PRIMARY RESEARCH PAPER

Biogeographic insights from a genomic survey of *Salmo* trouts from the Aralo-Caspian regions

Iraj Hashemzadeh Segherloo · **Seyedeh Narjes Tabatabaei** ·
Asghar Abdoli · **Jörg Freyhof** · **Eric Normandeau** · **Boris Levin** ·
Matthias F. Geiger · **Martin Laporte** · **Eric Hallerman** · **Louis Bernatchez**

Abstract The eastern-most members of the *Salmo trutta* species complex in the Aralo-Caspian Sea region were studied to infer their population genetic structure and biogeographic origin. A total of 68 individuals collected from Iranian endorheic inland basins (Namak and Urmia lakes), tributaries of the Caspian (Haraz, Kura, Samur, Volga, and Ural river drainages) and Aral (Amu River) seas, and the Baltic Sea basin were genotyped using 26,202 SNPs via Genotyping-by-Sequencing. The data were analyzed using admixture, discriminant analysis of principal components (DAPC), analysis of molecular variance (AMOVA), species tree, genetic differentiation (F_{ST}), allele frequency difference (AFD), and neighbor network approaches. Trout in the southern Caspian Sea basin differ from those of the western and northern Caspian Sea. Based on our results, the Lake Namak trout is divergent from the southern and western Caspian trout populations. Aral Sea and Lake Namak trouts likely originated from the northern and southern Caspian Sea populations, respectively. Although only few populations were considered in this study,

I. Hashemzadeh Segherloo (✉)
Department of Fisheries and Environmental Sciences, Faculty of Natural Resources and Earth Sciences, ShahreKord University, Shahr-e-Kord, Iran
e-mail: ihashem@sku.ac.ir

I. Hashemzadeh Segherloo · S. N. Tabatabaei ·
E. Normandeau · M. Laporte · L. Bernatchez
Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Quebec G1V 0A6, Canada

A. Abdoli
Department of Biodiversity and Ecosystem Management, Environmental Research Institute, University of Shahid Beheshty, G.C. Velenjak, Tehran, Iran

J. Freyhof
Museum Für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, 10115 Berlin, Germany

B. Levin
Papanin Institute of Biology of Inland Waters, Russian Academy of Sciences, Borok, Yaroslavl Region, Russia

B. Levin
A.N. Severtsov Institute of Ecology and Evolution of Russian Academy of Sciences, Moscow, Russia 119071

B. Levin
Cherepovets State University, Cherepovets, Vologda Region, Russia

M. F. Geiger
Museum Koenig Bonn, Leibniz Institute for the Analysis of Biodiversity Change, 53133 Bonn, Germany

M. Laporte
Ministère des Forêts, de la Faune et des Parcs (MFFP) du Québec, Quebec, QC, Canada

six conservation/management units of trouts are proposed.

Introduction

Trouts in the *Salmo trutta* Linnaeus, 1758 species complex have a broad native distribution extending from Iceland to the Aral Sea basin, and from northwestern Africa to Scandinavia and Russia (Bernatchez, 2001). Based on findings from the variation in the mitochondrial DNA (mtDNA) control region, ten large-scale phylogeographic groups within the *S. trutta* complex are known: the Atlantic, Marmoratus, Mediterranean, Adriatic, Balkan, Danube, Duero, North Africa, Dades, and Tigris lineages and sublineages (Bernatchez, 2001; Bardakci et al., 2006; Snoj et al., 2009; Suárez et al., 2001; Sanz, 2018; Tougard et al., 2018). These mtDNA-based divisions proposed for trouts are informative for the identification of major units in the *S. trutta* complex, but within each of the ten phylogeographic units, there are finer subdivisions and important questions regarding the geographic origin of local populations, which may not be clarified using mtDNA haplotypes only. The reasons for this shortcoming of mtDNA in detecting fine-scale structures are related to its maternal mode of inheritance, smaller effective population size, which causes loss of diversity at a faster pace compared to nuclear DNA in response to random genetic drift and population bottlenecks, and the extensive effort and large sample sizes needed to detect rare mtDNA diversity in nature. Nuclear DNA markers such as microsatellites or genome-wide SNPs are thus more informative for inferring regional biogeographic patterns.

Salmo trouts inhabiting the edge of their range—like trout populations in North Africa, the Persian Gulf, south Caspian and Aral Sea basins, and the inland Iranian lake basins—are mainly composed of nowadays isolated populations restricted to headwaters (Antunes et al., 2006; Hashemzadeh Segherloo et al., 2012; Snoj

E. Hallerman
Department of Fish and Wildlife Conservation, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

et al., 2021). Such populations may have diverged rapidly from their counterparts in geographic isolation due to the heightened effect of random genetic drift in small populations. This fragmentation of populations can be furthered by the loss of sea-trouts, anthropogenic modifications to trout habitats, and may become exacerbated by ongoing climate change, which is severe in peripheral habitats located in the southern or southeastern reaches of their distribution. In the context of management and conservation of such populations, there may be a need for transfer of fish originating from other genetically and ecologically similar populations from the same basins or from adjacent basins in severe cases of local extinction events or to effect demographic or genetic rescue (Miller & Kapuscinski, 2003; Frankham, 2015; Whiteley et al., 2015).

In the Caspian Sea and the Iranian inland lake basins, the few studies on conservation and management units of trouts were limited to regional studies considering only a few populations from a single river drainage in the south Caspian Sea (Tabatabaei et al., 2020a, b), a few populations in the southwest and south-central Caspian Sea (Vera et al., 2011), two populations in the southern Caspian Sea basin and three populations from lakes Urmia and Namak—two Iranian inland basins (Hashemzadeh Segherloo et al., 2012). In the north Caspian Sea drainages, a few populations in the Volga and the upper Ural rivers drainage have also been examined (Marić et al., 2016). Although these studies provided population genetics-based conservation guidelines or related insights, they provided little or no discussion of the geographic origins of the studied populations, which can be important for informed selection of source populations in severe conservation cases.

So far, no comprehensive study covering populations from the Aral and Caspian Sea basins and the adjacent inland populations in Iran have been performed. However, authors have proposed different hypotheses regarding the geographic origin of trouts in the Iranian inland lake basins and the Aral Sea basin. For example, Berg (1949) postulated a Mediterranean origin for the trout in the Lake Namak basin. Hashemzadeh Segherloo et al. (2012) and Saadati (1977) postulated a southwest Caspian origin of trouts in the Lake Urmia basin and a south Caspian Sea origin for the trout in the Lake Namak basin. Hashemzadeh et al. (2012) discussed a Caspian origin for the trout in the Aral Sea basin. These

hypotheses were based on limited genetic data only from the southern Caspian Sea and the Iranian inland basins (Hashemzadeh Segherloo et al., 2012), with no consideration of trouts in other parts of the Caspian Sea or based only on morphological and geographic data (Saadati 1977; Berg 1949). In addition to these hypotheses, there are questions about the fine-scale biogeographic relationships of Iranian and the Aral Sea trout, which have not been discussed before. For example, the alternative hypothesis that the Aral Sea basin trout originated either from the north or the south Caspian Sea drainages has never been tested. Here in order to test these alternative hypotheses we used a powerful set of genetic markers and with a sampling scheme that covers trout populations from the southern Caspian Sea, Iranian inland basins, west and north Caspian Sea and the Aral Sea drainages. Thus, we analyzed 64 trout individuals from 11 populations in the Caspian and Aral sea basins and the Iranian inland populations, plus four individuals from the Baltic Sea basin, using Genotyping-By-Sequencing (GBS).

