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A taxonomic revision of the butterfly genera *Esperarge* and *Kirinia* (Lepidoptera: Nymphalidae: Satyrinae)

VITALY M. SPITSYN[1]*, ALEXANDER V. KONDAKOV[1], ALENA A. TOMILOVA[1], MUNIRA KARAMKHUDOEVA[2], GRIGORY S. POTAPOV[1]

[1] *Federal Center for Integrated Arctic Research of the Russian Academy of Sciences, Northern Dvina Emb. 23, 163000, Arkhangelsk, Russia*
[2] *Pamir Biological Institute, Academy of Sciences of the Republic of Tajikistan, Khorog, Tajikistan*
** Corresponding author: Vitaly M. Spitsyn (spitsyn.v.m.91993@yandex.ru)*

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Abstract
The genus *Kirinia* was described from the Far East and contains two species, i.e. *Kirinia epimenides* and *Kirinia fentoni* (= *K. epaminondas*). Later, the other four species ranging in Central and Western Asia and on the Balkan Peninsula were transferred to this genus based on morphological features of the male genitalia. This action was incorrect and is not confirmed by our novel molecular data. Here, we restore the genus *Esperarge* Nekrutenko, 1988 **stat. rev.** with four species, i.e. *E. eversmanni* **comb. rev.**, *E. cashmirensis* **comb. rev.**, *E. roxelana* **comb. rev.**, and *E. climene* **comb. rev.** Additionally, the subspecies *Esperarge eversmanni shiva* **syn. nov.** is considered a junior synonym of *E. e. unicolor*.

Key words: DNA barcoding, integrative taxonomy, *Kirinia*, *Esperarge*, Kyrgyzstan, Tajikistan.

Introduction

Five genera were described for *Kirinia eversmanni* (Eversmann, 1847) and for two related species at the end of the XX and at the beginning of the XXI centuries. However, they were considered synonymous. Based on the morphology of the male genitalia, they were combined into one genus with two Far Eastern species, i.e. *Kirinia epimenides* (Ménétriés, 1859) and *K. fentoni* (Butler, 1877) (Bozano 1999; Lang 2017). However, these data were not confirmed by means of a molecular studies. During the accumulation of sequences in public genetic databases, it became clear that the western and eastern groups of these species are genetically far from each other. The type species of the genus *Esperarge* Nekrutenko, 1988, i.e. *Hipparchia eversmanni*, was described from Kazakhstan and, until recently, contains four subspecies including the nominative one. However, Tshikolovets and Pages (2016) stated that the subspecies *K. e. cashmirensis* (Moore, 1874) is a separate species, but they did not take into account the molecular data.

In this article, we present the first DNA-based taxonomic revision of the genera *Kirinia* Moore, 1893 and *Esperarge* Nekrutenko, 1988 with a review of the subspecies of *K. eversmanni* and an assessment of the validity of *K. cashmirensis*.

Materials and methods

The study is based on the material from the collection of the Russian Museum of Biodiversity Hotspots (RMBH), Federal Center for Integrated Arctic Research of the Russian Academy of Sciences, Arkhangelsk, Russia. The genitalia were dissected, mounted on temporary glass slides with 70% ethanol and photographed using a research stereomicroscope (AXIO Zoom.V16, Carl Zeiss, Germany). The genitalia are kept in micro-tubes with glycerin pinned to each specimen. The images of the specimens were taken with a Canon EOS 80D camera (Canon, Tokyo, Japan). Habitats were photographed with a Canon EOS 650D camera (Canon, Tokyo, Japan). The images of the type species of the genera *Lasiommata* and *Pararge* (Fig. 1) were taken from specimens keeping in the RMBH as follows: *Lasiommata megera* [Turkey, Avsallar village, pine forest, 36º37'45"N, 31º45'41"E, 02.IIX.2014, Spitsyn leg.], and *Pararge aegeria* [Russia, Arkhangelsk Region, glade in forest, 64º31'45"N, 40º45'49"E, 04-06.VI.2014, Spitsyn leg.].

The species distribution data was sampled using published sources (Bozano 1999; Lang 2017) and DNA barcoding data.

The molecular analysis (purification and PCR) was performed in the Federal Center for Integrated Arctic Research of the Russian Academy of Sciences. Total DNA was extracted from a single leg of each dry specimen according to standard phenol/chloroform procedures (Sambrook, Fritsch & Maniatis 1989). The mitochondrial *cytochrome c oxidase subunit I* gene (COI) was amplified and sequenced using primers LCO1490 (Folmer et al. 1994) and LepR (Hajibabaei et al. 2006). The PCR mix contained approximately 200 ng of total cell DNA, 10 pmol of each primer, 200 μmol of each dNTP, 2.5 μl of PCR buffer (with 20 mmol $MgCl_2$), 0.8 units Taq DNA polymerase (SibEnzyme Ltd., Russia), and H_2O was added for a final volume of 25 μl. Temperature cycling was as follows: 95 °C (5 min), 30-33 cycles of 95 °C (50 sec), 48 °C (50 sec), 72 °C (50 sec) and a final extension at 72 °C (5 min).

