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The karyology and the taxonomy of the blue butterflies of the genus *Agrodiaetus* HÜBNER, [1822] from the Crimea

(Lepidoptera: Lycaenidae)

by

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Summary: The following haploid chromosome numbers are found in the Crimean taxa of *Agrodiaetus: A. poseidon krymaeus* (SHELJUZHKO, 1928) (n = 26, asymmetric karyotype with 7 big chromosomes); *A. damone pljushtchi* (LUKHTANOV & BUDASHKIN, 1993) (n = 67, asymmetric karyotype with two macrochromosomes) and *A. ripartii budashkini* (KOLEV & DE PRINS, 1995) (n = 90, asymmetric karyotype with two macrochromosomes, one of them is always bigger than the other). As a result of the karyotype analysis the following taxonomic changes are proposed: *A. pljushtchi* (LUKHTANOV & BUDASHKIN, 1993) is considered as a subspecies of *A. damone* (EVERSMANN, 1841) and *A. budashkini* (KOLEV & DE PRINS, 1995) as a subspecies of *A. ripartii* (FREYER, 1830). It is suggested that *A. poseidon poseidon* (HERRICH-SCHÄFFER [1851]), with n = 19 and symmetric karyotype exists in the Crimea.

The genus *Agrodiaetus* HÜBNER, [1822] is a very difficult group for taxonomists as there are many species which are very close and a lot of them are prone to considerable geographic and individual variability. Even male genitalia are very similar and provide no reliable diagnostic character. Usually only the study of karyotypes can help to solve the taxonomic problems (DE LESSE, 1960a, 1960b; LUKHTANOV, 1989; MUNGUIRA et al., 1994).

The Crimean peninsula has received considerable attention to lepidopterologists. Even though the taxonomy of the Crimean butterflies is often regarded as settled, a great deal of research is still needed in the genus *Agrodiaetus*.

According to recent publications (NEKRUTENKO, 1985; BUDASHKIN & LUKHTANOV, 1993; KOLEV & DE PRINS, 1995), there are three species of *Agrodiaetus* in the Crimea: *A. poseidon krymaeus* (SHELJUZHKO, 1928), *A. pljushtchi* (LUKHTANOV & BUDASHKIN, 1993) and *A. budashkini* (KOLEV & DE PRINS, 1995). The karyotypes of these species have not been known so far. The author was interested in studying the karyotypes of these species to clarify their relationships with other species of the genus.

Material and Methods

Insects for this study were collected by the author and Dr. K. EFETOV in the Crimea. Only adult males were used. The testes of caught butterflies were extracted in the field and immediately fixated in a mixture of 96% alcohol and glacial acetic acid (3:1). Testis of each specimen was kept separately and was given the same code as the male. The material was stored in the fixative solution for 1–6 months. Then the testes were stained in 2% acetic orcein for 7–20 days. A stained testis was placed on a slide with a drop of 1:1 glacial acetic acid and lactic acid solution and then dissected with entomological pins. Thereupon the preparation was covered by a coverslide and squashed.

Chromosome numbers were counted at meiotic prometaphase 1 and meiotic metaphase 1. Photomicrographs were taken with Carl Zeiss Research microscope "Ergaval" External characters of corresponding specimens were studied with a binocular microscope.

The following abbreviations are used in this paper:
n – haploid number of chromosome;
Pr1 – meiotic prometaphase 1;
Me1 – meiotic metaphase 1;
ca – circa (lat.), approximately.

If it was not possible to count the chromosome number exactly (for instance, when one chromosome seemed to superimpose the other), then approximate numbers separated by a hyphen (table 1) are given.

Results

Three populations of *A. poseidon krymaeus* SHEL. were investigated. Two of them were situated near Kurortnoe closely to the type locality of *A. poseidon krymaeus* SHEL. (Crimea, Stary Crym) (table 1; fig. 1–4). Intraindividual variability in chromosome number was being found in these two populations. The mean chromosome number is n = 26; metaphase plates with n = 25 and n = 27 were found, but they were being met with scarcer than n = 26. No difference between two populations were found in both the mean chromosome number and the haploid number variability. During prometaphase and metaphase spermatocytes 1 had bivalents of different size: seven big bivalents were approximately four times larger than the other ones (figs. 2, 3).

