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First Record of Black Sea Trout (*Salmo labrax*) and Its Potential Hybrids with Endemic Munzur Trout (*Salmo munzuricus*) in Uzunçayır Dam Lake, Tunceli

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Abstract

The depletion of unique genetic resources and increased risk of extinction may result from hybridization between native and introduced species. In the present study, morphological and molecular analysis were performed on two putative natural interspecific hybrids of the genus *Salmo* and their parent species, *Salmo labrax* and *Salmo munzuricus*, collected from Uzunçayır Dam Lake, which is fed by Munzur and Pülümür streams. Molecular analyses revealed the presence of *S. labrax* in Uzunçayır Dam Lake for the first time. Phylogenetic analysis using the cytochrome b (*Cytb*) gene of mitochondrial DNA to determine the maternal origin revealed that hybrid individuals belonged to the same clades as *S. labrax* or *S. munzuricus*. Recombination activating gene 1 (*Rag1*) sequence of nuclear DNA was analyzed for paternal analysis and it was determined that hybrid individuals carried similar single nucleotide polymorphisms (SNPs) to *S. labrax* or *S. munzuricus*. These findings support that individuals derive from different parental species. Therefore, evidence from trout samples assumed to be natural hybrids in this study suggests interspecific hybridization between *S. labrax* and *S. munzuricus*, but further studies are required.

Introduction

Aquatic ecosystems are increasingly subject to anthropogenic disturbances such as climate change, pollution, habitat alteration, invasion of non-native species (failures in stocking programs, aquaculture escapees etc.,) that lead to rapid and often drastic environmental changes. If there is a close evolutionary relationship between alien and native species in nature, hybridization poses a threat. The interbreeding between introduced and native species leads to the transfer of genetic material into the native gene pool and is a major concern in the aquaculture of non-native species. Genetic mixing threatens the long term viability of native species, exacerbating their decline and destabilizing freshwater ecosystems in which these species play critical roles (Kim et al., 2025). The loss of genetic diversity and unique traits in native species due to hybridization can reduce their adaptability to environmental changes or impacts caused by human activities. Considering these issues, the challenge of hybridization is a key concern for conservation and management strategies (Allendorf et al., 2001; Olenin et al., 2010; Carugati et al., 2024).

Natural hybridization is an important aspect of the evolutionary process for many taxa in wild populations (Arnold, 1997). For instance, it is considered a harmful phenomenon and a significant threat to brown trout populations in conservation biology, as it leads to population decline, reduced genetic diversity, and even extinction (Leary et al., 1995; Huxel, 1999; Aparicio et al., 2005; Meldgaard et al., 2007; Kocabaş et al., 2018). In addition, the reproductive capacity of wild populations had decreased (Allendorf & Luikart, 2007; Frank & Baret, 2013). Moreover, hybridization with non-native *Oncorhynchus mykiss* is one of the greatest risks to the native *O. clarkia lewisi* a species to river systems in USA and Canada (Yau & Taylor 2013; Allen et al. 2016; COSEWIC, 2016). Due to the *O. clarkia lewisi* x *O. mykiss* hybrids are reproductive, natural habitats of genetic isolated populations of *O. clarkia lewisi* have been displaced, resulting in many populations losing conservation value because they are no longer recognized as pure (Rubidge & Taylor 2004; Muhlfeld et al. 2009; COSEWIC, 2016).

The Munzur trout (*Salmo munzuricus*), commonly known as the red-spotted trout, was previously not well-defined. It was formally described by Turan et al. (2017) and was accepted as an endemic fish species (Çiçek et al., 2018). This native Salmonid species has substantial economic and ecological importance, and is indigenous to the Munzur stream, a crucial freshwater source in Türkiye. However, only a very limited number of academic research articles have been encountered focusing on the evolution, ecology and conservation of this important species. Current studies are focused on its aquaculture and cultivation (Kocabaş et al., 2011a, b; Akgül & Can, 2020). Kocabaş et al. (2024) reported for the first time from Pülümür Stream-Roj Creek (Kutudere, Tunceli, Türkiye). On the other hand, *Salmo labrax* is native to the Eastern Black Sea area and exhibits an anadromous lifestyle (Kocabaş et al., 2018). The populations of this species have been negatively affected by overfishing (Çakmak et al., 2019). *S. labrax* has been assessed for The IUCN Red List of Threatened Species in 2022 and is listed as Least Concern (Freyhof, 2024). Due to its high potential for aquaculture, it is cultivated in commercial fish farms across Türkiye, with a production of 1440 tons annually across 19 farms (Çankırılıgil et al., 2017; Çankırılıgil & Berik, 2020; Turkish Statistical Institute, 2023).

