Genetic Diversity in *Labeo rohita* Population Based on Selected Mitochondrial and Nuclear DNA Markers

Ph.D. THESIS

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Genetic Diversity in *Labeo rohita* Population Based on Selected Mitochondrial and Nuclear DNA Markers

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Dedicated to my beloved parents
my loving sons
and my dear wife,
Umme Abdullah

Certification

This is to certify that the research presented in the thesis entitled 'Genetic Diversity in *Labeo rohita* Population Based on Selected Mitochondrial and Nuclear DNA Markers,' submitted by Md. Mahfuzur Rahman, Registration No. 52, Session: 2020-21, in partial fulfillment of the requirements for the Doctor of Philosophy (Ph.D.) degree at the University of Dhaka, Bangladesh, represents original research conducted under my direct supervision. I believe that the thesis meets the standards and complies with the regulations governing the award of this degree. The content of this thesis has not been previously submitted for any other degree or diploma at this or any other university.

We affirm that Md. Mahfuzur Rahman has fulfilled all requirements outlined by the university for the work presented in this thesis and certify that the content has not been the basis for the award of any prior degree, either to him or to any other individual.

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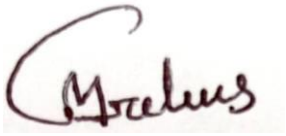
Declaration

I hereby declare that the research work presented in this thesis entitled 'Genetic Diversity in *Labeo rohita* Population Based on Selected Mitochondrial and Nuclear DNA Markers' was conducted by me under the supervision of Dr. Mohammad Shamimul Alam, Professor, Department of Zoology, University of Dhaka, Bangladesh. This thesis, or any part of it, has not been previously submitted for any degree or any other purpose to any university.



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The Author

Abstract

The genetic diversity of *Labeo rohita*, a commercially important freshwater fish in South Asia, was examined at specific DNA regions of the mitochondrial cytochrome b gene (*cytb*), the control region (D-loop), and the nuclear myostatin gene (*mstn*). Despite the significance of *L. rohita*, no prior studies have employed a combination of *cytb*, the control region, and *mstn* markers for genetic analysis. This research addresses that gap by using these as molecular markers to assess population structure and genetic diversity. The findings provide valuable insights into the effective conservation and management of *L. rohita* populations.

250 Samples were collected from Padma, Jamuna, and Halda rivers, as well as from hatchery-origin cultured stocks in Bangladesh. Following DNA extraction, PCR amplification, and sequencing of 200 fish samples, a total of 137 *cytb*, 162 D-loop, and 84 *mstn* sequences were analyzed after quality assessment. While *cytb* and D-loop sequences were analyzed for wild and cultured samples, *mstn1* analysis was limited to wild samples only. The *cytb* gene region (616 bp) exhibited 11 haplotypes with 98.4% sequence conservation, while the control region (598 bp) displayed 50 haplotypes with greater variability. The *mstn* gene region (569 bp) showed 18 haplotypes with 98.41% conservation. Additionally, the *mstn* sequences have shown homozygous (A_9/A_9) and heterozygous (A_9/A_{10}) genotypes at its microsatellite loci. The A_9 allele (with nine "A"s) was predominant (72%-89%) across all populations, with the highest occurrence of the homozygous A_9A_9 genotype in the Halda population (78%). Nucleotide polymorphisms were observed at 22 sites in the control region, 16 in the *cytb* gene, and 10 in the *mstn* gene. Notable regional variation was found, with specific haplotypes predominating in different populations, such as the haplotype HP04 of the control region found in 61.36% of the Halda River population.

Based on the control region DNA sequences, the cultured population showed the highest haplotype (0.951) and nucleotide diversity (0.00778) among all, and the Halda population had the lowest. Similarly, for *cytb*, the cultured population exhibited the highest diversity, while the Padma population had the lowest. Among the wild populations, the *mstn* gene sequences showed the highest diversity in the Halda population, with the lowest in the Jamuna population. AMOVA and pairwise FST analyses revealed significant genetic differentiation, particularly between the Halda and other populations. Phylogenetic and haplotype network analyses identified two major genetic clusters, with distinct separations between populations, particularly between the Halda and Padna

populations in all study markers. The genetic statuses of three river populations of *L. rohita* have been compared and discussed.

The causes of variation in genetic diversity may include differences in breeding practices, gene flow, and population history. Possibly, the cultured populations show higher diversity due to the mixing of different breeds in the hatchery. While the wild populations face anthropogenic and natural constraints. Additionally, varying mutation rates and population sizes may influence the observed differences in haplotype and nucleotide diversity.

Overall, the findings highlight significant genetic differentiation and regional variations, and document the genetic isolation of the Halda population and the mixed genetic background of the cultured stocks. These results underscore the need for targeted conservation strategies to protect wild populations and sustainably manage cultured stocks. The study provides valuable insights for future research and fisheries management practices in Bangladesh and similar regions facing anthropogenic pressures.

List of Abbreviations

% =Percentage

°C= Degree Celsius

µg= Microgram

µL = Microliter

cm= Centimeter

cytb = Cytochrome b

dNTP = Deoxy Nucleotide Triphosphate

e.g. = For example,

EDTA = Ethylene diamine tetra-acetic acid

et al.= And others

Fig.= Figure

g= gram

gDNA = Genomic DNA

i.e.= That is

L = Liter

M= Molar

min= Minute

ML= Maximum likelihood

mL = Milliliter

mm = Millimeter

mM= Millimole

mstn = myostatin

PCR= Polymerase Chain Reaction

rpm= Rotation per minute

TAE= Tris-acetate EDTA

V= Volume

1. Introduction

1.1 Background on *Labeo rohita*

One of Bangladesh's economically important freshwater fish species is *Labeo rohita* (Hamilton, 1822), commonly known as Rohu or Rui (Behera *et al.,* 2018). It is also common in other South Asian countries, including India, Pakistan, Nepal, and Myanmar. Belonging to the family Cyprinidae, *L. rohita* offers significant nutritional benefits and is widely cultivated in freshwater bodies due to its fast growth, delicious taste, and high market demand (Behera *et al.,* 2007; Hussain and Mazid, 2005). Its contribution (including capture and Culture) to the overall aquaculture production of Bangladesh increased from 8.83% in 2005 (Rahman, 2005) to 11.56% in 2023 (DoF, 2023). The quantity of cultured rohu fish in closed water bodies (e.g., ponds) is 22,72,667 metric tons which is 53.66% of total rohu fish production (DoF, 2023). The culture is dependent on isolated hatchery sources. So, there is a high chance of the occurrence of inbreeding, which can result in retardation in growth, development, and reproduction, decreased fitness in offspring, and reduction in yield (Neaves *et al.,* 2015; Piles *et al.,* 2022). Identifying genetically diversified wild populations and outcrossing can minimize such depression.

In recent years, the contribution of *L. rohita* to Bangladesh's total fish production has steadily increased, underlining its vital role in ensuring national food security. Unfortunately, this progress is being compromised by the widespread practice of hybridization in some hatcheries. The Catla-rohu hybrids are particularly concerning, as they closely resemble pure *L. rohita* and *Cirrhinus cirrhosus* (mrigal), making them difficult to identify and often misleading to consumers. The unchecked production of these hybrids threatens the genetic integrity of native stocks, necessitating urgent measures to preserve the purity of these species and safeguard the long-term sustainability of aquaculture.

Belonging to the Cyprinidae family, *L. rohita* is part of a diverse group of freshwater fish species that are recognized for their unique body structures and genetic characteristics. This family, one of the largest among fish, comprises numerous genera and species that inhabit varied environments across the globe (Crossman, 1996). The Cyprinidae family members, including rohu, display remarkable adaptability to different ecological niches, supported by their diverse body shapes, sizes, and specialized features like elongated jaws, distinctive fin arrangements, and unique

dentition (Crossman, 1996). These adaptations enable them to thrive in rivers, lakes, and floodplains, showcasing their evolutionary success.

Globally, the Cyprinidae family is distributed across North America, Europe, Asia, and parts of Africa, with their dietary and ecological significance being well-documented (Nelson et al., 2016). In Bangladesh, freshwater fish biodiversity is particularly rich, with over 200 species identified (Shafi & Quddus, 1982; Rahman, 1989). Among these, carps such as Catla, rohu, and mrigal are pivotal for both aquaculture and food production, underscoring their economic and nutritional importance.

The genus *Labeo*, to which rohu belongs, includes approximately 108 species distributed primarily across Africa and Asia (Zheng *et al.,* 2012; Tang *et al.,* 2009). These species exhibit distinctive physical traits, such as variations in lip structure and barbel arrangement, which aid in their identification and ecological adaptability (Tshibwabwa & Teugels, 1995; Sudasinghe *et al.,* 2018). For instance, some *Labeo* species have conical papillae on their lips, while others feature longitudinal folds, reflecting their evolutionary adaptations to specific feeding habits.

In terms of habitat, *Labeo* species, including rohu, are found in diverse aquatic environments such as rivers, lakes, and wetlands (Reid, 1985). Their specialized oral and pharyngeal structures allow them to efficiently graze on algae and biofilm growing on submerged surfaces (Reid, 1985). Morphological variations, such as differences in body depth and mouth structure, have been observed among populations of species like Labeo catla, further highlighting their adaptability to specific ecological conditions (Suresh *et al.,* 2023).

The taxonomic classification of *L. rohita* is deeply rooted in its evolutionary and morphological characteristics. It belongs to the kingdom Animalia, phylum Chordata, and class Actinopterygii, which encompasses ray-finned fishes. Within the order Cypriniformes, known for its freshwater diversity, *L. rohita* is part of the family Cyprinidae and subfamily Labeoninae. This subfamily is distinguished by specialized feeding adaptations, aligning with the ecological niches occupied by its members. The genus Labeo is characterized by unique features such as barbel pairs and distinct lip structures, making it an essential group for taxonomic and ecological studies (Howes, 1991; Nelson, 2006).

L. rohita holds a pivotal position in Bangladesh's aquaculture sector, serving as a vital source of nutrition and economic stability. However, the increasing prevalence of hybridization and the resultant genetic erosion present significant challenges to maintaining the genetic integrity of this species. Addressing these threats necessitates immediate and coordinated efforts to conserve its genetic purity and ecological importance. By incorporating advanced scientific research and implementing sustainable aquaculture practices, it is possible to safeguard the genetic diversity and population structure of *L. rohita*, thereby ensuring its sustained role in supporting food security and biodiversity conservation.

1.2 Contribution of *L. rohita* to food security

Rui fish, a traditional indigenous species, plays a crucial role in enhancing food security and nutrition, particularly in developing countries like those in South Asia. Its rich nutritional profile, including proteins, vitamins A and B12, iron, calcium, and zinc, makes it an essential dietary component. For economically disadvantaged and low-income populations, rui fish serves as an affordable and accessible source of high-quality protein (Bogard, 2017; Kawarazuka & Béné, 2011).

The unique geographical features and climatic conditions of Bangladesh significantly contribute to the cultivation and conservation of rui fish. The confluence of the Ganges, Brahmaputra, and Meghna rivers, combined with abundant rainfall and nutrient-rich soil, creates an ideal environment for aquaculture. Currently, fish provide approximately 80% of the country's animal protein supply, underscoring its critical role in ensuring food security for the population (DoF 2024).

The health and growth of rui fish are intricately linked to water quality and environmental conditions. Parameters such as temperature, dissolved oxygen (DO), and the presence of heavy metals are pivotal for its growth and survival. Studies indicate that low DO levels during transportation significantly increase mortality rates in rui fish (Das *et al.,* 2015). Additionally, the presence of heavy metals like lead and cadmium can induce oxidative stress and DNA damage, adversely affecting the overall health of the fish (Mahamood *et al.,* 2021).

Furthermore, the diet and water sources of rui fish play a critical role in its growth performance. For instance, fish fed on sunflower-based diets demonstrated superior growth in groundwater environments (Malik *et al.,* 2020). This highlights the intricate connection between nutritional quality and environmental conditions.

Rui fish contributes not only to nutrition and food security but also significantly impacts the national economy. The fisheries sector accounts for 3.52% of Bangladesh's GDP and 26.37% of the agricultural GDP, reflecting its economic potential (DoF 2024). Moreover, this sector plays a vital role in generating rural employment and alleviating poverty (Nilson, 1946; Weatherley and Gill, 1987; Ahmed *et al.,* 2012).

Despite its immense potential to enhance food security, challenges such as overexploitation and inadequate value chains hinder the optimal utilization of rui fish. Implementing effective conservation strategies, focusing on water quality management, and promoting sustainable aquaculture practices can address these challenges. With proper management, the significant contributions of rui fish to food security and economic development can be further expanded in the future.

1.3 *L. rohita* in aquaculture of Bangladesh

Rohu (*L. rohita*) holds a central place in Bangladesh's aquaculture, significantly contributing to the country's diet and economy. Through modern genetic improvement programs, the production efficiency of this species has increased, cementing its role in aquaculture development. The fish is highly valued for its taste and economic return, forming a vital part of the local diet and export potential (Rahman, 2005; Devi *et al.,* 2019).

The wild populations of *L. rohita* are primarily sourced from natural water bodies such as rivers, lakes, and floodplains. Among these, the Halda River is recognized as a natural breeding ground, officially declared a fish heritage site due to its unique tidal patterns and suitability for spawning Indian major carps, including rohu (Halda Gazette, 2020; The Financial Express, 2020). Other major rivers, such as the Jamuna and Padma, also contribute significantly to the collection of wild broodstock and eggs.

Genetic improvement initiatives led by the Bangladesh Fisheries Research Institute (BFRI) and WorldFish have emphasized selective breeding since 1999, focusing on individual and family-based selection methods. These efforts aim to enhance genetic purity and improve growth rates, which are vital for sustainable aquaculture. Additionally, research highlights that mixing rohu with native species like pabda and gulsha in polyculture systems has been shown to yield higher productivity and economic returns, with notable net profits and favorable benefit-cost ratios (Nahiduzzaman *et al.,* 2023).

Bangladesh, home to some of the world's largest floodplains, ranks third in Asia in terms of aquatic biodiversity, trailing only China and India. This biodiversity underpins the country's fisheries sector, where Indian major carps, including rohu, dominate production (Hossain & Mazid, 2005; Shamsuzzaman *et al.,* 2017). However, human activities, such as overfishing, habitat modification, and pollution, pose significant threats to these natural resources (Vandewoestijne *et al.,* 2008).

Rohu's market demand extends beyond Bangladesh, with imports from India and Myanmar filling production gaps. The imported fish, often referred to as "Indian rohu" or "Burmese rohu," underscores the high demand for this species both locally and internationally (Devi *et al.,* 2019). This demand is fueled by the fish's excellent taste, high protein content, and market value, making it a favorite among consumers (Rahman, 2005).

Environmental sustainability and genetic diversity are critical for the long-term viability of rohu aquaculture. Natural fry, preferred for their superior growth rates and disease resistance, face threats from declining water quality and genetic erosion in hatcheries. Hatchery practices, including limited broodstock diversity and negative selection, have resulted in reduced genetic variation compared to wild populations, emphasizing the need for robust breeding programs (Shah, 2004).

The conservation of natural spawning habitats, such as the Halda River, is essential for sustaining wild populations. Genetic studies and hybridization efforts have demonstrated that crosses between wild and hatchery stocks can enhance growth rates, with Padma wild stocks showing the highest growth potential (Shah *et al.,* 2012). These findings highlight the importance of preserving natural genetic resources to improve aquaculture outcomes.

In recent years, advancements in genomic research, including genome sequencing of *L. rohita,* have provided deeper insights into population structure and breeding strategies. These innovations pave the way for addressing challenges like disease management and environmental resilience, which are critical for the sector's growth (Arick, 2022; Arick *et al.,* 2022).

While Bangladesh's aquaculture sector continues to grow, balancing productivity with sustainability remains a challenge. By preserving natural breeding grounds, ensuring genetic diversity, and adopting advanced management practices, the country can secure the future of its fisheries sector and maintain rohu's status as a cornerstone of its aquaculture economy.

1.4 Problems in *L. rohita* culture and production

Rohu fish farming faces multiple challenges that directly impact the production process and the health of the fish. Natural disasters such as floods, which are common in various regions, pose a significant problem for farmers. These disasters can damage fish stocks, disrupting the production cycle (Samal *et al.,* 2022). Additionally, high stocking density is another major issue. When the fish density increases excessively, stress levels among the fish rise, which negatively affects growth rates and overall health (Minahl *et al.,* 2024). An ideal stocking density, such as 3.79 kg/m^3, ensures better growth (Minahl *et al.,* 2024).

The quality of fish feed is also a crucial factor in rohu farming. Insufficient or poorly prepared feed can hinder fish growth and health. Plant-based feed has proven to be an effective alternative to traditional fish feed (Arora & Parvez, 2024). However, improving only the feed quality will not solve all the farming problems. Poor water quality, such as high levels of ammonia and salinity, can lead to disease outbreaks, reducing the quality of the fish (Masud *et al.,* 2023). Therefore, regular monitoring of water parameters is vital to maintain a healthy farming environment (Masud *et al.,* 2023).

Human activities have caused the destruction and fragmentation of natural habitats, putting many species at risk of losing their genetic diversity. This not only reduces the survival chances of species but also weakens their ability to adapt to environmental changes. For instance, in India, the movement of gaur populations has been restricted due to road and land cover changes, resulting in high genetic divergence among them (Arora & Parvez, 2024). However, some species have shown resilience in adapting to adverse environments. For example, the southern damselfly has been able

to maintain genetic flow in urban central areas despite urbanization pressures (Minahl *et al.,* 2024). Nevertheless, relying solely on the species' resilience is not enough. It is essential to restore natural habitats and create migration corridors, which will help increase genetic flow among fragmented populations and maintain their genetic health.

The main causes of reduced genetic diversity in rohu fish production are breeding strategies, environmental changes, and human-induced impacts. Improper hatchery practices can lead to genetic introgression and allele loss, resulting in stock degradation (Ahmed *et al.,* 2022). To protect genetic diversity in rohu farming, selective breeding, and genomic methods can be used to enhance diversity. The Central Institute of Freshwater Aquaculture's initiatives in selective breeding have been successful, resulting in a 17% increase per generation (Mahapatra *et al.,* 2006). Moreover, creating a high-quality genome assembly for rohu fish has enabled the use of genomic tools to assist in breeding decisions. Genomic data can guide breeding programs, helping to reduce inbreeding depression and improve overall health (Chaudhary *et al.,* 2023).

