

Tarbiat Modares University Press  
Entomological Society of IranResearch Article  
Taxonomy<https://doi.org/10.61186/jibs.10.3.431>

ISSN: 2423-8112

<https://zoobank.org/urn:lsid:zoobank.org:E8F5B2EB-895E-4505-8AC4-534E75D55584>

## *Polyommatus (Agrodiaetus) asadii* Charmeux, 2016 stat. nov., recognized as a distinct species (Lepidoptera: Lycaenidae)

**Alireza Naderi**

National Natural History Museum & Genetic Resources, Tehran, Iran.

✉ [a2naderi@yahoo.com](mailto:a2naderi@yahoo.com)

 <https://orcid.org/0009-0003-5878-6333>

**Jean-François Charmeux**

67, rue de Tolbiac, F- 75013 Paris, France.

✉ [jfcharmeux@gmail.com](mailto:jfcharmeux@gmail.com)

 <https://orcid.org/0009-0007-0072-0568>

**Vazrick Nazari**

Department of Biology, University of Padova, Padova, Italy.

✉ [vazrick.nazari@unipd.it](mailto:vazrick.nazari@unipd.it)

 <https://orcid.org/0000-0001-9064-8959>

**ABSTRACT.** Based on new evidence from morphology, ecology, and DNA barcodes, the taxon originally described as *Polyommatus (Agrodiaetus) valiabadi asadii* Charmeux, 2016, is raised to a specific rank, *Polyommatus (Agrodiaetus) asadii* bona species. It is endemic to Northern Iran and confined to the Dalasm valley in the Alborz mountains. Its preferred larval host is the annual *Onobrychis mazanderanica* Reich. f., while the larvae of its sister species *P. valiabadi* feed on *O. talagonica* Reich. f. (Fabaceae). It can be diagnosed from *P. valiabadi* and other closely-related species by differences in wing pattern and male genitalia. Our time-calibrated phylogenetic hypothesis estimates that the common ancestor of *P. asadii* and *P. valiabadi* split around 750,000 years ago.

**Keywords:** Butterflies, DNA barcoding, Iran, gossamer-winged, species rank, taxonomy

**Received:**

21 February, 2024

**Accepted:**

16 March, 2024

**Available online:**

17 March, 2024

**Subject Editor:**

Hossein Rajaei

**Citation:** Naderi, A., Charmeux, J.-F. & Nazari, V. (2024) *Polyommatus (Agrodiaetus) asadii* Charmeux, 2016 stat. nov., recognized as a distinct species (Lepidoptera: Lycaenidae). *Journal of Insect Biodiversity and Systematics*, 10 (in press).

## INTRODUCTION

*Polyommatus* Latreille, 1804 is a large Holarctic butterfly genus that is represented in Iran by about 93 species (Rajaei et al. 2023). Within *Polyommatus*, subgenus *Agrodiaetus* – known as a separate genus for a long time – is among the most diverse groups. One local Persian endemic, “*Agrodiaetus rjabovi valiabadi*” Rose & Schurian, 1977 was described from the vicinity of Valiabad, a small montane village in the Chalus River valley in the Central Alborz Mountains (Rose & Schurian, 1977). It was later discovered that the name of “*Agrodiaetus rjabovi*”, a species known from the Talysh region in the Republic of Azerbaijan (Forster, 1960) was preoccupied, and thus it was replaced with *Agrodiaetus rjabovianus* by Koçak (1980). In the same publication, Koçak considered the nominotypical taxon *valiabadi* to be a distinct species. Recently, on the basis of a combination of chromosomal and DNA mitochondrial markers, Lukhtanov et al. (2015) have demonstrated that *Polyommatus valiabadi* is a triplet of cryptic species (*P. valiabadi* Rose & Schurian, 1977, *P. rjabovianus* Koçak, 1980 and *P. pseudorjabovi* Lukhtanov, Dantchenko, Vishnevskaya & Saifitdinova, 2015) within the subgenus *Agrodiaetus* that are strongly differentiated by their karyotypes as well as mitochondrial haplotypes. However, while *P. rjabovianus* and *P. valiabadi* belong to the *dolus*-group, the taxon *P. pseudorjabovi*, known only from the Talesh/Talysh region in NW Iran and the Republic of Azerbaijan, belongs to the *P. admetus* group (Eckweiler & Bozano, 2016).

