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Genetic divergence among native trout *Salmo trutta* populations from southern Balkans based on mitochondrial DNA and microsatellite variation

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The genetic structure and the phylogenetic relationships among five Balkan populations of trout *Salmo trutta* that have been classified earlier into five different taxa were studied, using microsatellite and mitochondrial DNA (mtDNA) analyses. The pattern of population differentiation observed at microsatellites differed to that depicted by mtDNA variation, yet both methods indicated a very strong partitioning of the genetic variation among sampling locations. Results thus suggest that conservation strategies should be directed towards preserving the genetic integrity and uniqueness of each population.

Key words: genetic diversity; Greece; Salmonidae; taxonomy.

INTRODUCTION

The considerable confusion regarding the taxonomy of brown trout *Salmo trutta* L. 1758 species complex originated from the complicated and incompletely described evolutionary history of the taxa. This is more evident in regions such as the Balkan Peninsula harbouring the most diverse phenotypic variation among *Salmo* spp. populations (Kottelat & Freyhof, 2007; Simonovic *et al*., 2007). Thus, numerous *Salmo* taxa have been described within this important region, and it is still debatable if these taxa should be considered as phenotypic variants or true Linnean species. On the other hand, it is well established that the use of molecular markers can provide clues regarding the evolutionary origin and useful tools for the conservation and management of populations and species (Apostolidis *et al*., 2008*a*).

Previous surveys, based on the analysis of allozymic loci, mitochondrial (mt) DNA and microsatellites, revealed that southern Balkans comprise a region where *Salmo*

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spp. populations exhibit particularly high levels of genetic variation (Apostolidis *et al*., 2008*a*; Snoj *et al*., 2009). Indeed, mtDNA analyses revealed that four of the five major evolutionary lineages identified throughout the species range (Bernatchez, 2001) were present in the region. Moreover, most of the populations examined were genetically highly divergent, possessing private genotypes, indicating that they may represent distinct and potentially locally adapted gene pools (Apostolidis *et al*., 2008*a*).

The aim of this work was to examine, using both bi-parentally and uni-parentally inherited molecular markers, the levels of genetic diversity and the patterns of genetic differentiation in two native wild *Salmo* spp. samples from Greece (Louros and Arapitsa) that have never been analysed at the molecular level so far. With respect to taxonomic status, the population from Louros was initially classified together with other Greek *Salmo* spp. populations from Epirus as *Salmo dentex* (Heckel 1851), then was considered to belong to a unique taxon (*Salmo* sp. Louros; Kottelat & Freyhof, 2007) and recently has been described as *Salmo lourosensis* (Delling, 2010). The taxonomic status of the Arapitsa population is unresolved. In order to juxtapose the present results with previous studies performed on *Salmo* spp. populations from the region, three more native wild populations, *i.e.* Agios Germanos (Lake Prespa), Aoos and Moravica were reanalysed at nuclear and mtDNA level, as well. These three *Salmo* spp. populations were selected on the basis that they inhabit waterbodies assumed to be unaffected by stocking activities, they represent three major mtDNA lineages, *i.e.* Adriatic (AD), Mediterranean (ME) and Danubian (DA) and, according to standard morphological methods of identification, they belong to three distinct taxa, namely, *Salmo peristericus* Karaman 1938, *S. dentex* and *Salmo labrax* Pallas 1814 (Kottelat & Freyhof, 2007) (Table I).

MATERIALS AND METHODS

Fin clips of 51 *Salmo* spp. originating from the Louros River (29) and the Arapitsa River (22) were collected in 2008. In addition, specimens originating from three locations (Aoos, Prespa and Moravica) previously analysed for mitochondrial and microsatellite variation (Apostolidis *et al*., 2008*a*, *b*) were reanalysed for this study, to increase the number of

TABLE I. Description of the southern Balkan *Salmo* spp. populations analysed

Number	Population	Sample code	Basin	mtDNA lineage	Taxon	Sample size	
						mtDNA	Microsatellite
1	Louros	LOU	Adriatic–Ionian Sea	AD	*lourosensis*	29	20
2	Arapitsa	NAO	Aegean Sea	AD	*pelagonicus?*	22	20
3	Agios Germanos*	PRE	Adriatic Sea	AD	*peristericus*	11	11
4	Aoos	AOO	Adriatic Sea	ME	*dentex*	22	14
5	Moravica	MOR	Danube River	DA	*labrax*?	22	11

*, Lake Prespa.

