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Research Article
Araştırma Makalesi

Genetic Diversity of Cultured Rainbow Trout (*Oncorhynchus mykiss*) Populations in Türkiye Based on Mitochondrial DNA Cytochrome b (cyt-b) Sequence Analysis

Türkiye'deki Kültür Gökkuşağı Alabalığı (*Oncorhynchus mykiss*) Popülasyonlarının Mitokondriyal DNA Sitokrom b (cyt-b) Dizi Analizine Dayalı Genetik Çeşitliliği

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Abstract: This study aimed to investigate the genetic diversity and structure of rainbow trout (*Oncorhynchus mykiss*) cultured populations in different provinces of Türkiye, based on the cytochrome b (cyt-b) gene region of mitochondrial DNA (mtDNA). Tissue samples were collected from a total of 98 fish (seven fish per province) across 14 provinces, followed by DNA isolation. The mtDNA cyt-b gene region (754 bp) was amplified using PCR. Genetic diversity indices, genetic structure, and phylogenetic analyses were calculated from the cyt-b gene sequence data. The average nucleotide frequencies of the four nucleotides cytosine (C), thymine (T), adenine (A), and guanine (G) were found to be 26.2%, 24.9%, 26.2%, and 22.8%, respectively, with a higher A + T content (51%) compared to G + C content (49%). A total of 645 polymorphic nucleotides were identified across the 14 populations, and 50 distinct haplotypes were defined. Haplotypic diversity ranged from 0.91 to 0.48, while nucleotide diversity (π) varied between 0.26 and 0.00. The highest genetic diversity was observed in the Tokat and Van populations, whereas the lowest was recorded in the Elazığ and Kahramanmaraş populations. AMOVA analyses revealed that 71.1% of the genetic diversity was found between populations, while 28.9% was found within populations. Pairwise F_{ST} values ranged from 0.38 to 0.99, with an average F_{ST} of 0.71. Phylogenetic analyses indicated that the populations clustered into two main groups, with further sub-groupings within these clusters. Notably, the Kahramanmaraş and Elazığ populations were found to be significantly differentiated from other populations. In conclusion, despite rainbow trout not being a native species in Türkiye, the findings of this study indicate that the genetic diversity of the populations is at a good level. However, in order to preserve the genetic diversity of cultured rainbow trout populations and to increase effective population size, it is recommended that breeders engage in fry exchanges and import new rainbow trout specimens to enhance genetic diversity.

Keywords
- Cytochrome b
- Genetic diversity
- Genetic structure
- mtDNA
- Phylogenetic analysis

Özet: Bu çalışmada, Türkiye'deki farklı illerdeki gökkuşağı alabalığı (*Oncorhynchus mykiss*) kültür popülasyonlarının mitokondriyal DNA (mtDNA) sitokrom b (cyt-b) gen bölgesine dayalı genetik çeşitliliğinin ve yapısının incelenmesi amaçlanmıştır. Çalışma kapsamında 14 ildeki toplam 98 balıktan (il başına yedi balık) alınan doku örnekleri ile DNA izolasyonu yapılmış ve mtDNA cyt-b gen bölgesi (754 bp) PCR ile çoğaltılmıştır. Cyt-b gen bölgesi sekans verilerinden genetik çeşitlilik indeksleri, genetik yapı ve filogenetik analizlerle ilgili hesaplamalar yapılmıştır. Çalışmada dört nükleotidin (sitozin (C), timin (T), adenin (A) ve guanin (G)) ortalama frekansları sırasıyla %26.2, %24.9, %26.2 ve %22.8 olarak bulunmuş; A + T içeriğinin (%51) G + C içeriğinden (%49) daha yüksek olduğu belirlenmiştir. 14 popülasyondan toplam 645 polimorfik nükleotid tespit edilmiş ve 50 farklı haplotip tanımlanmıştır. Haplotip

Anahtar kelimeler
- Sitokrom b
- Genetik çeşitlilik
- Genetik yapı
- mtDNA
- Filogenetik analiz

çeşitliliği 0.91 ile 0.48 arasında, nükleotid çeşitliliği ise 0.26 ile 0.00 arasında değişmiştir. En yüksek genetik çeşitlilik Tokat ve Van popülasyonlarında, en düşük ise Elazığ ve Kahramanmaraş popülasyonlarında gözlenmiştir. AMOVA analizleri, genetik çeşitliliğin %71.1'inin popülasyonlar arasında, %28.9'unun ise popülasyonlar içinde olduğunu göstermiştir. İkili F_{ST} değerleri 0.38 ile 0.99 arasında değişmiş ve ortalama F_{ST} değeri 0.71 olarak bulunmuştur. Filogenetik analizler, popülasyonların iki ana gruba ayrıldığını ve bu grupların kendi içinde farklı alt gruplar oluşturduğunu göstermiştir. Özellikle Kahramanmaraş ve Elazığ popülasyonlarının diğer popülasyonlardan belirgin şekilde farklılaştığı gözlenmiştir. Sonuç olarak gökkuşağı alabalıklarının gen kaynağı ülkemizde olmamasına rağmen çalışmada elde edilen bulgular popülasyonların genetik çeşitliliğinin iyi düzeyde olduğunu göstermektedir. Ancak gökkuşağı alabalığı kültür popülasyonlarının genetik çeşitliliğini korumak ve etkili popülasyon büyüklüğünü artırmak için yetiştiricilere yavru balıkları takas etmeleri ve genetik çeşitliliği artırmak için yeni gökkuşağı alabalıkları ithal etmeleri önerilebilir.