**Corresponding author:** Nazari, V., ✉ [vazrick.nazari@unipd.it](mailto:vazrick.nazari@unipd.it)

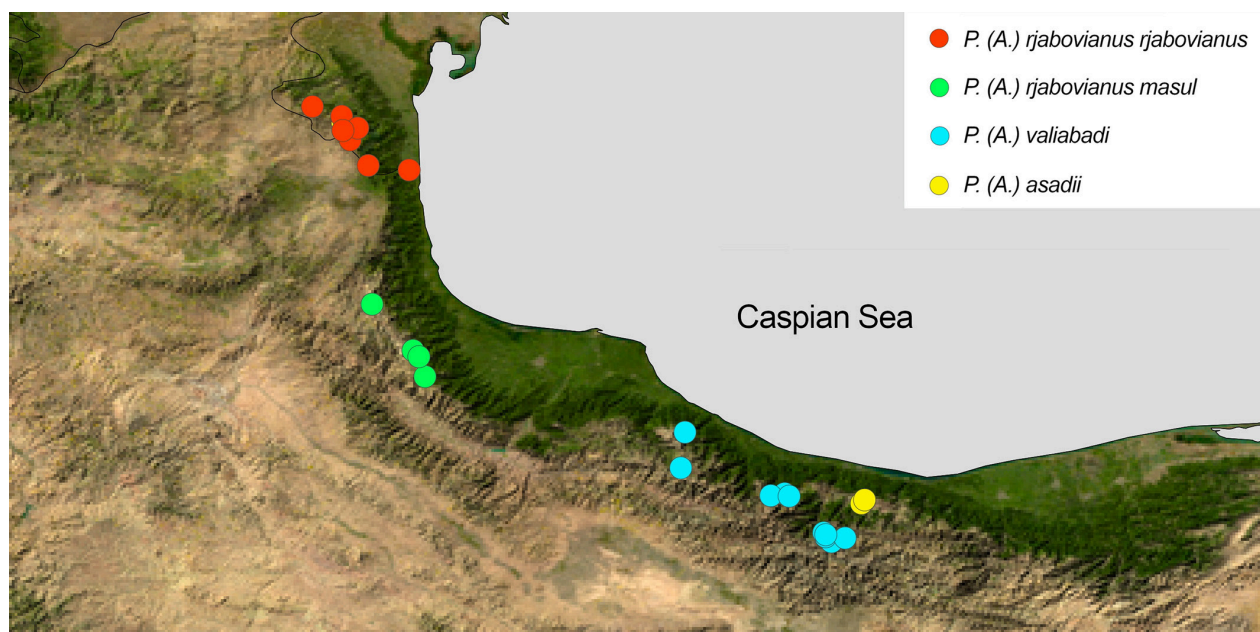
**Copyright** © 2024, Naderi et al. This is an open access article distributed under the terms of the Creative Commons NonCommercial Attribution License (CC BY NC 4.0), which permits Share - copy and redistribute the material in any medium or format, and Adapt - remix, transform, and build upon the material, under the Attribution-NonCommercial terms.

In 1999, another isolated population related to this triplet was discovered by Jean-François Charmeux in the vicinity of Dalasm, a small village further north from the type locality of *P. valiabadi* (Fig. 1). Besides the more pronounced wing markings and the slight shift in the position of the spot in cell 6 on the underside of the hindwing, the most striking difference between this population and *P. valiabadi* is the absence of the distinctive white disco-cellular streak on the underside of the hindwings. Based on the type series of nine males and one female, this population was described by Charmeux (2016) as a new subspecies, “*Polyommatus (Agrodiateus) valiabadi asadii*” (Fig. 2) (see Charmeux, 2016 for the original description). However, the morphology of genitalia, as well as molecular and karyotypic characteristics of this population have remained unknown to this day.

Recently, the first author revisited the type locality of *P. valiabadi asadii* and collected three additional individuals from this subspecies, as well as new information on its larval host plant (Fig. 3). Considering the remarkable morphological differences between *P. asadii* and the nominotypical *P. valiabadi*, we decided to re-examine the relationship between this unique taxon and other closely-related taxa using a combination of DNA barcodes, the morphology of the male genitalia, and new insights from their ecology.