FIG. 1. Map of the south-western Balkans showing *Salmo* spp. sampling locations: 1, Louros River; 2, Arapitsa (Aliakmonas River); 3, Agios Germanos (Lake Prespa); 4, Aoos River; 5, Moravica River (Danube River system).

character states and to allow standardization with previous studies. Detailed information of the samples is provided in Table I, and the approximate geographical location of the sampled populations is indicated in Fig. 1. Total genomic DNA was extracted using protocols reported in Apostolidis *et al.* (2008*a*).

Microsatellite polymorphism was quantified at nine loci using fluorescently labelled primers: *Ssad58*, *SsaD190*, *SsaD237* (King *et al.*, 2005), *SsaD85*, *SsaD170* (T. King, unpubl. data), *CA040261*, *CA053307*, *CA060177* (Vasemägi *et al.*, 2005) and *SSsp2213* (Paterson *et al.*, 2004). Loci were PCR amplified and analysed on an Applied Biosystems 3100 automated sequencer (www.appliedbiosystems.com).

Mitochondria1 DNA variation was analysed both by restriction fragment length polymorphism (RFLP) and sequencing performed on PCR amplified products. The RFLP analysis was performed on two adjacent PCR-amplified segments. One encompassed the complete ND5/6 region (*c.* 2·4 kb) and the other segment (*c.* 2·1 kb) comprised the cytochrome *b* gene and the control region (CR). The amplified ND5/6 segment was digested with the restriction endonucleases *Hae*III, *Msp*I, *Ava*II, *Hinf*I and *Alu*I and the CR segment with *Hae*III, *Alu*I, *Bcn*I and *Msp*I. Primers, amplifications, restriction digest and electrophoresis procedures were as described in Bernatchez & Osinov (1995). A 300 bp fragment at the 5′ end and a 441 bp fragment at the 3′ end of the CR were sequenced on representatives of each composite haplotype identified with the PCR–RFLP analysis. The primers LN20 and HN20 (Bernatchez, 2001), which amplify the whole CR, were used for PCR, whereas primers H2 (Bernatchez, 2001) and HN20 in conjunction with a newly designed internal sequencing primer H3 (5′-TGAATTCCAGAGAACCCATGT-3′) were used for sequencing the 5′ and 3′ end of the CR, respectively. Technical procedures of mtDNA purification, amplification and sequencing are detailed in Apostolidis *et al.* (2008*b*). The control region variants observed were deposited in the GeneBank database under accession numbers FJ821448–FJ821452.

Microsatellite allele frequencies, number of alleles per locus and observed and unbiased expected heterozygosities were determined using the programme GENETIX v.4.04 (Belkhir, 2000). Tests of conformation to Hardy–Weinberg equilibrium (HWE) and genotypic linkage disequilibrium were carried out using Markov chain methods. Tests for differences of allele frequencies between pairs of populations were conducted by Fisher's exact test. These analyses were all performed using GENEPOP 3.4 (Raymond & Rousset, 1995). Allelic richness (AR) and F_{IS} values were calculated using the programme FSTAT 2.9.3.2 (Goudet, 2001). Global and pair-wise F_{ST} and their associated P values were estimated using the θ estimator (Weir & Cockerham, 1984) of Wright's F_{ST}. The significance levels for the overall and the pair-wise values were determined after 10 000 permutations. As an alternative measure of the degree of genetic differences among populations, an assignment test was used to determine how unique individual *Salmo* spp. genotypes were to the locality from which they were sampled. Fishes were individually assigned according to the Bayesian method of Rannala & Mountain (1997), with a probability threshold of 5% using the software GeneClass2 version 2.0 (Piry *et al*., 2004). The occurrence of a population bottleneck was tested using the software BOTTLENECK 1.2 (Cornuet & Luikart, 1996), assuming an infinite allele model (IAM), a stepwise mutation model (SMM) or a two-phase model of mutation (TPM, with 90% SMM). The Wilcoxon signed-rank test was used to determine whether a population exhibited a significant number of loci with heterozygosity excess under the mutation model. The occurrence of a population bottleneck was tested for each locality separately. Genetic differences among individuals and populations were displayed by factorial component analysis (FCA) using Genetix v.4.04 (Belkhir, 2000).

