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Genetic characterization of the Balkan endemic species, *Merodon desuturinus* (Diptera: Syrphidae)

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Abstract. Variation of 15 nuclear allozyme genes and a 708 bp fragment of DNA sequence of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene were surveyed in a population of a hoverfly species, *Merodon desuturinus* Vujić, Šimić & Radenković, 1995 (Diptera, Syrphidae), endemic to the Balkan Peninsula. Based on phylogeography and taxonomy, *Merodon desuturinus* is of special interest from a biogeographical and conservation perspective. Characterization and determination of genetic relationships between *M. desuturinus* and taxa of the *M. aureus*, *M. avidus*, and *M. ruficornis* groups on the Balkan Peninsula were estimated based on molecular markers (allozymes and COI sequences). We observed that the mean number of alleles per allozyme locus was 1.1, frequency of polymorphic loci 0.067, and heterozygotes were absent. Our results showed an extremely low genetic variability in the *M. desuturinus* population sampled. We suggest that this taxon calls for a conservation management plan, as it is likely a vulnerable and threatened taxon, an endemic, spatially divided species and represents a unique genetic unit on the Balkan Peninsula.

INTRODUCTION

A high taxon diversity and high levels of endemism highlight the unique biota of the Balkan Peninsula within the Mediterranean hotspot (e.g. Crivelli & Maitland, 1995; Quézel & Médail, 1995). Genetic endemism is based on the delimitation of genetic units that are geographically discrete and characterized by nonoverlapping haplotype distribution and/or occurrence of unique allozyme markers. Conservation genetics has a primary focus on genetic diversity due to its importance in the maintenance of adaptive evolutionary potential (long term impact) and of reproductive fitness (short term impact) (Frankham, 2003). As Frankham (1995, 2005) pointed out, the observed low genetic diversity of endangered species in comparison to related, non-endangered species with large population sizes must be interpreted as loss of genetic diversity. Reduction in genetic diversity is associated with inbreeding that contributes to extinction risk in the long-term. The mechanisms responsible for a reduction in heterozygosity include many factors such as historical and current population size, population bottlenecks, breeding system, natural selection, different mutation rates and number of migrants, which may all affect the observed level of genetic diversity (e.g. Frankham, 2003).

Mitochondrial DNA (mtDNA) and allozyme nuclear loci are commonly used genetic markers in population studies, taxonomy, systematics and conservation genetics. Genetic diversity of many taxa of hoverflies (Diptera: Syrphidae) has been quantified for allozyme nuclear loci (Ludoški et al., 2002, 2004; Milankov et al., 2001, 2002, 2005) and for mtDNA cytochrome *c* oxidase subunit I (COI) (Pérez-Bañón et al., 2003; Rojo et al., 2006; Láska et al., 2006; Mengual et al., 2006; Milankov et al., 2008a, b, c).

The genus *Merodon* Meigen, 1803 with more than 170 species (Hurkmans, 1993), is one of the most speciose hoverfly genera. It is primarily distributed in the Palaearctic region, with the highest diversity in the Mediterranean area with only a few species occurring in the Afrotropical region (especially in South Africa). Numerous species are (steno)endemic, especially on Iberian and Balkan Peninsulas, in Turkey and SW Asia. Larval development and natural history remain poorly known (Marcos-García et al., 2007). Immature stages were described for only a few species, the first were larvae of *Merodon equestris* Fabricius that are internal feeders on tissues of plant bulbs (e.g. Liliaceae) (Rotheray, 1993). This is a good indication that (all) *Merodon* species feed on plant bulbs, although few observations exist to date.

The genus *Merodon* on the Balkan Peninsula has been the subject of faunistic and taxonomic studies since 1986, and from this area 30 species are recognized (Šimić et al., 1998). Based on phylogeography and taxonomy, *Merodon desuturinus* Vujić, Šimić & Radenković, 1995 is of special interest from a biogeographical and conservation perspective. The type locality of *M. desuturinus* is the western slopes of mountain Kopaonik in Serbia (Vujić et al., 1995). After the description of the taxon in 1995, one additional population was found on Durmitor Mt., Montenegro, approximately 160 km from the type locality. This is an early spring species with a short flight period (approx. 10 days) (Vujić et al., 1995). The known habitats of *M. desuturinus* are in the border zone between the biome of south-European mostly broad-leaved wood-

lands with oromediterranean elements and the biome of European mostly coniferous forests of boreal type (following Matvejev & Puncer, 1989). It is possible that populations of this species also occur on other high mountains in the southern Balkans.

