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A genomic perspective on an old question: *Salmo* trouts or *Salmo trutta* (Teleostei: Salmonidae)?

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ABSTRACT

There are particular challenges in defining the taxonomic status of recently radiated groups due to the low level of phylogenetic signal. Members of the *Salmo trutta* species-complex, which mostly evolved during and following the Pleistocene, show high morphological and ecological diversity that, along with their very wide geographic distribution, have led to morphological description of 47 extant nominal species. However, many of these species have not been supported by previous phylogenetic studies, which could be partly due to lack of significant genetic differences among them, the limited resolution offered by molecular methods previously used, as well as the often local scale of these studies. The development of next-generation sequencing (NGS) and related analytical tools have enhanced our ability to address such challenging questions. In this study, Genotyping-by-Sequencing (GBS) of 15,169 filtered SNPs and mitochondrial DNA (mtDNA) D-loop sequences were combined to assess the phylogenetic relationships among 166 brown trouts representing 21 described species and three undescribed groups collected from 84 localities throughout their natural distribution in Europe, west Asia, and North Africa. The data were analysed using different clustering algorithms (admixture analysis and discriminant analysis of principal components-DAPC), a Bayes Factor Delimitation (BFD) test, species tree reconstruction, gene flow tests (three- and four-population tests), and Rogue taxa identification tests. Genomic contributions of the Atlantic lineage brown trout were found in all major sea basins excluding the North African and Aral Sea basins, suggesting introgressive hybridization of native brown trouts driven by stocking using strains of the Atlantic lineage. After removing the phylogenetic noise caused by the Atlantic brown trout, admixture clusters and DAPC clustering based on GBS data, respectively, resolved 11 and 13 clusters among the previously described brown trout species, which were also supported by BFD test results. Our results suggest that natural hybridization between different brown trout lineages has probably played an important role in the origin of several of the putative species, including *S. marmoratus*, *S. carpio*, *S. farioides*, *S. pellegrini*, *S. caspius* (in the Kura River drainage) and *Salmo* sp. in the Danube River basin. Overall, our results support a multi-species taxonomy for brown trouts. They also resolve some species in the Adriatic-Mediterranean and Black Sea drainages as members of very closely related genomic clusters that may need taxonomic revision. However, any final conclusions pertaining to the taxonomy of the brown trout complex should be based on an integrative approach combining genomic, morphological, and ecological data. To avoid challenges in taxonomy and conservation of species complexes like brown trouts, it is suggested to describe species based on genomic clusters of populations instead of describing species based only on morphologically differentiated single type populations.

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1. Introduction

Delimitation of species is an obvious and essential pre-requisite for the conservation of biodiversity (De Queiroz, 2007). The past decades have witnessed many efforts to use molecular data including bi-parental (nuclear DNA, nDNA) and maternal (mitochondrial DNA, mtDNA) markers for this purpose (*e.g.*, the Barcode of Life project http://www.boldsystems.org/). However, species delimitations by means of molecular methods such as mtDNA barcoding may be problematic in recently diverged lineages characterised by incomplete lineage sorting (ILS), or when introgressive hybridization occurs between sister taxa (Alda et al., 2019; Fernández-Mazuecos et al., 2017; MacGuigan and Near, 2018; Palandačić et al., 2017; Roycroft et al., 2019). As a consequence, it is now generally accepted that using nDNA markers (*e.g.*, microsatellites, nuclear genes) is necessary to decipher relationships among recently diversified species.

Among fishes, one of the groups for which such challenges exist is the brown trout *Salmo trutta* species-complex, referred to as brown trouts hereafter. The brown trouts show a recent diversification history of 9.6–15.4 million years ago (Mya) for divergence of *S. trutta* lineages from *S. salar*, 3–9 Mya for the divergence of *S. ohridanus* and *S. obtusirostris* from *S. trutta*, around 4 Mya for divergence of *S. marmoratus* from *S. trutta*, and 0.5–2.5 Mya for the diversification of other brown trouts in the Mediterranean and Ponto-Caspian regions (Alexandrou et al., 2013; Bernatchez, 2001; Crête-Lafrenière et al., 2012; Lecaudey et al., 2018; Ninua et al., 2018; Osinov and Bernatchez, 1996; Pustovrh et al., 2014). The brown trouts have a wide native geographic distribution spanning from Iceland in the northwest to the Aral Sea basin in Afghanistan in the southeast, and from the Atlas Mountains in Morocco to northern Scandinavia and Russia (Bernatchez, 2001). Despite their relatively recent origin, the brown trouts exhibit a tremendous level of morphological and ecological diversity. For example, along with river-resident, lacustrine, and sea-run forms, which led to description of some species (Elliott, 1994; Kottelat and Freyhof, 2007), brown trouts comprise a broad array of morphotypes such as the Seyhan flathead trout *Salmo platycephalus* (once recognised as its own genus: *Platysalmo*), the softmouth trout *S. obtusirostris* (once considered as a monotypic genus: *Salmothymus*), the Ohrid trout *S. ohridanus* (once recognised as a monotypic genus, *Acantholingua*), the Dades trout *S. multipunctatus*, and the marble trout *S. marmoratus*, to name a few. The vast geographic distribution along with their high morphological and ecological diversity, mostly with no or shallow mtDNA divergence, along with natural or human-mediated introgressive hybridization among them have led to challenges to defining their taxonomy and defining conservation units (Kottelat and Freyhof, 2007; Lerceteau-Köhler et al., 2013; Machordom et al., 2000; Melville et al., 2017; Moran et al., 1995).

Two main schools of thoughts have promoted somewhat contrasting views pertaining to the taxonomy of brown trouts. The first school considers the high level of geographic, morphological and ecological diversity of brown trouts to be best represented by a correspondingly high level of specific diversity. Thus, proponents of this school have recognised 47 extant nominal species (Kottelat and Freyhof, 2007; Levin et al., 2018; Sanz, 2018; Delling and Doadrio, 2005; Doadrio et al., 2015; Snoj et al., 2011; Turan et al., 2009; 2011; 2014; 2017; 2020; www.catalogoflife.org). In contrast, proponents of the second school who mainly addressed the taxonomic issues of brown trouts using phylogenetics and phylogeography (mainly based on mitochondrial DNA analysis) consider all brown trouts as *Salmo trutta* being a widespread, polymorphic species (Apostolidis et al., 2011; Bardakci et al., 2006; Bernatchez, 2001; Berrebi, 2015; Hashemzadeh Segherloo et al., 2012; Lo Brutto et al., 2010; Osinov and Bernatchez, 1996; Sanz, 2018; Suárez et al., 2001; Tougard et al., 2018). More specifically, these authors defined trout diversity based on the distinct geographic distribution of ten mtDNA phylogenetic lineages and sub-lineages: namely the Danubian (DA in the Ponto-Caspian basins), Mediterranean (ME in the Mediterranean and Adriatic Basins), Adriatic (AD in the Mediterranean and Adriatic Basins), Marmoratus (MA in the Adriatic Basin), Atlantic (AT in the north Atlantic basin), Balkan (in the Balkan Peninsula), Tigris (TI in the Persian Gulf basin), North African (NA in Morocco, Algeria where it is extinct, and Sicily), Dadès (in the Dadès River, Morocco), and Duero (DU in the western Iberian Peninsula) phylogenetic groups (Bardakci et al., 2006; Bernatchez et al., 1992; Bernatchez, 2001; Sanz, 2018; Snoj et al., 2009; 2011; Suárez et al., 2001; Tougard et al., 2018). However, these lineages show considerable geographic overlap in some areas, and in some cases different mtDNA lineages are found within single populations, in the same geographic area, or in shared drainages (Apostolidis et al., 2008; 2011; Bernatchez, 2001; Berrebi et al., 2000; 2017; Duftner et al., 2003; Lerceteau-Köhler et al., 2013; Meraner et al., 2007; Weiss et al., 2001). In addition, mtDNA lineages were often in disagreement with morphological or geographic groups of populations. Furthermore, human-mediated transfers of Atlantic trout to habitats in the Mediterranean and the Ponto-Caspian regions have added to the complexity of taxonomic inferences on native brown trouts (Kottelat and Freyhof, 2007).

In order to resolve taxonomic uncertainties left unresolved by the analysis of mtDNA only, various nDNA approaches – including allozymes, single nucleotide polymorphisms (SNPs), and microsatellites – have been used in numerous studies (Apostolidis et al., 1996; Berrebi, 2015; Berrebi et al., 2013; 2019; Ferguson and Mason, 1981; Giuffra et al., 1996; Gratton et al., 2014; Marić et al., 2017; Pustovrh et al., 2014; Razpet et al., 2007; Snoj et al., 2002; 2010; 2011; Sušnik et al., 2006, 2007a, 2007b). Nuclear markers including allozymes and rDNA *ITS* had been in agreement with the 4–5 major mtDNA phylogenetic lineages of brown trout first defined by Bernatchez (2001) (García-Marín et al., 1999; Presa et al., 2002). However, most of the taxonomic studies that used nDNA markers were regional in scope, and because different sets of markers have been used in different studies, datasets cannot be combined in order to generate the broad picture of phylogenetic relationships of brown trouts throughout their range.

In this context, the goal of this study was to combine Genotyping-by-Sequencing and mitochondrial DNA sequence (mtDNA D-loop sequences) data to assess the phylogenetic relationships among 24 of previously described or potential brown trout species throughout its Eurasian and North African range, in order to: 1) identify and exclude admixed individuals resulting from Atlantic trout transfers to non-Atlantic basins to base subsequent analyses on pure native genetic background only, 2) identify genetic lineages according to genomic data and compare them to the major mtDNA lineages among the studied populations, and 3) identify genomic clusters and compare them with the proposed taxonomy of brown trouts, in order to 4) propose new species delimitations and conservation guidelines.

2. Material and methods

2.1. Sampling

Genotyping-by-Sequencing and mitochondrial data (15,169 SNPs and partial mtDNA D-loop sequences) were produced for 166 individuals (out of 209 individuals collected) representing 21 described nominal species and three undescribed groups (nearly half of the species recognised as valid by the Catalog of Fishes: https://www.calacademy.org/scientists/projects/eschmeyers-catalog-of-fishes) and *Salmo salar* collected from 84 localities over their natural distribution in Europe, west Asia, and North Africa (Table S1). The majority of specimens were collected in own field campaigns, with the aid of numerous colleagues, or stems from available collections (see Bernatchez, 2001; Geiger et al., 2014; Epitashvili et al., 2020 for further details). The species not covered in this study were usually from similar sea basins but found in different drainages, except for the sympatric species in Lake Ohrid, where we analysed only one species of the six described ones (Tables S1 and S2). The availability of samples, conservation status of the species, or

difficulty in collecting specimens in some geographic areas limited sample sizes for some species. All collected brown trouts were assigned scientific names based on morphology and geographic distribution following publications by Kottelat and Freyhof (2007) for Europe, Doadrio et al. (2015) and Delling and Doadrio (2005) for Morocco, and Turan et al. (2009; 2011; 2014; 2017; 2020) for western Asia. In cases when no reliable voucher material was available, the naming followed only the distributional areas given by these studies.

2.2. DNA extraction

Genomic DNA was extracted using the salt extraction method of Aljanabi and Martinez (1997) with an additional RNAse treatment to degrade RNA molecules (Benestan et al., 2015). With already-extracted DNA that was available from Bernatchez (2001), a RNAse treatment was performed using RNAse T and A (Qiagen) following the manufacturer's protocol with some modifications (www.qiagen.com). The quality of the extracted DNA was checked by electrophoresis through a 1% agarose gel, and degraded DNA samples were excluded. The extracted DNA was quantified using a NanoDrop™ 2000 (www.thermofisher.com) and normalized to around 20 ng/μl using Picogreen reads (Invitrogen: www.thermofisher.com).

2.3. Mitochondrial DNA control region

To determine the five major traditional phylogenetic grouping (mtDNA lineages) of brown trouts, namely the Danubian, Mediterranean, Adriatic, Marmoratus, and Atlantic lineages (Bernatchez, 2001), the 5′ end of the mitochondrial control region was amplified and sequenced using the primers and conditions detailed in Hashemzadeh Segherloo et al. (2012).

2.4. Genotyping-By-Sequencing

The libraries for Genotyping-By-Sequencing were prepared following Mascher et al. (2013). Genomic DNA was treated with the *Pst I* and *Msp I* restriction enzymes. The digested DNA samples were then barcoded using individual-specific oligonucleotide sequences and ligated to adaptors for amplification. Each set of 96 individuals was multiplexed and amplified in a single tube. Ninety-six individuals were included on each sequencing chip, for a total of 9 chips (3 chips for each set of 96 individuals). When several individuals were available for a given population, samples were dispersed randomly on different sequencing chips to avoid batch effects. Sequencing was performed using Ion Torrent technology at the IBIS sequencing platform, Université Laval, Canada (http://www.ibis.ulaval.ca/).

2.5. Bioinformatics and data processing

2.5.1. Mitochondrial DNA control region

The mtDNA control region sequences were edited using BioEdit v. 7.2.5 (Hall, 2005) and aligned using Muscle (Edgar, 2004) with default options as implemented in MEGA7 (Kumar et al., 2016). We then produced a rooted TCS haplotype network (Clement et al., 2000) using PopART-1.7 (http://popart.otago.ac.nz) and diversity indices, including haplotype and nucleotide diversities, were calculated with DnaSP V.6 0.10.03 (Rozas et al., 2017). In addition, sequences of Atlantic salmon (*S. salar*), which is the sister taxon of all brown trouts were produced in this study to root the network.

