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Phylogenetic status of brown trout *Salmo trutta* populations in five rivers from the southern Caspian Sea and two inland lake basins, Iran: a morphogenetic approach

I. Hashemzadeh Segherloo*†, H. Farahmand‡, A. Abdoli§, L. Bernatchez‖, C. R. Primmer¶, A. Swatdipong**, M. Karami‡ and B. Khalili‡

**Department of Aquaculture, Faculty of Natural Resources and Earth Sciences, University of Shahre Kord, 115, Iran, ‡Department of Fisheries and Environmental Sciences, Faculty of Natural Resources, University of Tehran, Karaj, 4111, Iran, §Department of Biodiversity and Ecosystem Management, Environmental Research Institute, University of Shahid Beheshty, G.C. Velenjak, Tehran, Iran, ‖Institut de Biologie Intégrative et des Systèmes, Pavillon Charles-Eugène-Marchand, Université Laval, Québec, QC, G1V 0A6, Canada ¶Department of Biology, 20014, University of Turku, Finland and **Department of Genetics, Faculty of Science, Kasetsart University, Bangkok, 10900, Thailand*

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Interrelationships, origin and phylogenetic affinities of brown trout *Salmo trutta* populations from the southern Caspian Sea basin, Orumieh and Namak Lake basins in Iran were analysed from complete mtDNA control region sequences, 12 microsatellite loci and morphological characters. Among 129 specimens from six populations, seven haplotypes were observed. Based on mtDNA haplotype data, the Orumieh and southern Caspian populations did not differ significantly, but the Namak basin–Karaj population presented a unique haplotype closely related to the haplotypes of the other populations (0·1% Kimura two-parameter, K2P divergence). All Iranian haplotypes clustered as a distinct group within the Danube phylogenetic grouping, with an average K2P distance of 0·41% relative to other Danubian haplotypes. The Karaj haplotype in the Namak basin was related to a haplotype (*Da26*) formerly identified in the Tigris basin in Turkey, to a *Salmo trutta oxianus* haplotype from the Aral Sea basin, and to haplotype Da1a with two mutational steps, as well as to other Iranian haplotypes with one to two mutational steps, which may indicate a centre of origin in the Caspian basin. In contrast to results of the mtDNA analysis, more pronounced differentiation was observed among the populations studied in the morphological and microsatellite DNA data, except for the two populations from the Orumieh basin, which were similar, possibly due to anthropogenic causes.

Key words: centre of origin; Danube phylogenetic grouping; microsatellite; morphology; mtDNA control region.

†Author to whom correspondence should be addressed. Tel.: +98 9125461127; email: ihashem@nres.sku.ac.ir

INTRODUCTION

The numerous forms of brown trout *Salmo trutta* L. 1758 have been classified under different taxonomic groupings. For example, *c*. 50 species have been described for varieties of *S. trutta*, including 10 species found only in the British Isles (Elliot, 1994). According to Berg (1948), S. *trutta* is represented by six subspecies within the former Soviet Union: *Salmo trutta trutta* L. 1758, *Salmo trutta labrax* Pallas 1814, S*almo trutta caspius* Kessler 1877, *Salmo trutta oxianus* Kessler 1874, *Salmo trutta aralensis* Berg 1908 and S*almo trutta ezenami* Berg 1948. Additionally, Kottelat & Freyhof (2007) referred to different populations of Caspian trout as *S. trutta* (northern Caspian basin), *Salmo ciscaucasicus* Dorofeeva 1967 (western Caspian basin), and *Salmo caspius* Kessler 1877 (southern Caspian basin).

These classifications are based mainly on morphological data and may reflect the phyletic and reproductive relationships of different populations, forms and species, poorly in some cases (Bernatchez, 1995; Osinov & Bernatchez, 1996). Many publications, including the IUCN Red List of Threatened Species (www.iucnredlist.org), have called for the identification of taxa (usually species and subspecies) that require conservation. Such identifications are generally based on morphological systematics, which can be misleading and may channel conservation activities toward genetically indistinct subspecies, rather than distinct lineages in need of conservation (Freeland, 2005).

Genetic markers, in association with morphologic and reproductive data, can frequently be helpful in resolving the taxonomy of particular groups of populations (Frankham *et al.,* 2002; Sušnik *et al.,* 2006, 2007). Bernatchez *et al*. (1992), Giuffra *et al*. (1994), Bernatchez & Osinov (1995), Bernatchez (1995), Apostolidis *et al*. (1997), Weiss *et al*. (2000) and Bernatchez (2001) revealed five major phylogenetic lineages in the *S. trutta* complex using mtDNA sequences, including Atlantic (AT), Mediterranean (ME), Adriatic (AD), Danube (DA) and Marmoratus (MA) phylogenetic lineages, throughout the range of this species. Additionally, Suárez *et al*. (2001) suggested another phylogenetic lineage of this complex inhabiting Duero (DU) in the Iberian Peninsula, and Marić *et al*. (2006) and Snoj *et al*. (2009) suggested a new phylogenetic group (Balkan cluster) in the Balkan peninsula. Among the *S. trutta* populations distributed in the former Soviet Union, only two phylogenetic groupings (DA and AT) were identified by Osinov & Bernatchez (1996), with DA being distributed in the basins of the Black, Caspian and Aral Seas.

The phylogenetic status of several regional *S. trutta* populations, including populations inhabiting Iranian inland basins and The (Persian) Gulf basin (Turkey) as well as North African populations, was previously unknown (Elliot, 1994). Recent reports place *S. trutta* populations from the Balikli and Fyrat Rivers (Bernatchez, 2001) and Catak Cay (Tigris basin) (Sušnik *et al.,* 2005) from The Gulf basin in the DA phylogenetic lineage, and the *S. trutta* populations from north-west Africa (Atlas Mountains) have recently been reported as members of the Atlantic lineage (Snoj *et al.,* 2011). This was the case even for morphologically defined species, such as *Salmo platycephalus* Behnke 1968, that were previously attributed to the Da lineage of *S. trutta* based on phylogenetic studies (Sušnik *et al.,* 2004).

Two inland lake basins (Namak and Orumieh) sustain *S. trutta* populations with an uncertain phylogenetic affinity. These lake systems are isolated by mountain chains stretching from the north-west to the south (Zagros Mountains) and from the

north-west to the north-east (Alborz Mountains) in Iran. These lakes are isolated from the southern Caspian basin in the northern part of the country, where resident and migratory forms of *S. trutta* occur (Abdoli, 2000). *Salmo trutta* inhabiting the Orumieh Lake basin, especially the Liqvan River, are a distinct phenotype, which has led some authors to propose them as a candidate subspecies of *S. trutta* (Abdoli, 2000). No detailed phylogenetic or morphological study, however, has been conducted on these populations to reveal their phylogeny and origin. A phylogenetic study using mtDNA sequences from five specimens identified as *S. trutta* collected from southern Caspian rivers in Iran showed that the individuals were actually rainbow trout *Oncorhynchus mykiss* (Walbaum 1792)(Osinov, 2009).

The origin and taxonomy of Iranian inland populations of *S. trutta* (Namak basin) have been placed in contention by referring to them as *S. t. macrostigma* (Duméril 1858) (Derzhavin, 1929; Berg, 1949; Coad, 2011), *S. t. caspius* (Saadti, 1977), *S. fario caspius* Kessler 1877 (Dadikyan, 1986; Coad, 2011), and *S. caspius* (Kottelat & Freyhof, 2007). Authors identifying the Namak basin trout as *S. trutta macrostigma* have presumed a Mediterranean origin for these populations via The Gulf and Zagros mountains (Coad, 2011), but Saadati (1977) refuted this assumption, because *S. trutta* populations are absent from the Iranian Persian Gulf basin and Zagros mountains. The fish inhabiting the Orumieh basin, particularly the Liqvan River population, are known to be a subspecies of *S. trutta* (Abdoli, 2000; Coad, 2011).

In this study the truss system of morphometric analysis (Turan, 1999) and both uniparentally (mtDNA control region complete sequences) and bi-parentally (microsatellite loci) inherited molecular markers were used to examine the phylogenetic and taxonomic status and origin of these *S. trutta* populations and their relationships to one another.

MATERIALS AND METHODS

SAMPLE COLLECTION

Salmo trutta ($n = 102$) were collected by electrofishing in four rivers from three basins during late summer 2006 (Table I and Fig. 1). Legal limitations restricted the sample sizes to a maximum of 30 fish per collection, and the numbers of some collections were further restricted by availability of fish (Table I). Electrofishing was conducted over river stretches >100 m in order to avoid the problem of family sampling (Hansen *et al*., 1997). Fish were anaesthetized and after tagging, the left side was photographed and the right pectoral fin was clipped, tagged as for the whole fish, and fixed in 96% ethanol. The fish was fixed in 4% formalin, after death from over-anaesthetization, for further analysis.

MORPHOLOGICAL ANALYSIS

Phenotypic characterization was based on 10 meristic and 120 metric traits. The meristic traits examined were the red spots on the left side, pectoral, pelvic, dorsal and anal fin soft rays, pored lateral-line scales, scales between the origin of the dorsal fin and the lateral line and between the origin of the adipose fin and the lateral line, the left branchiostegal rays, and the gill rakers on the first gill arch.