1.INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is one of the most widely farmed aquaculture species globally, with a long history of domestication (Longo et al., 2024). In recent years, Türkiye has emerged as one of the leading producers of this species, alongside Chile and Iran, driven by increasing global demand for rainbow trout (FAO, 2020). The extensive farming of rainbow trout has led to the development of numerous genetically distinct strains due to selective breeding practices aimed at enhancing desirable traits (Stanković et al., 2015; Wiens et al., 2018; D'Ambrosio et al., 2019; Kause et al., 2022). More than 75 rainbow trout subspecies have been identified worldwide, highlighting the genetic diversity within the species (Glover, 2008; Abdullah et al., 2019). In Türkiye, while some aquaculture companies import rainbow trout eggs to renew their genetic stocks, many rely on locally sourced broodstock, which may give rise to unique, region-specific genetic strains (Karataş, 2019). However, the lack of comprehensive records regarding the origins and sizes of the initial rainbow trout culture populations raises concerns about the genetic management of these stocks (Ağdamar, 2010; Oral, 2011).

The high fecundity of fish species, including rainbow trout, allows the establishment of populations from a limited number of breeding pairs (Saura et al., 2021; Kurta et al., 2023). However, prolonged reliance on a narrow genetic base can lead to inbreeding, which reduces genetic diversity and increases the likelihood of genetic problems within populations (Martsikalis et al., 2014; Paul et al., 2022). Inbreeding depression can decrease heterozygosity, result in the loss of important alleles, and reduce adaptability to environmental changes, ultimately impacting production efficiency and increasing the risk of deformities (Lind et al., 2012; Grant et al., 2017; Karataş, 2019). Therefore, understanding the genetic diversity in aquaculture populations is crucial for effective breeding programs and the long-term sustainability of the species (Lhorente et al., 2019; Houston et al., 2020). Genetic diversity is a critical factor influencing populations' resilience to environmental fluctuations and overall survival (Leitwein et al., 2017; Cossu et al., 2021). Higher genetic diversity increases the likelihood that some individuals within a population possess alleles that confer an advantage under changing conditions, facilitating the transmission of favorable traits to future generations (Wiens et al., 2018; D'Ambrosio et al., 2019). As a result, assessing the existing genetic diversity in target populations is essential before initiating any selective breeding program (Akhan and Canyurt, 2005; Chavanne et al., 2016; Grant et al., 2017). This evaluation is particularly important in rainbow trout aquaculture, where the potential for inbreeding depression requires careful genetic management to preserve diversity and adaptability (D'Ambrosio et al., 2019; Lhorente et al., 2019; Chu et al., 2020).

Despite the importance of genetic diversity in aquaculture, there is a notable lack of comprehensive studies focusing on the genetic structure of rainbow trout culture populations in Türkiye. Previous research has employed various markers, such as RFLP (Togan et al., 2002), RAPD (Akhan and Canyurt, 2005), microsatellites (Aksakal, 2009; Ağdamar, 2010; Oral, 2011), and mitochondrial DNA (mtDNA) (Sarmaşık et al., 2008; Karataş, 2019), to assess genetic diversity. However, these studies have often focused on limited geographic areas or

specific genetic markers, and most have not utilized sequence analysis to elucidate genetic differences. Consequently, while some research has been conducted to evaluate the genetic diversity of rainbow trout culture populations in Türkiye, no published studies have used sequence analysis to assess population structure using any region of the mitochondrial genome. Mitochondrial DNA is a powerful tool for analyzing population structure and genetic diversity due to its haploid nature and maternal inheritance (DeSalle et al., 2017; Baisvar et al., 2019; Sun et al., 2019; Peng et al., 2021). Numerous studies have successfully employed mtDNA sequence data to investigate the genetic diversity of various fish species, emphasizing the usefulness of the cytochrome b (cyt-b) gene region in understanding population structures (Ha et al., 2020; Sultana et al., 2022; Zhang et al., 2023).

Given the importance of genetic diversity for the sustainability of aquaculture, it is imperative to comprehensively evaluate the genetic diversity of rainbow trout culture populations in Türkiye using advanced molecular techniques. This study aims to address this knowledge gap by comparing the genetic diversity of rainbow trout culture populations in different provinces of Türkiye through sequence analysis of the mtDNA cyt-b gene region. By elucidating the genetic structure and diversity of these populations, this research will provide valuable insights for future breeding programs and conservation strategies, ultimately contributing to the sustainable management of rainbow trout aquaculture in Türkiye.

2.MATERIALS AND METHODS

2.1.Study Area, Sample Collection, and DNA Extraction

In this study, *Oncorhynchus mykiss* specimens were collected between October 1, 2023, and March 1, 2024, from 14 randomly selected provinces across Türkiye, covering all geographical regions, with two provinces from each region (Figure 1).