Material and methods

Library preparation

Genotyping-by-Sequencing data (involving 26202 SNPs) were produced for 68 *Salmo* trouts (64 specimens from the Ponto-Caspian drainages, and four specimens from the Baltic Sea basin) representing 11 populations in Iranian inland basins (Urmia and Namak), rivers of the south, west, and north Caspian Sea basin, as well as the Aral Sea basin (Table 1; Fig. 1). Trouts from the Baltic Sea basin were included to identify possible admixtures or cases of introgression from the Atlantic trout that historically had been introduced to the region (Derjavin 1941; Hashemzadeh Segherloo et al., 2021). For more details on sampling, see Hashemzadeh Segherloo et al. (2012, 2021) and Tabatabaei et al. (2020a, b). Genomic DNA was isolated using the salt extraction method of Aljanabi & Martinez (1997) with an additional RNAse treatment to degrade RNA molecules. The quality of the extracted DNA was checked by electrophoresis through a 1% agarose gel. The extracted DNA was quantified using a NanoDrop™ 2000 (www.thermofisher.com) and normalized to around 20 ng/μl using Quant-iT™ PicoGreen™ reads (Invitrogen: www.thermofisher.com).

The libraries for Genotyping-By-Sequencing were prepared following Mascher et al. (2013) (for more details, see Abed et al., 2019). Genomic DNA was digested with the *Pst I* and *Msp I* restriction enzymes. The digested DNA samples were then barcoded using individual-specific oligonucleotide sequences and ligation to adaptors before amplification. The individuals were multiplexed and amplified in a single tube (see Hashemzadeh Segherloo et al., 2021 for more details). Sequencing was

Table 1 Sampling details

River	N	Basin	Country	Coordinates decimal degree (WGS84)		Collection date
				Lat	Long	
Jajrud	5	Namak	Iran	35.933894	51.463427	2014
Karaj	9	Namak	Iran	36.016389	51.272778	2007
Haraz (Lar)	9	South Caspian	Iran	35.854444	52.056389	2007–2017
Haraz (Vararoo)	13	South Caspian	Iran	35.963370	52.002744	2017
Liqvan	7	Urmia	Iran	37.871667	46.465556	2007
Mardagh	5	Urmia	Iran	37.554722	46.436944	2007
Kura	3	Southwest Caspian	Turkey	41.083876	42.400155	2012
Samur	2	West Caspian	Russia	41.8455	47.8158	2004
Volga	2	North Caspian	Russia	NA	NA	2012
Ural	4	North Caspian	Russia	53.303889	58.246944	2008
Amu	5	Aral	Kyrgyzstan	39.5493	72.0861	2016
-	4	Baltic	Denmark	55.465133	10.02215	–

Fig. 1 Geographic distribution of the studied trout populations in the Aralo-Caspian region. Red dots denote sampling localities. The gray-colored section in the southern part of the Caspian Sea depicts the Alborz Mountains. The red dashed line isolates the Karaj and Jajrud rivers of Lake Namak basin from the west and east neighboring river drainages. As the map is produced using different resources in addition to DIVA-GIS free shape files (http://www.diva-gis.org/), there may be some extent of geographic inaccuracy in river shapes or boundaries

performed using Ion Torrent technology at the IBIS sequencing platform (Université Laval, Quebec City, Canada; http://www.ibis.ulaval.ca).

Data processing

The raw sequence reads were trimmed with Cutadapt (Martin, 2011) to remove the adapter sequences, and sequence quality was assessed using FastQC (Andrews, 2010). The sequences were extracted and trimmed (trimming length: 80 bp) using process_radtags in STACKS V.1.48 (Catchen et al., 2013). Trimmed sequence reads were aligned to the Atlantic salmon (*Salmo salar* Linnaeus, 1758) reference genome (PRJNA72713) with bwa (0.7.17-r1188, options: $-k=19$, $-c=500$, $-O=0,0$, $-E=2,2$, $-T=0$) and samtools (v. 1.9, options: $-S$, $-b$, $-q=1$, $-F=4$, $-F=2256$, $-F=2048$). Then, gstacks was performed to extract the stacks aligned to the reference genome (options: -max-clipped 0.1) and to identify SNPs at each locus in the individual samples. It is noteworthy that the size of the mitochondrial genome in *S. trutta* (16,677 bp; GenBank accession: NC_024032.1) compared to its nuclear genome (nDNA) size (2.37 gigabases; Hansen et al., 2021) is

negligible (~0.0007% of the nDNA size); hence, if any SNPs from mtDNA existed among the total data, they would have little effect on the results, and we did not exclude mtDNA SNPs (if any existed) from our data. Then the state of loci was written as variant call format (VCF) output using the populations program (options: $-r=0.5$, $-p=1$). The VCF output from the populations program was further filtered using 05_filter_vcf_fast.py and other scripts from STACKS workflow (see the documentation section on filtering here: https://github.com/enormandeau/stacks_workflow) with the following parameter values: m4 p70×0 S2. Each individual could have up to 30% of missing data at each SNP (Supplementary Table I). SNPs were then classified using HDplots improved from the method of McKinney et al. (2017) into singleton (canonical, two-allele SNPs), duplicated (SNPs that show patterns of having two copies), diverged (SNPs with two copies, each fixed for a different allele), and other categories for data which were not used: low MAF (minor allele frequency) and low confidence. The singleton SNPs were further treated to remove SNPs that showed linkage within 100,000 base pairs. A total of 26,202 SNPs was thus retained. In the case of heterologous loci, for SNP differences, IUPAC

codes were used (Emerson et al., 2010). The SNP data were converted to nexus sequence alignment using a ruby script (vcf_to_nexus.rb, available at: https://github.com/mmatschiner/tutorials).

Data analysis

To determine the genomic cluster specific to each population, identify admixed individuals, and clarify the geographic distributions of different genomic clusters, a Bayesian clustering analysis was performed on the SNP data using ADMIXTURE V.1.23 (Alexander et al., 2009). The admixture analysis was run for 200 bootstraps and the number of groups (K) was set from 1 to 8. K was selected according to the tenfold cross-validation error (CV), i.e., the K corresponding to the lowest values of cross-validation errors were selected. In addition, SNP data of all trouts were analyzed using discriminant analysis of principal components (DAPC) of geographically predefined groups using the Adegenet R package (Jombart & Collins, 2015) to provide insight into the differentiation and grouping of the studied populations in relation to discriminant functions. To visualize the clustering pattern of the studied populations and also to identify admixed/hybrid individuals, a neighbor-network was reconstructed with SplitsTree V. 414.6 (Huson & Bryant, 2005) using SNP sequences.

To quantify differentiation among the studied populations/population clusters, pairwise genetic differentiation (F_{ST}: Weir & Cockerham, 1984) were calculated using the STTAMP R Package (Pembleton et al., 2013). As sample sizes from each population were small, to avoid problems with asymmetrical and small sample sizes which can bias F_{ST} estimates of population differentiation, pairwise allele frequency differences (AFD) between each pair of populations were calculated (Berner, 2019). To further test population structure and differentiation, an analysis of the molecular variance (AMOVA) was conducted and diversity indices (Supplementary Table II) were calculated using the Poppr package (Kamvar et al., 2014) for the study populations. As the sample sizes for some populations from the same basins or river drainages were small, we pooled data for those populations based on their geographic region. We grouped populations as the: (1) Lar (Haraz drainage), (2) Vararoo (Haraz drainage), (3) Lake Namak basin, (4) Lake Urmia basin, (5) western Caspian Sea

drainages (Kura and Samur drainages), (6) Ural and Volga drainages, and (7) Aral Sea basin. In the south Caspian Sea basin, we had four sets of samples from the Haraz River drainage, two of which were from downstream of the Lar Dam where they were affected by non-native trout introduction, while samples from upstream of the dam had not been subject to any trout introductions. Hence, to avoid effects of introgression from Atlantic trout on the inferences related to natural history of the trouts in the studied region and also to decrease sample-size differences among the studied populations, we excluded the two sample sets belonging to the Haraz River with admixed individuals from further analyses and only included unaffected trout populations from the Vararoo and Lar tributaries of the Haraz River drainage.