2.5.2. Genomic data

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 (*S. salar*) reference genome (PRJNA72713) (Lien et al., 2016) with bwa (0.7.17-r1188, options: -k = 19, -c = 500, -O = 0,0, -E = 2,2, -T = 0) and samtools (1.9, options: -S, -b, -q = 1, -F = 4, -F = 256, -F = 2048). Then, pstacks was performed to extract the stacks aligned to the reference genome (options: -m = 1, –model_type = snp, –alpha = 0.05) and to identify SNPs at each locus. The minimum stacks for each locus was adjusted to 3. The loci were grouped together across individuals and cataloged using cstacks with a maximum between-loci mismatch parameter of 1, and then loci from each individual were matched against the catalog to clarify the alleles at each locus using sstacks. Then the state of loci was written as variant call format (VCF) output using the populations program (options: -r = 0.5, -p = 1, -m = 4). The VCF output from populations program was further filtered using STACKS workflow (https://github.com/enormandeau/stacks_workflow) with the following parameter values: -m = 5, -p = 80 and -H = 0.6. Each population thus had a maximum of 20% of missing data at each SNP. In the final VCF file, we kept only one SNP for each locus. The SNP data were converted to nexus sequence files with a Ruby script (vcf_to_nexus.rb available at: https://github.com/mmatschiner/tutorials). In cases where all individuals of the same population showed missing data for a locus, we left the locus as missing data; otherwise, we edited the missing data based on the respective genotype in other individuals of the same population. In case of heterologous loci, for the SNP differences, IUPAC codes were used (Emerson et al., 2010).

2.6. Species and gene tree reconstruction

To avoid errors in species names that we assigned to specimens based on the geographic origin or based on morphology, a Maximum Likelihood (ML) gene tree reconstructed for SNP sequence data with RaxMl v. 1.5 (Silvestro and Michalak, 2012) was used to assess whether all individuals classified under the same taxonomic name were monophyletic or not. The options for ML gene tree reconstruction were: ML + rapid bootstrap, 500 bootstrap replicates, and GTRGAMMA sequence divergence model (as determined by MEGA7 model test). A few individuals from the Khosta River (north east Black Sea; Russia) that did not nest in the predefined group were excluded to avoid possible errors in predefining members of different species for species tree reconstruction. To infer phylogenetic relationships of different trout taxa, while avoiding the effects of incomplete lineage sorting, we reconstructed a species tree under a multispecies coalescent model using the SVDQuartets method implemented in PAUP* V.4.0a (Swofford, 2002). To reconstruct the species tree, 100,000 quartets were evaluated and in cases where the number of quartets was less, all possible quartets were evaluated. A hundred bootstrap replicates were performed to assess branch support. The trees were selected using QFM quartet assembly. To avoid interpretation of IUPAC ambiguity codes as missing data, the "distribute" option under "handling of ambiguities" section was selected.

2.7. Analyses of admixture and introgression

To determine the genomic cluster specific to brown trouts of each geographic location and to clarify the distribution 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 1000 bootstraps and the number of groups (K) was set from 1 to 25. K was selected according to the 10-fold cross-validation error (CV), i.e. the K corresponding to the lowest values of cross-validation errors were selected. Admixed or pure individuals showing genetic cluster/s belonging to sea basins other than the basin from which they were sampled were excluded from further analyses, and pure indigenous individuals ($Q > 0.9$) with clusters observed only in their respective basins were kept in the analysis. To visualize the geographic range of each genotype, the different genomic clusters (Q values) that were identified were plotted on geographic map produced using DIVA-GIS 7.5.0 (http://www.diva-gis.org/). In addition to the Admixture

analysis, the SNP data of all trouts and also the pure native trouts, as defined above, were analysed using discriminant analysis of Principal Components (DAPC) using Adegenet R package (Jombart and Collins, 2015). To reduce the effect of missing data on DAPC, the missing data were imputed using the randomForest R package (Breiman, 2006) based on the genomic groups identified. To further assess admixture and past gene flow, all possible combinations of three population (f_3; A; B, C) (Patterson et al., 2012; Reich et al., 2009) and four population (f_4; A, B: C, D) (Keinan et al., 2007; Patterson et al., 2012) tests implemented in TreeMix (Pickrell and Pritchard, 2012) were performed on the data at the basin and taxonomic levels using 200- and 500-bp blocks of SNPs. In four-population tests, *S. salar* was used as the outgroup. In addition, the mitochondrial and GBS nuclear data were compared to assess cyto-nuclear discordance and thus identify cases of introgressive hybridization.

2.8. Molecular species delimitation

To provide statistical support to our inferences on taxa described in the literature as distinct species (mainly based on morphology) or observed as distinct assemblages in the DAPC, admixture, and phylogenetic analyses (this study), we performed a Bayes Factor Delimitation approach (BFD) on GBS data (Leaché et al., 2014). As a number of putative species from different localities represented very closely related DAPC clusters, we also assessed the statistical support for distinctiveness of their DAPC clusters in BFD analysis. In this approach, candidate species delimitation scenarios are compared and ranked according to the marginal likelihood estimates (MLE). We analysed the data using the SNAPP plug-in implemented in BEAST 2 (Bouckaert et al., 2014). To estimate the marginal likelihoods, we performed path sampling for 48 steps and a Markov Chain Monte Carlo (MCMC) chain of 100,000 with pre-burnin of 10,000 steps was used. The lambda for species delimitation was calculated based on the maximum pairwise sequence divergence (tree height) and the number of species or tree tips using the Python script pyule (available at: https://github.com/joaks1/pyule). XML files were set up following the instructions provided in the BFD* manual (Leaché and Bouckaert, 2018). Given the possibility that taxonomic assignment we used based on morphology or geographic origin of brown trouts (current taxonomy) might be erroneous in some cases, we also considered alternative species delimitation scenarios. In this way, we tried to further predict different possible groups regarding clusters resolved in DAPC and the monophyletic groups resolved on a ML gene tree (see species and gene tree reconstruction for details), in addition to a few additional species combinations in the Ponto-Caspian and Adriatic regions. In order to test the null hypothesis of a single species for each brown trout cluster (H0), we used *S. salar* as outgroup, since BFD cannot be run only with one species only. To circumvent the constraints imposed by the substantial computing resources required to run the BFD analysis, we ran the analysis using a maximum of 5–6 species within each monophyletic clade or sub-clade (personal communication with A. D. Leache, University of Washington).We thus defined speciation models for: a) brown trouts of the Caspian and Aral Sea sub-clade, b) brown trouts of the Black Sea sub-clade, c) brown trouts of the Mediterranean and Moroccan clade (two sets of models), d) brown trouts of the Atlantic clade plus *S. marmoratus*, and e) representatives of all major groups resolved on the phylogenetic tree (Table 1). Species delimitation models were compared with the single-species model for each cluster based on Bayes factor (BF) (Kass and Raftery, 1995). The *BF* statistic was calculated using the following formula:

$$BF = 2\mathrm{X}\left(MLE_{\mathrm{Mf}} - MLE_{\mathrm{Ma}}\right)$$

where MLE_{Mf} and MLE_{Ma} are the marginal likelihood estimates ($\log_e$) of the first-rank species model and the alternative models, respectively. Here, a positive *BF* statistic supports the first model (first- rank model) and a negative *BF* statistic provides support for the alternative model. The tested models are shown in Table 1.

2.9. Identification of rogue and hybrid taxa

To identify rogue/hybrid taxa and to infer past hybridization events, we performed a taxonomic jackknifing test (Russo and Selvatti, 2018) with RogueNarok (Aberer et al., 2011, 2012) and reconstructed a neighbor network using SNP data with SplitsTree V. 4.14.6 (Huson and Bryant, 2005). The following options were used for identification of rogue taxa, which decrease the bootstrap supports in the phylogenetic tree: strict consensus threshold of rogue taxon search (SC), maximum drop size = 1, and optimization of overall support.

3. Results

3.1. Mitochondrial DNA control region

A 571-bp fragment of the 5′ end of the mitochondrial control region was sequenced for 201 brown trouts and five *S. salar* analysed in this study and 47 haplotypes (45 haplotypes for trouts and 2 for *S. salar*) were found (Fig. 1). Several haplotypes were found to be endemic to the Aral, Caspian, Black, Atlantic, or Mediterranean Sea basins. Others were found only in the Oum Er-Rbia River drainage (Morocco) or in the Persian Gulf basin (Table 2). Brown trouts of the Mediterranean Sea basin were the most diverse group observed here (Table 2). Among the D-loop sequences used to reconstruct the haplotype network (Fig. 1), haplotypes belonging to the Marble trout *S. marmoratus* were the closest to *S. salar* on the mutation path from *S. salar* to all other trout haplotypes. In one *S. farioides* individual, we observed a haplotype typical of *S. marmoratus*.

3.2. Genomic data

Forty-three (three *S. salar* and 40 trouts) of the 209 individuals assayed did not have enough genomic sequence reads (<2,000,000 reads), and hence were excluded from the analysis, and genomic data from 166 individuals genotyped at 15,169 unlinked high quality SNPs were retained for subsequent analyses.

3.3. Clustering analyses

Based on the cross-validation (CV) values calculated, the most likely number of clusters (K) was 15 (CV = 0.1566). For $K = 15$, brown trout from the Atlantic basin (two pure and three admixed clusters), Mediterranean (six clusters), Black (one cluster; 39 individuals; average Q value: 0.68), Caspian and Aral Seas (one cluster; 22 individuals; average Q value: 0.95), and Morocco (one cluster; 5 individuals; average Q value: 1.0) had their own set of genomic clusters (Fig. 2). Three of the 15 clusters resolved in admixture analysis were not represented by pure individuals ($Q_{\mathrm{value}} \leq 0.9$); hence, we excluded them and kept 12 clusters (including one for *S. salar*) comprising individuals with Q values of over 0.9. We observed a contribution from Atlantic basin brown trout in all major basins (Supporting data) but no Atlantic brown trout contribution was observed in smaller basins such as the Aral Sea basin and Oum Er-Rbia (Morocco) (Supporting Data). After excluding 27 individuals identified as admixed with Atlantic brown trout *S. trutta* according to Q values calculated in Admixture analysis, the DAPC detected 13 groups along the first two discriminant functions (PC1 and 2:45.04% of SNP variation) (see Fig. 3 for more details).

3.4. Introgression detection

The three-population test (f_3) based on allele frequency data of all trouts analysed detected no significant admixture (Z-score > 0). In the three-population test performed at the inter-basin level, brown trouts from the Black and the Mediterranean sea basins showed highly

Table 1
Results for Bayes Factor Delimitation (BFD) in different geographic and phylogenetic groups of trouts.

a. **Models for the Caspian and Aral Sea basin trouts**

Model	Species combinations					MLE	BF	Rank
CAS1	*S. ciscaucasicus* (I)	*S. caspius* (II)	*Salmo* sp. (III)	*S. oxianus* (IV)		−18168.4		1
CAS5	I+III	*S. caspius*	–	*S. oxianus*		−18788.1	1239.3	2
CAS3	I+II	–	*Salmo* sp.	*S. oxianus*		−20175.5	4014.2	5
CAS4	*S. ciscaucasicus*	II+III	–	*S. oxianus*		−20263.2	4189.6	6
CAS2	I+II+III	–	–	*S. oxianus*		−20719.5	5102.1	8
CAS6	I+III+IV	*S. caspius*	–	–		−19401.0	2465.2	4
CAS7	I+IV	*S. caspius*	*Salmo* sp.	–		−18923.8	1510.8	3
CAS8	*S. ciscaucasicus*	II+IV	*Salmo* sp.	–		−20415.4	4494	7
CAS9	I+II+IV		*Salmo* sp.	–		−20959.3	5581.8	9
CAS10	*S. ciscaucasicus*	II+III+IV	–	–		−21103.8	5870.8	10
CAS0	I+II+III+IV	–	–	–		−21573.5	6810.2	11

b. **Models for the Black Sea basin trouts**

Model	Species combinations					MLE	BF	Rank
BL3	I+II+III	–	–	*S. abanticus*	*Salmo* sp.	−18335.4	–	1
BL6	*S. labrax*	II+III	–	*S. abanticus*	*Salmo* sp.	−18339	7.2	2
BL5	I+II+III+V	–	–	*S. abanticus*	–	−18533.8	396.8	3
BL4	I+IV	II+III	–	–	*Salmo* sp.	−18661.7	652.6	4
BL1	*S. labrax* (I)	*S. rizeensis* (II)	*S. coruhensis* (III)	*S. abanticus* (IV)	*Salmo* sp. (V)	−21495.3	6319.8	5
BL2	I+II+III+IV	–	–	–	*Salmo* sp.	−24395.3	12119.8	6
BL0	I+II+III+IV+V	–	–	–	–	−25107.8	13544.8	7

c. **Models for the Mediterranean basin trouts a***

Model	Species combinations						MLE	BF	Rank
MEa1	*S. peristericus* (I)	*S. chilo* (II)	*S. lourosensis* (III)	*S. farioides* (IV)	*S. opimus* (V)	*S. carpio* (VI)	−28941.503	–	1
MEa2	*S. peristericus*	*S. chilo*	III+IV		*S. opimus*	*S. carpio*	−30074.549	2266.092	2
MEa6	*S. peristericus*	II+III		*S. farioides*	*S. opimus*	*S. carpio*	−30767.142	3651.278	3
MEa3	*S. peristericus*	II+V	*S. lourosensis*	*S. farioides*		*S. carpio*	−31613.205	5343.404	4
MEa4	*S. peristericus*	II+V	III+IV			*S. carpio*	−32784.702	7686.398	5
MEa9	*S. peristericus*	II+III		*S. farioides*	V+VI		−33835.556	9788.106	6
MEa7	*S. peristericus*	II+III		IV+V+VI			−35004.211	12125.42	7
MEa5	I+II+III+IV+V					*S. carpio*	−36498.013	15113.02	8
MEa8	*S. peristericus*	II+III+IV+V+VI					−36541.313	15199.62	9
MEa0	I+II+III+IV+V+VI						−38698.408	19513.81	10

d. **Models for the Moroccan and Mediterranean basin trouts b***

Model	Species combinations					MLE	BF	Rank
MEb1	*S. carpio*(I)	*S. lumi* (II)	*Salmo* sp. Sardinia (III)	*S. platycephalus* (IV)	*S. pellegrini* (V)	−44621.128	–	1
MEb3	*S. carpio*	*S. lumi*	III+IV	–	*S. pellegrini*	−48267.394	7292.532	3
MEb2	I+II	–	*Salmo* sp. Sardinia	*S. platycephalus*	*S. pellegrini*	−48328.652	7415.048	2
MEb4	*S. carpio*	*S. lumi*	III+V	*S. platycephalus*	–	−48403.802	7565.348	4
MEb7	*S. carpio*	II+III	–	*S. platycephalus*	*S. pellegrini*	−48822.685	8403.114	5
MEb5	*S. carpio*	*S. lumi*	*Salmo* sp. Sardinia	IV+V	–	−49422.185	9602.114	6
MEb8	I+II	–	III+IV	–	*S. pellegrini*	−52031.647	14821.04	7
MEb10	I+II	–	III+V	*S. platycephalus*	–	−52219.780	15197.3	8
MEb6	*S. carpio*	*S. lumi*	III+IV+V	–	–	−52551.773	15861.29	9
MEb9	I+II	–	III+IV+V	–	–	−56461.835	23681.41	10
MEb0	I+II+III+IV+V	–	–	–	–	−61793.100	34343.94	11

