Description of a new species of the "brown" *Agrodiaetus* complex from South-East Turkey (Lycaenidae)

Vladimir A. Lukhtanov[1], Martin Wiemers[2] & Karen Meusemann[2]

[1] Laboratory of Entomology, Biological Institute, St. Petersburg State University, St. Petersburg, Russia; e-mail: lukhtanov@mail.ru
[2] Zoologisches Forschungsinstitut & Museum Alexander Koenig, Adenauerallee 160, D-53113 Bonn, Germany; e-mail: martin@wiemers1.de; meusemann@uni-bonn.de

Abstract. *Agrodiaetus dantchenkoi* sp. n. is described from Van Province in Turkey. The new taxon belongs to the "brown" complex of the genus *Agrodiaetus* Hübner, 1822. This complex includes several sibling species which are extremely uniform in their morphology but have distinct chromosome numbers. The karyotype of *Agrodiaetus dantchenkoi* sp. n. (n=40–42) is investigated. The new species is compared with *A. eriwanensis* Forster, 1960 (n=34), *A. humedasae* Toso & Balletto, 1976 (n=38) and *A. aroaniensis* Brown, 1976 (n=48).

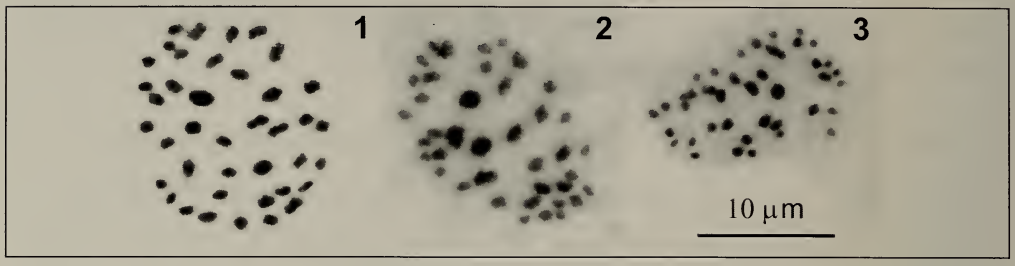
Zusammenfassung. *Agrodiaetus dantchenkoi* sp. n. wird aus der Provinz Van in der Türkei neu beschrieben. Das neue Taxon gehört zum "braunen" Artenkomplex der Gattung *Agrodiaetus* Hübner, 1822. Dieser Komplex enthält mehrere Zwillingsarten, die sich morphologisch äußerst ähnlich sind, aber verschiedene Chromosomenzahlen besitzen. Der Karyotyp von *Agrodiaetus dantchenkoi* sp. n. (n=40–42) wird untersucht und die neue Art wird mit *A. eriwanensis* Forster, 1960 (n=34), *A. humedasae* Toso & Balletto, 1976 (n=38) und *A. aroaniensis* Brown, 1976 (n=48) verglichen.

Key words. karyotype, chromosome number, *Agrodiaetus*, Lepidoptera, Lycaenidae, Turkey, biological species concept, reproductive isolation, chromosome rearrangement.

Introduction

The "brown" complex of the genus *Agrodiaetus* Hübner, 1822 is composed of sibling species in which both males and females have similar brown coloration of the upperside of the wings. This complex is a real stumbling block in the taxonomy of the genus. The species of this group are extremely similar in wing colour and pattern as well as in genitalia structure. In contrast to morphological uniformity, the complex possesses a great chromosome number diversity, and each species has a specific karyotype.

The following haploid chromosome numbers were found in the complex: n=19 in *A. alcestis karacetinae* Lukhtanov & Dantchenko, 2002 (Lukhtanov & Dantchenko 2002b), n=20–21 in *A. alcestis alcestis* (Zerny, 1932) (De Lesse 1960), n=29–32 in *A. eriwanensis interjectus* De Lesse, 1960 (De Lesse 1960), n=32–34 in *A. eriwanensis eriwanensis* Forster, 1960 (Lukhtanov & Dantchenko 2002a and unpublished data), n=38 in *A. humedasae* Toso & Balletto, 1976 (Troiano *et al.* 1979), n=48 in *A. aroaniensis* Brown, 1976 (Coutsis *et al.* 1999), n=66 in *A. galloi* Balletto & Toso, 1979 (Troiano & Giribaldi 1979), n=67–74 in *A. demavendi* (Pfeiffer, 1938) (De Lesse 1960), n=78–80 in *A. admetus* (Esper, [1783]) (De Lesse 1960), n=84 in *A. khorasanensis* Carbonell, 2001 (Carbonell 2001) and n=90 in *A. ripartii* (Freyer, 1830) and *A. fabressei* (Oberthür, 1910) (De Lesse 1960). The chromosome number is the



