NAT. CROAT. | VOL. 25 | No 1 | 119–129 | ZAGREB | June 30, 2016

original scientific paper / izvorni znanstveni rad
DOI: 10.20302/NC.2016.25.8

MOLECULAR DATA DO NOT CONFIRM THE GRECIAN ANOMALOUS BLUE *POLYOMMATUS (AGRODIAETUS) AROANIENSIS* (BROWN, 1976) AS A MEMBER OF THE CROATIAN FAUNA

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Lovrenčić, L., Podnar, M., Šašić, M., Koren, T. & Tvrtković, N.: Molecular data do not confirm the Grecian anomalous blue *Polyommatus* (*Agrodiaetus*) *aroaniensis* (Brown, 1976) as a member of the Croatian fauna. Nat. Croat., Vol. 25, No. 1, 119–129, 2016, Zagreb.

The presence of the Grecian anomalous blue, *Polyommatus (Agrodiaetus) aroaniensis* (Brown, 1976) in Croatia has been recently published based on external morphology of collected specimens. The aim of this study was to evaluate the credibility of these findings by using DNA barcoding approach as the species exhibit phenotypic variability and can easily be misidentified with morphologically similar species *Polyommatus (Agrodiaetus) ripartii*. Therefore, we performed a molecular analysis of the mitochondrial gene for cytochrome c oxidase subunit I (*COI*) of the samples previously published as *P. aroaniensis* from different localities in Croatia. The results showed that all analysed samples belong to *P. ripartii*. Additionally, all analysed samples from different Croatian localities (Mt. Mala Kapela, Mt. Lička Plješivica, Mt. Poštak, Mt. Troglav and Mt. Kamešnica) together with previously published population of *P. ripartii* from Mt. Mosor belong to the same Eurasian clade of *P. ripartii*.

Key words: *Polyommatus (Agrodiaetus) ripartii*, misidentification, Lycaenidae, Lepidoptera, *COI*, DNA barcoding

Lovrenčić, L., Podnar, M., Šašić, M., Koren, T. & Tvrtković, N.: Molekularni podaci nisu potvrdili grčkog plavca *Polyommatus* (*Agrodiaetus*) *aroaniensis* (Brown, 1976) kao pripadnika faune Hrvatske. Nat. Croat., Vol. 25, No. 1, 119–129, 2016, Zagreb.

Prisutnost vrste *Polyommatus (Agrodiaetus) aroaniensis* (Brown, 1976), leptira iz porodice plavaca, nedavno je zabilježena u Hrvatskoj na temelju vanjske morfologije prikupljenih jedinki. Cilj ovog rada bio je procijeniti vjerodostojnost tog nalaza korištenjem metode DNA barkodiranja budući da se radi o fenotipski varijabilnoj vrsti koja se lako može zamijeniti s morfološki sličnom vrstom *Polyommatus (Agrodiaetus) ripartii*. Provedena je molekularna analiza mitohondrijskog gena za citokrom c oksidazu podjedinica I (*COI*) na uzorcima objavljenih kao vrsta *P. aroaniensis* s različitih lokaliteta u Hrvatskoj. Rezultati su pokazali da svi analizirani uzorci pripadaju vrsti *P. ripartii*. Također svi uzorci s različitih lokaliteta (Mala Kapela, Lička Plješivica, Poštak, Troglav i Kamešnica) zajedno s ranije objavljenom populacijom *P. ripartii* s Mosora pripadaju istoj euroazijskoj grupi vrste *P. ripartii*.

Ključne riječi: *Polyommatus (Agrodiaetus) ripartii*, pogrešna identifikacija, Lycaenidae, Lepidoptera, *COI*, DNA barkodiranje

