Taxonomic interpretation of chromosomal and mitochondrial DNA variability in the species complex close to *Polyommatus* (Agrodiaetus) *dama* (Lepidoptera, Lycaenidae)

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Abstract

In this paper, by using combination of molecular and chromosomal markers, populations of *Polyommatus* (Agrodiaetus) *karindus* (Riley, 1921) from north-west and central Iran are analyzed. It has been found that taxon usually identified as *P.* (*A.*) *karindus* is represented in Iran by two geographically separated groups of individuals, strongly differentiated by their karyotypes and mitochondrial haplotypes. It is demonstrated that populations from NW Iran have the haploid chromosome number n = 68, while the haploid chromosome number of *P.* (*A.*) *karindus* from central Iran is found to be n = 73. Phylogenetic analysis revealed that these groups also differ by at least eight nucleotide substitutions in a 690 bp fragment of the mitochondrial COI gene and form separated groups of clusters in Bayesian inference tree. Thus, population entities from central Iran are described here as a new subspecies *Polyommatus* (Agrodiaetus) *karindus* *saravandi* ssp. n. Strong chromosomal and molecular differentiation are confirmed between *P.* (*A.*) *karindus* and its sister species, *P.* (*A.*) *dama* (Staudinger, 1892).

Keywords

COI, Iran, karyotype, molecular marker, chromosome number
Introduction

*Agrodiaetus* Hübner, 1822 is the most species-rich subgenus within the genus *Polyommatus* Latreille, 1804 (Talavera et al. 2013a, Lukhtanov et al. 2015a). It consists of approximately 130 species distributed in the western Palearctic (Vila et al. 2010, Lukhtanov et al. 2008, 2014, Vershinina and Lukhtanov 2010, Przybyłowicz et al. 2014, Lukhtanov and Tikhonov 2015). Today *Agrodiaetus* has become a model group in studies of speciation (Lukhtanov et al. 2005, 2015b), intraspecific differentiation (Dincă et al. 2013, Przybyłowicz et al. 2014, Lukhtanov et al. 2015a), and rapid karyotype evolution (Lukhtanov et al., 2005, Kandul et al. 2007). From the point of view of taxonomy, *Agrodiaetus* is a very complicated group. Many *Agrodiaetus* taxa display extremely similar phenotype (Hesselbarth et al. 1995) and, in contrast to other Lepidoptera taxa, genitalia offer only few distinctive features. Furthermore, many taxa represent allopatric populations which differ only slightly in morphology, and a conclusion on their status as distinct species or subspecies is controversial and can be misleading (Wiemers 2003, Lukhtanov et al. 2015a). This resulted in description of numerous polytypic species based on geographic distribution and classic morphological characters (Forster 1956, 1960a, b, 1961).

In particular, *Polyommatus* (*Agrodiaetus*) *dama* (Staudinger, 1892) was traditionally regarded as a polytypic species that included two subspecies: *Polyommatus* (*Agrodiaetus*) *dama dama* (Staudinger, 1892) (orig. comb. *Lycaena Dama*) and *Polyommatus* (*Agrodiaetus*) *dama karindus* (Riley, 1921) (orig. comb. *Lycaena dama* subsp. *karinda*). *P. (A.) dama dama* has only been found in South Anatolia (a few localities in Malatya, Maraş, and Mardin provinces (Turkey), while *P. (A.) dama karindus* distribution range is restricted to Zagros Mountains in Iran.

The karyotype studies of de Lesse (1957, 1959a, b, c, d, 1960a, b, 1961, 1962a, b, 1963a, b, 1964, 1966, 1968) revealed that *Agrodiaetus* species exhibit a wide diversity of karyotypes. Karyotyping may provide necessary diagnostic character for many *Agrodiaetus* species, and therefore become an important requirement for describing new taxa (de Lesse 1960a, b, Lukhtanov and Dantchenko 2002, 2003, Lukhtanov et al. 2008). Karyological investigations showed strong chromosomal differentiation between Turkish and Iranian populations of *P. (A.) dama* s. l.. De Lesse (1959a) described karyotype of *P. (A.) dama dama* from Kahramanmaraş and Olivier et al. (1999) confirmed his results from the type locality Malatya. It has an asymmetric karyotype with n = 41 chromosomes, about eleven of them are large, gradually decreasing in size, the others medium–sized; whereas the karyotype of Iranian taxon was determined as n = 68 (Wiemers 2003). Thus, on the basis of karyotype studies, *P. (A.) dama* s. l. was split into two species, *P. (A.) dama* and *P. (A.) karindus*, that can be characterized by species-specific haploid chromosome numbers.

However, the chromosome number of *P. (A.) karindus* was determined only for one population from NW Iran (Saqqez, Kordestan Province) (Wiemers 2003). Further investigations showed that Iranian species *P. (A.) karindus* has complicated genetic and
phylogeographic structure (Lukhtanov et al. 2015b). Here a combination of molecular mitochondrial (COI) and nuclear chromosomal (karyotype) markers are used to analyze different Iranian populations of *P. (A.*) karindus*. Our study demonstrates that butterflies from central Iran strongly differentiated by their karyotypes and mitochondrial haplotypes from NW Iranian populations. Thus, population entities from central Iran are described here as a separate subspecies *Polyommatus (Agrodiaetus) karindus saravandi* ssp. n.

