteristic of *P. icarus*. Additionally, in some specimens of the taxon *szabokyi,* the sequence ITS2 corresponded to *P. icadius*. To clarify the molecular genetic characteristics of taxa *fominae* and *szabokyi*, we have also studied the nuclear gene Ef-1a [Cho et al., 1995], which encodes the protein elongation factor 1-alpha in both *P. icarus* and *P. icadius*.

Material and methods

MATERIAL. *Polyommatus icarus*: ♀, Russia, Rostov-on-Don, Belokalitvensky District, 9.09.2008, B. Stradomsky (voucher ILL283, GenBank accession № MG834540); *P. icadius*: ♂, Kyrgyzstan, Ala-too, 02.07.2015, S. Korb (voucher ILL259, GenBank accession № MG834538); *P. fominae*: ♂, Russia, Karachaj-Cherkesia, Jamagat (1500 m), 25.07.2016, B. Stradomsky (voucher ILL281, GenBank accession № MG834539); *P. szabokyi*: ♂, SW Mongolia, Gobi-Altai aimak (1350 m), 62 km SSE Bugat, N slope of Adzh-Bogdo Range, Zoolon-Suuzhijn-Bulag spring, 28.06.2017, R.V. Yakovlev (voucher ILL279, GenBank accession № MG834541). We also used the sequence Ef-1a *P. icarus* from GenBank № AY496846, voucher NK-00-P562: Kazakhstan, Altai, Oktyabrsk.

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We amplified DNA 5' section of the nuclear gene elongation factor 1-alpha (Ef-1a) 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: forward, 5'- TAC CAT CGA GAA GTT CGA GAA G -3' with reverse, 5'- GCC ACC CCT TGA ACC AGG GCA T -3' [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].

Ef-1a nucleotide sequences were treated quantitatively using MEGA5 [Tamura et al., 2011] methods Minimum Evolution (ME) and were represented as ME-cladograms.

To produced the cladogram, we also used the sequences from GenBank [11] KF468771 (voucher ILL155), KF468770 (voucher ILL149), KJ671881 (voucher ILL162), KJ671884 (voucher ILL116), KJ671885 (voucher ILL143), KJ671888 (voucher ILL165) and KJ671889 (voucher ILL145).

Results and Discussion

The specimen nodes for *P. icarus* and *P. icadius* on the ME-cladogram of gene Ef-1a are sister, but completely independent (Fig. 1) indicating an independent status of these species. Concurrently, the taxa *fominae* and *szabokyi* belong uniquely to the clade corresponding to *P. icarus*.

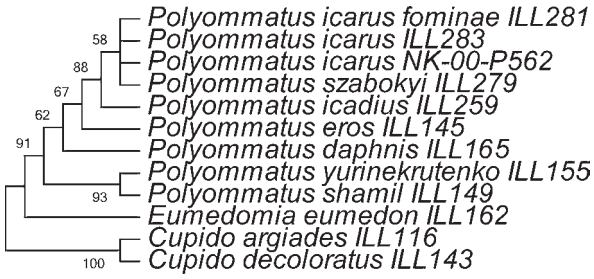

Fig. 1. ME-cladogram for Ef-1a DNA sequences.
Рис. 1. ME-кладограмма для ДНК-последовательности Ef-1a.

It should be also noted that the studied specimens of *fominae* and *szabokyi* had the mitochondrial COI gene characteristic of *P. icadius*. In this case, the nuclear sequence ITS2 in the specimen *fominae* corresponded to that of *P. icarus*, and in the specimen *szabokyi* — to *P. icadius* [Stradomsky, Yakovlev, 2018]. Thus, both specimens had more and less pronounced mixture of genes of both species: *fominae* (voucher ILL281) — COI from *P. icadius*, ITS2 and Ef-1a from *P. icarus*; *szabokyi* (voucher ILL279) — COI and ITS2 from *P. icadius*, Ef-1a from *P. icarus*.

Our study provides support that the taxa *fominae* and *szabokyi* are of a hybrid origin with the parental contribution of the species *P. icarus* and *P. icadius*.

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