Figs. 1–3. Karyotype of *Agrodiaetus dantchenkoi* sp. n. **1**: MI, n=40, paratype, ♂ NoVL01L344, Turkey, Prov. Van, 34 km N Çatak. **2**: MI, n=42, paratype, ♂ MW99319, Turkey, Prov. Van, 25–32 km N Çatak. **3**: MII, n=42, paratype, ♂ MW99274, Turkey, Prov. Van, Kurubas Geçidi.

same in the two latter species. However, there is a difference between them in karyotype structure: *A. ripartii* has one large, one medium and 88 small bivalents, and *A. fabressei* has 2 large, 2 medium and 86 small bivalents (De Lesse 1961).

As it follows from the investigations of De Lesse (1960), who first studied this group karyologically, the species description and the species determination is only possible on the basis of karyotype investigation. Our studies confirm this statement. Using karyological methods, we were able to find numerous mistakes in species identification even in cases, when species were determined by recognized experts of the group.

Abbreviations. ca – circa, approximately determined chromosome number, MCZH – Museum of Comparative Zoology (Harvard University, Cambridge, MA, USA), MI – first metaphase of meiosis, MII – second metaphase of meiosis, n – haploid chromosome number, SPSU – St. Petersburg State University, St. Petersburg, Russia, ZFMK – Zoologisches Forschungsinstitut und Museum Alexander Koenig (Bonn, Germany).

Methods

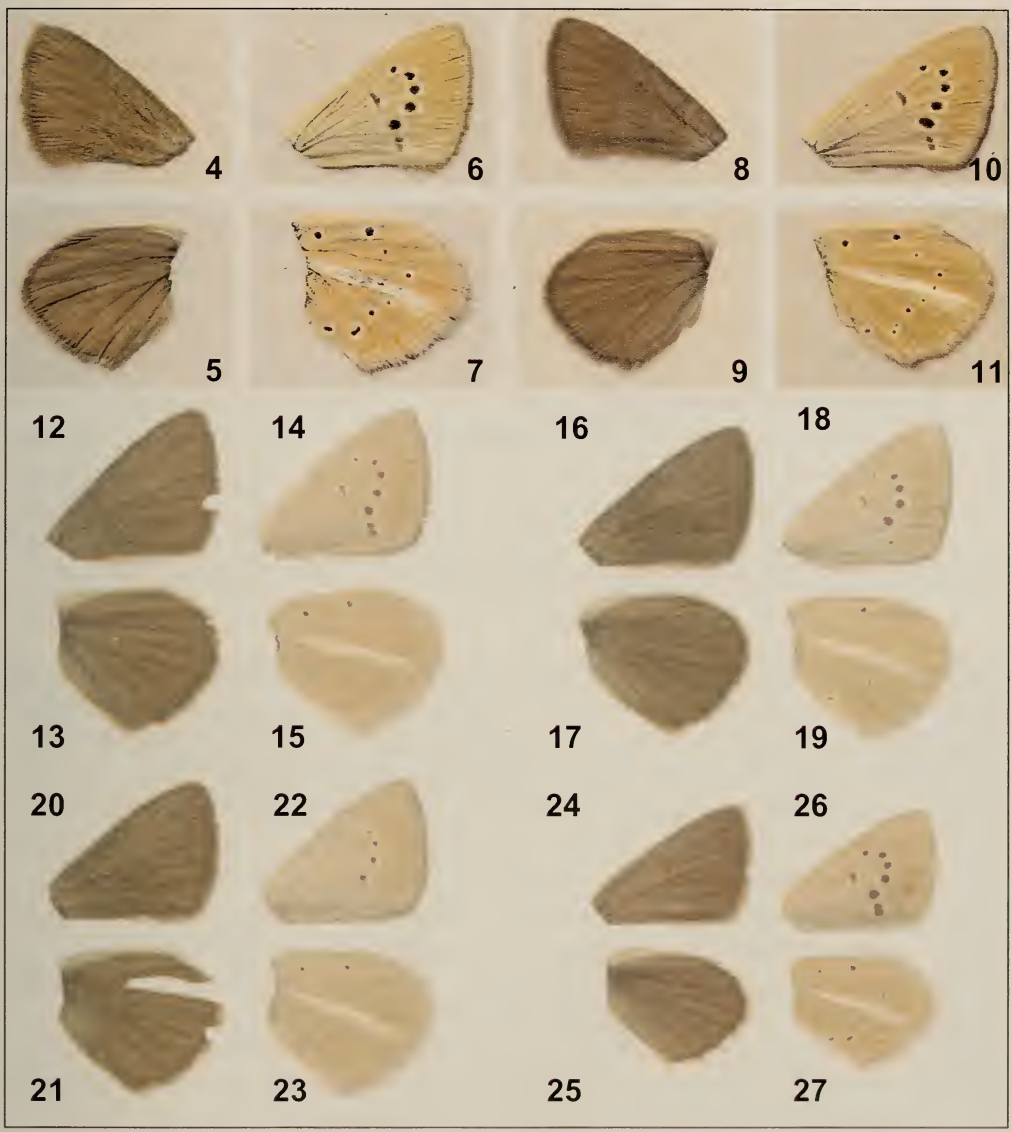
The methods described by Lukhtanov & Dantchenko (2002a) were used to investigate the karyotype in the specimens VL01L341, VL01L343, VL01L344, VL01L345 and 2001-372, and the methods applied to the specimens MW99319, MW99320 and MW99274 are described in Olivier *et al.* (2000).