To test the previously proposed hypotheses of possible origin of the Aral Sea and the Namak Lake trout from the south Caspian Sea, we reconstructed a species tree using a multi-species-coalescent model using SVDQuartets (Chifman & Kubatko 2014) implemented in PAUP*4. We assumed that the populations which nest in a single cluster on the species tree can be considered of the same geographic origin. For example, if the Aral Sea trout had originated from the south Caspian Sea trout populations, it should nest with the south Caspian Sea trout in a common clade; otherwise it should nest with the north Caspian Sea populations, which is the case also for the Namak Lake trout.

To reconstruct species tree, all possible quartets were evaluated and the trees were selected using QFM quartet assembly. The tree model was Multispecies coalescent. To provide support for the respective branches, 100 bootstrap replicates were performed. Further, in the "handling of ambiguities" section of SVDQuartets in PAUP*, the option "distribute" was selected. *Salmo trutta* from the Baltic Sea basin was used as the out-group for the species tree. To avoid the effects of introgression from the Atlantic or Black Sea trout, we excluded trouts from the Kura River drainage, since they were identified as introgressed with Atlantic and Black Sea trouts (Hashemzadeh Segherloo et al., 2021).

Results

Results of discriminant analysis of principal components (DAPC) revealed five clusters among the

analyzed populations along the first two discriminant functions (DFI and DFII; explaining together 27.28% of total genetic variation), which included trouts of the: (I) Aral Sea basin, (II) southern Caspian Sea basin (Haraz), (III) Lake Namak basin, (IV) Ural and Volga rivers, and (V) a cluster comprising trouts from the rivers Kura and Samur—named as west—as well as those from the Lake Urmia basin (Fig. 2). The cluster containing south (Haraz) Caspian Sea trout was separated from clusters of all other trout populations of the west and north Caspian Sea drainages, the populations from the Namak and Urmia lake basins, and the Aral Sea basin—all along the first discriminant function axis. Trouts from Lake Namak and Aral

Sea basins were also separated from Caspian Sea trout populations along the second discriminant function axis (Fig. 2).

According to the cross-validation error values calculated in the Admixture analysis, the best-supported numbers of clusters among the studied populations were six or seven clusters ($K = 6$ and 7; Fig. 3). For $K = 7$ with the lowest CV, trouts from the southern Caspian Sea basin (Haraz) formed a distinctive cluster which had apparent contributions to the western and northern Caspian Sea trouts and those in Lake Urmia basin. Lakes Namak and Urmia populations showed contributions from a third source shown in violet in Fig. 3. Within each cluster, a few
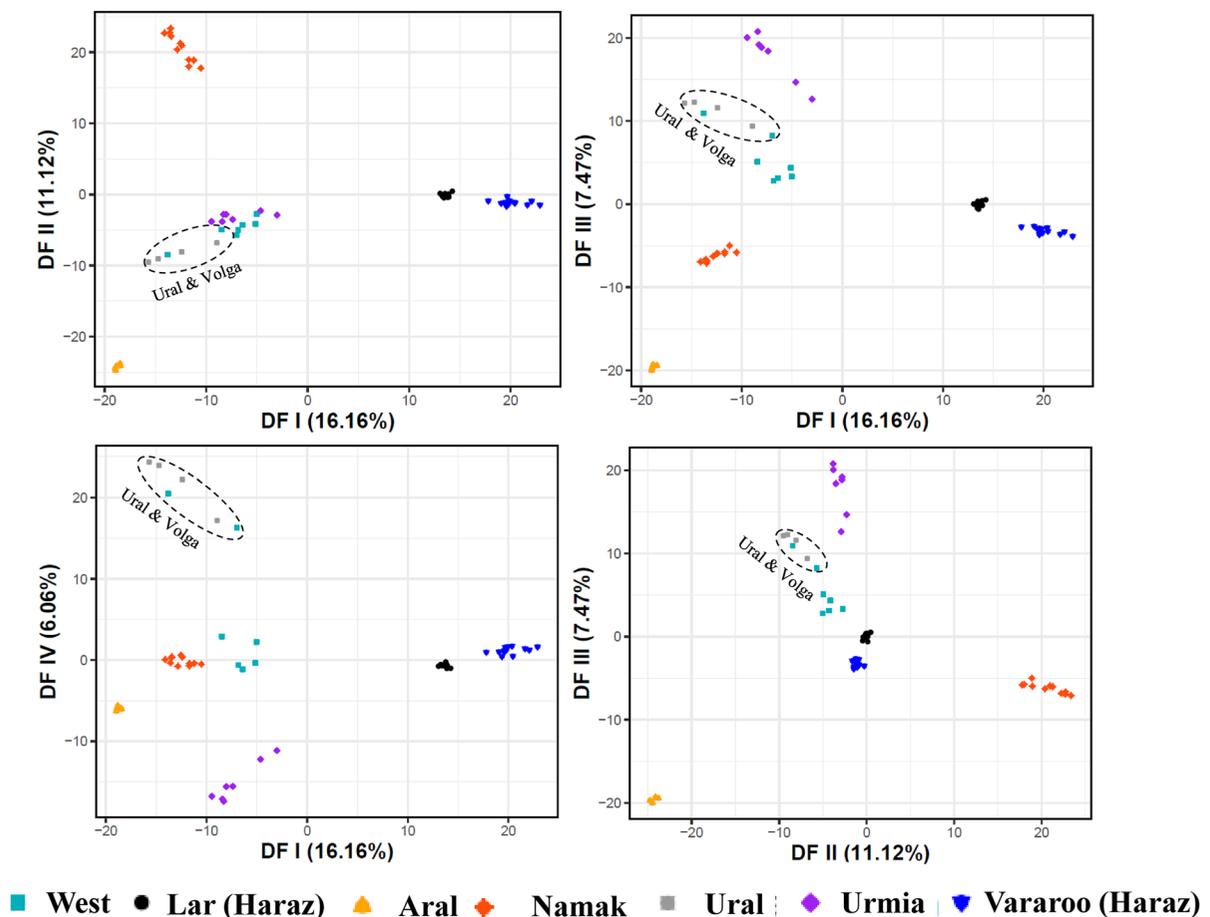
Fig. 2 Distributions of different trout populations along the first four discriminant functions (DF). As indicated in the legend, dots of each color denote a certain population or group of populations from a geographic area. The dashed ellipse denotes individuals from the Ural and Volga river drainages

of the analyzed individuals showed admixture from other genetic clusters. In a DAPC analysis in which a few Atlantic trout from the Baltic Sea basin were included, the admixed individuals deviated toward the Atlantic trout cluster along the first discriminant function (Supplementary Figure I).

Based on the groupings observed in the neighbor network (Fig. 4), the Aral basin trout nested among the north and west Caspian Sea trouts, and trouts from the Iranian inland basins clustered closer to the west and north Caspian Sea basin trout populations.

Among the southern Caspian Sea and the Iranian inland trout populations, a few individuals (three from Namak, five from Urmia, and three from upper Haraz; lower Haraz individuals were not included in this data set) did not assign to their respective source populations.