INTRODUCTION

The genus *Polyommatus* Latreille, 1804 is a large and diverse group of blue butterflies in the family Lycaenidae (Lepidoptera). *Agrodiaetus* Hübner, 1822 is one of its subgenera

distributed throughout the Palearctic region. The majority of the *Polyommatus* species display sexual dimorphism regarding wing colour, with the exception of „brown" type of *Agrodiaetus* taxa which usually have uniform brown wing upperside in both sexes. Species identification within this group is often difficult based solely on wing morphology, because they are prone to considerable geographic and individual variability in wing pattern (Kolev & De Prins, 1995; Kandul, 1997, 2004, 2007; Vila *et al*., 2010; Dincă *et al*., 2013). However, these species show extreme karyotypic diversity, ranging from n=10 to n=134 (de Lesse, 1960; Lukhtanov & Dantchenko, 2002; Lukhtanov *et al*., 2005; Kandul *et al*., 2007), so reliable taxonomic status is usually determined through chromosome number and/or DNA sequencing (Wiemers, 2003; Kandul *et al*., 2004; Lukhtanov *et al*., 2006; Vila *et al*., 2010; Dincă *et al*., 2013).

In the last century only two species belonging to the subgenus *Agrodiaetus* have been recorded in Croatia: *Polyommatus (Agrodiaetus) damon* (Denis & Schiffermüller, 1775) and *Polyommatus (Agrodiaetus) admetus* (Esper, 1783) (Lorković, 2009). *Polyommatus (Agrodiaetus) ripartii* (Freyer, 1830) was added to the List of Croatian butterfly fauna (Šašić & Mihoci, 2011) after Koren (2010) and Koren *et al.* (2011). Specimens of *P. ripartii* were recorded already in 1997 on Mt. Mosor (Croatia) by Marten Runquist and subsequently analysed with molecular methods which recovered this population within the Euroasian clade of the species (Dincă *et al*., 2013). After discovery of *Polyommatus (Agrodiaetus) aroaniensis* (Brown, 1976) south of the town of Gacko in neighbouring Bosnia and Herzegovina (Verovnik *et al*., 2015), the presence of this species in Croatia was recently reported by Koren & Lauš (2015), but this observation was based exclusively on external morphology of collected specimens (according to Tolman & Lewington, 2008). It is considered that *P. aroaniensis* can be distinguished from *P. ripartii* by the ground colour of the hindwing underside which is 'medium coffee brown' with reddish hue and without darker marking along the margin and the commonly lacking or indistinct white stripe on the underside of its hindwing (Kolev, 1994; Kolev & van der Poorten, 1997). With regard to the white stripe, the popular guide by Tolman & Lewington (2008) incorrectly claims that the stripe in question is always absent in *P. aroaniensis*. However, in terms of wing pattern, *P. aroaniensis* and *P. ripartii* specimens display considerable phenotypic variability regarding the presence, absence or intensity of the white stripe on the hindwings (Kolev, 1994; Kolev & de Prins, 1995; Kolev & van der Poorten, 1997; Dincă *et al*., 2013; Verovnik *et al*., 2015). This variability is pronounced even within the same population of *P. aroaniensis*: in the Greek population, from *locus typicus* of this species (Greece: Mt. Chelmos), the white stripe is present in about 50–60 % of all specimens (Kolev & van der Poorten, 1997). Additionally, the Croatian population of *P. ripartii* from Mt. Mosor includes specimens with and without the white stripe on the hindwing underside (Dincă *et al*., 2013). Male genitalia are a useful diagnostic character for distinguishing *P. ripartii* and *P. aroaniensis*: the Croatian populations of *P. ripartii* should have shorter valvae than *P. aroaniensis* and *P. admetus* (Kolev, 1994; Kolev & de Prins, 1995; Kolev & van der Poorten, 1997; Dincă *et al*., 2013), as well as the number of chromosomes: *P. ripartii* has a chromosome number of 2n = 90 (de Lesse, 1960; Wiemers, 2003) and *P. aroaniensis* 2n = 48 (Coutsis *et al*., 1999).

Available evidence from karyological, molecular and biogeographic studies shows that *P. ripartii* and *P. aroaniensis* belong not to one, as previously believed, but to two species groups which appear as sister clades in all published phylogenetic reconstructions: *P. ripartii* belongs to the so-called *admetus* group and *P. aroaniensis* to the *dolus* group (Wiemers, 2003; Kandul *et al*., 2004, 2007; Lukhtanov *et al*., 2005; Vila *et al*., 2010).

