

Study of mitochondrial COI and nuclear ITS2 sequences of Mongolian specimens of the *Polyommatus eros*-group (Lepidoptera: Lycaenidae)

Изучение митохондриальной COI и ядерной ITS2 нуклеотидных последовательностей ДНК монгольских представителей *Polyommatus eros*-группы (Lepidoptera: Lycaenidae)

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Abstract. Based on studying of molecular genetic characters (COI and ITS2 sequences) as well as morphological and genitalia ones within different populations of *Polyommatus aloisi* we consider the taxon as a subspecies of *P. eros* only. Hence, *Polyommatus eros aloisi* **stat. nov.** and *Polyommatus eros dividus* are stated.

Резюме. Изучение молекулярно-генетических (COI и ITS2 нуклеотидных последовательностей), а также морфологических и генитальных характеристик различных популяций *Polyommatus aloisi* свидетельствует о необходимости рассматривать этот таксон исключительно как подвид *P. eros*. Следовательно, необходимо констатировать: *Polyommatus eros aloisi* **stat. nov.** и *Polyommatus eros dividus*.

Introduction

Owing to the extended disjunctive transpalaeartic locality, isolated populations, considerable morphological and biological plasticity, a lot of taxa within the group *Polyommatus eros* gained their species rank and were described with minimal distinguishing characteristics. For the recent years an important part in establishing taxonomic status has belonged to the investigation of mitochondrial and nuclear DNA markers. Such methods allowed to considerably delimit the amount of real species within the genus *Polyommatus* with a large number of the described taxa reduced and some even restored to no more than subspecific rank.

Therefore, subspecies of *P. eros* (Ochsenheimer, 1808) at present are considered to include *P. eros eroides* (Frivaldszky, 1835), *P. eros erotides* (Staudinger, 1892), *P. eros menelaos* Brown, 1976, *P. eros kamtshadalis* (Sheljuzhko, 1933), *P. eros boisduvalii* (Herrich-Schäffer, 1844), *P. eros meoticus* (Zhdanko and Stshurov, 1998), *P. eros pacificus* (Stradomsky and Tuzov, 2006), *P. eros erotulus* (Nekrytenko, 1985), *P. eros tshetverikovi* (Nekrutenko, 1977), *P. eros napea* (Grum-Grshimailo, 1891), *P. eros kaabaky* (Korb, 2000), *P. eros yildizae* (Kočák, 1977), *P. eros extremiorientalis* (Kurentzov, 1970), *P. eros taimyrensis* Korshunov, 1982 [Vodolazhsky, Stradomsky, 2008; Settele

et al., 2008; Vodolazhsky et al., 2009; Wiemers et al., 2010; Vodolazhsky, Stradomsky, 2010].

Due to molecular genetic investigations, *P. eros* from the Eastern Asian area of Palaearctics is replaced with the sister species *P. amorata* (Alpheraky, 1897), which is significantly different from *P. eros* with mitochondrial and nuclear DNA markers [Vodolazhsky et al., 2009; Wiemers et al., 2010]. *P. amorata* also includes some subspecies previously recorded as separate species or within the taxa *P. amorata tartarus* (Fruhstorfer, 1916) and *P. amorata tsvetajevi* (Kurentzov, 1970). Mongolian populations of the group *Polyommatus eros* inhabit the border region between the habitats of *P. eros* and *P. amorata* and at present appear as a separate species *P. aloisi* Balint, 1988, presented [Churkin, 2003] with a pair of geographically isolated subspecies *P. aloisi aloisi* Balint, 1988 and *P. aloisi dividus* Churkin, 2003.

The nominative subspecies was recorded for the Alpien area of the Gurvan-Saikhan Mountains (the main mountain system in the Hobi Altai), when the subspecies *dividus* is widely spread all over the Mongolian Altai. The valley of Lake Alag-Nuur is the border region in distribution of some montane plants and animals, which divides the biota of the Mongolian and Hobi Altai [Yakovlev, 2006, 2012; Kamelin, 2010]. Later [Tshikolovets et al., 2009] *aloisi* and *dividus* were suggested as consubspecific. This work interprets *aloisi* as a bona species. The purpose of our research is molecular genetic investigation of members *P. aloisi* from various localities of the area (type locality of the nominative subspecies and various localities of the Mongolian Altai for *P. aloisi dividus*) in order to determine the status of the taxa mentioned above.