Pairwise F_{ST} estimates (Table 2) ranged from 0.1 between Urmia and west Caspian Sea trouts to 0.44 between Haraz and Aral Sea trout populations. It is noteworthy that distances to Atlantic trout (Baltic) were higher. The Aral trout was highly distant from
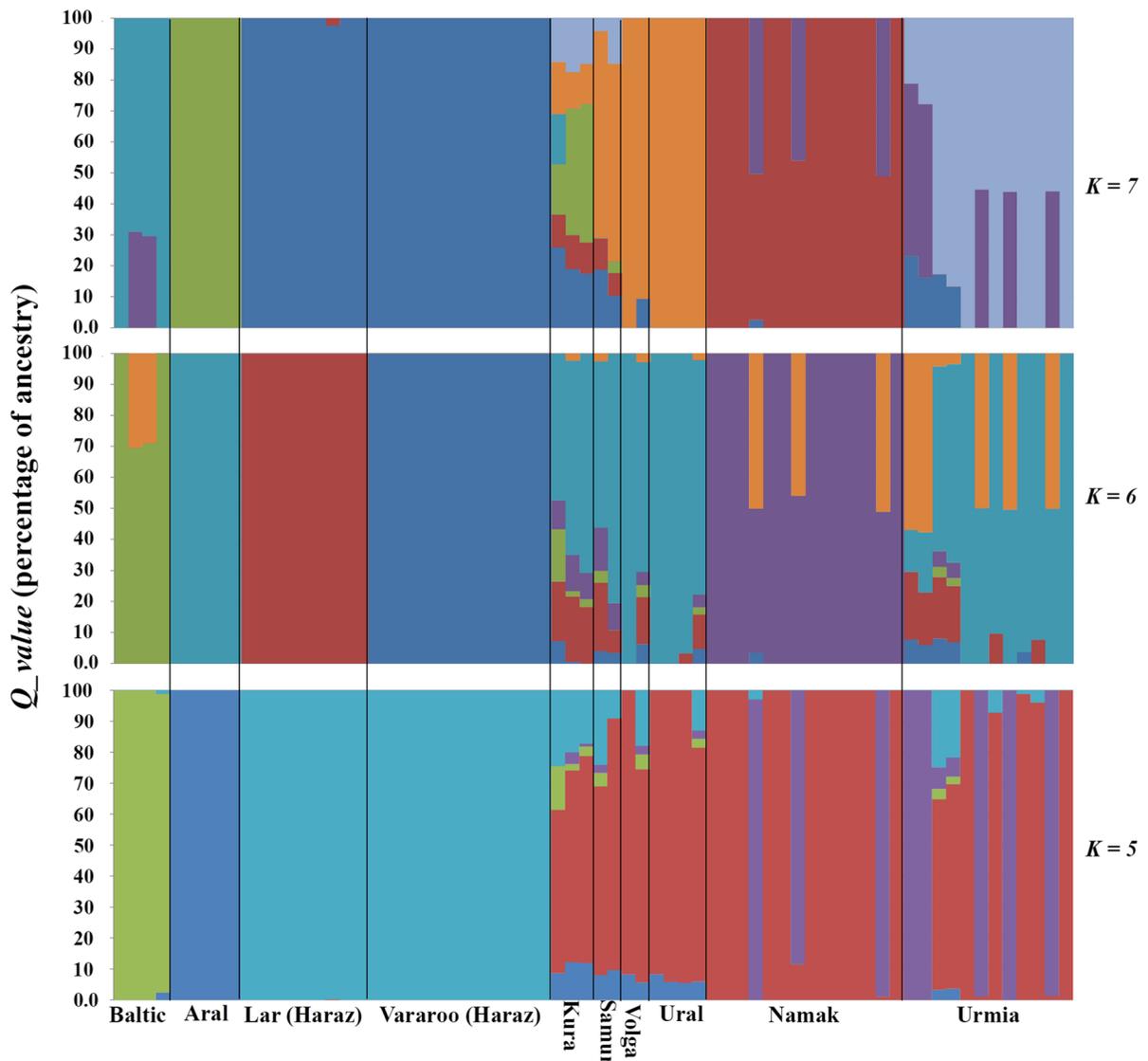
Fig. 3 Admixture clusters for the numbers (K) of multilocus genotypic clusters corresponding to the smallest cross-validation errors (*CV*)

Fig. 4 Neighbor-net reconstructed with SplitsTree using SNP genotypes. Each group of individuals is highlighted with a different color to show their geographic origin. The individuals from south Caspian Sea basin (Haraz River drainage) and Urmia and Namak lake basins highlighted in gray on the left side of the tree are admixed individuals
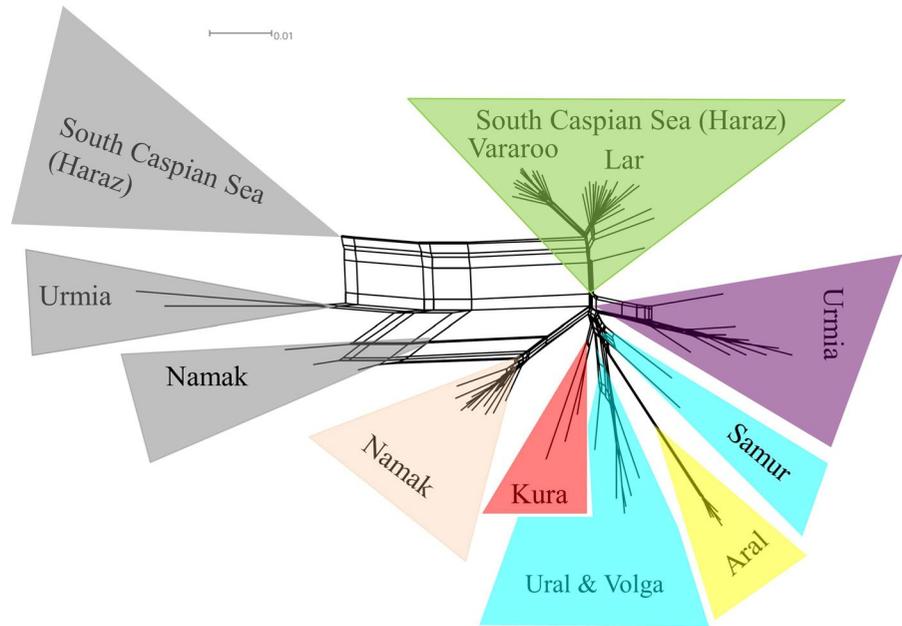
all Caspian Sea and Iranian inland trout populations. Further, allele frequency differences (AFD) ranged from 0.001 between Ural River and Aral Sea trout populations to 0.030 between Haraz River and Lake Urmia trout populations (Table 2). AMOVA based on geographic groups showed 23.74% variation among populations (Df = 6; ϕ = 0.28; $P < 0.05$), 4.57% within populations (Df = 57; ϕ = 0.05; $P > 0.05$), and 71.69% variation within samples (Df = 64; ϕ = 0.23; $P < 0.05$) (Table 3). Admittedly, as the sample sizes for the populations considered here were small, the genetic differentiation values and AMOVA results should be treated cautiously. On the species tree, Namak trout

nested with Haraz River trout (bootstrap support (BS) = 71%) and Aral Sea trout nested with north Caspian Sea trout (BS = 81%). Lake Urmia trout also nested with Namak and south Caspian Sea trout (BS = 89%; Fig. 5).

Discussion

Although a number of regional (Vera et al., 2011; Hashemzadeh Segherloo et al., 2012; Marić et al., 2016; Tabatabaei et al., 2020a, 2020b) or large-scale (Hashemzadeh Segherloo et al., 2021) studies have

Table 2 Pairwise F_{ST} differentiation (upper diagonal) between different population pairs in the Caspian, Aral, and the inland Iranian basins, and allele frequency differences (AFD: lower diagonal)

Population	Aral (Amu)	Baltic	Haraz (Lar and Vararoo)	Namak (Karaj and Jajrud)	Ural	Urmia (Liqvan and Mardagh)	West
Aral (Amu)	0	**0.59**	**0.44**	**0.31**	**0.39**	**0.26**	**0.28**
Baltic	0.104	0	**0.68**	**0.53**	**0.54**	**0.45**	**0.46**
Haraz (Lar and Vararoo)	0.007	0.112	0	**0.24**	**0.31**	**0.25**	**0.20**
Namak (Karaj and Jajrud)	0.010	0.094	0.017	0	**0.20**	**0.14**	**0.12**
Ural	0.001	0.103	0.009	0.008	0	**0.17**	**0.13**
Urmia (Liqvan and Mardagh)	0.022	0.081	0.030	0.012	0.021	0	**0.10**
West	0.003	0.101	0.010	0.006	0.0018	0.019	0