Different types of karyotype were discovered in the third population from near Angarsky pass. Some individuals had n = 19 and symmetric karyotype (t1; fig. 5), others had n = 23–26 (it was hard to count exactly) and asymmetric karyotype with 3–6 big bivalents (Table 1; fig. 6), sometimes with curved bivalents and trivalents. Usually in the latter case the bivalents were not formed in a regular equatorial plate (fig. 6).

The population of *A. pljushtchi* LUKHT. & BUD. from the type locality (Crimea, Ai-Petry mt.) was investigated. Intraindividual variability was being observed in some specimens. The haploid chromosome number n = 67 was being found more frequently than n = 66 and n = 68 (table 1; fig. 7). The karyotype was exactly asymmetric. There were the group of big bivalents (always 2) and the group of medium bivalents (usually 1 or 2) which differed in size from the other small ones. The big bivalents were usually situated in the centre of metaphase plates (fig. 7).

The chromosome number n = 90 of *A. budashkini* KOLEV & DE PRINS was determined in two populations (table 1; fig. 8). The appearance of other chromosome numbers (especially n = 87) could be explained by the difficulty of counting very small bivalents. The karyotype was exactly asymmetric. There were two big bivalent, one of them was always bigger than the other, which differed in size from the other small ones. The big bivalents were always located in the centre of metaphase plates and surrounded by small ones (fig. 9). At the moment it is difficult to say anything about intraindividual variability and polymorphism.

Table 1. Chromosome numbers of the studied taxa of the genus *Agrodiaetus*.

Investigated taxa of *Agrodiaetus*, localities and dates	code of specimen	chromosome number	quantity
A. poseidon krymaeus (SHELJUZHKO, 1928)			
Ukraine, Crimea, Sudak reg., 3–4 km N from Kurortnoe vill., Leghener mt., 400–600 m, 02.–05.VII.1995, N. P. KANDUL leg.	95012	n = 25	1 Me1
		n = 26	6 Me1
	95013	n = 26	6 Me1
	92030	n = ca 25–26	1 Me1
		n = 26	4 Me1
	95032	n = 26	1 Pr1
	95033	n = 25	2 Me1
		n = ca 25–26	2 Me1
		n = 26	6 Me1
		n = ca 26–27	3 Me1
	95034	n = 26	1 Me1
	95037	n = 26	8 Me1
		n = ca 26–27	1 Me1
	95040	n = 25	1 Me1
		n = 26	24 Me1
		n = 26	3 Pr1
		n = ca 26–27	1 Me1
Ukraine, Crimea, Sudak reg., 3–4 km N from Kurortnoe vill., Leghener mt., 400–600 m, 17.VII.1995, N. P. KANDUL leg.	95058	n = 26	4 Me1
	95060	n = 26	7 Me1
	95061	n = 25	2 Me1
		n = ca 25–26	2 Me1
		n = 26	6 Me1
	95062	n = 25	1 Me1
		n = 26	6 Me1
	95063	n = 26	4 Me1
	95066	n = 26	5 Me1
	95016	n = 26	7 Me1
	95017	n = 26	10 Me1
		n = ca 26–27	2 Me1
Ukraine, Crimea, Sudak reg., 3–4 km SW from Kurortnoe vill., Achke-dag mt., 600–800 m, 04.VII.1995, N. P. KANDUL leg.	95023	n = 25	3 Me1
		n = ca 25–26	1 Me1
		n = 26	15 Me1
	95024	n = 26	1 Pr1
	95025	n = ca 25–26	4 Me1
		n = 26	16 Me1
	95026	n = ca 25–26	1 Me1
		n = 26	4 Me1
	95027	n = ca 24–25	2 Me1
		n = 26	5 Me1
		n = 27	2 Me1

