

## Some nuances of molecular phylogeny of the genus *Satyrium* Scudder, 1876 (Lepidoptera: Lycaenidae)

### Некоторые нюансы молекулярной филогении рода *Satyrium* Scudder, 1876 (Lepidoptera: Lycaenidae)

© **B.V. Stradomsky**<sup>1</sup>, **E.S. Fomina**<sup>2</sup>  
© **Б.В. Страдомский**<sup>1</sup>, **Е.С. Фомина**<sup>2</sup>

<sup>1</sup>Rostov Branch of the Russian Entomological Society, PO Box 3318, Rostov-on-Don 344092 Russia. E-mail: bvstr@yandex.ru

<sup>2</sup>Federal Research Centre the Southern Scientific Centre of the Russian Academy of Sciences, Chekhov str., 41, Rostov-on-Don 344006 Russia

<sup>1</sup>Ростовское отделение Русского энтомологического общества, а/я 3318, Ростов-на-Дону 344092 Россия, E-mail: bvstr@yandex.ru

<sup>2</sup>Федеральный исследовательский центр Южный научный центр Российской академии наук, пр. Чехова, 41, Ростов-на-Дону 344006 Россия

**Key words:** Lepidoptera, Lycaenidae, *Satyrium*, *Fixsenia pruni*, molecular markers, developmental stages.

**Ключевые слова:** Lepidoptera, Lycaenidae, *Satyrium*, *Fixsenia pruni*, молекулярные маркеры, стадии развития.

**Abstract.** Molecular studies (based on COI of mtDNA and nuclear ribosomal ITS2 region) demonstrated that certain concepts of *Satyrium* Scudder, 1876 reflected by recent literature are not monophyletic. Two species are transferred to the genus *Fixsenia* Tutt, 1907: *Fixsenia pruni* (Linnaeus, 1758), **comb. resurr.** (from *Satyrium*), *Fixsenia herzi* (Fixsen, 1887), **comb. resurr.** (from *Satyrium*). This opinion is supported by differences between preimaginal stages of *pruni* and species of the genus *Satyrium*. The unusual structure of female genitalia of *pruni* should be also noted. The taxon *Fixsenia* Tutt, 1907, sensu nova (*F. pruni* and its closest relatives) is interpreted here as a genus to improve the classification.

**Резюме.** Молекулярные исследования на основе COI мДНК и ядерной рибосомальной области ITS2 показали, что таксон *Satyrium* Scudder, 1876, широко интерпретируемый в современной литературе, не является монофилетическим. Два вида перенесены в род *Fixsenia* Tutt, 1907: *Fixsenia pruni* (Linnaeus, 1758), **comb. resurr.** (из *Satyrium*) и *Fixsenia herzi* (Fixsen, 1887), **comb. resurr.** (из *Satyrium*). В пользу этого свидетельствуют различия преимагинальных стадий *pruni* и представителей рода *Satyrium*. Также необходимо отметить оригинальность строения гениталий самок *pruni*. Таксон *Fixsenia* Tutt, 1907, sensu nova (включающий *F. pruni* и наиболее близкие к нему виды) интерпретируется как род в целях усовершенствования классификации.

Currently, interpretation of taxonomic position of Palaearctic species of the genus *Satyrium* Scudder, 1876 is quite ambiguous. For one, many authors assign all these specimens directly to this genus [Gorbinov, 2001; Kudrna, 2002; Settele et al., 2008; Lafranchis et al., 2015]. At the same time there is a division of the genus *Satyrium* into *Nordmannia* Tutt, 1907 and *Strymonidia*

Tutt, 1908 [Higgins, Riley, 1978], or into *Armenia* Dubatolov et Korshunov, 1984, *Superflua* Strand, 1910 and *Nordmannia* [Tuzov et al., 2000], or into *Nordmannia* and *Fixsenia* Tutt, 1907 [Inomata, 1990, Korshunov, 2002], or *Nordmannia* and *Strymonidia* [Min, Xiaoling, 2002]. To clarify taxonomic position of Palaearctic specimens of the genus *Satyrium*, the authors performed their molecular genetic analysis with Nearctic specimens *Satyrium* s. str. involved. The authors examined mitochondrial and nuclear DNA sequences and structures of female genitalia and developmental stages.