One particular morphological character of *M. desuturinus* clearly differentiates this taxon from all other *Merodon* taxa occuring on the Balkan Peninsula, as the male is dichoptic (holoptic males in other *Merodon* taxa) (Vujić et al., 1995). In the course of a recent revision of the genus *Merodon* it was observed that morphologically this species and at least four other taxa form the *desuturinus* group (A. Vujić, unpubl.). The taxa belonging to this morphologically well-defined group occur in geographically distant areas, but are mainly found in South Africa and southern Palaearctic. They share the following characters: posterior side of mid coxa with hairs; anterior lobe of surstylus with curved distal prolongation; composition of hairs on anterior anepisternum variable in the *desuturinus* group; tip of lateral sclerite of aedeagus pointed and projected dorsally (e.g. Vujić et al., 1995). The species morphologically most similar to *M. desuturinus* is *M. cuthbertsoni* Curran, 1939, described from Rhodesia (Africa), the name that at an earlier period was used to refer to a larger region that corresponds to both Zimbabwe (Southern Rhodesia) and Zambia (Northern Rhodesia). The three additional species belonging to the same species group are *M. planifacies* Bezzi, 1915 (South Africa), *M. lydicus* Hurkmans (A. Vujić, unpubl.) (East Mediterranaean), and *M. cabanerensis* Marcos-García, Vujić & Mengual (Marcos-García et al., 2007), which was recently discribed from Spain.

Here we characterize and describe the genetic relationships of the endemic *M. desuturinus* taxon, and compare it with other genetically characterized taxa of *Merodon* from the Balkan Peninsula. Biochemical and molecular markers (allozyme loci and COI sequence) were used to (1) genetically characterize this taxon, (2) quantify genetic variability of the population in order to determine the long-term evolutionary success of the species analyzed, and (3) determine genetic relationships between *M. desuturinus* and taxa of the *M. aureus*, *M. avidus*, and *M. ruficornis* groups on the Balkan Peninsula.

MATERIAL AND METHODS

Sample collection

Specimens of *M. desuturinus* were identified using diagnostic features in the description (Vujić et al., 1995). For allozyme analysis specimens of one population of *M. desuturinus* were collected from Kopaonik Mt. (E 20°40′, N 43°15′, Serbia; collection date 6.vi.1998; leg. & det. Vujić, A.). A total of 17 specimens were analyzed by allozyme electrophoresis and out of these two individuals were used for mtDNA sequencing.

In order to analyse the evolutionary relationships between *M. desuturinus* and species of the *M. aureus*, *M. avidus*, and *M. ruficornis* groups and *M. funestus* taxon from the Balkan Peninsula, previously obtained sequences (Milankov et al., 2001, 2008a, b, c) were compared. Samples of 10 populations of the *Merodon aureus* group, 11 populations of the *M. ruficornis* group, seven populations of the *M. avidus* group and one of the *M. funestus* species were collected from five regions and nine localities on the Balkan Peninsula as follows (abbreviation in parenthesis): Vršačke planine Mt., 21°20′E, 45°08′N (Serbia; VP), Dubašnica Mt., 21°59′E, 44°01′N (Serbia; DUB); Kopaonik Mt., 20°40′E, 43°15′N (Serbia; KOP); Durmitor Mt., 19°00′E, 43°11′N (Montenegro; DUR); Morinj, 18°40′E, 43°29′30″N (Montenegro; MOR); Prokletije Mt., 19°50′E, 42°32′N (Serbia; PRO); Šar planina Mt., 21°05′E, 42°12′N (Serbia; ŠAR); Mavrovo Lake, 20°44′30″E, 41°38′ 30″N (FYR Macedonia; MAV) and Pindos Mt., 20°37′E, 39°14′N (Greece; PIN).

The following populations of the *M. aureus* group were assayed: *M. aureus* A (DUR, MOR), *M. aureus* B (KOP), *M. aureus* C (DUR, MOR), *M. cinereus* A (KOP), *M. cinereus* B (DUR, PRO), *M. cinereus* C (ŠAR), *M. funestus* (MOR); *M. ruficornis* group: *M. auripes* (VP, DUB, DUR), *M. armipes* (DUR), *M. trebevicensis* (DUB, VP, MAV), *M. loewi* (DUB, PIN), *M. ruficornis* (DUB, DUR), and the *M. avidus* group: *M. avidus* A (MOR, DUB, PIN) and *M. avidus* B (DUB, DUR, MAV, PIN).