For morphometric analysis, photographic images were made of the left side of each specimen. All specimens were placed in the same position for photography. Specimens were pinned to a white board using coloured pins to facilitate location of landmarks in the digital photographs. Sixteen landmark points were positioned on morphological points (Bookstein, 1991; Fig. 2).

TABLE I. Sample locations, basins, population codes, haplotype codes, counts and the number of *Salmo trutta* analysed for mtDNA, microsatellite (M_{sat}) and morphometric (Morph) variation, molecular diversity data and geographical co-ordinates

Population	Basin	Population code	Number of individuals assessed			Location	Nat. bar.		Molecular diversity data									
									mtDNA haplotype count							Msat diversity		
			mtDNA	Msat	Morph		Intra b.	Inter b.	H1	H2	H_{Co}	Ba	Ka	M1	M2	A_r	H_o	H_e
Haraz	Ca	Ha	20	20	20	35° 51′ N; 16″ 52° 03′ E 23″	No	Yes	1	1	18	0	0	0	0	3·83	0·313	0·342
Babolrud		Ba	24	24	—	36° 14′ N; 21″ 52° 36′ E 10″	No		0	0	23	1	0	0	0	1·58	0·071	0·072
Shirinrood		Sh	3	3	—	36° 9′ N; 04″ 53° 20′ E 52″	No		0	0	3	0	0	0	0	—	—	—
Karaj	Na	Ka	30	30	30	36° 00′ N; 59″ 51° 16′ E 22″	Yes	Yes	0	0	0	0	30	0	0	2·58	0·326	0·312
Liqvan	Or	Li	32	32	30	37° 52′ N; 18″ 46° 27′ E 56″	Yes	Yes	0	0	32	0	0	0	0	2·50	0·261	0·288
Mardagh		Ma	20	20	20	37° 33′ N; 17″ 46° 26′ E 13″	Yes		0	0	18	0	0	1	1	2·83	0·274	0·283

Ca, Caspian Sea; Na, Namak Salt Lake; Or, Orumieh Salt Lake, Nat. bar., natural barriers; Intra b., intra basin; Inter b., inter basin; A_r, allelic richness; H_O, observed heterozygosity; H_e, expected heterozygosity.

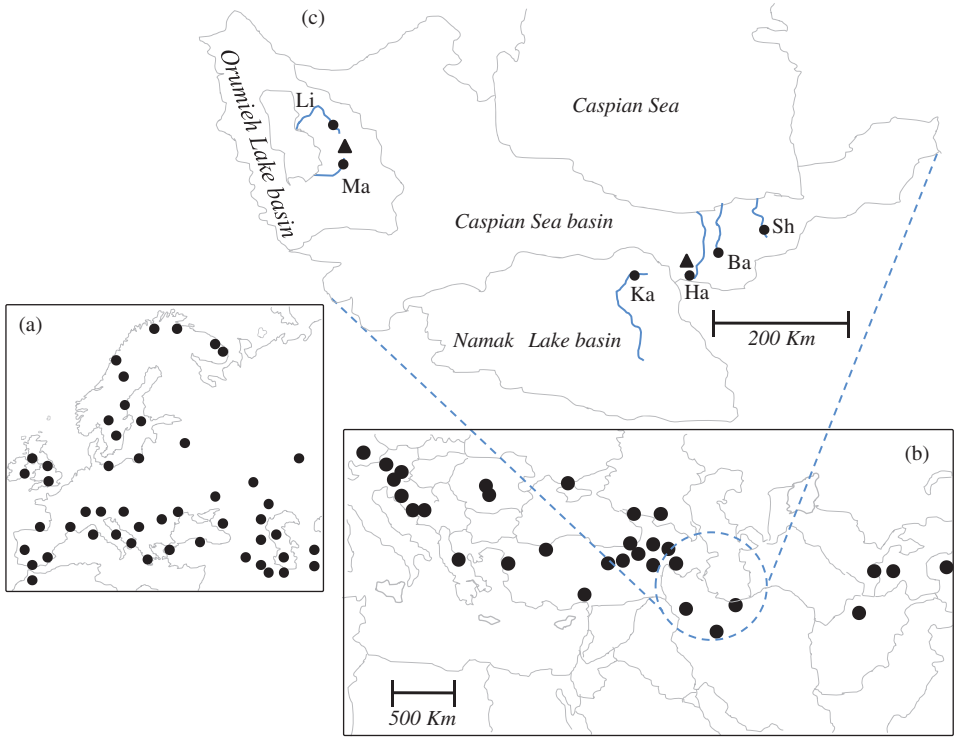

FIG. 1. (a) Distribution map of *Salmo trutta* lineages throughout their range, (b) detailed map of the distribution of the Danube lineage of *S. trutta* and (c) map showing the basins and rivers investigated in this study: Ka, Karaj River; Ha, Haraz River; Ba, Babolrud River; Sh, Shirinrud River; Li, Liqvan River; Ma, Mardagh River.

Landmarks were digitized using tpsDIG 2·10 software (Rohlf, 2006), and the X and Y co-ordinates of the landmarks were converted to linear interlandmark distances using the Pythagorean theorem as formulated in an Excel spread sheet (Turan, 1999). The extracted distance variables were processed using the ratio of each measured distance to the centroid size of the specimen (Bagherian & Rahmani, 2007; Ruehl & DeWitt, 2007) to eliminate size effects (Turan, 1999). Centroid size was calculated using tpsRelw 1·45 (Rohlf, 2007). Spearman (r_s) and Pearson (r) correlation tests were used for meristic and morphometric variables, respectively, to test whether any variables were significantly correlated with size.

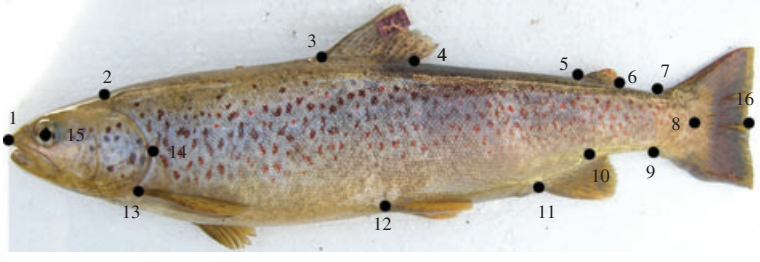

FIG. 2. The 16 landmarks (1–16) used for morphometric analysis of *Salmo trutta* populations.

The sex of the specimens was determined visually, and an ANOVA test was conducted to detect sex differences. Meristic and size-corrected metric data were subjected to discriminant function analysis (DFA). Forward stepwise DFA based on the Mahalanobis distance was used to determine the relationships among the populations studied (Turan, 2004). The resulting discriminant functions were used to assign individuals to particular samples. The relative importance of the morphometric and meristic characters in discriminating populations was assessed using the F-to-remove statistic (F-to-enter, 4; F-to remove, 3·9). Euclidian distance matrices of the morphometric and meristic data were compared to the allele sharing distance matrix (DAS) using a Mantle test to determine if there was a correlation between morphological and genetic distances (Froufe *et al.,* 2003). The statistical analyses were performed using SPSS v11·5 (www.ibm.com/spss statistics), SYSTAT v9 (www.systat.com) and XLSTAT v2010 (www.xlstat.com).

mtDNA ANALYSIS

DNA extraction was performed using the Chelex 100 method (Estoup *et al.,* 1996). The complete mtDNA control region was amplified in 129 individuals using the primers BrtD-F20 (5′-GAGATTTTAACTCCCACCCT-3′) and BrtD-R20 (5′-TAGGGTCCATCTTAACAGCT-3′), which were designed based on the sequences published in GenBank (NCBI) for *S. trutta* mtDNA. Two internal primers were also designed to amplify and sequence fragments of nearly 0·5 kb of the control region in order to correct any ambiguities in base pair reading resulting from the poly T region in the centre of the control region. These internal primers were InternalF (5′-ACGGGCAATAAGATTGACAC-3) and InternalR (5′-TCTTGAATTCCAGAGAACCC-3′). Polymerase chain reaction (PCR) amplifications were performed in 50 μl volumes. Each reaction contained 35·6 μl H_2O, 5 μl 10X Buffer, 1 μl 50 mM $MgCl_2$, 1 μl of a 10 mM solution of each primer, 1 μl 25 mM deoxynucleotide triphosphates (dNTP), 0·4 μl BioTaq DNA polymerase and 5 μl template (Estoup *et al.,* 1996). The PCR conditions used were: initial denaturation at 94° C for 10 min followed by denaturation at 94° C for 1 min, annealing at 52° C for 1 min, and extension at 72° C 90 s for 30 cycles with a final extension at 72° C for 15 min. All 129 amplified fragments were sequenced on an ABI-3130 DNA sequencer following the manufacturer's protocol (Applied Biosystems; www.appliedbiosystems.com) and aligned using BioEdit v7·0.0 (Applied Biosystems). The mtDNA sequences generated in this study have been deposited in GenBank under accession numbers JF276028–JF276034.