Furthermore, environmental pollution, such as agricultural chemicals, pesticides, and industrial waste, is a major cause of habitat degradation. Such pollution creates selective pressure, leading to a reduction in genetic diversity in wild populations (Hussein and Majid, 2002). When population size decreases, the genetic diversity of wild populations also decreases, increasing the risk of species extinction (Frankham *et al.,* 2004). To address these issues, research must seek advanced methods, such as evaluating the genetic diversity of rohu fish using microsatellite DNA markers (Alam *et al.,* 2012).

1.4.1 Influence of environmental stressors on genetic variation

Environmental factors such as pollution and other ecological stressors can significantly influence molecular-level results in fish populations. Exposure to contaminants can lead to reduced genetic diversity, altered allele frequencies, and increased mutation rates, thereby impacting the genetic structure of populations (Bickham et al., 2000). Such changes often arise due to selection pressure, genetic bottlenecks, or DNA damage induced by toxic substances present in polluted environments (Theodorakis & Shugart, 1998). For instance, Mulvey et al. (2002) demonstrated that populations of Fundulus heteroclitus from contaminated sites exhibited distinct genetic structures compared to those from reference sites, highlighting the role of environmental stress in shaping genetic variation. Although water quality analysis was not directly conducted in this study, sample

collection was performed from major river systems that are regularly used for aquaculture and fishing, and no severe pollution events were reported during the sampling period. However, future studies integrating environmental monitoring with molecular analysis would provide a more comprehensive understanding of how ecological conditions affect genetic diversity.

1.5 Significance of genetic diversity studies

Genetic diversity plays a pivotal role in the survival and productivity of aquatic species. It is not only essential for maintaining the health of populations but also crucial for adapting to long-term environmental changes. Higher genetic diversity enables fish populations to develop greater disease resistance, which is vital for sustaining healthy and productive aquaculture systems (Aminisarteshnizi & Moyo, 2024).

Diverse genetic backgrounds help populations adapt to changing environmental conditions. This is particularly important as the world faces rapid environmental changes. Mandal et al. (2024) emphasized that genetically diverse populations are better equipped to withstand environmental challenges. Previous studies have shown that understanding genetic differentiation among populations and preserving this diversity are critical for sustainable management. For instance, targeted conservation strategies have improved the survival prospects of species like Clarias gariepinus and Ompok bimaculatus (Sanda *et al.,* 2024; Mandal *et al.,* 2024).

Selective breeding programs can enhance desirable traits such as faster growth and increased disease resistance. For example, such programs have been successfully implemented in Nile tilapia populations (Robledo *et al.,* 2024). However, a lack of genetic diversity increases the risk of inbreeding, which negatively affects the health and fitness of populations. Martinez et al. (2023) highlighted that poor management practices can exacerbate genetic bottlenecks. Therefore, effective monitoring and regulatory measures are crucial for the survival of aquatic species.

The loss of genetic diversity not only threatens individual species but also poses a significant challenge to the health of ecosystems. It affects essential ecosystem functions like biomass production and stability. Fargeot et al. (2024) demonstrated a positive relationship between genetic diversity and ecosystem functions. For instance, high genetic diversity in wild fish populations contributes to more stable biomass over time, which is essential for maintaining ecosystem services (Prunier *et al.,* 2023).

Preserving genetic diversity is critical in combating the impacts of global environmental changes. Ruegg & Turbek (2022) demonstrated that mathematical modeling can predict genetic diversity loss and guide effective conservation strategies.

Advancements in biotechnology, particularly gene editing, have opened new possibilities for preserving genetic diversity. Fedoroff et al. (2022) suggested that gene editing can create genetic barriers to prevent the mixing of farmed and wild aquatic species, thereby safeguarding genetic integrity.

While the importance of genetic diversity is undeniable, the role of species diversity must also be considered. Fargeot et al. (2024) pointed out that while genetic diversity positively influences ecosystem functions, species diversity can sometimes have a complex or even negative correlation with these functions. This complexity underscores the need for integrated conservation approaches that address both genetic and species diversity.

Preserving genetic diversity is essential not only for the survival of aquatic species but also for the health and stability of ecosystems. Research has shown that effective management, conservation strategies, and the use of biotechnology can help protect this diversity. By working together, we can maintain genetic and species diversity to build a sustainable and thriving future.

1.6 Genetic diversity study

Genetic diversity research is a fascinating and crucial field that plays an essential role in biodiversity and environmental protection. Studies on the genetic diversity of fish populations influence each other and are necessary for the adaptation and survival of various species. One of the key components in this research is polymorphic and haplotypic diversity, which is highly beneficial for adapting to environmental changes. This diversity creates a genetic reservoir necessary for natural selection, helping fish species cope with challenges such as climate change, habitat destruction, and other environmental pressures.

For instance, specific haplotypes in redband trout were associated with local environmental conditions, providing evidence that genetic differentiation can drive the development of adaptive traits (Andrews *et al.,* 2022). Similarly, in Coilia nasus, chromosomal inversions facilitated rapid adaptation, highlighting the crucial role of haplotypic diversity in ecological divergence (Zong *et*

al., 2021). In this way, genetic diversity strengthens the adaptability of fish species and enhances their ability to cope with environmental changes.

However, despite genetic diversity being crucial, some populations face challenges in adapting to rapidly changing environments due to a lack of genetic variation. As a result, they become less resilient to environmental stress. In such cases, epigenetic modifications can help maintain resilience, allowing the populations to survive under environmental pressures (Bernatchez, 2016).

The preservation of genetic diversity in fish populations has become a critical issue, especially when we observe that some species are facing population size reductions due to environmental degradation. The use of high-resolution genetic markers is essential for assessing genetic health and guiding appropriate conservation strategies. For example, minor carp (*Labeo gonius*) has shown significant genetic diversity across different river systems, highlighting the need for precise conservation measures (Roy *et al.,* 2024).

Genetic diversity can be analyzed, and breeding programs can be managed using molecular markers such as microsatellites and mitochondrial DNA. Studies on African snakehead fish populations have shown that local adaptation can lead to significant genetic differentiation, suggesting that genetically distinct populations should be managed separately to preserve local adaptations (Amoutchi *et al.,* 2023).

While the emphasis on genetic diversity in conservation efforts is crucial, it is equally important to integrate environmental and ecological considerations. Sustainable fisheries management requires the integration of genetic research with habitat conservation and ecological health. Addressing habitat destruction and overfishing at the local level is essential (Batham & Garg, 2024).

Thus, genetic diversity is not only vital for the reproduction and natural selection of fish populations but also for the stability of ecosystems and long-term sustainability. Proper management of genetic diversity and ecosystem stability is crucial for effective and sustainable fisheries management.

1.7 Applications of molecular markers in fisheries and aquaculture research

Molecular markers are important tools for studying genetic diversity and biodiversity because they can detect changes at the DNA level with high accuracy and reproducibility. The use of molecular markers in fisheries and aquaculture has brought about a revolutionary change. There was a time when researchers relied on traditional methods to study fish diversity, population structure, and breeding processes. However, the advent of molecular markers like microsatellites, mitochondrial DNA (mtDNA), and single nucleotide polymorphisms (SNPs) has made this research more modern and accurate.

Microsatellites, which are highly polymorphic, are crucial for analyzing genetic diversity and population structure. They are also effective in parentage determination, although their development is time-consuming and labor-intensive. Ramya and Behera (2023) have demonstrated in their studies that microsatellites provide precise results in breeding and conservation efforts.

On the other hand, mitochondrial DNA offers valuable insights into maternal lineage and population history. It provides information about ancient population changes, though it has limitations in detecting recent changes. Okumuş and Çiftci (2003) have shown that mtDNA can effectively depict the historical trends of fish populations.

Currently, SNPs have revolutionized research. They enable high-throughput analysis and are widely used in marker-assisted selection. Maqsood and Ahmad (2017) highlighted in their research that SNPs are cost-effective to develop but require extensive genomic data.

Through research into the advantages and limitations of molecular markers, scientists have realized that these tools greatly enhance the evaluation of genetic diversity and increase the accuracy of breeding programs. Advanced sequencing technologies have made the use of these markers more accessible. However, Ramya and Behera (2023) and Okumuş and Çiftci (2003) noted that the complexity of marker selection and misinterpretation of terminologies among researchers remain significant challenges.

SNPs play a vital role in the conservation and management of aquatic species. They provide high-resolution data on genetic diversity, which is essential for understanding population structure and dynamics. Wenne (2023) and Ramya and Behera (2023) have shown that SNPs help identify at-risk populations and assist in developing restocking plans.

The application of molecular markers is not confined to research alone; it is also effective in monitoring the genetic structure of fish affected by environmental changes. These tools are powerful instruments in ensuring a sustainable future for aquaculture and fisheries. While further research and development are needed to enhance their effectiveness, the potential and contributions of molecular markers cannot be overlooked.

1.8 Types of molecular markers

Molecular markers have brought a major change in genetic research, making it easier to study genetic variations and diversity. These tools are widely used in areas like disease diagnosis,

breeding programs, and conservation efforts. Different types of molecular markers serve different purposes, each with its own strengths and limitations.

Single Nucleotide Polymorphisms (SNPs) are the most common type of molecular marker. They are small changes in DNA that are highly useful in genome-wide association studies (GWAS). SNPs help detect mutations early and are crucial for understanding genetic traits (Hasan & Ceyhan, 2021).

Short Tandem Repeats (STRs), also known as microsatellites, are used to study genetic diversity and determine parentage. Their repetitive nature makes them effective for identifying genetic relationships (Hasan & Ceyhan, 2021).

Restriction Fragment Length Polymorphisms (RFLPs), though not as commonly used today, still have applications in specific cases like diagnostic genetics and genetic mapping. They help detect variations in DNA and are useful for studying evolutionary patterns (Panchariya *et al.,* 2024).

For genetic diversity studies, Amplified Fragment Length Polymorphisms (AFLPs) and Randomly Amplified Polymorphic DNAs (RAPDs) are important tools. AFLPs are versatile and can be used in both plant and animal studies (Panchariya *et al.,* 2024; Hasan & Ceyhan, 2021). RAPDs are simpler and cost-effective, making them a good choice for basic genetic variation studies (Panchariya *et al.,* 2024; Hasan & Ceyhan, 2021).

Another important type is Quantitative Trait Loci (QTLs), which are linked to traits like milk production in cattle or disease resistance in crops. These markers are widely used in improving agricultural and animal breeding programs (Hasan & Ceyhan, 2021).

Molecular markers also have challenges. Markers like SNPs and SSRs provide reliable results, but their use often requires advanced technologies and expertise (Guler & Imamoglu, 2023). While markers like RAPDs and AFLPs can detect a wide range of genetic differences, they may lack the accuracy needed for more detailed studies (Bidyananda *et al.,* 2024; Bunjkar *et al.,* 2024).

Advanced techniques, such as next-generation sequencing, offer great potential but are costly and require specialized knowledge (Guler & Imamoglu, 2023). Additionally, some markers may be affected by environmental factors, which can influence their reliability.

Despite these challenges, molecular markers remain a powerful tool in genetic research. They have greatly improved our ability to study genetic traits, conserve biodiversity, and enhance breeding programs. However, as Assiri et al. (2024) point out, ethical concerns about genetic information and the complexity of data analysis require continuous improvement in technology and methods.

Molecular markers represent a remarkable step forward in science, helping researchers uncover the secrets of life and offering solutions for real-world problems. As technology advances, their role will only grow, supporting sustainable development in agriculture, healthcare, and conservation.

1.8.1 Allozyme markers

Allozyme markers are valuable for assessing genetic diversity and structure in carp populations. These enzyme variations, detected via electrophoresis, help analyze genetic variability within and between populations. Studies on Ukrainian carp breeds and Prussian carp have highlighted their role in identifying allelic diversity and polymorphic loci (Mariutsa *et al.,* 2023; Mezherin *et al.,* 2020). However, their lower resolution compared to modern molecular techniques, such as microsatellites, limits their application in complex genetic analyses (Kucuktas & Liu, 2007).

1.8.2 Restriction fragment length polymorphism (RFLP)

The genome revolution began with RFLP (Restriction Fragment Length Polymorphism) markers, following the discovery of restriction enzymes (1973) and DNA hybridization (1975) (Dodgson *et al.,* 1997). These codominant markers detect both alleles by cutting DNA at specific sites, generating fragment size variations observable via Southern blotting or PCR (Liu *et al.,* 2011). Though RFLPs exhibit low polymorphism (Liu & Cordes, 2004; Liu, 2011), they have been widely applied in species differentiation, population studies, and mitochondrial DNA analysis (Klinbunga *et al.,* 2005; Apte *et al.,* 2003; Alam *et al.,* 2021). Their role in genetic research remains significant, shaping our understanding of diversity and evolution.

1.8.3 Randomly amplified polymorphic DNA (RAPD)

The Random Amplification Polymorphic DNA (RAPD) technique is essential for assessing genetic diversity, phylogenetic relationships, and population structure in carp species. It has revealed significant polymorphism, with some populations showing up to 100% polymorphism (Rana *et al.,* 2018). Studies have demonstrated its effectiveness in constructing phylogenetic trees and analyzing genetic distances, aiding conservation and breeding programs (Wali et al., 2017; Muhammad *et al.,* 2017). Despite its limitations, such as reproducibility issues, RAPD remains valuable for identifying genetically distinct populations and improving genetic stock management (Butt *et al.,* 2023; Ikpeme *et al.,* 2015).

1.8.4 Simple Sequence Repeats (SSR)

Simple Sequence Repeats (SSRs), or microsatellites, are short tandem repeats in nuclear genomes, primarily in eukaryotes (Mtileni *et al.,* 2009). These repeats, classified by nucleotide length (mono- to hexanucleotide), are analyzed using PCR with primers targeting flanking sequences (Wang *et al.,* 2009; Medlin *et al.,* 2006). Electrophoresis reveals allele variations based on repeat length (Tautz, 1989). As co-dominant markers, SSRs are crucial for population studies, genetic mapping, marker-assisted selection, and phylogenetic analysis, aiding in genetic diversity and evolutionary research (Tambarussi *et al.,* 2009).

1.9 Mitochondrial DNA

Variability in mitochondrial DNA (mtDNA) is a crucial determinant of population structure and genetic diversity in fish species. Research shows that the analysis of mtDNA sequences provides valuable insights into genetic differentiation, haplotype diversity, and historical population dynamics. This information is essential for understanding the evolutionary processes, conservation needs, and management strategies of these species (Chakraborty *et al.,* 2024)).

mtDNA markers, such as cytochrome b and ATPase 6/8, have been used to identify genetic diversity among fish populations. A study on *Ompok bimaculatus* revealed distinct haplotypes and low nucleotide diversity, indicating unique genetic characteristics within different populations. The molecular variance analysis (AMOVA) showed a high level of genetic variation within populations, emphasizing the importance of mtDNA in conservation strategies (Chakraborty *et al.,* 2024).

Single Nucleotide Polymorphisms (SNPs) are high-resolution markers that provide essential data for understanding genetic diversity and population structure. SNP markers are particularly effective in genome-wide association studies (GWAS) and can enhance selection accuracy in breeding programs. SNP arrays ensure efficient genotyping across multiple species, facilitating comparative genomics and resource sharing, which plays a crucial role in managing aquatic species and their habitats (Rasal *et al.,* 2024; Wenne, 2023).

Understanding genetic diversity through DNA markers supports conservation efforts by identifying at-risk populations and guiding breeding programs to maintain genetic variability. Molecular markers also help assess the impacts of human activities, such as overfishing and habitat destruction, on fish populations, enabling targeted management strategies (Ramya & Behera, 2023; Singh *et al.,* 2022).

Although mtDNA markers significantly enhance the understanding of genetic diversity, it is also important to consider the potential harmful effects of genetic homogenization caused by aquaculture practices, which could threaten the genetic integrity of wild populations. Therefore, further research and development are necessary in both scientific and management approaches.

1.9.1 The mitochondrial *cytb* gene

The cytochrome b (Cyt b) gene is an essential tool in fish population studies. This mitochondrial gene provides researchers with valuable insights into migration patterns and dispersal. Acting as a genetic marker, the Cyt b gene facilitates the analysis of genetic diversity, population structure, and evolutionary relationships among species (Rahman *et al.,* 2024). Studies have shown that the Cyt b gene variation in Channa striata exhibits high similarity within populations in East Java (Khan *et al.,* 2025). In contrast, the presence of multiple haplotypes in *L. rohita* indicates

significant spatio-genetic variation, reflecting adaptation to diverse environmental conditions (Sarmah *et al.,* 2022).

In Randall's threadfin bream, high haplotype diversity has been observed, with notable genetic differentiation between populations along the east and west coasts of India due to geographic isolation (Raj *et al.,* 2024). Similarly, in common carp, Cyt b gene variation reflects both historical and anthropogenic influences (Tóth *et al.,* 2022). The diversity in the Cyt b gene is shaped not only by geographic isolation but also by historical demographic events and larval dispersal patterns.

For *Tenualosa ilisha*, distinct haplotypes identified through the Cyt b gene provided critical insights into the migratory behavior of this species in the Brahmaputra River (Choudhury, 2021). Conversely, studies on yellowfin snapper revealed an absence of genetic differentiation between populations. This lack of differentiation was attributed to historical demographic events and larval dispersal, further emphasizing the Cyt b gene's importance in understanding connectivity among populations (Rahman *et al.,* 2024).

The Cyt b gene's role is not confined to understanding connectivity alone; it is particularly effective in developing conservation strategies. Rochmatika et al. (2024) demonstrated that this gene helps identify genetically distinct populations, guiding targeted management strategies. Additionally, the Cyt b gene serves as a biomarker for monitoring the genetic impact of environmental pollutants on fish populations. Its utility extends to providing a comprehensive picture of evolutionary relationships and advancing conservation approaches.

However, alongside the Cyt b gene, other genetic markers and environmental factors must also be considered. This integrated approach is vital not only for understanding genetic structures but also for enhancing sustainable management and conservation strategies. The potential of the Cyt b gene continues to play a pioneering role in environmental and genetic studies of fish populations.

1.9.2 The mitochondrial control region

The D-loop, also known as the control region, serves as a critical marker for mitochondrial DNA variation and plays a fundamental role in assessing genetic diversity within fish populations. Its high mutation rate facilitates the identification of haplotypes and the evaluation of population

structure across various geographic regions (Yang *et al.,* 2022). Positioned between the phenylalanine tRNA (tRNA phe) and proline tRNA (tRNA pro), the D-loop is the most rapidly evolving region of the mitochondrial genome. As demonstrated by Hoelzel et al. (1991), this region is crucial for mitochondrial DNA replication and transcription. Measuring approximately 1 kilobase in length, the D-loop can be readily amplified using PCR, facilitating sequencing and genetic diversity assessments (Arif & Khan, 2009).