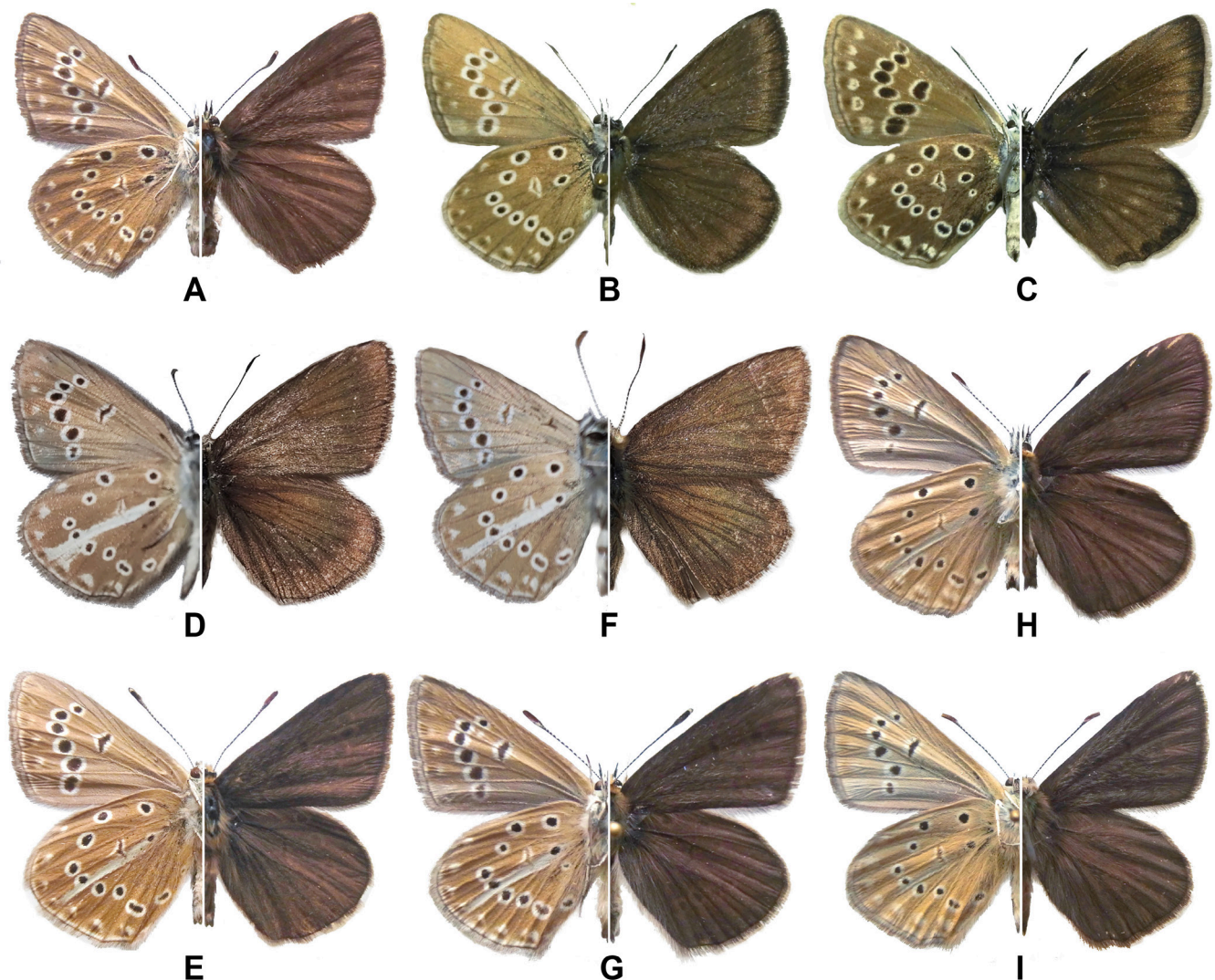
## MATERIAL AND METHODS

Genitalia dissections were carried out by Wolfgang ten Hagen on two males of *P. valiabadi* and one *P. asadii* using the methodology outlined by Higgins (1975) and photographed using standard equipment. Host plant data was collected by the first author during visits to the habitat of each species. Single specimens from several species and subspecies of *Polyommatus* subgenus *Agrodiateus* were selected for analysis, with an emphasis on the “brown” groups. Sequences for nearly all species were available from previous studies and were retrieved from GenBank. We added seven new sequences: One specimen of *P. demavendi ahmadi*, two specimens of *P. valiabadi*, and four specimens of *P. asadii* including two paratypes. Leg tissue and standardized procedures were used to obtain the barcodes at the Center for Biodiversity Genomics in Guelph, Canada, and the new sequences were deposited on GenBank (Accessions PP313483-PP313489). Specimen data is publicly available in the BOLD dataset “DS-ASADII” (<https://doi.org/10.5883/DS-ASADII>). *Polyommatus icarus* (Rottemburg, 1775) was selected as an outgroup and the sequence was retrieved from GenBank (Table 1).



**Figure 1.** Distribution map of the *Polyommatus* species discussed in this study.





**Figure 2.** Adults of *Polyommatus* (*Agrodiaetus*) species. **A–C.** *P. asadii* Charmeux, 2016 **stat. nov.**; **A.** Paratype, Iran: Mazandaran, environs de Dalasm, 1400m, 6.VII.1999, leg. et coll. J.-F. Charmeux; **B., C.** Iran: Mazandaran, S. Chalus, Dalasm village, 1500m, 20.VII.2020, leg. et coll. A.R. Naderi; **D–E.** *P. valiabadi* Rose & Schurian, 1977; **D.** Iran : Mazandaran, S. Ramsar, Tomol village, 13.VII.2006, leg. et coll. A.R. Naderi; **E.** Iran: Mazandaran, Valiabad, 20.VII.2019, local collector, coll. JFC; **F–G.** *P. rjabovianus* Koçak, 1980; **F.** Iran: Gilan, Heiran pass., 1700m- Jul.2009, leg. J. Bahrami, coll. A.R. Naderi; **G.** [JFC]; **H–I.** *P. pseudorjabovi* Lukhtanov, Dantchenko, Vishnevskaya & Saifitdinova, 2015. Azerbaijan Republic, Lerik, Rayonu, 1900 m, 8.VII.2023, leg. et coll. J.-F. Charmeux leg.

Alignment of sequences was done using MUSCLE modules implemented in AliView 1.28 (Larsson, 2014) and double-checked visually. A maximum likelihood tree was obtained using IQTree web server (Nguyen et al., 2015). Bayesian analysis was conducted in BEAST (Suchard et al., 2018) for 20 million generations and the results were tested using TRACER 1.7.1 (Rambaut et al., 2018). We calibrated the tree using a previously published age estimate for the subgenus *Agrodiaetus* (2.51–3.85 mya, Kandul et al., 2004). Most samples yielded full-length COI barcode sequences and the total length of our dataset was 658 base pairs. However, in order to avoid overestimation of genetic distances resulting from sequence length variation due to missing data, the Kimura 2-parameter distances were calculated in MEGA 11.0.8 (Tamura et al., 2021) on a trimmed dataset of 421 bp length with nearly completely overlapping fragments.

**Table 1.** List of samples used for phylogenetic analysis of *Polyommatus* and their GenBank accessions. Accession numbers highlighted in green were obtained through this study.

