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

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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

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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).

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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 *Agrodiaetus* 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).

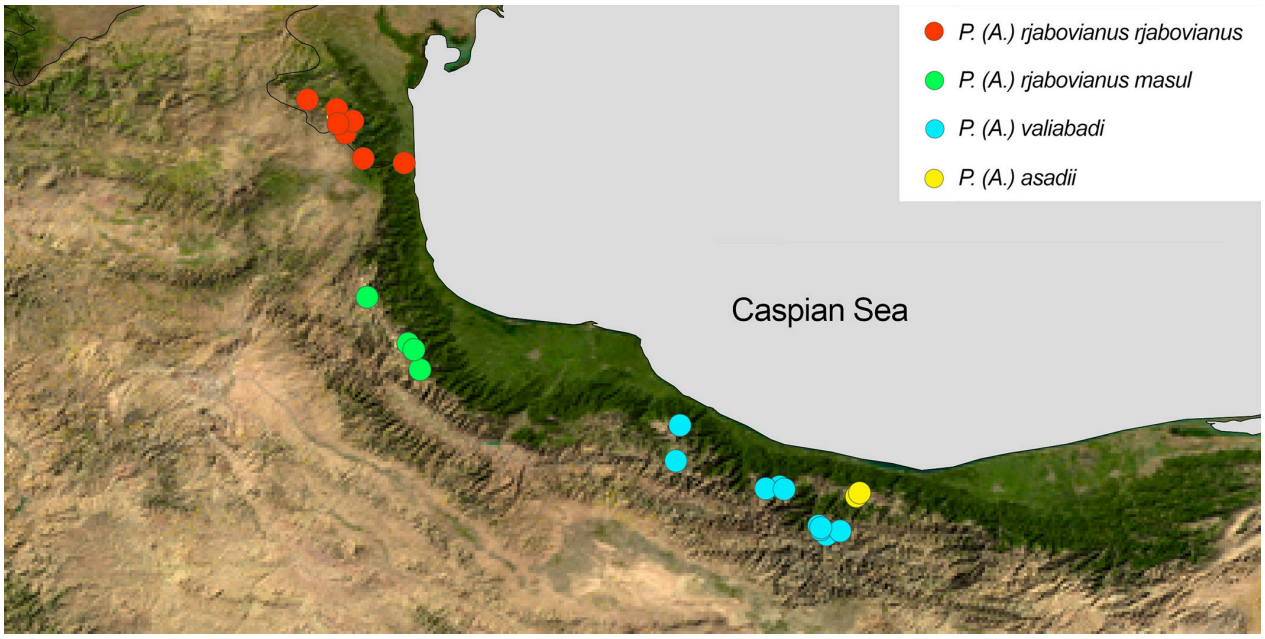

Figure 1. Distribution map of the *Polyommatus* species discussed in this study.