Significant genetic variation within the D-loop region has been observed across various fish species. For instance, in the minor carp (*Labeo gonius*), 58 haplotypes have been identified, indicating high haplotype diversity (0.954) and nucleotide diversity (0.01914) (Roy *et al.,* 2024). Similarly, in the far eastern catfish (*Silurus asotus*), 21 haplotypes were identified, with pronounced genetic differentiation among populations, particularly between the Yellow River and the Songhua River (Yang *et al.,* 2022). These findings underscore the importance of the D-loop in genetic monitoring, contributing to effective conservation and management strategies. For example, the unique haplotypes identified in *L. gonius* provide critical insights for stock differentiation and management planning (Roy *et al.,* 2024).

The D-loop has proven to be more effective than other markers, such as ATPase 6/8, in analyzing haplotype diversity and population structure. In *Labeo gonius*, the D-loop revealed 58 haplotypes with a haplotype diversity of 0.954, whereas ATPase 6/8 identified only 17 haplotypes with a diversity of 0.560 (Roy *et al.,* 2024). Additionally, in *Macrobrachium nipponense*, D-loop analysis revealed distinct population structures across various geographic locations, highlighting significant genetic differentiation (Zhang *et al.,* 2022).

However, relying solely on the D-loop has limitations. Environmental factors and anthropogenic activities can significantly influence genetic structures, potentially overshadowing the insights gained from D-loop analysis. Thus, a comprehensive approach that incorporates other genetic markers is essential for a more holistic understanding of population dynamics.

The information derived from the D-loop has substantial implications for conservation management. For instance, a fully sequenced mitochondrial genome can identify polymorphic sites, population-specific markers, haplogroups, and haplotypes, making the D-loop an invaluable

tool for phylogenetic studies due to its rich variability (Hofmann *et al.,* 1997). Monitoring genetic diversity through the D-loop can guide management practices, such as habitat restoration and species reintroduction, ensuring the preservation of genetic resources (Wakimura *et al.,* 2023).

This discussion highlights the potential and limitations of the D-loop in conservation genetics. While the D-loop is a powerful tool for understanding genetic diversity and informing conservation strategies, integrating additional genetic markers can enhance the precision and efficacy of these efforts.

1.10 Nuclear DNA

The nuclear DNA tool, particularly single nucleotide polymorphisms (SNPs), represents a remarkable advancement in modern genetic research for assessing genetic diversity in fish populations (Laconcha *et al.,* 2015). It provides precise insights into intraspecific variation and population structure, making it indispensable for conservation and management efforts. Tesfaye et al. (2021) and Mariani & Bekkevold (2014) highlighted the importance of high-resolution genetic markers in accurately evaluating genetic diversity, which plays a critical role in these fields.

With technological advancements, the transition from microsatellite analysis to SNP-based approaches has revolutionized our understanding of genetic diversity, offering a more comprehensive framework for monitoring fish populations (Laconcha *et al.,* 2015). The high-resolution capabilities of SNPs make them particularly effective in identifying natural populations, assessing the impact of domesticated species, and analyzing the effects of environmental changes. As Wenne (2023) demonstrated, SNP markers are highly efficient in detecting genetic diversity hotspots.

SNPs play a crucial role in understanding population structure and genetic connectivity. Research by Singh et al. (2022) and Wenne (2023) emphasized that SNP analysis is instrumental in identifying breeding populations and developing effective conservation strategies. For example, targeted plans to protect genetic diversity can be devised through the strategic use of specific breeding stocks.

Nuclear DNA tools, particularly SNPs, have brought transformative changes to the assessment of genetic diversity and conservation management in fish populations. While their utility in research and management is diverse, combining genetic insights with ecological considerations can further enhance their effectiveness. Studies such as those by Wenne (2023) shed light on both the potential and limitations of these tools, providing a roadmap for future research and applications.

However, reliance solely on genetic data may present certain limitations. Environmental factors and anthropogenic influences, which significantly impact fish populations, might be overlooked if ecological contexts are not integrated into management strategies. This underscores the need for a holistic approach that incorporates both genetic and environmental data.

1.10.1 Nuclear myostatin1 (*mstn)* gene

Myostatin (*mstn*) gene begins with scientists facing the challenge of understanding fish population dynamics to ensure their sustainability and conservation. This gene, pivotal in regulating growth, reproduction, and environmental adaptation, captured their attention due to its significant role in these processes (Song *et al.,* 2016). High levels of genetic diversity detected in the *Mstn* gene serve as indicators of population health and viability, making it an essential tool for conservation efforts (Song *et al.,* 2016). For instance, research on large yellow croaker highlighted that *Mstn* polymorphisms significantly influenced body length and weight, underlining the gene's relevance in conservation genetics (Qiao *et al.,* 2008). Such insights led scientists to use *Mstn* variations as markers in selective breeding programs, aiming to preserve genetic diversity while enhancing desirable traits.

The application of molecular markers like the *Mstn* gene extends beyond individual growth traits. These markers allow scientists to evaluate the effects of environmental changes and human activities on fish populations (Ramya & Behera, 2023). Single nucleotide polymorphisms (SNPs) within the *Mstn* gene have proven particularly valuable in understanding population differentiation. They reveal intraspecific variation, helping identify genetic diversity hotspots crucial for population structure analysis and adaptive evolution (Godoy, 2009; "Perspective Chapter: Molecular Approach for the Study of Genetic Diversity and Conservation Prioritization of Fish Population," 2022).

Despite the promise of the *Mstn* gene, scientists recognized the limitations of relying solely on a single genetic marker for comprehensive conservation assessments. Integrating *Mstn* with other molecular tools provides a robust understanding of genetic diversity and population dynamics (Song *et al.,* 2016). Such an approach ensures that ecological and genetic factors influencing fish populations are considered, leading to more effective management strategies (Song *et al.,* 2016).

The significance of *Mstn* research extends to aquaculture, where it helps mitigate risks like genetic homogenization caused by over-reliance on specific breeding stocks. Understanding *Mstn* polymorphisms enables scientists to balance conservation goals with sustainable fishery practices (Wenne, 2023). Furthermore, the gene's adaptability to environmental changes makes it a valuable resource for addressing challenges like climate change, and enhancing the resilience of fish populations (Hoey & Pinsky, 2018).

Ultimately, the *Mstn* gene's genetic insights empower scientists to inform breeding programs, develop targeted conservation strategies, and promote sustainable aquaculture. The collaborative use of *Mstn* with other molecular markers exemplifies a holistic approach to conservation genetics, bridging scientific discovery with the preservation of aquatic biodiversity for future generations (Song *et al.,* 2016).

1.11 Application of genetic diversity studies

Genetic diversity studies play an important role in conservation, selective breeding, and sustainable fisheries management. By assessing population structure and genetic variation, researchers can identify genetically resilient stocks, prevent inbreeding, and enhance breeding

programs for aquaculture. Additionally, these studies help in formulating conservation strategies to protect endangered populations and maintain biodiversity.

1.11.1 Enhancement of production

The enhancement of production in aquaculture, particularly in carp fish farming, has been significantly impacted by the application of genetic diversity data. By incorporating genetic information, breeding programs can achieve more precise selection and management of genetic traits, leading to the development of superior strains of carp that are better adapted to specific environments and production needs. This integration of genetic diversity has transformed breeding practices, enabling more efficient and sustainable aquaculture.

Genetic diversity analysis, including mitochondrial DNA sequencing and morphometric studies, plays a critical role in identifying distinct populations and their unique traits, which is essential for effective selective breeding programs. For instance, the work by Lalramnunsanga *et al.* (2024) emphasized how these methods help in distinguishing populations, providing valuable insights into their genetic characteristics. This knowledge allows for the selection of breeding stock that can enhance desirable traits such as growth rate and disease resistance. By understanding genetic variation within and among populations, breeding programs can make more informed decisions that lead to the development of healthier and more productive fish.

The use of advanced genomic tools has further revolutionized genetic selection. The application of Single-Nucleotide Polymorphism (SNP) arrays, as highlighted by Rasal et al. (2024), has significantly improved the accuracy of breeding values by offering high-throughput genotyping. These genomic tools help identify specific genetic markers associated with traits of economic importance, such as growth rate and disease resistance, enabling targeted breeding strategies. Furthermore, advances in genome editing technologies are expected to further enhance the efficiency of breeding programs, allowing for rapid improvements in aquaculture species, as noted by Houston et al. (2020). These developments hold the potential to increase productivity and sustainability in carp farming.

Genetic diversity data also plays a crucial role in enhancing disease resistance and growth rates in carp fish. The identification of beneficial alleles is vital for developing resilient populations. For example, disease-resistant grass carp (DR-GGC) exhibited higher genomic heterozygosity and

enriched immune pathways compared to common grass carp, a phenomenon that Tan et al. (2024) highlighted in their study. This increased genetic diversity is foundational for breeding programs aimed at improving disease resistance, as it enables the identification of specific alleles linked to immune response and resilience. Moreover, genomic approaches such as genome-wide association studies have facilitated the mapping of quantitative trait loci associated with disease resistance, offering a framework for selective breeding (Huang *et al.,* 2024).

Genetic diversity is equally essential for improving growth rates in carp. Studies on black carp populations, as discussed by Tang et al. (2024), revealed high genetic diversity, which is critical for selecting individuals with superior growth traits. The integration of genetic data into breeding programs can enhance growth rates by selecting for alleles that promote faster growth and better feed conversion efficiency. Lalramnunsanga et al. (2024) further emphasized how understanding genetic diversity aids in the conservation of carp species, ensuring that breeding programs maintain genetic variability, which is vital for long-term sustainability.

The genetic insights gained from diverse populations also inform management strategies that optimize breeding practices and enhance overall aquaculture productivity. By understanding the genetic makeup of different populations, breeders can make informed decisions that not only improve the efficiency of aquaculture operations but also contribute to the conservation of species, ensuring the resilience and adaptability of carp populations in the face of environmental changes (Sumana *et al.,* 2024).

While the focus on genetic diversity in breeding programs holds great promise, it is crucial to be mindful of the potential risks associated with reduced genetic diversity due to selective breeding practices. Such practices could lead to vulnerabilities in disease resistance and adaptability, particularly in the face of changing environmental conditions. Therefore, careful management of genetic diversity is essential to maintain the long-term health and sustainability of carp populations.

1.11.2 Conservation Implications for Wild Populations

The beauty of nature and the vibrancy of rivers, lakes, and oceans are inseparably tied to the role of fish. However, in modern times, habitat loss, fragmentation, and climate change are having devastating impacts on the genetic diversity of fish populations.

Habitat loss and fragmentation divide fish populations into small, isolated groups. This reduces gene flow and creates a pattern of distance-based diversity, known as isolation-by-distance. Fragmentation accelerates genetic drift, especially in smaller populations (Pinto *et al.,* 2023; "The evolution of the genetic load during habitat loss and population fragmentation", 2022).

In smaller groups, the level of inbreeding increases, leading to a loss of genetic diversity and the heightened expression of harmful mutations. These effects not only diminish genetic diversity but also threaten the long-term survival of species (Blanton *et al.,* 2019).

Climate change profoundly affects the habitats, distributions, and reproductive biology of fish species. Changes in temperature, precipitation, and ocean currents are altering the location and characteristics of fish habitats. For instance, in the Mekong River, it is predicted that approximately 84% of fish species will shift northward due to climate change (Nuon *et al.,* 2024). This is causing disruptions in local ecosystems, posing significant challenges for the fishing industry. Climate change also affects the timing and success rates of fish reproduction. For example, shifts in temperature can cause certain species to spawn earlier or later than usual, jeopardizing population stability (Bhagarathi *et al.,* 2024).

The abundance of economically significant fish species is likely to decline, while some secondary species may increase, creating new challenges for fisheries management (Liu *et al.,* 2023). While habitat loss and climate change pose significant challenges, they also offer opportunities for the development of adaptive management and conservation strategies. Through proper planning and conscious efforts, it is possible to protect fish populations and their ecosystems. Scientific research and sustainable management must work hand in hand to preserve these invaluable resources of nature, as fish play an unparalleled role in maintaining the balance of ecosystems.

1.12 Research gap and justification for the study

L. rohita, a vital aquaculture species in Bangladesh, has been widely studied using various genetic markers, yet significant gaps remain in understanding its genetic diversity and population structure. Previous studies primarily relied on RAPD, allozyme, and microsatellite markers to

assess genetic variation in *L. rohita* populations from major rivers like the Halda, Jamuna, and Padma. However, these markers have limitations in providing comprehensive insights into gene flow, and population-specific genetic structuring. The mitochondrial *cytb* and control region genes, along with the nuclear myostatin *(*mstn) gene, offer a more precise approach to evaluating genetic diversity and differentiation, but their application in *L. rohita* studies has been limited.

One major gap lies in the lack of extensive mitochondrial and nuclear DNA-based studies on wild and cultured populations of *L. rohita*. Although previous research has identified some genetic variation, the observed diversity has often been low, raising concerns about potential bottlenecks, habitat fragmentation, and anthropogenic influences on natural populations. The present study aims to address this by analyzing polymorphic sites, haplotype distribution, genetic distances, and nucleotide diversity across different riverine populations, which will help determine the underlying genetic structure and connectivity among populations.

Furthermore, while some studies have reported genetic differentiation among riverine populations, the extent of gene flow and the impact of artificial breeding on genetic integrity remain unclear. The inclusion of both mitochondrial and nuclear markers allows a more robust assessment of genetic variability, as mitochondrial markers reveal maternal inheritance patterns, while nuclear markers, such as the *mstn* gene, provide insights into biparental inheritance and adaptive genetic traits. By integrating these markers, this study will help clarify whether genetic differentiation is due to natural barriers, artificial selection in aquaculture, or a combination of both.

Additionally, comparisons of *L. rohita* populations in Bangladesh with those in neighboring regions, such as India, have shown lower genetic diversity in Bangladesh. The causes of this reduced diversity remain speculative, with factors such as geographic constraints, selective breeding practices, and habitat degradation being potential contributors. This study seeks to explore these aspects by employing advanced molecular techniques, including phylogenetic analysis, haplotype network construction, and molecular variance analysis, to provide a clearer picture of genetic structuring and evolutionary relationships.

This research is crucial in filling the existing knowledge gaps regarding the genetic diversity of *L. rohita* populations in Bangladesh. By employing mitochondrial (*cytb* and control region) and nuclear (*mstn*) DNA markers, this study will provide a more comprehensive understanding of population-specific variations, genetic connectivity, and potential conservation strategies. The

findings will not only contribute to genetic resource management but also aid in the sustainable breeding and conservation of this economically significant species.

1.13 Aims and objective

The primary objective of this study is to assess the genetic variation and population structure of *L. rohita* across different river systems and rearing conditions using molecular markers. Understanding the genetic diversity at both mitochondrial and nuclear levels is crucial for sustainable management, conservation, and selective breeding programs in aquaculture. This study aims to generate comprehensive genetic data that can support evidence-based strategies for maintaining genetic health and enhancing the productivity of *L. rohita* populations.

Specific Objectives

i. Investigating the genetic diversity within and between populations of *L. rohita* using mitochondrial (*cytb, D-loop*) and nuclear (*mstn*) DNA markers.
ii. Examine the population-specific haplotype distribution, nucleotide composition, genetic distances, and potential genetic clusters in *L. rohita*.
iii. Analysis of molecular data to provide insights into the genetic makeup of wild and cultured populations, that may help in the formulation of effective conservation and management strategies for this aquaculture species.

2 Materials and methods

This study employed a comprehensive approach to analyze the genetic diversity of Rohu fish (*L. rohita*) populations in Bangladesh. The workflow included sample collection from diverse sources, DNA extraction using two methods, PCR amplification of target genes, and downstream sequence analysis to evaluate genetic diversity and population structure. The methodology ensures robust data collection and analysis for meaningful insights.

2.1 Selection of studied fish and sampling sites

The studied fish, *L. rohita,* was selected because of its economic importance, widespread cultural and ecological significance, and the pressing need to preserve its genetic diversity. This species is one of the major carps contributing significantly to Bangladesh's fisheries sector, fulfilling about

60% of the country's protein demand (DOF, 2017). However, challenges like inbreeding depression, habitat degradation, and unplanned crossing in hatcheries have led to a decline in genetic diversity, growth performance, and disease resistance. The study aims to identify and conserve valuable genetic traits to sustain fish production and ensure food security.

The sampling sites (Fig. 2.1), such as rivers e.g., Padma, Jamuna and Halda and hatcheries, were chosen to represent both natural and managed populations. We selected the Padma, Jamuna, and Halda rivers for their importance as major natural habitats and breeding grounds of *L. rohita*, representing diverse ecological regions. The Halda is especially notable as the only natural carp spawning river in Bangladesh. A culture stock was included to compare wild and hatchery populations, providing insights into genetic variation and management impacts. These sites provide insights into the genetic structure of wild populations and hatchery stocks, essential for understanding and improving brood selection practices, enhancing production, and conserving genetic resources in the long term.

2.2 Sample Collection:

The study aimed to observe the genetic diversity of Rohu fish (*L. rohita*) species in Bangladesh, focusing on samples collected from both wild and cultured sources. A total of 250 (200 from wild and 50 from culture) fish samples were collected initially from Halda, Padma, and Jamuna rivers for wild fish, while culture fish were sourced from hatcheries across five distinct areas: Jessore, Satkhira, Gaibandha, Rangpur, and Mymensingh. These locations were chosen to ensure representation from various regions of Bangladesh, given that this species predominantly inhabits the Padma-Jamuna and Halda river systems. Sampling location and collection dates have been tabulated in **Table 2.1**. The locations of the sampling sites also shown in **Figure 2.1**

An up-to-date summary of the sample collection is as follows

1. Wild sample collection-

- from Halda source (twice from two nurseries of Chittagong)

- from Padma source (twice from two different nurseries of Rajshahi)
- from Jamuna source (one nursery of Rajshahi)

2. Cultured samples –

- Seven different culture ponds of different regions (Jessore, Satkhira, Gaibandha, Rongpur, and Mymensing).