Taxon	SampleID	Accession No.	Localities
<i>icarus</i>	NK-00-P562	AY496817	Kazakhstan: Altai, Oktyabrsk
<i>carmon</i>	VL01Q129	AY496723	Turkey: Gümüşhane
<i>muelleriae</i>	MUE03	OR413720	Pakistan: Chitral, Birmogh Lasht
<i>damone</i>	KN00799	MW500962	Russia: Orenburgskaya Oblast, S Ural, Kuvandyk 12 km S
<i>magnificus</i>	VL04A003	EF104619	Uzbekistan: Sangurdak
<i>erschoffii</i>	AD02L274	AY496743	Iran: Gorgan, Shahkuh
<i>damocles</i>	VL01B001	AY496729	Russia: South Ural, Verblyuzhka
<i>actis</i>	RV04G284	EF104606	Turkey: Sertevul Geçidi
<i>damon</i>	TLMF Lep 21694	MN142348	Austria: Niederoesterreich, Wollmannsberg, Waschberg
<i>demavendi demavendi</i>	VL02N685	AY953996	Iran: Tehran, Mobarahabad
<i>demavendi almadi</i>	ARLY-0231-002	PP313486	Iran: Qazvin, Alamut pass
<i>demavendi belovi</i>	050A07	KR265494	Armenia: Khosrov
<i>lorestanus</i>	VL02N575	AY953995	Iran: Lorestan, Razan Pass
<i>ripartii ripartii</i>	RV03H463	EF104603	Spain: Barcelona, El Brull
<i>ripartii ramonagenjo</i>	RVcoll.09-X021	KC581739	Spain: Catalonia, La Farga Vella (1100 m)
<i>ripartii exuberans</i>	RVcoll.14-O216	MN138512	Italy: Piedmont, Balbieres, South of Oulx
<i>ripartii galloi</i>	RE07G436	HM210167	Italy: Pollino, Serra del Prete, Mont Pollino
<i>ripartii pelopi</i>	JC00043	AY556858	Greece: Peloponnisos, Mt. Helmos (1350-1500 M)
<i>ripartii paralcensis</i>	VL01L166	AY496784	Turkey: Gümüşhane, Dilekyolu
<i>ripartii budashkini</i>	NK00P859	AY496779	Ukraine: Crimea, Karabi yaila
<i>ripartii colemani</i>	NK00P822	AY496781	Kazakhstan: West Tian-Shan
<i>ripartii sarkani</i>	NK00P829	AY496785	Kazakhstan: Dzhungarian Alatau mts., Kolba
<i>ripartii ovchinnikovi</i>	2005-LOWA-67	FJ663245	Kazakhstan: Semipalatinsk Region, W. Tarbagatai, Taskesken
<i>admetus admetus</i>	RVcoll14O392	KY322829	Hungary: Trizs
<i>admetus malievi</i>	VL03F903	EF104617	Azerbaijan: Talysh, Zuvand
<i>yeraniyani</i>	AD00P016	AY496711	Armenia: Aiodzor mts., Gnishyk
<i>pseudorjabovi</i>	VL03F820	AY954020	Azerbaijan: Talysh, Zuvand
<i>khorasanensis</i>	VL03F526	AY954013	Iran: Khorasan, Kopetdagh mts
<i>nephoiptamenos</i>	JC00045	AY556859	Greece: Macedonia, Mt. Orvilos (1200-2100 M)
<i>iphigenia</i>	MW98049	AY556984	Turkey: Fethiye, Salur Dagı (1700-1900 M)
<i>violetae violetae</i>	RVcoll.07-C687	GU677017	Spain: Andalusia, Granada, Sierra de Almijara
<i>violetae subbaeticus</i>	RVcoll.09-V909	HM901760	Spain: Andalusia, Granada, Sierra de la Sagra
<i>armaniensis</i>	JC00040	AY556856	Greece: Peloponnisos, Mt. Helmos (1350 M)
<i>luræ</i>	LP18_119_K88_PT	ON715904	Albania: Diber, Lure, Prej Lure
<i>timfristos</i>	LR-08-D247	KY066725	Greece: Karpenisis, Timfristos Mt
<i>humedasae</i>	RVcoll15H043	MW502349	Italy: Aosta, Pont d'Ael
<i>orphicus orphicus</i>	RVcoll12M015	MW501023	Bulgaria: Chepelare, Hvoyna (quarry)
<i>orphicus eleniae</i>	LR-08-D431	KY050599	Greece: Granitis
<i>fabressei</i>	RV03H596	EF104605	Spain: Castelló, Coll d'Ares
<i>ainsae</i>	MAT99Q894	AY496712	Spain: Lleida, Tremp
<i>fulgens fulgens</i>	MAT99Q910	AY496746	Spain: Santa Colomade de Queralt
<i>fulgens leonensis</i>	RVcoll.08-L865	GU676235	Spain: Castilla y Leon, Soria, Abejar
<i>fulgens pseudovirgilius</i>	MW01107	AY556963	Spain: Tarragona, Sta. Coloma De Queralt (700 M)
<i>dolus dolus</i>	LD-3505	MN138992	Italy: Liguria, Ventimiglia
<i>dolus gargano</i>	LEP-SS-00567	MW503058	Italy: Calabria, Timpone Montillo-Alessandria Del Carretto
<i>dolus vittatus</i>	RVcoll.12-P690	MN141207	France: Occitanie, Languedoc-Roussillon, Herault, Cirque de Navacelles
<i>dolus virgilius</i>	RVcoll.07-E105	MN139341	Italy: Abruzzi, L'Aquila, Rocca Pia, L'Aquila
<i>dolus paravirgilius</i>	LD-2653	MN139580	Italy: Salerno, Monte Faito
<i>menalcas</i>	VL01L122	AY496763	Turkey: Gümüşhane, Dilekyolu
<i>interjectus</i>	MW99164	AY557059	Turkey: Erzurum, Ciftlik (1900 M)
<i>alcestis</i>	MW98212	AY557008	Turkey: Adana, Saimbeyli Falls (1500 M)
<i>erivanensis</i>	AD00P303	AY496742	Armenia: Aiodzor mts.
<i>dantchenkoi</i>	VL01L342	AY496737	Turkey: Van, Çatak
<i>karacetinae</i>	MW99380	AY557090	Turkey: Hakkari, Yuksekova (1800 M)
<i>urmiaensis</i>	VL04E365	EF104631	Iran: Azarbayjan-e-Gharbi
<i>rjabovianus rjabovianus</i>	VL03F816	AY954019	Azerbaijan: Talysh, Zuvand
<i>rjabovianus masul</i>	VL02X474	AY954006	Iran: Gilan, Masuleh
<i>valiabadi</i>	MW00064	AY556882	Iran: Mazandaran, Pul-e Zanguleh (2400 M)
<i>valiabadi</i>	MW00498	AY556934	Iran: Mazandaran, 5 km S Valiabad (1900 m)
<i>valiabadi</i>	2007val02	KR265486	Iran: Mazandaran
<i>valiabadi</i>	2007val01	KR265495	Iran: Mazandaran
<i>valiabadi</i>	ARLY-101b-001	PP313489	Iran: Mazandaran, near Valiabad, Shahrak e Daryabar
<i>valiabadi</i>	ARLY-232f-001	PP313489	Iran: Mazandaran, S. Ramsar, Tomol village
<i>asadii</i>	JFC23001	PP313484	Iran: Mazandaran, environs de Dalasm
<i>asadii</i>	JFC23002	PP313485	Iran: Mazandaran, environs de Dalasm
<i>asadii</i>	JFC23003	PP313488	Iran: Mazandaran, 5 km SW Dalasm
<i>asadii</i>	Naderi2023-008	PP313487	Iran: Mazandaran, S. Chalus, Dalasm village