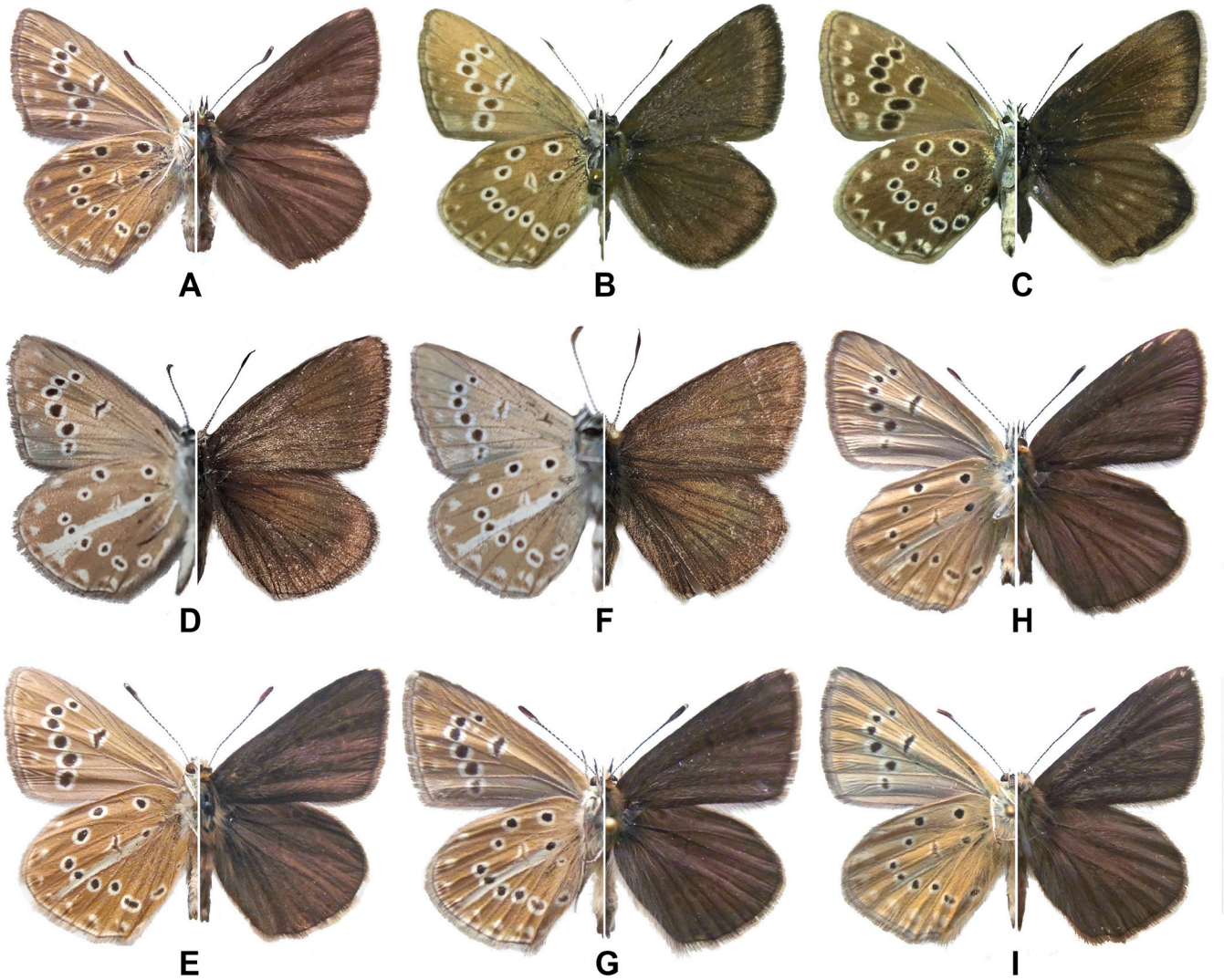

Figure 2. Adults of *Polyommatus* (*Agrodiaetus*) species. **A–C.** *P. asadii* Charmeux, 2016 **stat. nov.**; **A.** Paratype, Iran: Mazandaran, environs de Dalasm, 1400m, 6.VII.1999, leg. et coll. J.-F. Charmeux; **B., C.** Iran: Mazandaran, S. Chalus, Dalasm village, 1500m, 20.VII.2020, leg. et coll. 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
icarus	NK-00-P562	AY496817	Kazakhstan: Altai, Oktyabrsk
carmon	VL01Q129	AY496723	Turkey: Gümüshane
muellerae	MUE03	OR413720	Pakistan: Chitral, Birmogh Lasht
damone	KN00799	MW500962	Russia: Orenburgskaya Oblast, S Ural, Kuvandyk 12 km S
magnificus	VL04A003	EF104619	Uzbekistan: Sangurdak
erschoffii	AD02L274	AY496743	Iran: Gorgan, Shahkuh
damocles	VL01B001	AY496729	Russia: South Ural, Verblyuzhka
actis	RV04G284	EF104606	Turkey: Sertevul Geçidi
damon	TLMF Lep 21694	MN142348	Austria: Niederoesterreich, Wollmannsberg, Waschberg
demavendi demavendi	VL02N685	AY953996	Iran: Tehran, Mobarahabad
demavendi ahmadi	ARLY-0231-002	PP313486	Iran: Qazvin, Alamut pass
demavendi belovi	050A07	KR265494	Armenia: Khosrov
lorestanus	VL02N575	AY953995	Iran: Lorestan, Razan Pass
ripartii ripartii	RV03H463	EF104603	Spain: Barcelona, El Brull
ripartii ramonagenjo	RVcoll.09-X021	KC581739	Spain: Catalonia, La Farga Vella (1100 m)
ripartii exuberans	RVcoll.14-O216	MN138512	Italy: Piedmont, Balbieres, South of Oulx
ripartii galloi	RE07G436	HM210167	Italy: Pollino, Serra del Prete, Mont Pollino
ripartii pelopi	JC00043	AY556858	Greece: Peloponnisos, Mt. Helmos (1350-1500 M)
ripartii paralcestis	VL01L166	AY496784	Turkey: Gümüshane, Dilekyolu
ripartii budashkini	NK00P859	AY496779	Ukraine: Crimea, Karabi yaila
ripartii colemani	NK00P822	AY496781	Kazakhstan: West Tian-Shan
ripartii sarkani	NK00P829	AY496785	Kazakhstan: Dzhungarian Alatau mts., Kolba
ripartii ovchinnikovi	2005-LOWA-67	FJ663245	Kazakhstan: Semipalatinsk Region, W. Tarbagatai, Taskesken
admetus admetus	RVcoll14O392	KY322829	Hungary: Trizs
admetus malievi	VL03F903	EF104617	Azerbaijan: Talysh, Zuvand
yeraniyani	AD00P016	AY496711	Armenia: Aiodzor mts., Gnishyk
pseudorjabovi	VL03F820	AY954020	Azerbaijan: Talysh, Zuvand
khorasanensis	VL03F526	AY954013	Iran: Khorasan, Kopetdagh mts
nephohiptamenos	JC00045	AY556859	Greece: Macedonia, Mt. Orvilos (1200-2100 M)
iphigenia	MW98049	AY556984	Turkey: Fethiye, Salur Dagi (1700-1900 M)
violetae violetae	RVcoll.07-C687	GU677017	Spain: Andalusia, Granada, Sierra de Almijara
violetae subbaetuicus	RVcoll.09-V909	HM901760	Spain: Andalusia, Granada, Sierra de la Sagra
aroaniensis	JC00040	AY556856	Greece: Peloponnisos, Mt. Helmos (1350 M)
lurae	LP18_119_K88_PT	ON715904	Albania: Diber, Lure, Prej Lure
timfristos	LR-08-D247	KY066725	Greece: Karpenisis, Timfistos Mt
humedasae	RVcoll15H043	MW502349	Italy: Aosta, Pont d`Ael
orphicus orphicus	RVcoll12M015	MW501023	Bulgaria: Chepelare, Hvoyna (quarry)
orphicus eleniae	LR-08-D431	KY050599	Greece: Granitis
fabressei	RV03H596	EF104605	Spain: Castelló, Coll d'Ares
ainsae	MAT99Q894	AY496712	Spain: Lleida, Tremp
fulgens fulgens	MAT99Q910	AY496746	Spain: Santa Colomade de Queralt
fulgens leonensis	RVcoll.08-L865	GU676235	Spain: Castilla y Leon, Soria, Abejar
fulgens pseudovirgilius	MW01107	AY556963	Spain: Tarragona, Sta. Coloma De Queralt (700 M)
dolus dolus	LD-3505	MN138992	Italy: Liguria, Ventimiglia
dolus gargano	LEP-SS-00567	MW503058	Italy: Calabria, Timpone Montillo-Alessandria Del Carretto
dolus vittatus	RVcoll.12-P690	MN141207	France: Occitanie, Languedoc-Roussillon, Herault, Cirque de Navacelles
dolus virgilius	RVcoll.07-E105	MN139341	Italy: Abruzzi, L`Aquila, Rocca Pia, L`Aquila
dolus paravirgilius	LD-2653	MN139580	Italy: Salerno , Monte Faito
menalcas	VL01L122	AY496763	Turkey: Gümüshane, Dilekyolu
interjectus	MW99164	AY557059	Turkey: Erzurum, Ciftlik (1900 M)
alcestis	MW98212	AY557008	Turkey: Adana, Saimbeyli Falls (1500 M)
eriwanensis	AD00P303	AY496742	Armenia: Aiodzor mts.
dantchenkoi	VL01L342	AY496737	Turkey: Van, Çatak
karacetinae	MW99380	AY557090	Turkey: Hakkari, Yuksekova (1800 M)
urmiaensis	VL04E365	EF104631	Iran: Azarbayjan-e-Gharbi
rjabovianus rjabovianus	VL03F816	AY954019	Azerbaijan: Talysh, Zuvand
rjabovianus masul	VL02X474	AY954006	Iran: Gilan, Masuleh
valiabadi	MW00064	AY556882	Iran: Mazandaran, Pul-e Zanguleh (2400 M)
valiabadi	MW00498	AY556934	Iran: Mazandaran, 5 km S Valiabad (1900 m)
valiabadi	2007val02	KR265486	Iran: Mazandaran
valiabadi	2007val01	KR265495	Iran: Mazandaran
valiabadi	ARLY-101b-001	PP313489	Iran: Mazandaran, near Valiabad, Shahrak e Daryabar
valiabadi	ARLY-232f-001	PP313489	Iran: Mazandaran, S. Ramsar, Tomol village
asadii	JFC23001	PP313484	Iran: Mazandaran, environs de Dalasm
asadii	JFC23002	PP313485	Iran: Mazandaran, environs de Dalasm
asadii	JFC23003	PP313488	Iran: Mazandaran, 5 km SW Dalasm
asadii	Naderi2023-008	PP313487	Iran: Mazandaran, S. Chalus, Dalasm village

