

***Polyommatus dantchenkoi* (Lukhtanov & Wiemers, 2003) tentatively identified as new to Europe, with a description of a new taxon from the Balkan Peninsula (Lycaenidae)**

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Abstract. Karyological data have revealed the presence of a taxon corresponding to the recently described *Polyommatus (Agrodiaetus) dantchenkoi* (Lukhtanov & Wiemers, 2003) in the Rhodopi Mts. of Bulgaria. The find expands greatly the range of this karyospecies, previously known only from Van Province in SE Turkey. This population is identical in karyotype structure and haploid chromosome number ($n=41-42$) and has a generally similar external and genital morphology than *P. (A.) dantchenkoi*. On the other hand, the Rhodopi population differs morphologically from nominotypical *P. (A.) dantchenkoi* as well as from all other Balkan taxa of brown *Agrodiaetus*. For these reasons this population is here described as a separate taxon, *Polyommatus dantchenkoi orphicus* ssp. n.

Key words. karyotype, chromosome, *Polyommatus*, *Agrodiaetus*, *dantchenkoi*, *aroaniensis*, *ripartii*, *admetus*, Lycaenidae, Lepidoptera, Europe, Balkan Peninsula, Bulgaria, Rhodopi, *orphicus* new subspecies.

Introduction

The systematics of *Agrodiaetus* Hübner, 1822, a subgenus of *Polyommatus* Latreille, 1804 is presently in a state of upheaval. In addition to the steady flow of descriptions of new taxa, the first molecular phylogenies of the group (Wiemers 2003; Kandul et al. 2004) provided entirely new insights on the relationships within this highly complex and diverse subgenus. Most challenging is the taxonomy of the so-called monomorphic or ‘brown’ taxa, so named because of the uniform brown colour of the male upperside, similar to that of the female. The high species diversity of this group was brought to light in the 1960’s by the pioneering karyological research of H. De Lesse. Nowhere in the subgenus is reliance on karyology as important for taxonomy and systematics as in the brown taxa. Currently four species of brown *Agrodiaetus* are known from the Balkan Peninsula: *P. (A.) admetus* (Esper, [1783]), *P. (A.) ripartii* (Freyer, 1830), *P. (A.) aroaniensis* (Brown, 1976), and *P. (A.) nephohiptamenos* (Brown & Coutsis, 1978). The last two species are endemic to this region. Despite this diversity (the highest in Europe), karyological research on the Balkan brown *Agrodiaetus* has been sporadic to date and has concentrated almost exclusively on Greek populations (Brown 1976; Brown & Coutsis 1978; Coutsis et al. 1999; Wiemers 2003). The recent reports of *P. (A.) aroaniensis* and *P. (A.) nephohiptamenos* as new to Bulgaria (Kolev 1994) and *P. (A.) aroaniensis* as new to Former Yugoslav Republic of Macedonia (Kolev & Poorten 1997) have been based on morphological and anatomical characters. The only published karyological data from elsewhere in the peninsula remain those of H. de Lesse, who sampled *P. (A.) ripartii* and *P. (A.) admetus* in westernmost Bulgaria (Kalotina checkpoint) en route to Asia Minor (de Lesse 1960).

In 1999 a large mixed population of brown *Agrodiaetus* in the Rhodopi Mts. of southern Bulgaria has been found, which consists of two morphologically very similar taxa, *P. (A.) ripartii* and an unknown taxon. Analysis of karyological samples revealed that the latter differs from *P. (A.) ripartii* and *P. (A.) aroaniensis* in its karyotype as

well as morphology. The karyotype of the Rhodopi population is identical with that of the recently described *P. (A.) dantchenkoi* (Lukhtanov & Wiemers, 2003) which so far is known only from its type locality, the Van Province of south-eastern Turkey (Lukhtanov et al. 2003). Thus the Rhodopi population can be considered the first record of *P. (A.) dantchenkoi* for the whole of Europe. However, the morphological differences between nominotypical *P. (A.) dantchenkoi* and the Rhodopi population as well as their considerable geographical separation prompted me to offer the following description of a new subspecies.