The sequencing was carried out at the facilities of the Inter-Institution Center of Group Usage "Genom" at the Engelhardt Institute of Molecular Biology of the Russian Academy of Sciences, Moscow using the ABI PRISM® BigDye™ Terminator v. 3.1 reagents kit. Reaction products were analyzed using an automatic sequencer ABI PRISM® 3730 (Applied Biosystems). The resulting sequences were checked manually using a sequence alignment editor BioEdit version 7.2.5 (Hall 1999). In addition, 37 COI sequences were obtained from NCBI's GenBank and BOLD system, including four COI sequences of *Nymphalis antiopa*, *Erebia ligea*, *E. meolans* and *E. montanus* as outgroup for the phylogenetic analyses (Table 1).

The alignment of the COI sequences was performed directly using the ClustalW algorithm (Thompson et al., 1994). For the phylogenetic analyses, each COI sequence of aligned datasets was trimmed up to a 658-bp fragment. Then, identical COI sequences were removed from the dataset using an online FASTA sequence toolbox (FaBox1.41: Villesen, 2007), leaving a total of 37 haplotype sequences (including the four outgroup taxa). For phylogenetic analyses, we used the COI dataset with unique haplotypes. The best models of sequence evolution for each partition as suggested based on corrected Akaike Information Criterion (AICc) of MEGA6 (Tamura et al. 2013) were as follows: (1) 1st codon of the COI: GTR+G (G=1.35); (2) 2nd codon of the COI: TN93+G (G=0.24); (3) 3rd codon of the COI: HKY+G (G=0.05). Phylogenetic relationships were reconstructed based on Bayesian inference using MrBayes v. 3.2.6 (Ronquist et al. 2012). Four Markov chains, one cold and three heated (temperature = 0.1), were run simultaneously for 10,000,000 generations. The resulting phylogenies were constructed using a tree figure drawing tool Archaeopteryx v. 0.9901 beta (Han & Zmasek 2009).

Results

Our phylogeny revels that the genus *Kirinia* is divided into two separate clades for western and eastern species with high support values (Fig. 1). The COI genetic distances between members of those clades are 7.5–10%. These values are greater than the minimum distance between genera *Pararge* (7.4%) and *Lasiommata* (7.4%). Based on these data, we restore the genus *Esperarge* **stat. rev.** for the western clade and include into this genus four species ranging from the Balkan Peninsula to the Tien Shan Range and the Himalayas. Hence, the genus *Kirinia* contains two species belonging to the eastern clade inhabiting the Far East.

Table 1. Additional COI sequences used in this study with BOLD and NCBI's GenBank accession codes