**Figure 3.** Adult specimens, habitats, and larval food plants. **A., C., E.** *Polyommatus asadii* Charmeux, 2016 **stat. nov.**; **B., D., F.** *P. valiabadi* Rose & Schurian, 1977; **E.** *Onobrychis mazanderanica* Reich. f.; **F.** *O. talagonica* Reich. f. (Fabaceae).



## RESULTS

*Taxonomic hierarchy*

Class Insecta Linnaeus, 1785

Order Lepidoptera Linnaeus, 1758

Lycaenidae Leach, 1815

Polyommatinae Swainson, 1827

Genus *Polyommatus* Latreille, 1804Type species: *Papilio icarus* Rottemburg, 1775:21*Polyommatus (Agrodiaetus) asadii* Charmeux, 2016 stat. nov.

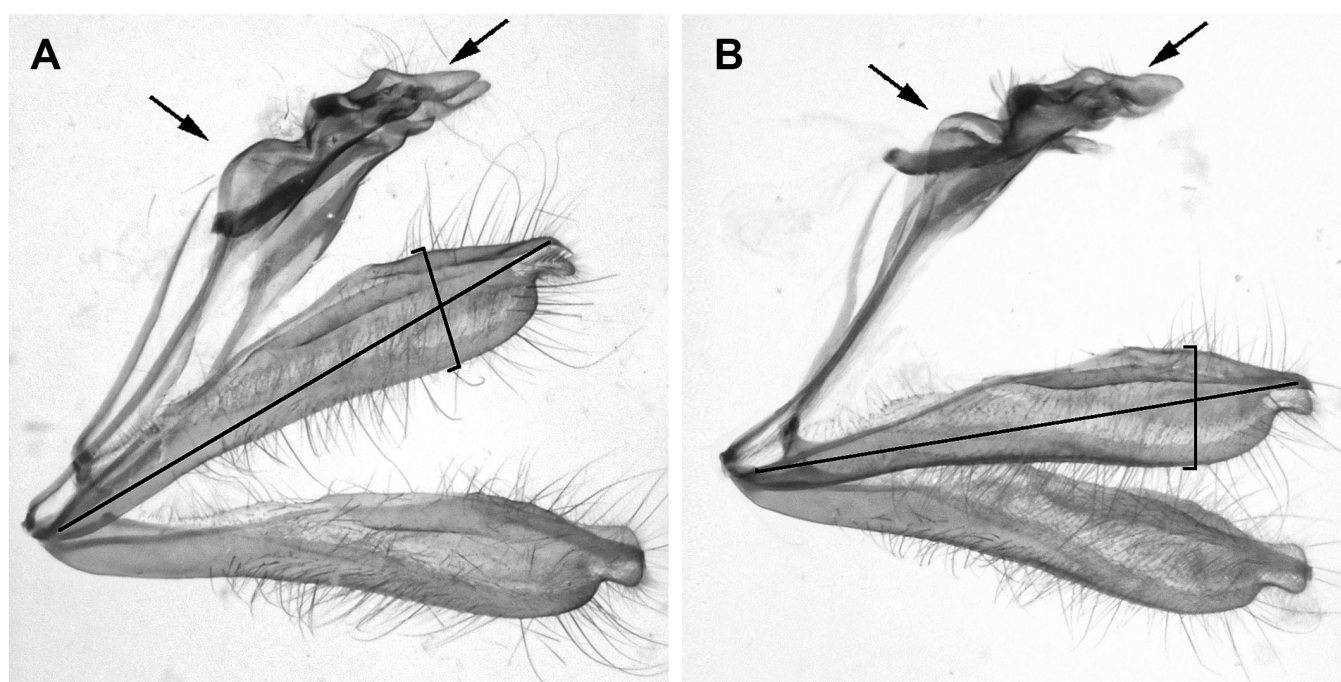
**Material examined.** **Holotype** ♂, (Muséum d'Histoire Naturelle de Paris - MNHN), Iran, Mazandaran, environs de Dalasm, 1400 m, 13.VII.1999, J.-F. Charmeux leg.; **Paratypes** (MNHN): 1 ♀, same data as holotype (designed as Allotype by J.-F. Charmeux); 5 ♂♂, same locality as holotype, 6.VII.1999, J.-F. Charmeux leg. & coll., 3 ♂♂, same locality as holotype; 13.VII.1999, J.-F. Charmeux leg. & coll.

**Additional material.** 2 ♂♂, and 1 ♀, (Research Collection of Alireza Naderi) Iran, Mazandaran, S. Chalus, Dalasm village, 20.VII.2020, Alireza Naderi leg.