Non-migratory forms of *S. labrax* are found in small streams and river branches that flow into the Black Sea, but anadromous ones are found in large streams and rivers that flow into the same sea (Çakmak et al., 2025). There is no record of Black Sea trout in the freshwater systems connected to the Munzur Stream Basin. Therefore, natural hybridization between Munzur trout and Black Sea trout cannot occur. It is thought that the Black Sea trout was brought to the Munzur Stream Basin by faulty stocking programs or for production purposes by aquaculture farmers in the region. Thus far, the assessment of phenotypic characteristics and genetic analysis has been conducted on hybridization and introgression among brown trout strains (Kruse et al., 2000; Aparicio et al., 2005; Kocabaş et al., 2018).

Mitochondrial DNA (mtDNA), which is used to determine inter- and intraspecific variation in fish, is a genetic system to study gene flow, hybrid zones, and population structure. (Saraswat et al., 2014). mtDNA Cytochrome b (*Cytb*) sequences have been utilized for molecular phylogenetic and population analyses. DNA markers indicate that intraspecific differences are generally less than 1%, while interspecific differences are higher than 10% for fish (Li et al., 2018). *Rag1* (Recombination Activating 1) which one of the nuclear DNA (nDNA) gene regions, encodes enzymes and is found in the V(D)J recombination of immunoglobulins and in T cell receptors (Bercovich et al., 2012). Thymus and head kidney are immune-related tissues, where T/B cells develop in fish. Additionally, lymphoid-specific recombinants encoded by *Rag1* and *Rag2* catalyze a site-specific V(D)J recombination reaction in the developing T/B cells (Mao et al., 2015). These gene regions are increasingly used in phylogenetic studies of fish (Li, C., 2007).

Within the scope of the study, the presence of *S. labrax* in the Uzunçayır Dam Lake and its potential hybrids with *S. munzuricus* were investigated. Based on the available information, no effort has been realized so far to assess interactions and determine the phenotypic and genetic characteristics in these *Salmo* species. The genetic structure of the parental species in this region was studied using sequences of one mitochondrial gene and one nuclear gene. Phenotypic and genotypic variation in *S. labrax*, *S. munzuricus* and their potential hybrids (*S. labrax* × *S. munzuricus*) were determined and compared.

Material and Methods

Sampling

Trout specimens were collected (n=8) by fishermen from Uzunçayır Dam Lake (Tunceli, Türkiye) and transported to the laboratory. The following morphometric and meristic characters were measured and recorded from every fish sample: 1. Total Length; 2. Standard Length; 3. Head Length; 4. Head Depth; 5. Eyes Diameter; 6. Snout Length; 7. Distance between eyes; 8. Preanal Length; 9. Body Depth; 10. Body Width; 11. Length of the base of ventral fin; 12. Caudal peduncle height; 13. Caudal peduncle length; 14. Dorsal Base Length; 15. Pectoral fin length; 16. Prepectoral length; 17. Anal Base Length; 18. Pre dorsal length; 19. Caudal fin length

Fish were photographed using a digital camera. The spotting patterns were analyzed according to Aparicio et al. (2005). A total of eleven quantitative variables were assessed: (1) the number of red spots located at lateral line; (2) number of red black spots on operculum; number of (3) black and (4) red spots above the lateral line; number of (5) black and (6) red spots below the lateral line; number of (7) black spots and (8) red spots on the adipose fin; number of (9) black spots; and (10) red spots on the dorsal fin, number of (11) black and (12) red spots below the lateral line;

For genetic studies, approximately 1–1.5 cm^2 tissue samples taken from the caudal fin of the specimens were placed in 1.5–2.0 ml tubes containing 98% ethanol and kept in a deep freezer at -20°C.

Diagnostic Characters

S. labrax is distinguished by its considerable size, reaching a maximum length of at least 800 mm SL. As the fish grows, the number of black and red spots increases. In large adult males, 4-17 black spots are typically found behind the eye, on the cheek and pre-opercle. Males over 200 mm display black spots along the back, sides, and mid-body, whereas females under 300 mm SL have very few such spots. Red spots appear in the central body region, each encircled by an irregular white ring. The maxilla is relatively short and narrow (Kocabaş et al., 2018). *S. munzuricus* is recognized for its unusually large adipose fin, which in adults is roughly the same length as the base of the anal fin. In a 400 mm SL male, this fin can even rival the size of both the dorsal and anal fins (Turan et al., 2017). In some trout samples thought to be hybrids, which were identified by a single conspicuous black spot behind the eye, and the number of black and red spots increased with increasing size, had never been described until this study.