Allozyme analysis

Genetic variability of the *M. desuturinus* population was studied by standard 5% polyacrylamide gel electrophoresis, following Munstermann (1979) (FUM, GPD, GPI, HAD, HK, IDH, MDH, ME, PGM, SOD) and Pasteur et al. (1988) (AAT), with slight modifications (Milankov, 2001). The Tris-boric-EDTA buffer system (pH 8.9) was used to assay glucosephosphate isomerase (5.3.1.9. GPI; *Gpi*), hexokinase (2.7.1.1. HK; two loci: *Hk-2, Hk3*), malic enzyme (1.1.1.40. ME; *Me*), phosphoglucomutase (2.7.5.1. PGM; *Pgm*), and superoxide dismutase (1.15.1.1. SOD; three loci: *Sod-1, Sod-2, Sod-3*). A Tris-citric buffer system (pH 7.1) was used to assay aspartat amino transferases (2.6.1.1. AAT; *Aat*), fumarate hydratase (4.2.1.2. FUM; *Fum*), glycerol β-phosphate dehydrogenase (1.1.1.8. GPD; *Gpd-2*), α-hydroxy acid dehydrogenase (3.1.1.31. HAD, *Had*), isocitrate dehydrogenase (1.1.1.42. IDH; *Idh-2*), and malate dehydrogenase (1.1.1.37. MDH; *Mdh-1, Mdh-2*).

Allozyme data of 12 loci (*Mdh-1*, *Sod-2*, and *Sod-3* loci were not available for comparison) available for taxa of the genus *Merodon* were used for calculating genetic relationships. Loci were numbered and alleles marked alphabetically with respect to increasing anodal migration. Genotype and allele frequencies were calculated directly from the observed banding patterns based on the genetic interpretation of zymograms. Specimens from analyzed taxa were run concurrently on all gels to facilitate comparison of electrophoretic mobility. Statistical analysis of allozyme data, including calculations of allelic frequencies, mean number of alleles per locus (*A*), frequency of polymorphic loci (*P*), mean heterozygosity (*H*), was performed using the computer program BIOSYS-2 (Swofford & Selander, 1989). Diagnostic value of allozymes was calculated after Ayala & Powell (1972). Legs and other parts of specimens that remained after allozyme analysis were deposited at the Department of Biology and Ecology, University of Novi Sad (Serbia).

DNA sequencing

Mitochondrial DNA COI sequences were obtained for two specimens of *M. desuturinus*, 22 of the *M. ruficornis* group (Milankov et al., 2008c), 22 of the *M. aureus* group (Milankov et al., 2008b), and 26 of the *M. avidus* group (Milankov et al., 2008a). Specimens came mainly from the Balkan Peninsula but one specimen from France and three from Spain of the *M. avidus* group were also used. DNA was extracted from legs or other parts of some insect specimens that remained after previous allozyme electrophoresis. DNA extraction used the Nucleospin Tissue DNA extraction kit (Machery-Nagel, Düren,

Germany), and followed the manufacturer's protocols and was re-suspended in 50 µl of ultra-pure water. Remains of specimens, mainly male genitalia and wings, used for the morphological studies are deposited at the Zoological Museum (MZH) of the Finnish Museum of Natural History (Helsinki, Finland), and Department of Biology and Ecology, University of Novi Sad (Serbia).