Significant F_{ST} values are indicated in bold

Table 3 Analysis of molecular variance (AMOVA) of 64 trouts from the studied regions

	Df	V%	SS	MS	ϕ	*P-value*
Variations Between pop	6	23.74	84,088.68	14,014.78	0.2830	0.01
Variations Between samples Within pop	57	4.57	128,169.30	2248.58	0.0597	0.09
Variations Within samples	64	71.69	127,671.00	1994.86	0.2374	0.01
Total variations	127	100	339,928.98	2676.60		

Df degree of freedom, *SS* sum of squres, *MS* mean squares, *V%* percentage of variance, *φ* degree of differentiation between different hierarchical divisions of populations

considered trout populations from the Caspian, Aral, and Iranian inland lake basins, each study had gaps of geographic sampling, which limited their inferences. Among these studies, the most geographically comprehensive one, i.e., Hashemzadeh Segherloo et al. (2021), discussed over 20 species of trouts from across their native range in Eurasia and the North Africa regarding their evolution, taxonomy, and conservation. However, Hashemzadeh Segherloo et al. (2021) did not cover the Iranian inland trout populations, and as a broad-scale study, the authors included only a limited number of individuals from the south Caspian Sea, which left the status of the Iranian inland trout populations or other south Caspian Sea basin populations unresolved. We here provide preliminary insight into the biogeography, conservation, and taxonomy of trouts in the Iranian inland, Caspian Sea, and Aral Sea basins, which is discussed in the following sections.

Geographic origins

Trout in the Lake Urmia basin have been hypothesized to have originated from the Caspian Sea basin, as Lake Urmia is geographically and biogeographically very close to the Aras River (Saadati 1977; Hashemzadeh Segherloo et al., 2012; Freyhof et al., 2021). Hashemzadeh Segherloo et al. (2012) hypothesized two colonization routes for Lake Urmia trout, one from the Aras River drainage and the other from the southern Caspian Sea drainages (e.g., the Sefidrud drainage). Our data showing a close affinity of the Urmia trout to the west and north Caspian populations compared to the Haraz River population, support the hypothesis of origin of Lake Urmia trout from the Aras River, likely during the post-glacial period when the Lake Urmia basin became connected to the Caspian Sea basin due to the increased water level (Hashemzadeh Segherloo et al., 2012).

Fig. 5 Species tree reconstructed for 22602-SNP data set using SVDQuartets method
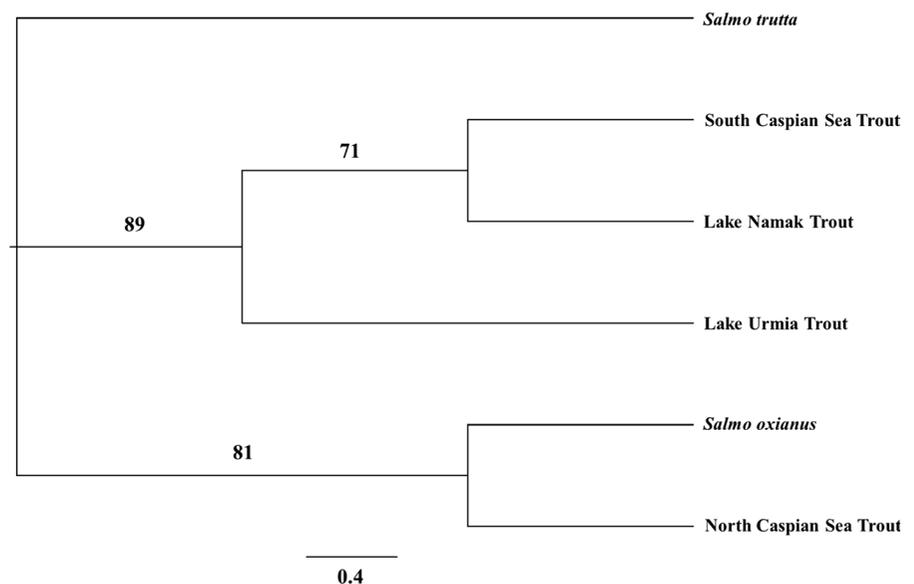
Further, trout in the Lake Urmia basin mostly have the mtDNA haplotype shared with Caspian trouts.

It has been hypothesized that the Lake Namak basin was colonized by trout alternatively from a Mediterranean wave reaching the Persian Gulf and Namak basins through the Zagros Mountains (Berg 1949) or from the Caspian Sea basin (Saadati 1977; Hashemzadeh Segherloo et al., 2012). Hashemzadeh Segherloo et al. (2012) provided only a general inference on possible mechanisms for colonization of the Lake Namak basin from the adjacent Caspian Sea drainages, especially from the southern Caspian Sea drainages. The results of this study regarding the Namak Lake basin trout were contradictory. For example, in DAPC graph the Namak population cluster was closer to the west and north Caspian Sea populations, but on the species tree it coalesced with the south Caspian Sea trout. As can be seen in Hashemzadeh Segherloo et al. (2012), the haplotype of the Namak Lake trout is a haplotype that has close mutational relationship to the common Caspian Sea haplotype and to a rare haplotype found in the Babolrud River of the south Caspian Sea. Hence, considering the geographic position of tributaries of the Karaj and Jajrud rivers that are close to the headwaters of the Lar River, their close relationship to the south Caspian Sea trout on the species tree, and the intermediate position of their haplotype in relation to the common Caspian and the rare Babolrud haplotypes, the most parsimonious inference on the geographic origin of the Namak trout may be south Caspian trout populations. However, this inference should be checked in follow-up studies in which a complete set of southeast, south-central and southwestern Caspian trout populations would be assessed using the same genomic approach to provide a clearer indication of population genetic clusters in the region.

The Aral trout also was inferred to have originated from the Caspian Sea (Hashemzadeh Segherloo et al., 2012, 2021). The Aral Sea basin is adjacent to both the north and south Caspian Sea drainages, and it is likely that their trout populations originated from either of the Caspian trout population clusters. The results of the DAPC, Admixture, Neighbor Network, and species tree analyses showed that Aral Sea trout is closely related to the west and north Caspian Sea basin trout population cluster, especially to the north Caspian Sea trout population in the Ural River drainage. Based on these results, we propose that the Aral

Sea basin was invaded from the northern Caspian Sea, probably through the Ural River, or from extinct drainages in the northeastern Caspian basin. Results of our DAPC and AFD analyses show a closer affinity of the Aral trout population to the Ural River trout population than to other Caspian trout populations. Further, on the species tree the Aral Sea basin trout nested with trout from the north Caspian Sea, which supports our inference about its origin from a north Caspian Sea trout population. Overall, our inference is contradictory to that hypothesized by Marić et al. (2016) and Hashemzadeh Segherloo et al. (2012). The latter proposed the southern Caspian Sea as a possible center of origin for Aral Sea trout, but our results imply a north Caspian origin for Aral trout. Based on observation of a single mtDNA haplotype in the Volga and Ural trouts, which was shared with the south Caspian Sea, Marić et al. (2016) hypothesized that the north Caspian Sea drainages had been colonized by trout originating from a south Caspian Sea refuge. Marić et al. (2016) based their inferences on possible extinction of pre-glacial trout populations in the north Caspian Sea drainages. However, our inferences of a north Caspian origin for the Aral trout is justified only with the pre-condition that pre-glacial trout populations of the north Caspian drainages survived during the last ice age. Existence of a single haplotype may have resulted from mtDNA having an effective population size of 25% of the nDNA effective size (Hallerman, 2003) and hence less-frequent haplotypes may be eliminated more quickly in response to bottleneck events and genetic drift. The survival of pre-glacial trout populations in the north Caspian Sea is possible, since the extent of the glaciers in the polar Ural Mountain region—the northern-most part of the Urals—during the last glacial maximum (LGM) differed only by around 1 km compared to the present-day surface area (Mangerud et al., 2008). Further, in the more southern reaches of the Ural Mountains toward the north Caspian Sea, the glaciers were very small or absent (Tudryn et al., 2016; personal communication with Jan Mangerud, University of Bergen, Norway). Assuming this situation during the LGM, it is unlikely for trouts to have gone extinct completely in the north Caspian Sea drainages during the last ice age. We do not aim to make a molecular clock calibration here, but the nucleotide differences in the mtDNA control region sequences of the Aral Sea trout and the Caspian trout