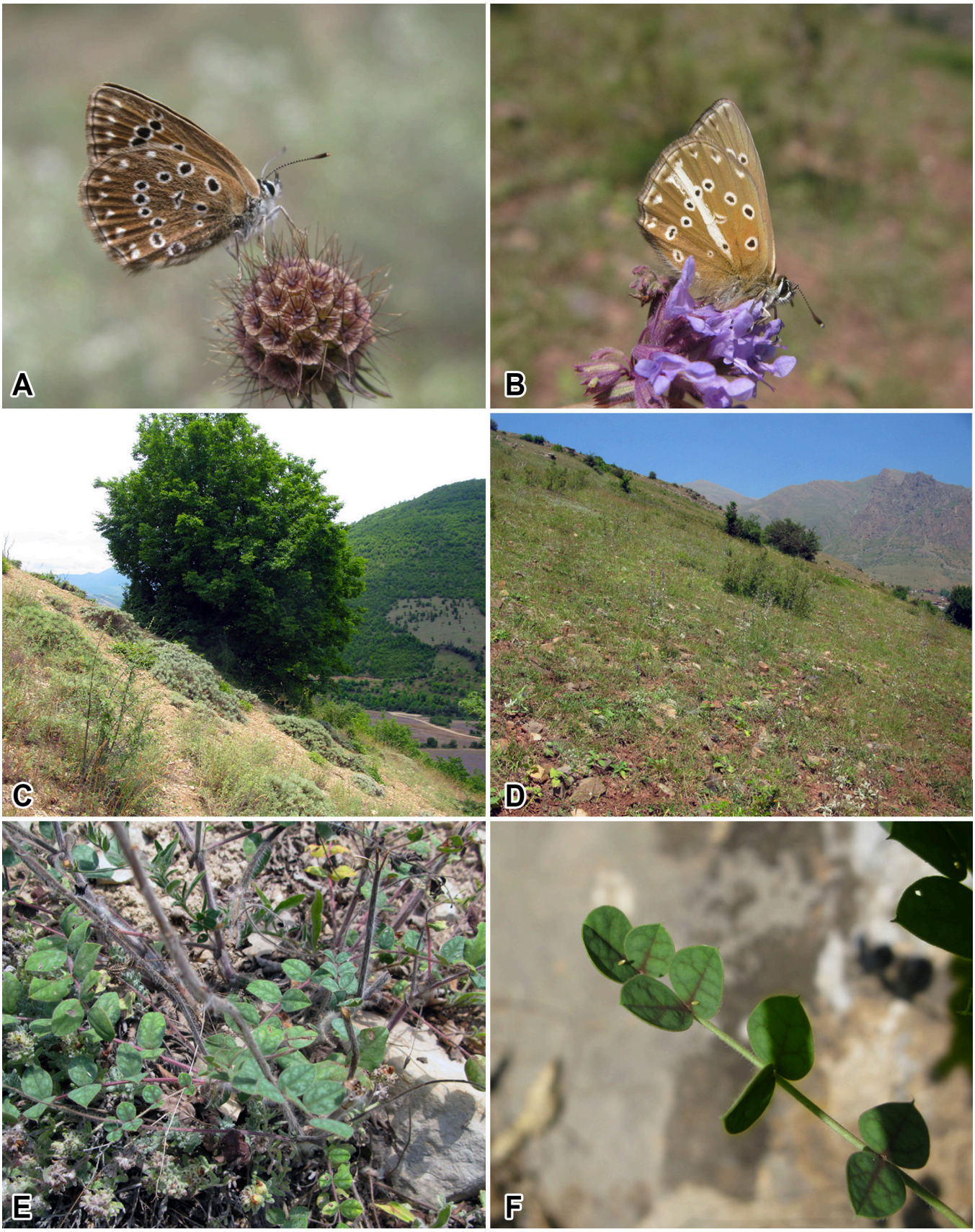

Figure 3. Adult specimens, habitats, and larval food plants. **A.**, **C.**, **E.** *Polyommatus asadii* Charmeux, 2016 **stat. nov**.; **B.**, **D.**, **F.** *P. valiabadi* Rose & Schurian, 1977; **E.** *Onobrychis mazanderanica* Reich. f.; **F.** *O. talagonica* Reich. f. (Fabaceae).

RESULTS

Taxonomic hierarchy

Class Insecta Linnaeus, 1785

Order Lepidoptera Linnaeus, 1758

Lycaenidae Leach, 1815

Polyommatinae Swainson, 1827

Genus *Polyommatus* Latreille, 1804

Type 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.VIL.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).

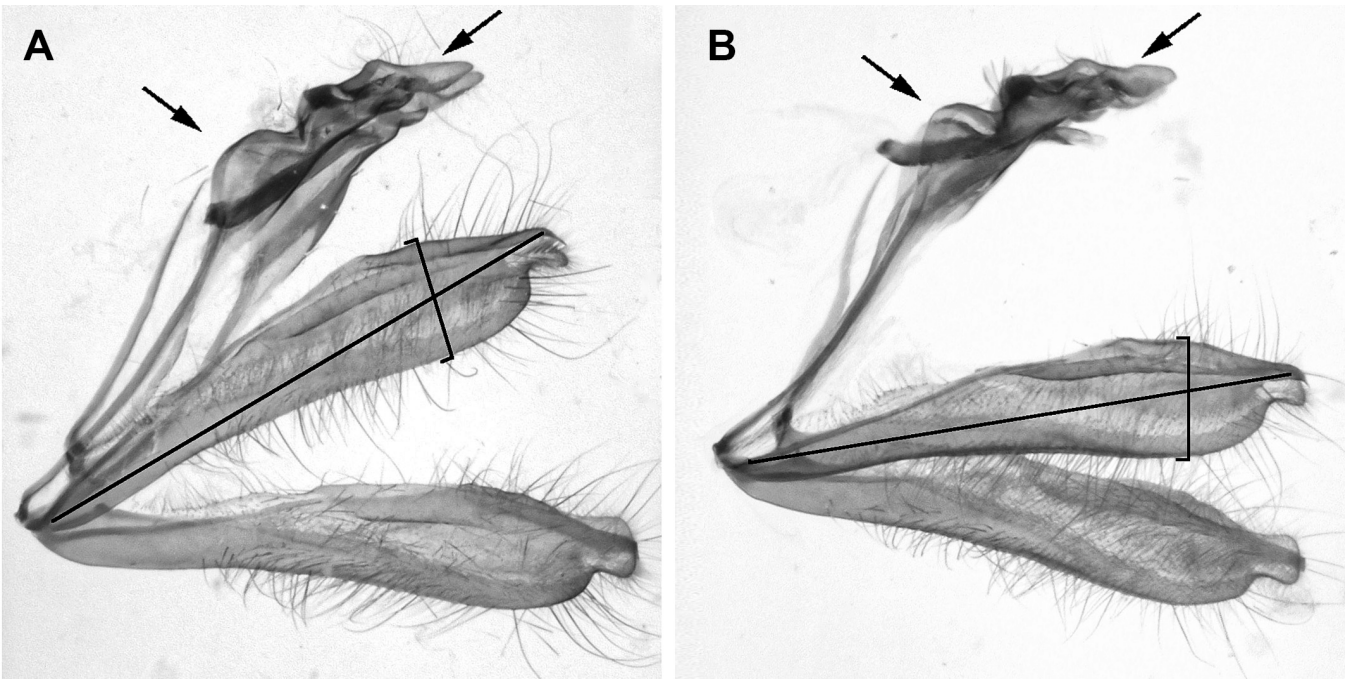

Figure 4. Male genitalia, lateral view. **A.** *Polyommatus asadii* Charmeux, 2016 **stat. nov**.; **B.** *P. valiabadi* Rose & Schurian, 1977. Arrows show subtle differences in the uncus and tegumen between the two species, and the lines show measures of the maximum length and width of the valvae.

