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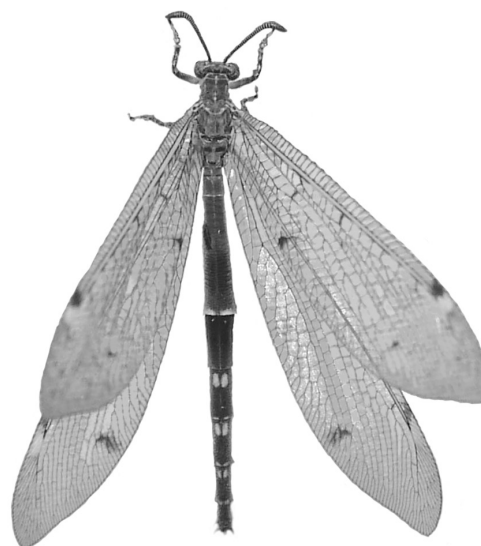


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**Molecular-genetic data for the better knowledge
of the identity of *Polyommatus elena* Stradomsky et Arzanov, 1999
(Lepidoptera: Lycaenidae)**

**Молекулярно-генетические данные к лучшему познанию
идентичности *Polyommatus elena* Stradomsky et Arzanov, 1999
(Lepidoptera: Lycaenidae)**

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Key words: Lepidoptera, Lycaenidae, *Polyommatus elena*, COI, ITS2 DNA sequences.

Ключевые слова: Lepidoptera, Lycaenidae, *Polyommatus elena*, COI, ITS2-последовательности ДНК.

Abstract. It is demonstrated that specimens of *Polyommatus elena* Stradomsky et Arzanov, 1999 form separate branches on cladograms of their mitochondrial COI and nuclear ITS2 sequences that are separate from specimens of *P. icarus* (Rottemburg, 1775) and *P. icadius* (Grum-Grshimailo, 1890). This may give evidence of species independence of *Polyommatus elena*.

Резюме. Показано, что экземпляры *Polyommatus elena* Stradomsky et Arzanov, 1999 на кладограммах митохондриальной COI и ядерной ITS2-последовательностей ДНК образуют отдельные, независимые ветви от представителей *P. icarus* (Rottemburg, 1775) и *P. icadius* (Grum-Grshimailo, 1890), что может свидетельствовать о самостоятельности вида.

Described about 20 years ago *Polyommatus elena* Stradomsky et Arzanov, 1999 had, until now, only morphological characteristics that determined its species status [Stradomsky, Arzanov, 1999; Polumordvinov et al., 2005; Stradomsky, 2006]. Yet nowadays the full characterization of a taxon should include molecular-genetic data as well. Carrying out a DNA analysis required fresh specimens of *P. elena*. The type locality of the species was recently completely destroyed by a construction project. However, three specimens which met related morphological criteria of *P. elena* (i.e. peculiar features of genitalia) were found on a virgin land in Belaya Kalitva District, Rostov Region, Russia (Fig. 1). The authors examined mitochondrial and nuclear DNA sequences, structures of genitalia and wing pattern characteristics of those specimens in comparison with related species *P. icarus* (Rottemburg, 1775) and *P. icadius* (Grum-Grshimailo, 1890).

Material and methods

The characteristic data of studied specimens of *P. elena* and the compared specimens of *P. icarus* and *P. icadius*

(place of collection, vouchers, accession numbers of COI and ITS2 sequences registered in GenBank) are presented in Table 1.

We amplified DNA 5' section of the mitochondrial gene Cytochrome Oxidase subunit I (COI) and the nuclear noncoding 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,



Fig. 1. Localities of *Polyommatus elena* in Rostov Region, Russia.
1 – type locality; 2 – locality of the new samples.

Рис. 1. Местонахождения *Polyommatus elena* в Ростовской области.
1 – типовое местонахождение; 2 – местонахождение новых экземпляров.

Table 1. List of material with voucher codes and GenBank accession numbers.

Таблица 1. Используемый материал с музейными номерами и номерами последовательностей в GenBank.