Control region sequences were aligned manually with the programme Bioedit version 7.0 (Hall, 1999). The attribution of the sequence haplotypes to each of the main *S. trutta* lineages, previously described by Bernatchez (2001), was performed based on the diagnostic sites characterizing these lineages. The data obtained from restriction analysis and sequencing were pooled, and a presence and absence matrix of restriction sites resolved from RFLP patterns and sequence variation was constructed. Because of the partial overlap of mutational sites detected from the two methods, restriction sites located in the sequenced region were omitted. Phylogenetic relationships between the identified haplotypes were determined by calculating an unrooted haplotype network with the software NETWORK version 4.1 (Fluxus Technology Ltd; www.fluxus–engineering.com). Network calculation was based on the median joining (MJ) and maximum parsimony (MP) algorithms (Bandelt *et al*., 1999), and the robustness of the network topology was tested by increasing the e value ($e = 0–10$), which attenuates the distance criterion.

RESULTS

Five significant deviations from HWE were observed. Three of these were present at the locus *CA06* (at Louros, Arapitsa and Aoos populations), and they were all associated with significant heterozygote deficiencies suggesting the presence of null alleles. Furthermore, for this locus, three DNA samples were not amplified, which could also be interpreted as evidence of a null allele in the form of a null homozygote. Therefore, this locus was removed from further analyses. For the remaining eight microsatellite loci, a total of 113 alleles were found in the five populations studied, ranging from five for locus D190 to 28 for locus *D237* (Table II). Private alleles (PA), *i.e*. found in only one population, were found in all the populations investigated (nPA = 77). Notably, the population of Arapitsa was fixed with specific private alleles at four loci (*D190*, *D58*, *2213* and *D170*), while the populations of Louros, Prespa and Moravica at one locus (*D190*, *D58* and *D190*, respectively). Expected heterozygosities (H_E) ranged from 0·346 to 0·536 and observed heterozygosity (H_O) values varied from 0·314 to 0·508. Genetic diversities for the five populations studied are summarized in Table II.

TABLE II. Summary of microsatellite genetic variability of five southern Balkan *Salmo* spp. populations: the total number of alleles observed at the loci (in square brackets) with allele richness (AR) in parentheses, observed numbers of alleles with AR, deviations from expected Hardy-Weinberg proportions (HW test), expected (H_E) and observed (H_O) heterozygosity, total number of alleles and F_{IS} values at nine microsatellite are given