* a and b denote models set for the members of the Mediterranean and the North African Clade (Clade II; Fig. 5) based on the clustering pattern of the phylogenetic tree, DPAC clusters, and also according to the taxonomy proposed by different authors (see the text).

e. **Models for the Atlantic basin trouts**

Model	Species combinations				MLE	BF	Rank
AT1	*S. trutta* 1 (I)	*S. trutta* 2 (II)	*S. cettii* (III)	*S. marmoratus* (IV)	−30798.4	–	1
AT4	I+III	*S. trutta* 2	–	S. marmoratus	−32872.5	4148.2	3
AT2	I+II	-	*S. cettii*	*S. marmoratus*	−30873.3	149.8	2
AT5	*S. trutta*1	II+III	–	*S. marmoratus*	−32831.1	40.65.4	
AT3	I+II+III	–	–	*S. marmoratus*	−33231.1	4865.4	4
AT0	I+II+III+IV	–	–	-	−37613.1	13629.4	5

f. **Models for representatives from the respective trout clades**

Model	Species combinations					MLE	BF	Rank
G1	*S. labrax* (I)	*S. carpio* (II)	*S. pellegrini* (III)	*S. trutta* (IV)	*S. marmoratus* (V)	−37414.7	–	1
G6	*S. labrax*	*S. carpio*	*S. pellegrini*	IV+V		−40213.1	5596.798	2
G4	I+II	–	*S. pellegrini*	*S. trutta*	*S. marmoratus*	−41123.3	7417.186	3
G5	*S. labrax*	II+III		*S. trutta*	*S. marmoratus*	−41366.2	7902.933	4
G3	I+II+III	–		*S. trutta*	*S. marmoratus*	−45472.7	16116	5
G2	I+II+III+IV	–			*S. marmoratus*	−49977.4	25125.48	6
G0	I+II+III+IV+V	–	–	–	–	−53775.5	32721.67	7

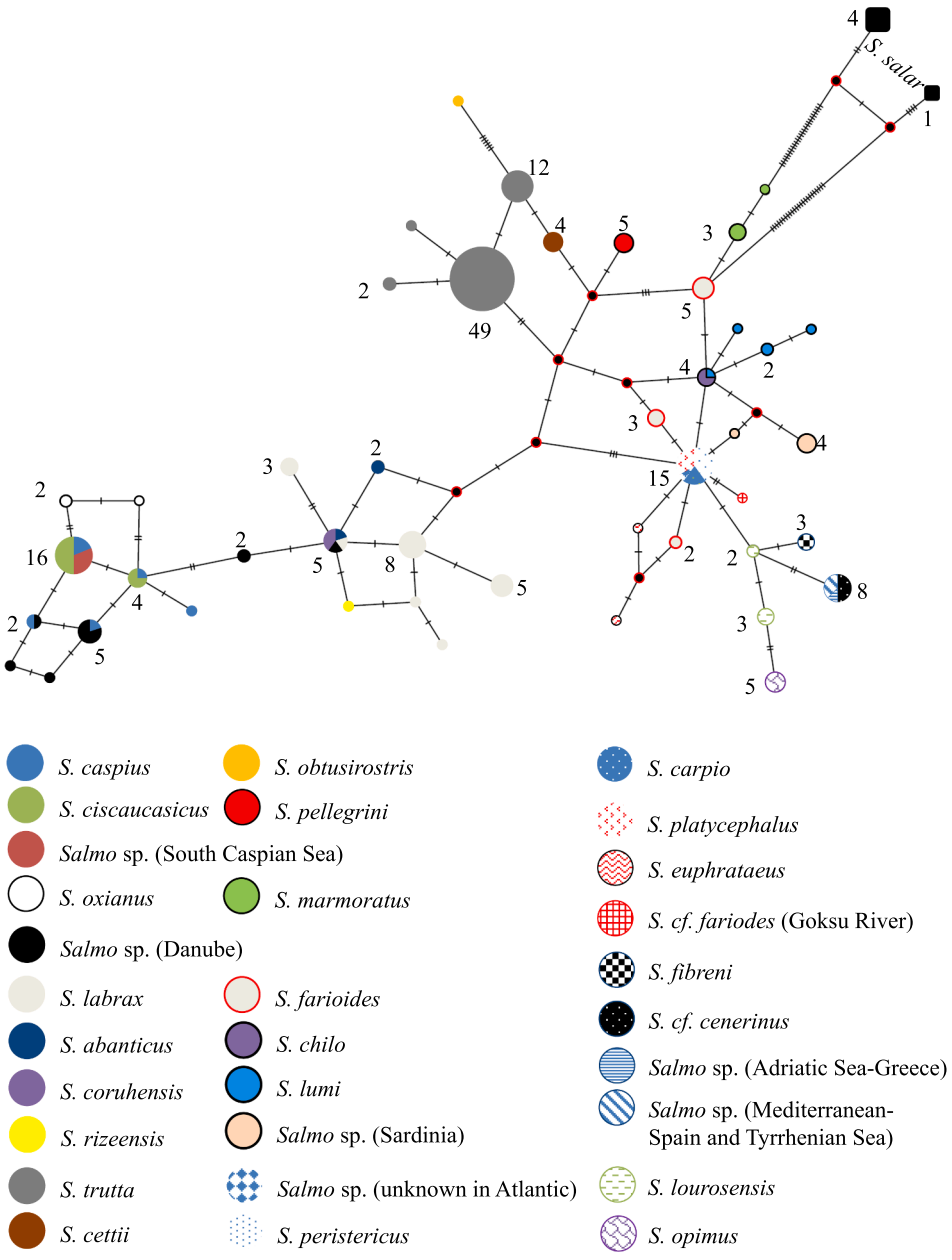

Fig. 1. Haplotype network reconstructed for a 571-bp sequence of the 5′ end of the mitochondrial control region with *S. salar* as the out-group. Cases of shared haplotypes between species are indicated by different colors and patterns in each pie graph. Relative frequency of each species is equivalent to the size of its related slice in the pie graph. Numbers of individuals > 1 are indicated beside each graph. Hatch marks on lines connecting haplotypes correspond to inferred mutational steps. Small black circles with red borders at bifurcating points are mutations that lead to different paths. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Diversity indices calculated for mtDNA control region (D-loop) sequences produced in this study.

Basin		Diversity indices				
	N	*nh*	*hd*	*pi*	*nss*	*pis*
Aral Sea	3	2	0.67	0.001	1	0
Caspian Sea	23	5	0.50	0.001	3	2
Black Sea	35	13	0.90	0.005	11	11
Mediterranean Sea	69	20	0.92	0.006	24	17
Atlantic	64	4	0.38	0.000	3	2
Oum Er-Rbia (Morocco)	5	1	0.00	0.000	0	0
Persian Gulf	2	2	1.00	–	2	0

Abbreviations: N: sample size for each basin, *nh*: number of haplotypes, *hd*: haplotype diversity, *pi*: nucleotide diversity, *nss*: number of segregating sites, *pis*: parsimony informative sites.

significant signs of admixture (Z-scores < -2). Brown trouts from the Black Sea basin showed significant admixtures with brown trout from the Atlantic, Caspian/Aral, and the Mediterranean sea basins (Table 3). Brown trout from the Persian Gulf basin (only two individuals analysed) did not show any sign of admixture in the f_3 test.

The four-population (f_4) test at the population level revealed significant signs of gene flow between different pairs of populations from a same basin or from closely related basins, such as in the Ponto-Caspian basins (Z-score $> |\pm 3|$) (Table 4). The results of the four-population test at the inter-basin level (pooling all non-admixed trouts from each basin) revealed significant gene flow mostly between geographically proximate basins (Table 4). The four-population test at the species level revealed highly significant gene flow among the Mediterranean species pairs. Due to the limited sample sizes that we had for some species or regions, we consider these observations as preliminary only, which should therefore be interpreted cautiously until confirmed with more samples.

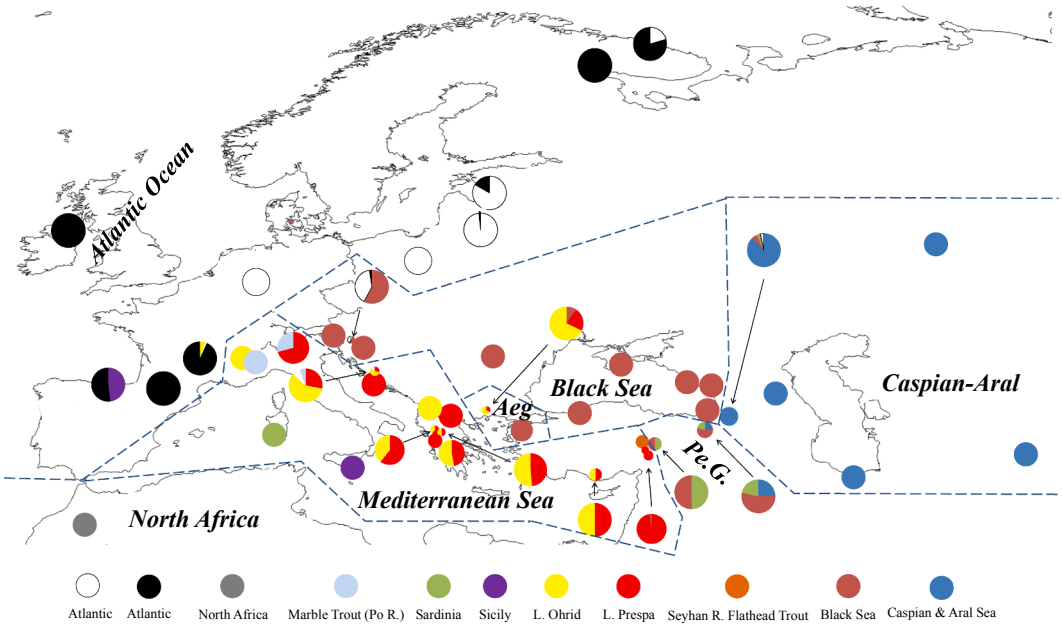

Fig. 2. Distribution of different admixture clusters within and among the studied basins. Colors of pie diagrams denote admixture clusters peculiar to each basin. Dashed lines delineate clusters observed in each basin and may not mirror with the exact basin boundaries. Clusters are named based on geographic region (mostly for the common clusters existing in several species) or species (*S. platycephalus* and *S. marmoratus*). Abbreviations are: L.: Lake, R.: River, Pe. G.: Persian Gulf.

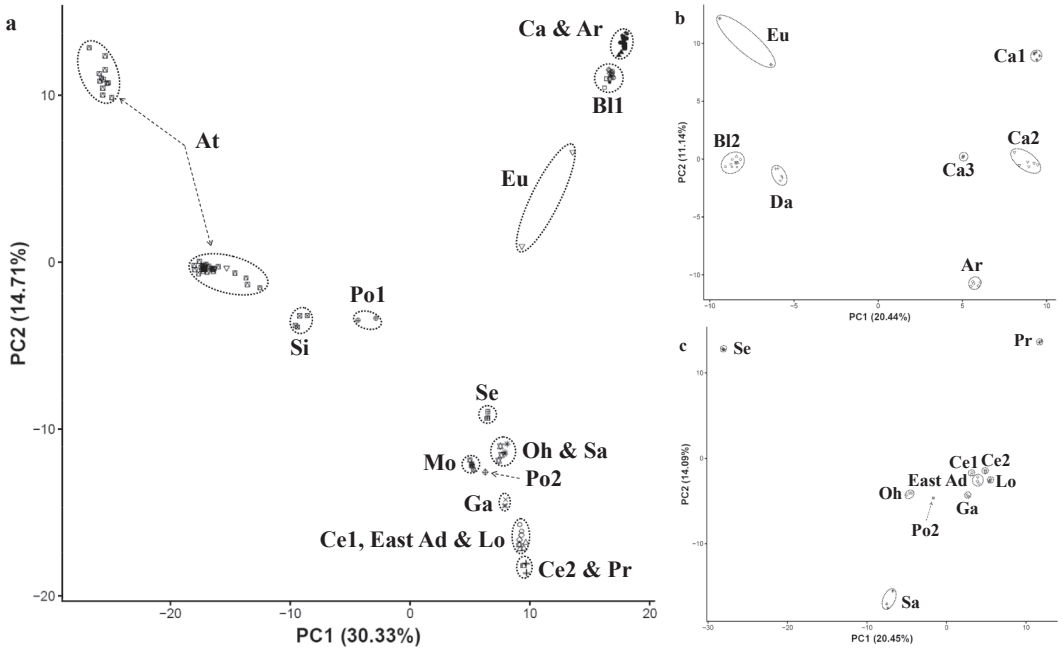

Fig. 3. DAPC graphs produced for SNP data of: a) all brown trouts from different basins (large-scale DAPC), b) brown trouts of the Ponto-Caspian and the Persian Gulf (fine-scale DAPC of the Ponto-Caspian brown trouts), and c) brown trouts of the Mediterranean related basins (except *S. cettii* and *S. marmoratus*) and North Africa (only *S. pellegrini*) along the first and second discriminant functions (fine-scale DAPC of the Mediterranean-Adriatic and North African brown trouts). The ellipses and circles around each cluster are only for the sake of visibility. The abbreviations are: At: Atlantic, North, Baltic, and Barents brown trouts (*S. trutta*), Bl1: Black Sea brown trouts (*S. labrax*, *S. abanticus*, *S. coruhensis*, *S. rizeensis*, and *Salmo* sp. (Danube)), Bl2: Black Sea brown trouts (*S. labrax*, *S. abanticus*, *S. coruhensis*, and *S. rizeensis*), Da: Danube brown trout (*Salmo* sp.), Ca & Ar: Caspian and Aral Sea brown trouts (*S. caspius*, *S. ciscaucasicus*, *Salmo* sp. (south Caspian Sea), and *S. oxianus*), Ca1: *Salmo* sp. (South Caspian Sea), Ca2: West and north Caspian Sea brown trout (*S. ciscaucasicus*), Ca3: Southwest Caspian Sea brown trout (*S. caspius*), Eu: Euphrates brown trout (*S. euphrataeus*), Po1: Po River Marble trout (*S. marmoratus*), Si: Sicilian brown trout (*S. cettii*), Oh: Ohrid Lake brown trout (*S. lumi*), Sa: Sardinian brown trout (*Salmo* sp.), Se: Seyhan River Flathead trout (*S. platycephalus*), Mo: Moroccan (North African) brown trout (*S. pellegrini*), Po2: Po River brown trout (*S. cf. cenerinus*), Ga: Garda Lake brown trout (*S. carpio*), Ce1: Ceyhan River brown trout (*S. opimus*), East Ad: East Adriatic brown trout (*S. farioides*), Lo: Louros River brown trout (*S. lourosensis*), Ce2: Ceyhan River brown trout (*S. chilo*), Pr: Prespa Lake brown trout (*S. peristericus*). As there was one non-admixed Po River brown trout (*Salmo cf. cenerinus*) its DAPC cluster was not considered.