The main goal of the present paper is to evaluate the credibility of the published findings of *P. aroaniensis* by additional analyses of the available voucher specimens (Koren & Lauš, 2015), as well as to confirm the identification of *P. ripartii* in Lika region including the source of Zrmanja river (Koren, 2010; Koren *et al*., 2011; Tvrtković *et al*., 2015; Koren *et al*., 2015; Koren & Lauš, 2015) and on the Dinara Massif (Mt. Dinara, Mt. Troglav, Mt. Kamešnica) (Koren & Lauš, 2013). Due to variability of external morphology and therefore limited reliability of the identification of these two 'brown' *Agrodiaetus* taxa, specimens were re-identified using the DNA barcoding approach. This molecular method uses a short, standardized region of the mitochondrial gene for cytochrome c oxidase subunit I (*COI*) for accurate species identification and it accelerates delimitation of morphologically similar species while often revealing cryptic diversity (Hebert *et al*., 2003).

MATERIAL AND METHODS

Material

Analyzed specimens belonging to the subgenus *Agrodiaetus* were collected in the period from 2011 to 2015 in several localities in Croatia (Tab.1, Fig.1) as described in Koren & Lauš (2013, 2015), Koren *et al*. (2015), and Tvrtković *et al*. (2015), with two additional specimens from Gacko in the south-eastern part of Bosnia and Herzegovina. Taxonomic identification by wing morphology was done according to Lafranchis (2004) and Tolman & Lewington (2008) for *P. admetus* and *P. damon* samples and in accordance

Fig. 1. Map of the surveyed Croatian locations of specimens belonging to the subgenus *Agrodiaetus*. 1 – Mt. Mala Kapela (Svračkovo Selo); 2 – Mt. Lička Plješivica (Kozja Draga); 3 – Mt. Lička Plješivica (Lisac); 4 – Mt. Poštak; 5 – Mt. Dinara (Kučeljak); 6 – Mt. Troglav (Ravno Vrdovo); 7 – Mt. Kamešnica (Rože); 8 – Mt. Kamešnica (Ćalete), 9 – Mt. Kamešnica (Arkačica). 10 – Locality from Mt. Mosor (Dincă *et al*., 2013).

Tab. 1. List of specimens analysed in the present study. Location numbers correspond to the numbers in Fig. 1. Croatian specimens identified as *P. aroaniensis* by Koren & Lauš (2015) are indicated by quotation marks in the species column.

	Locality	Species	White stripe	Sex	Publication //leg. /date	Sample code
1	Mt. Mala Kapela: Svračkovo Selo	*damon*	yes	f	Tvrtković *et al*., 2015	LDAMK1
1	Mt. Mala Kapela: Svračkovo Selo	*„aroaniensis"*	no	f	Koren & Lauš, 2015	LARMK1
2	Mt. Lička Plješivica: Kozja Draga	*ripartii*	yes	f	Koren & Lauš, 2015	LARLP1
2	Mt. Lička Plješivica: Kozja Draga	*„aroaniensis"*	no	m	Koren & Lauš, 2015	LARLP3
2	Mt. Lička Plješivica: Kozja Draga	*„aroaniensis"*	no	f	Koren & Lauš, 2015	LARLP4
3	Mt. Lička Plješivica: Lisac	*ripartii*	yes	f	Tvrtković *et al*., 2015	LRLIS1
3	Mt. Lička Plješivica: Lisac	*ripartii*	no	f	Tvrtković *et al*., 2015	LARLIS1
4	Mt. Poštak	*ripartii*	no	f	Koren *et al*., 2015	LARPO1
5	Mt. Dinara: Kučeljak	*admetus*	no	m	N. Tvrtković, 7.07.2011	LADDIN3
6	Mt. Troglav: Ravno Vrdovo	*ripartii*	yes	f	N. Tvrtković, 1.08.2014	LRTRO1
7	Mt. Kamešnica: Rože	*ripartii*	no	m	N. Tvrtković, 1.08.2014	LARKAM2
8	Mt. Kamešnica: Arkačica (Ćalete)	*ripartii*	yes	f	Koren & Lauš, 2015	LARKAM3
9	Mt. Kamešnica: Arkačica	*ripartii*	yes	f	N. Tvrtković, 1.08.2014	LRKAM2
	B&H: Gacko: Cernica Village	*aroaniensis*	no	m	M. Šašić, 19.07.2013	LARGA1
	B&H: Gacko: Cernica Village	*ripartii*	yes	f	T. Koren, 19.07.2013	LARGA2