To explore the relationships among the Iranian *S. trutta* populations, the Kimura two-parameter (K2P) distances among the haplotypes were calculated. The maximum parsimony (MP) method implemented in MEGA version 4 (Tamura *et al.,* 2007) and a quartet-puzzling maximum likelihood procedure (ML) implemented in Tree-Puzzle 5·2 (Schmidt *et al.,* 2002) were used to reconstruct the phylogeny of the studied fish, with the Atlantic salmon *Salmo salar* L. 1758 being used as an outgroup. In addition to the new sequences, 27 haplotypes representing previously described lineages (Bernatchez, 2001; Suárez *et al*., 2001) were analysed (Table SI, supporting information). For the MP analysis, a heuristic search (10 replicates) with a bootstrap test of the phylogeny with 1000 replicates was used. To implement the ML method of phylogenetic reconstruction, HKY + Γ (Hasegawa *et al.,* 1985) was selected using an online model test (Posada & Crandall, 1998). To visualize the relationships among haplotypes, a haplotype network was produced using the Templeton, Crandall and Sing method, TCS 1·21 (Clement *et al.,* 2000). To analyse the mitochondrial genetic differentiation among the Iranian populations, G_{ST} statistics (Nei, 1973) were calculated using DnaSP v. 5·0 (Librado & Rozas, 2009).

MICROSATELLITE ANALYSIS

Total genomic DNA was extracted from the fin clips using a salt extraction method (Aljanabi & Martinez, 1997). Samples were genotyped for 12 dinucleotide microsatellite loci (for more details on microsatellite loci see Tables SII and SIII, supporting information).

The samples were genotyped using single PCR reactions optimized for each primer set. Four fluorescent dyes were used, and unlabelled primers had a GTTT tail added to the 5′ end

to enhance the adenylation of the nascent DNA strand and facilitate accurate genotyping (Brownstein *et al.,* 1996). The primers for Str543INRA, Str15INRA, StruttaINRA, Strutta58 and Str85INRA were re-designed to overcome allele-size overlaps (Swatdipong, 2009). The single PCRs (10 μl) consisted of 1 μl (70-80 ng) template DNA, 1 μl (5 pmol) of both primers, 1 μl 10X PCR buffer, 0·3 μl $MgCl_2$(50 mM), 0·1 μl 25 mM dNTPs, 0·02 μl 5 $U\mu l^{-1}$ BioTaq DNA polymerase and 6·58 μl H_2O. Amplifications were carried out in a PTC100 thermal cycler (MJ Research; www.mjr.com), a 2720 Thermal Cycler (Applied Biosystems), or a Piko thermal cycler (Finnzymes Instruments; www.finnzymes.fi). PCR amplifications of all microsatellite loci, except Str85, Str543, Strutta58 and Str15, were run under the following thermal conditions: 3 min at 94° C followed by 30 cycles of 30 s at 94° C, 30 s at an annealing temperature specific to each target sequence, 30 s at 72° C, and 5 min at 72° C. For the other loci, the conditions were 3 min at 94° C followed by 30 cycles of 30 s at 94° C, 30 s at the annealing temperature, 1 min at 72° C, and final extension for 10 min at 72° C.

For all samples, the amplicons were diluted and denatured and subsequently subjected to electrophoresis on an ABI Prism 3130X1 genetic analyser (Applied Biosystems) with a GeneScan 600 LIZ size standard (Applied Biosystems). The sizes of the DNA fragments were identified using GeneMapper 4·0 (Applied Biosystems), and all genotypes were manually inspected. Some specimens from the Inari Lake Basin in the Barents Sea region in northern Europe (Nukk population) were included in the genotyping analysis as an outgroup.

Exact probability tests for deviations from Hardy–Weinberg equilibrium (HWE) across populations within loci and loci within populations and exact tests for deviations from genotypic linkage equilibrium (LE) across populations were performed using GenePop 4·0.10 (Rousset, 2008). A sequential Bonferroni correction (Holm, 1979) was used to correct for multiple testing.

Interpopulation genetic divergence was calculated with the multilocus F_{ST} estimator (Weir & Cockerham, 1984) using FSTAT 2.9.3.2 (Goudet, 1995). Tests for genetic differentiation among population pairs were conducted using GenePop 4.0.10 (Rousset, 2008). Population differentiation also was evaluated through assignment of individuals to their putative populations of origin based on multi-locus genotypes. The Bayesian assignment method (Rannala & Mountain, 1997) implemented in GeneClass2 (Piry *et al.,* 2004) was performed with the assignment threshold score set to 0·05 and with Monte–Carlo resampling using the simulation algorithm of Cornuet *et al*. (1999). Signals of recent population bottlenecks were assessed using the Wilcoxon sign-rank and mode-shift tests as implemented in the software BOTTLENECK 1.2.02 (Cornuet & Luikart, 1996) assuming the two-phase model (TPM) of mutation for microsatellite loci with 5% multi-step changes and variance of 12 (Gum *et al.,* 2003).

RESULTS

MORPHOMETRIC DATA

Some degree of statistical association was found between most meristic characters and the fish body size across the entire data set, but it was not linear and therefore not allometric. The size-corrected metric characters showing a statistically significant correlation ($P \leq 0{\cdot}05$) with the size of the fish were excluded from further analysis. No significant difference was found between sexes for most of the characters ($P \leq 0{\cdot}05$); thus, after excluding the characters that differed between the sexes, all specimens were pooled into population groups.

In the DFA of the meristic data, three canonical functions were produced. Plotting the first two functions (representing 77 and 21% of the total dispersal, respectively) revealed between-population differences. The 95% confidence ellipses of the two populations from the Orumieh Lake basin overlapped and were clearly distinct from the populations of the Namak Lake basin (Karaj River) and the Caspian basin (Haraz River) (Fig. 3). The DFA showed that the counts of red spots (RSp), gill

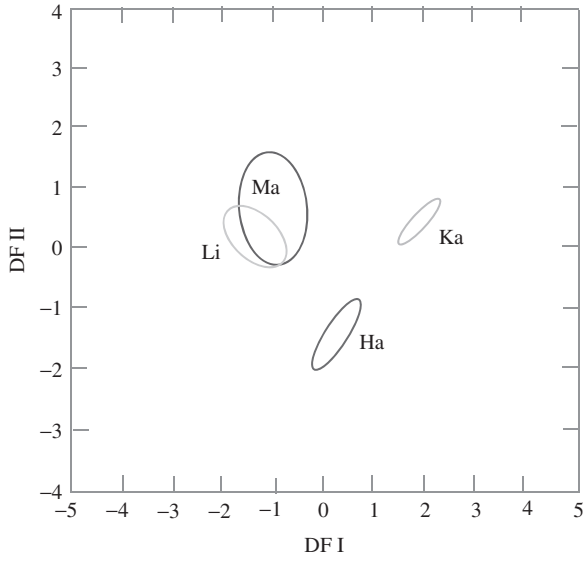

FIG. 3. Ninety-five per cent confidence ellipses of the discriminant function (DF) analysis scores for the meristic analysis of *Salmo trutta*. Ma, Mardagh; Li, Liqvan; Ka, Karaj; Ha, Haraz (see Fig. 1).

rakers of the first gill arch (GR), scales between the lateral line and adipose fin origin (LLAF) and lateral line scales (LL) were effective countable characters in discriminating the studied populations (Table II).

Discriminant function analysis of meristic characters produced three discriminant functions for morphometric variables. The first two function scores (representing 62 and 30% of total dispersal) were plotted against each other to visualize the dispersal of the populations (Fig. 4). The populations from the Orumieh Lake basin (Ma and Li) were closer to each other than to the populations from the other two studied basins. Among the morphometric variables analysed, L1-12, L5-6, L6-7, L6-12, L9-10 and L2-13 (Table III) were the effective variables for discriminating populations (Table III; $P < 0{\cdot}001$).

TABLE II. Summary of forward stepwise discriminant function analysis on meristic characters of *Salmo trutta*

Variable	Variable description	*F*-to enter	Wilks Lambda	App. *F**	*P*
RSp	Counts of red spots	34·26	0·472	34·26	<0·001
GR	Counts of gill rakers	17·46	0·229	25·07	<0·001
LLAF	Counts of scales between lateral line and the origin of adipose fin	8·64	0·233	19·96	<0·001
LL	Counts of pored scales on lateral line	4·53	0·202	16·27	<0·001

*Approximate *F*-statistic for Wilks Lambda.