Table 3
Significant results of inter-basin three-population (f_3) test.

Basins			f_3	SE	Z-score
Admixed basin	Source basin I	Source basin II			
Black	Aral	Atlantic	−0.00943	0.000507	−18.6017
Black	Atlantic	Caspian	−0.00659	0.000445	−14.7966
Black	Aral	Mediterranean	−0.00406	0.000395	−10.2662
Black	Caspian	Mediterranean	−0.0024	0.000369	−6.51007
Black	Aral	Morocco	−0.00243	0.000443	−5.47413
Black	Caspian	Morocco	−0.00208	0.000455	−4.565
Mediterranean	Atlantic	Morocco	−0.00254	0.000585	−4.34243
Mediterranean	Aral	Atlantic	−0.00173	0.00042	−4.12132
Mediterranean	Atlantic	Persian	−0.00128	0.000512	−2.50613

Table 4
Results of the significant inter-basin four-population (f_4) test with *Salmo salar* as out-group. The basins in blue denote the pairs of basins between which a probable gene flow existed. The out-group is not shown.

Basin A	Basin B	Basin C	f_4	Std err	Z-score	Basin A	Basin B	Basin C	f_4	Std err	Z-score
Aral	Atlantic	Caspian	0.043051	0.001627	26.4549	Aral	Atlantic	Mediterranean	0.008338	0.000878	9.49172
Aral	Mediterranean	Caspian	0.031845	0.00143	22.27	Aral	Atlantic	Morocco	0.007788	0.000985	7.91039
Aral	Persian Gulf	Caspian	0.015906	0.00077	20.6509	Mediterranean	Persian	Morocco	0.004214	0.000655	6.43248
Aral	Atlantic	Persian Gulf	0.026412	0.001295	20.3935	Atlantic	Black	Caspian	-0.03818	0.001531	-24.9333
Aral	Mediterranean	Black	0.026974	0.001326	20.3416	Atlantic	Black	Persian Gulf	-0.02618	0.001281	-20.4424
Black	Mediterranean	Caspian	0.026979	0.001344	20.073	Atlantic	Caspian	Persian Gulf	-0.02618	0.001375	-19.0435
Aral	Morocco	Caspian	0.031588	0.001594	19.8138	Atlantic	Morocco	Mediterranean	-0.01389	0.000935	-14.8634
Aral	Morocco	Black	0.026513	0.001444	18.3609	Atlantic	Mediterranean	Caspian	-0.01121	0.000765	-14.6423
Black	Morocco	Caspian	0.026722	0.001491	17.9182	Atlantic	Mediterranean	Black	-0.01147	0.000811	-14.1442
Black	Persian Gulf	Caspian	0.01104	0.000736	15.004	Atlantic	Mediterranean	Persian Gulf	-0.01112	0.000836	-13.3032
Aral	Mediterranean	Persian Gulf	0.015296	0.001079	14.1793	Atlantic	Black	Mediterranean	-0.00844	0.000794	-10.6348
Black	Mediterranean	Persian Gulf	0.015066	0.00107	14.0736	Black	Mediterranean	Morocco	-0.00499	0.000524	-9.51686
Aral	Persian Gulf	Black	0.011433	0.000865	13.2098	Atlantic	Caspian	Mediterranean	-0.00805	0.000885	-9.09576
Caspian	Mediterranean	Persian Gulf	0.015066	0.001155	13.0426	Caspian	Mediterranean	Morocco	-0.00559	0.000617	-9.06641
Black	Morocco	Persian Gulf	0.014306	0.001201	11.9081	Aral	Mediterranean	Morocco	-0.00529	0.000623	-8.48848
Aral	Morocco	Persian Gulf	0.014536	0.001247	11.6589	Black	Morocco	Mediterranean	-0.00545	0.000769	-7.0856
Aral	Black	Caspian	0.004866	0.00044	11.0518	Caspian	Morocco	Mediterranean	-0.00585	0.000849	-6.88789
Caspian	Morocco	Persian Gulf	0.014306	0.001299	11.0138	Aral	Mediterranean	Atlantic	-0.00334	0.000621	-5.37761

3.5. Rogue and hybrid taxa

The results of taxonomic jackknifing identified *S. pellegrini* and *Salmo* sp. (from the Danube drainage) as rogue taxa. Possible signatures of hybridization events were identified in the neighbor network for *S. marmoratus*, *S. pellegrini*, *S. cf. cenerinus*, *S. carpio*, *Salmo* sp. (Danube drainage), and *S. euphrataeus* (Fig. 4).

3.6. Phylogeny based on Genotype-by-sequencing data

Both the SVDQuartets Species and ML gene trees reconstructed for SNP data were similar in topology except for a few changes in the order of species within the Mediterranean brown trout clade. In the species tree, three clades with high bootstrap support values (BS = 98–100) were resolved, including the Atlantic (Clade I), Mediterranean and Moroccan (clade II), and Ponto-Caspian (Clade III) and marble trout (*S. marmoratus*) showed a sister position relative to these (Fig. 5). Brown trout from Sicily (Mediterranean Sea basin) nested with Atlantic brown trout in clade I and clades II and III showed a sister relationship to one another. Clade II included brown trouts from Oum Er-Rbia River (Morocco), Seyhan River (Turkey), and from Sardinia, as well as all other Mediterranean brown trouts included in this study. Clade III included brown trout from the Euphrates River, as well as from the Aral, Black, and Caspian Sea basins (BS = 100), with brown trout from the Euphrates River being sister to other brown trouts of the Ponto-Caspian basins. Two sub-clades including the Caspian-Aral and Black Sea sub-clades were resolved within clade III.

3.7. Molecular species delimitation

In most cases, Bayes factor delimitation (BFD) results were concordant with the current taxonomy proposed for brown trouts (Doadrio et al., 2015; Kottelat and Freyhof, 2007; Ninua et al., 2018; Turan et al., 2014). In the Caspian and Aral Sea basins, however, BFD results rejected all models tested here in favor of a four-species model (model Cas1), in which the southern Caspian brown trout were considered as a separate group (Table 1a). In the case of the Black Sea basin brown trouts, BFD results supported a three-species model (model BL6) in which *S. labrax*, *S. coruhensis* and *S. rizeensis* were lumped and trout from the Danube River drainage were considered as a group separate from *S. labrax* and *S. abanticus* (Table 1b). Among different species delimitation models proposed *S. farioides*, *S. lourosensis*, *S. chilo*, *S. opimus*, *S. peristericus*, and

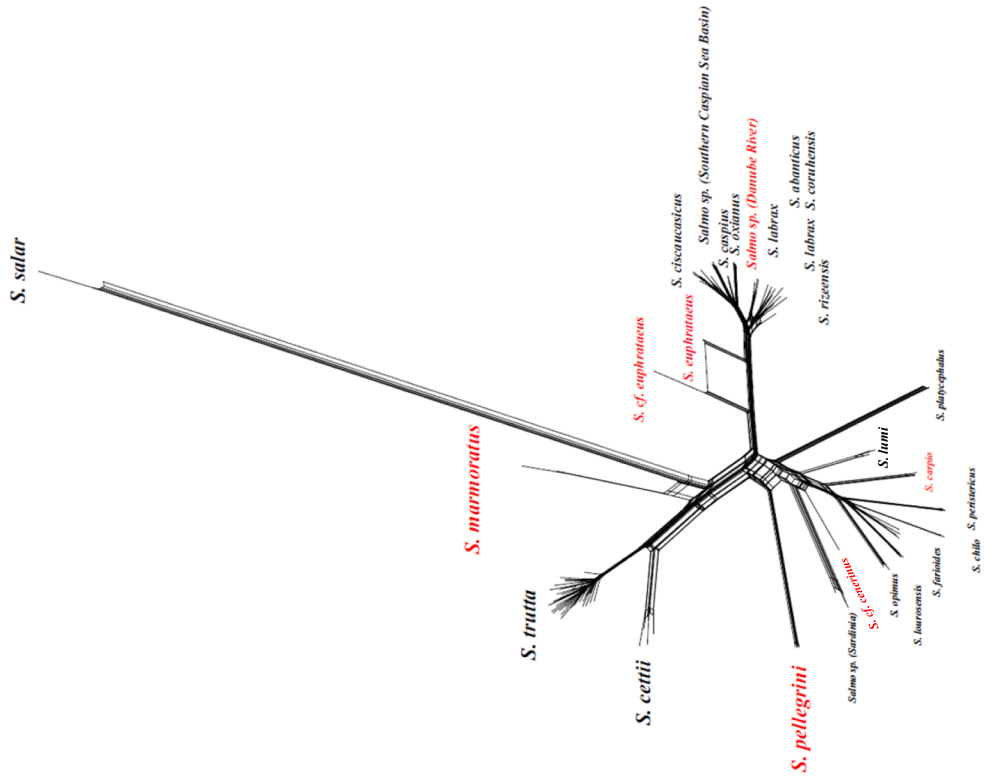

Fig. 4. Neighbor-Net reconstructed with SplitsTree using SNP sequences. The species that appear to be hybrids or claimed as hybrids are shown in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

S. carpio, as separate species (model MEa1) was better supported than other models lumping them in various groups (Table 1c). Among the second set of models proposed for the Mediterranean and the Moroccan trouts, the five-species model which proposes *S. carpio* (a representative from the previous set of the Mediterranean brown trouts shown in Table 1c), *S. lumi*, *Salmo* sp. (Sardinia), *S. pellegrini*, and *S. platycephalus* as separate groups (model Meb1) was better supported than other models (Table 1d). In a species delimitation scenario based on DAPC and admixture clustering patterns of the Atlantic brown trout *S. trutta* (two clusters), Sicilian brown trout *S. cettii* (one cluster associated with the Atlantic lineage), and marble trout *S. marmoratus* (a non-Atlantic cluster), the four-species model whereby Atlantic *S. trutta* was split into two groups, with *S. cettii* and *S. marmoratus* as separate species (model AT1), was better supported than the current taxonomy in which the Atlantic trout *S. trutta* is considered as a single species (model AT2) or the other models we tested (Table 1e). Finally, among the models tested for different clades, the four-clade model (model G1), with *S. marmoratus* (sister species to all other brown trouts), *S. trutta* (clade I), *S. pellegrini* and *S. carpio* (clade II), and *S. labrax* (clade III) as representatives of the clades identified on the species tree (Fig. 5), was supported as the most likely model describing the brown trout clades (Table 1f).

4. Discussion

Given their tremendous phenotypic diversity, brown trouts have been the subject of long-lasting debates regarding their taxonomy and species delineation. In this context, we combined Genotyping-by-Sequencing and mitochondrial DNA sequencing data to assess the phylogenetic relationships among 24 previously described or potential brown trout species in order to compare resolved genomic clusters with the current taxonomy, and to provide a perspective on phylogenetic, taxonomic, and conservation considerations of brown trouts from North Africa and Eurasia, as discussed below.

4.1. Atlantic brown trout stocked out of their natural range

The geographic distribution of brown trout genetic diversity has been impacted by recent anthropogenic activities, and particularly stocking for commercial and recreational purposes (Berrebi et al., 2019). Our results revealed traces of introgression from northern Atlantic brown trout in all major basins, except for the Aral Sea and Moroccan Atlantic basins. This is concordant with studies that previously documented stocking of northern Atlantic basin brown trout into other basins (Barbat-Leterrier et al., 1989; Bernatchez, 2001; Berrebi et al., 2019; Kohout et al., 2013; Osinov and Bernatchez, 1996). The identification and removal of admixed individuals from our analysis helped to filter out the consequences of these anthropogenic effects (*e.g.*, Atlantic basin brown trout transfers into non-native basins), which would have blurred the signal of natural genetic differentiation among different brown trout lineages.