Table 2.1 Origin of Rohu (*L. rohita*) populations

Population/rivers	Sampling site	Latitude	Longitude	Sampling year
Padma	Nouhata, Rajshahi	24.376	88.603	2021
Jamuna	Vuapur, Tangail	24.249	89.921	2021
Halda	Chittagong	22.356	91.783	2021
Culture	Jessore	23.166	89.220	2022
	Satkhira	22.709	89.084	2022
	Gaibandha	25.324	89.544	2021
	Rangpur	25.740	89.261	2021
	Mymensing	24.743	90.398	2023

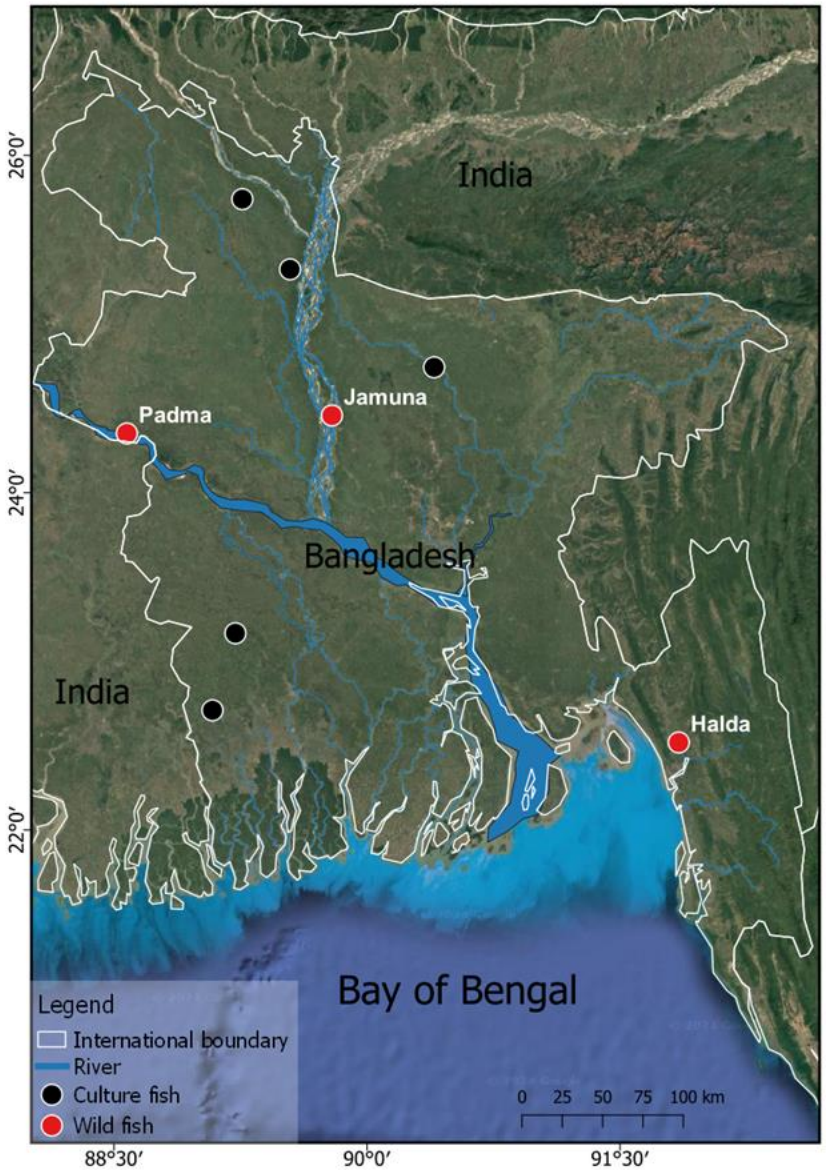

Fig2.1: A map of Bangladesh illustrating the sampling locations of *L. rohita*

2.3 Sample transportation and storage:

From 2021 to 2023, Rohu fish samples were collected from wild and cultured sources. Due to the prohibition on direct fishing in the Halda and Jamuna rivers, and scarcity in Padma, wild fish samples were collected from regional fish farmers who collected fish fries or eggs from respective rivers and raised those in their ponds separately. Thus, 250 river-origin dead, fresh fish samples were collected from the fish farmers located near Halda, Padma, and Jamuna rivers. Fifty fresh samples were commercially sourced from the local markets of Jessore, Satkhira, Gaibandha, Rangpur, and Mymensingh and verbally confirmed to be from cultured sources. Collected samples were primarily preserved in ice boxes to prevent deterioration during transport from the field to the laboratory. The samples were stored at -20°C. some representative pictures of the sample collection are shown in **Figure 2.2.**



Fig2.2: Representative images of sample collection

2.4 DNA Extraction

(Genomic DNA was extracted from all 250 *L. rohita* samples using a modified CTAB (cetyltrimethylammonium bromide) protocol and the Monarch® Genomic DNA Purification Kit (New England Biolabs, NEB), following established procedures (Alam et al., 2016; Begum et al., 2019). Based on DNA quality and concentration, 50 high-quality samples from each of the four populations, totaling 200 samples, were selected for sequencing. The remaining 50 samples were excluded due to suboptimal DNA yield or purity, ensuring that only reliable and consistent data were used for downstream genetic analyses. From the sequenced data, only high-quality sequences from each gene were retained for final analysis.)

2.4.1 CTAB Method

To extract high-quality genomic DNA suitable for large-scale genome sequencing, we employed the CTAB (cetyltrimethylammonium bromide) method. This widely used protocol is effective in isolating pure DNA, particularly from tissues with high levels of polysaccharides and proteins. The extraction process utilized a 2% CTAB buffer containing 100 mM Tris-HCl (pH 8.0), 20 mM EDTA, 1.4 M NaCl, and 0.2% β-mercaptoethanol (added fresh before use). Additional reagents included chloroform-isoamyl alcohol, 10 mM Tris-HCl, 10 mg/mL RNase, 70% ethanol, and 5 M NaCl. The required equipment comprised a centrifuge, micropipettes, Eppendorf tubes, a water bath, and agarose powder for subsequent gel electrophoresis.

Protocol of DNA extraction using CTAB buffer

Tissue samples (25–50 mg) were excised from the lateral line muscle of each fish specimen using sterile scalpel blades and forceps. The samples were finely minced to facilitate rapid enzymatic digestion and enhance DNA yield. The prepared tissue fragments were transferred into 1.5 mL Eppendorf tubes. Initially, 50 µL of CTAB buffer was added to each sample, followed by thorough maceration. An additional 400–600 µL of CTAB buffer was subsequently introduced, and the homogenization process was repeated. Proteinase K (10 µL, 10 mg/mL) was then added to each sample and gently mixed by inversion. To remove RNA contamination, 10 µL of RNase (10 mg/mL) was added, and the samples were briefly vortexed. The mixture was incubated at 37°C for 1–3 hours, with intermittent vortexing every 5–15 minutes to enhance tissue digestion.

Following incubation, the samples were centrifuged at 13,000 rpm for 5 minutes, and the supernatant was carefully collected. An equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) was added to the supernatant, followed by brief vortexing (5–10 seconds). The mixture was centrifuged again at 13,000 rpm for 5 minutes, and the resulting supernatant was carefully transferred to a new tube. To purify the DNA, 500 µL of 70% ethanol (at room temperature) was added, and the samples were centrifuged at 13,000 rpm for 1 minute. The supernatant was discarded, and any residual ethanol was removed before allowing the DNA pellet to air-dry for 10 minutes. The dried DNA pellet was resuspended in 40–80 µL of TE buffer and incubated at 37°C for 10 minutes to facilitate rehydration. Finally, the extracted DNA was stored at -20°C for future use.

2.4.2 Genomic DNA extraction

Genomic DNA was extracted from Rohu samples using the Monarch® Genomic DNA Purification Kit (New England Biolabs, NEB). The methodology for DNA extraction using this kit is outlined below.Tissue samples (25–50 mg) were collected from the lateral line muscle of each fish specimen using sterile scalpels and forceps. The excised tissue was finely minced to facilitate efficient lysis and optimize DNA yield. The prepared tissue was then transferred into 1.5 mL Eppendorf tubes. Each tube received 10 µL of Proteinase K and 200 µL of Tissue Lysis Buffer, followed by gentle vortexing. To promote tissue lysis, the samples were incubated at 56°C for 1–3 hours, with periodic vortexing every 5–15 minutes. Next, 3 µL of RNase A was added to each sample, followed by gentle mixing and incubation at 56°C for at least 5 minutes. A total of 400 µL of gDNA Binding Buffer was then added to the samples, which were mixed thoroughly by pulse vortexing for 5–10 seconds. The resulting mixture (~600 µL) was transferred into gDNA Purification Columns pre-inserted into collection tubes, ensuring that the upper portion of the column was not disturbed. The columns were first centrifuged at 1,000 × g for 3 minutes, followed by a second centrifugation at maximum speed (>12,000 × g) for 1 minute to clear the membrane. The flow-through and collection tubes were discarded.

The gDNA Purification Columns were then placed into fresh collection tubes, and 500 µL of gDNA Wash Buffer was added to each column. The columns were inverted several times to ensure even distribution of the buffer before being centrifuged at maximum speed (12,000 × g) for 1 minute, after which the flow-through was discarded. An additional 500 µL of gDNA Wash Buffer was added, and the columns were centrifuged again at maximum speed (>12,000 × g) for 1 minute. The collection tubes and flow-through were discarded once more.

The gDNA Purification Columns were subsequently transferred to DNase-free 1.5 mL Eppendorf tubes. Preheated (60°C) gDNA Elution Buffer (35–100 µL) was added to each column, and the samples were incubated at room temperature for 1 minute. The columns were then centrifuged at maximum speed (>12,000 × g) for 1 minute to elute the genomic DNA. The extracted DNA was stored at -20°C for further use.

2.5 Agarose gel electrophoresis for mtDNA quantification and quality analysis

DNA presence was determined using gel electrophoresis with an electric current. Since DNA has a negative charge, it moves toward the positive side (anode). A high concentration of agarose gel slows down the movement of molecules, while a lower concentration helps separate larger molecules. Genomic DNA was run on a 0.8% agarose gel, and the PCR-amplified DNA fragments were run on a 1% agarose gel. A 5µl fluorescent dye was added to allow the DNA fragments to be detected and seen in the gel. Below is the procedure for gel electrophoresis.

Agarose powder was dissolved in electrophoresis buffer (1xTAE) to achieve the required concentration and then heated in a microwave until fully melted. Midori dye (10 mg/mL) was incorporated into the gel to facilitate DNA visualization. After cooling the solution to approximately 60°C, it was poured into a casting tray with a sample comb and allowed to solidify at room temperature (25°C - 32°C). Once the gel had solidified, the comb was carefully removed without disrupting the wells.

The gel, still within its plastic tray, was placed horizontally into the electrophoresis chamber and covered with buffer. DNA samples, mixed with loading buffer, were pipetted into the wells, and the apparatus was sealed by attaching the lid and power leads. An electrical current was applied, and the flow of the current was confirmed by observing the formation of bubbles at the electrodes. DNA migration was monitored visually during the process.

Once the DNA had migrated sufficiently, the fragments were visualized using Gel Loading Buffer stain. To further visualize the DNA, the gel was exposed to ultraviolet light on a transilluminator, and a photograph was captured for documentation.

2.6 Primer designing

Proper primer design is important for the success of applications such as polymerase chain reaction (PCR) and DNA sequencing, as it ensures specificity, efficiency, and accuracy in amplifying or sequencing the target DNA. Relevant sequences were retrieved from the available online sources.

Then primers were designed by using bioinformatics tools for the amplification of selected genes from the selected species.

The features of an ideal primer are as follows:

- The melting temperature (Tm) of primers should ideally be between 55°C and 65°C to ensure optimal binding and specificity during amplification.
- The ability to dimerize is not present.
- There are no substantial hairpins (typically >3 bp).
- The template lacks secondary priming locations.
- To avoid mis-priming, the 3' end has a low specific binding.

A 20-24 nucleotide primer is usually sufficient. Primer Blast (and other programs) will automatically select good primers. However, many of these applications' "standard" parameters do not work with a particular sequence. Primers must be chosen manually in this scenario. The following suggestions should assist in this process:

2.6.1 List of primers

Designing primers by using bioinformatics tools for the amplification of selected genes from the selected species. The names of the selected primer, sequence, and G-C content have been presented in Table 2.2. This primer is designed by us manually.

Table 2.2: List of primers designed and being utilized in this study

Primer name	(Number of base pairs) of each primer sequence	Expected Product length	GC%	sequence	Annealing temperature
CR F	18	745	55.5	TTTAACTCCCACCCCTGG	61°C
CR R	18	745	55.5	CGCGCAAAAACCAAAGGG	61°C
cytb F	20	828	45.00	AACCGAGACCAATGACTTGA	61°C
cytb R	20	828	50.00	GGGTGAAGTTTTCTGGGTCT	61°C
mstn F	20	870	50	GTCAGTGTAGTCCCGAATGT	61°C
mstn R	20	870	50	AGGGAATCTTGCCGTAGATG	61°C

2.7 Basic components of PCR amplification

The following materials are required for the polymerase chain reaction (PCR):

- The template DNA refers to the DNA sample that contains the target sequence intended for amplification
- Primers are short, single-stranded DNA sequences that determine the initiation and termination sites for amplification.
- Taq DNA Polymerase (Master mix) – A heat-stable enzyme that synthesizes new DNA strands.
- The PCR buffer is a solution that preserves the optimal pH and ionic environment necessary for the reaction's efficiency.
- Sterile Nuclease-Free Water – Used to prepare the reaction mixture.
- Thermocycler – A machine that controls the temperature cycles necessary for DNA denaturation, annealing, and extension.

2.7.1 PCR reaction setting

Each 50 µl PCR reaction mixer contains of the following table,

Component	Volume
Taq Master mix	25 µl
Primer (forward) 10 µM	1.4 µl
primer (reverse) 10 µM	1.4 µl
DNA template	1-5µl
Nuclease free water	Up to 50µl

2.7.2 Thermal cycling parameters for PCR

The thermal cycling protocol for gene amplification via Polymerase Chain Reaction (PCR) was set for 35 cycles as follows:

Gene Region	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension
Cytb	95°C, 5 min	95°C, 45 s	60°C, 60 s	72°C, 45 s	72°C, 5 min
Control region	95°C, 5 min	95°C, 45 s	58°C, 60 s	72°C, 60 s	72°C, 5 min
Mstn	95°C, 5 min	95°C, 45 s	56°C, 60 s	72°C, 60 s	72°C, 5 min

2.8 Gel electrophoresis procedure

Electrophoresis was conducted using a horizontal slab gel apparatus. The gel was cast into a gel case with a comb to create sample wells and was allowed to solidify. Subsequently, 3–5 µl of a 1 kb DNA marker and 3–5 µl of the template DNA (sample) were mixed with 1–2 µl of loading dye and carefully loaded into the wells. The electrophoresis process was carried out at 110 mA and 120 V for 30 minutes to assess the quantity and fragment size of digested mtDNA. Following electrophoresis, the amplified or digested mtDNA bands were visualized using an ultraviolet transilluminator.

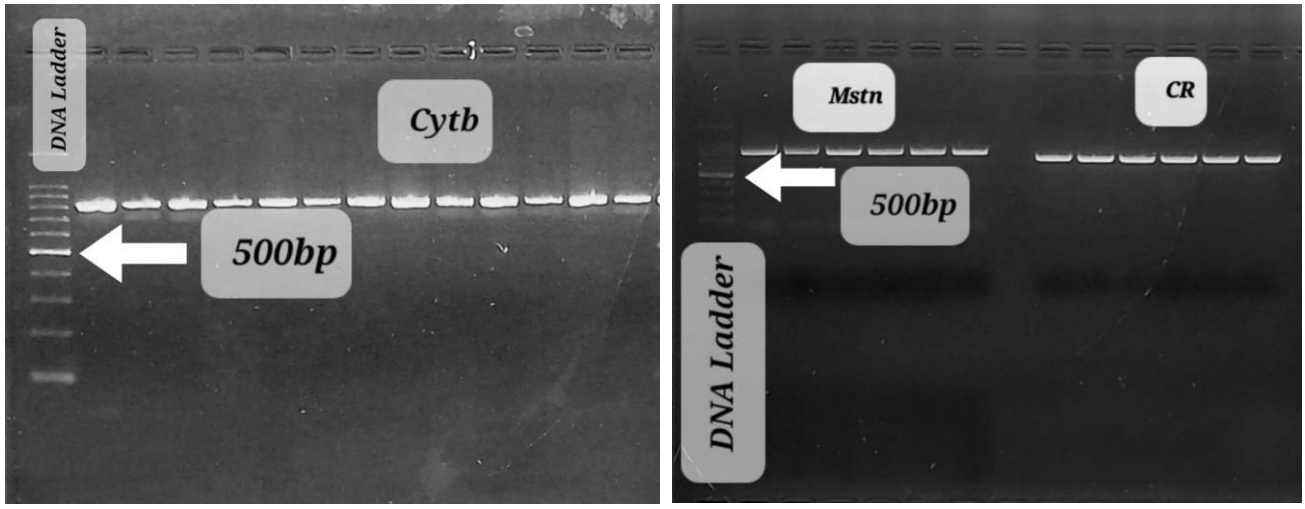

Fig 2.3: Gel image of PCR product from the respected gene of *L. rohita* samples.

2.9 PCR Purification Procedure

The PCR-amplified products were purified using the AxyPrep PCR Clean-up Kit, following the protocol outlined below.

Buffer Addition and Sample Preparation

A 3× reaction volume of Buffer PCR-A was added to each sample to facilitate purification. If the calculated volume was less than 100 µL, a minimum of 100 µL of Buffer PCR-A was used. The mixture was briefly vortexed to ensure homogeneity. Specifically, for a 20 µL PCR reaction (with or without an oil overlay), 100 µL of Buffer PCR-A was added, while for a 40 µL PCR reaction, 120 µL of Buffer PCR-A was used.

Sample Transfer and Centrifugation

A PCR purification column was placed into a 2 mL Microfuge tube, and the prepared reaction mixture was carefully transferred into the column. The column was then centrifuged at 12,000 × g for 1 minute. The filtrate collected in the Microfuge tube was discarded, and the PCR column was repositioned in the same tube for further processing.

Washing Steps

First Wash:

A volume of 700 µL of Buffer W2 was added to the PCR column, followed by centrifugation at 12,000 × g for 1 minute. The resulting filtrate was discarded, and the column was placed back into the same 2 mL Microfuge tube.

Second Wash:

A second washing step was performed by adding 400 µL of Buffer W2 to the column, followed by centrifugation at 12,000 × g for 1 minute.