PCR reactions were carried out in 25 µl reaction aliquots containing 2 µl of DNA extract, 1 µl of each primer (at 10 pmol/µl), 0.25 µl of DNA polymerase (5U/µl), 2 µl of 2.5 mM $MgCl_2$, 2.5 µl of 10× Buffer II (MBI Fermentas, St. Leon-Rot, Germany), 4 µl of 200 mM dNTP (GeneAmp, Applied Biosystems, Foster City, CA, USA) and balanced with ultra-pure water. Thermocycler conditions were initial denaturing at 95°C 2 min, 29 cycles of 30 s denaturing at 94°C, 30 s annealing at 49°C, 2 min extension at 72°C, followed by a final extension of 8 min at 72°C. The universally conserved primers used for amplifying and sequencing the COI fragment were the forward primer C1-J-2183 (5'-CAA CAT TTA TTT TGA TTT TTT GG-3') (alias JERRY) and two reverse primers TL2-N-3014 (5'-TCC AAT GCA CTA ATC TGC CAT ATT A-3') (alias PAT) (Simon et al., 1994) and C1-N-2735 (5'-AAA ATG TTG AGG GAA AAA ATG TTA-3') (alias INGER) (Lunt et al., 1996). PCR products were purified using the GFX PCR Purification Kit (GE Healthcare Biosciences, Little Chalfont, UK) and then sequenced (with the PCR primers) in both directions using the Big Dye Terminator Cycle Sequencing Kit vs. 1.1 (Applied Biosystems) at one-fourth of the recommended volumes on an ABI PRISM 377 (Applied Biosystems) semi-automated DNA sequencer. The sequences were edited for base-calling errors and assembled using Sequence Navigator™ (version 1.01) (Applied Biosystems).

Genetic analysis

Nei's genetic distance (1978) calculated from allozyme data was used to compare gene frequencies among species or among populations of the *M. aureus*, *M. ruficornis*, and *M. avidus* groups. Uncorrected pairwise divergences (*p*-distances) were calculated based on the COI sequences of two specimens of *M. desuturinus* and specimens from the above mentioned groups and additional specimens, totaling 70 specimens.

RESULTS

Allozyme variability

Out of 15 analysed loci only *Gpi* izozyme locus was polymorphic in the *M. desuturinus* population. A total of 16 alleles were registered. The mean number of alleles per locus (*A*) and frequency of polymorphic loci (*P*) were 1.1 (SE = 0.1) and 0.067 (SE = 0.134), respectively. The analysis of genotype frequencies showed a statistically important deviation from the expected values according to Hardy-Weinberg's law, and the absence of heterozygotes in the polymorphic locus.

Allozyme variability and genetic relationships of taxa of the genus *Merodon* were analysed based on the allelic frequencies of 12 isozyme loci: *Aat*, *Fum*, *Gpd*-2, *Gpi*, *Had*, *Hk*-2, *Hk*-3, *Idh*-2, *Mdh*-2, *Me*, *Pgm*; and *Sod*-1 (Milankov et al., 2001, 2008a, b, c). Alleles common to *M. desuturinus* and the other *Merodon* taxa analysed were: Aat^b (the common allele shared with *M. aureus* and *M. cinereus* complexes; *M. armipes*; *M. ruficornis*; *M. auripes* DUB, *M. auripes* DUR); Gpi^i (in *M. aureus* C MOR; *M. funestus*; *M. avidus* B; *M. avidus* A PIN); Gpi^j (in *M. aureus* and *M. cinereus* complexes, except *M. cinereus* B; *M. funestus*; *M. avidus* group); *Hk*-2^c and *Hk*-3^c (in *M. aureus* and *M. cinereus* complexes; *M. armipes; M. trebevicensis; M. auripes; M. ruficornis*); *Mdh*-2^e (in *M. aureus* and *M. cinereus* complexes; *M. armipes; M. loewi* DUB*; M. auripes; M. ruficornis; M. avidus* group); Pgm^c (in *M. aureus* B; *M. loewi; M. ruficornis; M. auripes* VP, *M. auripes* DUB) and *Sod*-1^e (in *M. cinereus* B; *M. funestus; M. aureus* C). The *Mdh*-1, *Sod*-2, and *Sod*-3 loci were monomorphic, with a common allele in the populations of the *M. aureus* group and *M. desuturinus*.

TABLE 1. A list of the allozyme loci[a] used to distinguish between *M. desuturinus* and taxa of the *M. aureus*, *M. ruficornis*, and *M. avidus* groups.