DNA Extraction

DNA isolation was done from the caudal fin of the samples stored in 98% ethanol. In order to obtain high quality and optimum DNA, the samples were homogenized in the homogenizer device with vials containing iron beads at 10-minute intervals for 5 minutes. DNA extraction from homogenized tissue samples was performed using the QIAGEN DNeasy Blood & Tissue commercial kit. The concentration and quality of double-stranded DNA (dsDNA) in the total DNA obtained after isolation were determined by reading the optical density at 260 and 280 nm wavelengths using Nanodrop (NanoDrop 8000, ThermoFisher, Massachusetts) and 1% electrophoresis agarose gel was used for DNA purity. The isolation was repeated for samples that could not be obtained in appropriate quality and quantity. According to the measurement results, dsDNA samples with approx. quality (OD 260/280 = 1.8, OD 260/230 = 2.02.2) were stored at -20°C until use.

Amplification and Sequence Analysis of mtDNA Gene Regions

Universal primer sets (F:5'-GGCTGATTCGGAATATG CAYGCNAAYGG-3' R: 5'-GGGAATGGATCGTAGAATTG CRTANGCRAA-3') were used for amplification of mitochondrial cytochrome b (*Cytb*) gene regions from the obtained total DNA. The PCR reaction was prepared with 10 µl of 2X Master mix (Qiagen), 10µM primers (F and R), 90–150 ng DNA and NFW, so that the total volume was 25µl. Samples prepared for PCR application were placed in Thermal Cycler (ABI Veriti) and firstly; 1 min denaturation at 94°C, second step 1 min denaturation at 94°C, 45 sec hybridizations between 58°C, and 1.5 min polymerization at 72°C were performed in 35 cycles, then 5 min final polymerization at 72°C and keeping the samples at 4°C in the last step. The PCR cycle was optimized according to the annealing temperature of the primers and optimization studies continued until a quality product was obtained. For the sequence of all gene regions, samples were purified and sequencing was performed on the ABI 3500 Genetic Analyzer (ThermoFisher) device using the BigDye v.3.1 Terminator Cycle Sequencing Kit.

Amplification and Sequence Analysis of nDNA Gene Regions

Primer sets (RAG1F:5'-GAAGCACAGCCGTCTCATC CTG-3'; RAG1R:5'-TGGACAARCAGCTGAGGAAGAAGA-3') were used for amplification of the *Rag1* gene region from the total DNA obtained (Shedko et al., 2012). The PCR reaction was prepared with 10 µl of 2X Master mix (Qiagen), 10µM primers (F and R), 90–150 ng DNA and NFW, so that the total volume was 25µl. Samples prepared for *Rag1* were placed in Thermal Cycler (ABI Veriti) and firstly; 1 min denaturation at 95°C, second step 30 sec denaturation at 95°C, 90 sec hybridizations at 59 ^{0}C, 30 sec polymerizations at 72°C were performed in 35 cycles and then 10 min final polymerization at 72°C and keeping the samples at 4°C in the last step. The PCR cycle was optimized according to the annealing temperature of the primers and optimization studies continued until a quality product was obtained. For the sequence of all gene regions, samples were purified and sequencing was performed on the ABI 3500 Genetic Analyzer (ThermoFisher) device using the BigDye v.3.1 Terminator Cycle Sequencing Kit.

Statistics

IBM SPSS Statistics 27.0 for Windows was used for analyzing the data. Results are presented as mean±standard deviation (SD). The nonparametric Kruskal-Wallis test was applied for differences between groups. A significance level of $P<0.05$ was used in all analyses.

Raw sequences of gene regions were edited and aligned using the ClustalW algorithm in BioEdit v.7.2.5. Low quality sequences were discarded. Species identification was performed by comparison with the reference dataset at NCBI (The National Center for Biotechnology Information). Phylogenetic relationships between species were established by applying the Maximum Likelihood method based on the Hasegawa-Kishino-Yano (HKY) model distance using the MEGA X phylogenetic analysis program. Median-Joining Network analysis was performed with PopArt 1.7 program.

Results

Morphometric measurements (mean±SD) of *S labrax*, *S. munzuricus* and their hybrids (*S. labrax* × *S. munzuricus*) are presented in Table 1. There were no significant differences in *S. labrax*, *S. munzuricus* and their hybrids. Numbers of black and red spots of *S. labrax*, *S. munzuricus* and their hybrids are given in Table 2. No significant difference was in spotting patterns. Operculum surface red spot was not observed in *S. labrax*, *S. munzuricus* and their hybrids.