Abbreviations

IZS	Institute of Zoology, Sofia
n	haploid chromosome number
NMNHS	National Museum of Natural History, Sofia
ZK	collection of Zdravko Kolev, Helsinki

Methods

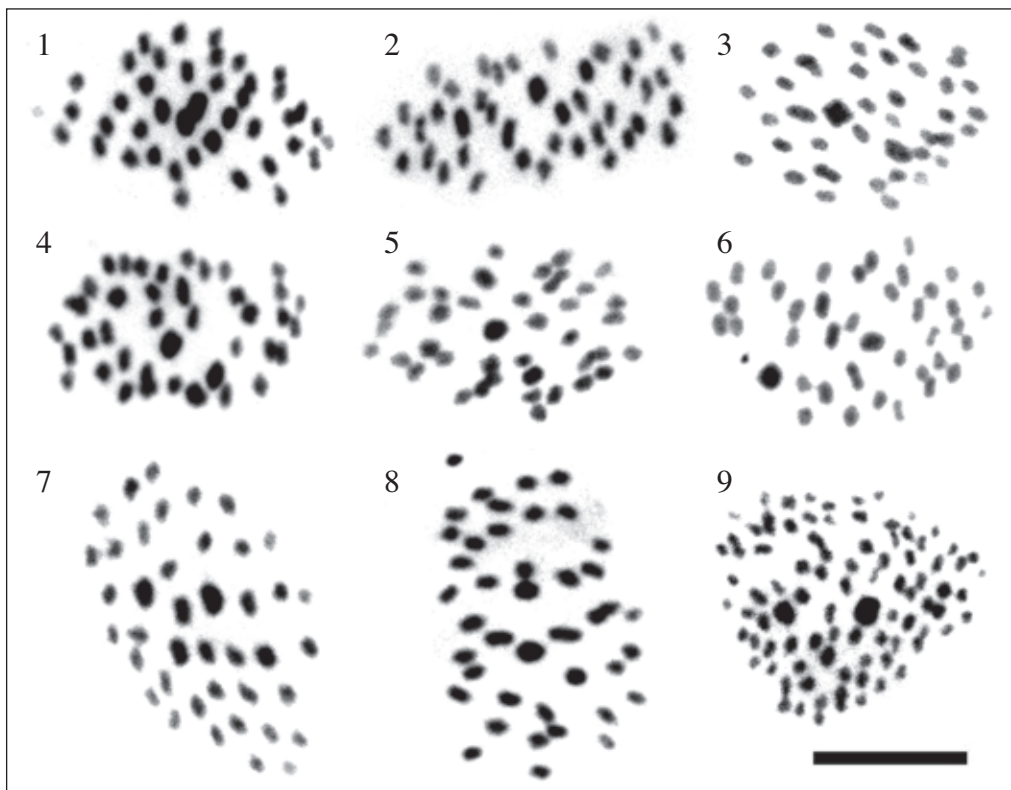
For karyological analysis males were collected and kept alive in moistened paperbags until the extraction of their testes was possible. Testes were fixed in a 3:1 solution of 96% ethanol and glacial acetic acid and refrigerated until further study. Preparations were made at the Department of Genetics, University of Turku (Finland) following an advanced methodology for studying holokinetic chromosomes developed by Dr. Seppo Nokkala (e.g. Grozeva & Nokkala 1996). Countable metaphase plates were photographed at a magnification of 1000× with an Olympus DP11 microscope digital camera system mounted on an Olympus BH-2 light microscope. The brightness and contrast of the digital images were enhanced for printing in Jasc Paint Shop Pro 7.04; no other image processing was applied.

For studying the genitalia, abdomens were macerated in a 10% solution of KOH, the genital structures were extracted, cleaned, measured, and drawn immersed at a magnification of 50× with the aid of camera lucida attached to a Wild stereo-microscope.

In attempting to establish the distribution of the new taxon and its relatives in Rhodopi, the public collections of the National Museum of Natural History (Sofia), the Institute of Zoology (Sofia), and the Museum of Natural History (Burgas) were examined. Additional material was received from the private collections of Mr. Julius Ganev (Sofia) and Dr. Stoyan Beshkov (Sofia).