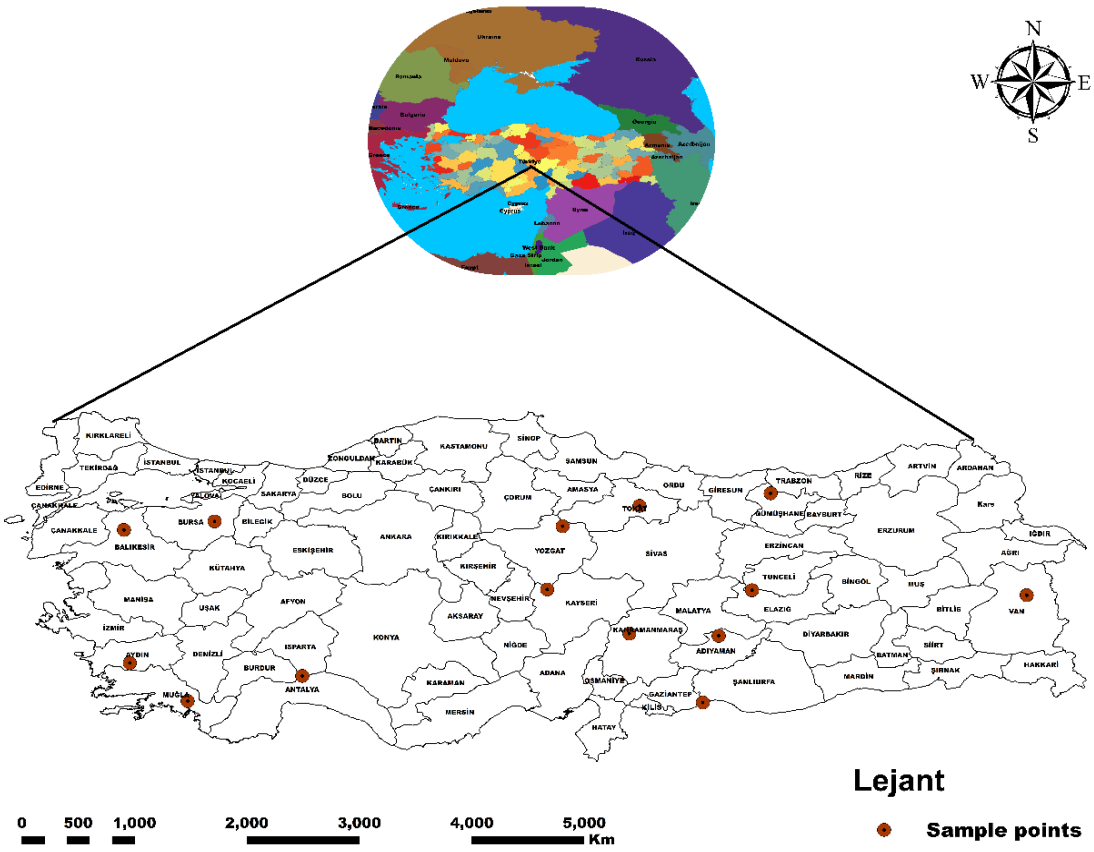

Figure 1. Sample points of rainbow trout culture populations.

A total of 98 rainbow trout (seven fish per province) were anesthetized using clove powder at a concentration of 200 mg/L (Naderi et al., 2017). The fish samples were transported under a cold chain to the Aquaculture Department Laboratory of Van Yüzüncü Yıl University's Faculty of Fisheries. Muscle tissue samples (25–50 mg) were taken from each fish, fixed in 96% ethanol, and stored until DNA extraction. Tissue samples were first homogenized using a tissue lyser, and DNA was extracted using the SuSpin Tissue DNA Isolation Kit (Sugenomics) according to the manufacturer's protocol, with modifications per Karataş (2019). The concentration and purity of the extracted DNA were determined using a NanoDrop 2000/2000c (Thermo Scientific) spectrophotometer at 230/260/280 nm. The DNA samples were stored at -20°C until further processing.

2.2.PCR Amplification

PCR amplification of the mtDNA cyt-b gene region was performed using a RotorGene 6000 (Qiagen) thermal cycler. The reaction used specific primers designed for the partial cyt-b gene region of rainbow trout mtDNA (GenBank accession no: NC_001717.1): forward primer 5'-AGAAACCTGGAATATCGGAGTTGTA-3' and reverse primer 5'-GATGGTGAAGTAAATTACAGAGGC-3'. The PCR mixture consisted of a total volume of 25 µL, containing 10 µL of PCR Master Mix (Sugenomics), 3 µL of forward and reverse primers, 4 µL of H2O, and 5 µL of template DNA. The PCR protocol began with an initial denaturation at 94°C for 10 minutes, followed by 45 cycles of denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 45 seconds. A final extension step at 72°C for 10 minutes completed the PCR process.