Species	The original identification of the species from BOLD and GenBank	*COI* haplo-type code	*COI* GenBank acc. no.	*COI* BOLD acc. no.	Specimen voucher	Locality	References
Esperarge eversmanni eversmanni	*Kirinia eversmanni*	EE3	-	EZHBA440-07	CSG08554	Kirgizstan, Tien-Shan	-
E. e. eversmanni	*Esperarge eversmanni*	EE4	FJ663541	LOWA037-06	2005-LOWA-37	Kazakhstan , Tien-Shan	Lukhtanov et al. 2009
E. e. eversmanni	*E. eversmanni*	EE5	FJ663540	LOWA038-06	2005-LOWA-38	Kazakhstan , Tien-Shan	Lukhtanov et al. 2009
E. cashmirensis	*E.eversmanni* photo – *Nymphalis xanthomelas*	ECa1	-	MABUT389-13	NIBGE BUT-00389	Pakistan, Punjab	-
E. cashmirensis	*E. eversmanni*	ECa2	-	MABUT390-13	NIBGE BUT-00390	Pakistan, Punjab	-
E. roxelana	*K. roxelana*	ER1	HQ004569	EZRMN001-08	RVcoll.08-M665	Romania	Dincă et al. 2010, 2015
E. roxelana	*K. roxelana*	ER2	HQ004570	EZROM225-08	RV-07-C959	Romania	Dincă et al. 2010, 2015
E. roxelana	*K. roxelana*	ER2	HQ004571	EZROM1034-08	RVcoll.08-M664	Romania	Dincă et al. 2010, 2015
E. roxelana	*K. roxelana*	ER2	HQ004568	EZRMN002-08	RVcoll.08-M666	Romania	Dincă et al. 2010, 2015
E. roxelana	*K. roxelana*	ER3	DQ338767	GBLN0685-06	CP10-09	Iran	Peña et al. 2006
E. roxelana	*K. roxelana*	ER4	DQ176348	GBLN0533-06	EW25-26	Greece	Weingartner et al. 2006
E. climene	*K. climene*	ECl1	-	IRANB247-08	VNLEP00059	Iran	-
E. climene	*K. climene*	ECl1	-	IRANB248-08	VNLEP00060	Iran	-
E. climene	*K. climene*	ECl2	-	IRANB246-08	VNLEP00058	Iran	-
E. climene	*E. climene*	ECl3	-	LOWAB049-07	LOWA-AR-49	Armenia	-
E. climene	*E. climene*	ECl4	-	LOWAB242-09	McGuire09-TR77	Turkey	-
E. climene	*K. climene*	ECl5	GQ357184	GBLN3137-10	CP10-08	Iran	Peña et al. 2011
Kirinia epimenides	*K. epimenides*	KE2	-	EZHBA428-07	CSG08539	Russia, Primorskiy Krai	-
K. epimenides	*K. epaminondas*	KE3	KM111628	GBMAB987-15	kiepa1	China	Yang et al. 2016
K. epimenides	*K. epaminondas*	KE4	KP735949	-	SAT-Kir-1-4	China	Yang et al. 2016
K. fentoni	*K. epimenides*	KF1	JX185852	GBLN5004-14	NymKIREPI	South Korea	Wan et al. 2013
K. fentoni	*K. epimenides*	KF2	AB855962	GBLN4448-14	J065	Japan	-
Pararge aegeria	*Pararge aegeria*	PA1	MH089765	BIBSA1599-16	12-R099	Italy	-
P.aegeria	*P. aegeria*	PA2	KJ547676	-	-	Portugal	Teixeira da Costa, 2016
P. xiphia	*P. xiphia*	PX1	KX042552	PHLPM051-11	TLMF Lep 04746	Portugal, Madeira	Mutanen et. al. 2016
Lasiommata deidamia	*Lasiommata deidamia*	LD1	JX185853	GBLN5005-14	NymLASDER	South Korea	Wan et al. 2013
L. deidamia	*L. deidamia*	LD1	MG880214	-	-	China	-
L. maera	*L. maera*	LMa1	-	ABOLD015-16		Austria	-
L. maera	*L. maera*	LMa2	GU669662	-	RVcoll.07-D062	Romania	-
L. megera	*L. megera*	LMe1	-	ABOLD064-16	TLMF Lep 21093	Austria	-
L. megera	*L. megera*	LMe2	KX049935	-	NHMO Lep2011.018	Norway	Mutanen et. al. 2016

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Species	The original identification of the species from BOLD and GenBank	*COI* haplo-type code	*COI* GenBank acc. no.	*COI* BOLD acc. no.	Specimen voucher	Locality	References
L. petropolitana	*L. petropolitana*	LP1	HM901705	EZSPC864-10	RVcoll.09-T078	Spain	Dincă et al. 2015
L. petropolitana	*L. petropolitana*	LP2	HM393202	-	BC ZSM Lep 30692	Germany	Hausmann et al. 2011
*Erebia ligea**	*Erebia ligea*	-	KR138769	-	HW2-3	Russia, Middle Ural, Chusovaya River	Peña et al. 2015
*E. montanus**	*E. montanus*	-	KP253472	-	TLMF Lep 14140	Austria	Huemer & Hebert 2015
*E. meolans**	*E. meolans*	-	KR138750	-	EW24-11	France, Languedoc	Peña et al. 2015
*Nymphalis antiopa**	*Nymphalis antiopa*	-	-	ABOLD582-17	TLMF Lep 21658	Austria	-

*Haplotypes of *Nymphalis antiopa*, *Erebia ligea*, *E. montanus*, and *E. meolans* were used as outgroup.