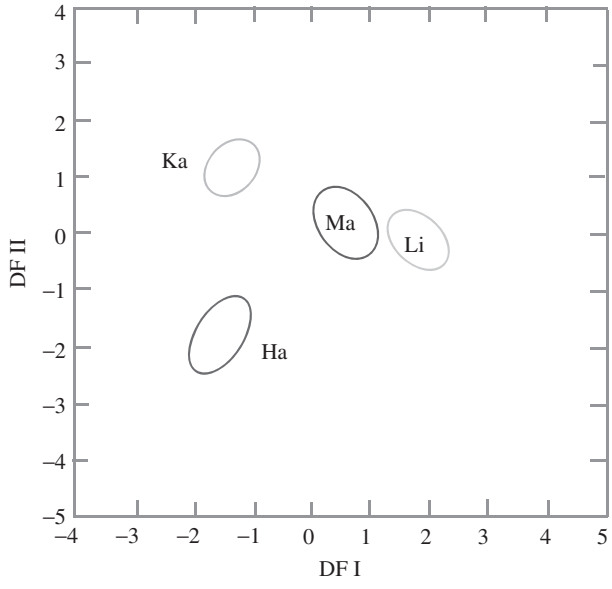

FIG. 4. Ninety-five percent ellipses of the discriminant function (DF) analysis scores for the morphometric analysis of *Salmo trutta*. Ka, Karaj; Ma, Mardagh; Li, Liqvan; Ha, Haraz (see Fig. 1).

In assignment tests using a jack-knifing method for the meristic and morphometric characters, the average proportions of correct assignments were 68% (Ha: 95%; Ka: 97%; Li: 38% and Ma: 42%) and 79% (Ha: 84%; Ka: 86%; Li: 72% and Ma: 74%), respectively. The lower assignment success observed for the populations of the Orumieh Lake basin was due to their similarity, and the individuals from these two populations were mostly mis-assigned to each other. When the Orumieh populations were treated as a single population and the assignment test was conducted at the interbasin level, the percentage of correct assignments for Orumian populations increased to 75%, with average correct assignment of 89% over all the studied

TABLE III. Summary of forward stepwise discriminant function analysis on morphometric variables of *Salmo trutta*

Variable	Variable description	*F*-to enter	Wilks Lambda	App. *F**	*P*
L1–12	Snout to pelvic fin base	26·092	0·548	26·092	<0·001
L5–6	Origin of adipose fin to its end	22·712	0·318	24·243	<0·001
L6–7	Adipose-fin end to upper origin of caudal fin	19·037	0·197	23·847	<0·001
L6–12	Adipose-fin end to pelvic-fin base	8·142	0·156	20·657	<0·001
L9–10	Lower origin of caudal fin to anal-fin end	5·671	0·131	18·199	<0·001
L2–13	Pelvic-fin base to pectoral-fin base	4·118	0·115	16·241	<0·001

*Approximate *F*-statistic for Wilks Lambda.

TABLE IV. Variable site positions in the control region among Iranian *Salmo trutta* haplotypes. Numbers refer to nucleotide positions in reference to Da24. As the sequences for the Da haplotypes published in GenBank were *c*. 994 bp in length, the 999th bp, which is variable in an Iranian haplotype, is not known in the Da haplotypes

Haplotype	177	233	234	389	529	541	542	547	549	562	663	839	878	902	994	999
Da24	C	G	A	C	T	G	G	C	T	–	C	A	T	C	A	N
H_{Ba}	T	·	·	·	·	A	C	T	·	T	T	·	·	T	·	A
H_{Ka}	T	·	·	·	·	A	C	T	·	T	T	·	·	·	·	A
H_{H1}	T	·	·	·	·	A	C	T	·	T	T	G	·	·	G	A
H_{Co}	T	·	·	·	·	A	C	T	·	T	T	·	·	·	G	A
H_{M2}	T	·	·	T	·	A	C	T	·	T	T	·	·	·	G	A
H_{M1}	T	·	·	·	·	A	C	T	·	T	T	·	·	·	G	G
H_{H2}	T	·	·	·	·	A	C	T	·	T	T	·	C	·	G	A

·, nucleotide identity with the Da24 haplotype. Ba, Babolrud River; Ka, Karaj River; H1 & H2, Haraz River; Co, Caspian Sea and Orumieh Lake; M1, Mardagh River.

populations, for the meristic and 92%, with average correct assignment of 88% over all the studied populations, for the morphometric variables.

MTDNA

The complete mtDNA control region (*c*. 1030 bp) was analysed to compare the Iranian populations to one another; whereas to compare the results for these populations to other published haplotypes, *c*. 995 bp of this region was used. A total of 129 specimens from six populations were used in sequencing, and seven haplotypes including H_{Ka} (Karaj River), H_{H1}, H_{H2} (Haraz River), H_{Ba} (Babolrud River), H_{Co} (the most frequent haplotype in all the studied rivers except the Karaj River), H_{M1} and H_{M2} (Mardagh River) were found among them. The haplotypes observed mainly comprised a common haplotype (H_{Co}) existing in the Caspian Sea and Orumieh Lake basins with an overall frequency of 95%. In contrast, in the Karaj River of the Namak Lake basin, all specimens exhibited a unique haplotype (H_{Ka}), which differed by 1 bp (0·01%) from the common haplotype. There was no significant sequence divergence found among fish from the Iranian basins except in the Karaj population, which was fixed for the *Ka* haplotype and showed pairwise G_{ST} values of 0·85-1·00 compared to the other populations. With the exception of one haplotype from the Caspian basin (H_{Ba}:2 bp), all other haplotypes were 1 bp distant from the common haplotype (Table IV).

The MP and ML methods used for phylogenetic reconstruction resulted in 50% bootstrap consensus trees with similar topologies. Therefore, only the ML tree is presented here, with the bootstrap values of the MP method shown on the branches (Fig. 5). As can be seen in the phylogram, the Iranian haplotypes are grouped into a cluster with the haplotypes of the Danubian lineage. Based on the phylogenetic relationships between the Iranian populations and the other populations of the Danubian lineage, it can be inferred that the Iranian populations, with a mean sequence divergence of 0·41%, are genetically distinct from the other populations. The minimum and maximum between group pairwise sequence divergence values of the Iranian haplotypes, compared to the other lineages were 1·2% (Adriatic) and 1·54% (Duero), respectively.

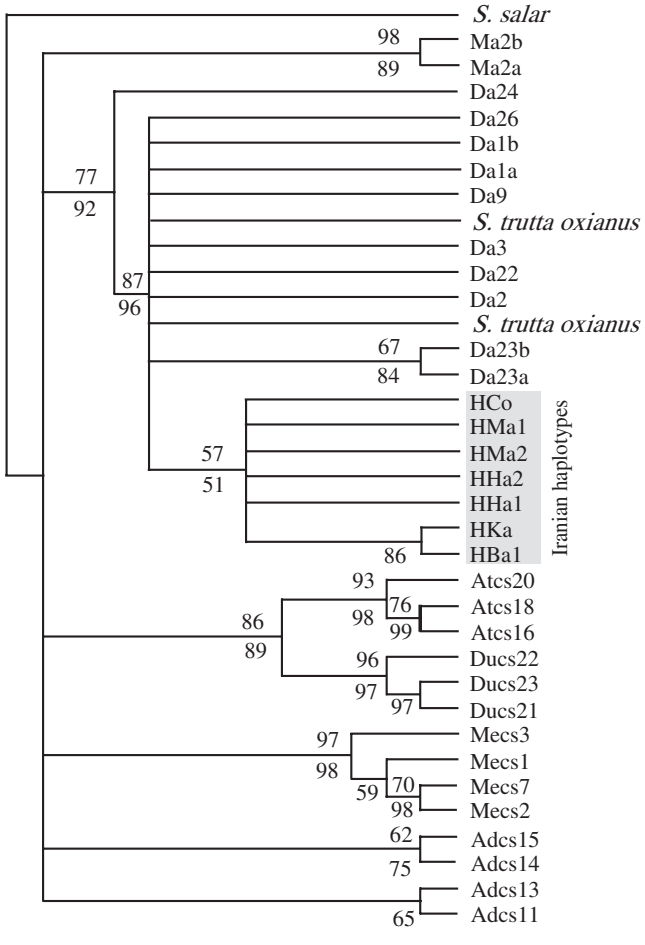

FIG. 5. Maximum likelihood phylogram of complete mtDNA control region haplotypes (997 bp) from *Salmo trutta*. Node support is shown by the percent bootstrap values for the maximum parsimony consensus (1000 replicates) above and for maximum likelihood below. Me, Mediterranean; Ad: Adriatic; Da, Danubian; Ma, Marmoratus; At, Atlantic; Du, Duero lineages within the *Salmo trutta* complex, as presented in Bernatchez (2001) and Suárez *et al*., (2001).

MICROSATELLITE DNA

All loci were found to be in HW equilibrium ($P \geq 0{\cdot}5$), except for Strutta58 in the Liqvan population. After using the correction for multiple tests, all loci were in HW equilibrium. The HWE test over all loci and all populations was not significant ($P > 0{\cdot}05$).