Taxa	N[b]	*M. desuturinus*
M. aureus A	7	*Fum, Gpd-2, Had, Idh-2, Me, Pgm, Sod-1*
M. aureus B	6	*Fum, Gpd-2, Had, Idh-2, Me, Sod-1*
M. aureus C	6	*Fum, Gpd-2, Had, Idh-2, Me, Pgm*
M. cinereus A	7	*Fum, Gpd-2, Had, Idh-2, Me, Pgm, Sod-1*
M. cinereus B	6	*Fum, Gpd-2, Had, Idh-2, Me, Pgm*
M. cinereus C	7	*Fum, Gpd-2, Had, Idh-2, Me, Pgm, Sod-1*
M. funestus	11	*Aat, Fum, Gpd-2, Had, Hk-2, Hk-3, Idh-2, Mdh-2, Me, Pgm, Sod-1**
M. armipes	8	*Fum, Gpd-2, Gpi, Had, Idh-2, Me, Pgm, Sod-1*
M. trebevicensis	10	*Aat, Fum, Gpd-2, Gpi, Had, Idh-2, Mdh-2, Me, Pgm, Sod-1*
M. loewi	12	*Aat, Fum, Gpd-2, Gpi, Had, Hk-2, Hk-3, Idh-2, Mdh-2*, Me, Pgm, Sod-1*
M. ruficornis	7	*Fum, Gpd-2, Gpi, Had, Idh-2, Me, Sod-1*
M. auripes	7	*Fum, Gpd-2, Gpi, Had, Idh-2, Me, Sod-1*
M. avidus A	8	*Aat, Fum, Gpd-2, Had, Idh-2, Me, Pgm, Sod-1*
M. avidus B	8	*Aat, Fum, Gpd-2, Had, Idh-2, Me, Pgm, Sod-1*

[a] Diagnostic loci allow correct diagnosis of the species and populations with a probability 100% and >90%*.
[b] N – number of diagnostic loci.

Diagnostic allozyme loci

Among the *Merodon* taxa, the species *M. desuturinus* could be identified using the unique alleles at the loci *Fum* (Fum^e), *Gpd-2* ($Gpd\text{-}2^h$), *Had* (Had^h), *Idh-2* ($Idh\text{-}2^c$) and *Me* (Me^h). The highest number of diagnostic loci that distinguish *M. desuturinus* from other species of *Merodon* taxa (with 100% probability) were recorded between *M. desuturinus* and *M. funestus* (11), and *M. desuturinus* and *M. loewi* (12) (Table 1).

Genetic relationships among *M. desuturinus* and taxa of the *Merodon* genus on the Balkan Peninsula

The highest values of genetic distance (Nei's *D*, 1978) calculated from allozyme data were recorded between *M. desuturinus* and *M. funestus* (*M. aureus* group), *M. loewi* (*M. ruficornis* group) and *M. avidus* A (*M. avidus* group). Genetically closest species to *M. desuturinus* were taxa of the *M. aureus* complex (Table 2).

TABLE 2. Genetic distances (*D*; Nei, 1978) between *M. desuturinus* and species of the *M. aureus*, *M. ruficornis*, and *M. avidus* groups calculated using allozyme data.

Group	Species	No. of popul.	D^a
M. ruficornis	*M. armipes*	1	1.189 (1.027–1.351)
	M. trebevicensis	3	1.751 (1.735–1.783)
	M. loewi	2	3.728 (3.265–4.190)
	M. ruficornis	2	1.005 (0.955–1.054)
	M. auripes	3	1.257 (1.226–1.303)
M. aureus	*M. aureus* A	2	1.029 (1.024–1.034)
	M. aureus B	1	0.822
	M. aureus C	2	1.009 (1.003–1.014)
	M. cinereus A	1	0.996
	M. cinereus B	2	1.055 (1.038–1.063)
	M. cinereus C	1	0.996
	M. funestus	1	3.348
M. avidus	*M. avidus A*	3	1.268 (1.132–1.369)
	M. avidus B	4	1.203 (1.093–1.274)

[a] Minimum and maximum values of genetic distance (*D*) are given in parenthesis.

COI variation

Merodon desuturinus

The sequences obtained for *M. desuturinus* are of different length (708 bp of VM357 and 553 bp of VM342). They are deposited in GenBank (Accession numbers: VM357-COI DQ387899; VM342-COI EF100776). Both sequences are identical with an A+T content of 72.34%. As we have observed the intrapopulation consistency of the COI variation within taxa of *M. aureus* (Milankov et al., 2008b) and *M. ruficornis* (Milankov et al., 2008c) groups, we expected a similar haplotype pattern within *M. desuturinus* species and analyzed only two specimens.

Other *Merodon* taxa

The sequences obtained for other *Merodon* taxa ranged from 708 to 770 bp for the Jerry+Pat fragment, and from 518 to 524 bp for the Jerry+Inger fragment. They were submitted to GenBank under accession numbers DQ 387900–DQ387921 (*M. aureus* group), DQ 387895–DQ 387898, DQ845109–DQ845136 (*M. avidus* group) and DQ885917–DQ885936 (*M. ruficornis* group).