with Kolev (1994), Kolev & van der Poorten (1997) and Dincă *et al*. (2013) in the case of *P. ripartii* and *P. aroaniensis*. Examination of genitalia was performed on three males among the specimens sequenced. The valva length was measured as in Dincă *et al*. (2013: Appendix, Figure S1). Male genitalia were prepared according to the protocol described in Higgins (1975). For molecular analysis we examined nine specimens of *P. ripartii* with or without a white stripe on the hindwing underside, four specimens published to be *P. aroaniensis* and one specimen of each *P. admetus* and *P. damon* (Tab. 1). Vouchers of all specimens (Tab. 1) are deposited either in the Central collection of Lepidoptera in the Croatian Natural History Museum in Zagreb or in T. Koren's private collection.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from ethanol-preserved legs (one or two) using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich) according to the manufacturer's specifications and eluted in 60 μl of elution buffer. A standard 658-bp barcode region of the mitochondrial gene *COI* was amplified by polymerase chain reaction (PCR) using the primers LepF1 (5'ATTCAACCAATCATAAAGATATTGG3') and LepR1 (5'TAAACTTCTGGA TGTCCAAAAAATCA3') (Hebert *et al*., 2004). DNA was amplified in 50 μl reactions containing PCR buffer with 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 μM of each primer, 1.25 U of Taq polymerase (Promega) and 5 μl of eluted DNA. The following

PCR thermocycling conditions were applied: initial denaturation at 95 °C for 120 s, followed by 35 cycles of 30 s at 95 °C denaturation, 30 s at 50 °C annealing, 90 s at 72 °C, and a final extension step of 7 min at 72 °C. The PCR products were purified and sequenced using the same primers by Macrogen Inc. (Amsterdam, Netherlands). Sequences were edited and aligned using ClustalW as implemented in BioEdit 7.2.4. (Hall, 1999).

Phylogenetic analysis

Data set for phylogenetic analysis involved 97 *COI* sequences of different taxa of the subgenus *Agrodiaetus* (*P. ripartii*, *P. aroaniensis*, *P. nephohiptamenos*, *P. admetus*, *P. humedasae*, *P. dantchenkoi*, *P. damon*) available in GenBank mainly from the studies of Wiemers (2003), Vila *et al.* (2010) and Dincă *et al.* (2013) together with our 15 newly sequenced samples which were submitted to GenBank (Tab. 2). The final alignment for *COI* was 623 bp long and contained 112 sequences representing seven taxa, including *Polyommatus icarus* as outgroup. Sequences were collapsed to unique haplotypes using FaBox v.1.41 (Villesen, 2007) and phylogenetic relationships were inferred using Maximum likelihood (ML) as implemented in MEGA6 (Tamura *et al*., 2013). MEGA6 was used to select optimal model of sequence evolution (T92+G+I) according to Bayesian Information Criterion (BIC) and to calculate genetic distances with Kimura 2-parameter model of base substitution.

RESULTS AND DISCUSSION

Phylogenetic analyses of specimens belonging to the subgenus *Agrodiaetus* based on mitochondrial DNA resulted in the ML tree characterised with three well supported main clades: A, B and C (Fig. 2). Clade A comprises specimens of *P. ripartii, P. nephohiptamenos*, *P. admetus* and the Croatian samples previously identified as *P. aroaniensis* by Koren & Lauš (2015). Clade B encompasses specimens of *P. aroaniensis* (from Greece and Bosnia and Herzegovina), *P. humedasae* and *P. dantchenkoi*. Clade C contains *P. damon* specimens. As in the previous study of Dincă *et al*. (2013), three main mitochondrial lineages of *P. ripartii* in Europe (Eurasian, Balkan and West European) are comprised within the *ripartii* clade (clade A). *P. ripartii* is resolved as paraphyletic assemblage with respect to *P. admetus* and *P. nephohiptamenos*, but also with respect to the Croatian specimens previously identified as *P. aroaniensis*. While the identification of *P. admetus* based on external morphology and chromosome number is considered reliable and its species status is not questionable, the taxonomic status of *P. nephohiptamenos* from Bulgaria and Greece is yet to be properly assessed (Wiemers, 2003; Dincă *et al*., 2013). The herein obtained DNA sequences of *P. admetus* and *P. damon* clustered within their respective species clades and represent the first published DNA barcodes of Croatian specimens for those two species.