Based on allele size distances (R_{ST}), the Iranian populations were highly differentiated from one another, except for those from the Liqvan and Mardagh Rivers in the Orumieh basin ($R_{ST} = 0{\cdot}071$). The F_{ST} values showed extremely high levels of differentiation among the Iranian populations, with the exception of the Orumieh populations ($P < 0{\cdot}001$; Table V). Signals suggestive of bottleneck events were also observed, with the Wilcoxon test revealing significant deviations ($P < 0{\cdot}01$)

TABLE V. Pairwise genetic differentiation (F_{ST}: θ_{ST}) values (lower diagonal) among the *Salmo trutta* populations based on 12 microsatellite loci and the results of mtDNA differentiation test (G_{ST}: upper diagonal). As the sample size from the Shirinrud River was small, the divergence values of this population are not shown here

	Babolrud	Haraz	Karaj	Liqvan	Mardagh
Babolrud	0	0·00	0·93	0·00	0·00
Haraz	0·72*	0	0·85	0·02	0·00
Karaj	0·70*	0·45*	0	1·00	0·85
Liqvan	0·73*	0·50*	0·49*	0	0·02
Mardagh	0·75*	0·47*	0·47*	0·04*	0

*, significant ($P < 0·01$).

from equilibrium heterozygosity in all populations, and the mode-shift test revealing deviations from L-shaped allele frequency distributions in all populations (Table SIV, Supporting information).

The NJ tree based on allele sharing distances (Fig. 6) revealed that all individuals from each population clustered into distinct groups representing their population of origin, except individuals from the Orumieh basin. Similarly, all individuals were assigned correctly to their population of origin except fish from the Orumieh Lake basin, where individuals were often reciprocally misassigned. When the Orumieh populations were considered a single population, they were 100% successfully assigned to the Orumieh basin.

The D_{AS} microsatellite distance matrix was highly correlated with the distance matrices of the meristic (Mantel's $r = 0·241$, $P < 0·001$) and morphometric characters (Mantel's $r = 0·168$, $P < 0·001$).

DISCUSSION

ORIGIN OF IRANIAN INLAND *S. TRUTTA*

Based on mtDNA analysis of the *S. trutta* populations studied here, it can be inferred that the populations of the Orumieh and southern Caspian basins are of the same maternal origin, as a common haplotype is found in these populations. In contrast, in the case of the Karaj River population (Namak basin), the observed haplotype is unique and was not observed in other populations. The K2P distances of this haplotype to other haplotypes were from 0·01 (to H_{Co}) to 0·02 % (to the rare haplotypes) (1–2 bp). In the Caspian basin, a rare haplotype (H_{Ba}) differing from the common haplotype by 2 bp was observed. As the mutation pathway from H_{Co} to haplotype H_{Ba} passes through haplotype H_{Ka}, it can be inferred that the haplotype H_{Ka} may have originated in the southern Caspian basin, or its intermediate position in the haplotypic network in relation to the other haplotypes could suggest that it represents a haplotype that might have been abundant in the Caspian region in the past. Therefore, previous conclusions suggesting the origination of the Karaj population from *S. t. macrostigma* (Berg, 1949; Coad, 2011) can be refuted.

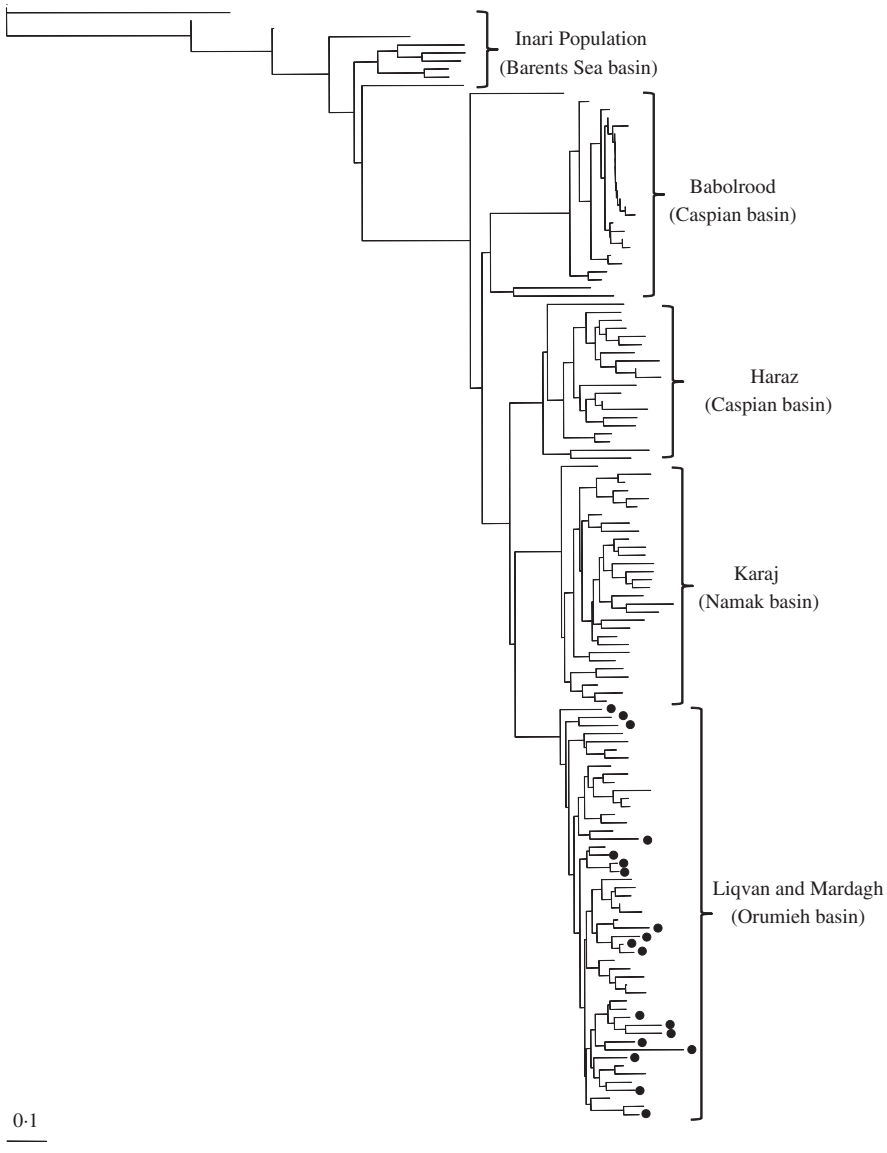

FIG. 6. Neighbour-joining tree based on individual microsatellite allele sharing (D_{AS}) values for all *Salmo trutta* sampled across five populations, with those from the Nukk population in the Barents Sea basin being used as an outgroup. All populations, except those from the Orumieh basin, clustered together, so only the population names are given. ●, individuals belonging to the Mardagh population from the Orumieh basin.

POTENTIAL PATHWAYS FOR THE ORIGIN OF THE OBSERVED HAPLOTYPES

The similarity found among *S. trutta* populations in the basins studied indicates that gene flow occurred among them in the past. Past gene flow among the basins of the Ponto-Caspian region has been previously reported based on haplotype

similarities among *S. trutta* inhabiting the Aral, Caspian and Black Sea basins (Osinov & Bernatchez, 1996). Possible connection corridors between the basins studied here could be interdrainage connections in the northern part of the Orumieh basin in Khoy (Ghara-Tappeh) *via* the Aras River, which drains into the Caspian Sea, or stream capture events occurring in the southeast of this basin, as the headwaters of some rivers draining to the Caspian Sea are located in proximity to the south-eastern Orumieh basin. Osinov and Bernatchez (1996) reported a molecular clock rate of 0·8 % per million years for the *S. trutta* control region. Assuming this clock rate, based on the negligible sequence divergence found among Iranian *S. trutta* populations (<1%), it can be inferred that the time elapsing since their isolation has not been long. This conclusion is supported by geological reports indicating the origin of the modern Orumieh Lake *c*. 10 000–30 000 years ago (Darvishzadeh, 2007). Additionally, it was noted by Coad (2011) that Orumieh Lake was formed during the late Pleiocene–Pleistocene and may have had a Pleistocene connection to the Caspian Sea. Saadati (1977) suggested two possible connections between the Caspian and Orumieh basins, including a Pliocene–early Pleistocene connection, giving rise to endemic species of this basin and a late Pleistocene connection resulting in species that are similar or subspecifically distinct from their Caspian counterparts. Stream capture is another mechanism that could have allowed the introduction of some species, such as *S. trutta*, into the Orumieh basin in recent times (Coad, 2011). This could also be the case for the Namak basin. A number of river drainages are close to those of the Caspian Sea basin, and the fauna in the Namak basin may be of recent origin (Berg, 1940). Derzhavin (1934) suggested a Pliocene origin for *S. trutta* in the Namak basin. Defensible inferences might be derived by screening samples from other rivers in these basins to provide new insights into historical relationships among *S. trutta* populations in these parts of their range.

INFERENCES FROM MORPHOLOGY

The counts of the meristic characters obtained in the populations studied are similar to the results of other studies of *S. trutta* populations in the southern Caspian Sea basin (Vatandoost, 2008) and to reports on freshwater fishes of Iran (Saadati, 1977). The most strikingly different meristic character among the studied populations was the higher mean ± S.D. count of red spots ($P < 0{\cdot}001$) in populations from the Orumieh Lake basin ($119{\cdot}17 \pm 39{\cdot}25$), which could have a genetic basis (Skaala & Jøstard, 1987; Blanc *et al*., 1994). Additionally, a meristic character for which counts were significantly higher ($P < 0{\cdot}001$) in the Karaj population, compared to the other three populations studied here, was the gill rakers on the first gill arch, which have been noted as an important character in the identification of lineages and subspecies of *S. trutta* (Hermida *et al.,* 2009). It is apparent from mtDNA data and from the overlapping ranges of the studied characters among populations that this character does not discriminate among lineages for the Iranian populations, except in indicating that the Karaj population is a distinct population of Iranian *S. trutta*. Derzhavin (1929), Nümann (1969) and Saadati (1977) also reported a Caspian origin for the Karaj *S. trutta* based on morphological characters. Therefore, no Iranian population can be considered significantly diverged from the others, including the Liqvan population, which some authors have suggested may be a subspecies of *S. trutta* (Abdoli, 2000).

