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Polyommatus fominae Stradomsky, 2005 и *P. szabokyi* Bálint, 1990 (Lepidoptera: Lycaenidae) — виды, произошедшие вследствие гибридизации *P. icarus* (Rottemburg, 1775) и *P. icadius* (Groum-Grshimaïlo, 1890)

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

ABSTRACT. Molecular-genetic study of blues *Polyommatus fominae* Stradomsky, 2005 and *P. szabokyi* Bálint, 1990 showed that they have the mitochondrial gene COI characteristic of *P. icadius* (Groum-Grshimaïlo, 1890). The nuclear gene ITS2 of all the studied *P. fominae* is identical to that of *P. icarus* (Rottemburg, 1775), the ITS2 in one specimen of *P. szabokyi* was characteristic of *P. icarus*, and in the other — to *P. icadius*. Basing on the external characters and the molecular-genetic data, it is supposed that the taxa *P. fominae* and *P. szabokyi* appeared as a result of hybridization of *P. icarus* and *P. icadius*.

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РЕЗЮМЕ. Молекулярно-генетические исследования голубянок *Polyommatus fominae* Stradomsky, 2005 и *P. szabokyi* Bálint, 1990 показали, что они имеют митохондриальный ген COI, характерный для *P. icadius* (Groum-Grshimaïlo, 1890). Ядерный ген ITS2 всех изученных экземпляров *P. fominae* идентичен *P. icarus* (Rottemburg, 1775), а ITS2 у одного экземпляра *P. szabokyi* была свойственной для *P. icarus,* а у другого — для *P. icadius*. На основании внешних признаков и молекулярно-генетических данных, предполагается, что *P. fominae* и *P. szabokyi* возникли вследствие гибридизации *P. icarus* и *P. icadius*.

Introduction

In the three last decades three taxa of blues (Lepidoptera: Lycaenidae) have been described in the genus *Polyommatus* Latreille, 1804 from southern Mongolia and the North Caucasus: *P. szabokyi* Bálint, 1990 (type locality — "Mongolei, Bayan Chongor Berge, Ih-Bogd, Orog-Nuur"), *P. elenae fominae* Stradomsky, 2005 (type locality — Russia, Karachay-Cherkess Republic, Mussa-Achitara Mountains Range), and *P. shchurovi* Stradomsky, 2006 (type locality — Russia, Karachay-Cherkess Republic, Mussa-Achitara Mountains Range) [Bálint, 1990; Stradomsky, 2005, 2006]. The status of these taxa were subsequently reappraised. *P. szabokyi* was recognized only as a reduced subspecies, *P. icarus szabokyi* [Tshikolovets et al., 2009] with the comment "According to our best knowledge the phenotype *szabokyi* is unique, and can be considered as a distinct and isolated taxon. This phenotype is most probably the result of the extreme ecological conditions typifying the Ih-Bogd region where there are vast saline steppes in the lake bed. Somewhat similar situation can be observed in Turkestan where the dwarf phenotype represented by "*Lycaena Icarus* Rott. var *turanica* Heyne, 1895" (Male and female syntypes: "Tura") occurs in arid and warmer regions".