Haplotype diversity

Based on the 708 bp mtDNA COI analysed, the number of different nucleotide sites between the *M. desuturinus* haplotype and observed haplotypes of the *M. aureus* group were calculated (Milankov et al., 2008b). The highest number was registered when comparing *M. desuturinus* with *M. aureus* C MOR (haplotype VI), and the lowest number in the comparison with *M. aureus* A MOR (haplotype II). Comparing haplotypes of *M. desuturinus* and species of *M. ruficornis,* the highest number was registered between *M. desuturinus* and *M. trebevicensis* DUB, VP (haplotype VIII), and the lowest number in the comparison with *M. trebevicensis* MAV (haplotype IX), *M. auripes* DUB and *M. auripes* DUR (these taxa share haplotype III of the group). Within the *Merodon* taxa analysed the greatest number of different sites on average was recorded between *M. desuturinus* and *M. avidus* group, and ranged from 71 to 74. Uncorrected pairwise COI divergences between *M. desuturinus* and haplotypes of the *Merodon* taxa ranged from 7.37% (*M. desuturinus* vs. *M. crymensis* of the *M. ruficornis* group) to 10.4% (*M. desuturinus* vs. *M. avidus* group; *M. desuturinus* vs. *M. aureus* C of the *M. aureus* group, haplotype VI) (Table 3).

DISCUSSION

Genetic variation

The level of genetic variation in *M. desuturinus* was low (no heterozygotes were detected, only the *Gpi* locus was polymorphic). Genetic variability this low is observed rarely, such as in populations of *Melanogaster nuda* (Ludoški et al., 2004), the *Cheilosia canicularis* group (Milankov et al., 2005) and certain species of the genus *Merodon* (Milankov et al., 2008a, b, c). Typically, much higher values are recorded for species from the genus *Cheilosia,* ranging from 0.250 to 0.600 (Ludoški et al., 2002; Milankov et al., 2002).

Low genetic diversity is associated with reduced reproduction and survival (reproductive fitness) (Frankham et al., 2002). Contrary to large populations that are relatively stable for molecular genetic markers, small populations typically lose genetic diversity over time due to stochastic processes, population substructuring and inbreeding (Frankham, 2005). In addition, an important aspect of a small population is that deleterious alleles are more likely to be fixed by lower efficiency of selection than in large populations, which increases the risk of extinction (Frankham et al., 2002). The putative small population size of the spatially isolated population of *M. desuturinus* and its probable narrow ecological niche associated with possible historical events, especially bottlenecks, could easily be the cause of the low genetic diversity observed. The *M. desuturinus* population is located in one of the numerous Pleistocen refugia in the Balkans. The impact of glaciations during the Quaternary on the contraction and fragmentation of species is well known (e.g. Hewitt 1996, 1999). Extraordinarily low variability can be attributed to epistatic interactions or some other form of interaction of allozyme loci as well. The host plant species *Ornithogalum* spp and *Scilla* spp (A. Vujić, pers. observ.) connected with development of the putatively phytophagous larvae and level of specialization could possibly explain the distribution and population structure of the species analysed.

Intraspecific variability of particular morphological features of the *M. desuturinus* specimens from Kopaonik and Durmitor Mts. were found: dichoptic eyes in males meet at only one point or over a very short distance that varies from 1 to 5 facets; tergites 2–5 with narrow dusted stripes that are sometimes absent from tergite 4 (in some males) or tergite 2 (in females) (A. Vujić, unpubl.). Furthermore, using a geometric-morphometric approach to the study of interpopulation phenotypic diversity revealed that males

TABLE 3. Raw fixed differences (*No*) and uncorrected distances (*p*) calculated from a comparison of mtDNA COI sequences of *M. desuturinus* and haplotypes of the *M. aureus* (Milankov et al., 2008b), *M. avidus* (Milankov et al., 2008a) and *M. ruficornis* (Milankov et al., 2008c) groups.