Population	Louros	Arapitsa	Prespa	Aoos	Moravica
Locus					
D190 [5] (4·25)					
Number of alleles (AR)	2 (1·74)	1 (1)	1 (1)	1 (1)	1 (1)
HW test	NS	—	—	—	—
H_E	0·139	0	0	0	0
H_O	0·150	0	0	0	0
D58 [18] (7·89)					
Number of alleles (AR)	4 (2·83)	1 (1)	1 (1)	8 (6·49)	8 (6·74)
HW test	*	—	—	NS	NS
H_E	0·341	0	0	0·766	0·831
H_O	0·150	0	0	0·615	0·818
D237 [28] (9·93)					
Number of alleles (AR)	8 (6·18)	6 (4·24)	5 (4·48)	10 (7·25)	12 (9·96)
HW test	NS	NS	NS	NS	NS
H_E	0·817	0·520	0·702	0·814	0·883
H_O	0·900	0·550	0·545	0·929	1·000
2213 [11] (7·49)					
Number of alleles (AR)	1 (1·00)	3 (2·91)	5 (3·91)	3 (2·89)	6 (5·30)
HW test	—	NS	NS	NS	NS
H_E	0	0·564	0·612	0·584	0·750
H_O	0	0·632	0·636	0·571	1·000
CA04 [6] (3·38)					
Number of alleles (AR)	1 (1·00)	1 (1·00)	3 (2·40)	2 (1·76)	3 (2·62)
HW test	—	—	NS	NS	NS
H_E	0	0	0·185	0·133	0·265
H_O	0	0	0·100	0	0·300
CA05 [10] (5·85)					
Number of alleles (AR)	2 (1·96)	6 (3·63)	3 (2·95)	4 (2·99)	2 (1·64)
HW test	NS	*	NS	NS	NS
H_E	0·289	0·605	0·483	0·331	0·087
H_O	0·250	0·950	0·091	0·308	0·091
CA06 [9] (5·98)					
Number of alleles (AR)	8 (6·29)	4 (3·37)	4 (3·60)	5 (4·73)	3 (2·63)
HW test	*	*	NS	*	NS
H_E	0·827	0·447	0·607	0·746	0·368
H_O	0·632	0·300	0·727	0·154	0·273
D170 [19] (8·34)					
Number of alleles (AR)	3 (2·94)	3 (2·58)	3 (2·76)	5 (4·15)	11 (8·60)
HW test	NS	NS	NS	NS	NS
H_E	0·508	0·466	0·314	0·671	0·876
H_O	0·579	0·400	0·182	0·571	0·727
D85 [16] (8·74)					
Number of alleles (AR)	4 (3·78)	3 (2·35)	4 (3·99)	7 (6·03)	4 (3·60)
HW test	NS	NS	NS	NS	NS
H_E	0·667	0·516	0·740	0·775	0·645
H_O	0·600	0·650	0·545	0·857	0·364
Total alleles	33	28	29	45	50
F_{IS}	0·117	−0·091	0·269	0·206	0·078
H_E	0·399	0·346	0·405	0·536	0·523
H_O	0·325	0·387	0·314	0·445	0·508

*$P < 0·05$ after using the sequential Bonferroni technique (Rice, 1989). NS, non-significant.

None of the 1800 randomization tests for significance of F_{IS} (for heterozygote deficiency–excess) and of the 7200 permutation tests for gametic phase equilibrium (linkage) resulted in significant outcomes after adjustment for multiple comparisons using a sequential Bonferroni correction (Rice, 1989). Wilcoxon tests were not significant for any of the five samples assuming IAM, TPM or SMM ($P > 0{\cdot}05$), suggesting that populations have neither recently declined nor recently expanded. All global and pair-wise populations tests for differences of allele frequency distributions were highly significant ($P < 0{\cdot}001$). This translated into highly significant partitioning of the genetic variation among sampling locations ($F_{ST} = 0{\cdot}55$; $0{\cdot}39 \leq 95\%$ C.I. $\leq 0{\cdot}72$). Furthermore, all pair-wise population differentiation estimates (pair-wise F_{ST}) were high, ranging from 0·29 (between Aoos and Moravica) to 0·65 (between Arapitsa and Louros), with all being significant ($P < 0{\cdot}01$) after Bonferroni correction for multiple tests. As a consequence, all *Salmo* spp. could be correctly assigned to their population of origin based on individual assignment test (quality index: 99·87%).

The factorial component analysis (FCA) plot showed that *Salmo* spp. sampled from different localities are clustering in three distinct groups (Fig. 2). The first two groups consisted, respectively, of individuals from Arapitsa and Louros and were mainly discriminated by the first axis (10·73% inertia). The third group, placed at the negative side of the second axis (inertia 9·18%), consisted of individuals from Prespa, Aoos and Moravica. Within this group, however, the populations of Prespa and Aoos could be discriminated regarding the third and fourth axes, respectively (inertia 7·35% and 6·01%, respectively). No apparent distribution of individuals among the three main groups was revealed, further illustrating the deep genetic differentiation among them. It is noteworthy that the third group of populations was composed of individuals that, according to mtDNA analyses, belonged to three different major mtDNA lineages (Fig. 3).