MICROSATELLITE PHYLOGENY OF IRANIAN *S. TRUTTA*

In contrast to observations of the mtDNA control region, microsatellite data showed extensive divergence among the studied populations. This discordance between mtDNA and nuclear phylogenies has been reported previously and can be caused by events such as historical introgression and selection (Redenbach & Taylor, 2002; Sanz *et al.,* 2006; Canino *et al.,* 2010; Hedtke & Hillis, 2010; Waters *et al.,* 2010). The discordance between mtDNA differentiation and microsatellite loci might be caused by population bottlenecks (Gonçalves *et al.,* 2009), past introgression between lineages (Pustovrh *et al*., 2011), or the higher mutation rate of microsatellite markers compared to mtDNA. Vera *et al*. (2011) using both microsatellite and mtDNA markers did not report such differing results in Iranian *S. trutta* populations from the Caspian Sea. They studied managed populations in which hatchery produced offspring were reared to 10-20 g in common facilities and subsequently released into managed rivers (P. Zanoosi, pers. comm.). Such pooling of the fish produced by spawners of various origins homogenizes any significant allele frequency differentiation or reduces the differences (Ton-e-Kabon and Sardabrud Rives being managed normally and Karganrud and Navrud Rivers augmented with hatchery produced offspring in the past). None of the populations studied here were managed, and the differentiation levels observed are likely to be natural. All of the studied populations, except those from the Orumieh basin, were separated in an individual NJ tree based on D_{AS} distances. This level of divergence found for nuclear loci is possibly due to the reproductive isolation of the populations resulting from existing natural barriers or ecological barriers to intra-basin migration at the southern boundaries of the distribution of these populations, which limits anadromy (Antunes *et al.,* 2006). This would result in a stronger influence of random genetic drift and, possibly, natural selection in altering allele frequencies in the absence of homogenizing migration (Hansen & Mensberg, 1998). The populations of the Orumieh basin are highly similar to each other with respect to their microsatellite loci and morphology, indicating recent strong gene flow between them. If the divergence of populations in the Orumieh basin (where the hypersaline Lake Orumieh represents a barrier between populations) is compared to the divergence in the Caspian basin, where there are no strong barriers except the geographical distances among river mouths, the similarity between Mardagh and Liqvan populations may be due to anthropogenic causes, specifically, unreported transfer of fish from the Mardagh to the Liqvan River. This may have led to the disequilibrium in allele frequency at locus Strutta58 in the Liqvan population.

PHYLOGENETIC STATUS OF IRANIAN *S. TRUTTA*

Based on the mtDNA phylogenetic tree constructed using Iranian haplotypes and published haplotypes representing major evolutionary lineages of the *S. trutta* complex, it is clear that the Iranian haplotypes, which present a mean intergroup K2P distance of 0·41% to the other Da haplotypes, are distinct members of the Danubian lineage (Fig. 5). This observation is in agreement with those of the phylogenetic structure of *S. trutta* in the Ponto-Caspian region by Osinov & Bernatchez (1996) and southern Caspian sea *S. trutta* populations by Vera *et al*. (2011), who showed that *S. trutta* inhabiting this region are of Danubian phylogeny. The *S. trutta* populations inhabiting the southern Caspian basin and the two inland basins studied here

(referred to as *S. caspius* by Kottelat & Freyhof, (2007)) can be regarded as distinct members of the Danubian lineage of *S. trutta* based on their mtDNA phylogeny, rather than as separate species or subspecies.

A POSSIBLE CENTRE OF ORIGIN

In the haplotype network drawn for the Da haplotypes, it can be observed that the nearest haplotypes to the Iranian haplotypes are found for Tigris *S. trutta* (The Gulf basin), *S. t. oxianus* (Amu–Darya: Afghanistan–Aral Sea basin), and Da1a (European; Duftner *et al.,* 2003). These differ by 3 bp from H_{Co} and by 2 bp from haplotype H_{Ka} (Fig. 7). This could indicate a relationship among these haplotypes and a possible common centre of origin. As the haplotype H_{Ka} is linked to the Amu–Darya haplotype in the east and the Tigris haplotype (Da26) in the west, as well as to the European haplotype Da1a *via* a third unknown haplotype, and as the mutation path from the above noted haplotypes to other haplotypes observed here passes through the haplotype H_{Ka}, it can be inferred that haplotype H_{Ka} may be the ancestral haplotype of the other Caspian haplotypes. The connection of haplotypes H_{Ka}, Da26, Amudarya, and Da1a to a central haplotype possibly existing in the

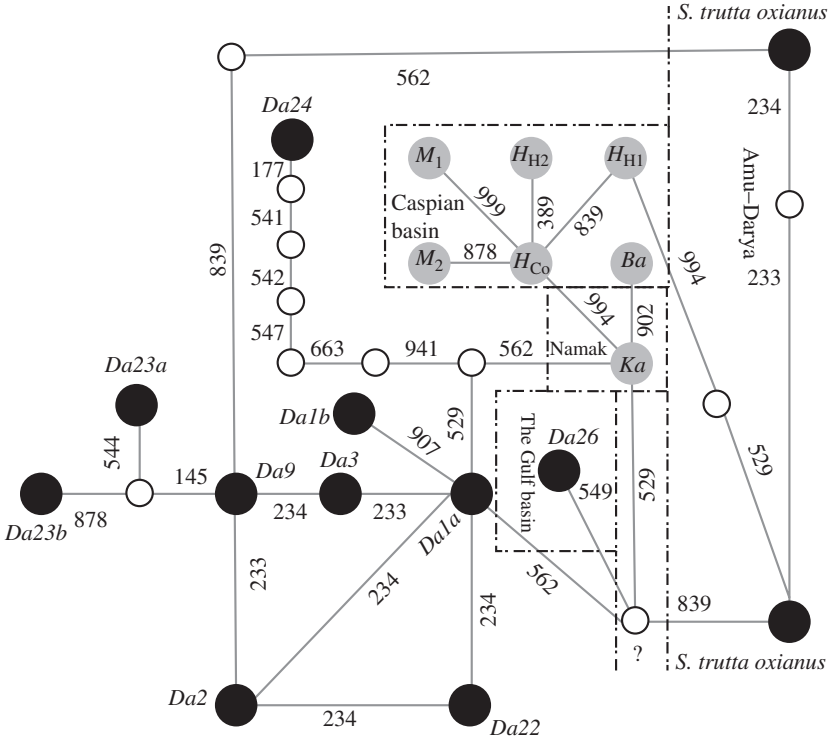

FIG. 7. Haplotypic network showing the relationships among different Danubian haplotypes based on point mutations and their position in complete D-loop sequences: Ka, Karaj haplotype; Ba, Babolrud haplotype; M1 and M2, haplotypes of Mardagh River; H_{H1} and H_{H2}, haplotypes of Haraz River; H_{Co}: the most frequent haplotype in the Caspian and Orumieh basins; _.._., separate the basins, ? on the haplotype connected to Ka denotes the uncertainty about its location.

Caspian region might indicate the role of Caspian basin haplotypes in the origin of the geographically and genetically close haplotypes Da26 and *S. t. oxianus*, although this could also present a case for some northern haplotypes such as Da1a and Da9 to be regarded as ancestors of Da26 and *S. t. oxianus*. The geographic proximity of the Caspian basin and the Tigris and Amu–Darya basins, compared to the areas where the northern haplotypes are found, might make it more probable for The Gulf (Da26) and Amu–Darya haplotypes to have originated in the Caspian basin. On the other hand, the similarity of the haplotype H_{H1} in its basic composition at position 839 to both the Amu–Darya (Aral Sea basin) haplotypes, which was not seen among the other haplotypes studied here, can be further indication of the possible origin of the Aral Sea basin haplotypes in the Caspian region.

GUIDELINES FOR CONSERVATION

Microsatellite data and morphometric characters showed high levels of divergence among the studied populations and basins. *Salmo trutta* of each basin and population showed unique alleles not observed in other populations. These observations, related to microsatellite and morphological data, are in accordance with the isolation of populations by natural barriers and their exposure to varying environmental conditions. Based on hydrochemical data reported for the rivers studied, different environmental conditions and, therefore, different selection regimes govern the evolutionary processes occurring in each basin and river. Such differences indicate that populations of the studied basins should be treated as evolutionarily significant units from an interbasin perspective and that each population in each basin should be considered as a management unit. To avoid the loss of local adaptations and to support the long-term stability of populations through management programmes, natural gene exchange among populations should be made possible (*via* improving the environmental conditions of rivers and considering fish paths in dams). Additionally, local or ecologically similar stocks should be used for restocking, managers must avoid interbasin fish transfers, and an adaptive management approach should be implemented to manage and conserve stocks.