Agrodiaetus dantchenkoi Lukhtanov &Wiemers sp. n.

Material. Holotype ♂: No VL01L342, n=42, Turkey, Prov. Van, 34 km N Çatak, 20.vii.2001, Dantchenko leg., MCZH. The mitochondrial genes COI and COII of the holotype specimen were sequenced (Kandul *et al.*, in press) and will be submitted to GenBank (http://www.ncbi.nlm.nih.gov/). – Paratypes. 5♂ same data as holotype, but VL01L341 (n=ca 40–42), VL01L343 (n=ca 40–42), VL01L344 (n=40,41), VL01L345 (n=ca 40–42), MCZH; 2001–372, (n=ca 40–42), SPSU. 2♂ MW99319 (n=42), MW99320 (n=ca 40–41), Prov. Van, 25–32 km N Çatak, 2100 m, 18.vii.1999, Wiemers leg., ZFMK. 1♂ MW99274 (n=42), ♀ MW99275 (found in copula with ♂ MW99274), Prov. Van, Kurubas Geçidi, 2200 m, 17.vii.1999, Wiemers leg., ZFMK.

Description ♂ (Figs 4–27). Forewing length 14–17 mm.

Upperside: Ground colour light brown with light yellow shimmer and with darker veins. Discoidal, submarginal and antemarginal marking completely absent on both fore- and hindwings. Forewings with a well developed sex brand and scale-tuft. Fringe brown.



Figs. 4–27. Wings of *Agrodiaetus dantchenkoi* sp. n. **4–7.** Holotype ♂ No VL01L342, Turkey, Prov. Van, 34 km N Çatak, 20.vii.2001, Dantchenko leg., MCZH (n=42). **8–11.** Paratype ♂ NoVL01L343, Turkey, Prov. Van, 34 km N Çatak, 2100 m, 20.vii.2001, Dantchenko leg., MCZH (n=ca 40–42). **12–15.** Paratype ♂ MW99319, Turkey, Prov. Van, 25–32 km N Çatak, 2100 m, 18.vii.1999, Wiemers leg., ZFMK (n=42). **16–19.** Paratype ♂ MW99320, Turkey, Prov. Van, 25–32 km N Çatak, 2100 m, 18.vii.1999, Wiemers leg., ZFMK (n=ca 40–41). **20–23.** Paratype ♂ MW99274, Turkey, Prov. Van, Kurubas Geçidi, 2200 m, 17.vii.1999, Wiemers leg., ZFMK (n=42). **24–27.** Paratype ♀ MW99275, Turkey, Prov. Van, Kurubas Geçidi, 2200 m, 17.vii.1999, found in copula with ♂ MW99274, Wiemers leg., ZFMK.
Colour differences between figs. 4–11 and figs. 12–27 are caused by different scanning techniques and do not reflect real differences.