4.2. Brown trout taxonomy

At least 34 species concepts have been proposed, each with different sets of criteria including reproductive isolation, ecological, evolutionary, phylogenetic, phenetic, and genotypic distinctiveness among many others, for delineating species (De Queiroz, 2007; Zachos, 2018). Recently diverged taxa may violate the conditions underlying many of these different species concepts due to weak phylogenetic signals resolved, incomplete lineage sorting, or naturally occurring introgressive hybridization (Alda et al., 2019; Fernández-Mazuecos et al., 2017; MacGuigan and Near, 2018; Palandačić et al., 2017; Roycroft et al., 2019). In the case of brown trouts, for instance, mtDNA data alone do not discriminate most morphologically described species because of these factors. Yet, private mtDNA haplotypes were observed among some taxa, although the divergence between them fell within the intraspecific range for many other fish species (April et al. 2011). These private haplotypes do not resolve monophyletic groups/lineages that

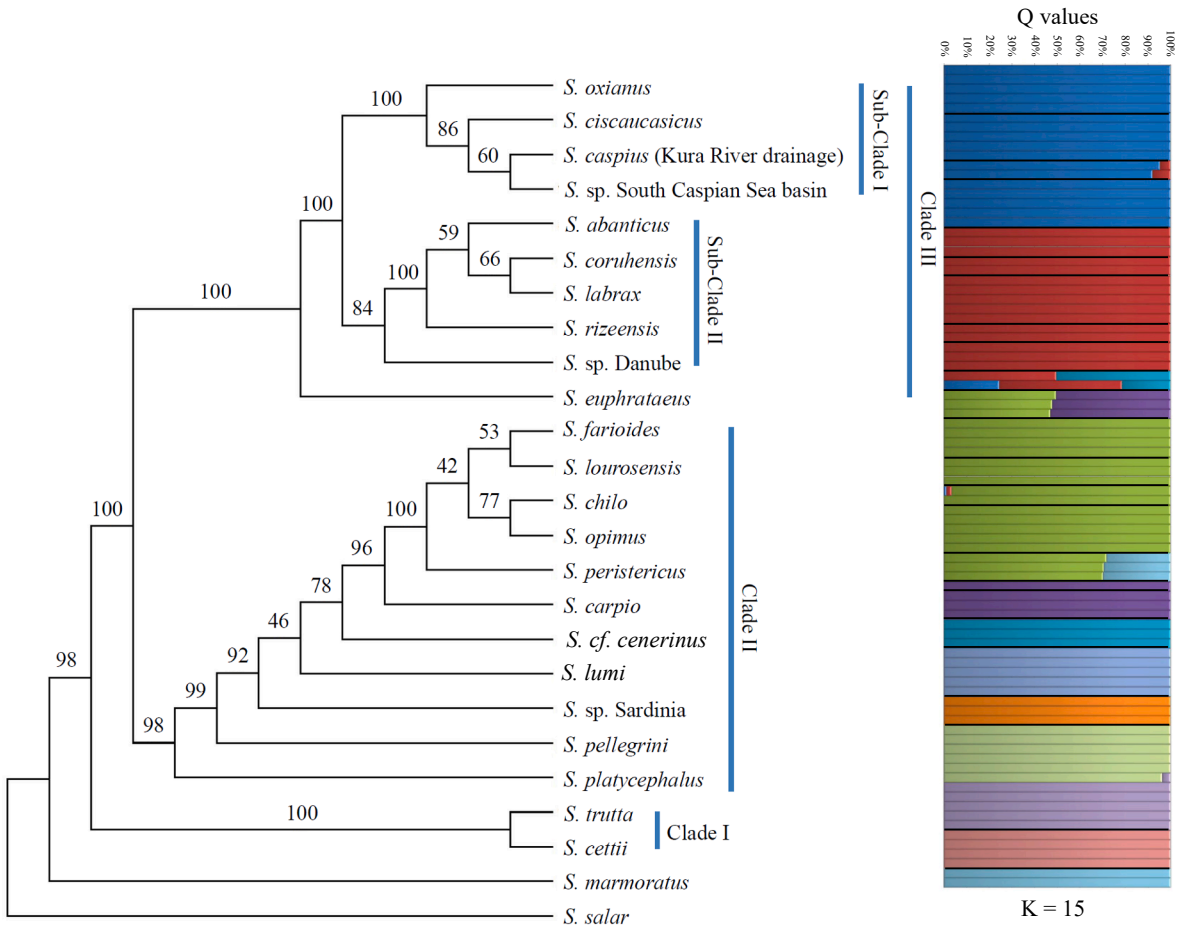

Fig. 5. Species tree (left panel) reconstructed for 15169-SNP data set using SVDQuartets method and bar plot (right panel) showing the admixture clusters ($K = 15$) of the species on species tree. The black lines delineating the bars determine clusters belonging to each species. The order of species on the bar plot is exactly the same as the order of species on the species tree. As we have excluded three of the clusters for which there were no pure genotypes, in the admixture graph shown, there are only 12 clusters.

can be considered as valid species based on the phylogenetic species concept. Moreover, some of the brown trout species included here can interbreed and produce viable hybrids (Delling et al. 2000; Jug et al. 2005; Meldgaard et al. 2007; Sušnik et al. 2007b, 2015), which does not accord with the biological species concept. Overall, except for the morphological species concept, brown trout diversity does not conform to the other most commonly applied species concepts. Delimiting brown trout species based on morphological attributes only could erroneously lead to taxonomic inflation, since these could be caused by phenotypic plasticity. On the other hand, recognition of brown trout diversity as comprising only one or a few species based on mtDNA phylogenetic lineages is also problematic because this cannot confirm or infirm the possibility of reproductive isolation among populations belonging to a same mtDNA lineage. Both taxonomic inflation (over-splitting of closely related populations into species) and reduced presentation of biodiversity (lumping of multiple valid species into one species) represent extreme cases of taxonomic delineation posing risks for conservation (Frankham et al., 2012; 2017; Frankham, 2015). This represents an important dilemma since on the one hand, taxonomy as a science is not meant to be at the service of conservation and on the other hand, the adequate conservation of the diversity of such a widespread and important species is a duty for stakeholders and biologists, which may partly depend on proper taxonomic recognition.

Previous studies (Doadrio et al., 2015; Kottelat and Freyhof, 2007; Ninua et al., 2018; Turan et al., 2009; 2011; 2014; 2017; 2020; Zaccara et al., 2015) provide a set of morphological species hypotheses that allowed recognition of conservation units. The species delimitations proposed in these studies are the best-science background based on morphological units of brown trout, which might also form evolutionarily significant units (Waples, 1991), as populations which belong to one species usually occur in adjacent geographical units and species have discrete distributional areas. This discrete distributional pattern is in contrast with several of the molecular lineages proposed before, which, in some cases, have no discrete distributional areas, and several lineages are sometimes found within a particular brown trout population.

Here, using GBS and mtDNA data, we could only compare the groups resolved and delimited by our analyses with reference to the morphological species of brown trout proposed by different authors (Doadrio et al., 2015; Kottelat and Freyhof, 2007; Ninua et al., 2018; Turan et al., 2011; 2009; 2014; 2017; Zaccara et al., 2015). However, for the full recognition of species, morphological and molecular analyses should be brought together in an integrative fashion, which is beyond the scope of this study, since the small sample sizes and incomplete taxon sampling used here hinder our ability to make robust inferences on recognition or rejection of the nominal species. In this context, our main goal was only to test whether previously described trouts can be supported or not as independent groups by the methods that we applied. This is the first survey including many of the existing taxa of the species complex from throughout most of its distribution range in a common analysis based on both mtDNA and nDNA markers. The confirmation of our results awaits more studies using larger sample sizes and more complete taxon

sampling.

The clustering pattern of our phylogenomic tree resolved four phylogenetic groups only, which agrees with four of the major mtDNA lineages proposed for brown trouts by Bernatchez (2001) (Danube, Mediterranean/Adriatic, Marmoratus, and Atlantic) and the lineages resolved using the nuclear rDNA *ITS* or allozyme markers (GarcÍa-MarÍn et al., 1999; Presa et al., 2002). In contrast, the results of admixture analysis or discriminant analysis of principal components discriminated 11 and 13 groups respectively, as discussed below for each studied basin or region. Thus, admixture clusters, DAPC clustering pattern, and BFD test results all supported the existing taxonomy for the Atlantic brown trout (*S. trutta*), marble trout (*S. marmoratus*), Sicilian brown trout (*S. cettii*), Seyhan flathead (*S. platycephalus*), and brown trout from Oum Er-Rbia (*S. pellegrini*). However, for other Adriatic and Mediterranean and the Ponto-Caspian species, these analyses grouped several species into common or very closely related clusters.

4.2.1. Atlantic brown trout

Mitochondrial DNA data, BFD, phylogenomic, DAPC, and admixture analyses were all concordant with the hypothesis of separate species status for the Atlantic brown trout *S. trutta*, which may be divided into more taxonomic units or lineages according to our data. Our admixture and DAPC analyses identified two distinct clusters for the Atlantic brown trout. One of these putative pure clusters comprise trouts from the White Sea, rivers in France and Ireland, whereas the other cluster mostly comprise trouts from the Baltic Sea drainages. These different clusters are probably concordant with clusters identified recently for *S. trutta* (Bekkevold et al., 2020) and/or with different groups of the Atlantic brown trout proposed by Bernatchez (2001). Based on these findings, we propose that Atlantic brown trouts comprise more than one species, but this hypothesis remains to be rigorously tested.

4.2.2. Italian and North African brown trouts

Our results showed that *S. marmoratus*, *S. cettii*, *S. carpio*, and the North African *S. pellegrini* belong to separate DAPC clusters. The pronounced distinctiveness of *S. marmoratus* is concordant with previous studies (Delling, 2002; Giuffra et al., 1994; Gratton et al., 2014; Lecaudey et al., 2018; Pustovrh et al., 2014; Sušnik et al., 2007a). Lobón-Cerviá et al. (2018) considered all brown trout populations found in insular and peninsular Italy to be *Salmo cettii*, whereas our results showed that *S. cettii* from the type locality (Sicily) is genetically very distinct from the Sardinian brown trout. In addition, Schöffmann et al. (2007), Snoj et al. (2011), Fruciano et al. (2014), and Tougard et al. (2018) reported a similarity between the Sicilian and the North African brown trout based on mtDNA phylogeny. Our mtDNA results are concordant with this interpretation, but not our nuclear GBS data which grouped the North African brown trout close to the Mediterranean and Adriatic brown trouts, but the Sicilian *S. cettii* close to the Atlantic *S. trutta*. Also, our GBS data discriminate *S. cettii* from Sicily from all other species analysed based on the clusters resolved both by admixture analysis and DAPC. This supports the recognition of *S. cettii* from Sicily as a valid species. Our GBS data resolved separate admixture and DAPC clusters for the only North African brown trout species that we analysed (*S. pellegrini*) from Morocco. Therefore, we propose that retaining separate species recognition for *S. pellegrini* is justified.

Salmo carpio is considered as one of the accepted endemic brown trout species of Italy (Bianco, 2014; Giuffra et al., 1994; Gratton et al. 2014; Lobón-Cerviá et al., 2018). Splendiani et al. (2017, 2019) refered to *S. carpio* only as an "ecotype" of Adriatic brown trout in Lake Garda, Italy. Our DAPC results showed that *S. carpio* formed a distinct cluster which appeared to be introgressed with *S. marmoratus* in the admixture analysis. Overall, our DAPC and BFD test results are concordant with the distinct taxonomic status of *S. carpio*.

Kottelat and Freyhof (2007) used the name *S. cenerinus* for brown trout from the upper reaches of the Po River drainage of Italy. But by reinterpreting published morphological and biological attributes, Bianco (2014) proposed considering the Po River brown trout as *S. farioides*. Here, the pure *S. cf. cenerinus* specimens from the Po River drainage we could analyse genetically, had the Adriatic mtDNA haplotype, its admixture cluster was shared with *S. lumi* from Lake Ohrid (see discussion for this taxon below), and it was also relatively closer to the *S. lumi* DAPC cluster than with *S. farioides* or any other Adriatic brown trouts. Based on our limited data we suggest considering a separate species status for the Po River brown trout, possibly *S. cenerinus*, but this remains to be tested using a larger sample size.

Different studies using morphology and molecular data concluded that brown trouts from Corsica and Sardinia, belong to the Adriatic-Mediterranean phylogenetic lineages (Berrebi et al., 2017; 2019; Delling et al., 2020; Sabatini et al., 2018; Tougard et al., 2018). These authors also refuted the suggestion that brown trouts from Corsica and Sardinia belong to *S. macrostigma*, which was originaly described from Algeria. Both our mtDNA and genomic data are concordant with these previous studies. However, since our results also showed that brown trout from Sardinia formed a distinct admixture cluster, we propose that it may be justified to recognise them with a distinct scientific name.

4.2.3. Balkan and Turkish Mediterranean brown trouts

Salmo lumi is a river-spawning brown trout from Lake Ohrid where non river-spawning brown trout are known as *S. letnica*. The admixture and fine-scale DAPC revealed separate clusters for *S. lumi*, which corroborates its distinct status. *Salmo peristericus* is a brown trout found in the Lake Prespa (Albania, Macedonia, and Greece). Based on morphological analysis, Delling (2010) proposed that recognising it as a distinct species was valid. However, Snoj et al. (2009) and Berrebi et al. (2013) subsequently refuted this view based on mtDNA and microsatellite DNA analyses and instead proposed to consider this taxon as a conservation unit within *S. trutta*. Our fine-scale DAPC results showed that *S. peristericus* formed a DAPC cluster which is highly distinct from all other Mediterranean-Adriatic species (excluding *S. cettii* and *S. marmoratus*) analysed here. Consequently, we propose that this brown trout should retain its taxonomic distinction as *S. peristericus*.

Based on mtDNA and nDNA sequence analyses, Sušnik et al. (2004), argued that *S. platycephalus* is a member of the Adriatic phylogenetic lineage of *S. trutta* but they could not infer its exact position within the Adriatic lineage. Later, Kara et al. (2011) reconfirmed the morphological uniquness of *S. platycephalus*. Our GBS results are concordant with the results of Kara et al. (2011). They showed that *S. platycephalus* form a distinct cluster occupying a basal position realtive to all other brown trouts (excluding *S. cettii* and *S. marmoratus*) from the Mediterranean and Adriatic sea drainages (Figs. 3 and 5). Hence, we propose to retain a distinct species status for *S. platycephalus*.