### Material and methods

We amplified mtDNA 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'-GTC AAC AAT CAT AAA ATA TTG G-3' (reserve forward 5'- TAG CGA AAA TGA CTT TTT TCT-3') with reverse, 5'-TTG CTC CAG CTA ATA CAG GTA A-3' (reserve reverse 5'- AAG AAT GAG GTA TTG AGG TTT C-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' [Wiemers et al., 2010; 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].

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 Таксон	Locality Местонахождение	Voucher No / Музейный номер	COI GenBank accession No	ITS2 GenBank accession No
<i>Satyrium abdominalis</i> (Gerhard, 1850)	Azerbaijan: Shemkir near Ganja (900 m)	ILL127	JX112883	JX122758
<i>Satyrium acaciae</i> (Fabricius, 1787)	Russia: Bolshekrepinskaya, Rostov Region	ILL123	JX112886	JX122758
<i>Satyrium ilicis</i> (Esper, 1779)	Russia: Kalach-on-Don, Volgograd Region	ILL128	JX112887	JX122759
<i>Satyrium w-album</i> (Knoch, 1782)	Russia: Belaya Kalitva Distr., Rostov Region	ILL125	JX112882	JX122756
<i>Satyrium spini</i> (Fabricius, 1787)	Russia: Yasinovskaya riv., Rostov Region	ILL126	JX112881	JX122757
<i>Satyrium pruni</i> (Linnaeus, 1758)	Russia: Bolshekrepinskaya, Rostov Region	ILL124	JX112880	JX122755
	Russia: Bolshekrepinskaya, Rostov Region	ILL285	MK343429	MK343431
<i>Satyrium hyrcanicum</i> (Riley, 1939)	Turkey: Kağızman (1400 m), Kars Province	MW99158	AY557057	AY556549
<i>Satyrium titus</i> (Fabricius, 1793)	USA: Washington County, Maryland	ILL290	MK410093	MK410094
<i>Thecla betulae</i> (Linnaeus, 1758)	Russia: Dzhamagat (1500 m), Karachay-Cherkessia	ILL122	KC676696	KC676698
<i>Quercusia quercus</i> (Linnaeus, 1758)	Russia: Gornaya Polyana, Volgograd Region	ILL161	KF918772	KF918772
<i>Neolycaena eckweileri</i> Lukhtanov, 1993	Kyrgyzstan: Kok-Jar, Osh Region	ILL286	MK343428	MK343430
<i>Neolycaena rhymnus</i> (Eversmann, 1832)	Russia: Belaya Kalitva Distr., Rostov Region	ILL099	JF810412	JF813098

COI and ITS2 nucleotide sequences were treated quantitatively using MEGA6 [Tamura et al., 2013] methods Minimum-Evolution (ME) and were represented as ME-cladograms.

Network for COI sequences constructed with the NETWORK: Version 4.6.1.6 program.

In order to elaborate the system of the genus *Satyrium* the authors used also some sequences from GenBank for comparison, namely COI sequences of *S. acaudata* (Staudinger, 1901) (accession No GenBank FJ664041) and *S. herzi* (Fixsen, 1887) (accession No GenBank GU372539). Other material is listed in the Table 1.

*Thecla betulae*, *Quercusia quercus*, *Neolycaena rhymnus* and *N. eckweileri* were used as an outgroup to root the tree.

## Results and discussion

The results of molecular genetic studies (Color plate 11: 1, 2) suggest that while members of the outgroup to the genus *Neolycaena* de Nicéville in Marshall et de Nicéville, 1890 are not an outgroup to specimens of the taxon *S. pruni*, they meet the criteria for an outgroup for the other specimens of the genus *Satyrium*, including the Nearctic species *S. titus* (Color plate 11: 1). This pattern is specific for both COI gene and ITS2 sequence, which is unlinked to COI gene (Color plate 11: 2).