DNA Elution

The PCR column was transferred to a clean 1.5 mL Microfuge tube. To elute the purified DNA, 25–30 µL of pre-warmed (65°C) Eluent was applied directly to the center of the membrane. The column was then incubated at room temperature for 1 minute before being centrifuged at 12,000 × g for 1 minute to collect the eluted DNA.

2.10 Sequence data analysis

The quality of the raw sequence chromatograms was evaluated, and the forward and reverse reads were aligned. High-quality base calls were extracted and saved as separate FASTA files using Finch TV software (version 1.4.0) (http://www.geospiza.com/Products/finchtv.shtml).The amplified fragment's origin was confirmed using NCBI's BLASTn search program. The ClustalW algorithm (Thompson *et al.,* 1994) within MEGA v.11 (Tamura *et al.,* 2021) and BioEdit 7.1.3.0 (Hall, 1999) software programs were used to accomplish multiple sequence alignment. The sequencing data were examined to ascertain the sequence composition, count the number of polymorphic sites, identify parsimony informative sites, and apply the maximum-likelihood (ML) and neighbor-joining (NJ) topology approaches with 1000 bootstrap replicates (Felsenstein 1985; Tamura *et al.,* 2021). The sequences were subsequently allocated to haplotypes.

The dataset was analyzed to construct haplotype networks using NETWORK 4.6.1.2 (Bandelt *et al.,* 1999). Haplotype and nucleotide diversities were calculated using DnaSP v. 5.1 (Librado and Rozas, 2009). Principle component analysis was conducted on haplotypes. Adegent R package was used to convert fasta files to genlight format, followed by eigenvalue estimation using snp variations (Jombart, 2008; Jombart and Ahmed, 2011).
Genetic variation within and among populations, genetic differentiation, and pairwise Fst values were assessed through molecular variance analysis (AMOVA) using Arlequin version 3.5 (Excoffier and Lischer, 2010). To examine the relationship between haplotypes and their geographical distribution, a minimum-spanning tree was generated using PopArt version 0.6 (Teacher and Griffiths, 2010).

3. Result

3.1 Molecular characterization of *L. rohita*

3.1.1 DNA isolation from *L. rohita*

In this study, we successfully extracted high-quality DNA from 200 samples of *L. rohita*, commonly known as Rohu, to investigate their genetic diversity. The extraction process yielded consistent and pure DNA, as confirmed by and agarose gel electrophoresis (**Fig. 3.1**). Agarose gel electrophoresis confirmed the integrity of the DNA, showing clear, high-molecular-weight bands without significant degradation. The DNA samples are now prepared for downstream applications, such as PCR amplification and genetic marker analysis, to evaluate genetic variability within and among populations. This comprehensive dataset will offer valuable insights into the population genetic structure of *Labeo rohita*, aiding in the formulation of effective conservation and management strategies for this economically significant aquaculture species

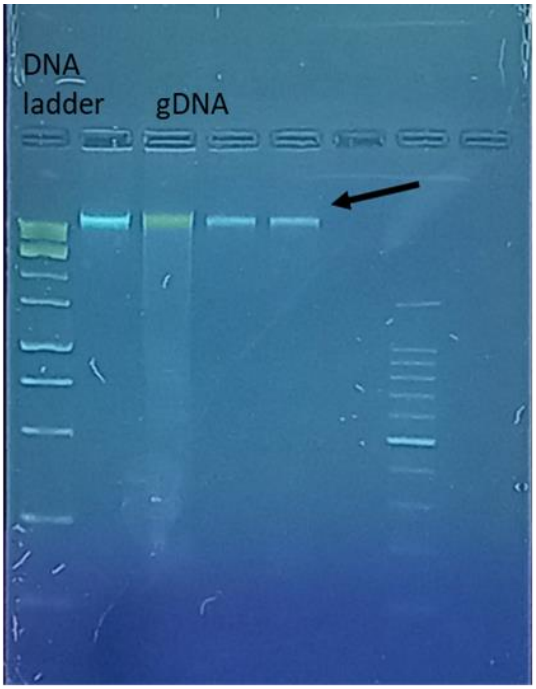

Figure 3.1. Gel electrophoresis of DNA extracted from fish tissue isolated From *Labeo rohita*

3.1.2 Primer selection and PCR amplification

To assess genetic diversity, three primers were selected: two targeting mitochondrial genes—*cytochrome b* (*cytb*) and the control region, and one targeting a nuclear gene, *myostatin 1* (*mstn*). The mitochondrial cytb gene and control region were selected for analysis due to their elevated mutation rates, which make them well-suited for examining genetic diversity at the population level (Song *et al.,* 2014; Zhao *et al.,* 2020; Han *et al.,* 2008; Liu *et al.,* 2020). Furthermore, nuclear DNA markers have been extensively utilized to detect high levels of polymorphism and rare alleles, making them valuable tools for assessing genetic variation in both wild and cultured fish populations (Norris *et al.,* 1999; DeWoody and Avise, 2000; Was and Wenne, 2002; Das *et al.,* 2005).

PCR amplification of the *cytb* gene, control region and *mstn* gene was successfully performed across all 200 DNA samples. The *cytb* gene amplification resulted in a 616bp fragment, while the control region yielded a fragment of approximately 598bp and *Mstn* has 569bp. Agarose gel electrophoresis confirmed the successful amplification of these target regions, with clear and distinct bands observed for each sample.

3.2 Analysis of *Cytb* gene

The representative sequences obtained from our study are given below, Fasta format of *cytb* gene obtained from *L. rohita* population.

>*cytb* sequence A08 size - 799

TTTAACCTAACAAGAACCAAATAATGGCAAGCCTACGAAAAACACACCCCCTCATTA
AAATCGCTAACGACGCACTAGTCGACCTACCAGCACCATCCAACATCTCTACATGATG
AAACTTTGGATCCCTCCTAGGATTATGTTTGATTACCCAAATCCTAACTGGACTATTTC
TAGCCATACATTACACCTCAGACATCTCAACCGCATTCTCATCAGTAACCCACATCTGC
CGTGACGTAAACTACGGCTGACTAATCCGTAATATACACGCCAACGGAGCATCATTCT
TCTTCATCTGTATTTACATACATATCGCCCGAGGTTTATACTACGGATCATACCTGTACA
AAGAAACCTGAAACATCGGTGTAGTCCTCCTACTATTAGTTATAATAACAGCCTTCGTC
GGCTATGTCCTCCCATGAGGGCAAATATCCTTCTGAGGTGCCACTGTTATTACAAACCT
GCTATCCGCCGTACCATACATAGGAGACATATTAGTCCAATGAATTTGAGGTGGCTTCT
CAGTAGACAATGCCACACTAACACGTTTCTTCGCATTCCACTTCCTACTACCATTTATC
ATTGCCGCCGCAACCCTTATTCACCTCCTATTCCTCCACGAAACAGGATCCAACAACC
CAATTGGACTAAACTCAGACGCAGACAAAATCTCCTTCCACCCATACTTTACATATAA
AGACCTTCTCGGATTCGTAATTATACTACTAGCCCTCACATTACTAGCACT

Sequence chromatogram of *cytb* gene has been presented as follows,

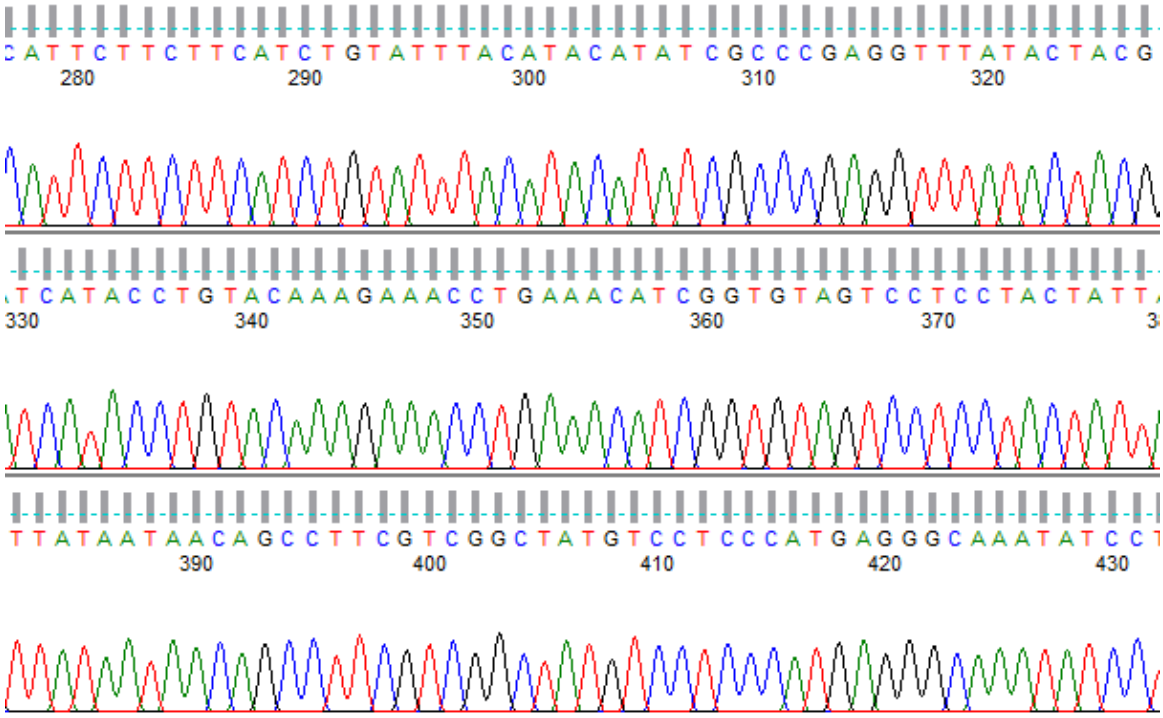

Figure 3.2 Representative chromatogram of the sequenced *cytb* gene region

3.2.1 Polymorphic sites in *cytb* gene sequences of *L. rohita* from Bangladesh

Out of 200 samples collected from the Halda, Jamuna, Padma, and cultured populations, *cytb* gene sequences from 137 samples successfully passed the quality check. Sequencing of an 829 bp fragment of the *cytb* gene revealed a total of 11 haplotypes (Accession number PQ458546 - PQ458556). Further analysis of the 616 bp region, based on high-quality sequence chromatograms and forward-reverse alignments, identified ten polymorphic sites, indicating a high level of sequence conservation (98.4%). Among these, two sites were singletons, and the remaining eight were parsimony-informative sites.

Table 3.1 summarizes the polymorphic sites in the *cytb* gene sequences across 11 different haplotypes identified in the *L. rohita* population from Bangladesh. A polymorphic site is a position in the DNA sequence where different nucleotides, adenine (A), thymine (T), cytosine (C), and guanine (G) occur among the different haplotypes, indicating genetic variation. The columns represent specific nucleotide positions (45, 99, 108, 267, 295, 435, 504, 507, 525, and 592) within the selected *cytb* gene where polymorphisms were observed. Each row corresponds to one of the 11 haplotypes, and the cells contain the nucleotide present at the respective polymorphic site for that haplotype. For instance, at position 45, Hap1BD has an 'A', while Hap3BD has a 'G'.

The presence of multiple haplotypes with different nucleotides at specific positions indicates a high level of genetic diversity in the *cytb* gene among the *L. rohita* population in Bangladesh. Each position with nucleotide variations across haplotypes represents a polymorphic site, highlighting genetic variability. For example, position 45 shows either 'A' or 'G' among different haplotypes.

Table 3.1 Polymorphic sites in 11 haplotypes of *cytb* gene sequences found in *L. rohita* population from Bangladesh.

	Nucleotide position									
Haplotypes	45	99	108	267	295	435	504	507	525	592
Hap1BD	A	C	C	G	G	T	T	C	T	A
Hap2BD	A	T	C	G	G	T	T	C	T	A
Hap3BD	G	C	C	G	G	T	T	C	T	A
Hap4BD	A	C	C	G	G	T	T	C	C	A
Hap5BD	A	C	C	G	A	T	T	C	T	A
Hap6BD	A	C	C	A	G	T	T	C	T	A
Hap7BD	A	C	T	G	G	C	T	T	T	A
Hap8BD	A	C	T	G	G	T	T	T	T	A
Hap9BD	G	C	T	G	G	C	T	T	T	A
Hap10BD	A	C	C	G	G	T	C	C	C	A
Hap11BD	A	C	C	G	G	T	T	C	T	C

Species/Abbrv										
1. HP 1 N=107	A	C	C	G	G	T	T	C	T	A
2. HP 2N=2	A	T	C	G	G	T	T	C	T	A
3. HP3 N=4	G	C	C	G	G	T	T	C	T	A
4. HP4 N=1	A	C	C	G	G	T	T	C	C	A
5. HP5 N=1	A	C	C	G	A	T	T	C	T	A
6. HP6 N=6	A	C	C	A	G	T	T	C	T	A
7. HP7 N=7	A	C	T	G	G	C	T	T	T	A
8. HP8 N=5	A	C	T	G	G	T	T	T	T	A
9. HP9 N=1	G	C	T	G	G	C	T	T	T	A
10. HP 10 N=1	A	C	C	G	G	T	C	C	C	A
11. HP11 N=2	A	C	C	G	G	T	T	C	T	C

Figure 3.3 Polymorphic sites in 11 haplotypes of *cytb* gene sequences found in *L. rohita* population from Bangladesh.

3.2.2 Percentage of different haplotypes

Table 3.2 shows the percentage distribution of different haplotypes among various populations of *L. rohita* in Bangladesh. The haplotypes are analyzed across four specific populations: Culture, Halda, Jamuna, and Padma. The data reveals the prevalence of each haplotype in these populations.

Altogether, 78% of the Bangladeshi *L. rohita* population belongs to haplotype Hap1BD. The other haplotypes are represented in a very low percentage such as Hap3BD (3%), Hap6BD (4.5%), Hap7BD (5.1%), Hap8D (3.64%), etc. (Table 3.2). Among the populations, the haplotype Hap1BD was found in 88% of individuals of the Padma River population, 86% in Jamuna, 72% in Halda, and 66% in the culture. Each group exhibits some uniqueness in the haplotype diversity. For instance, the Culture population has three distinct haplotypes (Hap9BD, Hap10BD, and Hap11BD) that were absent in other groups: the Halda population one (Hap8BD), the Jamuna population two (Hap4BD and Hap5BD), and the Padma population one (Hap2BD).

Table 3.2 Percentage of different haplotypes among the populations of *L. rohita* in Bangladesh.

Haplotypes	Total%	Culture%	Halda%	Jamuna%	Padma%
Hap1BD	78	66	72	86	88
Hap2BD	1.5	0	0	0	5
Hap3BD	3	0	0	3	8
Hap4BD	0.72	0	0	3	0
Hap5BD	0.72	0	0	3	0
Hap6BD	4.5	0	16	3	0
Hap7BD	5.1	21	0	3	0
Hap8BD	3.64	0	13	0	0
Hap9BD	0.72	3	0	0	0
Hap10BD	0.72	3	0	0	0
Hap11BD	1.6	7	0	0	0

3.2.3 Pairwise genetic distance analysis

The analysis of pairwise genetic distances among the 11 haplotypes (Hap1BD to Hap11BD) of *L. rohita* revealed varying degrees of genetic differentiation presented in **Table 3.3**. The genetic distances ranged from 0.00163 to 0.00983, indicating low to moderate genetic divergence among the haplotypes.

Haplotypes Hap1BD, Hap2BD, Hap3BD, Hap4BD, Hap5BD, and Hap6BD displayed minimal genetic distances among themselves, with values consistently at or around 0.00163 to 0.00326. This suggests a close genetic relationship within this group.

In contrast, Hap7BD exhibited greater divergence from the other haplotypes, with pairwise genetic distances ranging from 0.00489 to 0.00654 when compared to Hap1BD through Hap6BD. The highest genetic distance (0.00983) was recorded between Hap9BD and Hap10BD, indicating significant genetic differentiation. While Hap8BD and Hap9BD were more divergent than the initial cluster of haplotypes, they still displayed moderate genetic distances, ranging from 0.00326 to 0.00819. Similarly, Hap10BD and Hap11BD showed moderate genetic distances within this range.

The pairwise genetic distance analysis indicates the presence of several distinct genetic clusters within the *L. rohita* population, with some haplotypes being closely related and others showing more substantial genetic differentiation. This variation can be instrumental in understanding the genetic diversity and evolutionary dynamics of the species.

Table 3.3 Pairwise genetic distance among haplotype of *cytb* gene

Haplotypes	Hap1BD	Hap2BD	Hap3BD	Hap4BD	Hap5BD	Hap6BD	Hap7BD	Hap8BD	Hap9BD	Hap10BD	Hap11BD
Hap1BD											
Hap2BD	0.00163										
Hap3BD	0.00163	0.00326									
Hap4BD	0.00163	0.00326	0.00326								
Hap5BD	0.00163	0.00326	0.00326	0.00326							
Hap6BD	0.00163	0.00326	0.00326	0.00326	0.00326						
Hap7BD	0.00489	0.00653	0.00654	0.00653	0.00654	0.00654					
Hap8BD	0.00326	0.00489	0.0049	0.00489	0.0049	0.0049	0.00163				
Hap9BD	0.00654	0.00818	0.00489	0.00818	0.00819	0.00819	0.00163	0.00326			
Hap10BD	0.00326	0.00489	0.0049	0.00163	0.0049	0.0049	0.00817	0.00653	0.00983		
Hap11BD	0.00163	0.00326	0.00327	0.00326	0.00327	0.00327	0.00654	0.0049	0.0082	0.0049	

3.2.4 Nucleotide composition analysis among the haplotype of *cytb* gene

The nucleotide composition analysis of the 11 haplotypes (Hap1BD to Hap11BD) of *L. rohita* revealed consistent patterns in the percentages of thymine (T), cytosine (C), adenine (A), and guanine (G) across the haplotypes. The average nucleotide composition was 27.5% T, 29.7% C, 28.9% A, and 14% G.

Thymine (T) percentages ranged from 27.1% in Hap10BD to 27.8% in Hap8BD, indicating minor variability in T content among the haplotypes. Cytosine (C) percentages were slightly more variable, ranging from 29.4% in Hap8BD to 30% in Hap10BD. Adenine (A) content was relatively stable across haplotypes, with values between 28.7% in Hap3BD, Hap9BD, and Hap11BD to 29.1% in Hap5BD and Hap6BD. Guanine (G) content showed the least variation, with most haplotypes maintaining a consistent 14%, except for Hap3BD and Hap9BD, which had a slightly higher percentage of 14.1%.