In order to determine potential hybridization in individuals showing significant morphological differences among the trout species (*S. labrax* and *S. munzuricus*) caught in Uzunçayır Dam Lake, *Cytb* gene region of mtDNA were amplified. Polymerase Chain Reaction (PCR) products of these regions were run on 1% agarose gel using the grading marker. The *Cytb* gene region of mtDNA was sequenced and aligned with the ClustalW algorithm (Figure 1). For the *Cytb* gene region, clear nucleotide differences were determined between two species. The nucleotide difference for the *Cytb* gene region was determined as Pi: 0.00780, while the sequence variation was 7 bp in total, representing a variation rate of 1.6 percent. Maximum Likelihood method and Hasegawa–Kishino–Yano (HKY) model were used to construct the phylogenetic tree and NCBI data were also used. GenBank accession numbers of each species are given before the scientific names (Figure 2). Among the individuals thought to be hybrids, Hybrid1-Hybrid3 and Hybrid2-Hybrid4 individuals showed 100% sequence similarity. While Hybrid1-Hybrid3 individuals showed similarity to *S. munzuricus* samples, Hybrid2-Hybrid4 individuals showed similarity to *S. labrax* species. Figure 2 showed two monophyletic haplotype groups that definitely corresponded to the species from which the haplotype was obtained. This similarity is also shown by the distance-based approximation model created using the Median-Joining Network (Figure 3). Haplotype samples (Figure 3), indicated that both the mtDNAs of *S. munzuricus* leaked into *S. labrax* and *S. labrax* to *S. munzuricus*. Morever, when we assigned haplotypes as *S. munzuricus* (Hybrid2-Hybrid4) type or *S. labrax* (Hybrid1-Hybrid3) type based on genetic relatedness to the reference sequences of the two species, some of the *Cytb* haplotypes showed incompatibility (as appearance) between the species of lineage and the genetically assigned type (Figure 4).

The *Rag1* gene region of nuclear DNA was sequenced and aligned with the ClustalW algorithm (Figure 5 and 6). Analysis of a total of 350 bp of sequence amplified from the *Rag1* gene region of nuclear DNA revealed a total of 1 bp of sequence variation between the putative parental species, indicating a variation rate of approximately 0.31%.

Table 1. Morphometric measurements (mean±SD) of *Salmo labrax*, *Salmo munzuricus* and their potential hybrids (*Salmo labrax* × *Salmo munzuricus*)

	Salmo labrax	*Salmo munzuricus*	*Salmo labrax* X *Salmo munzuricus*	P value
Total length (cm)	46.95±8.27	32.45±7.58	28.95±2.31	0.127
Standard length (cm)	42.30±6.17	29.46±7.26	25.46±1.95	0.127
Head length (cm)	9.16±0.80	5.90±0.75	5.96±1.55	0.135
Head depth (cm)	6.67±0.92	5.23±1.55	4.62±0.65	0.210
Eye diameter (cm)	1.15±0.44	0.91±0.05	0.84±0.02	0.205
Snout length (cm)	2.45±0.40	1.23±0.10	1.31±0.28	0.135
Preanal distance (cm)	29.86±4.72	21.37±5.34	19.27±2.14	0.127
Body height (cm)	9.80±2.55	6.82±1.73	5.80±1.16	0.108
Ventral fin length (cm)	3.97±0.27	3.20±1.45	2.69±0.19	0.368
Caudal peduncle height (cm)	3.97±0.41	2.82±0.70	2.38±0.46	0.105
Caudal peduncle length (cm)	3.57±0.60	2.27±0.91	1.77±0.45	0.127
Dorsal fin length (cm)	6.30±1.92	4.14±1.24	3.63±0.86	0.210
Pectoral fin length (cm)	5.61±0.16	4.40±1.60	3.26±0.77	0.210
Prepectoral distance (cm)	15.07±0.22	10.67±2.97	11.66±3.95	0.346
Anal base length (cm)	5.79±1.05	3.96±1.08	3.05±0.84	0.105
Predorsal distance (cm)	19.322.47	12.93±3.19	12.352.03	0.135
Caudal fin length (cm)	6.75±1.06	4.71±1.02	4.12±1.35	0.105
Distance between eyes (cm)	3.60±0.01	2.15±0.49	2.50±0.77	0.124
Weight (g)	1029.00±1.86	352.50±2.01	352.50±3.84	0.243

Table 2. Mean±SD numbers of black and red spot of *Salmo labrax*, *Salmo munzuricus* and their potential hybrids (*Salmo labrax* × *Salmo munzuricus*)

	Salmo labrax	*Salmo munzuricus*	*Salmo labrax X Salmo munzuricus*	P value
Operculum surface black spot	8.00±9.90	11.50±14.85	6.00±6.06	0.827
Operculum surface red spot	0.00±0.00	0.00±0.00	0.00±0.00	-
Dorsal fin black spot	35.00±9.90	15.00±21.21	18.50±35.68	0.592
Dorsal fin red spot	1.00±1.41	5.50±3.54	3.00±4.76	0.355
Adipose fin black spot	0.50±0.71	0.00±0.00	0.25±0.50	0.558
Adipose fin red spot	1.50±0.71	3.00±1.41	1.25±0.50	0.171
Above lateral line red spot	25.00±35.36	10.00±14.14	10.75±4.79	0.979
Above lateral line black spot	39.00±8.49	0.00±0.00	23.25±31.34	0.100
Below lateral line red spot	7.50±10.61	13.50±2.12	8.75±5.74	0.590
Below lateral line black spot	59.00±15.56	1.50±2.12	9.50±7.72	0.077
Lateral line red spot	5.00±7.07	11.50±6.36	8.50±3.11	0.587
Lateral line black spot	6.50±4.95	4.50±6.36	6.00±6.22	0.764