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).

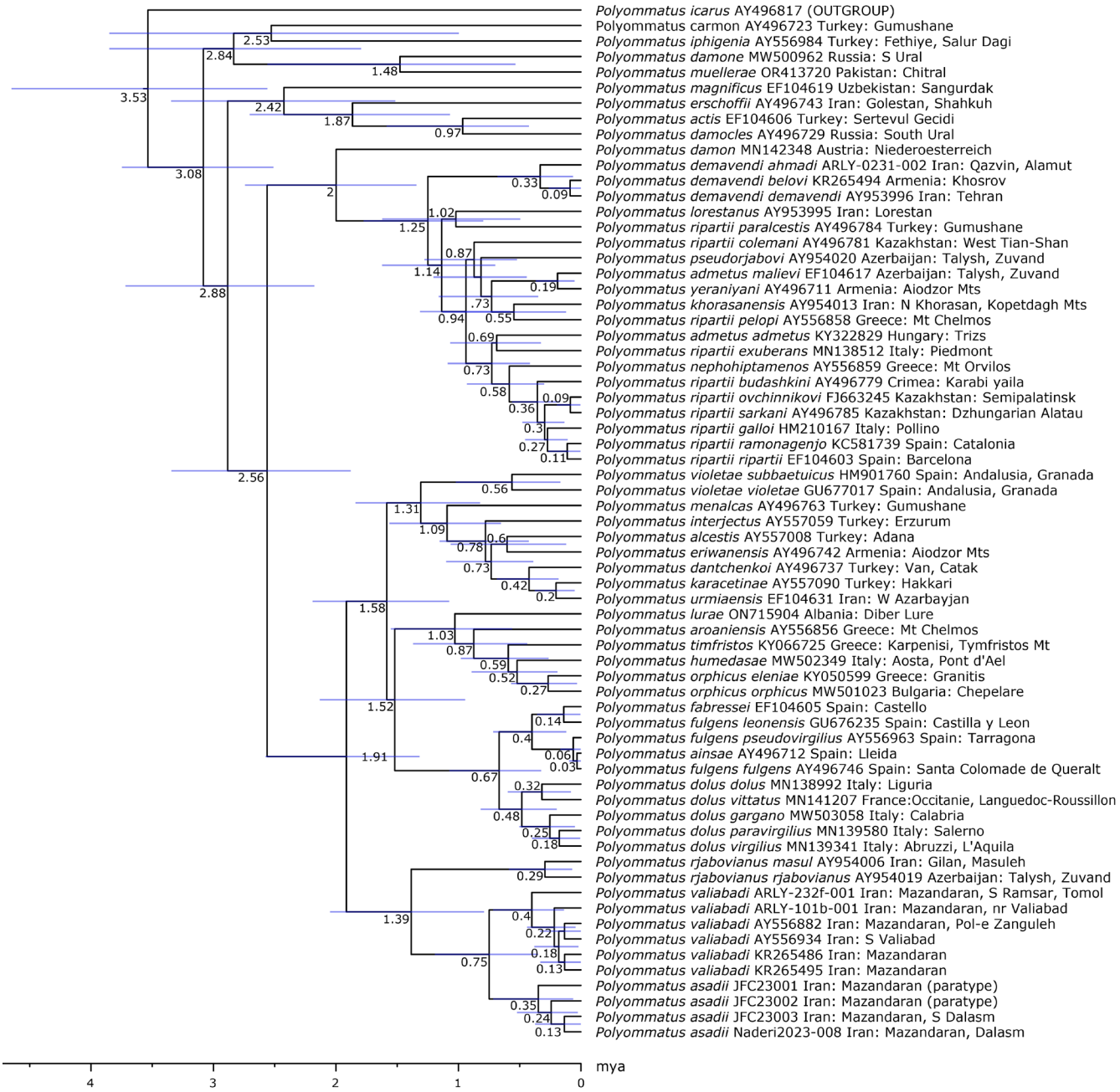

Figure 5. BEAST phylogeny of the selected species of *Polyommatus* (*Agrodiaetus*). Numbers indicate mean divergence time estimates millions of years ago.

Table 2. Average genetic distances between taxa in Fig. 4 arranged in the same phylogenetic order. The intensity of the shade denotes shallower (light) to deeper (dark) divergence between pairs of samples. Boxes denote intra-species variation between subspecies of the same species; the box with thick border shows the distances between *asadii* and *valiabadi*, examined in this study