Table 3.4 Nucleotide composition of 11 haplotypes. Letters denote one-letter abbreviations for standard nucleotides.

Haplotype	T%	C%	A%	G%
Hap1BD	27.4	29.7	28.9	14
Hap2BD	27.6	29.5	28.9	14
Hap3BD	27.4	29.7	28.7	14.1
Hap4BD	27.3	29.9	28.9	14
Hap5BD	27.4	29.7	29.1	13.8
Hap6BD	27.4	29.7	29.1	13.8
Hap7BD	27.6	29.5	28.9	14
Hap8BD	27.8	29.4	28.9	14
Hap9BD	27.6	29.5	28.7	14.1
Hap10BD	27.1	30	28.9	14
Hap11BD	27.4	29.9	28.7	14
Avg.	27.5	29.7	28.9	14

3.2.5 Genetic diversity analysis of *L. rohita* populations at the *cytb* gene

Based on the data presented in **Table 3.5**, diversity analysis at the mitochondrial *cytb* locus across four populations of *L. rohita* reveals significant variation. The Padma population, with 40 individuals, exhibited low haplotype diversity (0.232) and nucleotide diversity (0.00039), with only 2 polymorphic and 2 singleton sites. The Jamuna population, comprising 36 individuals, showed moderate haplotype diversity (0.262) and nucleotide diversity (0.00063), characterized by 7 polymorphic and singleton sites. In contrast, the Halda population, with 32 individuals, displayed higher haplotype diversity (0.494) and nucleotide diversity (0.00133), with 3 polymorphic sites but no singleton sites. The cultured population, consisting of 29 individuals, demonstrated the highest haplotype (0.539) and nucleotide diversity (0.0024), with 7 polymorphic sites, 3 singleton sites, and 4 parsimony sites. When all populations were combined, the overall haplotype diversity was 0.385, and nucleotide diversity was 0.00118, with 10 polymorphic sites and 8 parsimony sites. The haplotype diversity (Hd) for the Padma, Jamuna, Halda, and Culture populations was 0.232, 0.262 0.494, and 0.539, whereas nucleotide diversity (p) for the same populations was 0.00039, 0.00063, 0.00133 and 0.0024. Among the three river-origin populations, Halda had the highest haplotype diversity (hd, 0.494) and nucleotide diversity (nd, 0.00133) compared to the two others (Table 5). The cultured samples which were collected from different regions of the country had the highest haplotype and nucleotide diversity when all four groups were considered.

Table 3.5 Diversity analysis at mitochondrial *cytb* locus of four populations

Population	*No of individual*	*No of Haplotype*	*Haplotype diversity*	*Nucleotide diversity*	*Monomorphic site*	*Ppolymorphic site*	*Singleton site*	*Parsimony site*
Padma	40	3	0.232	0.00039	614	2	2	0
Jamuna	36	6	0.262	0.00063	609	7	7	0
Halda	32	3	0.494	0.00133	613	3	0	3
Culture	29	5	0.539	0.0024	609	7	3	4
All	137	11	0.385	0.00118	606	10	2	8

3.2.6 Analysis of Molecular Variance (AMOVA) for *cytb* sequence data

The Analysis of Molecular Variance (AMOVA) for the mitochondrial cytb sequence (616 bp) across four populations of *L. rohita* **(Table 3.6)** revealed that the majority of genetic variation occurs within populations rather than among them. Specifically, 90.35% of the total genetic variation was attributed to within-population differences, with a variance component of 0.33704. In contrast, only 9.65% of the variation was due to differences among populations, with a variance component of 0.03600. The fixation index (FST = 0.0965) indicated moderate but statistically significant genetic differentiation (P-value = 0.00000±0.00000). This suggests that while some genetic divergence exists among the Padma, Jamuna, Halda, and cultured populations, the majority of genetic diversity is retained within individual populations.

Table 3.6 AMOVA analysis of Padma, Jamuna, Halda, Culture *Labeo rohita* populations of *cytb* sequence 616bp.

Source of variation	df	Sum of square	Variance of component	Percentage of variation	FST	P-value
Among populations	3	4.692	0.03600Va	9.65	0.0965	0.00000±0.00000
Within populations	133	44.826	0.33704Vb	90.35		
Total	136	49.518	0.37304			

3.2.7 Population pairwise FST values and statistical significance at the *cytb* Locus

The population pairwise FST values and their statistical significance (p-values) for the mitochondrial *cytb* sequence (616 bp) among Bangladeshi *L. rohita* populations are presented in **Table 3.7**. The pairwise FST values below the diagonal indicate the level of genetic differentiation between populations, while the p-values above the diagonal denote the statistical significance of these differences. The analysis reveals that the Padma population exhibits low genetic differentiation with the Jamuna population (FST = 0.00376) and the Halda population (FST = 0.11987), but higher differentiation with the cultured population (FST = 0.17764). The p-values for these comparisons are 0.27027, 0.00000, and 0.00000, respectively, indicating significant differentiation with the Halda and cultured populations but not with the Jamuna population.The Jamuna population shows moderate differentiation with the Halda population (FST = 0.05632) and higher differentiation with the cultured population (FST = 0.11845). The corresponding p-values are 0.01802 and 0.00000, indicating significant differentiation with both populations.The Halda population exhibits low genetic differentiation with the cultured population (FST = 0.05701) with a p-value of 0.06306, indicating that the differentiation is not statistically significant.

Overall, the pairwise FST analysis highlights significant genetic differentiation between several population pairs, particularly involving the cultured population, suggesting varying degrees of genetic isolation and diversity among the Bangladeshi *Labeo rohita* populations.

Table 3.7 Population pairwise FST value (below diagonal) and FST p-value (above diagonal) among Bangladeshi *Labeo rohita* populations of *cytb* sequence 616bp.

	Padma	Jamuna	Halda	Culture
Padma	0	0.27027±0.0359	0.00000±0.0000	0.00000±0.0000
Jamuna	0.00376	0	0.01802±0.0121	0.00000±0.0000
Halda	0.11987	0.05632	0	0.06306±0.0194
Culture	0.17764	0.11845	0.05701	0

3.2.8 Haplotype diversity and population-specific variations in the *cytb* gene

This study analyzes haplotype diversity in the *cytb* gene **(Figure 3.4),** focusing on the distribution and unique characteristics of distinct haplotypes. The haplotype network analysis reveals that Hap1BD predominates, constituting 78% of the total haplotypes identified. Notably, population-specific haplotypes were observed, with Hap2BD exclusive to the Padma population, Hap4BD and Hap5BD specific to the Jamuna population, and Hap8BD unique to the Halda population. Hap9BD, Hap10BD, and Hap11BD were identified solely in the culture population. Furthermore, the study identified shared haplotypes between populations. Hap3BD was found to be common between Jamuna and Padma populations, Hap6BD between Halda and Jamuna, and Hap7BD between Culture and Jamuna. In terms of genetic variations, Hap2BD, Hap3BD, Hap4BD, Hap6BD, and Hap9BD each exhibit a single nucleotide substitution from the most common haplotype Hap1BD. Hap8BD differs by two substitutions from Hap1BD, while Hap7BD diverges by one substitution from Hap8BD and three substitutions from Hap1BD. Lastly, Hap11BD displays four substitutions relative to Hap1BD.

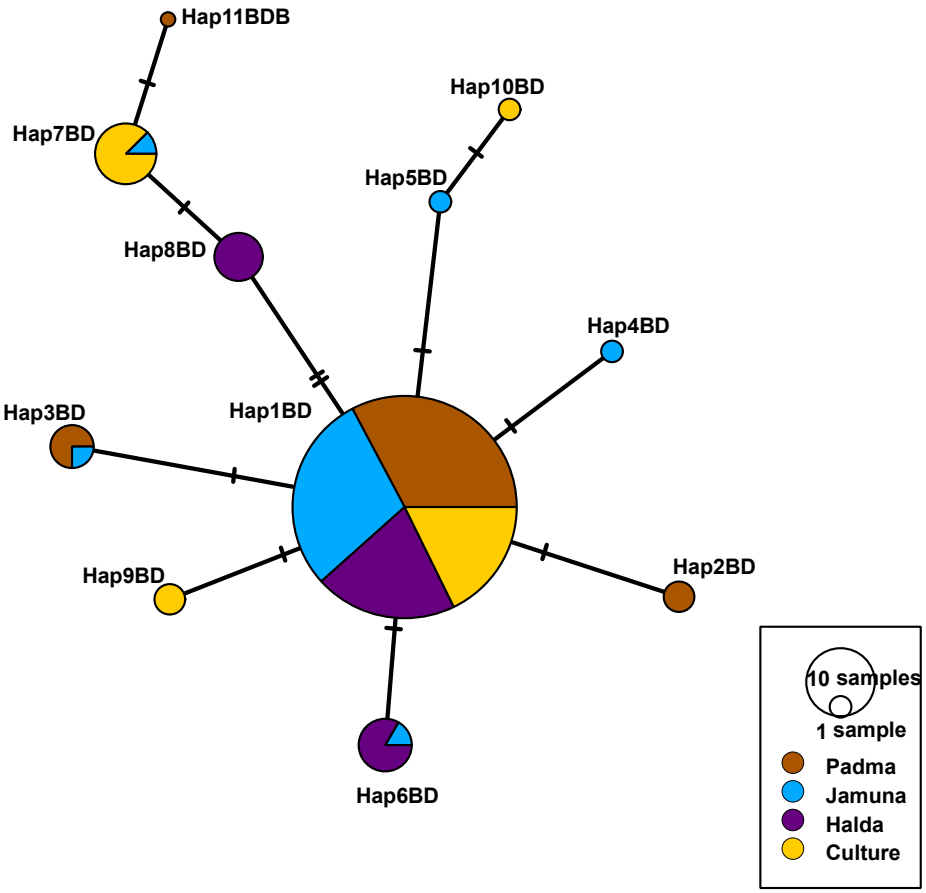

Figure 3.4 Haplotype networks for *L. rohita* population in Bangladesh. Size of the circles is proportional to the frequency of each haplotype

3.2.9 Phylogenetic analysis of *L. rohita cytb* sequences

The UPGMA dendrogram of the four *Labeo rohita* populations (**Figure 3.5**) identified two distinct genetic clusters. The cultured population formed a separate cluster, whereas the Padma, Jamuna, and Halda populations grouped together within the second cluster. Within this cluster, the Halda population was further distinguished as a distinct subgroup, while the Padma and Jamuna populations formed another subgroup. The genetic distance values corroborated this clustering pattern, with the greatest genetic distance observed between the Padma and cultured populations (0.0013), whereas the shortest genetic distance was recorded between the Padma and Jamuna populations (0.002). The UPGMA tree, constructed based on Nei's (1972) genetic distance, effectively demonstrated the genetic differentiation among these populations.

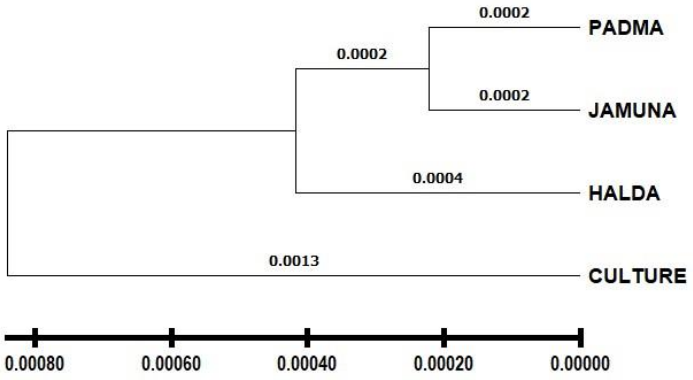

Figure 3.5 The UPGMA tree of four populations constructed with the ape package

3.3 Analysis of control region

The representative sequences obtained from our study are given below. The fasta format of the control region was obtained from the *L. rohita* population.

>Control region sequence A01. J01 size - 669 bp

AACCTACCCTATATGGTATAGTACATAATATGCATGATATTACATTAATGTATTAGTACAT
CTATGTATTATCACCAACTCATTATTTTAACCATAAAGCAAGTACTAACTATTAAGGTAG
ACATAAGACATAAAATTAAGACTCAAAAGTCCAATATTTAAACCTGAAAAATAGATTA
TTCCCCAAAAAATTGACCTCAAATATTTCCTTGAAATAACCAACTAAAATTCCACTAA
ACCATATTAATGTAGTAAGAGATCACCAACAATATAAATTAACAATATCATGCATGATA
GAATCAAGGACAATAACTGTGGGGGTAGCACAATATGAACTATTACTGGCATCTGGTT
CCTATTTCAGGTCCATAAAATGTAAACTTCCCCCCCCCAAATAATTATACTGGCATCTGA
TTAATGGTGTAGTACATACGTCTCGTTACCCCCCATGCCGAGCATTCTTTTATATGCATA
GGGTATCTTTTTCTGGTTTCCTTTCATCTGGCATCTCAGAGTGCAAGCTCAAATGTTAA
TCAAGGTTGAACATTTTCCTTGTATGTGATAATATATATGAATTATTGGAAGACATGAAT
TAAGCATTACATTCTTCTAATTCAAGTGCATAACATATCTATTCCTTATTCAACTATACTT
GCTATAATGCCCCCTT

The sequence chromatogram of the control region has been presented as follows,

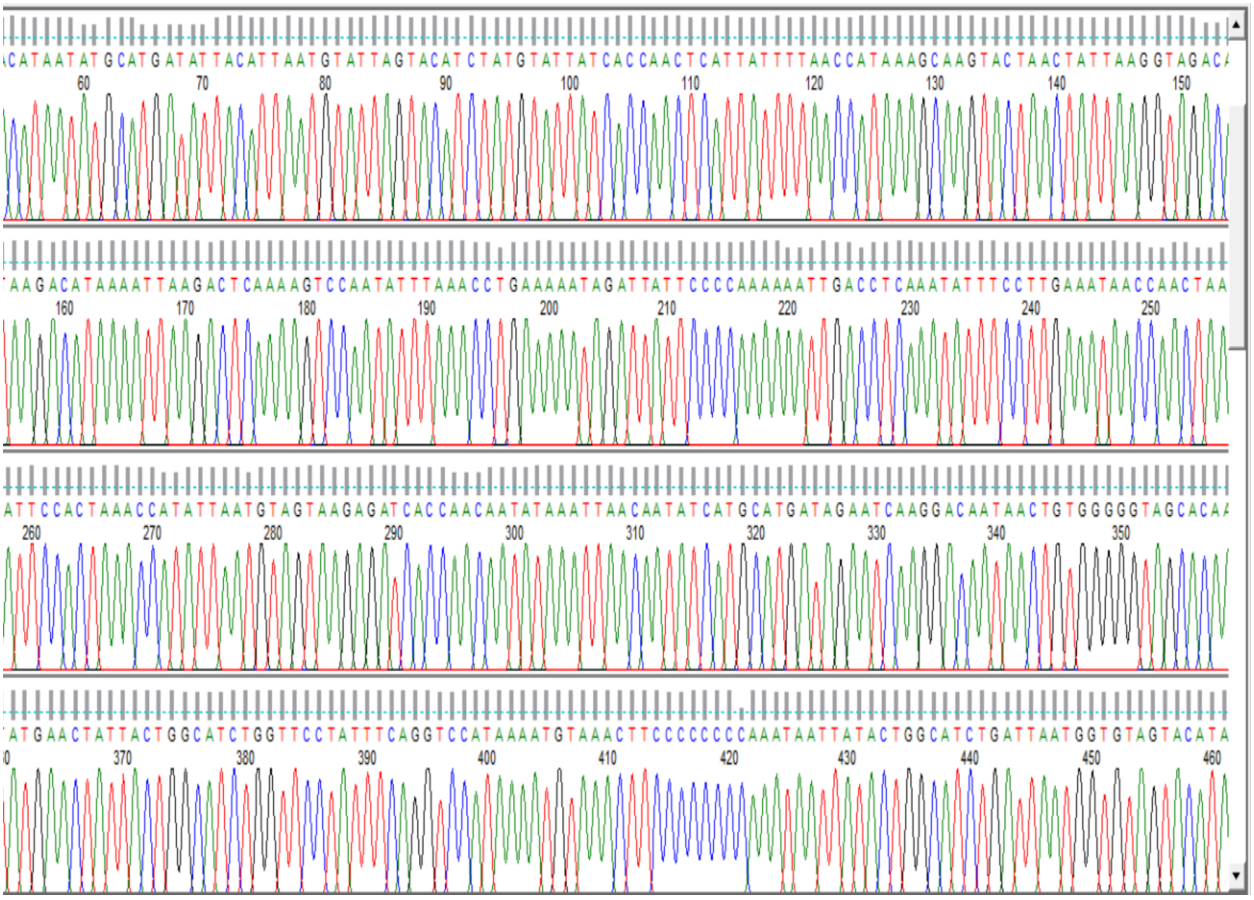

Figure 3.6 Representative chromatogram of the sequenced control region

3.3.1 Polymorphic sites in control region sequences of *L. rohita* population

Out of 200 samples collected from the Halda, Jamuna, Padma, and cultured populations, control region sequences from 162 samples successfully passed the quality check. Sequencing of an 873bp fragment of the control region revealed a total of 50 haplotypes. We used only 18 haplotypes out of a total of 50, focusing on those with frequencies greater than one. Further analysis of the 598 bp region, based on high-quality sequence chromatograms and forward-reverse alignments. The analysis of polymorphic sites in the control region sequences of *L. rohita* from Bangladesh revealed notable nucleotide variations across 18 identified haplotypes. Table 3.8 illustrates the nucleotide composition at 22 variable positions (positions 35, 57, 87, 90, 91, 115, 121, 131, 147, 197, 207, 292, 460, 464, 479, 518, 537, 548, 559, 567, 579, and 591). Haplotypes exhibited both transitions and transversions, with some positions showing high variability. For instance, position 35 shows a T/C variation, while position 91 varies between T and C. Similarly, position 537 has either T or C. Hap1BD, Hap3BD, and Hap6BD share identical sequences, indicating potential common ancestry or low mutation rates in these regions. Conversely, Hap11BD and Hap18BD exhibit unique polymorphisms at positions 147 and 292, respectively, suggesting distinct evolutionary pathways. This nucleotide diversity highlights the genetic variability within the L. rohita population in Bangladesh, which is crucial for understanding their evolutionary dynamics and managing their conservation effectively.