**Material and methods**

**Specimens sampling**

The butterflies were collected in the period of 2007–2014 in Iran (list of collected specimens is given in Table 1). In north–west Iran we collected material in two localities: 1) in the mountain range between Saqqez and Baneh (30–40 km SW of Saqqez), and 2) in the vicinity of Dare Dozdan (30–40 km W of Divandarreh). In central Iran we collected butterflies in the vicinity of Vennai (18 km W of Borujerd), in the vicinity of Saravand (15 km SE of Dorud), in the vicinity of Nahavand and in the vicinity of Darreh Takht (35 km NE of Dorud) (information about sampling localities is given in Figure 1 and Table 1).

![Figure 1. Distribution ranges of *P. (A.*) dama* (green circles), *P. (A.*) karindus karindus* (red circles) and *P. (A.*) karindus saravandi* (blue circles). The asterisk indicates the type locality of *P. (A.*) karindus karindus.*](image-url)
Table 1. List of studied material (129 specimens) with information on karyotype (48 specimens) and COI sequences (54 specimens). Collectors: V. Lukhtanov (VL), N. Shapoval (NS) and A. Barabanov (AB).

| Species | Sex | Sample ID | Chromosome number (n) | COI GenBank number | Province | Locality and coordinates | Altitude | Date       | Collectors |
|---------|-----|-----------|-----------------------|--------------------|----------|--------------------------|----------|------------|------------|
| karindus | M   | E391      | 70                    |                    | Kordestan | ca. 40 km SW Saqqez 36°06.18’N; 046°00.27’E | 1725 m   | 29 July 2004 | VL         |
| karindus | M   | E399      | 68                    |                    | Kordestan | ca. 40 km SW Saqqez 36°06.18’N; 046°00.27’E | 1725 m   | 29 July 2004 | VL         |
| karindus | M   | E400      | 68                    |                    | Kordestan | ca. 40 km SW Saqqez 36°06.18’N; 046°00.27’E | 1725 m   | 29 July 2004 | VL         |
| karindus | M   | E402      | 68                    |                    | Kordestan | ca. 40 km SW Saqqez 36°06.18’N; 046°00.27’E | 1725 m   | 29 July 2004 | VL         |
| karindus | M   | Z726      | 68                    | h02/GH5 KT582701   | Kordestan | ca. 40 km SW Saqqez 36°05.97’N; 045°59.63’E | 1720 m   | 30 July 2007 | VL & NS    |
| karindus | M   | Z727      | 68                    | h03/GH5 KT582702   | Kordestan | ca. 40 km SW Saqqez 36°05.97’N; 045°59.63’E | 1720 m   | 30 July 2007 | VL & NS    |
| karindus | M   | Z729      | 68                    |                    | Kordestan | ca. 40 km SW Saqqez 36°05.97’N; 045°59.63’E | 1720 m   | 30 July 2007 | VL & NS    |
| karindus | M   | Z749      | 68                    | h01/GH5 KT582703   | Kordestan | ca. 40 km SW Saqqez 36°04.82’N; 045°58.88’E | 1880 m   | 31 July 2007 | VL & NS    |
| karindus | M   | Z750      | 68                    | h01/GH5 KT582704   | Kordestan | ca. 40 km SW Saqqez 36°04.82’N; 045°58.88’E | 1880 m   | 31 July 2007 | VL & NS    |
| karindus | M   | Z753      | 68                    | h01/GH5 KT582705   | Kordestan | ca. 40 km SW Saqqez 36°04.82’N; 045°58.88’E | 1880 m   | 31 July 2007 | VL & NS    |
| karindus | M   | Z800      | 68                    | h01/GH5 KT582706   | Kordestan | ca. 40 km SW Saqqez 36°04.09’N; 045°58.82’E | 2050 m   | 31 July 2007 | VL & NS    |
| karindus | M   | Z809      | 68                    | h01/GH5 KT582707   | Kordestan | ca. 40 km SW Saqqez 36°04.09’N; 045°58.82’E | 2050 m   | 31 July 2007 | VL & NS    |
| karindus | M   | Z820      | 68                    | h01/GH5 KT582708   | Kordestan | ca. 40 km SW Saqqez 36°04.09’N; 045°58.82’E | 2050 m   | 31 July 2007 | VL & NS    |
| karindus | M   | Z843      | 68                    | h01/GH5 KT582709   | Kordestan | ca. 40 km SW Saqqez 36°04.64’N; 045°59.16’E | 1920–1950 m | 1 August 2007 | VL & NS    |
| karindus | M   | Z845      | 69                    | h01/GH5 KT582710   | Kordestan | ca. 40 km SW Saqqez 36°04.64’N; 045°59.16’E | 1920–1950 m | 1 August 2007 | VL & NS    |
| karindus | M   | W253      | 68                    |                    | Kordestan | ca. 40 km SW Saqqez 36°03.00’N; 045°58.54’E | 2027 m   | 29 July 2009 | VL & NS    |
| karindus | M   | W254      | 68                    |                    | Kordestan | ca. 40 km SW Saqqez 36°03.00’N; 045°58.54’E | 2027 m   | 29 July 2009 | VL & NS    |
| karindus | M   | W259      | 68                    |                    | Kordestan | ca. 40 km SW Saqqez 36°03.00’N; 045°58.54’E | 2027 m   | 29 July 2009 | VL & NS    |
| karindus | M   | W271      | 68                    |                    | Kordestan | ca. 40 km SW Saqqez 36°04.39’N; 045°59.06’E | 1869 m   | 29 July 2009 | VL & NS    |
| karindus | M   | W272      | 68                    |                    | Kordestan | ca. 40 km SW Saqqez 36°04.39’N; 045°59.06’E | 1869 m   | 29 July 2009 | VL & NS    |
| karindus | M   | W273      | 68                    |                    | Kordestan | ca. 40 km SW Saqqez 36°04.39’N; 045°59.06’E | 1869 m   | 29 July 2009 | VL & NS    |
| karindus | M   | W274      | 68                    |                    | Kordestan | ca. 40 km SW Saqqez 36°04.39’N; 045°59.06’E | 1869 m   | 29 July 2009 | VL & NS    |
| karindus | M   | W275      | 68                    |                    | Kordestan | ca. 40 km SW Saqqez 36°04.39’N; 045°59.06’E | 1869 m   | 29 July 2009 | VL & NS    |
| karindus | M   | W276      | 68                    |                    | Kordestan | ca. 40 km SW Saqqez 36°04.39’N; 045°59.06’E | 1869 m   | 29 July 2009 | VL & NS    |
| karindus | M   | W277      | 68                    |                    | Kordestan | ca. 40 km SW Saqqez 36°04.39’N; 045°59.06’E | 1869 m   | 29 July 2009 | VL & NS    |
| karindus | M   | W278      | 68                    |                    | Kordestan | ca. 40 km SW Saqqez 36°04.39’N; 045°59.06’E | 1869 m   | 29 July 2009 | VL & NS    |