Polyommatus dantchenkoi orphicus ssp. n. Figs. 1–8, 10–18, 33, 34, 39, 42

Material. Holotype ♂, white printed label ‘S BG [Bulgaria], Rhodopi Mts., l open dry rocky slopes above l Hvoyna village, 800-950 m | 7.vii.1999, Z. Kolev leg.’; white hand-written label ‘ZK99017’; red printed label ‘HOLOTYPE | *Polyommatus dantchenkoi orphicus* ssp. n. | Z. Kolev det.’; ZK. – Paratypes 6♂, 2♀: same white printed labels as holotype except 1♀ (Figs. 11, 34), ‘S BG [South Bulgaria], Rhodopi Mts., l open dry rocky slopes above l Hvoyna village, 800-950 m | 6.vii.1999, Z. Kolev leg.’; white hand-written labels (5♂) ‘ZK99001’, ‘ZK99004’, ‘ZK99010’, ‘ZK99018’ and ‘ZK99019’; red printed label ‘PARATYPE | *Polyommatus dantchenkoi orphicus* ssp. n. | Z. Kolev det.’; ZK. Part of the type material is to be deposited in NMNHS. – **Additional material.** 1♂: S Bulgaria, Rhodopi Mts., Lukovitsa gorge near Asenovgrad town, 400 m, 21.vi.1986, Beshkov leg., ZK; 1♀: same data but 21.vii.1998, Kolev leg., ZK; 1♀: Rhodopi Mts., “Persenk” mine, 25.vii.1977, Ganev leg., ZK; 1♀: Rhodopi Mts., vic. Gela village, 1500 m, 23.vii.1999, Kolev leg., ZK.



Figs. 1–8. Metaphase plates of *P. (A.) dantchenkoi orphicus* ssp. n. **1.** n=42, paratype (Fig. 16), prep. ZK99001-02. **2.** n=41, same specimen as Fig. 1, prep. ZK99001-15. **3.** n=42, same specimen as Fig. 1, prep. ZK99001-24. **4.** n=41, paratype (Fig. 14), prep. ZK99004-02. **5.** n=42, same specimen as Fig. 4, prep. ZK99004-14. **6.** n=42, holotype (Fig. 10), prep. ZK99017-04. **7.** n=41, same specimen as Fig. 6, prep. ZK99017-07. **8.** n=42, paratype (Fig. 15), prep. ZK99019-01.
Fig. 9. Metaphase plate of *P. (A.) ripartii* n=90, same specimen as Figs. 28 & 35, prep. ZK99011-02. Scale bar = 10 μ m.

Description. Measurements. Relatively large for a brown *Agrodiaetus*. Male forewing length 13.0–18.2 mm (holotype 17.0 mm), mean 16.65 mm (8♂), female forewing length 14.5–16.5 mm, mean 15.3 mm (5♀).

Upperside. Resembles *P. (A.) aroaniensis* closely in both sexes. Ground colour dark brown with silky sheen and, in males, an extensive androconial field on forewing. Veins relatively inconspicuous in both sexes, not markedly darker than ground colour, giving upperside a smooth appearance. Discoidal spot in females darker than ground colour. Fringe brown, on hindwing brown or brownish-grey on distal half.

Underside. Ground colour warm, light to medium yellowish-brown, with basal half of forewing tinged colder grey. **Forewing:** Postdiscal black ocelli prominent, forming strongly curved row in spaces M3, Cu1 and Cu2, with ocelli in spaces Cu1 and Cu2 on straight, or nearly so, line with discoidal spot. Space M2, along vein M3, with narrow, diffuse but well visible white streak connecting discoidal and postdiscal spots in space M2 and often extending a short distance distad from latter. **Hindwing:** Greenish iridescent basal suffusion absent or almost so. Postdiscal black ocelli usually

well-defined and in complete series, seldom some reduced. Ocelli in spaces M3, Cu1, Cu2 and A2 situated more basad than in *P. (A.) ripartii*, identical to those of *P. (A.) d. dantchenkoi* and *P. (A.) aroaniensis*. White streak in space M2 always prominent and sharply defined, its expression independent of degree of reduction of postdiscal ocelli. Submarginal markings diffuse and faint, only slightly darker than ground colour; reddish submarginal lunules absent or very faint and exceedingly small.