Based on DNA barcode sequence, the specimens of *P. aroaniensis* (from Greece and Bosnia and Herzegovina) and *P. ripartii* could be unambigously distinguished from each other and identified to a species level. All of the Croatian samples previously identified by Koren & Lauš (2015) as *P. aroaniensis* were recovered within the Euroasian clade of *P. ripartii* (clade A, Fig. 2), while the sample of *P. aroaniensis* from Gacko in Bosnia and Herzegovina clustered together with the sample of *P. aroaniensis* from Greece (clade B, Fig. 2). Furthermore, sequence divergences (Kimura two-parameter, K2P distances) between the samples from Croatia previously identified as *P. aroaniensis* (Koren & Lauš, 2015) and the rest of *P. ripartii* ranged from 0 to 2,49 % which is actually a usual range of intraspecific variability within *P. ripartii* (Dincă *et al*., 2013). The minimal interspecific genetic distances found between *P. ripartii* and *P. aroaniensis* specimens from Greece (Mt.

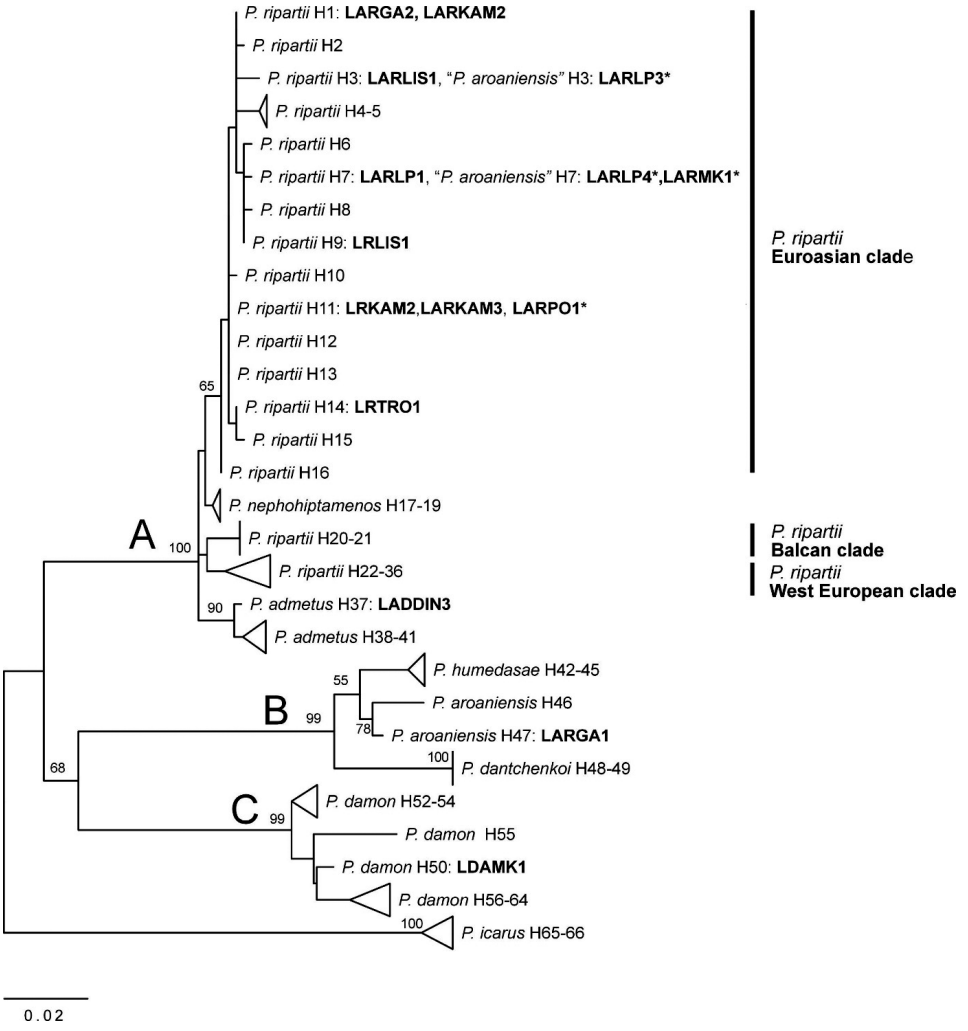

Fig. 2. Maximum likelihood (ML) tree of *Agrodiaetus* taxa based on the mitochondrial cytochrome c oxidase subunit I (*COI*) haplotypes (Tab. 2). Some clades were collapsed to traingles for clarity. ML bootstrap values higher than 50 are shown. Croatian specimens identified as *P. aroaniensis* based on morphological characters (Koren & Lauš, 2015) are indicated by „*".

Chelmos) and Bosnia and Herzegovina (Gacko) (6,24 – 7,25 %) were 2.5 times greater than the maximum intraspecific distances within *P. ripartii.* Therefore, the phylogenetic analysis implies that of all samples barcoded in this study, the sample from Gacko is the only one that actually belongs to *P. aroaniensis*. Based on the phylogenetic lines of evidence and sequence divergence values described above, we conclude that the specimens published as *P. aroaniensis* by Koren & Lauš (2015) were misidentified and actually belong to *P. ripartii*. As stated in the paper by Koren & Lauš (2015) the previous identification of specimens was based entirely on external morphology.