| karindus | M   | W279      | 68                    |                    | Kordestan | ca. 40 km SW Saqqez 36°04.39’N; 045°59.06’E | 1869 m   | 29 July 2009 | VL & NS    |
| Species | Sex | Sample ID | Chromosome number (n) | GenBank number | Province | Locality and coordinates | Altitude | Date | Collectors |
|---------|-----|-----------|-----------------------|----------------|----------|--------------------------|----------|------|------------|
| *karindus* | M   | W280      | 68                    | h01/GH5        | Kordestan | ca. 40 km SW Saqqez 36°04.39'N; 045°59.06'E | 1869m    | 29 July 2009 | VL & NS |
| *karindus* | M   | W281      | 68                    | h01/GH5        | Kordestan | ca. 40 km SW Saqqez 36°04.39'N; 045°59.06'E | 1869m    | 29 July 2009 | VL & NS |
| *karindus* | M   | W282      | 68                    | h01/GH5        | Kordestan | ca. 40 km SW Saqqez 36°04.39'N; 045°59.06'E | 1869m    | 29 July 2009 | VL & NS |
| *karindus* | M   | W283      | 68                    | h01/GH5        | Kordestan | ca. 40 km SW Saqqez 36°04.39'N; 045°59.06'E | 1869m    | 29 July 2009 | VL & NS |
| *karindus* | M   | W340      | h01/GH5 | KT582732 | Kordestan | Dare Dozdan 35°52.05'N; 046°33.03'E | 2066m    | 30 July 2009 | VL & NS |
| *karindus* | M   | W341      | h01/GH5 | KT582733 | Dare Dozdan 35°52.05'N; 046°33.03'E | 2066m    | 30 July 2009 | VL & NS |
| *karindus* | M   | W342      | h01/GH5 | KT582734 | Dare Dozdan 35°52.05'N; 046°33.03'E | 2066m    | 30 July 2009 | VL & NS |
| *karindus* | M   | W354      | h04/GH5 | KT582737 | Kordestan | Dare Dozdan 35°52.05'N; 046°33.03'E | 2277m    | 31 July 2009 | VL & NS |
| *karindus* | M   | W355      | h01/GH5 | KT582735 | Kordestan | Dare Dozdan 35°52.05'N; 046°33.03'E | 2277m    | 31 July 2009 | VL & NS |
| *karindus* | M   | W361      | h01/GH5 | KT582736 | Kordestan | Dare Dozdan 35°52.05'N; 046°33.03'E | 2066m    | 31 July 2009 | VL & NS |
| *karindus* | M   | W366      | h01/GH5 | KT582738 | Kordestan | Dare Dozdan 35°52.05'N; 046°33.03'E | 2066m    | 31 July 2009 | VL & NS |
| *karindus* | M   | V069      | h01/GH5 | KT582739 | Kordestan | Dare Dozdan 35°51.30'N; 046°42.60'E | 2200m    | 27 July 2014 | NS & AB |
| *karindus* | M   | V070      | h01/GH5 | KT582740 | Kordestan | Dare Dozdan 35°51.30'N; 046°42.60'E | 2200m    | 27 July 2014 | NS & AB |
| *karindus* | M   | W370      | 73                | h05/GH4       | Lorestan | Nahavand 34°02.57'N; 048°20.22'E | 2173m    | 2 August 2009 | VL & NS |
| *karindus* | M   | W371      | 73                | h05/GH4       | Lorestan | Nahavand 34°02.57'N; 048°20.22'E | 2173m    | 2 August 2009 | VL & NS |
| *karindus* | M   | W372      | 73                | h09/GH2       | Lorestan | Nahavand 34°02.57'N; 048°20.22'E | 2173m    | 2 August 2009 | VL & NS |
| *karindus* | M   | W373      | 73                | h05/GH4       | Lorestan | Nahavand 34°02.57'N; 048°20.22'E | 2173m    | 2 August 2009 | VL & NS |
| *karindus* | M   | W374      | h09/GH2 | KT582726 | Lorestan | Nahavand 34°02.57'N; 048°20.22'E | 2173m    | 2 August 2009 | VL & NS |
| *karindus* | M   | W375      | h09/GH2 | KT582727 | Lorestan | Nahavand 34°02.57'N; 048°20.22'E | 2173m    | 2 August 2009 | VL & NS |
| *karindus* | M   | W376      | h09/GH2 | KT582728 | Lorestan | Nahavand 34°02.57'N; 048°20.22'E | 2173m    | 2 August 2009 | VL & NS |
| *karindus* | M   | W388      | h08/GH2 | KT582731 | Lorestan | Nahavand 34°02.57'N; 048°20.22'E | 1950–2173m | 3 August 2009 | VL & NS |
| *karindus* | M   | W389      | h05/GH4 | KT582729 | Lorestan | Nahavand 34°02.57'N; 048°20.22'E | 1950–2173m | 3 August 2009 | VL & NS |
| *karindus* | M   | W390      | h09/GH2 | KT582730 | Lorestan | Nahavand 34°02.57'N; 048°20.22'E | 1950–2173m | 3 August 2009 | VL & NS |
| *karindus* | M   | W391      | Lorestan | Nahavand 34°02.57'N; 048°20.22'E | 1950–2173m | 3 August 2009 | VL & NS |
| *karindus* | M   | W392      | Lorestan | Nahavand 34°02.57'N; 048°20.22'E | 1950–2173m | 3 August 2009 | VL & NS |
| *karindus* | M   | U217      | Lorestan | Nahavand, 34°02.92'N; 48°20.40'E | 2161 m    | 19 July 2011 | VL & NS |
| *karindus* | M   | U218      | Lorestan | Nahavand, 34°02.92'N; 48°20.40'E | 2161 m    | 19 July 2011 | VL & NS |
| *karindus* | M   | U219      | Lorestan | Nahavand, 34°02.92'N; 48°20.40'E | 2161 m    | 19 July 2011 | VL & NS |
| *karindus* | M   | U220      | Lorestan | Nahavand, 34°02.92'N; 48°20.40'E | 2161 m    | 19 July 2011 | VL & NS |
| *karindus* | M   | U223      | Lorestan | Nahavand, 34°02.92'N; 48°20.40'E | 2161 m    | 19 July 2011 | VL & NS |
| Species | Sex | Sample ID | Chromosome number (n) | COI | GenBank number | Province | Locality and coordinates | Altitude | Date      | Collectors |
|---------|-----|-----------|-----------------------|-----|----------------|----------|--------------------------|----------|-----------|------------|
| karinda | M   | U228      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U229      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U230      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U231      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U232      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U233      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U234      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U235      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U236      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U237      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U238      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U239      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U240      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U256      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U257      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U262      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U263      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U264      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U265      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U266      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U267      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U278      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U279      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U280      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | U281      |                       |     |                | Lorestan | Nahavand, 34°02.91’N; 48°21.08’E | 2020 m   | 20 July 2011 | VL & NS    |