Fig. 28. *Agrodiaetus dantchenkoi*, paratype, ♂ genitalia (MW99274) (drawing by J. Coutsis).

Underside: Ground colour is warm yellowish brown. Greenish blue basal suffusion nearly absent. Basal black spots present on hindwings, but absent on forewings. Discoidal and postdiscal black marking well developed on both fore- and hindwings, but postdiscal black marking vestigial in some specimens. Submarginal and antemarginal marking completely absent on forewings. On the hindwings submarginal marking strongly reduced, nearly absent; antemarginal marking is presented by small inconspicuous strokes. White streak on hindwings clearly visible. Fringe grey.

Phenotypically similar to *A. eriwanensis eriwanensis* Forster, 1960 and *A. eriwanensis interjectus* De Lesse, 1960 but can be distinguished by the karyotype.

♀. One female was found in copula with a male of *A. dantchenkoi* whose karyotype could be established. Forewing length 14 mm.

Upperside: Ground colour is light brown with light yellow shimmer and with darker veins. Discoidal cell spot present on forewings, hindwings with faint yellowish brown submarginal lunules in S1–S3. Fringes warm yellowish brown.

Underside: Ground colour warm yellowish brown. Basal black spots present on hindwings, but absent on forewings. Discoidal and postdiscal black marking well developed on both fore- and hindwings. Faint brown submarginal marking present on forewings (S1–S3) and reduced orange submarginal marking on hindwings (S1–S4). White streak on hindwings clearly visible. Fringe greyish brown.

Karyotype (Figs. 1–3). In the holotype the chromosome number n=42 was found in 4 studied MII cells from one spermatocyst. In another spermatocyst, in each of 8 studied MI cells we have found 36 bivalents and 3 multivalents. Taking into account the fact that MII plates have 42 chromosomes, we can conclude that these MI cells were heterozygous for reciprocal translocation involving three chromosome pairs, i.e. these multivalents were tetravalents in reality. Thus, the chromosome

number of the specimen VL01L342 is n=42. The same chromosome number n=42 was precisely determined in one MII cell of paratype specimen MW99274 and in one MI cell of the paratype specimen MW99319.

In few well squashed MI cells from the paratype specimens VL01L344 and MW99320, we were able to count only 40 or 41 chromosome elements. In the last case, it remains unknown whether these counts reflect the real chromosome number in these specimens or, like in the holotype specimen, the decrease of chromosome elements was caused by undetected chromosome rearrangements. In three other paratype specimens, the counts of chromosome elements were made with approximation (n=ca 40–42) due to the fact that some of the bivalents overlapped.

Distribution. SE Turkey (Van) (map). Only known from the type locality (N Çatak) and from Kurubas Geçidi south of Van where it was found flying together with *A. ripartii* (n=90). *A. alcestis karacetinae* (n=19) was found a few km south of the type locality on 18.vii.1999 (MW) and *A. demavendi* (n=68–71) is known from Edremit near Van (VL). Therefore four species of the brown *Agrodiaetus* complex (identified by their karyotype) are now known to occur in Van Province.

Derivatio nominis. The new species is dedicated to A. Dantchenko, an expert in the taxonomy and biology of the Lycaenidae.

Map. Distribution of *Agrodiaetus eriwanensis* (◐), *A. interjectus* (◑), and *A. dantchenkoi* (●).