Material and methods

Four specimens of the taxon *aloisi* were compared with the previously examined members of *Polyommatus eros*-group (Table). COI and ITS2 nucleotide sequences of the specimen *Polyommatus amandus* (Schneider, 1792) were used as an external group when creating phylogenetic models.

DNA extraction, PCR and sequencing were carried out according to the protocols described in Vodolazhsky and Stradomsky [2008].

Table. List of material with voucher and GenBank accession numbers

Voucher	Taxon (ssp.)	Species Locality/Collection date	COI GenBank accession numbers	ITS2 GenBank accession numbers
<i>Polyommatus eros</i>				
ILL016	<i>taimyrensis</i>	Russia: Polar. Ural (300 m), Komi Republic, 18-Jul-2005	EU597137	JQ319889
ILL017	<i>eros</i>	Italy: Taufers (1200 m), S.Tirol, 06-Jul-2004	EU597138	GQ885155
ILL025	<i>eroides</i>	Bulgaria: Dibrinishte (1500-1600 m), Pirin Mts., 05-Jul-1999	EU597146	GQ885154
ILL040	<i>boisduvalii</i>	Russia: Ilovlya (100 m), Volgograd reg., 18-Jun-2004	FJ428804	GQ885152
ILL063	<i>orientalis</i>	Russia: Fedyashevo (200 m), Tula reg., 27-Jun-2008	FJ428804	GQ885156
ILL068	<i>erotides</i>	Russia: E. Sayan, Tissa River (1600 m), Burjatia, 17-Jul-2008	GQ885171	GQ885157
ILL081	<i>aloisi dividus</i>	Mongolia: Elt-Gol River (2100 m), Bayan-Ulegei aimak, 01-Jul-2005	JQ319881	JQ319885
ILL082	<i>aloisi dividus</i>	Mongolia: 50 km. S. Manhan (2300 m), Hovd aimak, 03-Jul-2004	JQ319882	JQ319886
ILL083	<i>aloisi dividus</i>	Mongolia: Bayan-Gol (2100 m), Hovd aimak, 25-Jun-2004	JQ319883	JQ319887
ILL085	<i>aloisi aloisi</i>	Mongolia: Tzikhor (2300 m.), Gurvan Mts., Omnegov aimak, 14-Jul-2002	JQ319884	JQ319888
RU02003	<i>kamtshadalis</i>	Russia: Sokol (250 m), Magadan reg., 10-Jul-2002	GU244492	GQ166184
<i>Polyommatus amorata</i>				
ILL050	<i>tsvetajevi</i>	Russia: Suchodol River (300 m), Primorsky Territory, 25-Jul-2004	GQ885170	GQ885151
ILL051	<i>amorata</i>	China: S.W. Sichuan (2500 m), 20-Jun-2004	GQ885168	GQ885149
ILL076	<i>tartarus</i>	China: Wudu District (2000 m), S. Gansu, 10-Jun-2005	GQ885169	GQ885150
<i>Polyommatus amandus</i>				
MW99047	<i>amandus</i>	Turkey: Koskkoy (1900 m), Erzurum, 07-Jul-1999	AY557035	AY556661

The following PCR primer pairs: PolF (forward, 5'-TAG CGA AAA TGA CTT TTT TCT A-3') with PolR2 (reverse, 5'-TTG CTC CAG CTA ATA CAG GTA A-3') were used to amplify COI. ITS2 was amplified with PiF (forward, 5'-GGG CCG GCT GTA TAA AAT CAT A-3') and PiR (reverse, 5'-AAA AAT TGA GGC AGA CGC GAT A-3').

The following cycling protocols were used: an initial 4 min denaturation at 94°C and 36 cycles of 40 s denaturation at 94°C, 40 s annealing at 58°C and 40 s extension at 72°C.

Amplified fragments were separated with using an automated sequencing machine ABI PRISM 3100 (Applied Biosystems).

Primary nucleotide sequences were determined using a software suit "DNA Sequencing Analysis Software", version 3.7. 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]. Differences in primary nucleotide sequences were established in terms of quantity with the use of the Kimura-2-parameter model [Kimura, 1980] and presented graphically as Neighbor-Joining (NJ)-cladograms.

Results

The localization of the examined specimens and specimens for comparison is given in Color plate 11: fig.1 and occupies almost all the Palearctic region.