Taxon	icarus AY496817 OUTGROUP	carmon AY496723 Turkey Gumushane	muellerae OR413720 Pakistan Chitral	damone MW500962 Russia S Ural	magnificus EF104619 Uzbekistan Sangurdak	erschoffii AY496743 Iran Gorgan Shahkuh	damocles AY496729 Russia South Ural	actis EF104606 Turkey Sertevul Ge idi	damon MN142348 Austria Niederoesterreich	demavendi ahmadi ARLY-0231-002 Iran Qazvin Alamut pass	demavendi belovi KR265494 Armenia Khosrov	demavendi demavendi AY953996 Iran Tehran	ripartii budashkini AY496779 Crimea Karabi yaila	ripartii colemani AY496781 Kazakhstan West Tian-Shan	ripartii exuberans MN138512 Italy Piedmont	ripartii galloi HM210167 Italy Pollino	ripartii ovchinnikovi FJ663245 Kazakhstan Semipalatinsk	ripartii paralcestis AY496784 Turkey Gumushane	ripartii pelopi AY556858 Greece Mt Chelmos	ripartii ramonagenjo KC581739 Spain Catalonia	ripartii ripartii EF104603 Spain Barcelona	ripartii sarkani AY496785 Kazakhstan Dzhungarian Alatau mts	lorestanus AY953995 Iran Lorestan	admetus admetus KY322829 Hungary Trizs	admetus malievi EF104617 Azerbaijan Talysh Zuvand	yeraniyani AY496711 Armenia Aiodzor mts	pseudorjabovi AY954020 Azerbaijan Talysh Zuvand	khorasanensis AY954013 Iran N Khorasan Kopetdagh Mts	nephohiptamenos AY556859 Greece Mt Orvilos	iphigenia AY556984 Turkey Fethiye Salur Dagi	violetae subbaeticus HM901760 Spain Andalusia Granada	violetae violetae GU677017 Spain Andalusia Granada
icarus AY496817 OUTGROUP																																
carmon AY496723 Turkey Gumushane	5.2																															
muellerae OR413720 Pakistan Chitral	5.7	3.8																														
damone MW500962 Russia S Ural	4.7	3.9	3.5																													
magnificus EF104619 Uzbekistan Sangurdak	5.0	2.7	4.3	3.9																												
erschoffii AY496743 Iran Gorgan Shahkuh	4.7	5.2	4.8	4.7	4.7																											
damocles AY496729 Russia South Ural	7.4	6.5	6.8	7.3	5.5	6.0																										
actis EF104606 Turkey Sertevul Ge idi	7.1	6.0	7.0	6.2	5.2	6.0	3.9																									
damon MN142348 Austria Niederoesterreich	5.8	4.7	6.7	5.4	6.0	7.0	8.4	6.5																								
demavendi ahmadi ARLY-0231-002 Iran Qazvin Alamut pass	6.8	5.2	5.9	5.7	6.0	6.8	7.6	7.0	6.0																							
demavendi belovi KR265494 Armenia Khosrov	6.9	5.2	5.9	5.8	6.1	6.5	7.6	7.1	6.0	0.5																						
demavendi demavendi AY953996 Iran Tehran	6.8	5.2	5.9	5.7	6.0	6.8	7.6	7.0	6.0	0.5	0.0																					
ripartii budashkini AY496779 Crimea Karabi yaila	5.5	3.5	5.7	5.0	5.0	5.5	6.3	5.8	4.7	2.5	2.5	2.5																				
ripartii colemani AY496781 Kazakhstan West Tian-Shan	6.5	4.4	5.9	5.2	6.0	6.5	7.6	5.7	5.4	3.2	3.2	3.2	2.2																			
ripartii exuberans MN138512 Italy Piedmont	6.0	3.2	5.4	5.2	5.2	6.0	6.8	6.0	4.4	2.4	2.4	2.4	1.0	2.2																		
ripartii galloi HM210167 Italy Pollino	6.0	3.9	5.9	5.2	5.7	6.0	6.8	6.2	5.2	2.7	2.7	2.7	0.2	2.4	1.2																	
ripartii ovchinnikovi FJ663245 Kazakhstan Semipalatinsk	6.0	3.9	5.9	5.2	5.7	6.2	6.8	6.2	5.7	3.2	3.2	3.2	0.7	2.7	1.7	1.0																
ripartii paralcestis AY496784 Turkey Gumushane	6.0	3.4	4.8	4.9	4.7	6.0	6.2	6.2	4.7	1.7	1.7	1.7	1.2	1.9	1.2	1.4	1.9															
ripartii pelopi AY556858 Greece Mt Chelmos	6.2	4.0	5.4	4.8	5.4	5.9	7.0	5.9	4.8	2.2	1.7	1.7	0.7	2.0	1.2	1.0	1.5	1.0														
ripartii ramonagenjo KC581739 Spain Catalonia	5.7	3.7	5.6	4.9	5.5	5.7	6.5	6.0	4.9	2.4	2.5	2.4	0.0	2.2	1.0	0.2	0.7	1.2	0.7													
ripartii ripartii EF104603 Spain Barcelona	6.0	3.9	5.4	4.7	5.7	6.0	6.8	6.2	5.2	2.4	2.4	2.4	0.2	2.2	1.2	0.5	1.0	1.2	1.0	0.2												