Fig. 3. Ventral view of the several of Croatian specimens of subgenus *Agrodiaetus* included in the present study. Scale bar is in twenty millimetres. 1 – Mt. Kamešnica (Arkačica), female; 2 – Mt. Kamešnica (Rože), male; 3,4 – Mt. Lička Plješivica (Lisac), females; 5 – Mt. Troglav (Ravno Vrdovo), female; 6 – Mt. Dinara (Kučeljak), male; 7 – Mt. Mala Kapela (Svračkovo Selo), female. 1-5 = *P. (A.) ripartii*; 6 = *P. (A.) admetus*; 7 = *P. (A.) damon*.

It is confirmed that Croatian sequenced specimens of *P. ripartii* exhibit phenotypic variability regarding wing pattern: even within a single population (Mt. Mosor: Dincă *et al*., 2013; Mt.Troglav, Mt. Kamešnica and Lika: the present paper) there are specimens with and without the white stripe on the hindwing underside, as well as with and without pale marginal markings, rarely with submarginal markings (Fig. 3). Variability of the hindwing underside markings of some sequenced specimens (Fig. 3) is probably larger than variability found on Mt. Mosor (Dincă *et al*., 2013: Fig. S3). In conclusion, the wing pattern including the presence and absence of white stripe, ground colour of the hindwing and the underside markings should be used with caution as they may lead to serious errors in species identification. Examination of male genitalia confirmed that two male specimens of *P. ripartii* had shorter valvae the single specimen of *P. aroaniensis* from Gacko had longer valvae like in Kolev (1994) and Dincă *et al*. (2013).

Tab. 2. List of COI sequences used in this study. Bold – samples sequenced in the present study.