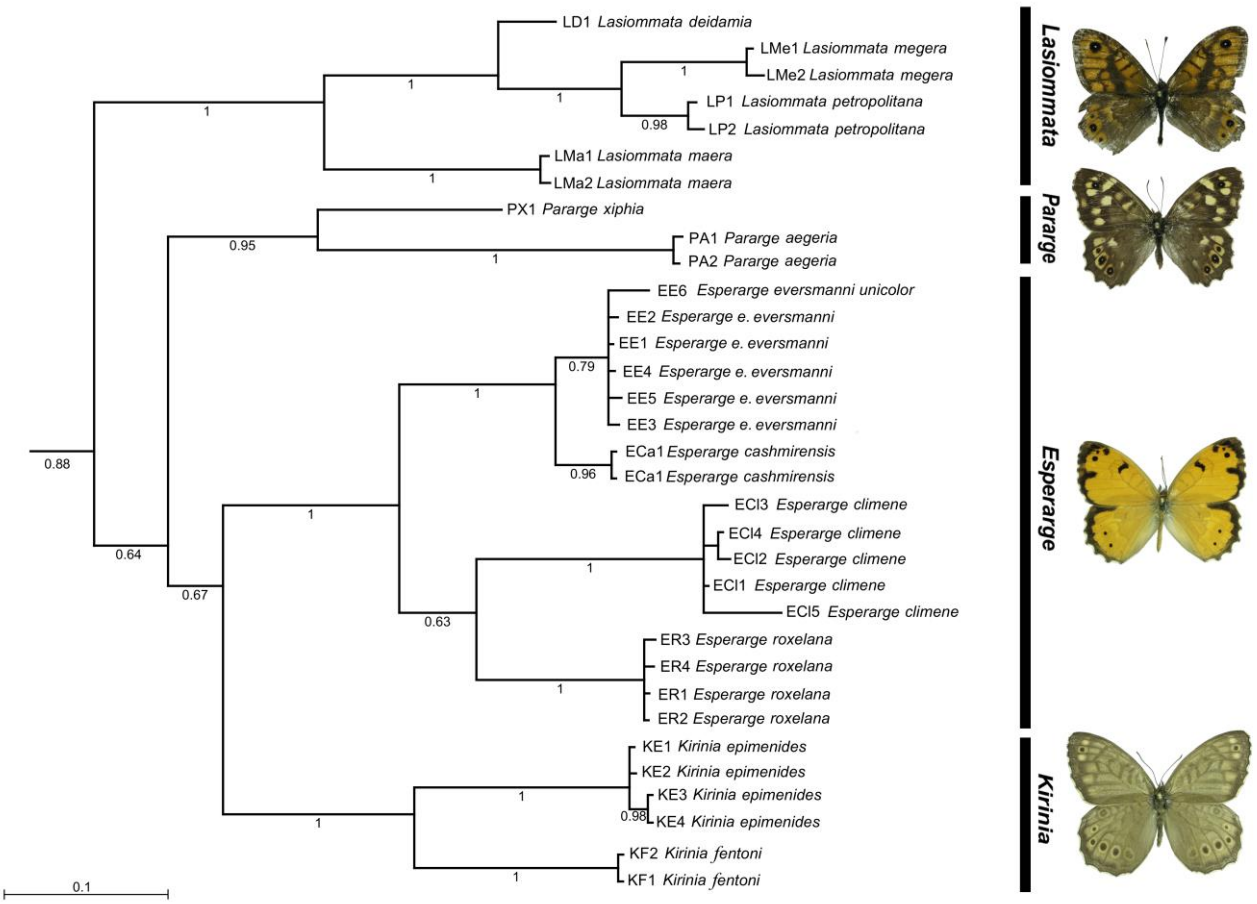

Figure 1. Phylogenetic tree recovered from Bayesian inference analysis based on the COI barcode sequence dataset of *Lasiommata*, *Pararge*, *Esperarge* **stat. rev.** and *Kirinia*. Sequences of *Nymphalis antiopa*, *Erebia ligea*, *E. meolans* and *E. montanus* were used as outgroup (not shown on the tree). Scale bar indicates the branch lengths. Black numbers near nodes are Bayesian Posterior Probabilities (BPP). (Photos: Vitaly M. Spitsyn).