ripartii sarkani AY496785 Kazakhstan Dzhungarian Alatau mts	5.8	4.2	6.2	5.5	6.0	6.5	7.0	6.5	6.0	3.4	3.5	3.4	0.7	2.9	1.9	1.2	0.2	2.2	1.7	1.0	1.2											
lorestanus AY953995 Iran Lorestan	6.8	4.9	6.2	6.0	5.7	6.8	7.3	6.8	6.0	2.7	2.2	2.2	2.5	3.4	2.7	2.9	3.4	1.9	2.0	2.7	2.7	3.7										
admetus admetus KY322829 Hungary Trizs	6.3	3.9	5.4	4.7	5.2	6.0	7.3	5.7	4.7	2.7	2.7	2.7	1.2	2.4	1.7	1.4	1.9	1.4	1.0	1.2	1.4	2.2	2.9									
admetus malievi EF104617 Azerbaijan Talysh Zuvand	6.3	3.7	5.4	4.7	5.0	6.5	6.5	5.4	5.4	2.7	2.7	2.7	1.7	2.4	2.2	1.9	1.9	1.4	1.5	1.7	1.7	2.2	2.9	1.9								
yeraniyani AY496711 Armenia Aiodzor mts	6.5	3.8	6.2	4.9	4.9	6.8	6.8	5.7	5.1	3.3	3.6	3.6	2.0	2.8	2.5	2.3	2.3	2.3	1.8	2.0	2.3	2.3	3.6	2.3	0.7							
pseudorjabovi AY954020 Azerbaijan Talysh Zuvand	6.0	4.2	5.6	5.5	5.2	6.0	6.8	6.0	4.7	2.2	2.2	2.2	1.5	2.2	1.7	1.9	2.4	1.0	1.5	1.7	1.7	2.7	1.9	1.9	1.9	2.5						
khorasanensis AY954013 Iran N Khorasan Kopetdagh Mts	6.8	5.2	5.9	5.2	6.0	6.8	7.6	6.0	6.0	2.9	2.5	2.4	2.0	2.7	2.4	2.2	2.7	1.7	1.2	1.9	1.9	2.9	2.7	2.2	2.2	3.1	2.2					
nephohiptamenos AY556859 Greece Mt Orvilos	6.0	3.6	5.1	4.6	4.9	5.7	6.5	5.7	4.6	2.0	2.0	2.0	0.5	1.8	1.0	0.7	1.3	0.5	0.2	0.5	0.7	1.5	2.3	0.7	1.3	1.6	1.2	1.5				
iphigenia AY556984 Turkey Fethiye Salur Dagi	4.6	3.3	3.8	3.5	3.8	5.1	6.1	5.6	5.8	4.8	4.8	4.8	4.3	4.8	4.0	4.5	4.5	3.5	4.6	4.3	4.3	4.8	5.1	5.1	4.3	5.3	4.5	4.3	4.1			
violetae subbaeticus HM901760 Spain Andalusia Granada	5.8	3.7	5.9	4.7	5.0	6.8	8.4	5.7	5.7	7.0	7.1	7.0	5.5	5.5	6.0	6.0	6.0	5.7	5.6	5.7	5.5	6.2	6.8	4.9	4.9	5.4	5.7	5.5	5.4	4.3		
violetae violetae GU677017 Spain Andalusia Granada	5.0	3.4	5.1	4.4	4.2	6.0	7.6	6.0	5.4	6.2	6.3	6.2	5.3	5.2	5.2	5.7	5.7	4.9	5.3	5.5	5.2	6.0	6.2	5.2	4.7	5.1	4.9	5.2	5.1	3.5	0.7	
aroaniensis AY556856 Greece Mt Chelmos	5.9	4.0	5.7	5.1	4.8	6.7	8.3	7.2	6.4	5.8	5.8	5.8	5.4	6.4	5.3	5.8	5.8	5.0	5.3	5.6	5.3	6.1	6.4	5.3	4.8	5.0	5.0	5.3	5.1	4.0	2.2	1.5
lurae ON715904 Albania Diber Lure	5.5	4.9	5.1	3.9	4.9	6.5	7.8	6.2	6.7	6.2	6.3	6.2	5.5	5.7	5.7	5.7	5.7	5.4	5.3	5.4	5.2	6.0	6.5	5.2	5.2	5.7	5.7	5.2	5.1	3.5	2.7	2.4
timfristos KY066725 Greece Karpenisis Timfistos Mt	5.7	5.0	6.2	4.9	5.7	7.0	8.1	6.0	5.7	6.2	6.3	6.2	4.7	5.2	5.2	5.2	5.2	4.9	4.8	4.9	4.7	5.4	6.2	5.2	4.7	4.9	4.7	4.7	4.6	4.0	2.2	1.9
humedasae MW502349 Italy Aosta Pont d Ael	6.0	5.2	6.5	5.2	6.0	7.0	8.4	6.2	5.4	6.0	6.0	6.0	4.5	4.9	4.9	4.9	5.4	4.7	4.5	4.7	4.4	5.7	6.0	4.9	4.9	5.4	4.4	4.4	4.3	4.3	2.4	2.2
orphicus eleniae KY050599 Greece Granitis	6.0	5.0	6.2	4.9	5.7	6.8	8.7	7.0	5.7	5.7	5.7	5.7	4.7	5.2	5.2	5.2	5.7	4.9	4.8	4.9	4.7	6.0	6.2	4.7	5.2	5.4	4.7	5.2	4.6	5.1	2.7	2.4
orphicus orphicus MW501023 Turkey Van atak	5.7	4.4	5.7	4.4	5.2	6.5	8.1	7.0	5.4	5.7	5.7	5.7	4.2	5.2	4.7	4.7	5.2	4.4	4.3	4.4	4.2	5.4	5.7	4.2	4.7	4.9	4.4	4.7	4.1	4.5	2.7	2.4
fabressei EF104605 Spain Castello	5.7	4.9	6.2	4.9	5.7	6.8	8.4	6.2	5.9	7.0	7.1	7.0	5.5	5.4	6.0	6.0	6.2	5.7	5.6	5.7	5.4	6.5	7.0	5.4	5.7	5.9	5.4	5.4	5.4	4.5	2.7	2.4
fulgens fulgens AY496746 Spain Santa Colomade de Queralt	5.5	4.9	6.2	4.9	5.7	6.5	8.4	6.0	5.7	7.0	7.1	7.0	5.5	5.4	6.0	5.9	6.2	5.7	5.6	5.7	5.4	6.5	7.0	5.4	5.7	5.9	5.4	5.4	5.4	4.5	2.7	2.4
fulgens leonensis GU676235 Spain Castilla y Leon	5.7	4.9	6.2	4.9	5.7	6.5	8.4	6.2	5.9	7.0	7.1	7.0	5.5	5.4	6.0	6.0	6.2	5.7	5.6	5.7	5.4	6.5	7.0	5.4	5.7	5.9	5.4	5.4	5.4	4.5	2.7	2.4
fulgens pseudovirgilius AY556963 Spain Tarragona Sta Coloma De Queralt	5.6	5.1	6.2	5.0	5.8	6.4	8.6	6.1	5.8	7.2	7.2	7.2	5.6	5.6	6.1	6.1	6.4	5.8	5.6	5.8	5.6	6.6	7.2	5.6	5.8	6.1	5.6	5.6	5.4	4.5	2.7	2.5
ainsae AY496712 Spain Lleida	5.5	4.9	6.2	4.9	5.7	6.5	8.4	6.0	5.7	7.0	7.1	7.0	5.5	5.4	6.0	5.9	6.2	5.7	5.6	5.7	5.4	6.5	7.0	5.4	5.7	5.9	5.4	5.4	5.4	4.5	2.7	2.4