Male genitalia (Figs. 39 a, b, c). As in *P. (A.) aroaniensis* (Fig. 40) and *P. (A.) d. dantchenkoi*, of the 'long type' (Kolev & De Prins 1995). Valva 3.00–3.20 mm long, mean 3.11 mm (6 ♂), being significantly ($P < 0.001$, two-tailed *t*-Test assuming equal variances) longer than in *P. (A.) ripartii* (Fig. 41): 2.36–2.71 mm, mean 2.59 mm (13 ♂). Within each taxon the length of valva generally increases with increasing individual size, as is only to be expected, but the morphometric difference between the male genitalia of *P. (A.) dorphicus* and *P. (A.) ripartii* is clearly not a function of of variable individual size. This is evident from Fig. 47, which shows a consistent gap between the ranges of variance in valva length of syntopic samples of the two taxa. This gap is remarkably wide, particularly in view of the almost total overlap of variance ranges in forewing length between the two taxa. Besides, the effect of individual variation on genital size can be eliminated by calculating and comparing individual values of the unit-less index FWL/VL, which is obtained by dividing the forewing length (FWL in mm) by the valva length (VL in mm). This index is 5.38–5.69 (mean 5.5683, 6 ♂) for *P. (A.) dorphicus* and 5.97–6.73 (mean 6.38, 13 ♂) for *P. (A.) ripartii*. The differences between the means of these non-overlapping ranges of variance is statistically significant ($P < 0.001$, two-tailed *t*-test assuming equal variances). In other words, the valva of *P. (A.) orphicus* is disproportionately longer, relative to individual size, than that of *P. (A.) ripartii*.

Female genitalia (Fig. 42). Henia similar to that of *P. (A.) aroaniensis* (Fig. 43), with minor differences in sclerotisation and dimensions of unknown significance due to very limited number of females available for dissection. Henia of *P. (A.) ripartii* clearly shorter and stouter (Fig. 44), that of *P. (A.) admetus* much longer and more slender (Fig. 45).

Karyotype (Figs. 1–8). Clear countable metaphase plates, mostly MI, were obtained in preparations of five males: ZK99001, ZK99004, ZK99010, ZK99017, and ZK99019. In all plates that could be counted precisely the haploid chromosome number was $n=41-42$. The karyotype is identical in structure and chromosome number to that of *P. (A.) d. dantchenkoi* (Lukhtanov et al. 2003: 66) and in structure also to that of *P. (A.) aroaniensis* (Coutsis et al. 1999).

Differential diagnosis. From nominotypical *P. (A.) dantchenkoi* the new taxon is distinguished by the presence of a white postdiscal streak on the forewing underside, a generally larger postdiscal ocelli on the hindwing underside, and less contrasting veins on the upperside. From *P. (A.) aroaniensis*, which it resembles extremely closely on the upperside, the new taxon is distinguished by the constant presence of a clear white postdiscal streak on the forewing underside, as a rule a complete series of larger postdiscal spots on the underside, and the constant presence on the hindwing underside of a white streak (completely absent in at least half of the individuals in any given population of *P. (A.) aroaniensis*) which is wider, better defined, and more contrasting than in *P. (A.) aroaniensis* individuals possessing such a streak. From the sympatric and syntopic *P. (A.) ripartii* the new taxon is distinguished by the presence on the forewing underside of a white postdiscal streak and on average a more strongly curved row of postdiscal spots, and on the upperside the veins are less pronounced and



Figs. 10–18. Undersides of *P. (A.) dantchenkoi orphicus* ssp. n. **10.** Holotype ♂, S Bulgaria, Rhodopi Mts., open dry rocky slopes above Hvoyna village, 800–950 m, 7.vii.1999, Z. Kolev leg., ZK. **11–18.** Paratypes, same data, Figs. 12–16, 18: ♂; Figs. 11, 17: ♀, and Fig. 11 6.vii.1999. – **Figs. 19–26.** Underside of *P. (A.) aroaniensis*. **19.** ♂, SW Bulgaria, S Pirin Mts., vic. Paril village, 850 – 950 m, 30.vi.1994. **20.** ♂, SW Bulgaria, Mt. Alibotush, Hambar Dere gorge, 1500–1600 m, 11.vii.1993. **21, 22.** ♀, same data as Fig. 20, but 3.vii.1994. **23, 24.** ♂, S Bulgaria, Rhodopi Mts., vic. ‘Trigradski skali’ chalet, ca. 1400 m, 11.vii.2003. **25.** ♀, same data, as Fig. 19, but 1.vii.2003. **26.** ♀, SE Bulgaria, Stara Planina Mts., vic. Sliven, ca. 1000 m, 1–2.viii.1999. – **Figs. 27–32.** Undersides of *P. (A.) ripartii*. **27–30.** ♂, same data as Figs. 10–18. **31–32.** ♀, same data as Figs. 10–18. Scale bar = 1 cm.

concolorous with the ground colour or only slightly darker. Worn individuals of the two taxa may be indistinguishable externally. A character that appears useful for separation of *P. (A.) ripartii* and *P. (A.) orphicus* is the longer valva in the male genitalia of *P. (A.) orphicus* which in the studied samples shows no numerical overlap with *P. (A.) ripartii* (see above). However, the samples studied are small and a further research into the significance of this character is therefore necessary. From the sympatric *P. (A.) admetus* the new taxon is very easily separated on account of the very distinct appearance of *P. (A.) admetus*, especially its strongly marked underside which almost never has a hindwing streak in the western part of the species' range.