| karinda | M   | Z381      | 73                    | h09/GH2 | KT582691    | Lorestan | W of Borujerd, Kuh-e Garin mount, Vennnai, 33°53.89’N; 48°34,03’E | 2150 m   | 21 July 2007 | VL & NS    |
| karinda | M   | Z382      | 73                    | h09/GH2 | KT582692    | Lorestan | W of Borujerd, Kuh-e Garin mount, Vennnai, 33°53.89’N; 48°34,03’E | 2150 m   | 21 July 2007 | VL & NS    |
| karinda | M   | Z396      | 73                    | h09/GH2 | KT582693    | Lorestan | W of Borujerd, Kuh-e Garin mount, Vennnai, 33°53.89’N; 48°34,03’E | 2150 m   | 21 July 2007 | VL & NS    |
| Species | Sex | Sample ID | Chromosome number (n) | COI    | GenBank number | Province | Locality and coordinates                                                                 | Altitude | Date           | Collectors |
|---------|-----|-----------|----------------------|--------|----------------|----------|------------------------------------------------------------------------------------------|----------|----------------|------------|
| karindae | M   | Z397      | 73                   | h10/GH2 | KT582694       | Lorestan | W of Borujerd, Kuh-e Garin mount, Vennnai, 33°53.89’N; 48°34.03’E                      | 2150m    | 21 July 2007   | VL & NS    |
| karindae | M   | Z398      | 73                   | h09/GH2 | KT582695       | Lorestan | W of Borujerd, Kuh-e Garin mount, Vennnai, 33°53.89’N; 48°34.03’E                      | 2150m    | 21 July 2007   | VL & NS    |
| karindae | M   | Z399      | 73                   | h11/GH1 | KT582696       | Lorestan | W of Borujerd, Kuh-e Garin mount, Vennnai, 33°53.89’N; 48°34.03’E                      | 2150m    | 21 July 2007   | VL & NS    |
| karindae | M   | Z400      | 73                   | h10/GH2 | KT582697       | Lorestan | W of Borujerd, Kuh-e Garin mount, Vennnai, 33°53.89’N; 48°34.03’E                      | 2150m    | 21 July 2007   | VL & NS    |
| karindae | M   | Z408      | 73                   | h10/GH2 | KT582698       | Lorestan | W of Borujerd, Kuh-e Garin mount, Vennnai, 33°53.89’N; 48°34.03’E                      | 2150m    | 21 July 2007   | VL & NS    |
| karindae | M   | Z412      | 73                   | h11/GH1 | KT582700       | Lorestan | W of Borujerd, Kuh-e Garin mount, Vennnai, 33°53.89’N; 48°34.03’E                      | 2150m    | 22 July 2007   | VL & NS    |
| karindae | M   | Z413      |                      |        |                | Lorestan | W of Borujerd, Kuh-e Garin mount, Vennnai, 33°53.89’N; 48°34.03’E                      | 2150m    | 22 July 2007   | VL & NS    |
| karindae | M   | Z416      | h09/GH2              |        | KT582699       | Lorestan | W of Borujerd, Kuh-e Garin mount, Vennnai, 33°53.89’N; 48°34.03’E                      | 2150m    | 22 July 2007   | VL & NS    |
| karindae | M   | V331      |                      |        |                | Lorestan | W of Borujerd, Kuh-e Garin mount, Vennnai, 33°53.89’N; 48°34.03’E                      | 2150m    | 2 August 2014  | NS & AB    |
| karindae | M   | V335      |                      |        |                | Lorestan | W of Borujerd, Kuh-e Garin mount, Vennnai, 33°53.89’N; 48°34.03’E                      | 2150m    | 2 August 2014  | NS & AB    |
| karindae | M   | V336      |                      |        |                | Lorestan | W of Borujerd, Kuh-e Garin mount, Vennnai, 33°53.89’N; 48°34.03’E                      | 2150m    | 2 August 2014  | NS & AB    |
| karindae | M   | W061      | 73,74,75             | h12/GH1 | KT582711       | Lorestan | Saravand, 33°22.39’N; 49°10.25’E                                                    | 2070m    | 21 July 2009   | VL & NS    |
| karindae | M   | W062      | ca73                 |        |                | Lorestan | Saravand, 33°22.39’N; 49°10.25’E                                                    | 2070m    | 21 July 2009   | VL & NS    |
| karindae | M   | W063      | 71                   | h12/GH1 | KT582712       | Lorestan | Saravand, 33°22.39’N; 49°10.25’E                                                    | 2070m    | 22 July 2009   | VL & NS    |
| karindae | M   | W064      | 73                   | h12/GH1 | KT582713       | Lorestan | Saravand, 33°22.39’N; 49°10.25’E                                                    | 2070m    | 22 July 2009   | VL & NS    |
| karindae | M   | W065      | ca73                 | h12/GH1 | KT582714       | Lorestan | Saravand, 33°22.39’N; 49°10.25’E                                                    | 2070m    | 22 July 2009   | VL & NS    |
| karindae | M   | W072      | ca73                 | h12/GH1 | KT582715       | Lorestan | Saravand, 33°22.39’N; 49°10.25’E                                                    | 2070m    | 22 July 2009   | VL & NS    |
| karindae | M   | W073      |                      |        |                | Lorestan | Saravand, 33°22.39’N; 49°10.25’E                                                    | 2070m    | 22 July 2009   | VL & NS    |
| karindae | M   | W074      |                      |        |                | Lorestan | Saravand, 33°22.39’N; 49°10.25’E                                                    | 2070m    | 22 July 2009   | VL & NS    |
| karindae | M   | W075      |                      |        |                | Lorestan | Saravand, 33°22.39’N; 49°10.25’E                                                    | 2070m    | 22 July 2009   | VL & NS    |
### Table 1: Sample Information