dolus dolus MN138992 Italy Liguria	5.7	4.7	5.9	4.7	5.5	6.5	8.1	6.5	5.9	6.8	6.8	6.8	5.3	5.7	5.7	5.7	6.0	5.4	5.3	5.4	5.2	6.2	6.7	5.2	5.4	5.7	5.4	5.2	5.1	4.3	2.9	2.7
dolus gargano MW503058 Italy Calabria	5.7	4.9	6.2	4.9	5.7	6.5	8.4	6.2	5.9	7.0	7.1	7.0	5.5	5.4	6.0	6.0	6.2	5.7	5.6	5.7	5.4	6.5	7.0	5.4	5.7	5.9	5.4	5.4	5.4	4.5	2.7	2.4
dolus paravirgilius MN139580 Italy Salerno	5.7	4.7	5.9	4.7	5.5	6.5	8.1	6.5	5.9	6.8	6.8	6.8	5.3	5.7	5.7	5.7	6.0	5.4	5.3	5.4	5.2	6.2	6.7	5.2	5.4	5.7	5.4	5.2	5.1	4.3	2.9	2.7
dolus virgilius MN139341 Italy Abruzzi L Aquila	5.7	4.7	5.9	4.7	5.5	6.5	8.1	6.5	5.9	6.8	6.8	6.8	5.3	5.7	5.7	5.7	6.0	5.4	5.3	5.4	5.2	6.2	6.7	5.2	5.4	5.7	5.4	5.2	5.1	4.3	2.9	2.7
dolus vittatus MN141207 France Occitanie Languedoc-Roussillon	5.7	4.9	6.2	4.9	5.7	6.8	7.8	6.2	5.7	6.5	6.5	6.5	5.0	5.4	5.4	5.4	5.7	5.2	5.1	5.2	4.9	6.0	6.5	5.4	5.2	5.4	5.2	4.9	4.9	4.0	3.2	2.9
menalcas AY496763 Turkey Gumushane	5.2	4.4	6.2	5.5	4.7	6.3	7.0	6.2	5.4	6.2	6.3	6.2	4.7	5.4	5.2	5.2	5.7	4.9	5.3	4.9	4.7	6.0	6.2	5.7	4.7	5.1	4.4	5.7	5.1	4.5	2.9	2.2
interjectus AY557059 Turkey Erzurum	5.7	3.5	5.1	5.1	4.8	6.5	7.4	6.5	5.7	6.5	6.5	6.5	5.5	5.4	5.4	5.9	6.0	4.8	5.4	5.7	5.4	6.2	6.5	5.4	4.5	5.0	5.1	6.0	5.1	3.7	2.9	2.1
alcestis AY557008 Turkey Adana	5.4	3.5	4.6	4.9	4.9	6.2	7.3	5.9	5.4	6.2	6.2	6.2	5.2	5.1	5.1	5.6	5.6	4.6	5.1	5.4	5.1	5.9	6.2	5.1	4.6	5.1	4.6	5.6	4.9	3.5	2.5	1.7
eriwanensis AY496742 Armenia Aiodzor mts	5.7	3.6	4.8	4.9	4.4	6.8	7.7	6.5	6.0	6.0	6.3	6.3	5.4	5.7	5.2	5.7	5.7	4.9	5.3	5.4	5.2	5.7	6.3	5.2	4.1	4.6	4.9	5.7	5.1	3.9	2.3	1.5
dantchenkoi AY496737 Turkey Van	6.0	3.7	5.1	5.2	4.9	7.0	7.8	6.7	5.9	6.5	6.5	6.5	5.5	5.4	5.4	6.0	6.0	5.2	5.6	5.7	5.4	6.2	6.5	5.4	4.4	4.9	5.2	6.0	5.4	4.3	2.4	1.7
karacetinae AY557090 Turkey Hakkari	6.7	4.0	5.4	5.1	5.3	7.2	7.7	6.6	6.4	6.9	6.9	6.9	5.4	5.3	5.8	5.8	5.8	5.6	5.3	5.6	5.3	6.1	6.9	5.3	4.2	4.7	5.6	5.8	5.1	4.5	2.7	2.5
urmiaensis EF104631 Iran W Azarbayjan	6.8	4.4	5.9	5.5	6.3	7.6	8.1	6.0	6.2	7.0	7.1	7.0	5.5	4.7	5.7	6.0	6.0	5.7	5.6	5.7	5.4	6.2	7.0	5.4	4.9	5.4	5.4	6.0	5.4	5.1	3.2	2.9
rjabovianus masul AY954006 Iran Gilan Masuleh	5.5	4.4	5.7	5.5	5.2	5.2	5.4	5.2	6.0	6.0	6.0	6.0	4.5	5.2	4.7	4.9	4.9	4.7	5.1	4.7	4.4	5.2	6.0	5.4	4.4	4.9	4.7	5.4	4.9	4.5	4.2	3.4
rjabovianus rjabovianus AY954019 Azerbaijan Talysh Zuvand	5.5	4.7	5.9	5.7	5.5	5.7	6.2	5.4	6.0	6.8	6.8	6.8	5.3	5.4	5.5	5.7	5.7	5.4	5.9	5.4	5.2	6.0	6.8	6.2	5.2	5.7	5.2	6.2	5.7	4.8	3.9	3.2
asadii JFC23003 Iran Mazandaran S Dalasm	5.7	5.2	5.9	5.2	6.0	6.8	7.0	5.4	5.7	7.0	7.1	7.0	5.5	4.9	6.0	6.0	6.0	5.7	5.6	5.7	5.5	6.2	6.5	6.0	4.9	4.9	5.4	6.0	5.4	4.8	3.4	3.2
asadii Naderi2023-008 Iran Mazandaran Dalasm	5.7	5.2	5.9	5.2	6.0	6.8	7.0	5.4	5.7	7.0	7.1	7.0	5.5	4.9	6.0	6.0	6.0	5.7	5.6	5.7	5.5	6.2	6.5	6.0	4.9	4.9	5.4	6.0	5.4	4.8	3.4	3.2
valiabadi ARLY-232f-001 Iran Mazandaran S Ramsar Tomol	5.9	4.2	5.6	5.0	5.3	6.1	7.6	5.8	5.3	7.0	7.0	7.0	5.1	4.7	5.6	5.6	6.1	5.3	5.3	5.3	5.0	6.4	6.7	5.3	5.9	5.8	5.3	5.9	5.0	4.5	2.9	2.9
valiabadi AY556882 Iran Mazandaran Pul-e Zanguleh	6.2	5.1	5.9	5.4	5.9	6.5	7.6	6.2	5.9	7.3	7.3	7.3	5.7	5.1	6.2	6.2	6.7	5.6	5.6	5.9	5.6	7.0	6.7	5.6	5.6	5.6	5.6	6.2	5.4	5.1	3.6	3.3
valiabadi AY556934 Iran S Valiabad	6.2	5.3	5.9	5.3	6.1	6.4	7.7	6.1	5.8	7.2	7.2	7.2	5.7	5.0	6.1	6.1	6.6	5.8	5.6	5.8	5.6	6.9	6.6	5.6	5.6	5.5	5.6	6.1	5.4	5.3	3.5	3.3
valiabadi KR265486 Iran Mazandaran	6.1	5.3	5.9	5.2	6.0	6.3	7.6	6.0	5.7	7.1	7.1	7.1	5.6	5.0	6.0	6.0	6.5	5.7	5.6	5.7	5.5	6.8	6.5	5.5	5.5	5.4	5.5	6.0	5.4	5.3	3.5	3.2
valiabadi KR265495 Iran Mazandaran	6.1	5.3	5.9	5.2	6.0	6.3	7.6	6.0	5.7	7.1	7.1	7.1	5.6	5.0	6.0	6.0	6.5	5.7	5.6	5.7	5.5	6.8	6.5	5.5	5.5	5.4	5.5	6.0	5.4	5.3	3.5	3.2