Derivatio nominis. The adjective *orphicus* has two meanings: 'without apparent significance to the senses nor obvious to the intelligence; beyond ordinary understanding'; and 'ascribed to Orpheus'. Both meanings apply to *P. (A.) orphicus*: the former alludes to the highly cryptic appearance of the new taxon which can easily be confused with the sympatric *P. (A.) ripartii*, while the latter meaning refers to the range of *P. (A.) orphicus*: the Rhodopi Mountains, home to the mythical Thracian poet and musician Orpheus.

Life history. *P. (A.) orphicus* inhabits xerothermic and xeromontane calcareous localities. The vegetation of the type locality is sparse and dominated by low-growing xerophytous calciphilous species, with scattered *Juniperus* bushes and low *Pinus nigra* trees. In all known localities *P. (A.) orphicus* ssp. n. is syntopic with *P. (A.) ripartii*, which is widespread and abundant in calcareous habitats in the western Rhodopi (pers. observ.). The lowest known locality of *P. (A.) orphicus*, the gorge of Lukovitsa river, is a xerothermic submediterranean habitat supporting a very large population of *P. (A.) admetus* and a very small population of *P. (A.) ripartii*. In the type locality the ratio *ripartii* : *orphicus* equals 2.6 based on the collected unbiased sample of 32 specimens. There are as yet no observations regarding the larval host-plant of the new taxon.

Distribution. Collection material from three further localities in the western Rhodopi agrees well with *P. (A.) orphicus*, but this additional material (see above) is not included in the type series because no karyological data from these localities are available so far. Three known localities of *P. (A.) orphicus* (including the type locality) are situated in the gorge of the Chepelarska river at altitudes between 400 and 1100 m, the fourth (vic. Gela village) is on the northern slope of the Mursalitsa ridge at 1500 m. The habitats are situated on mostly karstified Proterozoic marbles of the Dobrostan formation (Zagorchev 1995) which are widespread in the western Rhodopi. Thus, it is to be expected that this taxon occurs elsewhere in Rhodopi as well.

Records of *P. (A.) aroaniensis* from Rhodopi have been very scarce so far. In Greece only two localities are known at low altitude in the southern foothills of the massif (Kolev & van der Poorten 1997; Coutsis & Ghalalás 2001). In the collection of IZS are preserved 1♂ from the 'Skalni Mostove' karst arches and 1♀ from the 'Kolarovski Livadi' locality, both specimens completely lacking a white streak on the hindwing underside. The first of these records corroborates a record of *P. (A.) aroaniensis* from "Čudnite mostovi" ['Chudnite Mostove' karst arches very close to Skalni Mostove] (Bálint 1995), based on specimens lacking a streak (Z. Bálint, in litt.). This locality is in immediate proximity to the known range of *P. (A.) orphicus*. In addition, during a short visit to the vicinity of 'Trigradski skali' chalet on 11.vii.2003 I collected a small sample (3♂, 1♀) with various degrees of reduction of the streak on the hindwing underside and lacking postdiscal streak on the forewing underside (Figs. 23, 24). Thus these specimens correspond well to *P. (A.) aroaniensis* and differ from *P. (A.) orphicus*