Investigated taxa of *Agrodiaetus*, localities and dates	code of specimen	chromosome number	quantity
Ukraine, Crimea, Sudak reg., 3–4 km SW from Kurortnoe vill., 600–800 m, 01.–05.VII.1996, N. P. KANDUL leg.	96001	n = 25	1 Me1
		n = 26	1 Pr1
	96005	n = 26	4 Me1
	96006	n = 26	10 Me1
		n = 26	3 Pr1
	96007	n = 25	1 Me1
		n = ca 25–26	1 Me1
		n = 26	3 Me1
Ukraine, Crimea, Simferopol reg., Angarsky pass, 700–800 m, VII.1992, K. EFETOV leg.	E92011	n = ca 24–26	4 Me1
	E92016	n = 23	9 Me1
		n = 25	3 Pr1
		n = ca 26–27	2 Me1
A. poseidon poseidon (HERRICH-SCHÄFFER, [1851])			
Ukraine, Crimea, Simferopol reg., Angarsky pass, 700–800 m, VII.1992, K. EFETOV leg.	E92003	n = 19	2 Me1
	E92012	n = 19	8 Me1
	E92014	n = 19	4 Pr1
	E92015	n = 19	2 Me1
A. damone pljushtchi (DANTCHENKO & LUKHTANOV, 1993)			
Ukraine, Crimea, Yalta reg., Ai-petry mt., 900–1400 m, 14.VII.1995, N. P. KANDUL leg.	95050	n = 66	2 Me1
		n = 67	3 Me1
	95051	n = 65	1 Me1
		n = 67	3 Me1
	95054	n = 66	4 Me1
		n = 67	3 Me1
		n = 68	2 Me1
	95055	n = ca 65	4 Me1
		n = 67	1 Me1
Ukraine, Crimea, Yalta reg., Ai-petry mt., 900–1400 m, 10.VII.1996, N. P. KANDUL leg.	96009	n = ca 65–66	2 Me1
	96010	n = 67	1 Me1
	96011	n = ca 65	2 Me1
	96012	n = 66	1 Me1
		n = 67	1 Me1
	96017	n = ca 66–68	1 Me1

Investigated taxa of *Agrodiaetus*, localities and dates	code of specimen	chromosome number	quantity
A. ripartii budashkini (KOLEV & DE PRINS, 1995)			
Ukraine, Crimea, Sudak reg., env. of Kurortnoe vill., 02.VII.–10.VII.1995, N. P. KANDUL leg.	95001	n = ca 87	1 Me1
	95004	n = ca 88–90	1 Me1
		n = ca 90–92	1 Me1
	95005	n = ca 89–90	1 Me1
		n = 90	1 Me1
	95011	n = ca 86–88	1 Me1
		n = 90	2 Me1
		n = ca 89–91	1 Me1
Ukraine, Crimea, Yalta reg., Ai-Petry mt., 900–1400 m, 01.VII.1996, N. P. KANDUL leg.	96020	n = ca 88–90	1 Me1
	96021	n = ca 88–90	2 Me1