Taxon of the genus <i>Polyommatus</i> / Таксон рода <i>Polyommatus</i>	Locality / Местонахождение	Voucher No / Музейный номер	COI GenBank accession No	ITS2 GenBank accession No
<i>icarus</i>	Russia: Sochi, Krasnodar Region	ILL030	FJ428821	GQ885166
<i>icarus</i>	Greece: Dodoni (450 m), near Igoumenista	ILL027	FJ428819	GQ885162
<i>icarus</i>	Russia: Dugino, Azov Distr., Rostov Region	ILL041	FJ428822	GQ885165
<i>icarus fuchsi</i>	Russia: Novaya Chara, former Chita Region (now Zabaykalskiy Region)	ILL043	FJ428818	GQ885161
<i>icarus</i>	Russia: Belaya Kalitva Distr., Rostov Region	ILL283	MG779475	MG779476
<i>icadius</i>	Tajikistan: Lake Dunkeldyk (4100 m), SE Pamir	ILL022	EU597143	GQ885159
<i>icadius</i>	Iran: Ambarkesh (2900 m), Qazvin	ILL071	GQ885172	GQ885160
<i>icadius</i>	Afghanistan: 10 km S Bamian, 2800 m	ILL096	JQ026942	JQ026945
<i>icadius</i>	Kyrgyzstan: Ala-too	ILL259	KX247291	KX247293
<i>elena</i>	Russia: Belaya Kalitva Distr., Rostov Region	ILL054	GQ885173	GQ885164
<i>elena</i>	Russia: Belaya Kalitva Distr., Rostov Region	ILL075	MF943245	MF943246
<i>elena</i>	Russia: Belaya Kalitva Distr., Rostov Region	ILL284	MH006697	MH006698

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].

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].

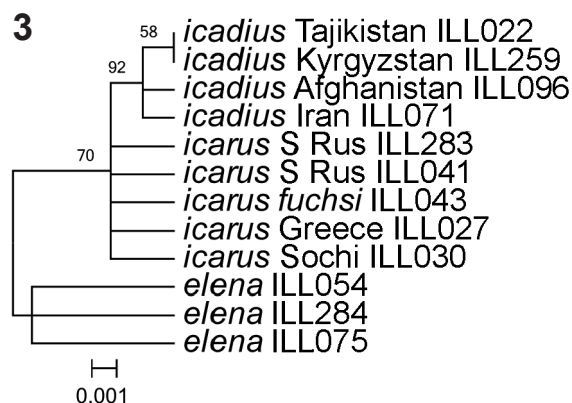
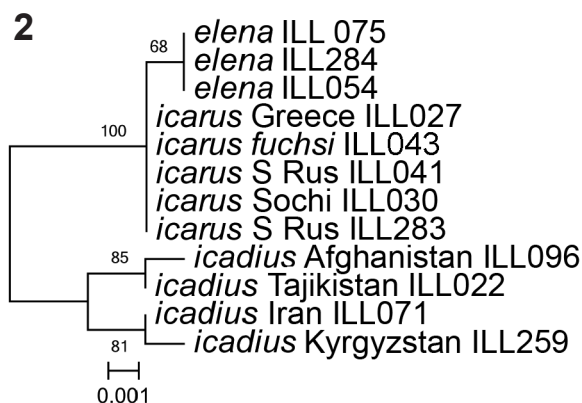
COI and ITS2 nucleotide sequences were treated quantitatively using MEGA5 [Tamura et al., 2011] methods Minimum Evolution (ME) and were represented as ME-cladograms.

Results and discussion

The results of our molecular-genetic studies are presented in form of MP cladograms of DNA sequences for mitochondrial COI gene (Fig. 2) and nuclear nucleotide sequence ITS2 (Fig. 3), not linked together.

The data presented suggest that both mitochondrial and nuclear DNA sequences of *P. icarus*, *P. icadius* and *P. elena* form distinct independent branches in cladograms.