Discussion

A. dantchenkoi sp. n. is genetically (Kandul *et al.*, in press) and phenotypically most similar to *A. eriwanensis eriwanensis* and A. *eriwanensis interjectus*. All these three taxa are allopatric in their distribution (map). In most cells of *A. dantchenkoi* we found metaphase plates showing 42 bivalents; in few metaphase plates 40 or 41 chromosome elements were detected. A variable chromosome number of n=29–32 was found by De Lesse (1960) in *A. eriwanensis interjectus*. In MI cells of *A. eriwanensis eriwanensis* from 29 up to 34 chromosome elements (bivalents, multivalents and univalents) were counted (Lukhtanov & Dantchenko 2002a). This variability in *A. eriwanensis eriwanensis* is due to different chromosome rearrangements of the main chromosome set consisting of 34 bivalents (Lukhtanov & Dantchenko, unpublished). The cytological nature of the rearrangements in *A. eriwanensis eriwanensis* as well as in *A. eriwanensis interjectus* and *A. dantchenkoi* remains unknown. However it is evident that the difference in the number of visible chromosome elements in MI plates, which was found within the above mentioned taxa, does not reflect the real variation of their diploid number. Heterozygosity for different chromosome rearrangements may result in multivalent formation in the MI stage and consequently in change of number of recognizable chromosome units even if the diploid chromosome number remains constant. More investigations are necessary to clarify this complex situation.

Thus, at the minimum there is a difference of 6 chromosome pairs (12 chromosomes) between *A. eriwanensis* (n=34) and *A. dantchenkoi* (n=40–42). Therefore these two taxa represent different karyospecies. The application of the biological species concept (BSC) is complicated in this case because of their allopatric distribution. Theoretically we can not exclude that these two chromosome forms could be genetically compatible and produce fertile hybrids. However according to our knowledge about the characters of intra- and interspecific karyotype variability in Lepidoptera (Lukhtanov & Dantchenko 2002b) the hypothesis about their nonconspecifity seems to be much more likely.

The pattern of geographical distribution can provide one more evidence of nonconspecifity of chromosomal races. If multiple chromosome rearrangements would be no barrier to gene flow (i.e. unimportant as isolating mechanisms), chromosome races should hybridize freely and should form a broad transition zone, in which extensive chromosome polymorphism will be expected. A free circulation of chromosome rearrangements in accordance to the Hardy-Weinberg equilibrium should be also expected in such zones. In reality, the chromosomal races, differing in multiple fixed rearrangements, have discrete distribution (even if there is no geographical border between them) and, if a contact zone is present, it is narrow, and hybridization is either absent, or present but with very limited chromosomal introgression (see review: King, 1993). Such a pattern of distribution is usually considered as a characteristic of species but not of subspecies (Kitching & Cadiou 2000).

Similar chromosome numbers, n=38 and n=48, were found in two other species of the "brown *Agrodiaetus*-complex" – *A. humedasae* and *A. aroaniensis* correspondingly (Troiano *et al.* 1979; Coutsis *et al.* 1999). *A. humedasae* has a very restricted distribution in Val d'Aosta in NW Italy. *A. aroaniensis* is locally distributed in Greece.

One could speculate that *A. dantchenkoi* may be conspecific with *A. humedasae* or *A. aroaniensis*. However this supposition seems to be improbable due to karyological differences, geographical distribution and the morphology of the taxa. Phenotypically *A. humedasae* and *A. aroaniensis* differ by the total absence of the white streak in the hindwing underside in all (*A. humedasae*) or most individuals (ca. 60–70% of *A. aroaniensis*; Coutsis pers. comm.) and by the postdiscal series of points that are nearer to the discal spot than to the margin.