2.3.Sequencing and Data Analysis of the Cyt-b Gene Region of mtDNA

Genetic diversity analyses of the mtDNA cyt-b gene region were performed to assess the genetic variation and population structure of rainbow trout populations from different provinces. PCR amplicons were sequenced using the Sanger sequencing method on an Applied Biosystems 3500/3500 xL Genetic Analyzer. The raw sequence data were obtained in Ab1 format for further analysis. Genetic diversity indices, including base composition, nucleotide diversity (π), haplotype diversity (Hd), haplotype number (H), polymorphic nucleotides count (Ps), average number of nucleotide differences (k), allele number (Na), and expected heterozygosity, were calculated for the cyt-b gene region using standard procedures (Tajima, 1983). Calculations were performed using Arlequin v3.5.2.2 (Excoffier et al., 2005) and DnaSP v6.12.03 (Rozas et al., 2017) software, with a 95% confidence interval and 1000 bootstrap iterations. Population differentiation (F_{ST}) was calculated pairwise using Arlequin v3.5.2.2 (Weir and Cockerham, 1984) with 1000 random permutations for significance testing. Additionally, molecular variance (AMOVA) was computed to test the significance of population structure and analyze differentiation within and between populations using Arlequin. Principal Coordinate Analysis (PCoA) based on F_{ST} values was analyzed using GenAlEx ver. 6.5 (Peakall and Smouse 2006). Evolutionary distance analyses were performed using the Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods in MEGA X, following established methodologies (Kimura, 1980; Felsenstein, 1985; Saitou and Nei, 1987; Tamura, 1992; Kumar et al., 2018). To identify the optimal model, the MEGA X program was utilized, and the Tamura 3-Parameter (T92-G) model, which assumes a higher rate of transitions compared to transversions, was selected as the most suitable (Tamura, 1992; Tamura et al., 2021). The gamma distribution was applied to account for variation in substitution rates among sites. Bootstrap analyses were performed with 1,000 replicates for each method to assess branch support. A single phylogenetic tree was constructed using the ML method, with bootstrap values from both ML and NJ methods annotated at the respective branches to indicate consistency across methods.

3.RESULTS

3.1.Genetic Diversity Indices

A total of 754 base pairs (bp) of the mtDNA cyt-b gene region were amplified, and after correction and alignment, 726 bp fragments were analyzed to determine the genetic diversity among rainbow trout culture populations. The average frequencies of the four nucleotides cytosine (C), thymine (T), adenine (A), and guanine (G) across the 98 samples collected from 14 different provinces were found to be 26.2%, 24.9%, 26.2%, and 22.8%, respectively. The A + T content (51%) was higher than the G + C content (49%).

As shown in Table 1, a total of 645 polymorphic nucleotides were identified among

98 individuals from 14 different rainbow trout culture populations. The highest number of polymorphic nucleotides was observed in the Tokat population, while the lowest number was found in the Kahramanmaraş population. Based on these polymorphic regions, a total of 50 haplotypes were identified. The highest number of haplotypes was found in the Tokat and Van populations. The haplotype diversity (Hd) ranged from 0.91 ± 0.10 to 0.48 ± 0.17, and nucleotide diversity (π) ranged from 0.26 ± 0.05 to 0.00 ± 0.00. Both haplotype and nucleotide diversity were highest in the Tokat and Van populations and lowest in the Elazığ and Kahramanmaraş populations. Additionally, the average number of nucleotide differences (k) was highest in the Tokat population (185.86) and lowest in the Kahramanmaraş population (0.95).

Table 1. Number of polymorphic nucleotides (Ps), number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π), and average number of nucleotide differences (k) in rainbow trout culture populations.

Populations	n	Ps	h	Hd	π	k
Adıyaman	7	56	4	0.81±0.13	0.04±0.00	29.52
Aydın	7	198	4	0.71±0.18	0.13± 0.05	94.19
Balıkesir	7	240	2	0.57±0.12	0.19±0.04	137.14
Bursa	7	216	3	0.67± 0.16	0.17±0.03	119.52
Elazığ	7	12	3	0.52±0.20	0.01±0.00	3.81
Gümüşhane	7	174	3	0.67±0.16	0.14±0.03	98.19
Isparta	7	27	3	0.76±0.12	0.02±0.01	13.24
Kahramanmaraş	7	2	2	0.48±0.17	0.00±0.00	0.95
Kayseri	7	49	4	0.71±0.18	0.03±0.01	23.33
Muğla	7	94	4	0.86±0.10	0.07±0.01	52.76
Şanlıurfa	7	275	4	0.81±0.13	0.18±0.06	131.33
Tokat	7	328	5	0.91±0.10	0.26±0.05	185.86
Van	7	269	5	0.86±0.14	0.17± 0.06	122.14
Yozgat	7	75	4	0.81± 0.13	0.04±0.01	31.71

3.2.Genetic Structure

AMOVA analysis of the mtDNA cyt-b gene sequences of rainbow trout showed that genetic variation among populations accounted for 71.1% of the total variation, while genetic variation within populations accounted for 28.9% of the total variation (Table 3). As shown in Table 4, pairwise F_{ST} values, which indicate the degree of genetic differentiation between populations based on the mtDNA cyt-b gene region, ranged from 0.38 to 0.99, with an average F_{ST} value of 0.71. The PCoA results revealed that PC1 (11.62%) distinguished the Kahramanmaraş, Gümüşhane, Tokat, Balıkesir, Şanlıurfa, Aydın, and Muğla populations from those in Kayseri, Bursa, Van, Yozgat, Adıyaman, Elazığ, and Isparta. Meanwhile, PC2 (10.69%) further separated the populations into two groups: Kahramanmaraş, Gümüşhane, Kayseri, and Bursa, and Tokat, Balıkesir, Şanlıurfa, Aydın, Muğla, Van, Yozgat, Adıyaman, Elazığ, and Isparta (Figure 2).