Species group	Haplotype [a]	Taxa	No. of specimens	*No*	*p* (%)
M. aureus	I	*M. aureus* A MOR, *M. aureus* A DUR	3	65	9.16
	II	*M. aureus* A MOR	1	64	9.14
	III	*M. aureus* B KOP	4	67	9.44
	IV	*M. aureus* C DUR	1	73	10.28
	V	*M. aureus* C DUR	1	72	10.00
	VI	*M. aureus* C MOR	1	74	10.42
	VII	*M. aureus* C MOR, *M. cinereus* C ŠAR, *M. cinereus* B DUR, *M. cinereus* B PRO	7	72	10.14
	VIII	*M. cinereus* A KOP	2	70	10.14
	IX	*M. funestus* MOR	1	64	9.01
	X	*M. funestus* MOR	1	65	9.16
M. avidus	I	*M. avidus* A MOR	1	72	10.16
	II	*M. avidus* A MOR	1	73	10.30
	III [b]	*M. avidus* A MOR	1	58	11.11
	IV	*M. avidus* A DUB, *M. avidus* B MAV, *M. avidus* B DUR, *M. avidus* A Lesvos	3	72	10.17
	V	*M. avidus* B MAV	1	73	10.16
	VI	*M. avidus* A Lesvos	1	72	10.17
	VII	*M. avidus* A PIN, *M. avidus* A DUB, *M. avidus* B DUB	3	73	10.31
	VIII	*M. avidus* B DUB, *M. avidus* A Lesvos	2	72	10.13
	IX	*M. avidus* B DUR	1	73	10.30
	X	*M. avidus* B DUR	1	73	10.30
	XI	*M. avidus* B PIN	1	71	10.02
	XII	*M. avidus* A MOR	1	72	10.17
	XIII	*M. avidus* B DUB	1	74	10.45
	XIV	*M. avidus* B DUB, *M. avidus* A MOR	2	72	10.15
	XV	*M. avidus* B (France)	1	72	10.17
	XVI	*M. avidus* A MOR	1	74	10.45
	XVII	*M. avidus* A Lesvos	1	73	10.30
	XVIII	*M. bicolor* (Spain) [c]	1	73	10.19
	XIX	*M. bicolor* (Spain) [c]	1	71	10.04
	XX	*M. bicolor* (Spain) [c]	1	73	10.32
M. ruficornis	I	*M. ruficornis* DUB	1	56	8.44
	II	*M. ruficornis* DUB, *M. ruficornis* DUR	2	55	8.27
	III	*M. auripes* DUB, *M. auripes* DUR	4	53	7.89
	IV	*M. ruficornis* DUR	1	55	8.27
	V	*M. loewi* DUB	1	55	8.23
	VI	*M. loewi* DUB	2	56	8.40
	VII	*M. loewi* PIN, *M. loewi* DUB, *M. armipes* DUR	5	57	8.35
	VIII	*M. trebevicensis* VP, *M. trebevicensis* DUB	4	56	8.60
	IX	*M. trebevicensis* MAV	1	53	7.37
	X [b]	*M. trebevicensis* MAV	1	40	7.86

[a] Sequences ranged from 708 to 770 bp in length; [b] sequences of 524 bp in length; [c] previously morphologically defined as *M. avidus* B (Marcos-García et al., 2007).

of allopatric populations from Kopaonik and Durmitor Mts could not be significantly separated based on wing size and wing shape although the discriminant analysis gave correct classification scores of 100% (Lj. Francuski, unpubl.).

Genetic relationships between *M. desuturinus* and taxa of the *Merodon* genus on the Balkan Peninsula

Parsimony analysis of the COI sequences (haplotypes) of 44 *Merodon* species showed that *M. desuturinus*, *M. planifacies*, and *M. cabanerensis* belong to a larger clade of species (putative subgenus), all possessing particular

morphological traits, a hairy posterior side of mid coxa and anterior lobe of surstylus with curved distal prolongation (A. Vujić, unpubl.). In this clade, the *desuturinus* group forms a particular and well-supported lineage. Morphologically, *M. desuturinus* is similar to species from *albifrons* and *aureus* groups (A. Vujić, unpubl.), but with some important differences in male genitalia (anterior lobe of surstylus reduced or undeveloped and lateral sclerite of aedeagus partly or completely reduced in *aureus* group). We argue that this species belongs to the oromediterranean relicts, especially because of the extreme geographical distance between this and phylogenetically closely related species in South Africa and on the Iberian Peninsula (A. Vujić, unpubl.).