**Diagnosis.** Wing markings more pronounced than *P. valiabadi*; spot in cell 6 on the underside of the hindwing slightly shifted; white disco-cellular streak on the underside of the hindwings absent. While the male genitalia in *P. valiabadi* and *P. asadii* are very similar, we note subtle differences in the shape of the uncus, the tegumen, and the ratio of maximum length to maximum width of the valvae (Fig. 4).

**Full description.** – See Charmeux (2016).

**Host plant.** The host plant of *P. asadii*, discovered by the first author in July 2020 at the type locality, is the annual *Onobrychis mazanderanica* Rech. f. (Fabaceae) that grows only in steep meadows and forest clearings. The female was observed laying eggs on this plant, while the females of *P. valiabadi* oviposit on *Onobrychis talagonica* Rech. f. (Fabaceae)(Alireza Naderi, personal observation).



**Figure 4.** Male genitalia, lateral view. **A.** *Polyommatus asadii* Charmeux, 2016 stat. nov.; **B.** *P. valiabadi* Rose & Schurian, 1977. Arrows show subtle differences in the uncus and tegumen between the two species, and the lines show measures of the maximum length and width of the valvae.

**Molecular analysis.** Of the four DNA barcode sequences obtained for *P. asadii*, the two older paratypes yielded only shorter barcode fragments (203 and 306bp), which nevertheless matched perfectly with the two full-length barcode sequences (658bp) from more recent material. Similarly, one of the two new *P. valiabadi* sequences was shorter (296bp), but both new sequences of this taxon matched closely with others available on GenBank. In our phylogenetic inferences, *P. asadii* always appeared as a sister taxon to *P. valiabadi* but with a notable distance (Fig. 5). The uncorrected *p*-distance between *P. asadii* and *P. valiabadi* was found to be between 0.5-1.0% (average: 0.6%) (Table 2). Together with *P. rjabovianus*, the three formed a well-supported clade (BPP:0.99, ML: 77) that was sister to all other taxa in the *dolus*-group (Lukhtanov et al., 2023 group 9). The split between *P. valiabadi* and *P. asadii* in our analysis is estimated to have occurred around 750,000 years ago, while their common ancestor separated from *A. rjabovianus* much earlier, around 1.39 million years ago (Fig. 5).



**Figure 5.** BEAST phylogeny of the selected species of *Polyommatus* (*Agrodiaetus*). Numbers indicate mean divergence time estimates millions of years ago.





## DISCUSSION

The ranges of the allopatric species *P. rjabovianus*, *P. valiabadi* and *P. asadii* are all restricted to the mountains south of the Caspian Sea. The three species have never been recorded in sympatry and their ranges are not known to overlap. All three are found in similar habitats, in forest clearings. Our DNA barcode analysis demonstrates a genetic distinction between *P. asadii* and its sister taxa. Comparing our male genitalia dissections of *P. valiabadi* and *P. asadii* with those previously published for *P. pseudorjabovi* and *P. rjabovianus* (Lukhtanov et al., 2015), we note a high degree of similarity between all four taxa. It is well known that the species in the subgenus *Agrodiaetus* do not always exhibit pronounced, easy-to-check differences (Coutsis, 1986). Despite nearly identical male genitalia, Lukhtanov et al. (2015) indicated that the valvae in *P. pseudorjabovi* were “slightly wider than in *P. rjabovianus*”. Similarly, we also note subtle differences in the male genitalia between *P. valiabadi* and *P. asadii* (Fig. 4). Geographic isolation from populations of *P. valiabadi*, distinctive wing-pattern elements, different larval host plant species, differences in male genitalia, and divergent DNA barcode haplotypes all suggest that *P. asadii* should be regarded as a separate species. The Kimura-2 parameter genetic (barcode) distance between *P. asadii* and *P. valiabadi* (0.5–1.0%; average: 0.6%) is well within the range observed between many species in the subgenus *Agrodiaetus*, and in some cases even higher (Table 2). Low genetic divergence is common in *Agrodiaetus*, e.g. among the species in the *admetus* group that show great karyotypic differences (chromosome numbers indicated between brackets): For example, the species pairs *P. ainsae* ( $n=108-110$ ) and *P. fulgens* ( $n=103$ ), or *P. eriwanensis* ( $n=34$ ) and *P. dantchenkoi* ( $n=40-42$ ), are either barcode-identical or show very small degrees of DNA barcode divergence. The chromosome number of *P. valiabadi* is  $n=23$  (Wiemers 2003), *P. rjabovianus rjabovianus*  $n=49$  (Lukhtanov et al., 2005, 2015) and *P. rjabovianus masul*  $n=43$  (Lukhtanov et al., 2015). The chromosome number of *P. asadii* remains unknown, but we predict that it will be notably different from both *P. valiabadi* and *P. rjabovianus*.

The host plants of *P. asadii* and *P. valiabadi* (i.e. *Onobrychis mazanderanica* and *O. talagonica*) are sister species in section *Hymenobrychis* of the genus *Onobrychis* (Safaei Chaei Kar et al., 2014; Amirahmadi et al., 2015). They are both Persian endemics with limited ranges in northern Iran (Ranjbar et al., 2011, 2012). *Onobrychis mazanderanica* has a wide distribution in Alborz, Golestan and Mazandaran provinces and is found in altitudes between 500–2400m (Ghorbanalizadeh & Akhane, 2022). On the other hand, *O. talagonica* seems to have a more limited range in Tehran and Mazandaran provinces (Ranjbar et al., 2011, Talebi et al., 2020). The type locality of *P. asadii* is located in the Hyrcanian forest ecotone zone. The area is a semi dry plateau with a fair amount of annual precipitation and is surrounded by dense humid forests. In late summer, this area is very hot and dry with xerophytic vegetation. Some of the accompanying species flying together with *P. asadii* are *P. marcidus* (Lederer, 1870), *P. Icarus* (Rottemburg, 1775), *P. thersites* (Cantener, 1834), *Lysandra bellargus* (Rottemburg, 1775), and *Aricia agestis* (Denis & Schiffermüller, 1775). The climate in this habitat is fairly humid with lush vegetation consisting of different species (AN, personal observations). The newly collected specimens are the first series to be found after the description of this taxon. Further studies are needed to determine the chromosome number of *P. asadii* and its genomic characteristics in the wider context of the phylogeny of *Polyommatus* blues.