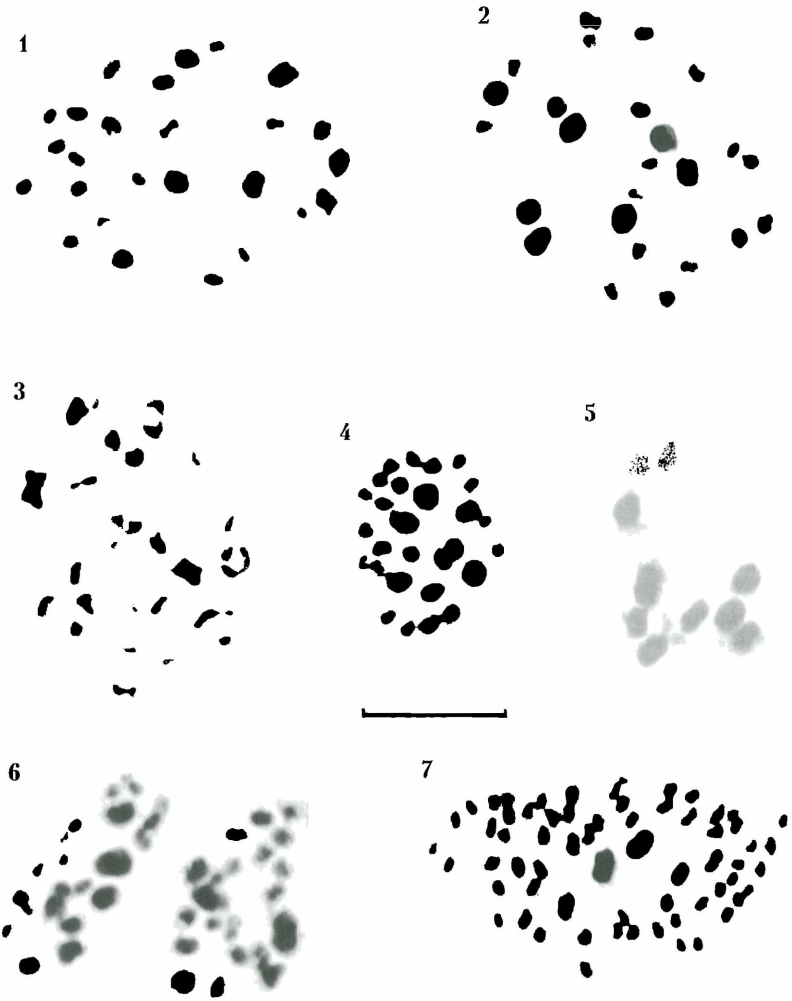
Discussion

1. A. *poseidon krymaeus* (SHELJUZHKO, 1928)

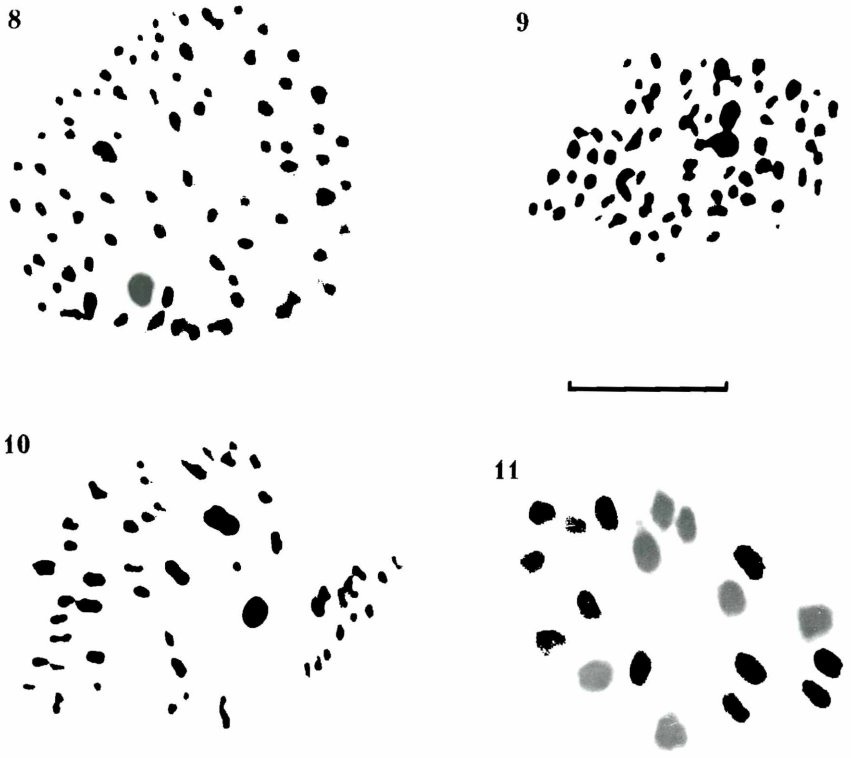
At first the taxon *krymaeus* SHEL. was described as the subspecies of the East-European species *A. damone* (EVERSMANN, 1841) with which it has some external similarity. It was not until 1961 that *krymaeus* SHEL. was transferred to *A. poseidon* H.-S. by FORSTER (1961) as a subspecies. According to our data, *A. damone damone* EV. has a different chromosome number (n = 67) and karyotype structure (fig. 10). Therefore, it is already obvious that *krymaeus* SHEL. and *damone* EV. do not belong to the same species.

At the same time populations of *krymaeus* SHEL. which were similar externally were found to be heterogeneous karyologically. Individuals from two populations from the environs of Kurortnoe, near the type locality of *A. poseidon krymaeus* SHEL. (Crimea, Stary Crym) were cytogeneticly close to the taxa of *poseidon*'s group with high chromosome numbers (from n = 24 to n = 26) (KANDUL & LUKHTANOV, 1997). They also had the same karyotype structure. A part of the butterflies from the locality near Angarsky pass could be approached with the taxa of *poseidon*'s group with low chromosome numbers (from n = 19 to n = 20) (DE LESSE, 1963). The latter had the same symmetric karyotype as *A. poseidon poseidon* (HERRICH-SCHÄFFER, [1851]) from Artvin, Turkey (fig. 11). The other part of the specimens from this locality (table 1: E92011, E92016) had a karyotype displaying features of probable interspecies numerical hybrid: some bivalents were being seen outside a regular equatorial plate, trivalents and conjugation between two bivalents were found (fig. 6). Anyway karyologically these specimens (E92011, E92016) more closely related with *A. poseidon krymeaus* SHEL. than with *A. posidon poseidon* H.-S.