Table 3.8 Polymorphic sites in 18 haplotypes of control region sequences found in *L. rohita* population from Bangladesh.

	Nucleotide position																					
Haplotypes	35	57	87	90	91	115	121	131	147	197	207	292	460	464	479	518	537	548	559	567	579	591
Hap1BD	T	C	A	A	T	T	C	C	A	C	T	T	T	C	C	T	T	A	G	G	A	T
Hap2BD	C	T	A	A	T	T	C	C	A	C	T	T	T	C	C	T	T	A	G	G	A	T
Hap3BD	T	C	A	A	T	T	C	C	A	C	T	T	T	C	C	T	T	A	G	G	A	T
Hap4BD	C	T	A	A	T	C	C	C	A	C	T	T	T	C	C	T	T	A	A	G	A	T
Hap5BD	C	T	A	A	T	C	C	C	A	C	T	T	C	C	C	T	T	G	A	T	A	T
Hap6BD	T	C	A	A	T	T	C	C	A	C	T	T	T	C	C	T	T	A	G	G	A	T
Hap7BD	T	C	A	A	T	T	T	C	A	C	T	T	T	C	C	C	T	A	G	G	A	T
Hap8BD	C	T	A	A	T	C	C	C	A	T	T	T	C	C	C	T	T	G	G	T	A	T
Hap9BD	C	T	A	A	T	C	C	C	A	C	T	T	C	T	C	T	T	G	G	T	A	T
Hap10BD	C	T	A	A	T	C	C	C	A	C	T	T	C	C	T	T	T	G	G	T	A	T
Hap11BD	C	T	A	A	T	C	C	C	G	C	T	T	C	C	C	T	T	G	G	T	A	T
Hap12BD	C	C	A	A	C	T	C	C	A	C	T	T	T	C	C	T	T	G	A	G	A	T
Hap13BD	C	T	A	G	T	C	C	C	A	C	T	T	C	C	C	T	T	A	G	G	A	T
Hap14BD	C	T	A	A	T	C	C	C	A	C	T	T	C	C	C	T	T	G	G	T	G	C
Hap15BD	C	T	A	A	T	C	C	C	A	C	T	T	C	C	C	T	C	A	G	T	A	T
Hap16BD	T	C	A	A	T	T	C	T	A	C	T	T	T	C	C	T	T	A	G	G	A	T
Hap17BD	T	C	A	A	T	T	C	C	A	C	C	T	T	C	C	T	T	A	G	G	A	T
Hap18BD	C	C	A	A	C	T	C	C	A	C	T	C	T	C	C	T	T	G	G	G	A	T

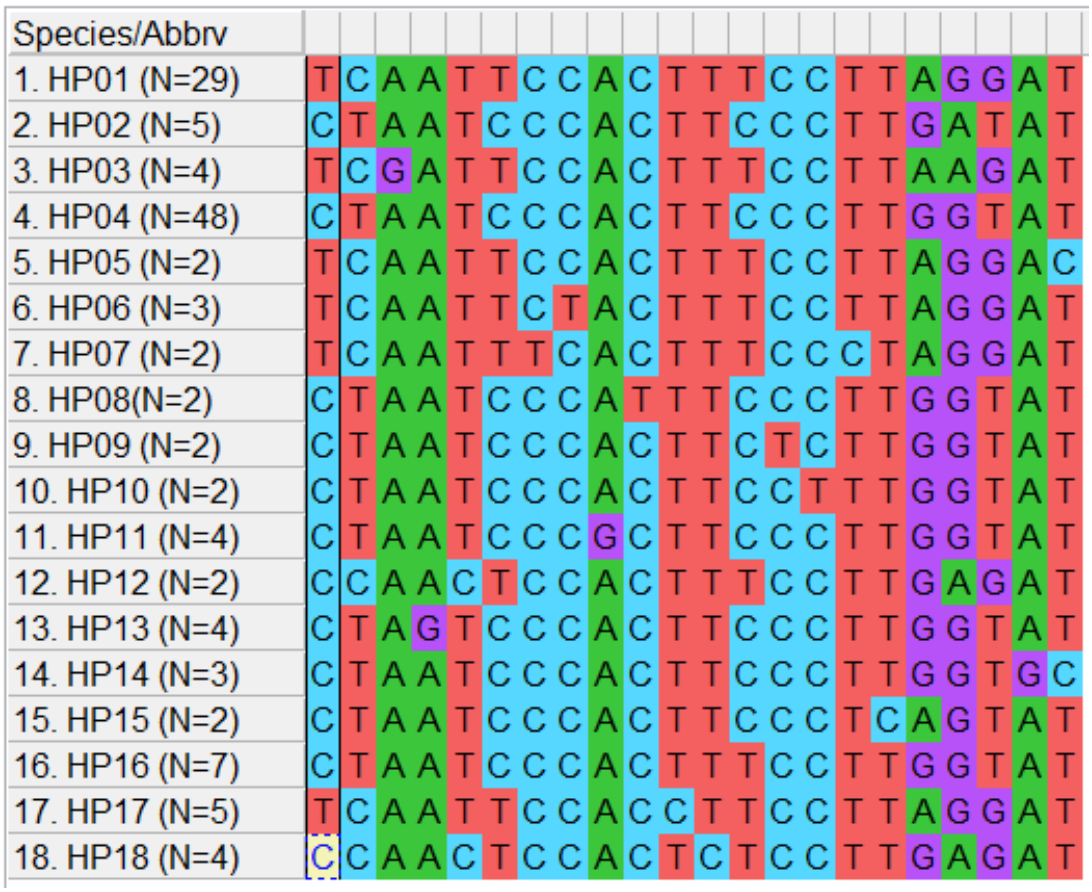

Figure 3.7 Polymorphic sites in 18 haplotypes of control region sequences found in *L. rohita* population from Bangladesh.

3.3.2 Percentage of different haplotypes

The analysis of the percentage of different haplotypes among the populations of *L. rohita* in Bangladesh reveals significant variability and distinct regional differences as illustrated in **Table 3.9**. Haplotype Hap04BD is the most prevalent, constituting 29.62% of the total, and is particularly dominant in the Halda River population (61.36%) but less so in the Padma (17.94%), Jamuna (9.67%), and cultured populations (22.91%). Hap01BD is the second most common haplotype overall (17.90%) and shows notable presence in the Padma (33%), Jamuna (20.83%), and cultured populations (19.35%). Interestingly, Hap01BD is absent in the Halda population. Unique haplotypes such as Hap08BD, Hap09BD, Hap13BD, Hap14BD, and Hap15BD are found exclusively in specific populations. Hap08BD and Hap09BD are unique to the cultured population, each constituting 6.45%. Hap13BD, Hap14BD, and Hap15BD are unique to the Jamuna population with frequencies of 8.33%, 6.25%, and 4.16%, respectively. Notably, several other haplotypes, such as Hap02BD, Hap03BD, Hap05BD, Hap06BD, and Hap07BD have lower overall frequencies, with specific distributions varying across different populations. Noteworthy is the absence of several haplotypes in particular populations; for instance, Hap01BD, Hap02BD, and Hap03BD are absent in the Halda population, and several haplotypes such as Hap07BD, Hap13BD, Hap14BD, and Hap15BD are not found in cultured populations. The presence and absence of certain haplotypes in specific populations underscore the genetic diversity and regional differentiation within *L. rohita* populations in Bangladesh.

Table 3.9 Percentage of different haplotypes among the populations of *L. rohita* in Bangladesh

Haplotypes	Total%	Padma%	Jamuna%	Halda%	Culture%
Hap01BD	17.90%	33%	20.83%	0%	19.35%
Hap02BD	3.08%	2.56%	2.08%	0%	9.67%
Hap03BD	2.46%	7.69%	0%	0%	3.22%
Hap04BD	29.62%	17.94%	9.67%	61.36%	22.91%
Hap05BD	1.23%	2.56%	0%	2.27%	0%
Hap06BD	1.85%	2.56%	2.08%	0%	3.22%
Hap07BD	1.23%	5.12%	0%	0%	0%
Hap08BD	1.23%	0%	0%	0%	6.45%
Hap09BD	1.23%	0%	0%	0%	6.45%
Hap10BD	1.23%	0%	2.08%	0%	3.22%
Hap11BD	2.46%	0%	6.25%	0%	3.22%
Hap12BD	1.23%	0%	2.08%	0%	3.22%
Hap13BD	2.46%	0%	8.33%	0%	0%
Hap14BD	1.85%	0%	6.25%	0%	0%
Hap15BD	1.23%	0%	4.16%	0%	0%
Hap16BD	4.32%	0%	4.16%	11.36%	0%
Hap17BD	3.08%	0%	0%	11.36%	0%
Hap18BD	2.46%	0%	0%	9.09%	0%

3.3.3 Nucleotide composition analysis

Table 3.10 presents the nucleotide composition of 18 haplotypes of *L. rohita*, revealing a consistent pattern across the haplotypes. The average composition is 31.8% thymine (T), 18.6% cytosine (C), 36.2% adenine (A), and 13.4% guanine (G). Individual haplotypes exhibit minor deviations from these averages. For instance, haplotypes Hap06BD and Hap16BD display the highest thymine content at 32.1%, whereas Hap18BD shows the lowest at 31.4%. The cytosine content ranges from 18.2% in Hap06BD and Hap16BD to 18.9% in Hap14BD and Hap15BD. Adenine percentages are relatively stable, fluctuating slightly between 36% in Hap11BD, Hap13BD, and Hap14BD, and 36.3% in several other haplotypes. Guanine content is notably consistent, predominantly at 13.4%, with minor deviations observed in Hap02BD and Hap15BD at 13.2%, and Hap11BD at 13.5%.

Table 3.10 Nucleotide composition of 18 haplotypes. Letters denote one-letter abbreviations for standard nucleotides

Haplotypes	T%	C%	A%	G%
Hap01BD	31.9	18.4	36.3	13.4
Hap02BD	31.8	18.7	36.3	13.2
Hap03BD	31.9	18.4	36.3	13.4
Hap04BD	31.8	18.7	36.1	13.4
Hap05BD	31.8	18.6	36.3	13.4
Hap06BD	32.1	18.2	36.3	13.4
Hap07BD	31.9	18.4	36.3	13.4
Hap08BD	31.9	18.6	36.1	13.4
Hap09BD	31.9	18.6	36.1	13.4
Hap10BD	31.9	18.7	36.1	13.4
Hap11BD	31.8	18.7	36	13.5
Hap12BD	31.6	18.7	36.3	13.4
Hap13BD	31.8	18.7	36	13.4
Hap14BD	31.6	18.9	36	13.4
Hap15BD	31.6	18.9	36.3	13.2
Hap16BD	32.1	18.2	36.3	13.4
Hap17BD	31.8	18.6	36.3	13.4
Hap18BD	31.4	18.7	36.3	13.4
Avg.	31.8	18.6	36.2	13.4

3.3.4 Genetic diversity analysis of *L. rohita* populations at the control region sequence

Table 3.11 presents a diversity analysis of the mitochondrial control region sequences among four populations of *Labeo rohita* in Bangladesh. The analysis reveals variations in haplotype and nucleotide diversity across the populations. The Padma population, consisting of 39 individuals, has 18 haplotypes with a haplotype diversity of 0.861 and nucleotide diversity of 0.00773. This population shows 574 monomorphic sites, 24 polymorphic sites, 10 singleton sites, and 14 parsimony sites. The Jamuna population, with 48 individuals, exhibits 20 haplotypes, a haplotype diversity of 0.898, and nucleotide diversity of 0.0072, with 570 monomorphic sites, 28 polymorphic sites, 13 singleton sites, and 15 parsimony sites. The Halda population, comprising 44 individuals, has the lowest haplotype diversity (0.601) and nucleotide diversity (0.00488) among the four populations, with 586 monomorphic sites, 12 polymorphic sites, 2 singleton sites, and 10 parsimony sites. The cultured population, consisting of 31 individuals, shows the highest haplotype diversity (0.951) and a nucleotide diversity of 0.00778, with 572 monomorphic sites, 26 polymorphic sites, 17 singleton sites, and 9 parsimony sites. When considering all 162 individuals together, the overall haplotype diversity is 0.876, and nucleotide diversity is 0.00706, with 550 monomorphic sites, 48 polymorphic sites, 19 singleton sites, and 29 parsimony sites. This data indicates significant genetic diversity among the populations, with the cultured population exhibiting the highest diversity and the Halda population the lowest.

Table 3.11 Diversity analysis at mitochondrial control region sequence among four populations in Bangladesh

Population	No of individual	No of Haplotype	Haplotype diversity	Nucleotide diversity	Monomorphic site	Ppolymorphic site	Singleton site	Parsimony site
Padma	39	18	0.861	0.00773	574	24	10	14
Jamuna	48	20	0.898	0.0072	570	28	13	15
Halda	44	7	0.601	0.00488	586	12	2	10
Culture	31	20	0.951	0.00778	572	26	17	9
All	162	50	0.876	0.00706	550	48	19	29

3.3.5 Analysis of Molecular Variance (AMOVA) for control region sequence data

Table 3.12 presents the results of the Analysis of Molecular Variance (AMOVA) for the *Labeo rohita* populations from Padma, Jamuna, Halda, and Culture, based on the control region sequence. The AMOVA results show that the majority of genetic variation is within populations, with 95.30% of the total variation observed within populations and only 4.70% among populations. Specifically, among populations, the sum of squares is 18.196 with a variance component of 0.10032, and the percentage of variation attributed to differences among populations is 4.70%. The fixation index (FST) for the variation among populations is 0.04698, and the P-value is 0.000, indicating that the observed differentiation among populations is statistically significant. Within populations, the sum of squares is 321.576 with a variance component of 2.03529. The total sum of squares is 339.772, with a total variance component of 2.13562. These results suggest that while there is some genetic differentiation among the populations, the predominant genetic variation is within individual populations.

Table 3.12 AMOVA analysis of Padma, Jamuna, Halda, Culture *L. rohita* populations of control region sequence.

Source of variation	df	Sum of square	Variance of component	Percentage of variation	FST	P-value
Among populations	3	18.196	0.10032	4.69770	0.04698	0.000±0.000
Within populations	158	321.576	2.03529	95.30230		
Total	161	339.772	2.13562			

3.3.6 Population pairwise FST values and statistical significance at the control region sequence

Table 3.13 presents the pairwise *FST* values and *p*-values for genetic differentiation among Bangladeshi *Labeo rohita* populations based on the control region sequence. The highest *FST* value (0.14804) was observed between the Halda and Padma populations, with a highly significant *p*-value of 0.00000, indicating substantial genetic divergence. In contrast, the cultured and Padma populations exhibited the lowest *FST* value (0.00677) and a *p*-value of 0.25225, suggesting minimal and statistically non-significant differentiation. These results highlight strong genetic differentiation primarily between the Halda population and others, while the cultured population remains relatively undifferentiated from most other populations.

Table 3.13 Population pairwise FST value (below diagonal) and FST p-value (above diagonal) among Bangladeshi *Labeo rohita* populations of control region sequence.

	Culture	Halda	Jamuna	Padma
Culture	0	0.07207±0.0227	0.45045±0.0450	0.25225±0.0353
Halda	0.04645	0	0.08108±0.0252	0.00000±0.0000
Jamuna	0.00685	0.02123	0	0.02703±0.0139
Padma	0.00677	0.14804	0.05105	0

3.3.7 Phylogenetic analysis of *L. rohita* control region sequences

The phylogenetic tree presented in **Figure 3.8** illustrates the genetic relationships among various haplotypes (Hap) of *L. rohita* based on mitochondrial gene sequences. The tree, constructed using the neighbor-joining method, displays distinct clustering of haplotypes with significant bootstrap values supporting the nodes. Haplotype Hap01BD (N=29) forms a well-supported cluster with Hap06BD (N=3), Hap16BD (N=7), and Hap17BD (N=5) at a bootstrap value of 72%. Another prominent cluster includes Hap03BD (N=4), Hap05BD (N=2), and Hap07BD (N=2) with a bootstrap value of 61%. Hap12BD (N=2) and Hap18BD (N=4) show a close relationship with Hap02BD (N=5), supported by a high bootstrap value of 99%. The largest haplotype, Hap04BD (N=48), clusters with Hap11BD (N=4), Hap13BD (N=4), Hap08BD (N=2), Hap09BD (N=2), and Hap10BD (N=2), albeit with lower bootstrap values ranging from 33% to 43%. These results suggest a substantial genetic diversity among the *L. rohita* populations, indicating potential sub-structuring within the species.