Due to molecular genetic investigations, the cladogram, obtained after processing ITS2 sequence, almost completely conforms to COI-cladogram (Color plate 11: fig. 2). The analysis of the cladograms showed that the taxon *aloisi* with its mitochondrial and nuclear markers belongs to the cluster *P. eros*. Besides, *aloisi* bears minimal

distinguishing characters from the nominative species *P. eros eros*, 0.2–0.4% in COI-gene sequence and 0.2–0.9% in ITS2-sequence, when the specimen *aloisi* ILL085 from the type locality is closely related to *P. eros eros* in nuclear ITS2 sequence.

Comparative study of male genitalia showed their complete similarity to those of the other members of *P. eros*-group (Color plate 11: fig. 3–13).

Besides, we did not find considerable differences in the habitus of the taxon *aloisi* from that of the majority of members within the species *P. eros*, and the remote nominative subspecies in particular (Color plate 12: fig. 14–25). Some minimal differences in morphology are just infraspecific variation.

Thus, taking into account morphology, genitalia and molecular genetic structures as well as primary descriptions of external characters [Balint, 1988; Churkin, 2003] taxa *aloisi* and *dividus* should be considered only as the subspecies *P. eros*. Minimal distinguishing characters of the studied parameters from the nominative taxon eliminate the possibility for *aloisi* to be an independent species. Thus, *Polyommatus eros aloisi* **stat. nov.** and *Polyommatus eros dividus* are established.

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References

- Balint Z. 1988. A New *Polyommatus* Species from Southern Mongolia: *Polyommatus aloisi* spec. n. (Lepidoptera: Lycaenidae) // *Atalanta*. 18: 385–394.

- Churkin S.V. 2003. New taxa of butterflies (Lepidoptera, Rhopalocera) from Mongolia // *Helios*. 4: 160–178.
- Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT // *Nucleic Acids Symposium*. 41: 95–98.
- Kamelin R.V. 2010. Mongolia on the map of the phytogeographical division of Palaearctics // *Turczaninowia*. 13(3): 5–11.
- Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences // *Journal of Molecular Evolution*. 16: 111–120.
- Settele J., Kudrna O., Harpke A., Kühn I., van Swaay C., 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 Publishers 710 p.
- Tshkolovets V.V., Yakovlev R.V., Balint Z. 2009. *The Butterflies of Mongolia*. Brno–Kyiv. 320 p.
- Vodolazhsky D.I., Stradomsky B.V. 2008. Phylogenetic analysis of subgenus *Polyommatus* (s. str.) Latreille, 1804 (Lepidoptera: Lycaenidae) based on mtDNA markers. Part I // *Caucasian Entomological Bulletin*. 4(1): 123–130.
- Vodolazhsky D.I., Stradomsky B.V. 2010. Molecular Phylogeny of the Subgenus *Polyommatus* (s. str.) (Lepidoptera: Lycaenidae) based on the Sequence of the COI Mitochondrial Gene // *Moscow University Biological Sciences Bulletin*. 65(4): 158–160.
- Vodolazhsky D.I., Wiemers M., Stradomsky B.V. 2009. A comparative analysis of mitochondrial and nuclear DNA sequences in blue butterflies of the subgenus *Polyommatus* (s. str.) Latreille, 1804 (Lepidoptera: Lycaenidae: *Polyommatus*) // *Caucasian Entomological Bulletin*. 5(1): 115–120.
- 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.
- Yakovlev R.V. 2006. About biogeographical structure of Mongolian Altai // *Entomological investigations in the Northern Asia*. Novosibirsk: 165–168.
- Yakovlev R.V. 2012. Checklist of Butterflies (Papilionoidea) of the Mongolian Altai Mountains, including descriptions of new taxa // *Nota lepidopterologica*. 35(1): 51–96.