Our results also suggested that several additional taxa may be grouped together within a same fine-scale DAPC cluster suggesting their relatively weak genetic differentiation may not warrant recognising them as valid species. This is namely the case for *S. farioides*, *S. opimus*, *S. chilo*, and *S. lourosensis*. Admittedly, increased sample sizes and taxon sampling will be required to more rigorously assess the validity of retaining the species distinction of these taxa, although their genetic differentiation is clearly weaker than that we observed between other recognised brown trout species elsewhere.

4.2.4. Ponto-Caspian and Mesopotamian brown trouts

The Ponto-Caspian brown trouts include *S. labrax*, *S. coruhensis*, *S. rizeensis*, and *S. abanticus* (in the Black Sea basin), *S. caspius*, *S. ciscaucasicus*, *S. ezenami*, and *Salmo ischchan* (in the Caspian Sea basin), and *S. oxianus* (in the Aral Sea basin) (Kottelat and Freyhof, 2007; Levin et al., 2018; Ninua et al., 2018; Turan et al., 2009, 2011). Ninua et al. (2018) analysed mtDNA control region and *Cyt*-b sequences of brown trout species from the Black and Caspian Sea basins. They suggested considering only *S. rizeensis* and *S. labrax* as valid species in the Black Sea basin and *S. caspius* and *S. ciscaucasicus* in the Caspian Sea basin. Our large-scale DAPC and admixture clusters based on GBS data

grouped the Black Sea species into one genomic cluster. On the other hand, DAPC performed on the Ponto-Caspian and Mesopotamian brown trouts only (fine-scale DAPC) discriminated *Salmo* sp. of the Danube from other trouts of the Black Sea basin. A DAPC performed only on the Black Sea basin brown trouts could also discriminate *S. abanticus* and *Salmo* sp. from the Danube drainage but *S. rizeensis* and *S. coruhensis* apeared very closely related with *S. labrax* (concordant with BFD test results), suggesting that their species distinction may not be waranted whereas it may be the case for *S. abanticus* and *Salmo* sp. from the Danube drainage.

Regarding the brown trouts from the Caspian Sea (*S. caspius*, *S. ciscaucasicus*) and Aral Sea (*S. oxianus*) basins that we analysed, the large-scale DAPC clustering resolved only one group, which is not in line with their current taxonomy. On the other hand, the clustering pattern when performing a DAPC with the Ponto-Caspian and Mesopotamian brown trouts only, is in agreement with the taxonomy proposed for the Caspian and Aral Sea brown trouts (Kottelat and Freyhof, 2007; Levin et al., 2018; Ninua et al., 2018; Turan et al., 2011). In particular, Ninua et al. (2018) suggested using *S. caspius* for the Kura River and southern Caspian Sea brown trouts and *S. ciscaucasicus* for the western and northern Caspian Sea brown trouts. But, our fine-scale DAPC clustering pattern showed that the southern Caspian brown trout (*Salmo* sp.) differs from the Kura River brown trout *S. caspius* and might thus qualify for a separate species status. Brown trout from the Kura River drainage are closely related to the west and north Caspian brown trout (*S. ciscaucasicus*), and also realtively closer to the Black Sea brown trouts in the DAPC space, suggesting possible historical gene flow between them. In the Balik Golu Lake of the Aras River drainage a haplotype similar to the haplotypes that exist in the Danube River drainage was observed in our study, which may justify the relation of brown trout in the Aras River drainag to the Black Sea brown trout. Overall, our results sugest that *S. ciscaucasicus* could be synonymized with *S. caspius* and that *S. caspius* could be kept only for the brown trouts from the west and north Caspian Sea drainages, which should be assessed into more details with additional morphological and genetic analyses of type specimens. The Aral Sea brown trout (*S. oxianus*) formed a unique fine-scale DAPC cluster concordant with a previous report based on its divergent mtDNA haplotype (Griffiths et al., 2009). We thus propose to keep using *S. oxianus* for the Aral Sea brown trout.

Bernatchez (2001) previously showed that both the Danube and Mediterranean brown trout mtDNA lineages were found in the Persian Gulf basin. Here we only found Mediterranean-related haplotypes in a few specimens from the Euphrates River, probably *Salmo euphrataeus*. These specimens formed their own DAPC cluster and we suggest to consider the brown trouts of the Euphrates as a separate taxonomic unit, as proposed by Turan et al. (2014). As we had only two specimens from the Euphrates River, we cannot draw firm conclusions on the intra-basin taxonomic divisions of brown trout in Mesopotamia, which should be clarified using more specimens from different populations and nominal species.

4.3. Brown trouts originated via hybridization

Like previous studies (*e.g.*, Bernatchez, 2001), our study also revealed the major role of geographic isolation in diversification of brown trout species complex. Furthermore, our study also suggested a role for hybridization in the evolution of some trout species, which had previously been hypothesized. (Namely, this has been proposed for *S. marmoratus* (Templeton, 2004) and *S. carpio* (Giuffra et al., 1994, 1996; Gratton et al., 2014). A few of our tests also showed signatures of hybridization for *S. marmoratus* and *S. carpio*. Among other species with no previous report on their hybrid origin, our results showed signatures of hybridization in one or more of the analyses we performed, and thus suggested that possibly natural hybridization between different brown trout lineages has been involved in the origin of the following taxa: i) *S. pellegrini* in Morocco may have originated via hybridization between *S. trutta/S. cettii* and Mediterranean-Adriatic lineage of brown trout; ii) *S. farioides* from east Adriatic Sea drainages via hybridization between the Ohrid and Prespa lineages revealed by our admixture clusters; iii) *Salmo* sp. from the Danube River drainage via hybridization between the Black Sea and the Caspian Sea brown trouts; iv) *S. caspius* from the Kura R. drainage in Southwest Caspian Sea via hybridization between the Caucasian and the Black Sea brown trouts and v) *S. euphrataeus* from the Euphrates River drainage via hybridization between the Mediterranean-Adriatic and the Ponto-Caspian brown trouts. Therefore, we propose that these taxa should be the main research targets of future studies aiming at investigating more rigorously than could be achieved in this study the role of introgressive hybridization in the diversification of the brown trout species complex.

5. Management and conservation considerations

As noted before, there is no globally accepted or applicable species concept to describe brown trout diversity. Splitting brown trout diversity into numerous species based on morphology or considering different brown trouts as members of one or very few species can have adverse effects on conservation. In the case of the fragmented small populations that suffer from low genetic diversity, genetic rescue via transferring individuals from other conspecific populations is considered as an efficient conservation practice to reduce the adverse effects of inbreeding (Frankham, 2015; Frankham et al., 2017; Whiteley et al., 2015). Over-splitting each morphologically distinct population into a different species without phylogeographic considerations could have adverse effects on their conservation, since for genetic rescue of such populations there would be no alternative source population to use. On the other hand, considering many different populations as members of one or a few species only could also have serious conservation risk for local brown trout gene pools, since transfer of individuals among highly diverged populations could raise the problem of mixing gene pools at different points on the adaptive landscape, with possible outbreeding depression and loss of genetic identity (Hallerman, 2003; Frankham et al., 2012). This latter mechanism is particularly evident with the historic stocking of Atlantic-basin brown trout into the Mediterranean and the Black sea drainages, which led to introgression, thus eroding local trout diversity. Splendiani et al. (2019) proposed isolating conservation decision making from taxonomic consideration, i.e., to pursue management at the population level, since the taxonomy of brown trouts is subject to continuous changes. On the other hand, in order to provide a better framework for conservation, it may be necessary for taxonomists and conservationists to work jointly (Mace, 2004). In taxonomic studies, species are usually described by considering mostly morphological attributes of single populations (type populations) with no attention to metapopulations theory or population clusters. Based on our study, which should be followed up using more complete taxon sampling and larger sample sizes to cover all trout diversity, it appears that a number of described brown trout species in each basin are members of closely related population clusters in the East Adriatic, in northeast Mediterranean Turkish, and in the Ponto-Caspian sea drainages that may be considered as conservation units within a unified management regime for each region. Other such units may be defined in other regions. The common general feature of nearly all species concepts is a cluster of populations with a specific evolutionary pathway (De Queiroz, 2007). It appears that paying attention to this aspect of species concepts in taxonomy and conservation of brown trouts can be a useful alternative to avoid taxonomic inflation, while promoting conservation of brown trout diversity within each basin. Hence, in order to ensure long-term sustainability of brown trout diversity, we propose that taxonomic studies in fish species complexes including brown trouts should be based on population genomic clusters in each basin or geographic region rather than describing species using specimens only from a morphologically differentiated single population.

CRediT authorship contribution statement

Iraj Hashemzadeh Segherloo: Conceptualization, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Visualization, Funding acquisition. **Jörg Freyhof:** Conceptualization, Resources, Writing - review & editing, Funding acquisition. **Patrick Berrebi:** Resources, Writing - review & editing. **Anne-Laure Ferchaud:** Formal analysis, Writing - review & editing. **Matthias Geiger:** Resources, Writing - review & editing. **Jérôme Laroche:** Formal analysis, Writing - review & editing. **Boris A. Levin:** Resources, Writing - review & editing. **Eric Normandeau:** Formal analysis, Software, Data curation, Writing - review & editing. **Louis Bernatchez:** Conceptualization, Resources, Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Demultiplexed DNA sequences are available at SRA database (SUB5746961). NCBI Accession numbers for mtDNA control region sequences: MW251415-MW251472.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2021.107204.

References

Aberer, A.J., Krompaß, D., Stamatakis, A., 2011. RogueNaRok: An efficient and exact algorithm for rogue taxon identification. Heidelberg Institute for Theoretical Studies: Exelixis-RRDR-2011–10.

Aberer, A.J., Krompass, D., Stamatakis, A., 2012. Pruning rogue taxa improves phylogenetic accuracy: an efficient algorithm and webservice. Syst. Biol. 62, 162–166. https://doi.org/10.1093/sysbio/sys078.

Alda, F., Tagliacollo, V.A., Bernt, M.J., Waltz, B.T., Ludt, W.B., Faircloth, B.C., Alfaro, M. E., Albert, J.S., Chakrabarty, P., Near, T., 2019. Resolving deep nodes in an ancient radiation of neotropical fishes in the presence of conflicting signals from incomplete lineage sorting. Syst. Biol. 68 (4), 573–593. https://doi.org/10.1093/sysbio/syy085.

Alexander, D.H., Novembre, J., Lange, K., 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome Res. 19 (9), 1655–1664. https://doi.org/10.1101/gr.094052.109.

Alexandrou, M.A., Swartz, B.A., Matzke, N.J., Oakley, T.H., 2013. Genome duplication and multiple evolutionary origins of complex migratory behavior in Salmonidae. Mol. Phylogen. Evol. 69 (3), 514–523.

Aljanabi, S.M., Martinez, I., 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res. 25, 4692–4693. https://doi.org/10.1093/nar/25.22.4692.

Andrews, S., 2010. FastQC: a quality control tool for high throughput sequence data. [WWW document]. URL http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Apostolidis, A., Karakousis, Y., Triantaphyllidis, C., 1996. Genetic divergence and phylogenetic relationships among *Salmo trutta* L. (brown trout) populations from Greece and other European countries. Heredity 76 (6), 551–560. https://doi.org/10.1038/hdy.1996.80.

Apostolidis, A., Stoumboudi, M.T., Kalogianni, E., Cote, G., Bernatchez, L., 2011. Genetic divergence among native trout *Salmo trutta* populations from southern Balkans based on mitochondrial DNA and microsatellite variation. J. Fish Biol. 79, 1950–1960. https://doi.org/10.1111/j.1095-8649.2011.03136.x.

Apostolidis, A.P., Loukovitis, D., Tsigenopoulos, C.S., 2008. Genetic characterization of brown trout (*Salmo trutta*) populations from the southern Balkans using mtDNA sequencing and RFLP analysis. Hydrobiologia 600 (1), 169–176.

April, J., Mayden, R.L., Hanner, R.H., Bernatchez, L., 2011. Genetic calibration of species diversity among North America's freshwater fishes. Proc. Natl. Acad. Sci. 108 (26), 10602–10607.

Barbat-Leterrier, A., Guyomard, R., Krieg, F., 1989. Introgression between introduced domesticated strains and Mediterranean native populations of brown trout (Salmo trutta L.). Aquat. Living Resour. 2 (4), 215–223.

Bardakci, F., Degerli, N., Ozdemir, O., Basibuyuk, H.H., 2006. Phylogeography of the Turkish brown trout *Salmo trutta* L.: mitochondrial DNA PCR-RFLP variation. J. Fish Biol. 68 (A), 36–55. https://doi.org/10.1111/jfb.2006.68.issue-a10.1111/j.0022-1112.2006.00948.x.

Bekkevold, D., Höjesjö, J., Nielsen, E.E., Aldvén, D., Als, T.D., Sodeland, M., Kent, M.P., Lien, S., Hansen, M.M., 2020. Northern European *Salmo trutta* (L.) populations are genetically divergent across geographical regions and environmental gradients. Evol. applications 13 (2), 400–416.

Benestan, L., Gosselin, T., Perrier, C., Sainte-Marie, B., Rochette, R., Bernatchez, L., 2015. RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the American lobster (*Homarus americanus*). Mol. Ecol. 24 (13), 3299–3315. https://doi.org/10.1111/mec.13245.

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. https://doi.org/10.1111/evo.2001.55.issue-210.1111/j.0014-3820.2001.tb01300.x.

Bernatchez, L., Guyomard, R., Bonhomme, F., 1992. DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout *Saltmo trutta* populations. Mol. Ecol. 1, 161–173. https://doi.org/10.1111/j.1365-294X.1992.tb00172.x.