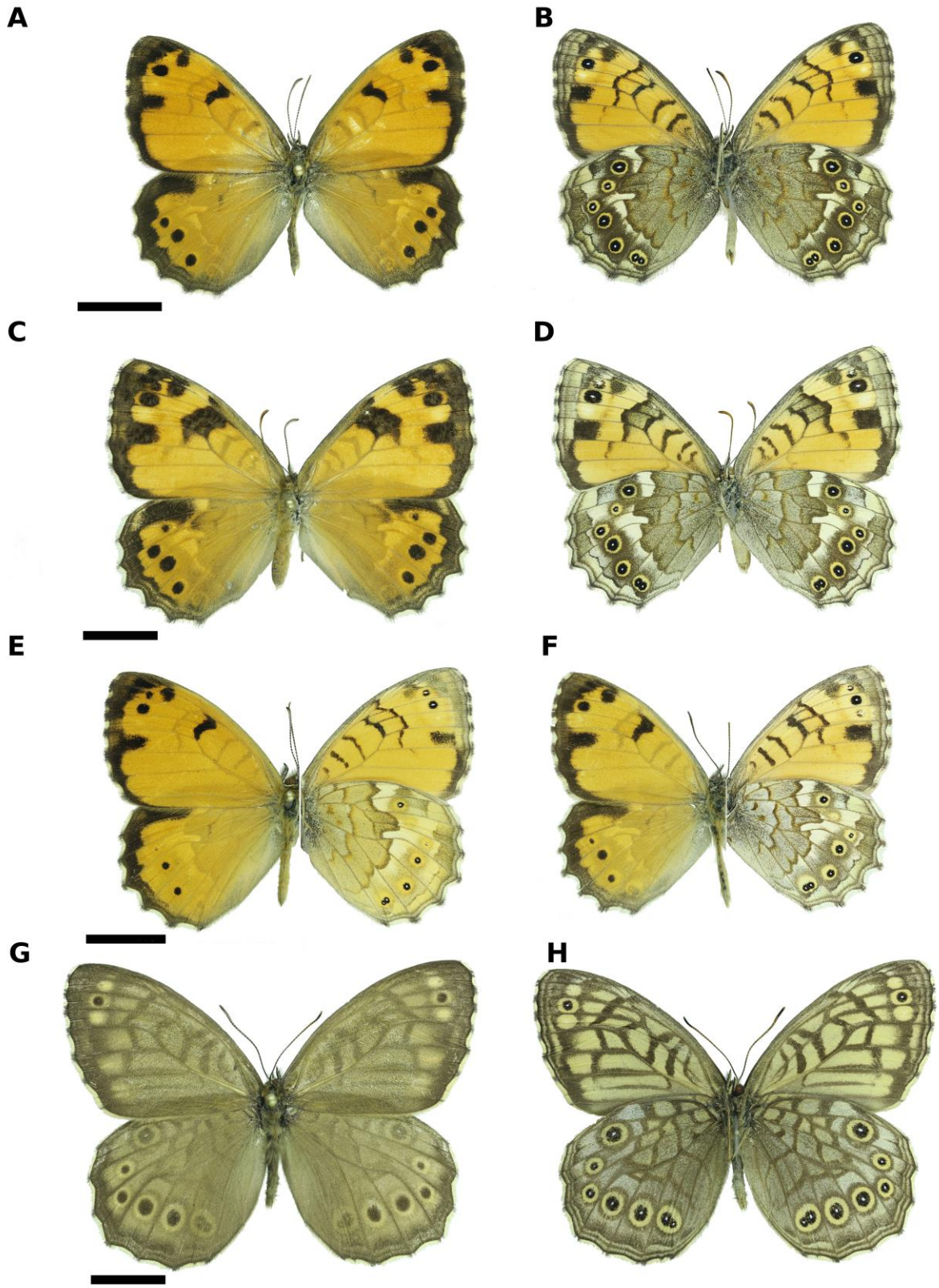

Figure 2. Type species of the genus *Esperarge* **stat. rev.** and *Kirinia*. A-F – *E. eversmanni* **comb. rev.**: A-D – ssp. *eversmanni*: A – male upperside; B – male underside, C – female upperside, D – female underside; E – ssp. *unicolor* – male upperside\underside; F – ssp. *shiva* **syn. nov.** [topotype] male upperside\underside; G-H – *K. epimenides*: G – male upperside, H – male underside.

The COI distance between *Esperarge cashmirensis* **comb. rev.** and *E. eversmanni* **comb. rev.** is 1.87–2.18%, and the first taxon should be considered a valid species. It should be noted that the subspecies *E. eversmanni unicolor* Grum-Grshimailo, 1892 **comb. rev.**, the range of which is geographically most close to that of *Esperarge cashmirensis* **comb. rev.**, has the largest genetic distance from the latter taxon of 2.18%. This fact indicates the absence of transitional forms between these taxa.

Samples of the subspecies *E. eversmanni unicolor* **comb. rev.** and *E. e. shiva* Wyatt, 1961 **syn. nov. & comb. rev.** share the same COI haplotype (Table 1). Hence, *E. e. shiva* **syn. nov. & comb. rev.** should be considered a junior synonym of *E. eversmanni unicolor* **comb. rev.** The COI genetic distance between the nominative subspecies and *E. e. unicolor* **comb. rev.** is 0.76–1.06% that supports the validity of the latter subspecies.