| Species | Sample ID | Sex | GenBank number | Chromosome number (n) | Province | Locality and coordinates | Altitude | Date       | Collectors |
|---------|-----------|-----|----------------|-----------------------|----------|--------------------------|----------|------------|------------|
| karinda | W081      | M   |                |                       | Lorestan | Saravand, 33°22.39'N; 49°10.25'E | 2070m    | 22 July 2009 | VL & NS    |
| karinda | W082      | M   |                |                       | Lorestan | Saravand, 33°22.39'N; 49°10.25'E | 2070m    | 22 July 2009 | VL & NS    |
| karinda | W083      | M   |                |                       | Lorestan | Saravand, 33°22.39'N; 49°10.25'E | 2070m    | 22 July 2009 | VL & NS    |
| karinda | W084      | M   |                |                       | Lorestan | Saravand, 33°22.39'N; 49°10.25'E | 2070m    | 22 July 2009 | VL & NS    |
| karinda | W085      | M   |                |                       | Lorestan | Saravand, 33°22.39'N; 49°10.25'E | 2070m    | 22 July 2009 | VL & NS    |
| karinda | W086      | M   |                |                       | Lorestan | Saravand, 33°22.39'N; 49°10.25'E | 2070m    | 22 July 2009 | VL & NS    |
| karinda | W087      | M   |                |                       | Lorestan | Saravand, 33°22.39'N; 49°10.25'E | 2070m    | 22 July 2009 | VL & NS    |
| karinda | W088      | M   |                |                       | Lorestan | Saravand, 33°22.39'N; 49°10.25'E | 2070m    | 22 July 2009 | VL & NS    |
| karinda | W089      | M   |                |                       | Lorestan | Saravand, 33°22.39'N; 49°10.25'E | 2070m    | 22 July 2009 | VL & NS    |
| karinda | W090      | M   |                |                       | Lorestan | Saravand, 33°22.39'N; 49°10.25'E | 2070m    | 22 July 2009 | VL & NS    |
| karinda | W091      | M   |                |                       | Lorestan | Saravand, 33°22.39'N; 49°10.25'E | 2070m    | 22 July 2009 | VL & NS    |
| karinda | W092      | F   |                |                       | Lorestan | Saravand, 33°22.39'N; 49°10.25'E | 2070m    | 22 July 2009 | VL & NS    |
| karinda | U168      | M   | KT582741       |                       | Lorestan | Darreh Takht, 33°21.19'N; 49°22.34'E | 2000–2100m | 18 July 2011 | VL & NS    |
| karinda | U169      | F   | KT582744       |                       | Lorestan | Darreh Takht, 33°21.19'N; 49°22.34'E | 2000–2100m | 18 July 2011 | VL & NS    |