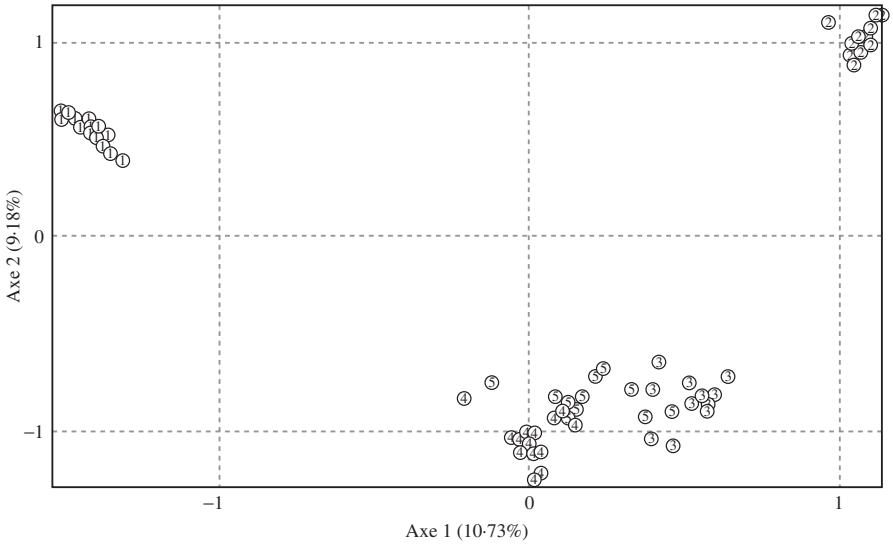

FIG. 2. A plot of individual *Salmo* spp. on axes 1 and 2 of the correspondence analysis. Numbers indicate each of the five southern Balkan populations as presented in Fig. 1.

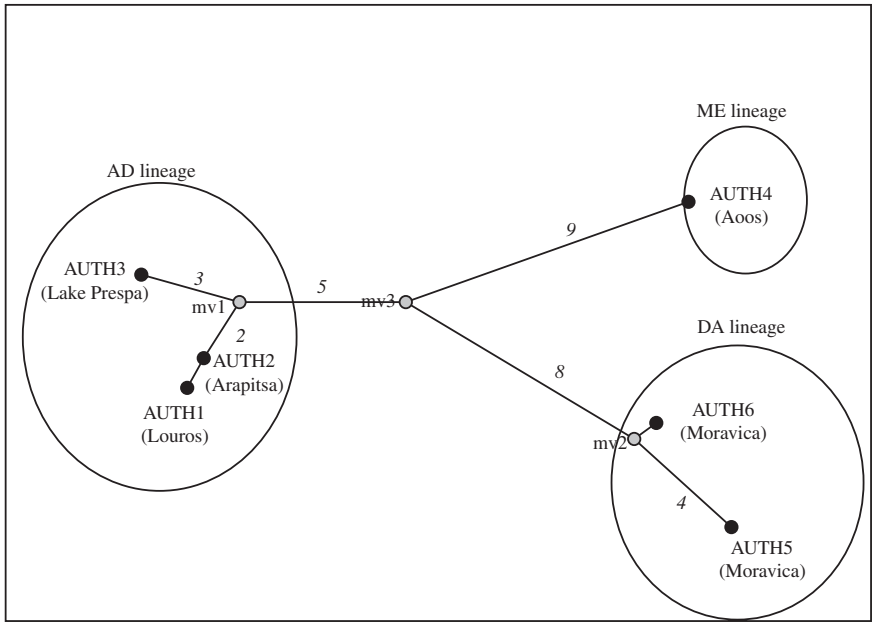

FIG. 3. Median-joining network showing the phylogenetic relationships among mtDNA haplotypes (AUTH1–AUTH6) of southern Balkan *Salmo* spp. populations (with $e = 0$). Each haplotype is represented by a different (●), while the (○) show the missing intermediate haplotypes (mv1–mv3). The mutational step for each branch between haplotypes is indicated by italicised numerals, except for the lines with a single mutational step. AD, Adriatic; DA, Danubian; ME, Mediterranean.

The RFLP analysis generated a total of 55 restriction sites of which 22 were variable. Polymorphism was found in both regions examined. Fragment patterns generated by each enzyme and restriction site differences between genotypes are available (unpubl. data). Five different haplotypes were found in the individuals examined. The pair-wise sequence divergence estimates among the five haplotypes were determined using the REAP computer package (McElroy *et al*., 1991) and varied from 0·82 to 4·55%. It is interesting to note that all samples but Moravica were fixed with only one haplotype, while the populations from Louros and Arapitsa were fixed with the same haplotype. Therefore, intra-population haplotype and nucleotide diversity was zero for four populations (Louros, Arapitsa, Prespa and Aoos). Contrary to the generally low intra-population diversity, the mean inter-population nucleotide diversity observed was high (mean = 2·79%) and translated in a very high mean F_{ST} estimate of 0·95.