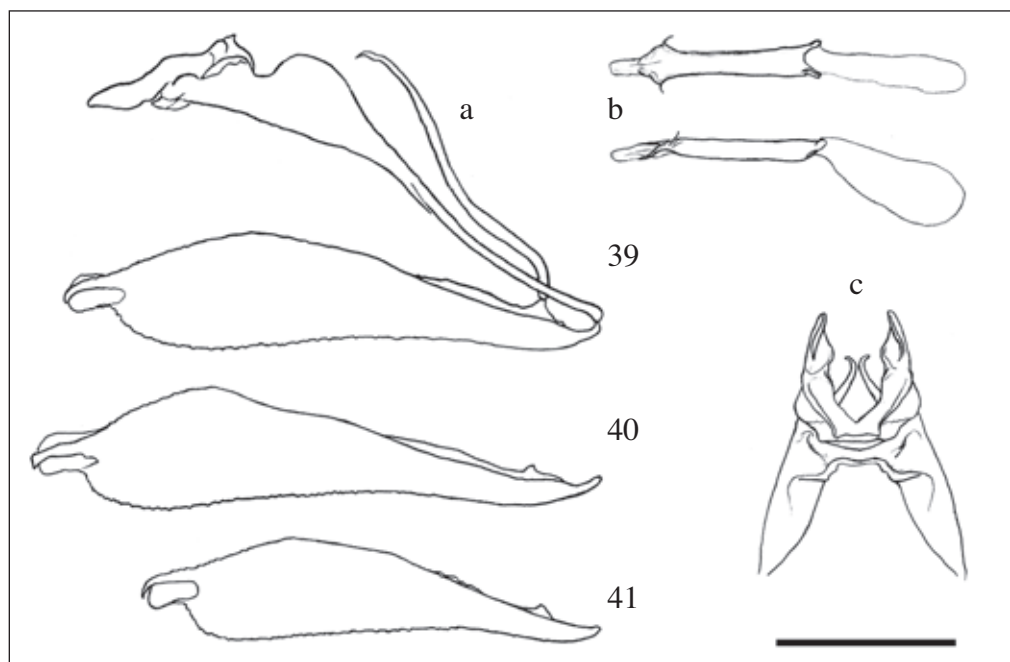
Figs. 33–34. Uppersides of *P. (A.) dantchenkoi orphicus* ssp. n. **33.** Paratype ♂, S Bulgaria, Rhodopi Mts., open dry rocky slopes above Hvoyna village, 800–950 m, 6.–7.vii.1999 (same specimen as Fig. 13). **34.** Paratype ♀, same data as Fig. 33 (same specimen as Fig. 11). – **Figs. 35–36.** Uppersides of *P. (A.) aroaniensis*. **35.** ♂, SW Bulgaria, S Pirin Mts., vic. Paril village, 850–950 m, 30.vi.1994 (same specimen as Fig. 19). **36.** ♀, same data as Fig. 35, but 1.vii.2003 (same specimen as Fig. 25). – **Figs. 37–38.** Uppersides of *P. (A.) ripartii*. **37.** ♂, same data as Fig. 33 (same specimen as Fig. 28). **38.** ♀, same data as Fig. 33 (same specimen as Fig. 31). Scale bar = 1 cm.

(samples for karyological analysis could not be obtained). The known records of *P. (A.) orphicus* and *P. (A.) aroaniensis* in Bulgaria are shown in Fig. 46. Syntopy of these two taxa has not been established so far, but is very likely in view of the very close proximity of their known ranges.

Discussion

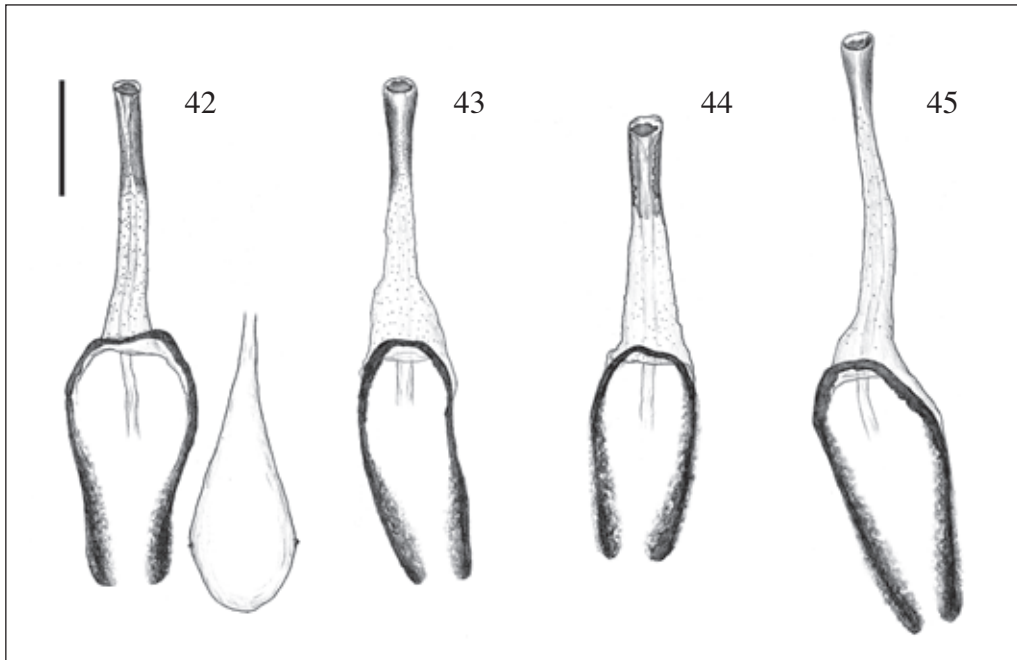
Morphologically *P. (A.) dantchenkoi orphicus* combines characters of *P. (A.) aroaniensis* (smooth dark brown upperside with inconspicuous veins) with characters of *P. (A.) ripartii* and nominotypical *P. (A.) dantchenkoi* (white streak on hindwing underside always present and very prominent; postdiscal ocelli usually prominent). The most conspicuous external character that separates *P. (A.) dorphicus* from these and all other *Agrodiaetus* taxa examined is the constant presence in the studied material of *P. (A.) dorphicus* of a whitish postdiscal streak along vein M3 in space M2 on the forewing underside. Based on examined material and photographs of brown *Agrodiaetus*, such a streak also occurs at very low frequencies in some taxa that have an underside hindwing streak. Thus, I have found only one specimen of Bulgarian *P. (A.) ripartii* with a forewing streak among more than 60 specimens examined.