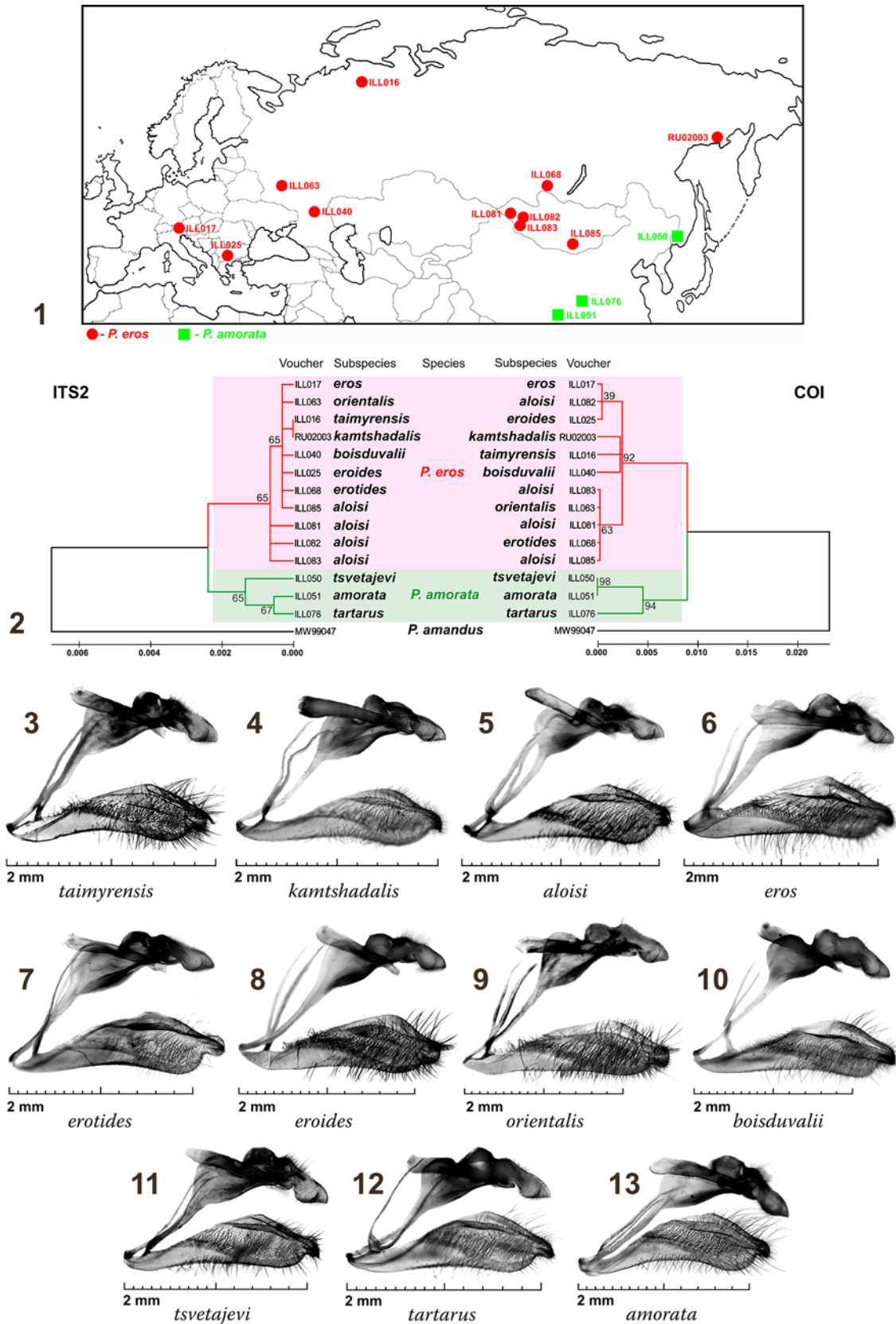


Fig. 1–13. *Polyommatus eros* and *P. amorata*. 1 – Localization of examined specimens; 2 – NJ-cladograms based on the Neighbor-Joining method of analysis of distances for COI and ITS2 DNA sequences; 3–13 – Male genitalia, lateral view: 3 – *P. eros taimyrensis*; 4 – *P. eros kamtshadalis*; 5 – *P. eros aloisi*; 6 – *P. eros eros*; 7 – *P. eros erotides*; 8 – *P. eros eroides*; 9 – *P. eros orientalis*; 10 – *P. eros boisduvalii*; 11 – *P. amorata tsvetajevi*; 12 – *P. amorata tartarus*; 13 – *P. amorata amorata*.

Рис. 1–13. *Polyommatus eros* и *P. amorata*. 1 – Локализация изученных экземпляров; 2 – NJ-кладограммы, полученные методом ближайших соседей при анализе COI и ITS2 последовательностей ДНК; 3–13 – Гениталии самцов, латеральная проекция.



Fig 14–25. *Polyommatus eros* and *P. amorata*, habit of imago: 14 – *P. eros taimyrensis*; 15–16. *P. eros dividus*; 17 – *P. eros aloisi*; 18 – *P. eros eros*; 19 – *P. eros erotides*; 20 – *P. eros eroides*; 21 – *P. eros orientalis*; 22 – *P. eros boisduvalii*; 23 – *P. amorata tsvetajevi*; 24 – *P. amorata tartarus*; 25 – *P. amorata amorata*.

Рис. 14–25. *Polyommatus eros* и *P. amorata*, внешний вид имаго.