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SUPPORTING INFORMATION

Supporting Information may be found in the online version of this paper:

Table SI. List of complete mtDNA control region haplotypes used in phylogenetic analysis of *Salmo trutta*

Table SII. Characterization of microsatellite loci in *Salmo trutta*. Locus, primer References, fluorescent dye, sequences annealing temperatures (T_a) used in single PCRs,

range of allele sizes, number of observed alleles (n_A), average expected heterozygosity (H_e) and allele size differentiation (R_{ST})

Table SIII. Microsatellite diversity indices of *Salmo trutta* populations. Sample size (N), average number of alleles/locus (A), number of private alleles (A_{pr}), expected (H_e) and observed (H_o) heterozygosity, result of Hardy–Weinberg probability

Table SIV. BOTTLENECK test statistics in the *Salmo trutta* populations

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References

Abdoli, A. (2000). *The Inland Water Fishes of Iran*. Tehran: Iranian Museum of Nature and Wildlife (in Persian).

Aljanabi, S. M. & Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research* **25,** 4629–4693. doi: 10.1093/nar/25.22.4692

Antunes, A., Faria, R., Johnson, W. E., Guyomard, R. & Alexandrino, P. (2006). Life on the edge: persistence and contrasting spatial genetic structure of distinct brown trout life histories at their ecological limits. *Journal of Heredity* **97,** 193–205. doi: 10.1093/jhered/esj014

Apostolidis, A. P. Triantaphyllidis, C., Kouvatsi, A. & Economidis, P. S. (1997). Mitochondrial DNA sequence variation and phylogeography among *Salmo trutta* L. (Greek brown trout) populations. *Molecular Ecology* **6,** 531–542. doi: 10.1046/j.1365-294X.1997.d01-176.x

Bagherian, A. & Rahmani, H. (2007). Morphological differentiation between two populations of the Shemaya, *Chalcalburnus chalcoides*: a geometrical morphometric approach. *Zoology in the Middle East* **40,** 53–62.

Berg, L. S. (1940). Zoogeografiya presnovodnykh ryb Perednei Azii (Zoogeography of freshwater fish of the Near East). *Uchenye Zapiski leningradskogo gosudarstvennogo Universiteta Seriya Geograficheskikh Nauk* **3,** 3–31.

Berg, L. S. (1948). *Ryby presnykh vod SSSR i sopredel'nykh stran (Freshwater Fishes of the Soviet Union and Adjacent Countries).* Moscow: Akad Nauk.

Berg, L. S. (1949). Presnovodnye ryby Irana i sopredel'nykh stran (Freshwater fishes of Iran and adjacent countries). *Trudy Zoologicheskogo Instituta Akademii Nauk SSSR* **8,** 783–858.

Bernatchez, L. & Osinov, A. (1995). Genetic diversity of trout (genus *Salmo*) from its most eastern native range based on mitochondrial DNA and nuclear gene variation. *Molecular Ecology* **4,** 285–297. doi: 10.1111/j.1365-294X.1995.tb00222.x

Bernatchez, L., Guyomard, R. & Bonhomme, F. (1992). DNA sequence variation of the mitochondrial control region among morphologically and geographically remote Europian brown trout *Salmo trutta* populations. *Journal of Molecular Ecology* **1,** 161–173. doi: 10.1111/j.1365-294X.1992.tb00172.x

Bernatchez, L. (1995). A role for molecular systematics in defining evolutionarily significant units in fishes. *Evolution and AquaticEcosystem: Defining Units in Population Conservation*, (Neilsen, J. L., ed.), pp. 114–132. *American Fisheries Society Symposium* **17**.

Bernatchez, L. (2001). The evolutionary history of brown trout (*Salmo trutta* L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. *Evolution* **55,** 351–379. doi: 10.1111/j.0014-3820.2001.tb01300.x

Blanc, J. M., Chevassus, B. & Krieg, F. (1994). Inheritance of the number of red spots on the skin of the brown trout. *Aquatic Living Resources* **7,** 133–136.

Bookstein, F. L. (1991). *Morphometric Tools for Landmark Data: Geometry and Biology.* Cambridge University Press: Cambridge.

Brownstein, M. J., Carpten, J. D. & Smith, J. R. (1996). Modulation of non-templated nucleotide addition by *Taq* DNA polymerase: primer modifications that facilitate genotyping. *Biotechniques* **20,** 1004–1010.

Canino, M. F., Spies, I. B. & Lowe, S. A. (2010). Highly discordant nuclear and mitochondrial DNA diversities in Atka mackerel. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* **2**: 375–387 doi: 10.1577/C09-024.1

Clement, M., Posada, D. & Crandall, K. A. (2000). TCS: a computer program to estimate gene genologies. *Molecular Ecology* **9**(10), 1657–1660. doi: 10.1046/j.1365-294x.2000.01020.x

Cornuet, J. M. & Luikart, G. (1996). Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144,** 2001–2014.

Corneut, J. M., Piry, S., Luikart, G., Estoup, A. & Solignac, M. (1999). New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* **153,** 1989–2000.

Dadikyan, M. G. (1986). *Ryby Armenii (Fishes of Armenia).* Yerevan: Izdatel'stvo Akademiya Nauk Armyanskoi SSR.

Darvishzadeh, A. (2007). *Geology of Iran*. Tehran: Amir-Kabir Publications (in Persian).

Derzhavin, A. N. (1929). A note on fishes of the River Karaj (North Persia) *News of Baku Ichthyological Laboratory* **2,** 69–79 (in Russian).

Derzhavin, A. N. (1934). Presnovodnye rybyyuzhnogo poberezh'ya Kaspiya.Vstuplenie [Freshwater fishes of the southern shore of the Caspian Sea.Introduction]. *Trudy Azerbaidzhanskogo Otdeleniya Zakavkazskogo Filiala Akademii Nauk SSSR, Sektor Zoologii, Bak* **7,** 91–126.

Duftner, N., Weiss, S., Medgyesy, N. & Sturmbauer, C. (2003). Enhanced phylogeographic information about Austrian brown trout populations derived from complete mitochondrial control region sequences. *Journal of Fish Biology* **62,** 427–435. doi: 10.1046/j.0022-1112.2003.00038.x

Elliot, J. M. (1994). *Quantitative Ecology and the Brown Trout.* Oxford: Oxford University Press.

Estoup, A., Largiadèr, C. R., Perrot, E. & Chourrout, D. (1996). Rapid one tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. *Molecular Marine Biology and Biotechnology* **5,** 295–298.

Frankham, R., Ballou, J. D. & Briscoe, D. A. (2002). *Introduction to Conservation Genetics.* Cambridge: Cambridge University Press.

Freeland, J. R. (2005). *Molecular Ecology.* Chichester: John Wiley & Sons, Ltd.

Froufe, E., Knizhin, I., Koskinen, M. T., Primmer, C. R. & Weiss, S. (2003). Identification of reproductively isolated lineages of Amur grayling (*Thymallus grubii* Dybowski 1869): concordance between phenotypic and genetic variation. *Molecular Ecology* **12,** 2345–2355. doi: 10.1046/j.1365-294X.2003.01901.x

Giuffra, E., Bernatchez, L. & Guyomard, R. (1994). Mitochondrial control region and protein coding genes sequence variation among phenotypic forms of brown trout *Salmo trutta* from northern Italy. *Molecular Ecology* **3,** 1161–1171. doi: 10.1111/j.1365-294X.1994.tb00117.x

Gonçalves, H., Martínez-Solano, I., Pereira, R. J., Carvalho, B., García-París, M. & Ferrand, N. (2009). High levels of population subdivision in a morphologically conserved Mediterranean toad (*Alytes cisternasii*) result from recent, multiple refugia: evidence from mtDNA, microsatellite and nuclear genealogies. *Molecular Ecology* **18,** 5143–5160. doi: 101111/j.1365-294X.2009.04426.x

Goudet, J. (1995). FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of Heridity* **86,** 485–486.

Gum, B., Gross, R., Rottmann, O., Schröder, W. & Kühn, R. (2003). Microsatellite variation in Bavarian populations of European grayling (*Thymallus thymallus*): Implications for conservation. *Conservation Genetics* **4,** 659–672. doi: 10.1023/B:COGE.0000006106.64243.e6

Hansen, M. M., Nielsen, E. E. & Mensberg, K. L. D. (1997). The problem of sampling families rather than populations: relatedness among individuals in samples of juvenile brown trout *Salmo trutta* L. *Molecular Ecology*, **6**:469–474. doi: 10.1046/j.1365-294X.1997.t01-1-00202.x

Hansen, M. M. & Mensberg, K. L. (1998). Genetic differentiation and relationship between genetic and geographical distance in Danish sea trout (*Salmo trutta* L.) populations. *Heridity* **81**, 493–504. doi: 10.1046/j.1365-2540.1998.00408.x

Hasegawa, M., Kishino, H. & Yano, T. (1985). Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22,** 160–174. doi: 10.1007/BF02101694

Hedtke, S. M. & Hillis, D. M. (2010). The potential role of androgenesis in cytoplasmic-nuclear phylogenetic discordance. *Systematic Biology* **60,** 87–96. doi: 10.109/sysbio/syq070

Hermida, M., Miguel, E. S., Bouza, C., Castro, J. & Martinez, P. (2009). Morphological variation in a secondary contact between divergent lineages of brown trout (*Salmo trutta*) from the Iberian Peninsula. *Genetics and Molecular Biology* **32,** 42–50. doi: 10.1590/S1415-47572009005000014

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* **6,** 65–70.