Discussion

The risk of extinction may increase due to hybridization, as it can cause outbreeding depression. This can manifest through genetic swamping, where the native gene pool is entirely replaced, or through demographic swamping, where hybrids are either infertile or maladapted (Rhymer & Simberloff, 1996; Todesco et al., 2016; Brauer et al., 2023). The risk associated with these issues is likely to be context-dependent and may be less pronounced if hybridization happens naturally over a prolonged period. Introgression refers to the introduction of new genetic material into a population and is only recently being widely acknowledged as a way to increase a species' ability to evolve, especially in animals (Taylor & Larson, 2019; Brauer et al., 2023). As a consequence, the importance of conservation policies and management

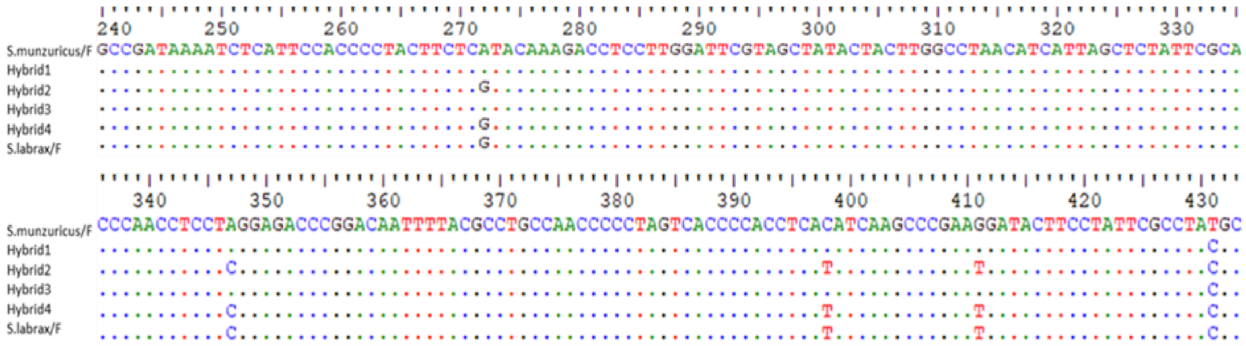

Figure 1. Nucleotide sequence alignment of the *Cytb* gene of *Salmo labrax, Salmo munzuricus* and their potential hybrids.

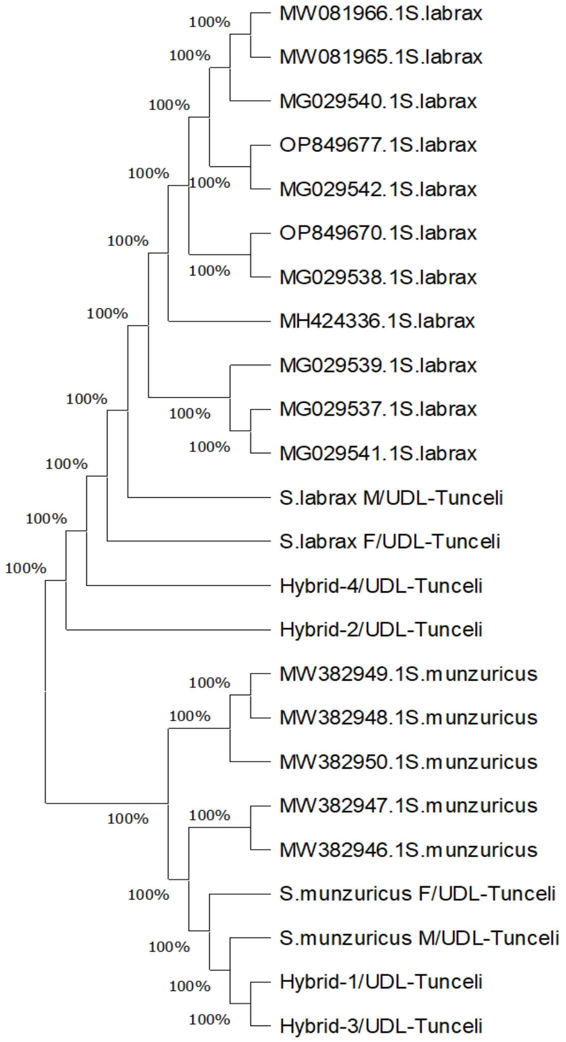

Figure 2. Dendogram generated using the Maximum Likelihood method and the Hasegawa, Kishino–Yano (HKY) model for the *Cytb* gene region for potential hybrid samples (UDL: Uzunçayır Dam Lake).