In addition, we used the following sequences from GenBank:

- COI: AY577145
- P. (A.) karrindas (holotype): KT58724
- P. (A.) dama: AY557007
- P. (A.) birunii: AY556887

P. (A.) karrindas (holotype): KT58724

P. (A.) dama: AY557007

P. (A.) birunii: AY556887
Fresh (not worn) adult males were used to investigate the karyotypes. After capturing a butterfly in the field, it was placed in a glassine envelope for 1–2 hours to keep it alive until processed. Butterflies were killed by pressing the thorax. Testes for karyotype analysis were removed from the abdomen and placed into a 0.5 mL vial with a freshly prepared fixative (ethanol and glacial acetic acid 3:1). Then each wing was carefully removed from the body using forceps and placed into glassine envelope. The wingless body was placed into a plastic, 2 mL vial with pure 100% ethanol (for DNA analysis). Each vial with ethanol has already been numbered. This ID number was also used to label a vial with the fixative and a glassine envelope, in which the wings are preserved. Thus, each specimen was individually fixed. All collected specimens are kept in the Zoological Institute of the Russian Academy of Science (St. Petersburg) (ZIN RAS). All the testes are kept in the Department of Karyosystematics (ZIN RAS).

Chromosome preparation and karyotyping

Testes were stored in the fixative for 1–12 months at 4 °C. Then the gonads were stained in 2% acetic orcein for 30–60 days at 18–20 °C. Chromosome preparations were obtained as previously described (Talavera et al. 2013b). Different stages of male meiosis were examined by using a light microscope (Amplival, Carl Zeiss). An original two-phase method of chromosome analysis was used (Lukhtanov et al. 2006).

DNA Extraction and Sequencing

A fragment of the mitochondrial cytochrome c oxidase subunit I gene (first 690 positions) served as a mitochondrial molecular marker. Thoracic muscles and first abdominal segments were used for DNA extraction. The segments were homogenized in CTAB buffer and digested with proteinase K (10 mg/mL) for three hours at 60 °C. DNA was purified through successive ethanol precipitations and stored in dd H₂O at -20 °C.

For DNA amplification of COI we used primers K698 and Nancy (Caterino and Sperling 1999). PCR reactions (50 µl) contained 10 pmol each of forward and reverse primer, 1 mM dNTPs, 10x PCR Buffer (0.01 mM Tris-HCl, 0.05 M KCl, 0.1% Triton X–100: pH 9.0), 1 unit Taq DNA Polymerase (Fermentas), 5 mM MgCl₂, and were conducted using the following profile: initial 4 min denaturation at 94 °C and 30 cycles of 30 sec denaturation at 94 °C, 1 min annealing at 55 °C, 1 min extension at 72 °C and 5 min final elongation at 72 °C. PCR products were analyzed on 1.5% agarose gel, and purified using GeneJET PCR purification kit (Fermentas). Sequencing of double-stranded product was carried out at the Research Resource Center for Molecular and Cell Technologies (St. Petersburg State University).
Sequence alignments and phylogeny inference

The sequences were edited and aligned using CHROMAS 2.4.3 (http://www.techne-lysium.com.au/), Geneious 8.1.6 (Kearse et al. 2012), and BioEdit 7.0.3 (Hall 2011) software. The alignment was unambiguous, as all the sequences were of equal length and included no insertions/deletions. Primer sequences were cropped. This resulted in final alignment of 690 bp COI fragments. The analysis involved COI sequences inferred from 54 P. (A.) karindus specimens. Additional sequences of the P. (A.) dama (accession number AY557007) and P. (A.) karindus (accession number AY557145) were found in GenBank (Wiemers 2003) and were included into analysis, since these sequences completely overlapped with our fragment. We used sequence of P. (A.) birunii (Eckweiler & ten Hagen, 1998) (accession number AY556558) as an outgroup to root the phylogeny (according to available data, this species does not belong to the group closely related to P. (A.) dama). Thus, the final analysis included in total 57 COI sequences. A Bayesian approach for estimating phylogeny was used. Bayesian analyses were performed using the program MrBayes 3.2 (Ronquist et al. 2012), with the nucleotide substitution model GTR+G+I as suggested by jModelTest (Posada 2008). TRACER, v. 1.4 was used for summarizing the results of Bayesian phylogenetic analyses (http://beast.bio.ed.ac.uk/Tracer). A maximum–parsimony haplotype network was built using TCS v. 1.21, with a 99% parsimony connection limit (Clement et al. 2000).

Results

Analysis of karyotypes

Meiotic karyotypes were studied in 48 specimens of P. (A.) karindus from different Iranian localities. Depending on karyotypes and localities, 2 groups of individuals can be distinguished (Table 1 and see below).

Group I (P. (A.) karindus from NW Iran)

The haploid chromosome number n = 68 was found in meiotic metaphase I (MI) and meiotic metaphase II (MII) cells. The MI karyotype displayed 5 large bivalents in the center of metaphase plate and 63 smaller bivalents in the periphery (Fig. 2A).