haplotypes is around 0.2–0.3% sequence difference (Hashemzadeh Segherloo et al., 2012); this indicates pre-glacial isolation of Aral trout from Caspian trouts, as a divergence rate of 0.8% sequence difference per million years was reported for the brown trout mtDNA control region (Osinov & Bernatchez, 1996). Hence, considering the sequence divergence of the Aral Sea trout and position of its DAPC cluster relative to the west and north Caspian Sea cluster, it would be less probable for them to have originated via a recent colonization from the Caspian Sea basin during 17–21 kyr BP via the Uzboy River at the east of the Caspian Sea (Tudryn et al., 2016). An alternative scenario which seems more fitting to the mtDNA sequence divergence levels between the Caspian and Aral trouts could be colonisation of the Aral Sea 90–80 kyr BP, *i.e.,* when large ice-dammed lakes overflowed into the Caspian and Aral seas (Mangerud et al., 2004). However, all these hypotheses must be tested in follow-up studies with larger sample sizes, since the limited sample sizes used in this study may provide only preliminary insights to the relationships of the trouts in the studied region.

Caspian trout clusters

During glacial times, the water level of the Caspian Sea was considerably lower than today (Kislov et al., 2014) and parts of the Caucasus and Alborz Mountains were covered by glaciers (Ferrigno, 1991; Gobejishvili et al., 2011). The sea itself was freshened and its rivers were much colder than today due to a 5–8 °C lower annual mean air temperature (Ferrigno, 1991). These environmental conditions were optimal for trouts compared to the present-day situation; however, precipitation was lower during glacial times, and many streams and rivers might have not reached the sea or many river sections were isolated from each other. Caspian trouts are known to include sea-trouts as a life-history form (Berg 1949). Therefore, we would have expected one single trout population to roam all of the Caspian basin at the end of the glacial period. The finding of two divergent molecular groups, however, refutes this hypothesis and suggests the possible existence of two glacial refugia: (1) a south-central refugium, and (2) a south-western refugium, which should be tested by further studies. Vera et al. (2011) also detected two population clusters: one in the south-central Caspian Sea

(Sardabrood and Tonekabon rivers) and one in south-west Caspian Sea (Karganrud and Navehrud rivers). Complex genetic structure was also detected in other fishes and invertebrates inhabiting the Ponto-Caspian region. For instance, the genetic structure of populations of Kura barbel *Barbus cyri* De Filippi, 1865 has been significantly influenced by climatic oscillations, Caspian Sea level fluctuations, and tectonic activity resulting in repeated secondary contacts, as well as temporary isolation of some populations during the Pleistocene–Holocene (Gandlin et al., 2022). Based on genetic structure, three glacial refugia were suggested for freshwater crabs *Potamon ibericum* in the southern Caspian Sea during the last glacial maximum (western, central, and eastern refugia; Parvizi et al., 2018). The reason for two population clusters of the Caspian Sea trout is not clear, but might be related to a combination of different factors including geological and climatic conditions ruling in the south Caspian Sea during the past. On the other hand, this observation may simply be a product of sampling two ends of a trout population genetic cline along the south Caspian Sea. These possible reasons need to be studied in more detail using more populations from the south Caspian Sea and paleo-ecological modeling studies. Assuming two population clusters in the south Caspian Sea, there are geological features along with the climatic conditions during the ice age that might have played a role in shaping the current structure of south Caspian Sea trout. For example, the distance between the Alborz Mountains and the Caspian Sea shoreline is rather little (~3–10 km) for a stretch of around 130 km (between geographic coordinates N37.05, E50.42 and N36.57, E51.80) from where we did not have any specimens. During the ice age, the southern Caspian Sea shoreline was likely similar to todays within the noted stretch (see Fig. 4 in Kislov et al., 2014) and the snow line was as much as 1800 m lower than the present snow line (Krinsley, 1970). These geological and climatic conditions along other habitat features might have affected suitable habitat area available for trout during the ice age and might have fragmented the south Caspian Sea trouts into eastern and western populations. However, the mentioned reasons need testing in follow-up population genetic studies with more populations from the south Caspian Sea and ecological modeling of current and past distribution of suitable trout habitats in the region.

Taxonomic notes

In the Aralo-Caspian region, a number of morphologically defined *Salmo* trout species have been recognised as valid, including *Salmo caspius* Kessler, 1877 described from the Kura River, *Salmo ciscaucasicus* Dorofeeva, 1967 described from the Keyranchay River (Dagestan region), *Salmo ezenami* Berg, 1948 from Kezenoi-Am lakes, four species (*S. aestivalis* Fortunatov 1926, *S. danilewskii* Gulelmi 1888, *S. gegarkuni* Kessler 1877, *S. ischchan* Kessler, 1877) in the *Salmo ischchan* complex in the Lake Sevan basin of the Caucasus, and *Salmo oxianus* Kessler, 1874 and *Salmo aralensis* Berg, 1908 in the Aral Sea basin (Griffiths et al., 2009; Kottelat and Freyhof, 2007; Levin et al., 2018; Ninua et al., 2018; Kuljanishvili et al., 2020; Levin et al., 2022). Trouts in the rivers flowing to the Caspian Sea north of the Caucasus Mountains were identified as *S. ciscaucasicus*—native to the Caspian slope of the Caucasus Mountains and north Caspian drainages—and trouts in rivers flowing to the southwest and south Caspian Sea were identified as *S. caspius* (Kottelat and Freyhof, 2007). Trout populations in the lakes Namak and Urmia basins also have been identified as *S. caspius* (Saadati 1977; Hashemzadeh Segherloo et al., 2012). Our genome-wide SNP data, however, do not accord with reported taxonomic boundaries for *S. caspius* and *S. ciscaucasicus*. As evident from the data presented in Hashemzadeh Segherloo et al. (2021), the Caspian and Aral Sea basin trouts are members of a single genomic cluster in the global genetic landscape of trouts over their distribution in Eurasia and the North Africa, but at regional level, they have a fine-scale genetic structure, which is also evident in present study. In this fine-scale genetic structure, trouts from Lake Namak and the south Caspian Sea can be clearly separated from trouts in the Lake Urmia, and the Kura, Samur, Volga, and Ural rivers drainage. Overall, as these trouts cannot be discriminated as separate clusters in the global clustering patterns of trouts, we suggest considering them as populations or sub-species of *S. caspius* along with trouts from the north and west Caspian Sea, except those from Lake Sevan in Armenia (Levin et al., 2022), which we did not analyze here. It is noteworthy that *Salmo caspius* and *Salmo ciscaucasicus* were described based on the sea trout morphs. Indeed, the case needs further studies, as we were only able to study resident trouts. Preliminarily, we consider resident and migratory trouts to be regionally conspecific (Elliott, 1994) and recommend identifying trouts of Lake Urmia, Lake Namak, and all trouts in the Caspian Sea drainages as *S. caspius* until the opposite would be clearly demonstrated.

As expected based on haplotype features (private haplotypes) and geographic isolation from the Caspian trouts, our genomic data also showed Aral basin trouts to be different from the Caspian basin trouts in fine-scale clustering pattern. Based on such nuclear genetic divergence, along with differentiated mtDNA haplotypes and geographic isolation, we suggest using *S. oxianus* for the Aral Sea basin trout.