Table 2. List of sequenced specimens of *Esperarge eversmanni* and *Kirinia epimenides*. Materials are deposited in the Russian Museum of the Biodiversity Hotspots (RMBH) of the Federal Center for Integrated Arctic Research of the Russian Academy of Sciences, Arkhangelsk, Russia

Species	*COI* haplo-type code	*COI* GenBank acc. no.	Specimen Voucher	Locality
Esperarge eversmanni eversmanni	EE1	MK530656	Sph763	Kyrgyzstan, Tien-Shan, Chet-Baysorun Village
E. e. eversmanni	EE1	MK530657	Sph764	Kyrgyzstan, Tien-Shan, Chet-Baysorun Village
E. e. eversmanni	EE2	MK530658	Sph765	Kyrgyzstan, Tien-Shan, Sary-Chelek
E. e. eversmanni	EE1	MK530659	Sph766	Kyrgyzstan, Tien-Shan, Sary-Chelek
E. e. eversmanni	EE1	MK530660	Sph767	Kyrgyzstan, Tien-Shan, Chychkan Gorge
E. e. unicolor	EE6	MK530661	Sph768	Tajikistan, Navobod Village
E. e. unicolor	EE6	MK530662	Sph769	Tajikistan, Navobod Village
E. e. unicolor	EE6	MK530663	Sph770	Tajikistan, Pamir, Khorog Town
E. e. unicolor	EE6	MK530664	Sph771	Tajikistan, Pamir, Khorog Town
Kirinia epimenides	KE1	MK530665	Sph772	Russia, Khabarovsk Krai

Taxonomy

Family Nymphalidae Rafinesque, 1815
Subfamily Satyrinae Boisduval, [1833]

Genus ***Esperarge*** **Nekrutenko, 1988 stat. rev.**
= *Esperia* Nekrutenko, 1987. Vest. zool. 1987(2): 83 [homonym]
= *Esperella* Nekrutenko, 1987. Vest. zool. 1987(3): 62 [homonym]
= *Marginarge* Korb, 2005. A catalogue of butterflies: 34
= *Urrusia* Zhdanko, 2005. Tethys ent. Res. 11: 81.
Type species *Hipparchia eversmanni* Eversmann, 1847

Esperarge eversmanni **(Eversmann, 1847) comb. rev.**

Distribution. Kazakhstan, Kyrgyzstan, China (Xinjiang), Turkmenistan, Uzbekistan, Tajikistan, Afghanistan.

E. e. eversmanni **(Eversmann, 1847) comb. rev.**

Distribution. Uzbekistan (?), Kazakhstan, Kyrgyzstan (Tien-Shan), China (Xinjiang).

Material examined. Kyrgyzstan, Tien-Shan, Chychkan Gorge, mountain meadows and coniferous-deciduous forests at the bottom of the gorge, 2006 m, 41º09’19”N, 72º52’07”E, 18-19 July 2018, Spitsyn leg. – 1 ♂; Kyrgyzstan, Tien-Shan, Biosphere Reserve Sary-Chelek, mountain forests with walnuts, apple trees, cherry plums, coniferous trees and meadows, 1285-1392 m, 41º48’59”N, 71º57’34”E – 41º49’12”N, 71º58’20”E, 13 July 2018, Spitsyn leg. – 1♂,1♀; Kyrgyzstan, Tien-Shan, Chet-Baysorun Village, mountain

slopes covered with meadows and shrubs, 1959 m, 42º48’01”N, 77º39’50”E, 23-24 July 2018, Spitsyn leg. – 1♂, 1♀.