Table 3. AMOVA analysis based on mtDNA cyt-b gene sequences.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p value
Among populations	13	8716.122	90.52403 Va	71.10	0.0000±0.0000
Within populations	84	3091.429	36.80272 Vb	28.90	
Total	97	11807.551	127.32675		
Fixation index (F_{ST})			0.71096		

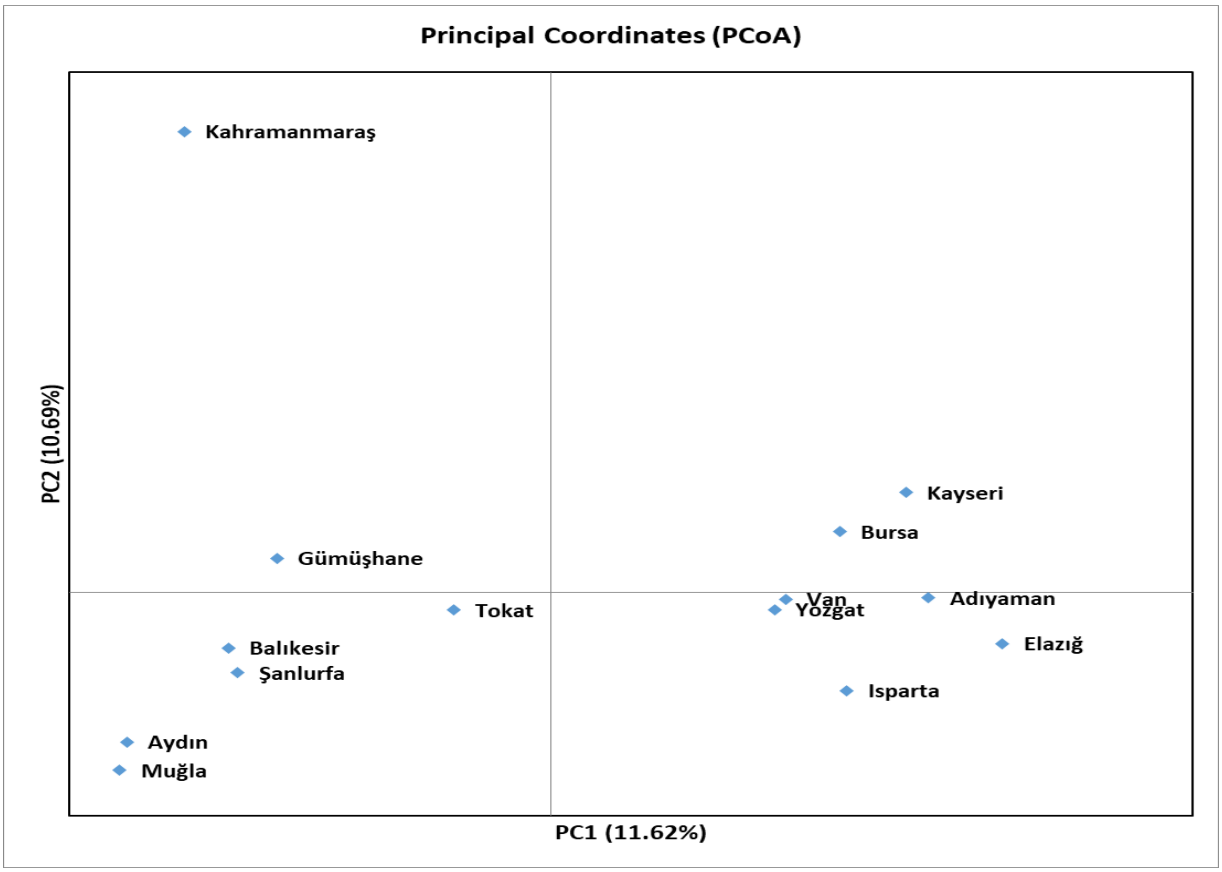

Figure 2. PCoA results are based on F_{ST} values.

Table 4. Pairwise F_{ST} (below diagonal) and p-values (above diagonal) based on the mtDNA cyt-b gene region.

	Adıyaman	Aydın	Balıkesir	Bursa	Elazığ	Gümüşhane	Isparta	Kahramanmaraş	Kayseri	Muğla	Şanlurfa	Tokat	Van	Yozgat
Adıyaman		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Aydın	0.78		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Balikesir	0.70	0.47		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Bursa	0.64	0.65	0.55		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Elazığ	0.92	0.83	0.74	0.70		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Gümüşhane	0.75	0.60	0.41	0.58	0.79		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Isparta	0.87	0.80	0.71	0.71	0.95	0.76		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Kahramanmaraş	0.94	0.83	0.72	0.74	0.99	0.72	0.97		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Kayseri	0.87	0.80	0.71	0.65	0.93	0.75	0.91	0.94		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Muğla	0.84	0.58	0.58	0.69	0.89	0.70	0.87	0.89	0.86		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Şanlurfa	0.75	0.53	0.44	0.63	0.78	0.52	0.75	0.78	0.76	0.64		0.00±0.00*	0.00±0.00*	0.00±0.00*
Tokat	0.60	0.44	0.38	0.46	0.65	0.47	0.62	0.63	0.63	0.52	0.46		0.00±0.00*	0.00±0.00*
Van	0.66	0.62	0.55	0.49	0.72	0.61	0.67	0.77	0.70	0.67	0.60	0.44		0.00±0.00*
Yozgat	0.89	0.79	0.73	0.73	0.94	0.80	0.92	0.95	0.90	0.86	0.75	0.64	0.71	