Conservation

Our DAPC results showed up to seven population clusters along different discriminant axes (DF I-IV). The AMOVA showed ~23% of genetic variance at the between-population level. As the sample sizes used in this study were small, inferences derived from AMOVA analysis should be treated cautiously. Overall, based on results of our genetic differentiation statistics, DAPC, and AMOVA analyses, those of previous studies of Aralo-Caspian trout (Griffiths et al., 2009; Hashemzadeh Segherloo et al., 2012; Marić et al., 2016; Tabatabaei et al., 2020b; Hashemzadeh Segherloo et al., 2021) and geographic isolation of the respective basins and drainages, and also to be conservative, we propose six management/conservation units including the: (1) southern Caspian Sea basin (management units), (2) Lake Urmia (conservation unit), (3) western Caspian Sea (management units), (4) northern Caspian Sea drainages (management units), (5) Lake Namak basin (conservation unit), and (6) the Aral Sea basin (conservation unit), which is in agreement with the results of other studies on the Caspian and Aral Sea trout populations (Griffiths et al., 2009; Hashemzadeh Segherloo et al., 2012; Marić et al., 2016; Tabatabaei et al., 2020b; Hashemzadeh Segherloo et al., 2021). These results should be tested in follow-up studies with larger sample sizes collected from more populations existing within the Caspian and Aral Sea region. Tabatabaei et al. (2020a, 2020b) studied the Caspian Sea trout populations in the Lar River drainage in the southern Caspian Sea basin and found significant fine-scale population genetic structure, which they proposed

should be considered as different management units. Hashemzadeh Segherloo et al. (2012) considered the Iranian inland trout populations and the southern Caspian trout as separate conservation units. Marić et al. (2016), in a study on population structure of the Caspian Sea trout from the Volga and Ural river drainages, proposed considering each population as a separate management unit, which is in agreement with our results.

As noted in the Introduction, in severe conservation cases, as when populations in a basin become extinct or severely depleted, there may be a need for inter-basin transfers of fish. Identifying suitable source populations for the inland trout populations in the lakes of Iran and the Aral Sea basin appears important, since these populations face serious threats caused by human activity and effects of global warming. Based on the results of this study, ignoring taxonomy, the genomically closer source populations to the Aral Sea, Lake Namak, and Lake Urmia trout populations for inter-basin conservation-oriented fish transfers—in cases where no alternative intra-basin sources exist—would be the Ural, Haraz, and Aras river trout populations, respectively. We reemphasize that our results are preliminary and general, and in each case detailed, specific population genetic and ecological studies must be conducted before taking such conservation actions.

References

Abed, A., G. Légaré, S. Pomerleau, J. St-Cyr, B. Boyle & F. J. Belzile, 2019. Genotyping-by-sequencing on the ion torrent platform in barley. Barley. In Harwood, W. (ed), Barley: Methods in Molecular Biology, Vol. 1900. Humana Press, New York, NY: 233–252.

Alexander, D. H., J. Novembre & K. Lange, 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome Research 19(9): 1655–1664.

Aljanabi, S. M. & I. Martinez, 1997. Universal and rapid salt-extraction of high-quality genomic DNA for PCR-based techniques. Nucleic Acids Research 25(22): 4692–4693.

Andrews, S., 2010. FastQC: A Quality Control Tool for High Throughput Sequence Data. Babraham Bioinformatics, Babraham Institute, Cambridge, United Kingdom.

Antunes, A., R. Faria, W. E. Johnson, R. Guyomard & P. Alexandrino, 2006. Life on the edge: the long-term persistence and contrasting spatial genetic structure of distinct brown trout life histories at their ecological limits. Journal of Heredity 97(3): 193–205.

Bardakci, F., N. Degerli, O. Ozdemir & H. Basibuyuk, 2006. Phylogeography of the Turkish brown trout *Salmo trutta* L.: mitochondrial DNA PCR-RFLP variation. Journal of Fish Biology 68(A):36–55.

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., 2001. The evolutionary history of brown trout (*Salmo trutta* L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. Evolution 55(2):351–379.

Berner, D., 2019. Allele frequency difference AFD–an intuitive alternative to FST for quantifying genetic population differentiation. Genes 10(4): 308.

Catchen, J., P. A. Hohenlohe, S. Bassham, A. Amores & W. A. Cresko, 2013. Stacks: an analysis tool set for population genomics. Molecular Ecology 22(11): 3124–3140.

Chifman, J. & L. Kubatko, 2014. Quartet inference from SNP data under the coalescent model. Bioinformatics 30(23): 3317–3324.

Derjavin A.N. 1941. Caspian salmon stock reproduction. Baku, AzFAN Publishing (in Russian).

Elliott, J. M., 1994. Quantitative Ecology and the Brown Trout, Oxford University Press:

Emerson, K. J., C. R. Merz, J. M. Catchen, P. A. Hohenlohe, W. A. Cresko, W. E. Bradshaw & C. M. Holzapfel, 2010. Resolving postglacial phylogeography using high-throughput sequencing. Proceedings of the National Academy of Sciences USA 107(37): 16196–16200.

Ferrigno, J.G., 1991. Glaciers of Iran. Glaciers of the Middle East and Africa: Satellite Image Atlas of Glaciers of the World. U.S. Geological Survey Professional Paper 1386-G-2, pp. G31-G47. https://pubs.usgs.gov/pp/p1386g/iran.pdf.

Frankham, R., 2015. Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. Molecular Ecology 24(11): 2610–2618.

Freyhof, J., C. Kaya, G. Epitashvili & M.F. Geiger, 2021. *Oxynoemacheilus phasicus*, a new nemacheilid loach from the eastern Black Sea basin with some remarks on other Caucasian *Oxynoemacheilus* (Teleostei: Nemacheilidae). Zootaxa 4952(1): zootaxa-4952.

Gandlin, A. A., B. Japoshvili, G. Epitashvili, N. J. Mustafaev, H. R. Roubenyan & B. A. Levin, 2022. Phylogeography of the Kura Barbel *Barbus cyri* De Filippi as Inferred from mtDNA Data. Inland Water Biology 15(1): 11–22.

Gobejishvili, R., N. Lomidze & L. Tielidze, 2011. Late Pleistocene (Würmian) glaciations of the Caucasus. Developments in Quaternary Sciences 15: 141–147.

Griffiths, A., D. Bright & J. Stevens, 2009. Complete mitochondrial control region sequences indicate a distinct variety of brown trout *Salmo trutta* in the Aral Sea. Journal of Fish Biology 74(5): 1136–1142.

Hallerman, E. M., 2003. Population Genetics: Principles and Applications for Fisheries Scientists, American Fisheries Society, Bethesda, MD:

Hansen, T., P. G. Fjelldal, S. Lien, M. Smith, C. Corton, K. Oliver, J. Skelton, E. Betteridge, J. Doulcan, O. Fedrigo, J. Mountcastle, E. Jarvis, S. A. McCarthy, W. Chow, K. Howe, J. Torrance, J. Wood, Y. Sims, L. Haggerty, R. Challis, J. Threlfall, D. Mead, R. Durbin & M. Blaxter, 2021. The genome sequence of the brown trout, *Salmo trutta* Linnaeus 1758. Wellcome Open Research 6: 108.

Hashemzadeh Segherloo, I., H. Farahmand, A. Abdoli, L. Bernatchez, C. Primmer, A. Swatdipong, M. Karami & B. Khalili, 2012. 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. Journal of Fish Biology 81(5): 1479–1500.

Hashemzadeh Segherloo, I., J. Freyhof, P. Berrebi, A. L. Ferchaud, M. Geiger, J. Laroche, B. A. Levin, E. Normandeau & L. Bernatchez, 2021. A genomic perspective on an old question: *Salmo* trouts or *Salmo trutta* (Teleostei: Salmonidae)? Molecular Phylogenetics and Evolution 162: 107204.

Huson, D. H., & D. Bryant, 2005. Estimating phylogenetic trees and networks using SplitsTree 4. www splitstree org.