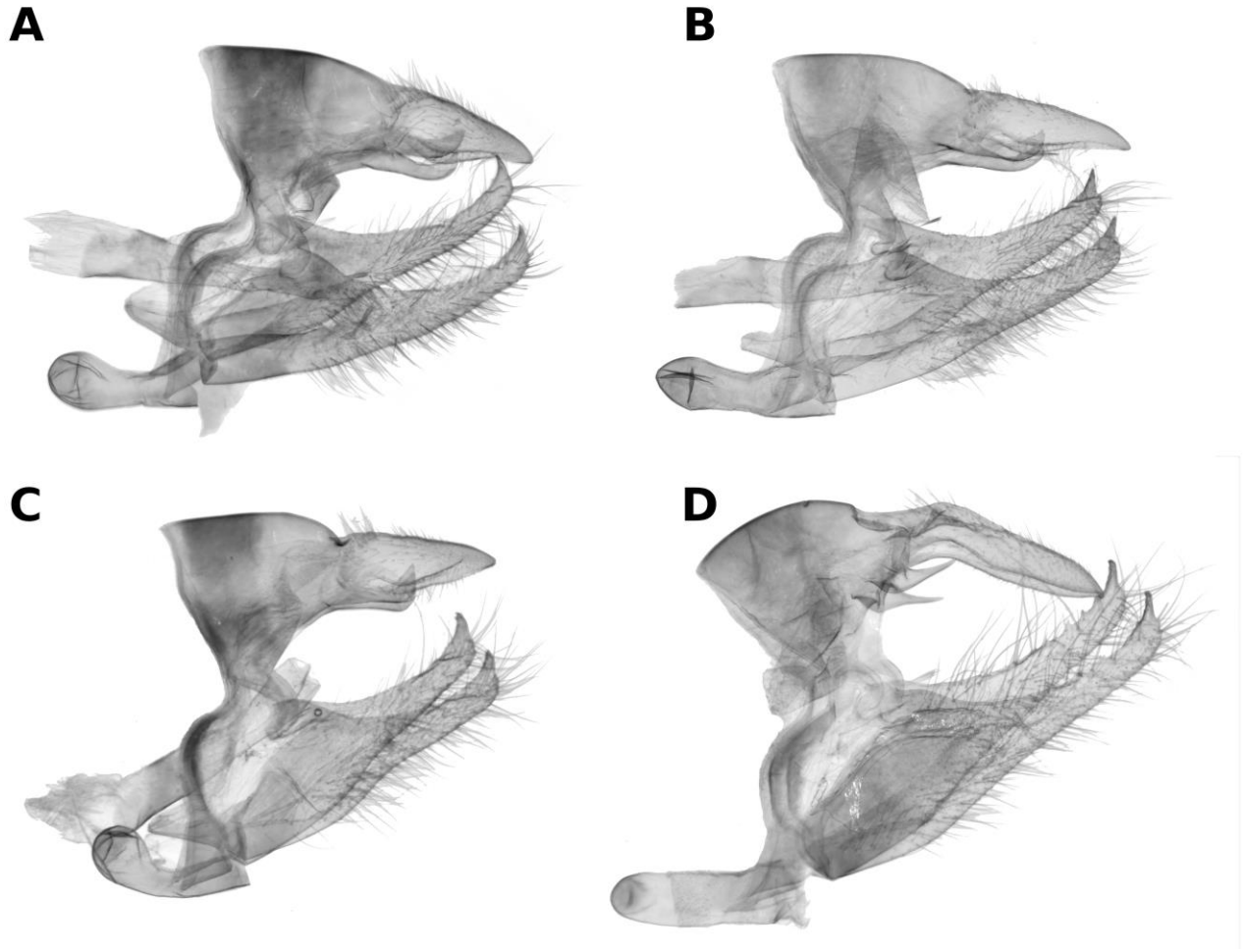

Figure 3. Male genitalia of *Esperarge* **stat. rev.**, and *Kirinia*: A-C – *E. eversmanni* **comb. rev.**: A – ssp. *eversmanni*, B – ssp. *unicolor*, C – ssp. *shiva* **syn. nov.**; D – *K. epimenides*.

E. e. unicolor **Grum-Grshimailo, 1892 comb. rev.**

=***E. e. shiva*** **Wyatt, 1961 syn. nov. & comb. rev.**; J. Lep. Soc. 15 (1): 9.

Distribution. Turkmenistan (?), S. Kyrgyzstan, Tajikistan, Afghanistan.

Material examined. (**ssp. *unicolor***) Tajikistan, Navobod Village, meadows on the mountain slopes, 1639 m, 38º57’38”N, 70º02’32”E, 21 June 2018, Spitsyn leg. – 4♂♂; (**ssp. *shiva* syn. nov.**) (topotype, collected less than 20 km from the type locality) Tajikistan (border with Afghanistan), Pamir, Khorog Town, Botanical Garden, 2249 м, 37º28’49”N, 71º35’59”E, 25-28 June 2018, Spitsyn leg. – 2♂♂.

Esperarge cashmirensis **(Moore, 1874) comb. rev.**

Distribution. Afghanistan, N. Pakistan, India (Kashmir).

Esperarge climene **(Esper, 1783) comb. rev.**

Distribution. Bulgaria, Romania, Serbia, Macedonia, Turkey, Syria, Iraq, Iran, Southern European Russia.

Esperarge roxelana **(Cramer, [1777]) comb. rev.**

Distribution. Asia Minor, Cyprus, Syria, Iraq.

Genus ***Kirinia*** **Moore, 1893**
Type species *Lasiommata epimenides* Ménétriés, 1859

Kirinia epimenides **(Ménétriés, 1859)**

Distribution. Russia: Primorsky and Khabarovsk Krai, China (Heilongjiang, Jilin, Liaoning, Beijing, Shandong, Shanxi, Henan, Zhejiang, Fujian, Shaanxi, Gansu, Sichuan), Korea, Japan (?).