Consequently, two taxa of *poseidon*'s group, externally the same, were found in the Crimea. It is quite a pity, that we did not succeed in collecting additional material from Angarsky pass in 1996. Further karyologic study in blue butterflies of *Agrodiaetus* from the Angarsky pass locality should be done. It is necessary because the Crimea can be the single sympatric



Figs. 1–7: Photomicrographs of spermatocytes 1:
1 – *A. poseidon krymaeus* (SHELJUZHKO, 1928) N 95066, n = 26, Me1.
2 – *A. poseidon krymaeus* (SHELJUZHKO, 1928) N 95040, n = 25, Me1.
3 – *A. poseidon krymaeus* (SHELJUZHKO, 1928) N 95040, n = 26, Pr1 (late diplotene).
4 – *A. poseidon krymaeus* (SHELJUZHKO, 1928) N 95027, n = 27, Me1.
5 – *A. poseidon poseidon* (HERRICH-SCHÄFFER, [1851]) N E92012, n = 19, Me1.
6 – *A. poseidon krymaeus* (SHELJUZHKO, 1928) N E92016, n = 23 and n=25, Me1.
7 – *A. damone pljushtchi* (LUKHTANOV & BUDASHKIN, 1993) N 95054, n = 66, Me1.
Scale bar for all figures represents 10 mkm.



Figs. 8–11: Photomicrographs of spermatocytes 1:
8 – *A. ripartii budashkini* (KOLEV & DE PRINS, 1995) N 95011, n = 90, Me1.
9 – *A. ripartii budashkini* (KOLEV & DE PRINS, 1995) N 95011, general view of the karyotype, Me1.
10 – *A. damone damone* (EVERSMANN, 1841) Saratov, Russia, n = 67, Me1.
11 – *A. poseidon poseidon* (HERRICH-SCHÄFFER, [1851]) Artvin, Turkey, n = 19, Me1.
Scale bar for all figures represents 10 mkm.

inhabitation of taxa from different chromosome groups of *A. poseidon* H.-S. known today and it may help to investigate taxonomic relationships inside the *poseidon* group.

2. *A. damone pljushtchi* (LUKHTANOV & BUDASHKIN, 1993)
According to karyologic data, the karyotype of *A. pljushtchi* LUKHT. & BUD. is quite similar to that of *A. damone damone* (EVERSMANN, 1841) (n = 67, rarely n = 66 or n = 68; asymmetric structure) (fig. 10). This taxon is known from a small population in the slope of Ai-Petry's yaila, far away from the type locality of *A. damone* EV. and its subspecies: *tanais* (DANTCHENKO &

PLJUSHTCH, 1993) (DANTCHENKO & LUKHTANOV, 1993) and *altaica* (ELWES, 1899). Nevertheless, it has some features approaching it to *A. damone* EV., especially with subspecies *altaica* ELW., for instance the green-blue dusting on the hindwings which can cover about one-fourth of the wings' area. Therefore, it would be more logical to consider this taxon as a separate subspecies of the species *A. damone* EV.

3. *A. ripartii budashkini* (KOLEV & DE PRINS, 1995)

The species of the "brown *Agrodiaetus*" complex, the *admetus-ripartii* group by DE LESSE (1960b), are the most complicated to classify. Cytogenetic information was often the most significant evidence, although not always sufficient, for taxonomy of some butterflies in this complex.

In the first catalogue of butterflies of the Soviet Union, KORSHUNOV (1972) mentioned three brown *Agrodiaetus* species which, according to him, were found in the Crimea: *admetus* ESP., *fabressei* OBTH. and *ripartii* FRR. It was not until 1985 that NEKRUTENKO (1985) found that only one polymorphic species of the brown *admetus-ripartii* group occurs in the Crimea. NEKRUTENKO decided that it was more reasonable to retain the oldest available name of this butterfly. On the other hand each of these three species has its own distinguished karyotype: *A. admetus* (ESPER, 1785) n = 80 with two macrochromosomes, *A. fabressei* (OBERTHÜR, 1910) n = 90 with four or three macrochromosomes, two of them always bigger than the other and *A. ripartii* (FREYER, 1830) n = 90 with two macrochromosomes, one of them always bigger than the other (DE LESSE,1960b; MUNGUIRA et al., 1994).

