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Callophrys rubi (Linnaeus, 1758) and *C. chalybeitincta* Sovynski, 1905 (Lepidoptera: Lycaenidae): a comparative analysis of mitochondrial and nuclear DNA sequences

Callophrys rubi (Linnaeus, 1758) и *C. chalybeitincta* Sovynski, 1905 (Lepidoptera: Lycaenidae): сравнительный анализ последовательностей митохондриальной и ядерной ДНК

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Ключевые слова: *Callophrys rubi*, *chalybeitincta*, *nigra*, последовательности ДНК COI и ITS2

Abstract. A study of a genetic marker, namely the nuclear internal transcribed spacer 2 (ITS2), demonstrated significant distinctions between specimens of *Callophrys chalybeitincta* Sovynski, 1905 and *Callophrys rubi* (Linnaeus, 1758). On this ground and taking into consideration morphological and genital differences between *C. rubi* and *C. chalybeitincta* it is necessary to reinstate the taxonomic status to the following taxa:

- *Callophrys chalybeitincta* Sovynski, 1905 **stat. rev.**
- *Callophrys chalybeitincta nigra* Stradomsky, 2005 **stat. rev.**

Резюме. Изучение ядерной нуклеотидной последовательности ДНК – ITS2 показало значимые различия между представителями *Callophrys chalybeitincta* Sovynski, 1905 и *Callophrys rubi* (Linnaeus, 1758). На основании этих данных и с учетом морфологических и генитальных отличий *C. rubi* от *C. chalybeitincta* необходимым является возвращение таксономического статуса следующим таксонам:

- *Callophrys chalybeitincta* Sovynski, 1905 **stat. rev.**
- *Callophrys chalybeitincta nigra* Stradomsky, 2005 **stat. rev.**:

Ten Hagen and Miller in their work [2010] on comparative study of nucleotide sequences of mtDNA COI gene demonstrated a close similarity between studied sequences of taxa *Callophrys rubi* (Linnaeus, 1758) and *C. chalybeitincta* Sovynski, 1905. Relying on data obtained, the authors reduced *C. chalybeitincta* to subspecies of *C. rubi*, in spite of pronounced morphological and genital differences between these taxa. To clarify the validity of taxonomic argumentations in question, we examined a genetic marker, namely the nuclear internal transcribed spacer 2 (ITS2), for taxa *C. rubi* (Linnaeus, 1758), *C. chalybeitincta* Sovynski, 1905 и *C. chalybeitincta nigra* Stradomsky, 2005, and repeated the study of the mitochondrial COI gene.

Material and methods

All specimens examined in this study are archived at the museum of the Institute of Arid Zones (SSC RAS, Rostov-on-Don) as voucher specimens.

Material:

C. rubi, ♂, Russia: Belokalitvensky District, Rostov-on-Don area, 27.04.2005, B. Stradomsky – voucher ILL080, accession №№ GenBank JF810413 (COI), JF813097 (ITS2);

C. chalybeitincta, ♂, Russia: Mts. Mussa-Achitara (2000 m), 02.06.2006, B. Stradomsky – voucher ILL084, accession №№ GenBank JF810410 (COI), JF813096 (ITS2);

C. chalybeitincta nigra, ♂, Russia: Kumzhenskaya grove, Rostov-on-Don area, 26.04.2004, B. Stradomsky – voucher ILL095, accession №№ GenBank JF810411 (COI), JF813099 (ITS2);

Neolycaena rhymnus (Eversmann 1832), ♂, Russia: Belokalitvensky District, Rostov-on-Don area, 23.05.2009, B. Stradomsky – voucher ILL099, accession №№ GenBank JF810412 (COI), JF813098 (ITS2).

Parameters for methods of DNA extraction, amplification and sequencing of COI and ITS2 sequences are described by Vodolazhsky et al., [2009].

Differences of primary nucleotide sequences were evaluated quantitatively using Kimura-2 parameter model [Kimura, 1980] and presented graphically as ME (Minimum Evolution) cladograms.

In the construction of phylogenetic models nucleotide sequences COI and ITS2 of specimen of *N. rhymnus* were used as an outer group.

Results and discussion

Our studies confirmed that nucleotide sequences COI loci of taxa *C. rubi*, *C. chalybeitincta* and *C. chalybeitincta nigra* are identical and form one branch on a cladogram (Fig.). At the same time nucleotide sequences of ITS2 of *C. rubi* and subspecies *C. chalybeitincta* differ widely: *C. rubi* and *C. chalybeitincta* differ by 2.7%, *C. rubi* and *C. chalybeitincta nigra*, by 3.0%. As this takes place, specimens of *C. chalybeitincta* in the cladogram form a branch separately from *C. rubi* (Fig.).

Therefore, we have good reason to believe that taxonomic conclusions on the status of the taxon *C. chalybeitincta* and its subspecies *nigra* made by ten Hagen and Miller (2010) just on the basis of analysis of mitochondrial COI gene are likely to be rather hasty ones.

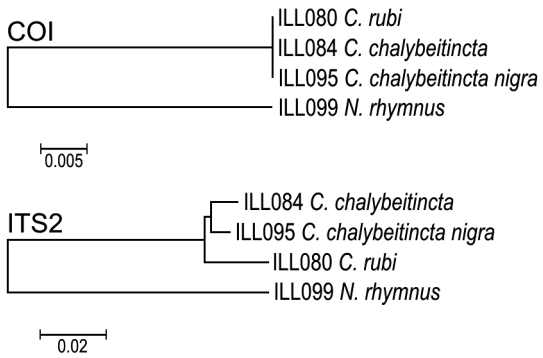

Fig. *Callophrys* spp.: phylogenetic trees based on the Minimum Evolution (ME) method of analysis of distances for COI and ITS2 DNA sequences.

Рис. *Callophrys* spp.: филогенетические деревья на основе анализа различий последовательностей ДНК генов COI и ITS2 с применением метода минимальной эволюции (ME).

In this connection we consider it necessary to reinstate the taxonomic status to the following taxa:

- *Callophrys chalybeitincta* Sovynski, 1905 **stat. rev.**:
«Callophrys rubi L. var. chalybeitincta nova» [Sovynski, 1905: 109];
«Callophrys chalybeitincta Sovynski, 1905» [Korshunov, 1972: 359];
«*chalybeitincta* (stat. n.) … als Unterarten von *C. rubi*» [ten Hagen, Miller, 2010: 188].

- *Callophrys chalybeitincta nigra* Stradomsky, 2005 **stat. rev.**:
«*Callophrys chalybeitincta nigra* subsp. nov.» [Stradomsky, 2005: 85];
«*C. rubi chalybeitincta* synonymisiert werden (syn. n. für *nigra*) … *Callophrys rubi chalybeitincta* Sovinsky, 1905 = *nigra* Stradomsky, 2005 syn. n.» [ten Hagen, Miller, 2010: 188, 195].

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