Taxon	Sample ID	GenBank accession number	COI haplotype	Country
P. ripartii	RE07-G437	HM210168	H1	Italy
P. ripartii	RVcoll.11-G197	KC581743	H1	Croatia
P. ripartii	RVcoll.11-G194	KC581742	H1	Croatia
P. ripartii	RVcoll.11-G189	KC581741	H1	Croatia
P. ripartii	**LARGA2**	**KX377637**	H1	B&H
P. ripartii	**LARKAM2**	**KX377631**	H1	Croatia
P. ripartii	RE07-G436	HM210167	H2	Italy
P. ripartii	**LARLP3***	**KX377624**	H3	Croatia
P. ripartii	**LARLIS1**	**KX377628**	H3	Croatia
P. ripartii	2005-LOWA-767	FJ663244	H4	Kazakhstan
P. ripartii	2005-LOWA-67	FJ663245	H4	Kazakhstan
P. ripartii	2005-LOWA-66	FJ663246	H4	Kazakhstan
P. ripartii	2005-LOWA-768	FJ663243	H5	Kazakhstan
P. ripartii	MW01105	AY556962	H6	Spain
P. ripartii	**LARLP1**	**KX377623**	H7	Croatia
P. ripartii	**LARLP4***	**KX377625**	H7	Croatia
P. ripartii	**LARMK1***	**KX377626**	H7	Croatia
P. ripartii	RVcoll.11-G193	KC581732	H8	Croatia
P. ripartii	RVcoll.11-G191	KC581731	H8	Croatia
P. ripartii	RVcoll.11-G198	KC581733	H8	Croatia
P. ripartii	RVcoll.11-G188	KC581730	H8	Croatia
P. ripartii	RVcoll.09-V363	KC581729	H8	Croatia
P. ripartii	**LRLIS1**	**KX377629**	H9	Croatia
P. ripartii	RV03-H463	EF104603	H10	Spain
P. ripartii	RVcoll.11-G195	KC581740	H11	Croatia
P. ripartii	RVcoll.09-X021	KC581739	H11	Spain
P. ripartii	RVcoll.09-V724	KC581738	H11	Spain
P. ripartii	RVcoll.09-V723	KC581737	H11	Spain
P. ripartii	RVcoll.08-M934	KC581736	H11	Spain
P. ripartii	**LRKAM2**	**KX377630**	H11	Croatia
P. ripartii	**LARPO1***	**KX377633**	H11	Croatia
P. ripartii	**LARKAM3**	**KX377632**	H11	Croatia
P. ripartii	RVcoll.07-C089	GU677029	H11	Spain
P. ripartii	RVcoll.08-P614	HM901664	H11	Spain
P. ripartii	RVcoll.08-M935	GU676152	H11	Spain
P. ripartii	RVcoll.07-F038	HM901559	H11	Spain
P. ripartii	RVcoll.08-M697	GU676158	H11	Spain
P. ripartii	RVcoll.07-F035	GU677012	H12	Spain
P. ripartii	RVcoll.08-P316	GU676039	H13	Spain
P. ripartii	RVcoll.11-G192	KC581744	H14	Croatia
P. ripartii	**LRTRO1**	**KX377627**	H14	Croatia
P. ripartii	RVcoll.11-G190	KC581734	H15	Croatia
P. ripartii	RVcoll.11-G196	KC581735	H15	Croatia
P. ripartii	RVcoll.08-P615	GU675760	H16	Spain
P. nephohiptamenos	RVcoll.09-V964	KC581745	H17	Bulgaria
P. nephohiptamenos	JC00045	AY556859	H18	Greece