Kirinia fentoni **(Butler, 1877)**
= *Kirinia epaminondas* (Staudinger, 1887)

Distribution. Russia: Primorsky Krai, China (Heilongjiang, Jilin, Hubei, Chongqing), Korea, Japan.

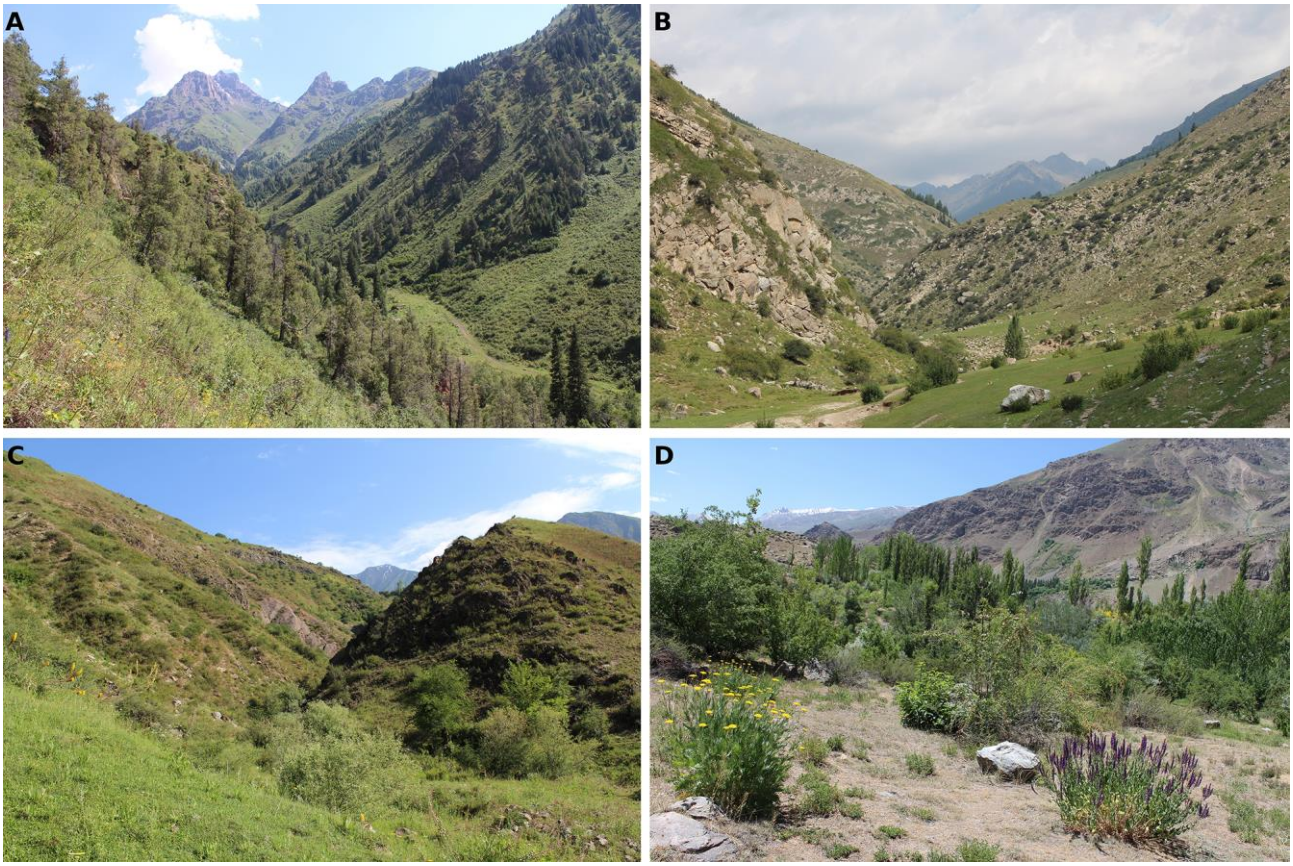

Figure 4. Habitats of selected subspecies of *Esperarge eversmanni* **comb. rev.**: A – Kyrgyzstan, Tien-Shan, Chychkan Gorge (ssp. *eversmanni*); B – Kyrgyzstan, Tien-Shan, Chet-Baysorun Village (ssp. *eversmanni*); C – Tajikistan, Pamir-Alay Mountain, Navobod Village (ssp. *unicolor*); D –Tajikistan (border with Afghanistan), Pamir, Khorog Town (ssp. *shiva* **syn. nov.**).

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