Acknowledgements

We thank Prof. Dr. Can Bilgin (Middle East Technical University, Ankara, Turkey), Prof. Dr. Naomi Pierce (Harvard University, USA), Dr. Cagan Hakki Sekercioglu (Stanford University, USA) for their invaluable help during the organization and realization of the collecting trips 2001 to the Van Region in Turkey, and Willy De Prins, Dirk van der Poorten and Alain Olivier (Antwerpen, Belgium) for help in organizing the joint collecting trip 1999 to Eastern Turkey. Thanks to Dr. A. Hausmann and Mr. U. Buchsbaum (Zoologische Staatssammlung München, Germany), late Prof. Dr. G. Bernardi, Dr. M. J. Pierre and Mr. F. Carbonell (Muséum National d'Histoire Naturelle, Paris, France) for the possibility to study the types of *A. eriwanensis* and *A. interjectus*. We thank Prof. Dr. C. Naumann (ZFMK, Bonn, Germany) for unlimited access to the facilities at the ZFMK and his invaluable all-out support and Dr. Jurate De Prins (Royal Museum for Central Africa, Tervuren, Belgium) for teaching her method of permanent chromosome preparations. We thank John Coutsis (Athens) for providing the genital drawing. We gratefully acknowledge the financial support from the William F. Milton Fund and Putnam Expedition Committee (Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA), the Russian Research Foundation (Grants RFFI 02-04-49138 and 00-15-97934) and the Russian Federal Programmes "Universities of Russia" (Grant UR 07.01.056) and "Scientific schools" (Grant 2232.2003.4) to VL and the support of the Deutsche Forschungsgemeinschaft (Grants 436 RUS 17/90/02 and Na 90/14).

References

Carbonell, F. 2001. Contribution à la connaissance du genre *Agrodiaetus* Hübner (1822), *A. ahmadi* et *A. khorasanensis* nouvelles espèces dans le Nord de l'Iran (Lepidoptera, Lycaenidae). – Linneana Belgica **18** (2): 105–110.

De Lesse, H. 1960. Les nombres de chromosomes dans la classification du groupe d'*Agrodiaetus ripartii* Freyer (Lepidoptera, Lycaenidae). – Revue française d'Entomologie **27**: 240–264.

De Lesse, H. 1961. Cohabitation en Espagne d'*Agrodiaetus ripartii* Freyer et *A. fabressei* Oberthür (Lepidoptera, Lycaenidae). – Revue française d'Entomologie **28**: 50–53.

Eckweiler, W. & C. Häuser 1997. An illustrated checklist of *Agrodiaetus* Hübner, 1822, a subgenus of *Polyommatus* Latreille, 1804 (Lepidotera: Lycaenidae). – Nachrichten des entomologischen Vereins Apollo, Suppl. **16:** 113–168.

Kandul, N. P., V. A. Lukhtanov, A. V. Dantchenko, J. W. S. Coleman, C. H. Sekercioglu, D. Haig & N. E. Pierce (in press). Phylogeny of *Agrodiaetus* Hübner 1822 (Lepidoptera: Lycaenidae) inferred from mtDNA Sequences of *COI* and *COII*, and Nuclear Sequences of *EF1-a*: Karyotype Diversification and Species Radiation. – Systematic Biology.

King, M. 1993. Species evolution. – Cambridge University Press, Cambridge, UK. 336 pp.

Kitching, I. J. & J.-M. Cadiou 2000. Hawkmoths of the world. An annotated and illustrated revisonary check-list (Lepidoptera: Sphingidae). – Cornell University Press, Ithaca and London. 226 pp.

Lukhtanov, V. A. & A. D. Dantchenko 2002a. Principles of the highly ordered arrangement of metaphase I bivalents in spermatocytes of *Agrodiaetus* (Insecta, Lepidoptera). – Chromosome Research **10** (1): 5–20.

Lukhtanov, V. A. & A. V. Dantchenko 2002b. Descriptions of new taxa of the genus *Agrodiaetus* Hübner, [1822] based on karyotype investigation (Lepidoptera, Lycaenidae). – Atalanta **33** (1/2): 81–107, 224–225, colour plate I.

Olivier, A., D. van der Poorten, J. Puplesiene & W. de Prins 2000. *Polyommatus (Agrodiaetus) artvinensis* stat. nov. and *P. (A.) sigberti* sp. nov., two vicariant species known so far only from Turkey (Lepidoptera: Lycaenidae). – Phegea **28** (2): 57–74.

Troiano, G., E. Balletto & G. G. Toso 1979. The karyotype of *Agrodiaetus humedasae* Toso & Balletto, 1976. – Bollettino della Società Entomologica Italiana **111** (7–10): 141–143.

Troiano, G. & M. A. Giribaldi 1979. Karyotypic analysis. – Nota lepidopterologica **2** (1–2): 22–23.