Berrebi, P., 2015. Three brown trout *Salmo trutta* lineages in Corsica described through allozyme variation. J. Fish Biol. 86 (1), 60–73. https://doi.org/10.1111/jfb.12534.

Berrebi, P., Caputo Barucchi, V., Splendiani, A., Muracciole, S., Sabatini, A., Palmas, F., Tougard, C., Arculeo, M., Marić, S., 2019. brown trout (*Salmo trutta* L.) high genetic diversity around the Tyrrhenian Sea as revealed by nuclear and mitochondrial markers. Hydrobiologia 826 (1), 209–231.

Berrebi, P., Jesenšek, D., Crivelli, A.J., 2017. Natural and domestic introgressions in the marble trout population of Soca River (Slovenia). Hydrobiologia 785, 277–291.

Berrebi, P., Povz, M., Jesensek, D., Cattaneo-Berrebi, G., Crivelli, A.J., 2000. The genetic diversity of native, stocked and hybrid populations of marble trout in the Soca river, Slovenia. Heredity 85, 277–287. https://doi.org/10.1046/j.1365-2540.2000.00753.x.

Berrebi, P., Tougard, C., Dubois, S., Shao, Z., Koutseri, I., Petkovski, S., Crivelli, A.J., 2013. Genetic diversity and conservation of the Prespa trout in the Balkans. Int. J. Mol. Sci. 14 (12), 23454–23470. https://doi.org/10.3390/ijms141223454.

Bianco, P.G., 2014. An update on the status of native and exotic freshwater fishes of I taly. J. Appl. Ichthyol. 30 (1), 62–77. https://doi.org/10.1111/jai.12291.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., Prlic, A., 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comp. Biol. 10 (4), e1003537. https://doi.org/10.1371/journal.pcbi.1003537.

Breiman, L., 2006. randomForest: Breiman and Cutler's random forests for classification and regression. URL http://stat-www.berkeley.edu/users/breiman/RandomForests, R package version.

Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A., Cresko, W.A., 2013. Stacks: an analysis tool set for population genomics. Mol. Ecol. 22 (11), 3124–3140. https://doi.org/10.1111/mec.12354.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x.

Crête-Lafrenière, A., Weir, L.K., Bernatchez, L., Fontaneto, D., 2012. Framing the Salmonidae family phylogenetic portrait: a more complete picture from increased taxon sampling. PLoS ONE 7 (10), e46662. https://doi.org/10.1371/journal.pone.0046662.