Recent DNA research (Wiemers 2003; Kandul et al. 2004) indicates that the closest relatives of *P. (A.) d. dantchenkoi* are the Turkish taxa *P. (A.) alcestis* (Zerny, 1932) with n=19–21 and *P. (A.) [eriwanensis] interjectus* (de Lesse, 1960) with n=29–32 (Wiemers 2003), and the Transcaucasian *P. (A.) eriwanensis eriwanensis* (Forster, 1960) with n=29–34 (Lukhtanov et al. 2003; Kandul et al. 2004). Lukhtanov et al. (2003) stated that nominotypical *P. (A.) dantchenkoi* is also phenotypically most similar to *P. (A.) e. eriwanensis* and *P. (A.) e. interjectus*. According to Wiemers (2003) these taxa form a



Figs. 39–41. Male genitalia. **39.** *P. (A.) dantchenko* *orphyicus* ssp. n., paratype (ZK99001) (same specimen as Fig. 16). **a.** lateral view, aedeagus removed, setae and membranous parts omitted. **b.** dorsoventral (above) and lateral (below) view of aedeagus including caecum. **c.** uncus and tegumen, dorsal view. **40.** Valva of *P. (A.) aroaniensis*, SW Bulgaria, Mt. Alibotush, Hambar Dere gorge, 1500–1600 m, 3.vii.1994 (same specimen as Fig. 22). **41.** Valva of *P. (A.) ripartii* (ZK99005), S Bulgaria, Rhodopi Mts., open dry rocky slopes above Hvoyna village, 800–950 m, 6–7.vii.1999. Scale bar = 1 mm.

cluster whose sister group is formed by the closely related *P. (A.) aroaniensis* and *P. (A.) humedasmae* (Toso & Baletto 1976), the latter endemic to the Aosta valley in northern Italy. The taxonomic status of *P. (A.) orphyicus* as presently proposed is tentative. It is possible that this taxon is a distinct species despite the identical karyotypes of *P. (A.) d. dantchenko*. Conspecificity of *P. (A.) orphyicus* and *P. (A.) aroaniensis* is unlikely considering the difference of six chromosome pairs in their karyotypes (Lukhtanov et al. 2003), the morphological differences, and the fact that these taxa occur in close proximity, possibly sympatrically and syntopically, in the western Rhodopi. Conspecificity with *P. (A.) dantchenko* might be challenged by the morphological differences between the two taxa and the considerable distance between their ranges. This distance, over 1500 km, is particularly great considering the very small ranges of many *Agrodiaetus* taxa. It must also be stressed that nominotypical *P. (A.) dantchenko* is so far only known from a region characterized by very high butterfly endemism, especially among Lycaenidae. This region has very little zoogeographical affinity with the central Balkan Peninsula in general and the Rhodopi Mts. in particular. While the occurrence of *P. (A.) dantchenko* further west in Turkey is not impossible, it is notable that so far other researchers have not come across such karyologically distinct populations in central Turkey. Thus, for the time being *P. (A.) dorphicus* is not given species status owing to the fact that its karyotype is identical to that of *P. (A.) dantchenko*. Molecular studies are needed to resolve the status of this new taxon.