* $P < 0.05$

3.3.Phylogenetic Analysis

The Neighbor-Joining (NJ) dendrogram based on the mtDNA cyt-b gene region revealed that the rainbow trout culture populations clustered into distinct groups. According to the NJ tree, the 14 populations formed two major groups, each further divided into subgroups. In the first group, the Kahramanmaraş population formed its distinct subgroup. Another subgroup consisted of the Tokat, Balıkesir, and Gümüşhane populations, with the Tokat and Balıkesir populations being closely related. In the subgroup containing the Şanlıurfa, Aydın, and Muğla populations, the Aydın and Muğla populations were more closely related to each other. In the second major group, the Elazığ population separated from all other populations to form its group. Another subgroup included the Isparta and Van populations, while a third subgroup consisted of the Bursa, Kayseri, Yozgat, and Adıyaman populations, with Bursa and Kayseri showing a closer relationship. The Maximum Likelihood (ML) dendrogram based on the mtDNA cyt-b gene region demonstrated that the cultured rainbow trout populations clustered into distinct groups. The ML tree revealed that the 14 populations formed two main groups, each further subdivided into subgroups. In the first group, the Kahramanmaraş population was distinctly separated from the others, while Muğla-Aydın and Balıkesir-Tokat populations formed closely related binary subgroups. Şanlıurfa and Gümüşhane populations were also included in this group, though their similarity to the other populations was lower. The second group comprised seven populations divided into two subgroups. The Elazığ population was distinct from all other populations, forming its subgroup. The Bursa-Kayseri populations, which exhibited a very close relationship, were grouped and shared similarities with Yozgat, Adıyaman, Van, and Isparta populations, collectively forming the second subgroup.

The phylogenetic tree, displayed in Figure 3, illustrates the genetic relationships among the studied populations. Bootstrap values from both the NJ and ML methods are shown at each branch as ML/NJ, highlighting the high consistency and reliability of the two methods.

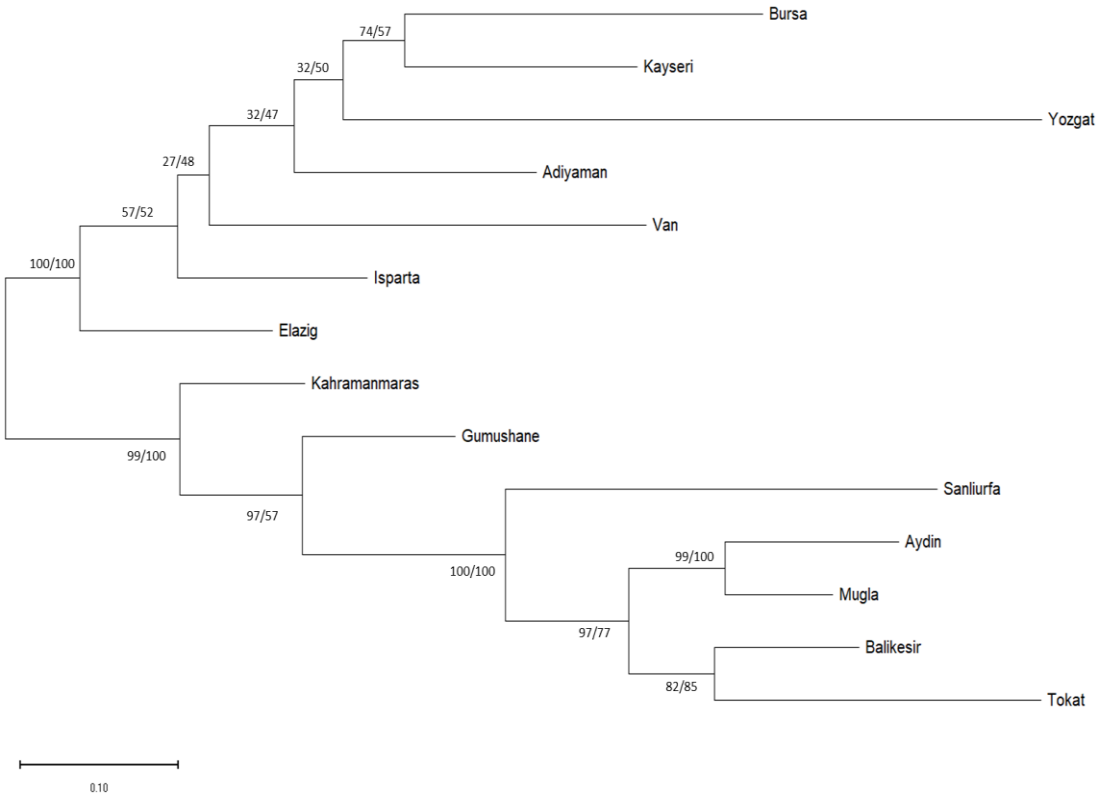

Figure 3. Phylogenetic tree constructed using the Maximum Likelihood (ML) method. Bootstrap values derived from both the ML and Neighbor-Joining (NJ) methods are indicated at each branch as ML/NJ. Branch lengths are proportional to genetic distance, and the scale bar represents substitutions per site.