Kottelat, M. & Freyhof J. (2007). *Handbook of European Freshwater Fishes.* Berlin and Cornol: Kottelat and Freyhof.

Librado, P. & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25,** 1451–1452. doi: 10.1093/bioinformatics/btp187

Marić, S., Sušnik, S., Simonović, P. & Snoj, A. (2006). Phylogeographic study of brown trout from Serbia, based on mitochondrial DNA control region analysis. *Genetics Selection Evolution* **38,** 411–430. doi: 10.1051/gse:2006012

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America* **70,** 3321–3323.

Nümann, W. (1969). Untersuchungen zur fischereilichen Bewirtschaftung iranischer Stauseen und Fließgewässer. *Verhandlungen der internationalen Vereinigung für theoretische und angewandte Limnologie* **17,** 705–713.

Osinov, A. G. & Bernatchez, L. (1996). "Atlantic" and "Danubian" Phylogenetic groupings of brown trout *Salmo trutta* complex: genetic divergence, evolution, and conservation. *Journal of Ichthyology*, **36,** 723–746.

Osinov, A. G. (2009). Iranian samples of brown trout *Salmo trutta* or rainbow trout *Onorhynchus mykiss*: comparison of evidence from allozyme and mitochondrial DNA control region sequences analysis. *Journal of Ichthyology* **49,** 825–828.

Piry, S., Alapetite, A., Cornuet, J. M., Paetkau, D., Baudouin, L. & Estoup, A. (2004). GeneClass2: a software for genetic assignment and first-generation migrant detection. *Journal of Heridity* **95,** 536–539. doi: 10.1093/jhered/esh074

Posada, D. and Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* **14,** 817–818. doi: 10.1093/bioinformatics/14.9.817

Pustovrh, G., Sušnik, S. & Sonj, A. (2011). Evolutionary relationship between marble trout of the northern and southern Adriatic basin. *Molecular Phylogenetics and Evolution* **59,** 761–766. doi: 10.1016/j.ympev.2011.03.024

Redenbach, Z. & Taylor, E. B. (2002). Evidence for historical introgression along a contact zoone between two species of Char (Pisces: Salmonidae) in northwestern North America. *Evolution* **56**(5): 1021–1035. doi: 10.1111/j.0014-3820.2002.tb01413.x

Rannala, B. & Mountain, J. L. (1997). Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences of the United States of America* **94,** 9197–9201.

Rohlf, F. J. (2006). *tpsDig Version 2.10*. Story Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F. J. (2007). *tpsRelw Version 1.45*. Story Brook, NY:Department of Ecology and Evolution, State University of New York at Stony Brook.

Rousset, F. (2008). GENEPOP'007: a complete reimplementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* **8,** 103–106. doi: 10.1111/j.1471-8286.2007.01931.x

Ruehl, C. B. & DeWitt, J. (2007). Trophic plasticity and foraging performance in red drum, *Sciaenops ocellatus* (Linnaeus). *Journal of Experimental Marine Biology and Ecology* **349,** 284–294. doi: 10.1016/j.jembe.2007.05.017

Saadati, MAG. (1977). Taxonomy and Distribution of the Freshwater Fishes of Iran. MS Thesis. Colorado State University: Fort Collins, Co, USA.

Sanz, N., Cortey, M., Pla, C. & García-Marín JL. (2006). Hatchery introgression blurs ancient hybridization between brown trout (*Salmo trutta*) lineages as indicated by complementary allozymes and mtDNA markers. *Biological Conservation* **130,** 278–289. doi: 10.1016/j.biocon.2005.12.023

Schmidt, H. A., Strimmer, K., Vingron, M. & Haeseler, A. V. (2002). TREE-PUZZLE: maximum likelihood Phylogenetic analysis using quartets and parallel computing. *Bioinformatics* **18,** 502–504. doi: 10.1093/bioinformatics/18.3.502

Skaala, Ø. & Jørstad, K. E. (1987). Inheritance of the fine spotted pigmentation pattern of brown trout. *Polskie Archiwum Hydrobiology* **35,** 295–304.

Snoj, A., Marić, S., Berrebi, P., Crivelli, A. J., Shumka, S. & Sušnik, S. (2009). Genetic architecture of trout from Albania as revealed by mtDNA control region variation. *Genetics Selection Evolution* **41,** 22. doi: 10.1186/1297-9686-41-22

Snoj, A., Marić, S., Sušnik, B. S., Berrebi, P., Janjani, S. & Schöffmann, J. (2011). Phylogeographic structure and demographic patterns of brown trout in North-West Africa. *Molecular Phylogenetics and Evolution* **61,** 203–211. doi: 10.1016/j.ympev.2011.05.011

Suárez, J., Bautista, J. M., Almodóvar, A. & Machordom, A. (2001). Evolution of the mitochondrial control region in Palaearctic brown trout (*Salmo trutta*) populations: the biogeographical role of the Iberian Peninsula. *Heredity* **87,** 198–206. doi: 10.1046/j.1365-2540.2001.00905.x

Sušnik, S., Schöffmann, J. & Snoj, A. (2004). Phylogenetic position of *Salmo* (*Platysalmo*) *platycephalus* Behnke 1968 from south-central Turkey, evidenced by genetic data. *Journal of Fish Biology* **64,** 947–960. doi: 10.1111/j.1095-8649.2004.00363.x

Sušnik, S., Schöffmann, J. & Weiss, S. (2005). Genetic verification of native brown trout from the Persian Gulf (Catak Cay River, Tigris basin). *Journal of Fish Biology* **67,** 879–884. doi: 10.1111/j.1095-8649.2005.00780.x

Sušnik, S., Knizhin, I., Snoj, A. & Weiss, S. (2006). Genetic and morphological characterization of a Lake Ohrid endemic, *Salmo* (*Acantholingua*) *ohridanus* with a comparison to sympatric *Salmo trutta*. *Journal of Fish Biology* **68**(Suppl. A), 2–23. doi: 10.1111/j.1095-8649.2005.00902.x

Sušnik. S., Weiss, S., Odak, T., Delling, B., Treer, T. & Sonj, A. (2007). Reticulate evolution: ancient introgression of the Adriatic brown trout mtDNA in softmouth trout *Salmo obtusirostris* (Teleostei: Salmonidae). *Biological Journal of the Linnean Society* **90,** 139–152. doi: 10.1111/j.1095-8312.2007.00717.x

Swatdipong, A. (2009). *Conservation Genetics of Exploited Finnish Salmonid Fishes*. PhD Thesis, University of Turku, Finland.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetic Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24,** 1596–1599. doi: 10.1093/molbev/msm092

Turan, C. (1999). A note on the examination of morphometric differentiation among fish populations: the Truss system. *Turkish Journal of Zoology* **23,** 259–263.

Turan, C. (2004). Stock identification of Mediterranean horse mackerel (*Trachurus mediterraneus*) using morphometric and meristic characters. *ICES Journal of Marine Sciences* **61,** 774–781. doi: 10.1016/j.icesjms.2004.05.001

Vatandost, S., Vosoghi, Gh. H., Nezami, Sh. A., Abdoli, A. & Matinfar, A. (2008). A study on diversity of morphological characters of brown trout (*Salmo trutta fario*) in Tajan River and Babolrud River in Mazandaran province. *Journal of Fisheries* **2,** 57–70 (in Persian).

Vera, M., Sourinejad, I., Bouza, C., Vilas, R., Pino-Querido, A., Kalbasi, M. R. & Martínez, P. (2011). Phylogeography, genetic structure, and conservation of the endangered Caspian brown trout, *Salmo trutta caspius* (Kessler, 1877), from Iran. *Hydrobiologia*, **664,** 51–67. doi: 10.1007/s10750-010-0581-4

Waters, J. M., Rowe D. L., Burridge, C. P. & Wallis, G. P. (2010). Gene trees versus species trees: reassessing life-history Evolution in a freshwater fish radiation. *Systematic Biology* **59,** 504–517. doi: 10.1093/sysbio/syq031

Weir, B. S. & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38,** 1358–1370.

Weiss, S., Antunes, A., Schlötterer, C. & Alexandrino, P. (2000). Mitochondrial haplotype diversity of brown trout *Salmo trutta* L. in Portuguese rivers supports a simple broad-scale model of the Pleistocene recolonization of northern Europe. *Molecular Ecology* **9,** 691–698. doi: 10.1046/j.1365-294x.2000.00910.x

Electronic Reference

Coad, B. (2011). *Freshwater Fishes of Iran*. Available at http://www.briancoad.com/contents.htm