Genetic relationships derived from allozyme data between *M. desuturinus* and *Merodon* taxa of the *M. aureus*, *M. ruficornis*, and *M. avidus* groups are incongruent with the mtDNA COI sequences. Indeed, the trees obtained from both markers presented different placement of *M. desuturinus*. In contrast to the unweighted pair group method with arithmetic average dendrogram (UPGMA) constructed using allozyme data, the mtDNA gene cladogram suggests that the closest relationships are between *M. desuturinus* and species of the *M. ruficornis* group. However, based on the Nei's *D* value and morphological traits, the closest group to *M. desuturinus* from the Balkan Peninsula is the *M. aureus* group (figures not shown). Both evolutionary rate variation of allozyme nuclear and COI mtDNA genes, and parsimony analysis of these four evolutionary well-defined groups of the *Merodon* genus on the Balkan Peninsula will be presented elsewhere (V. Milankov, G. Ståhls & A. Vujić, unpubl.). This incongruence is not surprising because of the maternal inheritance of mtDNA and fourfold smaller effective population size than in nuclear allozymes (Moore, 1995), which renders mtDNA more sensitive to the effects of genetic drift, incomplete lineage sorting and introgression following hybridization (Moritz & Cicero, 2004). Partial incongruence between mitochondrial gene trees and nuclear genes was also observed in the *M ruficornis* group (Milankov et al., 2008c).

Comparing values of mtDNA sequence divergence (uncorrected *p* divergence in %) of *M. desuturinus* species vs. the *M. avidus*, *M. ruficornis*, and *M. aureus* groups, and calculated *p* values among species within the *Merodon* groups, we found different levels of divergences. Contrary to the obtained high levels of divergence reported herein (p = 7.4%–10.4%), sequence divergences among species within the *M. ruficornis* (Milankov et al., 2008c) and *M. aureus* groups (Milankov et al., 2008b; Mengual et al., 2006) were from 0% to 5%. Comparison of levels of divergence of *M. funestus* and *M. avidus* species with members of the *M. aureus* group showed mean values of 8.87% and 10.37%, respectively (Milankov et al., 2008b), and these results are similar to the present results. Therefore, in addition to morphological traits, the level of mtDNA sequence divergence clearly defined the evolutionary independent lineage of *M. desuturinus* among *Merodon* taxa on the Balkan Peninsula. Additionally, comparison of *M. desuturinus* with a sequence of another member of the *desuturinus* group, *M. cabanerensis* from Spain, obtained from GenBank (Accession Number DQ386316; Mengual et al., 2006) showed uncorrected pairwise COI divergence of 7.32%, which is in the range of the difference obtained here between *M. desuturinus* and species of the *M. ruficornis* group .

Finally, ongoing investigations of taxonomy and systematics of the genus *Merodon* will give further insights into the evolutionary history and phylogenetic position of the Balkan endemic species, *M. desuturinus*.

Conservation implication

Conservation of genetic diversity is one of three global conservation priorities according to IUCN – International Union for the Conservation of Nature (McNeely et al., 1990). Due to the effect of the genetic factor on the reduction of genetic diversity and its role in species extinction, the observed extremely low genetic variability in the *M. desuturinus* population should be addressed in any future potential conservation management plan for this taxon. Very low levels of allozyme heterozygosity have been frequently found in threatened natural populations (for a review see: Frankham, 1995) indicating the important impact of inbreeding, which is presumed to increase the risk of extinction. Additionally, lower average heterozygosity in threatened taxa compared to closely related nonthreatened taxa is recorded (Spielman et al., 2004). *M. desuturinus* as a species with a low genetic variation and evolutionary potential would be expected to have a reduced ability to cope with environmental change and to survive climatic extremes, diseases and parasites. The future of *M. desuturinus* populations is associated not only with the current genetic diversity, as an evolutionary potential, but also with the influence of the anthropogenic factors responsible for possible habitat degradation and habitat fragmentation.

In the light of the recent debate on conservation priorities and the focus of conservation (evolutionary novelty or evolutionary potential), *M. desuturinus* could also be used as an example of the importance of protection of historically isolated lineages (Evolutionary Significant Units, Moritz, 1994). Bearing this in mind, research integrating phenotypic diversity clearly discriminated males of allopatric populations, which implied high phenotypic structuring and a separate and distinct evolutionary history of *M. desuturinus* (Lj. Francuski, unpubl.).

Consequently, we would like to propose that values of genetic diversity and also putative host plant habitat protection should be included when planning conservation strategies for preserving *Merodon* diversity. Thus, the endemic and spatially fragmented *M. desuturinus* needs to be incorporated into management plans to preserve the presently known habitats.

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