In order to preserve the principle of monophyly in this case there is the only appropriate solution: to recognize the taxon *pruni* as not belonging to the genus *Satyrium*. The another solution is to unite all the genera involved under *Satyrium*. But that not reflects the reality mirrored by genitalia and early stage characters.

The cladogram on Color plate 11: 3 demonstrates that the taxon *herzi*, the type species of the genus *Fixsenia*, is a sister taxon of *pruni*. Therefore, both taxa have to be recognized as valid: *Fixsenia herzi*, and *F. pruni*. Higgins et al. [1991] included *Satyrium pruni* to the genus *Fixsenia*.

Gorbunov [2001] placed *Fixsenia* as a subgenus of the genus *Satyrium*. In his view morphological similarity between both *S. (F.) herzi* and *S. (F.) pruni* and other specimens of the genus *Satyrium* based on the structure of genitalia: “Ventral cornutus in vesica reduced, short” [Gorbunov, 2001]. Korshunov [2002] listed the genus *Fixsenia* with species *F. herzi* and *F. pruni*, however without any substantial explanations.

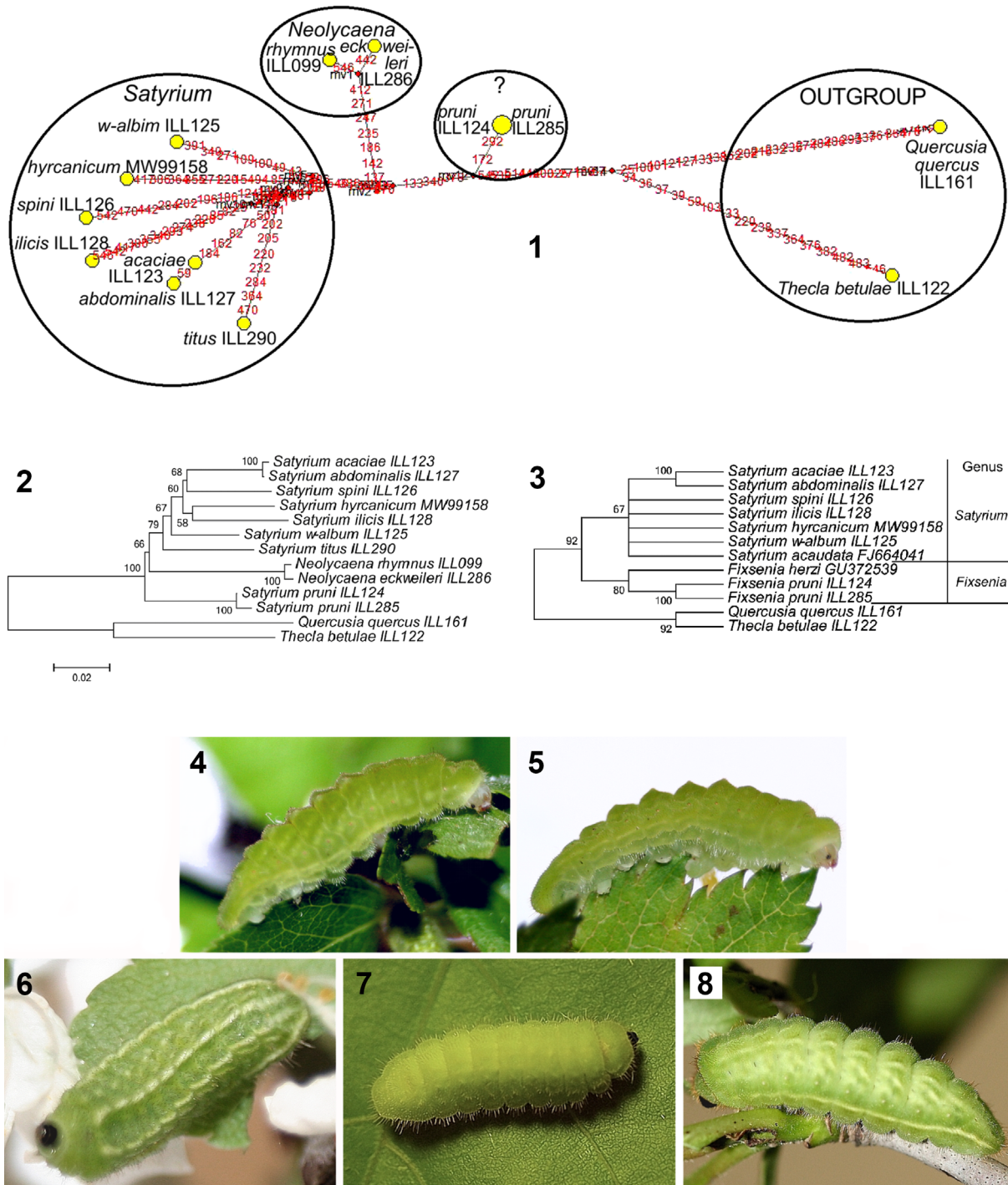
The distinctness of *F. pruni* is highlighted also by specific characters of its developmental stages which are different from those of species placed in the genus *Satyrium*: final instar larvae of the *F. pruni* have yellowish-green heads (Color plate 11: 4, 5), while those of the genus *Satyrium* have black heads (Color plate 11: 6–8). More significant differences are found between pupae. The pupa of *F. pruni* is black, with very large white spots; abdominal region very convex, with conical lumps on dorsal and lateral sides (Color plate 12: 9, 10). Pupae of the genus *Satyrium* are uniformly convex, with smooth, brownish surface with numerous small dark spots (Color plate 12: 11–16).