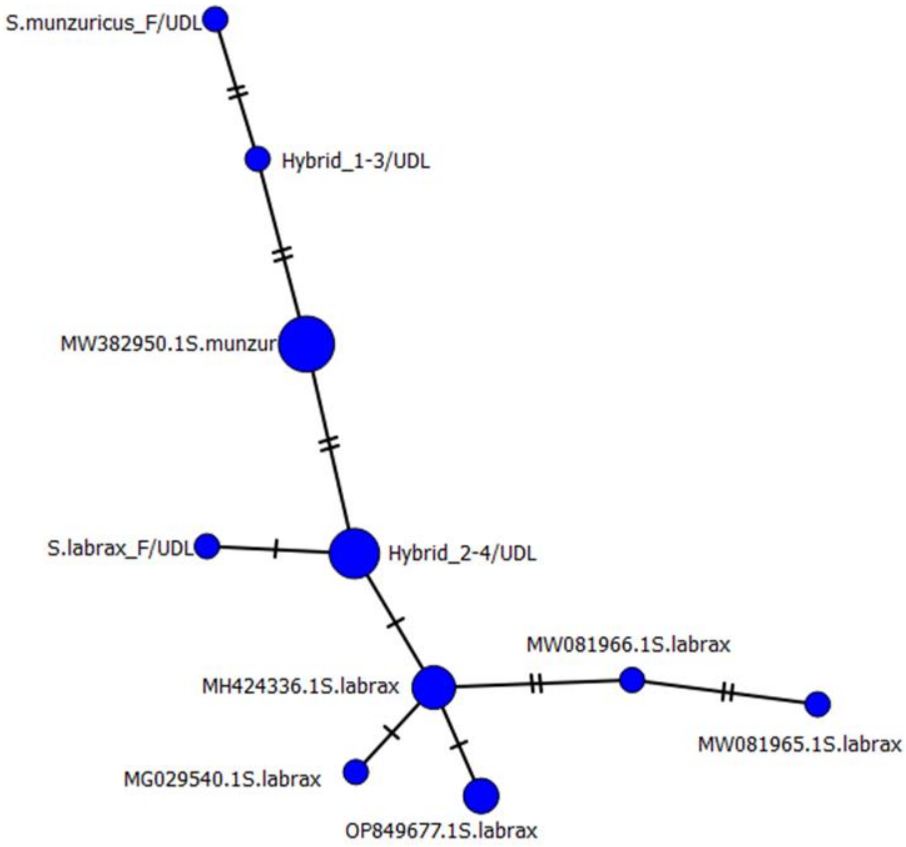

Figure 3. Distance based approximation model for *Cytb* gene region using Median-Joining Network (UDL: Uzunçayır Dam Lake).

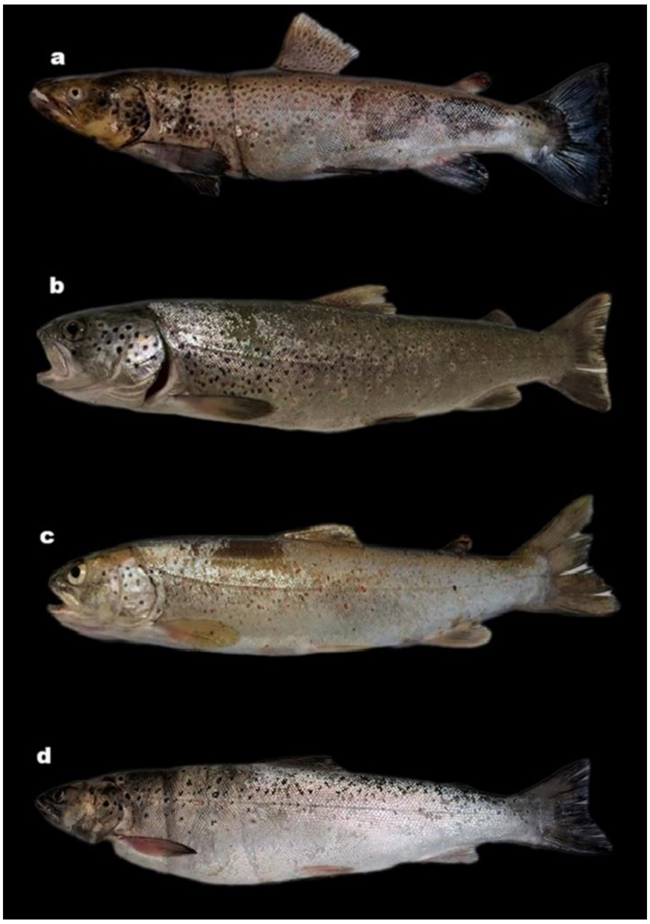

Figure 4. a) *Salmo labrax*, b) *Salmo munzuricus*, c) Hybrid2 and d) Hybrid1.