Group II (will be described below as P. (A.) karindus saravandi from central Iran)

The haploid chromosome number n = 73 was found in meiotic MI and MII cells of studied individuals (Fig. 2B). The MI karyotype was strongly asymmetric with 5–6 larger bivalents in the center of the MI plate and 67–68 smaller bivalents in the periphery.
Phylogenetic analysis of molecular data

A Bayesian inference recovered *P. (A.) karindus* as a strongly supported monophyletic clade characterized by a specific set of fixed nucleotide substitutions (Fig. 3). Specimens of *P. (A.) karindus* were divided into several clusters: one cluster united specimens of *P. (A.) karindus* collected in north–west Iran (Fig. 3, GH5, highlighted in pink) and the others (Fig. 3, GH1–GH4, highlighted in blue) included specimens of central Iran populations (described here as a novel subspecies *P. (A.) karindus saravandi*). Most parsimonious COI haplotype network demonstrated similar pattern (Fig. 4). *P. (A.) dama* differs from *P. (A.) karindus* by at least 20 fixed nucleotide substitutions. Specimens of *P. (A.) karindus* form several haplotypes clustered in five different haplogroups. In general, composition of each haplogroup reflects geographical distribution of butterflies. Thus, majority of the specimens from easternmost (Saravand and Darreh Takht) and central west (Vennai, Nahavand) localities form two distinct haplogroups: GH1 and GH2. Nevertheless, two specimens from Vennai (approx. 80 km NW from Saravand) were found to have mitochondrial haplotype similar to that in easternmost populations, which has led to the suggestion that there is no complete isolation (reproductive or/and geographical) between population from Vennai and easternmost populations. The third haplogroup (GH3) consists of only three specimens, which were collected in Saravand and Darreh Takht. Interestingly, the third haplogroup differs drastically (by 10–12 fixed nucleotide substitutions) from the haplotypes, which comprise all other specimens from Saravand and Darreh Takht (group GH1). The fourth haplogroup (GH4) unites four specimens from Nahavand. Finally, all the haplotypes found in NW Iran constituted a subset of the distinct haplogroup (GH5). Thus, most parsimonious COI haplotype network reflects complex phylogeographic pattern of *P. (A.) karindus.*
Figure 3. The Bayesian tree of *Polyommatus (Agrodiaetus) dama* and *Polyommatus (Agrodiaetus) karindus* based on analysis of the cytochrome c oxidase subunit I gene from 57 specimens. Numbers at nodes indicate Bayesian posterior probability. *Agrodiaetus karindus karindus* and *Agrodiaetus karindus saravandi* clusters highlighted in pink and blue respectively.
**Figure 4.** COI Haplotype analysis. A geographical distribution of haplogroups. Number of studied individuals sharing the same haplogroup is given in parentheses B most parsimonious COI haplotype network; h01–h12 are COI haplotypes; GH1–GH5 are COI haplogroups. Number of studied individuals sharing the same haplotype is given in parentheses.

**Discussion**

We have found that a taxon usually identified as *P. (A.) karindus* is represented in Iran by two geographically separated groups of individuals. The first group unites specimens collected in NW Iran, while the second group comprises specimens from central Iran. The representatives of these groups have different chromosome numbers, n = 68 and n = 73 respectively. They also have at least eight fixed nucleotide differences in 690 bp fragment of mitochondrial COI gene. The first group is monophyletic with respect to
both COI gene and karyotype (n = 68). The second group has complicated genetic structure, comprises several differentiated populations and is paraphyletic with respect to the COI gene. Despite this gene paraphyly, it appears as a clearly monophyletic group with respect to its karyotype (n = 73). Thus, the NW and central Iranian groups are differentiated by at least five fixed chromosome fusions/fissions. Fixed chromosome differences are often considered as characters associated with reproductive isolation (King 1993). From this point of view, the NW and central Iranian groups could be theoretically treated as a different species. However, our recent studies on Agrodiaetus demonstrated that multiple chromosome fusions and fissions did not block fertility in chromosomal hybrids (Lukhtanov et al. 2015b). In other words, differentiation by five fixed chromosome rearrangements would not guarantee impossibility of blending populations together when they occur in sympatry. Thus, NW and central Iranian groups of populations should be considered as a subspecies rather than separate species.

Since Polyommatus (Agrodiaetus) karindus (Riley, 1921) (orig. comb. Lycaena dama subsp. karinda) was described from NW Iran (type locality is “Harir, Karind, and Karind Gorge, N.W. Persia” according to original description, and “N.W. Persia, Karind Gorge, 6000 ft” according to lectotype designation made by Bálint (1999) (not from central Iran), the name P. karindus karindus should be attributed to the NW Iranian group of populations. The formal description and naming of the central Iranian group is provided below.

Description of the novel taxon

Polyommatus (Agrodiaetus) karindus saravandi ssp. n.
http://zoobank.org/ADC4F3C8-B804-4869-955A-9F7C7164B0C5
Fig. 1 – map, Fig. 2B karyotype, Figs 3–4 phylogeny, Fig. 5 – Holotype of P. (A.) karindus saravandi, Fig. 6 A, B – Underside and upperside of the male and female wings

Holotype. ♂. Forewing length 34.0 mm. Iran, Lorestan province, Zagros Mt., vicinity of Saravand village, 33°22.39’N; 49°10.25’E, 2070 m, 22.07 2009. N. Shapoval and V. Lukhtanov leg. In the Zoological Institute of the Russian Academy of Sciences (St. Petersburg). Specimen field code W064, GenBank code for mitochondrial cytochrome c oxidase subunit I (COI) gene (partial cds) is KT582713.