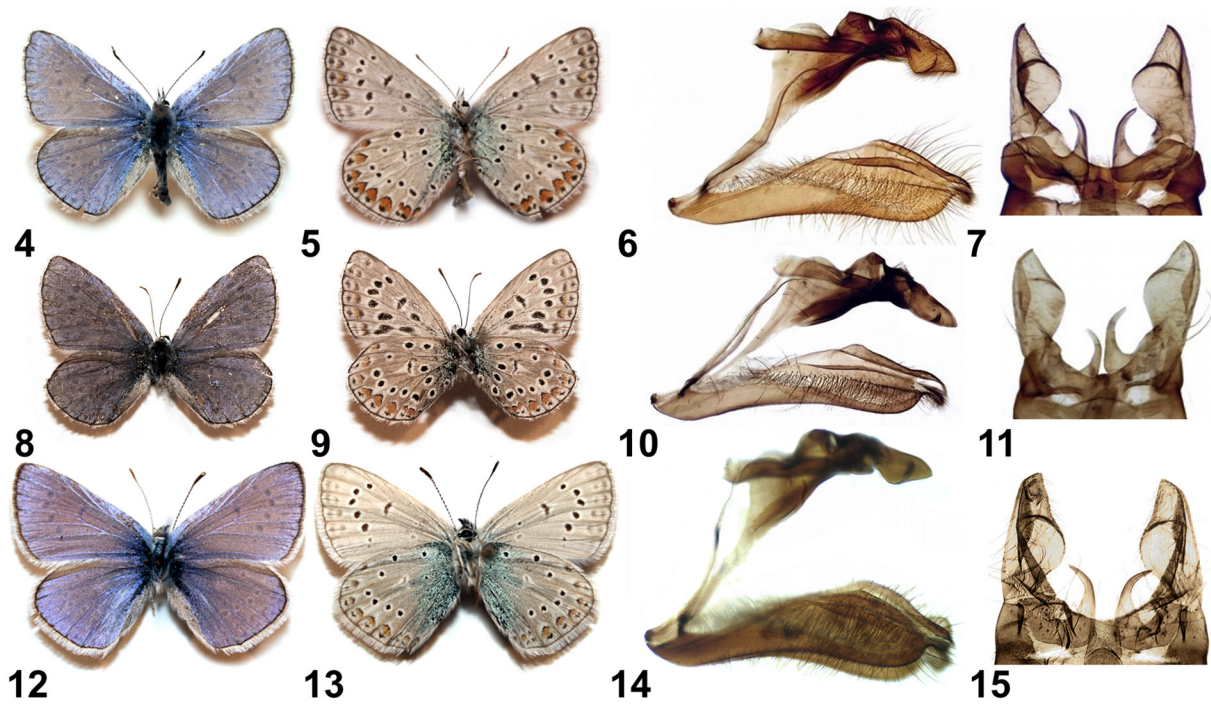
The results of molecular and genetic analysis correlate well with the results of comparison of genitalia structure characteristics. In lateral projection, unci of *P. icarus* and *P. icadius* have an expanding at their basal part (Color plate 8: 6, 14), while unci of *P. elena* is narrowed along the entire length (Color plate 8: 10). In ventral projection, unci lobes of *P. icarus* and *P. icadius* have a conical form (Color plate 8: 7, 15), while unci lobes of *P. elena* have a blade form (Color plate 8: 11). A distinctive feature of the structure of female genitalia of *P. elena* is a sclerotized part at the top of lamellae postvaginalis (Color plate 8: 21), which is absent in *P. icarus* (Color plate 8: 18). Also the habitus of imago of *P. elena* (Color plate 8: 8, 9, 19, 20) has very characteristic features in comparison with specimens of *P. icarus* and *P. icadius* (Color plate 8: 4, 5, 12, 13, 16, 17). It should be taken into account that the specimens of *P. icarus*

Figs 2–3. *Polyommatus* spp., ME-cladograms.

2 – for COI DNA sequences; 3 – for ITS2 DNA sequences.

Рис. 2–3. *Polyommatus* spp., ME-кладограммы.

2 – для COI-последовательностей ДНК; 3 – для ITS2-последовательностей ДНК.

Figs 4–15. *Polyommatus* spp., males.

4–7 – *P. icarus* (Voucher No ILL041); 8–11 – *P. elena* (Voucher No ILL075); 12–15 – *P. icadius* (Voucher No ILL022). 4, 8, 12 – upperside; 5, 9, 13 – underside; 6, 10, 14 – male genitalia, lateral view; 7, 11, 15 – uncus and gnathos, ventral view.

Рис. 4–15. *Polyommatus* spp., самцы.

4–7 – *P. icarus* (музейный № ILL041); 8–11 – *P. elena* (музейный № ILL075); 12–15 – *P. icadius* (музейный № ILL022). 4, 8, 12 – верх; 5, 9, 13 – испод; 6, 10, 14 – гениталии самца в латеральной проекции; 7, 11, 15 – ункус и гнатос в вентральной проекции.

Figs 16–21. *Polyommatus* spp., females.

16–18 – *P. icarus* (Voucher No ILL283); 19–21 – *P. elena* (Voucher No ILL284). 16, 19 – upperside; 17, 20 – underside; 18, 21 – lamellae postvaginalis. Scale bars 1 mm.

Рис. 16–21. *Polyommatus* spp., самки.

16–18 – *P. icarus* (музейный № ILL283); 19–21 – *P. elena* (музейный № ILL284). 16, 19 – верх; 17, 20 – испод; 18, 21 – поствагинальная пластинка. Масштабные линейки – 1 мм.

(Vouchers Nos. ILL041, ILL283) and *P. elena* (Vouchers Nos. ILL075, ILL284) both originate from Rostov Region, i.e. the specimens are in sympatry.

It can be concluded that specimens, which by their morphological characters are categorized as *P. elena*, also have segments of unlinked mitochondrial and nuclear DNA sequences different from those of related species. It could be assumed that this is a specific feature of a particular population of *P. icarus*, but then it would be difficult to explain sympatric habitation of populations which have different sets of morphological characters as well as specific structure of both mitochondrial and nuclear markers. According to an expert's opinion, "combined analysis of mitochondrial and nuclear markers is a simple and efficient way to identify the cryptic species in sympatry" [Lukhtanov, Shapoval, 2008: 436].

It would be logical to assume, therefore, that *P. elena* is a very young species in a state of formation.

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