strategies increased for native species (vonHoldt et al., 2018; Brauer et al., 2023). In the present study, *S. labrax* and its hybrids were recorded for the first time in Uzunçayır Dam Lake (Tunceli) which is located on the Munzur Basin. Therefore, we examined the phenotypic and genotypic variation in *S. labrax*, *S. munzuricus* and their hybrids (*S. labrax* × *S. munzuricus*). As far as we are aware, this is the first study about the genotypic evidence for hybridizing Black Sea trout (*S. labrax*) and Munzur trout (*S. munzuricus*).

The distribution of *S. labrax* does not correspond to Munzur Basin stream systems or even dam lakes, an unusual pattern for Black sea trout. Thus, the question arises of this distribution is anthropogenic in origin. This has increased concerns about the future of Munzur trout. For example, Cussac et al. (2014) reported that the introduction of salmonids to Patagonia has had significant impacts on lakes and rivers. Almost all native fish species have been displaced by rainbow trout. McDonald et al. (2008) suggested that phenotype generally served as a dependable indicator of nuclear DNA genotype for all parents and their hybrids. Various techniques, including image analysis and geometric morphometric methods, were used for morphometric analysis of populations in the 1990s (Rohlf & Marcus, 1993; Cadrin & Friedland, 1999; Márquez et al. 2010; Kocabaş et al., 2018). *S. munzuricus* can be most easily distinguished from *S. labrax* by its color pattern. Unlike *S. labrax*, *S. munzuricus* lacks black spots on the head, though some may be present. When black spots appeared, they are irregularly shaped, unlike the circular spots found in *S. labrax*. Additionally, *S. munzuricus* differed from *S. labrax* in that males have a longer distance between the adipose fin and the base of the caudal fin. The male of *S. munzuricus* also have a smaller mouth opening compared to *S. labrax*. *S. labrax* × *S. munzuricus* hybrid fish was identified by black spots on the head and adipose fin red spot. In this study, due to the small number of samples, no significant variation was found in the morphological and phenotypic traits for specimens. In addition, a larger number of fish need to be examined to better observe the possibility of hybridization from morphological and phenotypic perspective. It is recommended to define species by genomic clusters of populations rather than by morphologically distinct single-type populations, in order to overcome challenges in the taxonomy and conservation of species complexes such as brown trouts (Segherloo et al., 2021).

In many studies, both mtDNA and nDNA markers are used to determine whether the captured species are natural hybrids and to identify the parent species (Young & Harig, 2001; Weigel et al., 2003; McKelvey et al., 2016; Jang, et al., 2024). Mitochondria and mtDNA exhibit

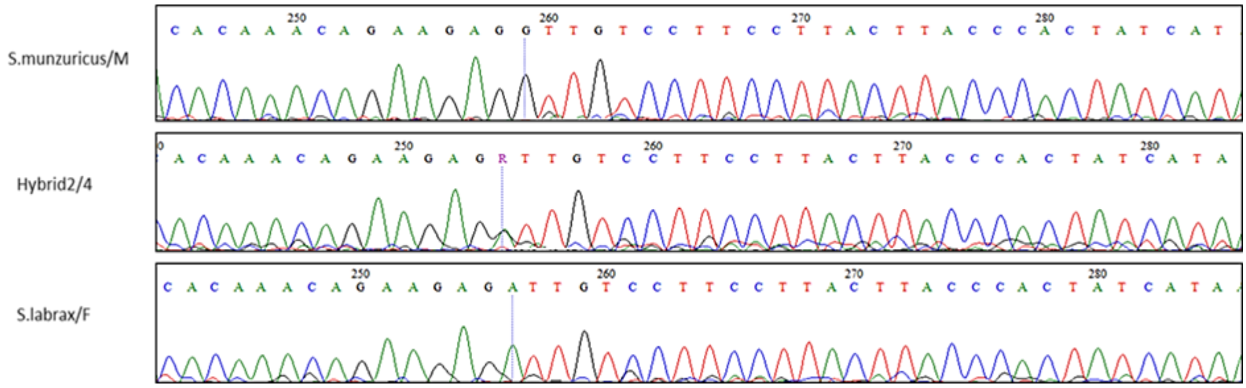

Figure 5. Electropherograms of the *Rag1* gene, showing possible signal of hybrid origin of parental SNP variation in Hybrid2/Hybrid4 individuals (SNP variations are shown with blue bars)..