Paratypes. 87 ♂♂, field codes W061, W062, W063, 21.07.2009; W065, W072, W073, W074, W075, W081, W082, W083, W084, W085, W086, W087, the same locality, date and collectors as the holotype. Field codes W093, W094, W095, W096 23.07.2009, the same locality and collectors as the holotype. Field codes W377, W378, W379, W380, W381, W382, W383, W386, W387 03.08.2009, the same locality and collectors as the holotype. Field codes W370, W371, W372, W373, W374, W375, W376, Iran, Lorestan province, Zagros Mt., vicinity of Nahavand village, 34°02.57’N; 048°20.22’E, 2170 m, 02.08.2009, the same collectors as the holotype. Field codes W388, W389, W390, W391, W392, Iran, Lorestan province, Zagros Mt., vicinity
Figure 5. Holotype of *P. (A.) karindus saravandi*, sample W064. Upperside (left) and underside (right) of the male wings.

Figure 6. Underside and upperside of the *P. (A.) karindus saravandi* ssp. n. wings. **A** upperside (left) and underside (right) of the male wings **B** upperside (left) and underside (right) of the female wings.
of Nahavand village, 34°02.57'N; 048°20.22'E, 2170 m, 02.08.2009, the same
collectors as the holotype. Field codes U217, U218, U219, U220, U223, Iran, Lorestan
province, Zagros Mt., vicinity of Nahavand village, 34°02.57'N; 048°20.22'E, 2170
m, 19.07.2011, the same collectors as the holotype. Field codes U228, U229, U230,
U231, U232, U233, U234, U235, U236, U237, U238, U239, U240, U256, U257,
U262, U263, U264, U265, U266, U267, U278, U279, U280, U281, Iran, Lorestan
province, Zagros Mt., vicinity of Nahavand village, 34° 02.92'N; 48° 20.40'E, 2160 m,
20.07.2011 the same collectors as the holotype. Field codes Z381, Z382, Z396, Z397,
Z398, Z399, Z400, Z408, Iran, Lorestan province, Zagros Mt., W of Borujerd, Kuh-
e Garin mount mount., Vennnai, 33°53.89'N; 48°34.03'E, 2150 m, 21.07.2007, the
same collectors as the holotype. Field codes V331, 335, V336, Iran, Lorestan province,
Zagros Mt., W of Borujerd, Kuh-e Garin mount., Vennnai, 33°53.89'N; 48°34.03'E, 2150
m, 22.07.2007, the same collectors as the holotype. Field codes V331, 335, V336,
Iran, Lorestan province, Zagros Mt., W of Borujerd, Kuh-e Garin mount., Vennnai,
33°53.89'N; 48°34.03'E, 2150 m, 02.08.2014, N. Shapoval and A. Barabanov leg.
Field codes U169, U178, U179, Iran, Lorestan province, Zagros Mt., Darreh Takht,
33° 21.19'N; 49° 22.34'E, 2000–2100 m, 18.07.2011, the same collectors as the holo-
type. 1 ♀, field code U169 Iran, Lorestan province, Zagros Mt., Darreh Takht, 33°
21.19'N; 49° 22.34'E, 2000–2100 m, 18.07.2011, the same collectors as the holotype.
All paratypes are kept in the Zoological Institute of the Russian Academy of
Sciences (St. Petersburg). GenBank accession numbers of the paratypes are presented in the Table 1.

Derivatio nominis. The new taxon is named after the village Saravand, one of the
places where it was found.

Description. Male upperside. Forewing length 30–36 mm, ground colour bright
blue with azure tint. Discoidal, submarginal and antemarginal marking absent on both
fore- and hindwings. Black outer marginal line on forewings and hindwings very nar-
row; forewing hind margin with long white pubescence. Fringes of both wings dark
grey; tips of hindwings veins indicated with fine black.

Male underside. Ground colour light grey, white streak on the hindwings absent.
Basal black spots present only on hindwings. Discoidal series of spots present on fore-
and hindwings, although the black spots composing it are minute. Postdiscal black
marking very narrow, longitudinal, present only on forewings. Submarginal and mar-
ginal lunules only faintly indicated.

Female upperside. Ground colour brown with vastly darker veins. Discoidal black
spots present on forewings. Submarginal markings dark brown with orange submargi-
nal lunules well developed on forewing and hindwing. Fringe greyish-brown.

Female underside. General design as in males, but ground colour slightly darker.

Genitalia. The male genitalia have a structure typical for other species of the subge-
nus Agrodiaetus (Coutsis 1986). No specific characters in genitalia are found.

Diagnosis. Genetically P. (A.) karindus saravandi differs from all other taxa of
Agrodiaetus by fixed substitutions in mitochondrial gene COI. Phenotypically the new
taxon is extremely similar to P. (A.) karindus karindus from north-west Iran, but they
have different chromosome numbers, n=73 and n = 68 respectively.
**Distribution.** Central part of Zagros Mountains, Iran.

**Flight period.** From July to August.

**Ecology.** Dry slopes, gorges and plateaus with xerophyte or steppe vegetation, sometimes wooded areas from 1800 up to 2800 m. Butterflies fly together with *P. (A.) alcestis* (Zerny, 1932), *P. (A.) cyaneus* (Staudinger, 1899), *P. (A.) hamadanensis* (de Lesse, 1959), *P. (A.) lorestanus* (Eckweiler, 1997) and *P. (A.) zarathustra* (Eckweiler, 1997).

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