There are also differences in structure of female genitalia. Lamella postvaginalis of *F. pruni* (Color plate 12: 17) is broadened apically, while the lamella of specimens representing the genus *Satyrium* is smoothly narrowed apically (Color plate 12: 18–21).

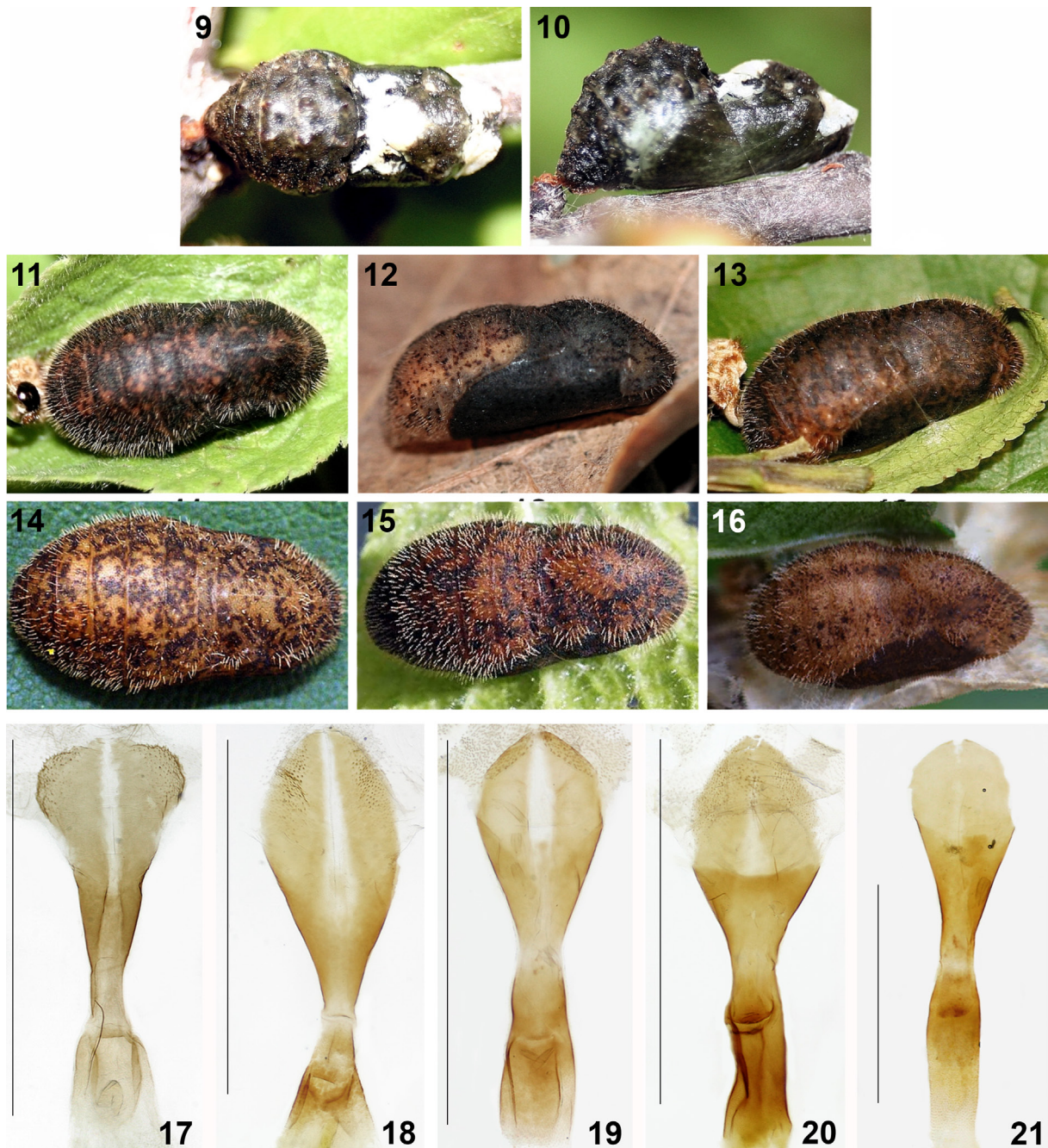
Consequently, on the basis of combination of morphological and molecular genetic characteristics the taxon *pruni* and its relatives should be considered as not belonging to the genus of *Satyrium*, but representing the genus *Fixsenia*: genus *Fixsenia* Tutt, 1907, sensu nova (not as a subgenus of the genus *Satyrium*), *Fixsenia pruni* (Linnaeus, 1758), **comb. resurr.** (from *Satyrium*), *Fixsenia herzi* (Fixsen, 1887), **comb. resurr.** (from *Satyrium*).

## References

- Gorbunov P.Y. 2001. The Butterflies of Russia (Lepidoptera: Hesperioidea and Papilionoidea): classification, genitalia, keys for identification. Ekaterinburg: Tesis. 320 p.



Figs 1–8. DNA sequences (network scheme and phylogenetic trees) and larvae of Theclinae.  
 1 – network of mDNA COI sequences; 2 – ME-cladogram for mDNA COI and rDNA ITS2 sequences; 3 – ME-cladogram for mDNA COI sequences with GenBank-sequences; 4–8 – larvae: 4–5 – *Fixsenia pruni*, 6 – *Satyrium acaciae*, 7 – *S. ilicis*, 8 – *S. spini*.  
 Рис. 1–8. Анализ последовательностей ДНК (сеть и филогенетические деревья) и гусеницы Theclinae.  
 1 – сеть последовательностей COI мДНК; 2 – ME-кладограмма для COI мДНК и ITS2 рДНК; 3 – ME-кладограмма для COI ДНК с использованием последовательностей из GenBank; 4–8 – гусеницы: 4–5 – *Fixsenia pruni*, 6 – *Satyrium acaciae*, 7 – *S. ilicis*, 8 – *S. spini*.