4.DISCUSSION AND CONCLUSION

Genetic studies are of great importance as they enhance our understanding of genetic diversity and evolution, thereby providing valuable guidance for the conservation and management of genetic resources and the development of breeding programs (Zhang et al., 2018; Fang et al., 2022). The genetic structure of populations essentially defines the total genetic diversity and its distribution within and among a set of populations (Sultana et al., 2022). Molecular markers serve as effective tools for investigating and monitoring the genetic status of populations, including both differentiation and variation (Kumar et al., 2017). In this context, mtDNA is widely used to assess genetic diversity and structure in fish species (Zhang et al., 2018). This study presents a comprehensive analysis of the genetic diversity and structure of rainbow trout (*Oncorhynchus mykiss*) cultured populations from different provinces in Türkiye, using the mtDNA cyt-b gene region. The findings underscore substantial genotypic differences among populations, along with notable variations in nucleotide diversity, haplotype diversity, and the frequency of polymorphic nucleotides. When examining the sequence composition of the mtDNA cyt-b gene region in rainbow trout populations, a higher observed A+T content (51%) compared to G+C content (49%) is consistent with the typical base composition of mtDNA in many fish species, including rainbow trout. The genetic differences among the rainbow trout culture populations, as presented in Table 1, reveal a total of 645 polymorphic nucleotides across 14 different populations. Additionally, the presence of various haplotypes in rainbow trout culture populations was observed. Based on these polymorphic regions, a total of 50 haplotypes were identified. The occurrence of distinct haplotypes within the studied populations indicates the existence of genetic diversity. Moreover, the large number of polymorphic nucleotides serves as a strong indicator of genetic diversity within these populations (Nei, 1987; Whitmore, 1990). Therefore, the presence of distinct haplotypes and a high number of polymorphic nucleotides among rainbow trout culture populations from different provinces in Türkiye suggests the existence of significant genetic diversity.

The study found that haplotype diversity ranged from 0.91±0.10 to 0.48±0.17, while nucleotide diversity varied between 0.26±0.05 and 0.00±0.00 (Table 1). Indeed, previous studies on the genetic diversity of rainbow trout have reported similar findings regarding haplotype and nucleotide diversity. For instance, a study on the genetic structure of rainbow trout farmed in Greece reported haplotype diversity ranging from 0.87±0.04 to 0.59±0.06 and nucleotide diversity from 0.36±0.19 to 0.18±0.10 (Martsikalis et al., 2014). Similarly, Colihueque et al. (2019) estimated the genetic diversity of rainbow trout stocks in Chile, reporting haplotype diversity between 1.00±0.50 and 0.00±0.00, and nucleotide diversity ranging from 0.011±0.00 to 0.00±0.00.

The AMOVA analysis for the mtDNA cyt-b gene sequences in rainbow trout revealed that 71.1% of the total genetic diversity was attributed to variation between populations, while 28.9% of the total diversity was due to within-population variation (Table 3). These results indicate that the primary source of diversity lies between populations. In previous studies, Martsikalis et al. (2014) reported that 5.65% of the genetic diversity in rainbow trout populations farmed in Greece was attributable to between-population differences, while 94.35% was due to within-population variation. Similarly, Colihueque et al. (2019) found that 38.35% of the genetic diversity in rainbow trout stocks in Chile was due to between-population variation, with 61.65% attributed to within-population diversity. Another study examined the population structure and genetic diversity of rainbow trout broodstock in Brazil using SNP markers, revealing that 17.29% of the genetic diversity was attributed to differences between populations, while 86.51% was attributed to variation within populations (de Araújo Júnior et al., 2023). Devaa et al. (2024) reported that 44.30% of the genetic diversity in trout populations in India was due to between-population variation, while 55.70% was due to within-population diversity.

An examination of the F_{ST} values for the 14 rainbow trout culture populations revealed high genetic differentiation between populations (Table 4). The pairwise F_{ST} values based on the mtDNA cyt-b gene region ranged from 0.38 to 0.99, with an average F_{ST} value of 0.71 (Tables 3 and 4). These results suggest a high level of genetic diversity among the studied populations. When compared to previous studies, the F_{ST} values observed in this study differ significantly. In studies on rainbow trout culture populations in Türkiye, Ağdamar (2010) and Oral (2011)