The nucleotide sequence of a 300 bp segment at the 5′-end of CR and of a 441 bp segment at the 3′-end, adjacent to tRNA Phe gene, was determined for at least two representatives of each sample and each haplotype (20 individuals in total). Pair-wise sequence comparisons of the haplotypes analysed identified seven variable nucleotide positions for the 5′-end and five for the 3′-end segment of the control region. Seven of them (four and three for the two CR ends, respectively), however, were observed exclusively at the two haplotypes from Moravica (DA-Mo1 and DA-Mo2, GeneBank Acc No FJ821451 and FJ821452). By combining the variable positions for both CR segments, five haplotypes were found (AD-s1, AD-s8, Me-s1, DA-Mo1 and

DA-Mo2). Notably, the samples from Arapitsa and Prespa share the same haplotype (AD-s1). This haplotype is identical to the ADcs1 haplotype (Cortey *et al*., 2004), which is the central and presumably ancestral haplotype in the lineage (Cortey *et al*., 2004; Susnik *et al*., 2007) and it is one mutational step distant (position 162 in Bernatchez, 2001) from the single haplotype (AD-s8, GeneBank Acc No FJ821449) found in Louros samples. This haplotype, however, differs from Ad1 described in Bernatchez *et al*. (1992) in a set of three mutational sites identified in the 3′-end segment of the control region (positions 6, 27 and 44 in Bernatchez *et al*., 1992), and consequently, none of the individuals sequenced possessed the Ad1 haplotype. Pair-wise sequence comparison revealed that this set of the three mutational sites is also shared by the rest of the haplotypes reported in this study, as well as by all Balkan and Iberian haplotypes reported so far (Cortey *et al*., 2004; Susnik *et al*., 2007; Snoj *et al*., 2009; Lo Brutto, *et al*., 2010). Pair-wise sequence divergence estimates among mtDNA variants detected in the 741 bp CR segment varied from 0·14 to 1·37% and are comparable with those reported by Bernatchez *et al*. (1992) for a 640 bp of the CR (0·16–1·92%) (*i.e.* for the same 640 bp segment, the values in this study varied from 0·16 to 1·61%).

The combination of both RFLP and sequence data sets added one additional haplotype to the five identified by RFLP or sequence analysis alone and differentiated the samples from Louros and Arapitsa, which were identical in RFLP analyses. The MJ network constructed from the combined data sets showed a relatively high number of mutations among most of the haplotypes (ranging from 1 to 21), and the relationships among haplotypes were in some cases defined by the introduction of missing intermediate haplotypes (Fig. 3). It is noteworthy that haplotypes AUTH2 and AUTH3, which were identical in the CR sequence region (Ads1), differed by five mutational steps according to RFLP analyses (Fig.3).

DISCUSSION

There was no correlation between levels of intra-population diversity observed in the mitochondrial and nuclear genome, since all populations except Moravica were fixed with one mtDNA haplotype. The population from Moravica, which was the only one found polymorphic at the mtDNA level, had also the highest value of nuclear heterozygosity ($H_O = 0{\cdot}508$; Table II). Contrary to the relative low levels of intra-population variability, very strong genetic differentiation among populations was evident from all pair-wise comparisons of allele or haplotype frequency distributions and F_{ST} values computed by each method. The F_{ST} estimates found in the present study are among the highest values reported between fish populations at a micro or even macro-geographical scale (Fumagalli *et al*., 2002) and are comparable with previous allozyme, microsatellite and mtDNA analyses of Greek *Salmo* spp. populations (Apostolidis *et al*., 2008*a*).