De Queiroz, K., 2007. Species concepts and species delimitation. Syst. Biol. 56, 879–886. https://doi.org/10.1080/10635150701701083.
Delling, B., 2002. Morphological distinction of the marble trout, *Salmo marmoratus*, in comparison to marbled *Salmo trutta* from River Otra, Norway. Cybium 26 (4), 283–300.
Delling, B., 2010. Diversity of western and southern Balkan trouts, with the description of a new species from the Louros River, Greece (Teleostei: Salmonidae). Ichthyol. Explor. Freshw. 21 (4), 331–344.
Delling, B., Crivelli, A.J., Rubin, J.-F., Berrebi, P., 2000. Morphological variation in hybrids between *Salmo marmoratus*, and alien *Salmo* species in the Volarja stream, Soca River basin, Slovenia. J. Fish Biol. 57 (5), 1199–1212. https://doi.org/10.1111/jfb.2000.57.issue-510.1111/j.1095-8649.2000.tb00481.x.
Delling, B., Doadrio, I., 2005. Systematics of the trouts endemic to Moroccan lakes, with description of a new species (Teleostei: Salmonidae). Ichthyol. Explor. Freshwat. 16, 49–64.
Delling, B., Sabatini, A., Muracciole, S., Tougard, C., Berrebi, P., 2020. Morphologic and genetic characterisation of Corsican and Sardinian trout with comments on *Salmo* taxonomy. Knowl. Manag. Aquat. Ecosyst. 421, 21. https://doi.org/10.1051/kmae/2020013.
Doadrio, I., Perea, S., Yahyaoui, A., 2015. Two new species of Atlantic trout (Actinopterygii, Salmonidae) from Morocco. Graellsia 71 (2), e031. https://doi.org/10.3989/graellsia.2015.v71.i210.3989/graellsia.2015.v71.142.
Duftner, N., Weiss, S., Medgyesy, N., Sturmbauer, C., 2003. Enhanced phylogeographic information about Austrian brown trout populations derived from complete mitochondrial control region sequences. J. Fish Biol. 62, 427–435. https://doi.org/10.1046/j.1095-8649.2003.00038.x.
Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32 (5), 1792–1797. https://doi.org/10.1093/nar/gkh340.
Elliott, J.M., 1994. Quantitative Ecology and the brown trout. Oxford University Press.
Emerson, K.J., Merz, C.R., Catchen, J.M., Hohenlohe, P.A., Cresko, W.A., Bradshaw, W. E., Holzapfel, C.M., 2010. Resolving postglacial phylogeography using high-throughput sequencing. Proc. Natl. Acad. Sci. 107 (37), 16196–16200. https://doi.org/10.1073/pnas.1006538107.
Epitashvili, G., Geiger, M., Astrin, J., Herder, F., Japoshvili, B., Mumladze, L., 2020. Towards retrieving the Promethean treasure: a first molecular assessment of the freshwater fish diversity of Georgia. Biodivers. Data J. 8 https://doi.org/10.3897/BDJ.8.e57862.
Ferguson, A., Mason, F.M., 1981. Allozyme evidence for reproductively isolated sympatric populations of brown trout *Salmo trutta* L. in Lough Melvin. Ireland. J. Fish Biol. 18 (6), 629–642. https://doi.org/10.1111/j.1095-8649.1981.tb03805.x.
Fernández-Mazuecos, M., Mellers, G., Vigalondo, B., Sáez, L., Vargas, P., Glover, B.J., 2017. Resolving recent plant radiations: power and robustness of genotyping-by-sequencing. Syst. Biol. 67, 250–268. https://doi.org/10.1093/sysbio/syx062.
Frankham, R., 2015. Genetic rescue of small inbred populations: Meta-analysis reveals large and consistent benefits of gene flow. Mol. Ecol. 24 (11), 2610–2618. https://doi.org/10.1111/mec.13139.
Frankham, R., Ballou, J.D., Dudash, M.R., Eldridge, M.D.B., Fenster, C.B., Lacy, R.C., Mendelson, J.R., Porton, I.J., Ralls, K., Ryder, O.A., 2012. Implications of different species concepts for conserving biodiversity. Biol. Conserv. 153, 25–31.
Frankham, R., Ballou, J.D., Ralls, K., Eldridge, M., Dudash, M.R., Fenster, C.B., Lacy, R. C., Sunnucks, P., 2017. Genetic Management of Fragmented Animal and Plant Populations. Oxford University Press.
Fruciano, C., Pappalardo, A.M., Tigano, C., Ferrito, V., 2014. Phylogeographical relationships of Sicilian brown trout and the effects of genetic introgression on morphospace occupation. Biol. J. Linn. Soc. 112 (3), 387–398. https://doi.org/10.1111/bij.12279.
García-MarÍn, J.L., Utter, F.M., Pla, C., 1999. Postglacial colonization of brown trout in Europe based on distribution of allozyme variants. Heredity 82, 46–56. https://doi.org/10.1046/j.1365-2540.1999.00447.x.
Geiger, M.F., Herder, F., Monaghan, M.T., Almada, V., Barbieri, R., Bariche, M., Berrebi, P., Bohlen, J., Casal-Lopez, M., Delmastro, G.B., Denys, G.P.J., Dettai, A., Doadrio, I., Kalogianni, E., Kärst, H., Kottelat, M., Kovačić, M., Laporte, M., Lorenzoni, M., Marčić, Z., Özuluğ, M., Perdices, A., Perea, S., Persat, H., Porcelotti, S., Puzzi, C., Robalo, J., Šanda, R., Schneider, M., Šlechtová, V., Stoumboudi, M., Walter, S., Freyhof, J., 2014. Spatial heterogeneity in the Mediterranean Biodiversity Hotspot affects barcoding accuracy of its freshwater fishes. Mol. Ecol. Resour. 14 (6), 1210–1221. https://doi.org/10.1111/1755-0998.12257.
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. Mol. Ecol. 3, 161–171. https://doi.org/10.1111/j.1365-294X.1994.tb00117.x.
Giuffra, E., Guyomard, R., Forneris, G., 1996. Phylogenetic relationships and introgression patterns between incipient parapatric species of Italian brown trout (Salmo trutta L. complex). Mol. Ecol. 5 (2), 207–220.
Gratton, P., Allegrucci, G., Sbordoni, V., Gandolfi, A., 2014. The evolutionary jigsaw puzzle of the surviving trout (*Salmo trutta* L. complex) diversity in the Italian region. A multilocus Bayesian approach. Mol. Phylogen. Evol. 79, 292–304. https://doi.org/10.1016/j.ympev.2014.06.022.
Griffiths, A.M., Bright, D., Stevens, J.R., 2009. Complete mitochondrial control region sequences indicate a distinct variety of brown trout Salmo trutta in the Aral Sea. J. Fish Biol. 74 (5), 1136–1142.
Hall, T., 2005. BioEdit. Version 7.2. 5 Biological sequence alignment editor for Win95/98/NT/2K/XP.
Hallerman, E.M., 2003. Population genetics: principles and applications for fisheries scientists.
Hashemzadeh Segherloo, I., Farahmand, H., Abdoli, A., Bernatchez, L., Primmer, C., Swatdipong, A., Karami, M., Khalili, B., 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. J. Fish Biol. 81, 1479–1500. https://doi.org/10.1111/j.1095-8649.2012.03428.x.
Huson, D.H., Bryant, D., 2005. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23, 254–267. https://doi.org/10.1093/molbev/msj030.
Jombart, T., Collins, C., 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. https://adegenet.r-forge.r-project.org/files/tutorial-dapc.pdf.
Jug, T., Berrebi, P., Snoj, A., 2005. Distribution of non-native trout in Slovenia and their introgression with native trout populations as observed through microsatellite DNA analysis. Biol. Conserv. 123 (3), 381–388.
Kara, C., Alp, A., Gürlek, M.E., 2011. Morphological variations of the trouts (*Salmo trutta* and *Salmo platycephalus*) in the rivers of Ceyhan, Seyhan and Euphrates, Turkey. Turkish J. Fish. Aquat. Sci. 11 (1), 77–85.
Kass, R.E., Raftery, A.E., 1995. Bayes factors. J. American Statistical Association 90, 773-795. https://doi.org/10.1080/01621459.1995.10476572.
Keinan, A., Mullikin, J.C., Patterson, N., Reich, D., 2007. Measurement of the human allele frequency spectrum demonstrates greater genetic drift in East Asians than in Europeans. Nat. Genet. 39 (10), 1251–1255. https://doi.org/10.1038/ng2116.
Kohout, J., Šedivá, A., Apostolou, A., Stefanov, T., Marić, S., Gaffaroğlu, M., Šlechta, V., 2013. Genetic diversity and phylogenetic origin of brown trout Salmo trutta populations in eastern Balkans. Biologia 68 (6), 1229–1237.
Kottelat, M., Freyhof, J.R., 2007. Handbook of European Freshwater Fishes. Publications Kottelat, Cornol, Switzerland.
Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33 (7), 1870–1874. https://doi.org/10.1093/molbev/msw054.
Leaché, A.D., Bouckaert, R.R., 2018. Species trees and species delimitation with SNAPP: a tutorial and worked example. Workshop on Population and Speciation Genomics, Český Krumlov, Czech Republic. http://evomicsorg.wpengine.netdna-cdn.com/wp-content/uploads/2018/01/BFD-tutorial-1.pdf.
Leaché, A.D., Fujita, M.K., Minin, V.N., Bouckaert, R.R., 2014. Species delimitation using genome-wide SNP data. Syst. Biol. 63, 534–542. https://doi.org/10.1093/sysbio/syu018.
Lobón-Cerviá, J., Esteve, M., Berrebi, P., Duchi, A., Lorenzoni, M., Young, K.A., 2018. Trout and char of central and Southern Europe and Northern Africa. In: Kershner, J., Williams, J., Lobón-Cerviá, J., Gresswell, B. (Eds.), Trout and char of the world. American Fisheries Society, Bethesda, Maryland, pp. 1–32.
Lecaudey, L.A., Schliewen, U.K., Osinov, A.G., Taylor, E.B., Bernatchez, L., Weiss, S.J., 2018. Inferring phylogenetic structure, hybridization and divergence times within Salmoninae (Teleostei: Salmonidae) using RAD-sequencing. Mol. Phylogen. Evol. 124, 82–99. https://doi.org/10.1016/j.ympev.2018.02.022.
Lerceteau-Köhler, E., Schliewen, U., Kopun, T., Weiss, S., 2013. Genetic variation in brown trout *Salmo trutta* across the Danube, Rhine, and Elbe headwaters: a failure of the phylogeographic paradigm? BMC Evol. Biol. 13 (1), 176. https://doi.org/10.1186/1471-2148-13-176.
Levin, B., Simonov, E., Rastorguev, S., Boulygina, E., Sharko, F., Tsygankova, S., Gabrielyan, B., Roubenyan, H., Mayden, R., Nedoluzhko, A., 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. https://doi.org/10.1007/s10750-018-3688-7.
Lien, S., Koop, B.F., Sandve, S.R., Miller, J.R., Kent, M.P., Nome, T., Hvidsten, T.R., Leong, J.S., Minkley, D.R., Zimin, A., Grammes, F., Grove, H., Gjuvsland, A., Walenz, B., Hermansen, R.A., von Schalburg, K., Rondeau, E.B., Di Genova, A., Samy, J.K.A., Olav Vik, J., Vigeland, M.D., Caler, L., Grimholt, U., Jentoft, S., Inge Våge, D., de Jong, P., Moen, T., Baranski, M., Palti, Y., Smith, D.R., Yorke, J.A., Nederbragt, A.J., Tooming-Klunderud, A., Jakobsen, K.S., Jiang, X., Fan, D., Hu, Y., Liberles, D.A., Vidal, R., Iturra, P., Jones, S.J.M., Jonassen, I., Maass, A., Omholt, S. W., Davidson, W.S., 2016. The Atlantic salmon genome provides insights into rediploidization. Nature 533 (7602), 200–205. https://doi.org/10.1038/nature17164.
Lo Brutto, S., Hristovski, N., Arculeo, M., 2010. Genetic divergence between morphological forms of brown trout *Salmo trutta* L. in the Balkan region of Macedonia. J. Fish Biol. 76, 1220–1227. https://doi.org/10.1111/j.1095-8649.2010.02595.x.
Mace, G.M., 2004. The role of taxonomy in species conservation. Philos. Trans. R. Soc. Lond. B. Biol. Sci., 359(1444), 711-719. https://doi.org/10.1098/rstb.2003.1454.
MacGuigan, D.J., Near, T.J., Cormack, J.M., 2019. Phylogenomic signatures of ancient introgression in a rogue lineage of darters (Teleostei: Percidae). Syst. Biol. 68 (2), 329–346. https://doi.org/10.1093/sysbio/syy074.
Machordom, A., Suarez, J., Almodovar, A., Bautista, J.M., 2000. Mitochondrial haplotype variation and phylogeography of Iberian brown trout populations. Mol. Ecol. 9 (9), 1324–1338. https://doi.org/10.1046/j.1365-294x.2000.01015.x.
Marić, S., Bajec, S.S., Schöffmann, J., Kostov, V., Snoj, A., 2017. Phylogeography of stream-dwelling trout in the Republic of Macedonia and a molecular genetic basis for revision of the taxonomy proposed by S. Karaman. Hydrobiologia 785 (1), 249–260. https://doi.org/10.1007/s10750-016-2930-4.
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. J. 17, 10–12. https://doi.org/10.14806/ej.17.1.200.
Mascher, M., Wu, S., Amand, P.S., Stein, N., Poland, J., Candela, H., 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. https://doi.org/10.1371/journal.pone.0076925.
Meldgaard, T., Crivelli, A.J., Jesensek, D., Poizat, G., Rubin, J.-F., Berrebi, P., 2007. Hybridization mechanisms between the endangered marble trout (Salmo marmoratus) and the brown trout (Salmo trutta) as revealed by in-stream experiments. Biol. Conserv. 136 (4), 602–611.
Melville, J., Haines, M.L., Boysen, K., Hodkinson, L., Kilian, A., Smith Date, K.L., Potvin, D.A., Parris, K.M., 2017. Identifying hybridization and admixture using SNPs: application of the DArTseq platform in phylogeographic research on vertebrates. R. Soc. Open Sci. 4 (7), 161061. https://doi.org/10.1098/rsos.161061.
Meraner, A., Baric, S., Pelster, B., Dalla Via, J., 2007. Trout (*Salmo trutta*) mitochondrial DNA polymorphism in the centre of the marble trout distribution area. Hydrobiologia 579 (1), 337–349. https://doi.org/10.1007/s10750-006-0479-3.
Moran, P., Pendas, A.M., Garcia-Vazquez, E., Izquierdo, J.I., Lobon-Cervla, J., 1995. Estimates of gene flow among neighbouring populations of brown trout. J. Fish Biol. 46 (4), 593–602. https://doi.org/10.1111/j.1095-8649.1995.tb01099.x.
Ninua, L., Tarkhnishvili, D., Gvazava, E., 2018. Phylogeography and taxonomic status of trout and salmon from the Ponto-Caspian drainages, with inferences on European brown trout evolution and taxonomy. Ecol. Evol. 8 (5), 2645–2658. https://doi.org/10.1002/ece3.3884.
Osinov, A., Bernatchez, L., 1996. Atlantic and Danubian phylogenetic groupings of brown trout *Salmo trutta* complex: genetic divergence, evolution, and conservation. J. Ichthyol. 36, 723–746.
Palandačić, A., Naseka, A., Ramler, D., Ahnelt, H., 2017. Contrasting morphology with molecular data: an approach to revision of species complexes based on the example of European *Phoxinus* (Cyprinidae). BMC Evol. Biol. 17, 184. https://doi.org/10.1186/s12862-017-1032-x.
Patterson, N., Moorjani, P., Luo, Y., Mallick, S., Rohland, N., Zhan, Y., Genschoreck, T., Webster, T., Reich, D., 2012. Ancient admixture in human history. Genetics 192, 1065–1093. https://doi.org/10.1534/genetics.112.145037.
Pickrell, J.K., Pritchard, J.K., 2012. Inference of population splits and mixtures from genome-wide allele frequency data. PLoS Genet. 8, e1002967 https://doi.org/10.1038/npre.2012.6956.1.
Presa, P., Pardo, B., Martínez, P., Bernatchez, L., 2002. Phylogeographic congruence between mtDNA and rDNA ITS markers in brown trout. Mol. Biol. Evol. 19, 2161–2175. https://doi.org/10.1093/oxfordjournals.molbev.a004041.
Pustovrh, G., Snoj, A., Bajec, S., 2014. Molecular phylogeny of *Salmo* of the western Balkans, based upon multiple nuclear loci. Genet. Sel. Evol. 46 (1), 7. https://doi.org/10.1186/1297-9686-46-7.
Razpet, A., Sušnik, S., Jug, T., Snoj, A., 2007. Genetic variation among trout in the River Neretva basin. Bosnia and Herzegovina. J. Fish Biol. 70 (sa), 94–110. https://doi.org/10.1111/j.1095-8649.2007.01392.x.
Reich, D., Thangaraj, K., Patterson, N., Price, A.L., Singh, L., 2009. Reconstructing Indian population history. Nature 461 (7263), 489–494. https://doi.org/10.1038/nature08365.
Roycroft, E.J., Moussalli, A., Rowe, K.C., 2019. Phylogenomics uncovers confidence and conflict in the rapid radiation of Australo-Papuan todents. Syst. Biol. 69 (3), 431–444. https://doi.org/10.1093/sysbio/syz044.
Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., Sánchez-Gracia, A., 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol. Biol. Evol. 34, 3299–3302. https://doi.org/10.1093/molbev/msx248.
Russo, C.A.d.M., Selvatti, A.P., Tamura, K., 2018. Bootstrap and rogue identification tests for phylogenetic analyses. Mol. Biol. Evol. 35 (9), 2327–2333. https://doi.org/10.1093/molbev/msy118.
Sabatini, A., Podda, C., Frau, G., Cani, M.V., Musu, A., Serra, M., Palmas, F., 2018. Restoration of native Mediterranean brown trout Salmo cettii Rafinesque, 1810 (Actinopterygii: Salmonidae) populations using an electric barrier as a mitigation tool. Eur. Zool. J. 85 (1), 137–149.
Sanz, N., 2018. In: Brown Trout: Biology, Ecology and Management. John Wiley & Sons, Ltd, Chichester, UK, pp. 15–63. https://doi.org/10.1002/9781119268352.ch2.
Schöffmann, J., Sušnik, S., Snoj, A., 2007. Phylogenetic origin of *Salmo trutta* L 1758 from Sicily, based on mitochondrial and nuclear DNA analyses. Hydrobiologia 575 (1), 51–55. https://doi.org/10.1007/s10750-006-0281-2.
Silvestro, D., Michalak, I., 2012. raxmlGUI: a graphical front-end for RAxML. Org. Divers. Evol. 12 (4), 335–337. https://doi.org/10.1007/s13127-011-0056-0.
Snoj, A., Glamuzina, B., Razpet, A., Zablocki, J., Bogut, I., Lerceteau-Köhler, E., Pojskić, N., Sušnik, S., 2010. Resolving taxonomic uncertainties using molecular systematics: *Salmo dentex* and the Balkan trout community. Hydrobiologia 651 (1), 199–212. https://doi.org/10.1007/s10750-010-0297-5.
Snoj, A., Marić, S., Bajec, S.S., Berrebi, P., Janjani, S., Schöffmann, J., 2011. Phylogeographic structure and demographic patterns of brown trout in North-West Africa. Mol. Phylogen. Evol. 61 (1), 203–211. https://doi.org/10.1016/j.ympev.2011.05.011.
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. Genet. Sel. Evol. 41 (1), 22. https://doi.org/10.1186/1297-9686-41-22.
Snoj, A., Melkič, E., Sušnik, S., Muhamedagić, S., Dovč, P., 2002. DNA phylogeny supports revised classification of *Salmothymus obtusirostris*. Biol. J. Linn. Soc. 77 (3), 399–411. https://doi.org/10.1046/j.1095-8312.2002.00130.x.
Splendiani, A., Fioravanti, T., Giovannotti, M., Olivieri, L., Ruggeri, P., Nisi Cerioni, P., Vanni, S., Enrichetti, F., Caputo Barucchi, V., 2017. Museum samples could help to reconstruct the original distribution of Salmo trutta complex in Italy. J. Fish Biol. 90 (6), 2443–2451.
Splendiani, A., Palmas, F., Sabatini, A., Caputo Barucchi, V., 2019. The name of the trout: considerations on the taxonomic status of the Salmo trutta L., 1758 complex (Osteichthyes: Salmonidae) in Italy. Eur. Zool. J. 86 (1), 432–442. https://doi.org/10.1080/24750263.2019.1686544.
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 (2), 198–206. https://doi.org/10.1046/j.1365-2540.2001.00905.x.
Susnik, 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*. J. Fish Biol. 68 (A), 2–23. https://doi.org/10.1111/j.0022-1112.2006.00902.x.
Sušnik Bajec, S., Pustovrh, G., Jesenšek, D., Snoj, A., 2015. Population genetic SNP analysis of marble and brown trout in a hybridization zone of the Adriatic watershed in Slovenia. Biol. Conserv. 184, 239–250.
Susnik, S., Schoffmann, J., Snoj, A., 2004. Phylogenetic position of *Salmo* (*Platysalmo*) *platycephalus* Behnke 1968 from south-central Turkey, evidenced by genetic data. J. Fish Biol. 64 (4), 947–960. https://doi.org/10.1111/j.1095-8649.2004.0363.x.
Sušnik, S., Snoj, A., Wilson, I.F., Mrdak, D., Weiss, S., 2007a. Historical demography of brown trout (*Salmo trutta*) in the Adriatic drainage including the putative *S. letnica* endemic to Lake Ohrid. Mol. Phylogen. Evol. 44 (1), 63–76. https://doi.org/10.1016/j.ympev.2006.08.021.
Sušnik, S., Weiss, S., Odak, T., Delling, B., Treer, T., Snoj, A., 2007b. Reticulate evolution: ancient introgression of the Adriatic brown trout mtDNA in softmouth trout *Salmo obtusirostris* (Teleostei: Salmonidae). Biol. J. Linn. Soc. 90 (1), 139–152.
Swofford, D.L., 2002. Phylogenetic Analysis Using Parsimony (* and other methods). Version.
Templeton, A.R., 2004. Using haplotype trees for phylogeographic and species inference in fish populations. Environ. Biol. Fishes. 69 (1-4), 7–20. https://doi.org/10.1023/B:EBFI.0000022870.76912.0c.
Tougard, C., Justy, F., Guinand, B., Douzery, E.J., Berrebi, P., 2018. *Salmo macrostigma* (Teleostei, Salmonidae): Nothing more than a brown trout (*S. trutta*) lineage? J. Fish Biol. 93, 302–310. https://doi.org/10.1111/jfb.13751.
Turan, D., Kalaycı, G., Bektaş, Y., Kaya, C., Bayçelebi, E., 2020. A new species of trout from the northern drainages of Euphrates River, Turkey (Salmoniformes: Salmonidae). J. Fish Biol. 96 (6), 1454–1462. https://doi.org/10.1111/jfb.v96.610.1111/jfb.14321.
Turan, D., Kottelat, M., Bektas, Y., 2011. *Salmo tigridis*, a new species of trout from the Tigris River, Turkey (Teleostei: Salmonidae). Zootaxa 2993, 23–33.
Turan, D., Kottelat, M., Engin, S., 2009. Two new species of trouts, resident and migratory, sympatric in streams of northern Anatolia (Salmoniformes: Salmonidae). Ichthyol. Explor. Freshwat. 20, 333–364.
Turan, D., Kottelat, M., Engin, S., 2014. Two new species of trouts from the Euphrates drainage, Turkey (Teleostei: Salmonidae). Ichthyol. Explor. Freshwat. 24, 275–287.
Turan, D., Kottelat, M., Kaya, C., 2017. *Salmo munzuricus*, a new species of trout from the Euphrates River drainage, Turkey (Teleostei: Salmonidae). Ichthyol. Explor. Freshwat. 28, 55–63.
Waples, R.S., 1991. Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. Can. J. Fish. Aquat. Sci. 48 (S1), 124–133. https://doi.org/10.1139/f91-311.
Weiss, S., Schlotterer, C., Waidbacher, H., Jungwirth, M., 2001. Haplotype (mtDNA) diversity of brown trout *Salmo trutta* in tributaries of the Austrian Danube: massive introgression of Atlantic basin fish—by man or nature? Mol. Ecol. 10 (5), 1241–1246. https://doi.org/10.1046/j.1365-294X.2001.01261.x.
Whiteley, A.R., Fitzpatrick, S.W., Funk, W.C., Tallmon, D.A., 2015. Genetic rescue to the rescue. Trends Ecol. Evol. 30 (1), 42–49. https://doi.org/10.1016/j.tree.2014.10.009.
Zaccara, S., Trasforini, S., Antognazza, C.M., Puzzi, C., Britton, J.R., Crosa, G., 2015. Morphological and genetic characterization of Sardinian trout *Salmo cettii* Rafinesque, 1810 and their conservation implications. Hydrobiologia 760 (1), 205–223. https://doi.org/10.1007/s10750-015-2322-1.
Zachos, F.E., 2018. (New) Species concepts, species delimitation and the inherent limitations of taxonomy. J. Genetics 97 (4), 811–815. https://doi.org/10.1007/s12041-018-0965-1.