We also noticed that our single specimen of *P. valiabadi* from Tomol, South of Ramsar, showed a notable genetic distance from the remaining populations of this species in Central Alborz, and further investigation may be needed to determine if it merits a separate status as a subspecies of *P. valiabadi*. In addition, we obtained for the first time a full-length barcode for *P. demavendi ahmadi*, which appears as sister to other subspecies of *P. demavendi* in our analysis. However, since this taxon is not the focus of the present study, we leave the discussion on its phylogenetic position for a future investigation.

## AUTHOR'S CONTRIBUTION

The authors confirm their contribution to the paper as follows: A. Naderi: Conceptualization, methodology, investigation, writing—original draft preparation, visualization; J.-F. Charmeux: writing—review and editing, visualization; V. Nazari: methodology, software, validation and formal analysis, investigation, resources and data curation, writing—review and editing, visualization, supervision, project administration, funding acquisition for DNA barcoding; All authors read and approved the final version of the manuscript.

## FUNDING

This research received no specific grant from any funding agencies.

## AVAILABILITY OF DATA AND MATERIAL

The data for specimens examined in this study are publicly available in the BOLD dataset “DS-ASADII” (<https://doi.org/10.5883/DS-ASADII>). Specimens directly examined in this study are deposited in the Muséum d’Histoire Naturelle de Paris (MNHN) and the Research Collection of Alireza Naderi (Tehran, Iran), and are available from the curator, upon request.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## CONSENT FOR PUBLICATION

Not applicable.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

## ACKNOWLEDGMENTS

We thank Gholamreza Naderi and Ehsan Jannati for accompanying the first author in his excursions, Wolfgang ten Hagen for dissections and photography of genitalia, Valiollah Mozaffarian for identification of the larval host plants, and Payam Zehzad for intellectual support. We are also grateful to anonymous reviewers for their invaluable comments that helped improve this paper.

## REFERENCES

- Amirahmadi, A., Kazempour-Osaloo, S., Kaveh, A., Maassoumi, A.A. & Naderi, R. (2016) The phylogeny and new classification of the genus *Onobrychis* (Fabaceae-Hedysareae): evidence from molecular data. *Plant Systematics and Evolution*, 302 (10), 1445–1456. <https://doi.org/10.1007/s00606-016-1343-1>
- Charmeux, J.F. (2016) *Agrodiaetus valiabadi asadii* nova subspecies d’Iran (Lepidoptera : Lycaenidae). *Lépidoptères - Revue des Lépidoptéristes de France*, 25 (65), 102–103.
- Coutsis, J.G. (1986) The blue butterflies of the genus *Agrodiaetus* Hübner (Lep. Lycaenidae): Symptoms of taxonomic confusion. *Nota Lepidopterologica*, 9 (3–4), 159–169.
- Eckweiler, W. & Bozano, G.C. (2016) *Guide to the butterflies of the Palearctic region, Lycaenidae part IV*. Omnes Artes, Milan. 132 p.
- Forster, W. (1960) *Agrodiaetus rjabovi* sp. nov. *Entomologische Zeitschrift*, 70 (14), 157.
- Ghorbanalizadeh, A. & Akhiani, H. (2022) Plant diversity of Hyrcanian relict forests: An annotated checklist, chorology and threat categories of endemic and near endemic vascular plant species. *Plant Diversity*, 44, 39–69. <https://doi.org/10.1016/j.pld.2021.07.005>
- Higgins, L.G. (1975) *The Classification European Butterflies*. Collins, London, UK. 320 p.
- Kandul, N.P., Lukhtanov, V.A., Dantchenko, A.V., Coleman, J.W.S., Sekercioglu, C.H., Haig, D. & Pierce, N.E. (2004) Phylogeny of *Agrodiaetus* Hübner 1822 (Lepidoptera: Lycaenidae) inferred from mtDNA sequences of COI and COII and Nuclear Sequences of EF1- $\alpha$ : Karyotype diversification and species radiation. *Systematic Biology*, 53 (2), 278–298. <https://doi.org/10.1080/10635150490423692>
- Koçak, A.Ö. (1980) On the nomenclature of some genus- and species-group names in Lepidoptera. *Nota Lepidopterologica*, 2 (4), 139–146.
- Larsson, A. (2014) AliView: a fast and lightweight alignment viewer and editor for large data sets. *Bioinformatics*, 30, 3276–3278. <https://doi.org/10.1093/bioinformatics/btu531>
- Lukhtanov, V.A., Kandul, N.P., Plotkin, J.B., Dantchenko, A.V., Haig, D. & Pierce, N.E. (2005) Reinforcement of pre-zygotic isolation and karyotype evolution in *Agrodiaetus* butterflies. *Nature*, 436, 385–389. <https://doi.org/10.1038/nature03704>