Taxon	aroaniensis AY556856 Greece Mt Chelmos	lurae ON715904 Albania Diber Lure	timfristos KY066725 Greece Karpenisis Timfistos Mt	humedasae MW502349 Italy Aosta Pont d Ael	orphicus eleniae KY050599 Greece Granitis	orphicus orphicus MW501023 Turkey Van atak	fabressei EF104605 Spain Castello	fulgens fulgens AY496746 Spain Santa Colomade de Queralt	fulgens leonensis GU676235 Spain Castilla y Leon	fulgens pseudovirgilius AY556963 Spain Tarragona Sta Coloma De Queralt	ainsae AY496712 Spain Lleida	dolus dolus MN138992 Italy Liguria	dolus gargano MW503058 Italy Calabria	dolus paravirgilius MN139580 Italy Salerno	dolus virgilius MN139341 Italy Abruzzi L Aquila	dolus vittatus MN141207 France Occitanie Languedoc-Roussillon	menalcas AY496763 Turkey Gumushane	interjectus AY557059 Turkey Erzurum	alcestis AY557008 Turkey Adana	eriwanensis AY496742 Armenia Aiodzor mts	dantchenkoi AY496737 Turkey Van	karacetinae AY557090 Turkey Hakkari	urmiaensis EF104631 Iran W Azarbayjan	rjabovianus masul AY954006 Iran Gilan Masuleh	rjabovianus rjabovianus AY954019 Azerbaijan Talysh Zuvand	asadii JFC23003 Iran Mazandaran S Dalasm	asadii Naderi2023-008 Iran Mazandaran Dalasm	valiabadi ARLY-232f-001 Iran Mazandaran S Ramsar Tomol	valiabadi AY556882 Iran Mazandaran Pul-e Zanguleh	valiabadi AY556934 Iran S Valiabad	valiabadi KR265486 Iran Mazandaran
lurae ON715904 Albania Diber Lure	2.0																														
timfristos KY066725 Greece Karpenisis Timfistos Mt	1.5	1.9																													
humedasae MW502349 Italy Aosta Pont d Ael	2.2	2.7	0.7																												
orphicus eleniae KY050599 Greece Granitis	2.0	2.4	1.0	1.2																											
orphicus orphicus MW501023 Turkey Van atak	1.5	1.9	1.0	1.2	0.5																										
fabressei EF104605 Spain Castello	3.0	2.9	2.4	2.4	2.7	2.7																									
fulgens fulgens AY496746 Spain Santa Colomade de Queralt	3.0	2.9	2.4	2.4	2.7	2.7	0.5																								
fulgens leonensis GU676235 Spain Castilla y Leon	3.0	2.9	2.4	2.4	2.7	2.7	0.2	0.2																							
fulgens pseudovirgilius AY556963 Spain Tarragona Sta Coloma De Queralt	3.0	3.0	2.5	2.5	2.7	2.7	0.5	0.0	0.2																						
ainsae AY496712 Spain Lleida	3.0	2.9	2.4	2.4	2.7	2.7	0.5	0.0	0.2	0.0																					
dolus dolus MN138992 Italy Liguria	2.7	2.7	2.7	2.7	2.9	2.4	0.5	0.5	0.2	0.5	0.5																				
dolus gargano MW503058 Italy Calabria	3.0	2.9	2.4	2.4	2.7	2.7	0.2	0.2	0.0	0.2	0.2	0.2																			
dolus paravirgilius MN139580 Italy Salerno	2.7	2.7	2.7	2.7	2.9	2.4	0.5	0.5	0.2	0.5	0.5	0.0	0.2																		
dolus virgilius MN139341 Italy Abruzzi L Aquila	2.7	2.7	2.7	2.7	2.9	2.4	0.5	0.5	0.2	0.5	0.5	0.0	0.2	0.0																	
dolus vittatus MN141207 France Occitanie Languedoc-Roussillon	3.0	2.9	2.4	2.4	3.2	2.7	0.7	0.7	0.5	0.7	0.7	0.2	0.5	0.2	0.2																
menalcas AY496763 Turkey Gumushane	2.5	3.4	2.7	2.4	2.7	2.4	3.4	3.4	3.4	3.5	3.4	3.4	3.4	3.4	3.4	3.2															
interjectus AY557059 Turkey Erzurum	2.1	2.9	2.9	3.2	3.5	2.9	2.6	2.6	2.6	2.6	2.6	2.4	2.6	2.4	2.4	2.6	2.6														
alcestis AY557008 Turkey Adana	2.3	2.8	2.3	2.5	2.8	2.8	2.3	2.3	2.3	2.3	2.3	2.5	2.3	2.5	2.5	2.8	2.5	1.0													
eriwanensis AY496742 Armenia Aiodzor mts	1.3	2.0	2.3	2.8	2.8	2.3	2.8	2.8	2.8	2.9	2.8	2.5	2.8	2.5	2.5	2.8	2.0	1.1	0.8												
dantchenkoi AY496737 Turkey Van	1.7	2.7	2.7	2.9	3.2	2.7	3.2	3.2	3.2	3.2	3.2	2.9	3.2	2.9	2.9	3.2	2.2	0.8	1.0	0.2											
karacetinae AY557090 Turkey Hakkari	2.5	3.0	3.0	3.3	3.5	3.0	3.5	3.5	3.5	3.5	3.5	3.2	3.5	3.2	3.2	3.5	3.0	1.6	1.8	1.0	0.7										
urmiaensis EF104631 Iran W Azarbayjan	3.5	3.4	2.9	3.2	3.4	3.4	3.4	3.4	3.4	3.5	3.4	3.7	3.4	3.7	3.7	3.9	3.7	2.4	1.8	2.0	1.7	1.0									
rjabovianus masul AY954006 Iran Gilan Masuleh	3.5	3.9	3.4	3.7	4.0	3.4	3.9	3.9	3.9	4.0	3.9	3.7	3.9	3.7	3.7	3.4	2.4	2.9	2.8	3.1	3.2	4.0	3.9								
rjabovianus rjabovianus AY954019 Azerbaijan Talysh Zuvand	3.8	4.2	3.2	3.4	3.7	3.7	3.7	3.7	3.7	3.8	3.7	3.9	3.7	3.9	3.9	3.7	2.4	3.2	2.5	3.3	3.4	4.3	3.7	0.7							
asadii JFC23003 Iran Mazandaran S Dalasm	3.8	3.7	2.2	2.4	2.7	2.7	2.7	2.9	2.7	3.0	2.9	2.9	2.7	2.9	2.9	2.7	2.9	2.9	2.5	3.3	3.4	3.8	3.7	2.7	2.4						
asadii Naderi2023-008 Iran Mazandaran Dalasm	3.8	3.7	2.2	2.4	2.7	2.7	2.7	2.9	2.7	3.0	2.9	2.9	2.7	2.9	2.9	2.7	2.9	2.9	2.5	3.3	3.4	3.8	3.7	2.7	2.4	0.0					
valiabadi ARLY-232f-001 Iran Mazandaran S Ramsar Tomol	3.4	3.1	2.3	2.1	1.8	1.8	2.1	2.3	2.1	2.3	2.3	2.3	2.1	2.3	2.3	2.6	2.9	3.0	2.6	3.6	3.7	4.0	4.0	3.4	3.2	1.0	1.0				
valiabadi AY556882 Iran Mazandaran Pul-e Zanguleh	3.8	3.8	2.8	2.5	2.3	2.3	2.5	2.8	2.5	2.8	2.8	2.8	2.5	2.8	2.8	3.0	3.0	2.9	2.5	3.5	3.6	3.8	3.8	3.3	3.0	0.5	0.5	0.5			
valiabadi AY556934 Iran S Valiabad	3.8	3.8	2.7	2.5	2.2	2.2	2.5	2.7	2.5	2.7	2.7	2.7	2.5	2.7	2.7	3.0	3.0	2.9	2.5	3.4	3.5	3.8	3.8	3.3	3.0	0.5	0.5	0.5	0.0		
valiabadi KR265486 Iran Mazandaran	3.8	3.7	2.7	2.5	2.2	2.2	2.4	2.7	2.4	2.7	2.7	2.7	2.4	2.7	2.7	2.9	2.9	2.9	2.5	3.4	3.5	3.8	3.7	3.2	3.0	0.5	0.5	0.5	0.0	0.0	
valiabadi KR265495 Iran Mazandaran	3.8	3.7	2.7	2.5	2.2	2.2	2.4	2.7	2.4	2.7	2.7	2.7	2.4	2.7	2.7	2.9	2.9	2.9	2.5	3.4	3.5	3.8	3.7	3.2	3.0	0.5	0.5	0.5	0.0	0.0	0.0

0.0	1.0
1.1	2.0
2.1	3.0
3.1	4.0
4.1	5.0
5.1	6.0
6.1	7.0
7.1	8.0
8.1	9.0

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; Amirahamadi 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 & Akhani, 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.

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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

This study only included plants and arthropod material, and all required ethical guidelines for the treatment and use of animals were strictly adhered to in accordance with international, national, and institutional regulations. No human participants were involved in any studies conducted by the authors for this article.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTERESTS

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

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

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چکیـــده: 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. گونهٔ *P. asadii* را می‌توان بر اساس الگوی متفاوت بال و اندام تناسلی نر از گونهٔ *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.

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