Figs. 42–45. Female genitalia. **42.** *P. (A.) dantchenkoi orphicus* ssp. n., paratype (same specimen as Fig. 17). **43.** *P. (A.) aroaniensis*, SW Bulgaria, S Pirin Mts., vic. Paril village, 850–950 m, 30.vi.1994. **44.** *P. (A.) ripartii* (ZK99005), S Bulgaria, Rhodopi Mts., open dry rocky slopes above Hvoyna village, 800–950 m, 6.–7.vii.1999. **45.** *P. (A.) admetus*, S Bulgaria, Rhodopi Mts., vic. Asenovgrad, Asenova krepost, 400–500 m, 17.vii.1992. Scale bar = 1 mm.

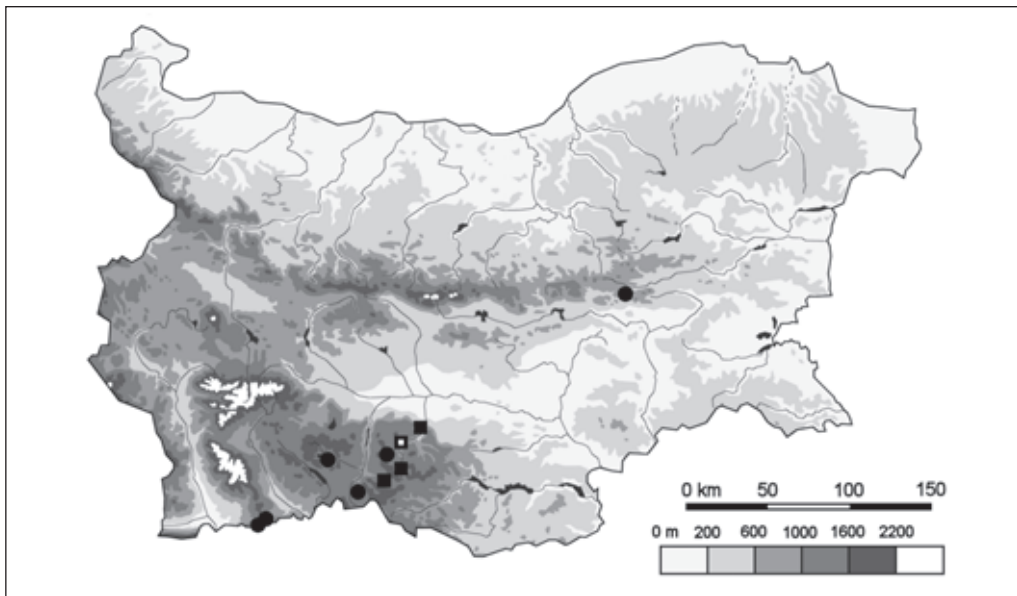


Fig. 46. Map of Bulgaria with records of *P. (A.) dantchenkoi orphicus* (squares; white-centred: type locality) and *P. (A.) aroaniensis* (circles).

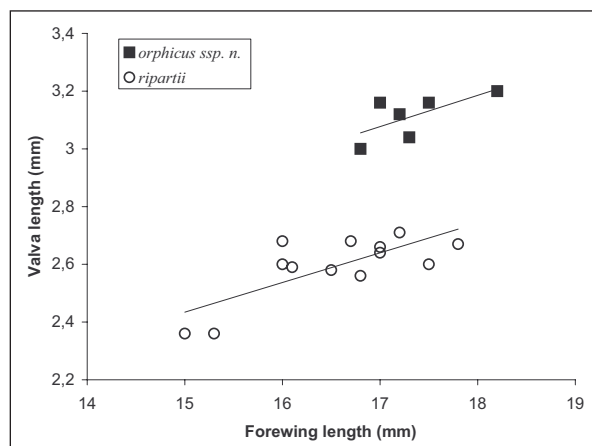


Fig. 47. Scatter diagram of individual values of forewing and valva length in *P. (A.) orphicus* (squares) and sympatric and syntopic *P. (A.) ripartii* (circles). All specimens determined from chromosome counts. Note the virtually complete overlap of forewing lengths of the two taxa but the lack of overlap between their valva length.

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