- Lukhtanov, V.A., Dantchenko, A.V., Vishnevskaya, M.S. & Saifitdinova, A.F. (2015) Detecting cryptic species in sympatry and allopatry: analysis of hidden diversity in *Polyommatus* (*Agrodiaetus*) butterflies (Lepidoptera: Lycaenidae). *Biological Journal of the Linnean Society*, 116, 468–485. <https://doi.org/10.1111/bij.12596>
- Lukhtanov, V.A., Shapoval, N.A., Dantchenko, A.V. & Eckweiler, W. (2023) Phylogenetic structure revealed through combining DNA barcodes with multi-gene data for *Agrodiaetus* blue butterflies (Lepidoptera, Lycaenidae). *Insects*, 14:769. <https://doi.org/10.3390/insects14090769>
- Nguyen, L.T., Schmidt, H.A., von Haeseler, A. & Minh, B.Q. (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution*, 32, 268–274. <https://doi.org/10.1093/molbev/msu300>
- Rajaei, H., Aarvik, L., Arnscheid, W.R., Baldizzone, G., Bartsch, D., Bengtsson, B.Å., Bidzilya, O., Buchner, P., Buchsbaum, U., Buszko, J. et al. (2023) Catalogue of the Lepidoptera of Iran. In: Rajaei, H. & Karsholt, O. (eds) *Lepidoptera Iranica. Integrative Systematics*, 6 (Special Issue), pp. 121–459. <https://doi.org/10.18476/2023.997558.7>
- Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67, 901–904. <https://doi.org/10.1093/sysbio/syy032>
- Ranjbar, M., Hajmoradi, F. & Karamian, R. (2011) Meiotic chromosome number and behaviour of *Onobrychis alborzensis* (Fabaceae): a new species from northern Iran. *Feddes Repertorium*, 122, 1–12. <https://doi.org/10.1002/fedr.201100028>
- Rose, K. & Schurian, K. (1977) Beitrage zur Kenntnis der Rhopaloceren Irans – 7. Beitrag: Eine Unterart von *Agrodiaetus rjabovi* Forster. *Journal of Entomological Society of Iran*, 4 (1,2), 61–64.
- Safaei Chaei Kar, S., Ghanavati, F., Naghavi, M.R., Amirabadi-zade, H. & Rabiee, R. (2014) Molecular phylogenetics of the *Onobrychis* genus (Fabaceae: Papilionoideae) using ITS and trnL-trnF DNA sequence data. *Australian Journal of Botany*, 62, 235–250. <https://doi.org/10.1071/BT13279>
- Suchard, M.A., Lemey, P., Baele, G., Ayres, D.L., Drummond, A.J. & Rambaut, A. (2018) Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution*, 4, vey016. <https://doi.org/10.1093/ve/vey016>
- Talebi, S.M., Azizi, N., Yadegari, P. & Matsyura, A. (2020) Analysis of pollen morphological characteristics in Iranian *Onobrychis* Miller (Fabaceae) taxa. *Brazilian Journal of Botany*, 43, 609–632. <https://doi.org/10.1007/s40415-020-00623-6>
- Tamura, K., Stecher, G. & Kumar, S. (2021) MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution*, 38, 3022–3027. <https://doi.org/10.1093/molbev/msab120>
- Wiemers, M. (2003) Chromosome differentiation and the radiation of the butterfly subgenus *Agrodiaetus* (Lepidoptera: Lycaenidae: *Polyommatus*): A molecular phylogenetic approach. PhD Thesis, University of Bonn, Bonn, Germany. 203 p. Available at: <https://hdl.handle.net/20.500.11811/1944>

شناسایی *Polyommatus (Agrodiaetus) asadii* Charmeux, 2016 stat. nov. (Lepidoptera: Lycaenidae)

به عنوان گونه‌ای متمایز

علیرضا نادری<sup>۱</sup>، ژان فرانسوا شارمو<sup>۲</sup> و وازریک نظری<sup>۳\*</sup>

۱ موزه ملی تاریخ طبیعی و ذخایر ژنتیکی، تهران، ایران

۲ خیابان تولبیاک ۶۷، کد پستی ۷۵۰۱۳ پاریس، فرانسه

۳ گروه زیست‌شناسی، دانشگاه پادوا، ایتالیا

\* پست الکترونیک نویسنده مسئول مکاتبه: [vazrick.nazari@unipd.it](mailto:vazrick.nazari@unipd.it)

تاریخ دریافت: ۰۲ اسفند ۱۴۰۲ | تاریخ پذیرش: ۲۶ اسفند ۱۴۰۲ | تاریخ انتشار: در حال چاپ |

**چکیده:** آرایه‌ای که در ابتدا در سطح زیرگونه، تحت عنوان *Polyommatus (Agrodiaetus) valiabadi asadii* Charmeux, 2016 توصیف شده بود، بر اساس شواهد جدید ریخت‌شناسی، اکولوژی و بارکدهای DNA به سطح گونه مختص خود با نام *Polyommatus (Agrodiaetus) asadii bona sp.* ارتقا داده شد. این گونه بومی شمال ایران و محدود به دره دلسم در رشته کوه‌های البرز است. گیاه میزبان لارو آن *Onobrychis mazanderanica* Reich. f. است، در حالی که لارو گونه خواهری آن، *P. valiabadi*، از گیاه *O. talagonica* Reich. f. (تیره بقولات) تغذیه می‌کند. گونه *P. asadii* را می‌توان بر اساس الگوی متفاوت بال و اندام تناسلی نر از گونه *P. valiabadi* و دیگر گونه‌های نزدیک تشخیص داد. فرضیه فیلوژنتیک کالیبره شده ما تخمین می‌زند که نیای مشترک *P. asadii* و *P. valiabadi* در حدود ۷۵۰۰۰۰ سال پیش انشقاق یافته است.

**واژگان کلیدی:** پروانه‌های آبی، روزپرک‌ها، ایران، بارکدگذاری دی‌ان‌آ، سطح گونه، تاکسونومی