Figs 9–21. *Fixsenia* and *Satyrium* pupae, female genitalia (ductus and lamella postvaginalis).  
 9–16 – pupae; 17–21 – female genitalia. 9–10, 17 – *Fixsenia pruni*; 11, 21 – *Satyrium acaciae*; 12, 18 – *S. ilicis*; 13, 20 – *S. spini*; 14 – *S. ledereri* (photo by V. Tikhonov); 15 – *S. abdominalis*; 16, 19 – *S. w-album*. Scale bars: 17–20 – 2 mm, 21 – 1 mm.

Рис. 9–21. Куколки и гениталии самок (ductus и lamella postvaginalis) видов *Fixsenia* и *Satyrium*.

9–16 – куколки; 17–21 – гениталии самок. 9–10, 17 – *Fixsenia pruni*; 11, 21 – *Satyrium acaciae*; 12, 18 – *S. ilicis*; 13, 20 – *S. spini*; 14 – *S. ledereri* (фото В. Тихонова); 15 – *S. abdominalis*; 16, 19 – *S. w-album*. Масштабные линейки: 17–20 – 2 мм, 21 – 1 мм.

- Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*. 41: 95–98.
- Higgins L., Hargreaves B., L'Honore J. 1991. Guide complet des Papillons d'Europe et d'Afrique du Nord. Paris: Delachaux & Niestle. 270 p.
- Higgins L., Riley N.D. 1978. Die Tagfalter Europas und Nordwestafrikas. Hamburg, Berlin: Parey. 377 p.
- Inomata T. 1990. Keys to the Japanese Butterflies in Natural Color. Tokyo: Hokuryakan. 223 p. (in Japanese).
- Korshunov Yu.P. 2002. Bulavousye cheshuekrylye Severnoy Azii [Rhopaloceran lepidopterans of Northern Asia]. Moscow: KMK Scientific Press Ltd. 424 p. (in Russian).
- Kudrna O. 2002. The Distribution Atlas of European Butterflies. *Oedippus*. 20: 1–342.
- Lafranchis T., Jutzeler D., Guilloson J.-Yv., Kan P., Kan B. 2015. La vie des papillons: écologie, biologie et comportement des Rhopalocères de France. Paris: Diatheo. 751 p.
- Min W., Xiaoling F. 2002. Butterflies Fauna Sinica: Lycaenidae. Zhengzhou: Henan Science and Technology Publishing House. 440 p., 28 pls. (in Chinese).
- Settele J., Kudrna O., Harpke A., Kühn I., van Swaay Ch., Verovnik R., Warren M., Wiemers M., Hanspach J., Hickler T., Kühn E., van Halder L., Veling K., Vliegenthart A., Wynhoff I., Schweiger O. 2008. Climatic Risk Atlas of European Butterflies. Sofia, Moscow: Pensoft. 710 p.
- Stradomsky B.V. 2016. A molecular phylogeny of the subfamily Polyommatae. *Caucasian Entomological Bulletin*. 12(1): 145–156. DOI: 10.23885/1814-3326-2016-12-1-145-156
- Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*. 30(12): 2725–2729. DOI: 10.1093/molbev/mst197
- Tuzov V.K., Bogdanov P.V., Churkin S.V., Dantchenko A.V., Devyatkin, A.L., Murzin V.S., Samodurov G.D., Zhdanko A.B. 2000. Guide to the Butterflies of Russia and Adjacent Territories. Vol. 2. Sofia, Moscow: Pensoft. 580 p.
- Wiemers M., Stradomsky B.V., Vodolazhsky D.I. 2010. A molecular phylogeny of *Polyommatus* s. str. and *Plebicula* based on mitochondrial COI and nuclear ITS2 sequences (Lepidoptera: Lycaenidae). *European Journal of Entomology*. 107(3): 325–336. DOI: 10.14411/eje.2010.041

Received / Поступила: 8.02.2019

Accepted / Принята: 28.02.2019