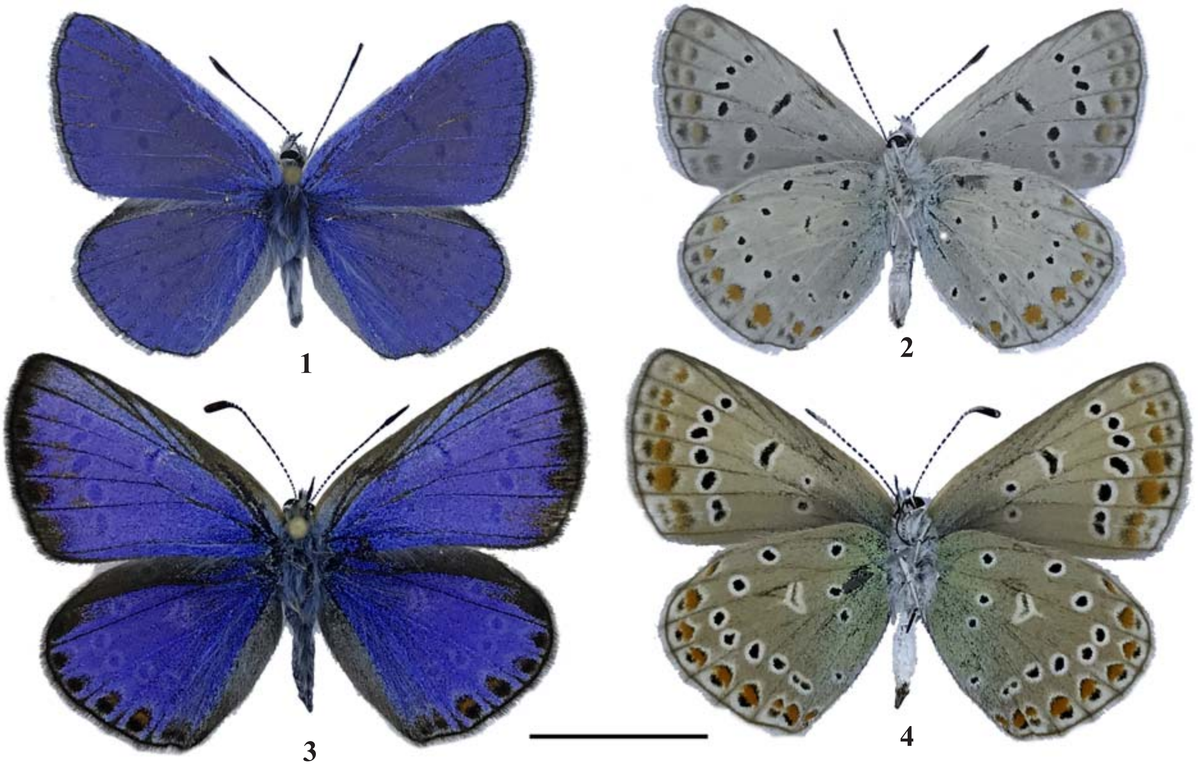
The sympatrically inhabiting taxa *P. fominae* and *P. shchurovi* based on the study of the mitochondrial DNA of the gene COI were synonymized and reassigned to *P. icadius* (type locality "Ferghana"), which has significant differences in the COI gene sequence from *P. icarus* [Vodolazhsky, Stradomsky, 2008]. The additional molecular-genetic studies of these taxa is bases for this work.

Material and methods

New specimens of the taxon *P. szabokyi* — 35 males, 12 females, from SW Mongolia, Gobi-Altai aimak, 62 km SSE Bugat, N slope of Adzh-Bogdo Range, Zoolon-Suuzhijn-Bulag spring, 45°05’25”N, 94°48’05”E, h = 1350 m, 28.06.2017, R.V. Yakovlev leg.), paratypes and additional specimens of *P. fominae* (Figs 1–5), *P. icarus*

Table 1. List of material with voucher codes and GenBank accession numbers
Таблица 1. Список экземпляров-ваучеров и номеров ГенБанка

Taxon of *Poly-ommatus*	Locality	Voucher No.	GenBank accession numbers	
			COI	ITS2
amandus	Russia: Gonachkhir (1900 m), Karachay-Cherkessia	ILL170	KF860851	KF894393
icarus	Israel: Mt Hermon (2050 m)	DB08003	GU244491	GQ166170
icarus	Italy: near Trento (500-700 m)	ILL018	EU597139	GQ885163
icarus	Russia: Sochi (100 m) Krasnodar	ILL030	FJ428821	GQ885166
icarus	Russia: Nov. Chara, Chita	ILL043	FJ428818	GQ885161
icarus	Russia: Dugino, Azov, Rostov-on-Don	ILL041	FJ428822	GQ885165
icarus	Greece: Dodoni (450 m), near Igoumenista	ILL027	FJ428819	GQ885162
icarus	Russia: Zav`yalovsky, Udmurtia	ILL049	FJ428825	GQ885167
icarus	Russia: Belokalitvensky District, Rostov-on-Don	ILL054	GQ885173	GQ885164
icadius	Tajikistan: Lake Dunkeldyk (4100 m), S.-E. Pamir	ILL022	EU597143	GQ885159
icadius	Iran: Ambarkesh (2900 m), Qazvin	ILL071	GQ885172	GQ885160
icadius	Afghanistan: 10 km S.Bamian, 2800 m	ILL096	JQ026942	JQ026945
icadius	Kyrgyzstan: Ala-too	ILL259	KX247291	KX247293
fominae	Russia: Gonachkhir (1900 m), Karachaj-Cherkesia	ILL003	EU597127	MF872678
fominae	Russia: Mts. Mussa-Achitara (2400 m), Karachaj-Cherkesia	ILL005	EU597129	MF872679
fominae	Russia: Jamagat (1500 m),, Karachaj-Cherkesia	ILL281	MF872681	MF872680
szabokyi	Mongolia: Gobi-Altai aimak (1350 m)	ILL278	MF803704	MF803707
szabokyi	Mongolia: Gobi-Altai aimak (1350 m)	ILL279	MF803705	MF803708