Recently this insect was described as a new species by KOLEV & DE PRINS (1995). They presented rather slight features for this new species but a strict character is its locality. The other measuring characters could have been more statistically accurate, if they had examined not only single individuals. *A. budashkini* (KOLEV & DE PRINS) and *A. agenjoi* (FORSTER) are approached each other by them, according to their external similarity.

The butterflies of the taxon *budashkini* KOLEV & DE PRINShave the same karyotype (chromosome number n = 90 and karyotype structure) (figs. 8, 9) as the species *A. ripartii* FRR. from Spain, France and Turkey (DE LESSE, 1960b; MUNGUIRA et al., 1994). These blue butterflies are both different from *A. admetus* ESP. and *A. fabressei* OBTH., forming the "short valva type" species group, and are morphologically very similar even by KOLEV & DE PRINS (1995). They also occur allopatricly. So today it would be more correct to place this brown butterfly in the species *A. ripartii* FRR. as a subspecies. Now it would be interesting to study *A. agenjoi* FORSTER karyologically.

As a conclusion of this study, the Crimean *Agrodiaetus* with their correct names and chromosome numbers are presented: *A. poseidon krymaeus* (SHELJUZHKO, 1928) (n = 26, asymmetric karyotype with 7 big chromosomes), *A. poseidon poseidon* (HERRICH-SCHÄFFER, [1851]) (n = 19 and symmetric karyotype), *A. damone pljushtchi* (LUKHTANOV & BUDASHKIN, 1993) (n = 67, asymmetric karyotype with two macrochromosomes) and *A. ripartii budashkini* (KOLEV & DE PRINS, 1995) (n = 90, asymmetric karyotype with two macrochromosomes, one of them always bigger than the other).

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References

BUDASHKIN, YU. & V. LUKHTANOV (1993): Eine neue Art der Untergattung *Agrodiaetus* von der Krim (Lepidoptera, Lycaenidae). – Atalanta **24**: 85–87.

DANTCHENKO, A. & V. LUKHTANOV (1993): Zur Systematik und Verbreitung der Arten der *Polyommatus (Agrodiaetus) damone*-Gruppe Südosteuropas und Südwestsibiriens (Lepidoptera, Lycaenidae). – Atalanta **24**: 75–83.

FORSTER, W. (1961): Bausteine zur Kenntnis der Gattung *Agrodiaetus* SCUDD. (Lep. Lycaen.). – Zeitschr. Wien. Ent. Ges. **46**: 8–13, 38–47, 74–79, 88–94, 110–116.

KANDUL, N. & V. LUKHTANOV (1997): Analysis of the karyotypes variability and the systematics of the blue butterflies of the *Poliommatus (Agrodiaetus) poseidon* and *P. (A.) dama* species groups (Lepidoptera, Lycaenidae.). – Zool. Zh. **76**: 63–69 (in russian).

KOLEV, Z. & W. DE PRINS (1995): A new species of the "brown *Agrodiaetus*" complex from the Crimea (Lepidoptera: Lycaenidae). – Phegea **23**: 119–132.

KORSHUNOV, YU. (1972): Catalogue of diurnal butterflies (Lepidoptera, Rhopalocera) of the fauna of the USSR. – Revue d'Entomologie de'URSS **51**: 136–154, 352–368 (in russian).

LESSE DE, H. (1960a): Speciation et variation chromosomique chez les Lepidopteres Rhopaloceres. – Annls Sci. nat., zool. **12**: 1–223.

LESSE DE, H. (1960b): Les nombres de chromosomes dans la classification du groupe d'*Agrodiaetus ripartii* FREYER. – Revue franc. Entomol. **27**: 240–262.

LESSE DE, H. (1963): Variation chromosomique chez les *Agrodiaetus* (Lep. Lycaenidae). – Revue franc. Entomol. **30**: 182–189.

LUKHTANOV, V. (1989): Karyotypes of some blue butterflies of the *Agrodiaetus* species groups (Lepidoptera, Lycaenidae). – Annls entomol. fenn. **55**: 137–144.

MUNGUIRA, M., MARTIN, J. & M. PEREZ-VALIENTE (1994): Karyology and distribution as tools in the taxonomy of Iberian *Agrodiaetus* butterflies (Lepidoptera: Lycaenidae) – Nota lepid. **17**: 125–140.

NEKRUTENKO, YU. (1985): Rhopalocera of the Crimea. – Naukova dumka, Kiev, 152 pp (in russian).

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