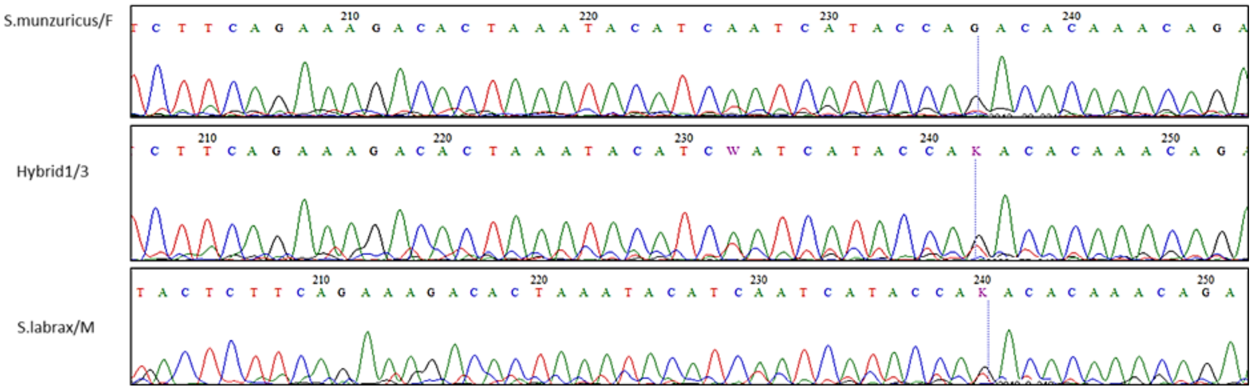

Figure 6. Electropherograms of the *Rag1* gene provide possible signal of hybrid origin that the double peaks are also present in Hybrid1/Hybrid3 in the variation region of the parent species (SNP variations are shown with blue bars).

strict maternal inheritance (Guangfu et al., 2014). Whereas, nDNA is stably transmitted to the offspring and is characterized by biparental inheritance (Yoo et al., 2024). Li et al. (2018) stated that *Cytb* sequences have been used in molecular phylogenetic and population analyses of many fish species. In our study, we used both mtDNA gene region (*Cytb*) and nuclear gene region (*Rag1*) to determine which species the morphologically different individuals were more similar to one another. mtDNAs of *S. munzuricus* infiltrated into *S. labrax* and *S. labrax* to *S. munzuricus*. Some of the *Cytb* haplotypes showed inconsistency between the species of origin and the genetically assigned type. Results showed that all the haplotypes of the nuclear locus were clearly discriminated by the species from which they were ensured. In contrast, all of which are thought to be hybrids showed either complete or partial incompatibility between the species-types assigned by the *Cytb* haploid genotype and the morphological species identification. Moreover, in the electropherogram, double peaks in both single nucleotide polymorphism (SNP) regions are also found in some hybrid species in the variation region of the parent species, and this situation increases the opinion that the parent carrying different alleles transfers the allele that is different from this other parent to the hybrid species. This can be considered as a possible signal of hybrid origin.

Conclusions

Here, we used both meristic procedure and sequences from two genetic markers to identify hybrids. Including molecular data overcame some of the uncertainties of the meristic approach, particularly in identifying parental *Salmo* species and highlights the need to apply an integrative approach to hybrid detection. Hybrid individuals, inherit half the genes from each parental species, are often morphologically indistinctive from their parents (Leary et al., 1996). Because of this, the nuclear and mitochondrial diagnostic markers identified during this study can be used as a hybrid sign. These tools will benefit future hybrid research for hybridization between these two species. However, given our small sample size (n = 8), this needs further analysis, as this means that factors such as detailing parental contributions to hybrids, determining the degree of introgression, understanding behavioral and molecular mechanisms are needed. Thus, the first record of preliminary evidence of possible hybridization between Black Sea trout and Munzur trout were determined by the molecular evaluation of individuals with intermediate morphology, caught in areas of sympatry. Besides, this study contributes to the understanding of the ecological and conservation needs of Munzur trout, highlighting the importance of implementing effective conservation strategies and further research to ensure the persistence of this endemic fish species in the face of hybridization.

Ethical Statement

All field and laboratory procedures followed the recommended ethical guidelines and legislation regarding animal capture, manipulation, and experimentation for scientific purposes, and were conducted under permits obtained from the ethics committee of the Munzur University (Tunceli, Türkiye, Protocol No: 2025/47-01).

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Author Contribution

Abdullatif ÖLÇÜLÜ: Conceptualization, Investigation, Supervision, Writing-original draft; Zehra Duygu DÜZGÜNEŞ: Investigation, Methodology, Resources, Writing – review and editing; Filiz KUTLUYER KOCABAŞ: Investigation, Writing – review and editing; Volkan KIZAK: Investigation, Writing – review and editing; Mehmet KOCABAŞ: Investigation, Writing – review and editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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