reported F_{ST} values of 0.06±0.18 and 0.06±0.04, respectively. Karataş (2019), in a study of rainbow trout hatcheries in Van, Türkiye, based on 10 mtDNA primers, reported an F_{ST} value of 0.21. In another study, Carcamo et al. (2015) reported an F_{ST} value of 0.222 in rainbow trout populations in Chile. Faccenda et al. (2018) reported F_{ST} values ranging from 0.12 to 0.06 in rainbow trout stocks from Trentino, Italy. Colihueque et al. (2019) reported an F_{ST} value of 0.38 in rainbow trout stocks in Chile. Devaa et al. (2024) found F_{ST} values ranging from 0.59 to 0.22 in trout populations in India. In a study analyzing the genetic differentiation and assignment of commercial rainbow trout strains using a SNP panel, Liu et al. (2017) reported Fst values ranging from 0.056 to 0.195. Another study assessed the population structure and genetic diversity of rainbow trout broodstock in Brazil using SNP markers, reporting an Fst value of 0.172 (de Araújo Júnior et al., 2023). The differences in F_{ST} values observed between this and other studies may be attributed to variations in the molecular markers used or differences in the geographic regions studied. As Glover (2008) noted, it is challenging to directly compare genetic variation across studies that use different marker sets. In comparison to other studies, the pairwise F_{ST} values obtained in this study are significantly higher. However, when considering studies on genetic diversity in other fish species based on the mtDNA cyt-b gene region, similar F_{ST} values have been reported (Habib et al., 2011; Zhu et al., 2016; Kumar et al., 2017; Ha et al., 2020). Thus, it is plausible that the mtDNA cyt-b gene region is conserved in rainbow trout populations.

The Neighbor-Joining (NJ) and Maximum Likelihood (ML) dendrograms based on the mtDNA cyt-b gene region revealed that the rainbow trout populations were grouped into distinct clusters (Figures 3). These dendrograms, constructed using genetic distance data, demonstrated that the populations formed two primary groups, each further subdivided into subgroups, reflecting the genetic relationships among the populations. Similar results were observed in previous studies on rainbow trout culture populations in Türkiye, where Ağdamar (2010) and Oral (2011) also reported the division of populations into two major groups. Additionally, a study conducted in Norway, which compared the genetic characterization of rainbow trout populations, found that the populations were split into two main groups based on the dendrogram (Glover, 2008). Another study by Devaa et al. (2024) on trout populations in India also reported the division of populations into two major groups.

The PCoA results revealed that PC1 (11.62%) and PC2 (10.69%) together explain only 22.31% of the total variation. Although these results account for only a small portion of the genetic variation, it is evident that the Kahramanmaraş population is genetically distinct from the others, forming an independent group. This finding, supported by high F_{ST} values and phylogenetic analyses, indicates that Kahramanmaraş should be considered a separate genetic group.

When the 14 rainbow trout culture populations sampled from different provinces in Türkiye were evaluated according to the aforementioned criteria, the findings indicated that, despite rainbow trout not being a native species in the country, the genetic diversity of these populations is at a good level. In particular, the high levels of haplotype and nucleotide diversity observed in the Van and Tokat populations suggest that these populations may have exchanged fish with other populations or imported new rainbow trout specimens, leading to higher genetic diversity compared to others. The high level of genetic diversity and differentiation among rainbow trout culture populations in Türkiye suggests that these populations have adapted to their local environments due to geographic isolation and varying ecological conditions. The distinct genetic structures observed in populations such as those from Kahramanmaraş and Elazığ highlight the importance of considering local adaptations in conservation and management strategies. The F_{ST} ratio of rainbow trout culture populations suggests that there is genetic differentiation among the populations. However, it is not sufficient to assess rainbow trout culture populations in Türkiye solely based on the high F_{ST} value. A high F_{ST} value does not guarantee that inbreeding issues will not arise in these populations over time. Therefore, to fully understand the situation, all genetic parameters should be evaluated together.

In conclusion, this study, which compared the genetic diversity of 14 rainbow trout culture populations in Türkiye, provided valuable insights into the genetic structure of these populations. One of the challenges in rainbow trout farming is estimating genetic diversity.

Since populations in confined environments like hatcheries are generally smaller, they are more susceptible to genetic variations compared to wild populations. As a result, reductions in genetic variation due to inbreeding and genetic drift are common in cultured populations. The loss of genetic variation corresponds to a loss of genetic potential for stock improvement and adaptation to environmental changes. Therefore, monitoring changes in the genetic structure of cultured populations, relative to wild populations, is essential. In general, the impact of reduced genetic diversity in cultured populations is the loss of adaptive capacity to environmental changes. The results of this study are expected to contribute to better management of production capacities in rainbow trout farms. Indeed, applying genetic diversity models in breeding programs is highly beneficial, providing useful information for improved management practices. It is recommended that rainbow trout breeders exchange fry or import new specimens to enhance genetic diversity and increase the effective population size. Furthermore, future studies covering a broader range of distribution areas with more samples will undoubtedly contribute to rainbow trout aquaculture in Türkiye. In this context, future research should prioritize expanding genetic analyses to incorporate nuclear DNA markers, RAD-seq, SNP analyses, and a broader range of populations, offering a more comprehensive understanding of the genetic diversity and structure of rainbow trout in Türkiye. Long-term monitoring and conservation programs should be implemented to preserve the genetic diversity and integrity of these populations and ensure the sustainability of rainbow trout culture in the region.

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CONFLICT OF 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.

AUTHOR CONTRIBUTIONS

Fiction: İB, BK; Literature: İB, BK; Methodology: İB, BK; Performing the experiment: İB, BK; Data analysis: İB, BK; Manuscript writing: İB, BK, Supervision: İB, BK. All authors approved the final draft.

ETHICAL STATEMENTS

This study was conducted with the approval of Animal Experiments Local Ethics Committee of Van Yuzuncu Yıl University (protocol no: 2023/05–07).

DATA AVAILABILITY STATEMENT

The data used in the present study are available upon request from the corresponding author. Data is not available to the public due to privacy or ethical restrictions.

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