Taxon	Sample ID	GenBank accession number	COI haplotype	Country
P. nephohiptamenos	JC00046	AY556860	H19	Greece
P. ripartii	RVcoll.12-M017	KC581752	H20	Bulgaria
P. ripartii	RVcoll.12-M016	KC581751	H20	Bulgaria
P. ripartii	RVcoll.12-M014	KC581750	H20	Bulgaria
P. ripartii	RVcoll.12-M011	KC581748	H20	Bulgaria
P. ripartii	RVcoll.12-M012	KC581749	H20	Bulgaria
P. ripartii	RVcoll.10-A998	KC581746	H20	Bulgaria
P. ripartii	RVcoll.11-J903	KC581747	H20	Bulgaria
P. ripartii	JC00043	AY556858	H21	Greece
P. ripartii	RE07-G266	HM210171	H22	France
P. ripartii	RVcoll.11-I869	KC581725	H22	France
P. ripartii	RVcoll.11-I703	KC581724	H22	France
P. ripartii	RVcoll.10-B712	KC581723	H22	France
P. ripartii	RVcoll.11-I851	KC581726	H23	France
P. ripartii	RE07-G229	HM210172	H24	Italy
P. ripartii	RE07-G255	HM210164	H24	Italy
P. ripartii	RE07-G254	HM210163	H24	Italy
P. ripartii	RVcoll.12-L128	KC581721	H25	Spain
P. ripartii	RVcoll.08-L945	GU676213	H26	Spain
P. ripartii	RVcoll.12-L137	KC581722	H27	Spain
P. ripartii	RVcoll.12-L140	KC567884	H28	Spain
P. ripartii	RVcoll.12-Q455	KC581728	H29	Spain
P. ripartii	RVcoll.12-Q453	KC581727	H29	Spain
P. ripartii	RVcoll.12-L129	KC567885	H30	Spain
P. ripartii	RVcoll.12-L138	KC581719	H30	Spain
P. ripartii	RVcoll.12-L133	KC567883	H31	Spain
P. ripartii	RVcoll.11-I888	KC581720	H32	France
P. ripartii	MW01014	AY556944	H33	Spain
P. ripartii	MW01072	AY556957	H34	Spain
P. ripartii	RVcoll.08-L946	KC617794	H35	Spain
P. ripartii	RVcoll.08-L948	KC617793	H35	Spain
P. ripartii	RVcoll.08-L892	GU676220	H35	Spain
P. ripartii	RVcoll.12-L130	KC581718	H36	Spain
P. ripartii	RVcoll.08-L390	KC581717	H36	France
P. admetus	**LADDIN3**	**KX377634**	H37	Croatia
P. admetus	RVcoll.09-V962	KC581753	H38	Bulgaria
P. admetus	RVcoll.09-V963	KC581754	H39	Bulgaria
P. admetus	JC01014	AY556867	H40	Greece
P. admetus	MW98084	AY556986	H41	Turkey
P. humedasae	RE07G191	HM210169	H42	Italy
P. humedasae	MW99605	AY557128	H43	Italy
P. humedasae	MW99591	AY557127	H44	Italy
P. humedasae	RE07G203	HM210170	H45	Italy
P. aroaniensis	JC00040	AY556856	H46	Greece
P. aroaniensis	**LARGA1**	**KX377636**	H47	B&H
P. dantchenkoi	MW99319	AY557081	H48	Turkey
P. dantchenkoi	MW99274	AY557072	H48	Turkey
P. dantchenkoi	MW99276	AY557073	H49	Turkey
P. damon	**LDAMK1**	**KX377635**	H50	Croatia

Taxon	Sample ID	GenBank accession number	COI haplotype	Country
P. damon	MW99613	AY557131	H51	France
P. damon	RVcoll.08-L895	GU676224	H52	Spain
P. damon	RVcoll.08-L894	GU676223	H53	Spain
P. damon	MW99546	AY557121	H54	Turkey
P. damon	MW99613	AY496731	H55	France
P. damon	RVcoll.07-F023	GU677042	H56	Spain
P. damon	RVcoll.08-P326	HM901317	H57	Spain
P. damon	RVcoll.08-R289	GU675795	H58	Spain
P. damon	RVcoll.08-P001	GU675971	H59	Spain
P. damon	RVcoll.08-L167	GU676405	H60	Spain
P. damon	RVcoll.08-L994	HM901305	H61	Spain
P. damon	RVcoll.07-W228	GU676752	H62	Spain
P. damon	RVcoll.08-L230	GU676393	H63	Spain
P. damon	RVcoll.09-X513	HM901513	H64	Spain
P. damon	RVcoll.08-P629	HM901325	H64	Spain
P. icarus	JC00063	AY556866	H65	Greece
P. icarus	RVcoll.10-C178	KM517840	H66	Spain

Since the herewith presented molecular analysis proves that the *Agrodiaetus* specimens reported as *P. aroaniensis* on morphological grounds (Koren & Lauš, 2015) actually belong to *P. ripartii,* the aforementioned report on the presence of *P. aroaniensis* in Croatia must be discounted. However, as both species occur in Bosnia and Herzegovina, it is impossible to exclude the presence of *P. aroaniensis* in Croatia, even within known localities of *P. ripartii*. Therefore, further identification of this species reporting on new localities and should be confirmed through additional analysis like genital morphometry of the males, molecular and/or karyological data.

Based on the present knowledge, additional findings of *P. ripartii* populations can be expected in Croatia but detailed studies on the biology and ecology of this species are needed to determine its habitat requirements and possibly the need for conservation actions, as the species has a restricted distribution.

Received June 2, 2016

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