Figs 1–4. *P. szabokyi* Bálint, 1990, adult specimens from Zoolon-Suuzhijn-Bulag spring: 1–2 — male; 3–4 — female; 1, 3 — upperside; 2–4 — underside.

Рис. 1–4. *P. szabokyi* Bálint, 1990, имаго Zoolon-Suuzhijn-Bulag spring: 1–2 — самец; 3–4 — самка; 1, 3 — сверху; 2–4 — снизу.

and *P. icadius* (Table 1) were sequenced in this study.

We amplified the DNA 5' section of the mitochondrial gene Cytochrome Oxidase subunit I (COI) and the nuclear non-coding sequence internal transcribed spacer 2 (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: forward, 5'-TAG CGA AAA TGA CTT TTT TCT A-3' with reverse, 5'-TTG CTC CAG CTA ATA CAG GTA A-3' were used to amplify COI. ITS2 was amplified with forward, 5'-GGG CCG GCT GTA TAA AAT CAT A-3' and reverse, 5'-AAA AAT TGA GGC AGA CGC GAT A-3' [Stradomsky, 2016].

The amplified fragments were separated using an automated sequencing machine (Applied Biosystems 3500). The analysis of primary nucleotide sequences was made with the application of BioEdit Sequence Alignment Editor, version 7.0.5.3 [Hall, 1999].

COI and ITS2 nucleotide sequences were treated quantitatively using MEGA5 [Tamura et al., 2011] methods Maximum Likelihood (ML).

Results

The obtained results of molecular-genetic study are presented in the form of ME-cladograms of DNA sections for non-linked mitochondrial COI gene (Fig. 6) and nuclear sequence ITS2 (Fig. 7). The presented data show that both mitochondrial and nuclear DNA sequences in *P. icarus* and *P. icadius* form independent branches on cladograms. Additionally, both clades if *P. icadius* occur closer to the outgroup — *P. amandus*, which indicates a more ancient origin of the taxon *P. icadius* of a mountainous Central Asian origin. The placement location of *P. fominae* and *P. szabokyi* on the clades was unexpected. The COI gene of *P. fominae* is close to and may be

Fig. 5. *Polyommatus* spp., ventral view: *icarus* vouchers ILL018, ILL041, ILL043; *fominae* vouchers ILL003, ILL005, ILL281; *icadius* vouchers ILL259, ILL022, ILL071, ILL096; *szabokyi* vouchers ILL278, ILL279.

Рис. 5. *Polyommatus* spp., снизу: ваучеры *icarus* ILL018, ILL041, ILL043; ваучеры *fominae* ILL003, ILL005, ILL281; ваучеры *icadius* ILL259, ILL022, ILL071, ILL096; ваучеры *szabokyi* ILL278, ILL279.

identical to that of *P. icadius*. Similarly the nuclear sequences ITS2 in all the studied *P. fominae* specimens were identical to *P. icarus*. For the *P. szabokyi,* the result was even more complex. Both examined specimens have, as well as *P. fominae*, the mitochondrial COI gene characteristic of *P. icadius*. The nuclear sequence ITS2 in one specimen was characteristic of *P. icarus,* in the other specimen of *P. icadius*.