Jombart, T. & C. Collins, 2015. A tutorial for discriminant analysis of principal components (DAPC) using adegenet 2.0. 0. London: Imperial College London, MRC Centre for Outbreak Analysis and Modelling.

Kamvar, Z. N., J. F. Tabima, & N. J. Grünwald, 2014. Poppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2: 281.

Kislov, A. V., A. Panin & P. Toropov, 2014. Current status and palaeostages of the Caspian Sea as a potential evaluation tool for climate model simulations. Quaternary International 345: 48–55.

Kottelat, M. & J. Freyhof, 2007. Handbook of European Freshwater Fishes, Publications Kottelat, Cornol, Switzerland:

Krinsley, D.B., 1970. A geomorphological and paleoclimatological study of the playas of Iran. U.S. Geological Survey Interagency Report IR-Military-1, 329 p. https://apps.dtic.mil/sti/citations/AD0721849.

Kuljanishvili, T., G. Epitashvili, J. Freyhof, B. Japoshvili, L. Kalous, B. Levin, N. Mustafayev, S. Ibrahimov, S. Pipoyan & L. Mumladze, 2020. Checklist of the freshwater fishes of Armenia, Azerbaijan and Georgia. Journal of Applied Ichthyology 36(4): 501–514.

Levin, B., E. Simonov, S. Rastorguev, E. Boulygina, F. Sharko, S. Tsygankova, B. Gabrielyan, H. Roubenyan, R. Mayden & A. Nedoluzhko, 2018. High-throughput sequencing of the mitochondrial genomes from archived fish scales: an example of the endangered putative species flock of Sevan trout *Salmo ischchan*. Hydrobiologia 822(1): 217–228.

Levin, B., E. Simonov, B. K. Gabrielyan, R. L. Mayden, S. M. Rastorguev, H. R. Roubenyan, F. S. Sharko & A. V. Nedoluzhko, 2022. Caucasian treasure: genomics sheds light on the evolution of half-extinct Sevan trout, *Salmo ischchan*, species flock. Molecular Phylogenetics and Evolution 167: 107346.

Mangerud, J., M. Jakobsson, H. Alexanderson, V. Astakhov, G. K. Clarke, M. Henriksen, C. Hjort, G. Krinner, J. P. Lunkka, P. Möller & A. Murray, 2004. Ice-dammed lakes and rerouting of the drainage of northern Eurasia during the Last Glaciation. Quaternary Science Reviews 23(11–13): 1313–1332.

Mangerud, J., J. Gosse, A. Matiouchkov & T. Dolvik, 2008. Glaciers in the Polar Urals, Russia, were not much larger during the Last Global Glacial Maximum than today. Quaternary Science Reviews 27(9–10): 1047–1057.

Marić, S., O. Askeyev, A. Askeyev, S. Monakhov, N. Yanybaev, I. Askeyev, D. Galimova & A. Snoj, 2016. Lack of mtDNA variation among remote middle Volga and upper Ural brown trout suggests recent and rapid recolonization. Journal of Applied Ichthyology 32(5): 948–953.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. Embnet Journal 17(1): 10–12.

Mascher, M., S. Wu, P. S. Amand, N. Stein & J. Poland, 2013. Application of genotyping-by-sequencing on semiconductor sequencing platforms: a comparison of genetic and reference-based marker ordering in barley. PLoS One 8(10): e76925.

McKinney, G. J., R. K. Waples, L. W. Seeb & J. E. Seeb, 2017. Paralogs are revealed by proportion of heterozygotes and deviations in read ratios in genotyping-by-sequencing data from natural populations. Molecular Ecology Resources 17(4): 656–669.

Miller, L. M. & A. R. Kapuscinski, 2003. Genetic guidelines for hatchery supplementations programs. In Hallerman, E. M. (ed), Population Genetics: Principles and Applications

for Fisheries Scientists American Fisheries Society, Bethesda, MD, USA: 329–355.

Ninua, L., D. Tarkhnishvili & E. Gvazava, 2018. Phylogeography and taxonomic status of trout and salmon from the Ponto-Caspian drainages, with inferences on European brown trout evolution and taxonomy. Ecology and Evolution 8(5): 2645–2658.

Osinov, A. G. & L. Bernatchez, 1996. Atlantic and Danubian phylogenetic groupings of brown trout *Salmo trutta* complex: genetic divergence, evolution, and conservation. Journal of Ichthyology 36(9): 723–746.

Parvizi, E., R. Naderloo, A. Keikhosravi, S. Solhjouy-Fard & C. D. Schubart, 2018. Multiple Pleistocene refugia and repeated phylogeographic breaks in the southern Caspian Sea region: insights from the freshwater crab *Potamon ibericum*. Journal of Biogeography 45(6): 1234–1245.

Pembleton, L. W., N. O. Cogan & J. W. Forster, 2013. St AMPP: an R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. Molecular Ecology Resources 13(5): 946–952.

Saadati, M. A. G. 1977. Taxonomy and distribution of the freshwater fishes of Iran. MS Thesis. Colorado State University: Fort Collins, Co, USA.

Sanz, N., 2018. Phylogeographic history of brown trout: a review, Brown Trout: Biology Ecology and Management. Wiley, New York: 17–63.

Snoj, A., S. Marić, P. Berrebi, A. J. Crivelli, S. Shumka & S. Sušnik, 2009. Genetic architecture of trout from Albania as revealed by mtDNA control region variation. Genetics Selection Evolution 41(1): 1–11.

Snoj, A., J. Bravničar, S. Marić, S. Sušnik Bajec, H. Benaissa & J. Schöffmann, 2021. Nuclear DNA reveals multiple waves of colonisation, reticulate evolution and a large impact of stocking on trout in north-west Africa. Hydrobiologia 848(15): 3389–3405.

Suárez, J., J. M. Bautista, A. Almodóvar & A. Machordom, 2001. Evolution of the mitochondrial control region in Palaearctic brown trout (*Salmo trutta*) populations: the biogeographical role of the Iberian Peninsula. Heredity 87(2): 198–206.

Tabatabaei, S. N., A. Abdoli, F. Ahmadzadeh, C. R. Primmer, A. Swatdipong & I. H. Segherloo, 2020a. Mixed stock assessment of lake-run Caspian Sea trout *Salmo caspius* in the Lar National Park. Iran. Fisheries Research 230: 105644.

Tabatabaei, S. N., A. Abdoli, I. H. Segherloo, E. Normandeau, F. Ahmadzadeh, F. Nejat & L. Bernatchez, 2020b. Fine-scale population genetic structure of endangered Caspian Sea trout *Salmo Caspius*: implications for conservation. Hydrobiologia 847(16): 3339–3353.

Tougard, C., F. Justy, B. Guinand, E. J. Douzery & P. Berrebi, 2018. *Salmo macrostigma* (Teleostei, Salmonidae): nothing more than a brown trout (*S. trutta*) lineage? Journal of Fish Biology 93(2):302–310.

Tudryn, A., S. A. Leroy, S. Toucanne, E. Gibert-Brunet, P. Tucholka, Y. A. Lavrushin, O. Dufaure, S. Miska & G. Bayon, 2016. The Ponto-Caspian basin as a final trap for southeastern Scandinavian Ice-Sheet meltwater. Quaternary Science Reviews 148: 29–43.

Vera, M., I. Sourinejad, C. Bouza, R. Vilas, A. Pino-Querido, M. R. Kalbassi & P. Martínez, 2011. Phylogeography, genetic structure, and conservation of the endangered Caspian brown trout, *Salmo trutta caspius* (Kessler, 1877), from Iran. Hydrobiologia 664(1): 51–67.

Weir, B. S. & C. C. Cockerham, 1984. Estimating F-statistics for the analysis of population structure. Evolution 38(6): 1358–1370.

Whiteley, A. R., S. W. Fitzpatrick, W.C. Funk, & D.A. Tallmon, 2015. Genetic rescue to the rescue. Trends in Ecology & Evolution 30(1): 42–49.