The present results showed lack of genetic differentiation in association with distinct lineages defined by mtDNA, implying discrepancies between mitochondrial and microsatellite-based phylogenies. For example, the phylogenetic analyses using mtDNA data placed the Arapitsa, Louros and Prespa haplotypes within the Adriatic phylogeographical lineage of the *S. trutta* complex, suggesting that these rivers were initially colonized by a common Adriatic haplotype (Ads1/AD-cs1, Bernatchez,

2001; Cortey *et al*., 2004) and then *S. trutta* populations underwent a subsequent differentiation. On the other hand, in the FCA analysis used, the populations of Arapitsa and Louros grouped separately, while the remaining three populations (which according to mtDNA data represent pure Adriatic, Mediterranean and Danubian lineages) clustered in a closest group.

Discrepancies in patterns of population differentiation between mitochondrial and nuclear-based markers could potentially be caused by several factors (Lu *et al*., 2001) and have frequently been reported for *Salmo* spp. populations in the literature (Bernatchez & Osinov, 1995; Fumagalli *et al*., 2002; Apostolidis *et al*., 2008*a*). Also, in the present study, the potential stochastic effects of low sample sizes, which could be important for microsatellite analyses and impose some limitations to the interpretation of the results, though these probably are, at least partly, compensated by the low number of alleles per sample cannot be ignored. Thus, sample sizes could be responsible for the high numbers of private alleles observed among populations and affect some of the estimates, such as absolute F_{ST} values. Nevertheless, this cannot explain all differences observed. For instance, analyses based on individual tests (assignment and correspondence analyses in Fig. 2) clearly show that there is little variation among individuals, which are all tightly clustered to their location of origin without sign of admixture.

McKeown *et al*. (2010) highlighted the importance of examining a large section of the mtDNA genome to infer phylogeographic structure, as genealogical information in different segments of the mtDNA genome is additive rather than duplicated. Here, however, it should be emphasized that most of the phylogenetic studies on the *S. trutta* complex were based on CR sequences. Yet, in a number of studies in salmonids, including the genus *Salmo*, the CR appears to evolve at a lower rate than some other regions of mtDNA (Apostolidis *et al*., 1997; Susnik *et al*., 2006). The reason for this discrepancy between intraspecific or interspecific divergences based on the CR appears to be evolutionary constraints on the CR within the genus *Salmo* (Susnik *et al*., 2006). Similar species-specific constraints on the CR, which lead to underestimated interspecific divergences compared to coding segments, have been also demonstrated in other salmonid genera such as *Thymallus* and *Hucho* (Froufe *et al*., 2005). Thus, the actual divergence among the three Adriatic *Salmo* spp. samples (Louros, Arapitsa and Prespa) must be considerably greater than the phylogenetic analyses based on, or including, the CR indicate.

Assessment of the species status of the *Salmo* spp. populations studied based on genetic data alone is rather complex. Microsatellite data support a clear distinction of the Louros and Arapitsa samples from the rest of the trout populations. The comparison of their mtDNA haplotypes with *S. trutta* lineages throughout Europe, however, results in paraphyly, suggesting that each of these trout populations has evolved from within the Adriatic lineage of *S. trutta*. Apart from allele homoplasy that could compromise the phylogenetic informativeness of microsatellite analyses (Paetkau *et al*., 1997), microsatellite differentiation is maybe a reflection of the extent of genetic drift in small isolated populations and irrelevant from a phylogenetic perspective. Indeed, the populations of Louros and Arapitsa had considerable fewer alleles overall than those of Aoos and Moravica (Table II), suggesting that they could have been subjected to greater genetic drift than the latter two populations. Instead, mitochondrial introgression among lineages and taxa in the apparent absence of nuclear introgression could be another possible explanation. This phenomenon

has been reported in several fish species including salmonids (Glemet *et al*., 1998). Since the present results are based on a small number of populations and exclude several taxonomically critical proposed species, nomenclatural conclusions cannot yet be drawn. A wider geographical range of sampled *Salmo* spp. populations with larger sample sizes and an increased phylogeographic resolution will be required for the validation of the proposed classification and for understanding the processes of evolution that have occurred in the genus *Salmo*, particularly in the Balkan Peninsula. On the other hand, from an evolutionary or a population biology point of view, it is genetic integrity rather than purely taxonomic status that is of interest. The results provide evidence that the populations studied may be on independent evolutionary trajectories, thus suggesting that effective conservation measures should be focusing on the preservation of the genetic integrity of each population separately rather than solely of each evolutionary lineage or defined taxon.

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