Discussion

Our following hypotheses for the above results are that the archaic taxon of the mountainous Caucasus was *P. icadius*. Migrating males of a more recent taxon, *P. icarus* dispersed into new areas mating with females of the closely related species, *P. icadius*. The descendants of these hybrids had mitochondrial genes (including COI) of *P. icadius*. Further dispersals of males *P. icarus* did not change the haplotype of mitochondrial DNA but contributed to a gradual and complete replacement of nuclear DNA of *P. icadius* by DNA of *P. icarus*. This process resulted in the appearance of a hybrid taxon as *P. fominae* with mitochondrial gene of *P. icadius* and the nuclear one of *P. icarus*. Furthermore, the habitus of the *P. fominae* specimens widely varies from the typical to *P. icarus* (Fig.5, voucher ILL003) to the phenotype of *P. icadius* (Fig. 5, vouchers ILL005, ILL281), supporting a hybrid origin.

Apparently, a similar phenomenon may be the cause for the appearance of the *P.szabokyi*. This hybridization may have occurred at an earlier period with the presence of hybrids as respresented by the type of *P. fominae*. There are also specimens with the genomic characteristic of only *P. icadius*. The habitus of *P. szabokyi*-like specimens are similar to *P. icadius,* supporting a hybridization process occurring during relatively recent times and is not yet complete.

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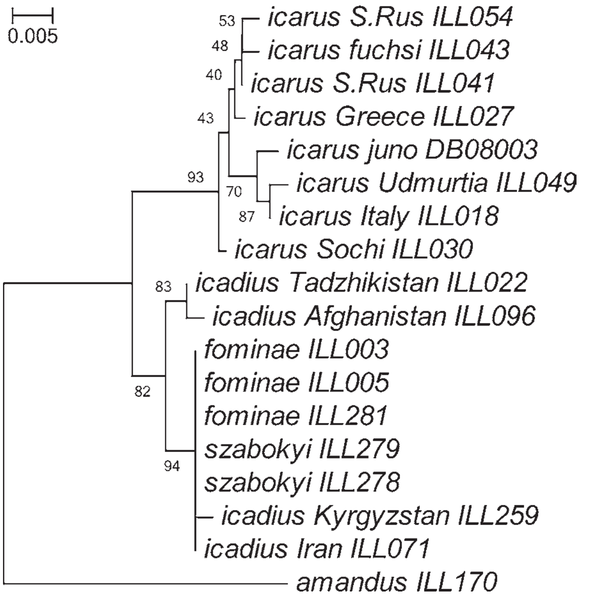

Fig. 6. *Polyommatus* spp.: ME-cladogram for COI DNA sequences

Рис. 6. *Polyommatus* spp.: кладограмма для COI ДНК сиквенсов.

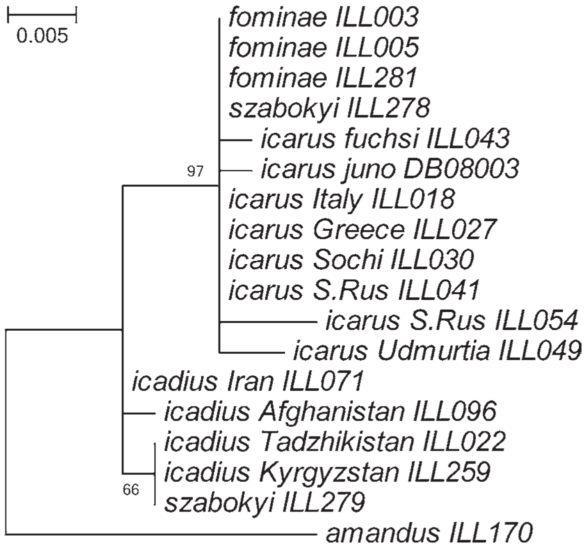

Fig. 7. *Polyommatus* spp.: ME-cladograms for ITS2 DNA sequences

Рис. 7. *Polyommatus* spp.: кладограмма для ITS2 ДНК сиквенсов.