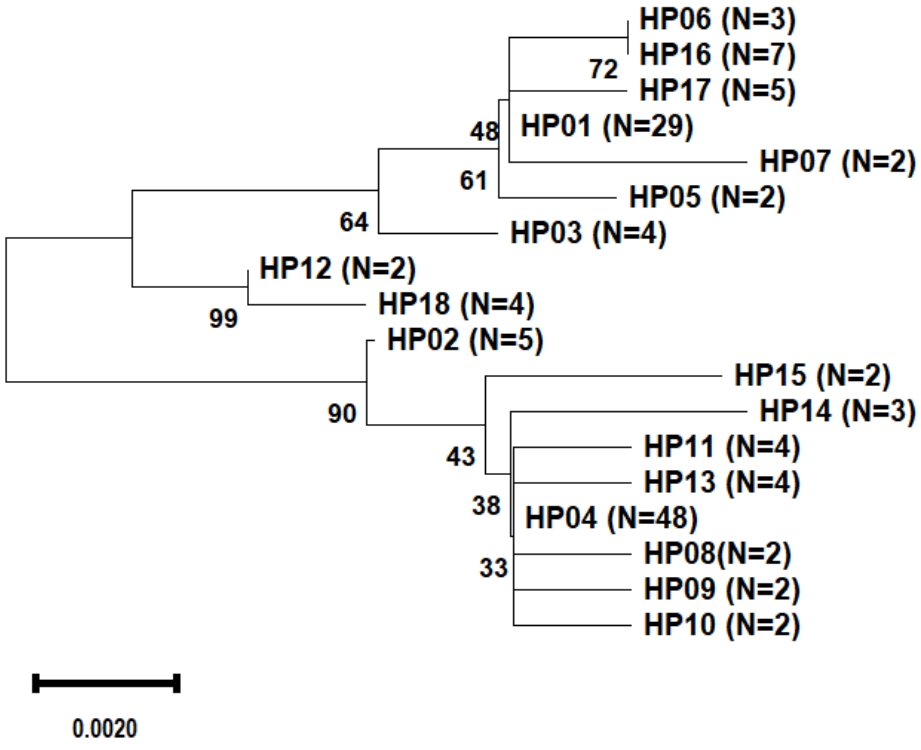

Figure 3.8 The Phylogenetic tree of the selected control region haplotypes using the Maximum likelihood method of *L. rohita* based on the control region

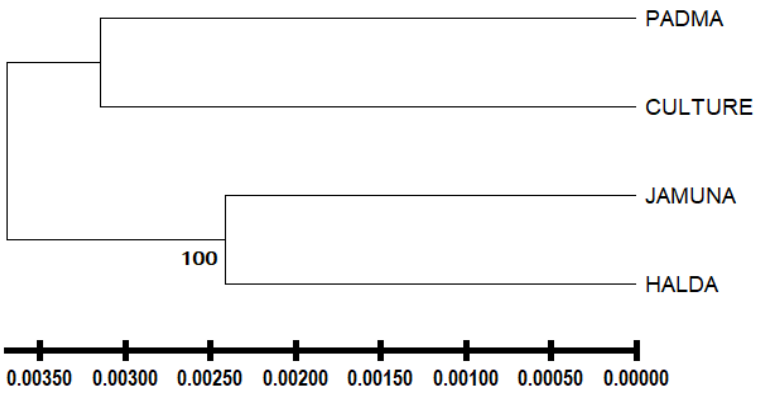

Figure 3.9 The UPGMA tree of four populations based on control region sequence

Figure 3.9 presents a UPGMA (Unweighted Pair Group Method with Arithmetic Mean) tree, illustrating the genetic relationships among four populations of *Labeo rohita* based on control region sequences. The tree indicates that the Jamuna and Halda populations are closely related, forming a distinct cluster supported by a high bootstrap value of 100. This strong support suggests a significant genetic similarity between these two populations. On the other hand, the Padma population appears more distantly related to the other populations, forming a separate branch. The cultured population also forms an independent branch, indicating its genetic distinction from the wild populations. The genetic distances are represented on the scale bar, which shows a range from 0.00000 to 0.00350. The closer genetic relationship between the Jamuna and Halda populations is evident from their shorter branch lengths compared to the Padma and cultured populations. Overall, the UPGMA analysis demonstrates clear genetic differentiation among the four *L. rohita* populations, with notable genetic closeness between the Jamuna and Halda populations and distinct separation of the Padma and cultured populations.

3.3.8 Haplotype diversity and population-specific variations in the control region sequence

The haplotype network **Figure 3.10** for the *L. rohita* populations in Bangladesh highlights the genetic relationships and diversity among the four populations (Padma, Culture, Jamuna, and Halda) based on the mitochondrial control region. Larger haplotypes (e.g., the central ones with larger circles) suggest these are more common and shared among multiple populations, indicating ancestral or widely distributed haplotypes. The distinct proportions of pie chart colors in some haplotypes suggest that certain haplotypes are population-specific. The Halda population (purple) displays several unique or less-shared haplotypes, indicating potential genetic isolation or distinct evolutionary pressures in this population. The Culture population (green) overlaps with other populations, which might reflect gene flow due to artificial stocking practices or interbreeding. Shared haplotypes among the populations (evidenced by mixed pie charts) suggest gene flow or a common ancestral lineage. Haplotypes with contributions from both the Padma and Jamuna populations indicate ongoing connectivity between these river systems. Conversely, the limited overlap between the Halda population and others may reflect restricted migration or unique environmental factors influencing genetic variation.

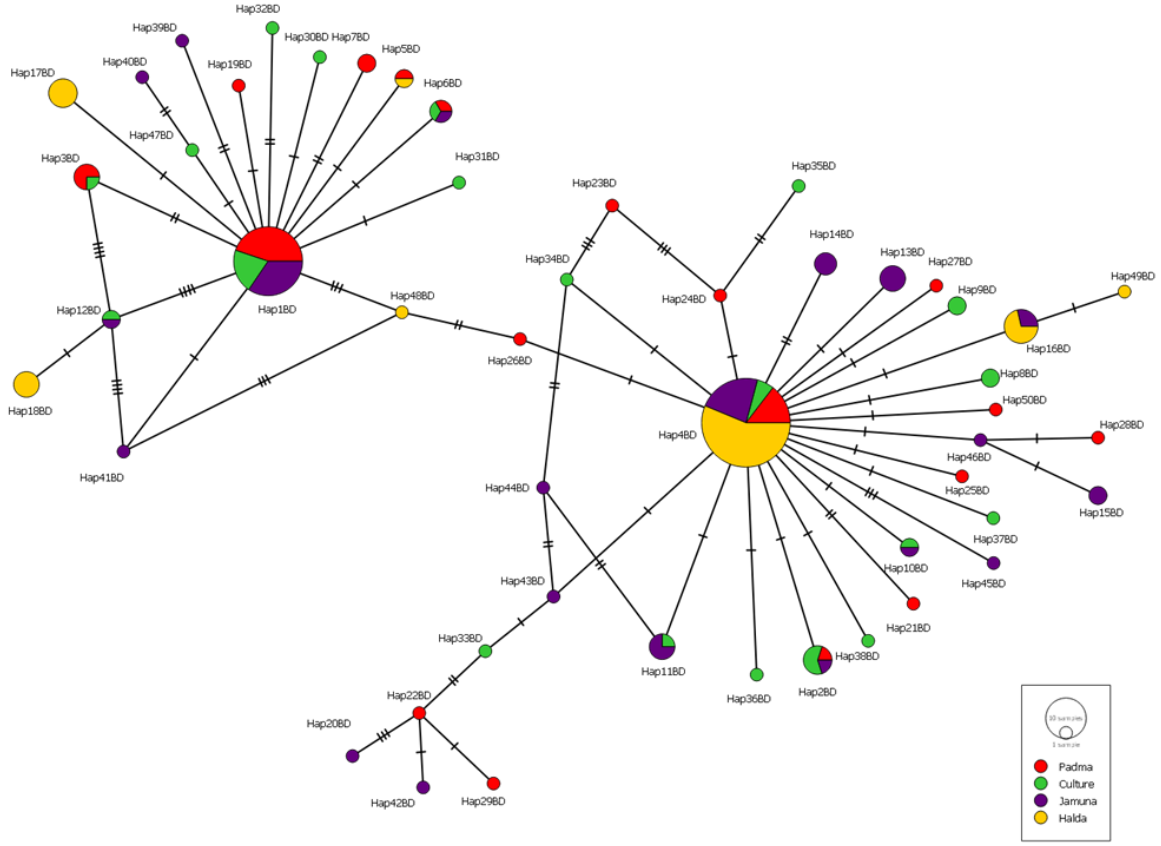

Figure 3.10: Haplotype network among Bangladesh's four *L. rohita* populations.

3.4 Analysis of nuclear *mstn* gene

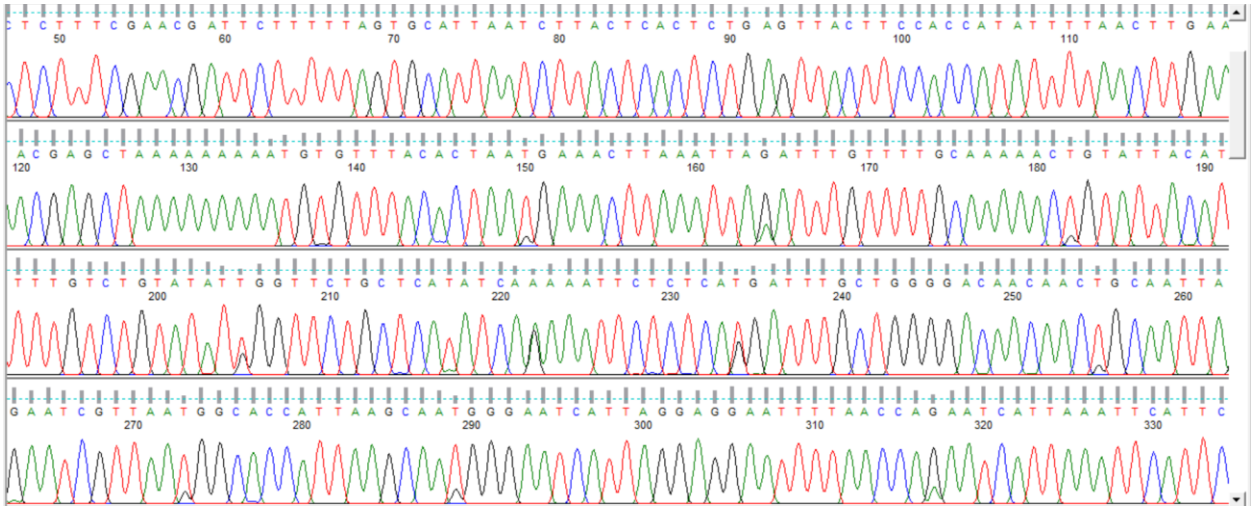

Figure 3.11 Sequence chromatogram of nuclear *mstn* gene (9A/9A) homozygous

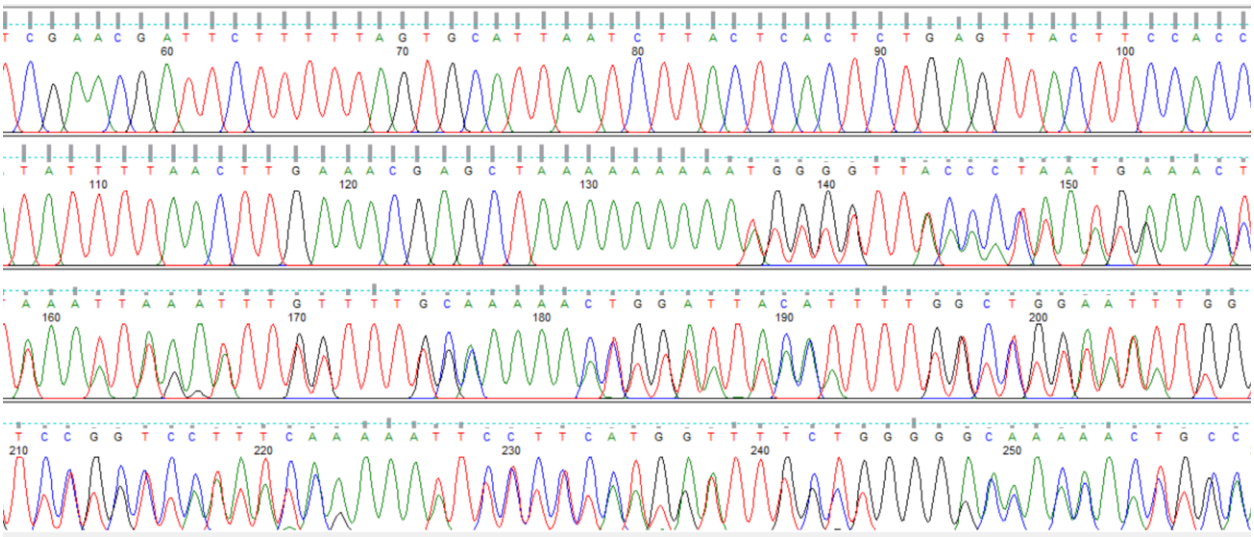

Figure 3.12 Sequence chromatogram of nuclear *mstn* gene (9A/10A) heterozygous

3.4.1 Genotyping and allelic frequencies of nuclear *mstn* gene

Table 3.14 Frequencies of genotypes and alleles for four single nucleotide polymorphisms (SNPs) of myostatin (*mstn*) gene in the *L. rohita* populations

Populations	Allele Frequencies (%)			Genotype Frequencies (%)				
	A9	*A10*	*A8*	*A9/A9*	*A9/A10*	*A8/A8*	*A8/A9*	*A10/A10*
Halda	89%	9%	2%	78%	18%	0	4%	0%
Jamuna	77%	20%	3%	58%	33%	0	6%	3%
Padma	72%	24%	4%	43%	48%	0	9%	0

Table 3.14 presents the allele and genotype frequencies for four single nucleotide polymorphisms (SNPs) in the myostatin (*mstn*) gene across *Labeo rohita* populations from three major rivers in Bangladesh: Halda, Jamuna, and Padma. The *A9* allele is predominant in all populations, with frequencies of 89%, 77%, and 72% in Halda, Jamuna, and Padma, respectively. The *A10* allele shows a higher presence in the Jamuna (20%) and Padma (24%) populations compared to Halda (9%). The *A8* allele has the lowest frequency across all populations, ranging from 2% in Halda to 4% in Padma. Overall, when combining all populations, the *A9* allele is the most common (79.33%), followed by *A10* (17.66%), and *A8* (3%).

In terms of genotype frequencies, the *A9/A9* genotype is dominant across all populations, with the highest occurrence in Halda (78%), followed by Jamuna (58%), and Padma (43%). The heterozygous genotype *A9/A10* shows a higher frequency in Padma (48%) and Jamuna (33%) compared to Halda (18%), suggesting a trend toward increased genetic variability in these populations. The *A8/A9* genotype appears at low frequencies in all populations, while the *A10/A10* genotype is only observed in the Jamuna population (3%). Notably, no individuals were homozygous for the *A8* allele. These findings suggest significant genetic differentiation among the river populations, indicating possible environmental influences or selective pressures shaping the genetic structure of *L. rohita*.

3.4.2 Genetic diversity analysis of *L. rohita* populations of nuclear *mstn* gene

The diversity analysis of the *mstn* gene across three populations of *L. rohita* from Bangladesh—Padma, Jamuna, and Halda—revealed significant variation in genetic diversity indices **(Table 3.15)**. The haplotypes ranged from 7 in the Jamuna and Halda populations to 8 in the Padma population, with 18 haplotypes identified across all populations. Haplotype diversity was highest in the Halda population (0.800) and lowest in the Jamuna population (0.345), with an overall diversity of 0.609.

Nucleotide diversity showed a similar trend, with the Halda population exhibiting the highest value (0.00296), followed by the Padma population (0.00281) and the Jamuna population (0.0011). Across all populations, the nucleotide diversity was 0.00244. The analysis of polymorphic sites indicated a total of 20 across all populations, with individual population values of 9, 10, and 6 for Padma, Jamuna, and Halda, respectively. The number of singleton sites was highest in the Jamuna population (10), while Padma and Halda exhibited 3 and 2 singleton sites, respectively. Parsimony sites were detected in all populations, with the highest number observed in the Padma population (6), followed by Halda (4), and none in Jamuna.

Monomorphic sites were predominant, with counts ranging from 549 across all populations to 563 in the Halda population, indicating the prevalence of conserved regions in the *mstn* gene. These findings highlight the variability in genetic diversity and polymorphism across the studied populations of *L. rohita*.

Table 3.15 Diversity analysis at myostatin (*MSTN*) gene in the *L. rohita*populations of Bangladesh.

Population	Padma	Jamuna	Halda	All
No of individual	32	32	20	84
No of Haplotype	8	7	7	18
Haplotype diversity	0.663	0.345	0.800	0.609
Nucleotide diversity	0.00281	0.0011	0.00296	0.00244
Monomorphic sites	560	559	563	549
Polymorphic sites	9	10	6	20
Singleton sites	3	10	2	13
Parsimony sites	6	0	4	7

3.4.3 Percentage of different haplotypes

Table 3.16 shows the percentage distribution of different haplotypes among three *Labeo rohita* populations sampled from Bangladesh's Padma, Jamuna, and Halda rivers. A total of 18 haplotypes (Hap01BD to Hap18BD) were identified, and their frequencies were calculated for each population as well as overall. Haplotype Hap05BD was the most dominant, constituting 61.90% of the total population and showing the highest prevalence in the Jamuna population (81.25%), followed by Padma (56.25%) and Halda (40%). Hap01BD and Hap02BD were moderately common, with their highest frequencies observed in Halda (10% and 20%, respectively). Hap04BD was exclusively present in Padma at a frequency of 15.62%.Rare haplotypes (Hap06BD to Hap18BD) displayed population-specific distributions. Hap09BD to Hap14BD were detected only in the Jamuna population, each contributing 3.12%. In contrast, the Halda population exhibited unique haplotypes (Hap15BD to Hap18BD) with frequencies ranging from 5% to 15%. These rare haplotypes were absent in both Padma and Jamuna populations.The findings highlight that the Jamuna population is highly dominated by a single haplotype (Hap05BD), whereas the Halda population exhibits greater diversity with several unique haplotypes. The Padma population shows a mix of moderately common haplotypes and some rare haplotypes. This variation in haplotype distribution underscores the genetic diversity and potential population structuring among the riverine populations of *L. rohita* in Bangladesh.

Table 3.16 Percentage of different haplotypes among the gene of *mstn* of *L. rohita* populations

Haplotypes	Total%	Padma%	Jamuna%	Halda%
Hap01BD	3.57%	3.12%	0%	10%
Hap02BD	8.34%	9.37%	0%	20%
Hap03BD	2.38%	6.25%	0%	0%
Hap04BD	5.95%	15.62%	0%	0%
Hap05BD	61.90%	56.25%	81.25%	40%
Hap06BD	1.20%	3.12%	0%	0%
Hap07BD	1.20%	3.12%	0%	0%
Hap08BD	1.20%	3.12%	0%	0%
Hap09BD	1.20%	0%	3.12%	0%
Hap10BD	1.20%	0%	3.12%	0%
Hap11BD	1.20%	0%	3.12%	0%
Hap12BD	1.20%	0%	3.12%	0%
Hap13BD	1.20%	0%	3.12%	0%
Hap14BD	1.20%	0%	3.12%	0%
Hap15BD	3.57%	0%	0%	15%
Hap16BD	1.20%	0%	0%	5%
Hap17BD	1.20%	0%	0%	5%
Hap18BD	1.20%	0%	0%	5%

3.4.4 Haplotype diversity and population-specific variations in the *L. rohita* population

The haplotype network of *L. rohita* populations from the Padma, Jamuna, and Halda rivers reveals distinct genetic structures and shared haplotypes among the populations **(Fig. 3.13).** The network shows a central haplotype shared by all three populations, suggesting a degree of genetic connectivity. However, unique haplotypes are also observed in each population, indicating population-specific genetic diversity. The Padma population (red) exhibits a higher proportion of unique haplotypes compared to the Jamuna (green) and Halda (purple) populations, which are represented by fewer distinct haplotypes. The mutational steps connecting haplotypes suggest a close genetic relationship among the populations, with limited differentiation. Overall, the network highlights a balance between shared genetic lineages and population-specific variability, likely shaped by historical gene flow and localized adaptation.

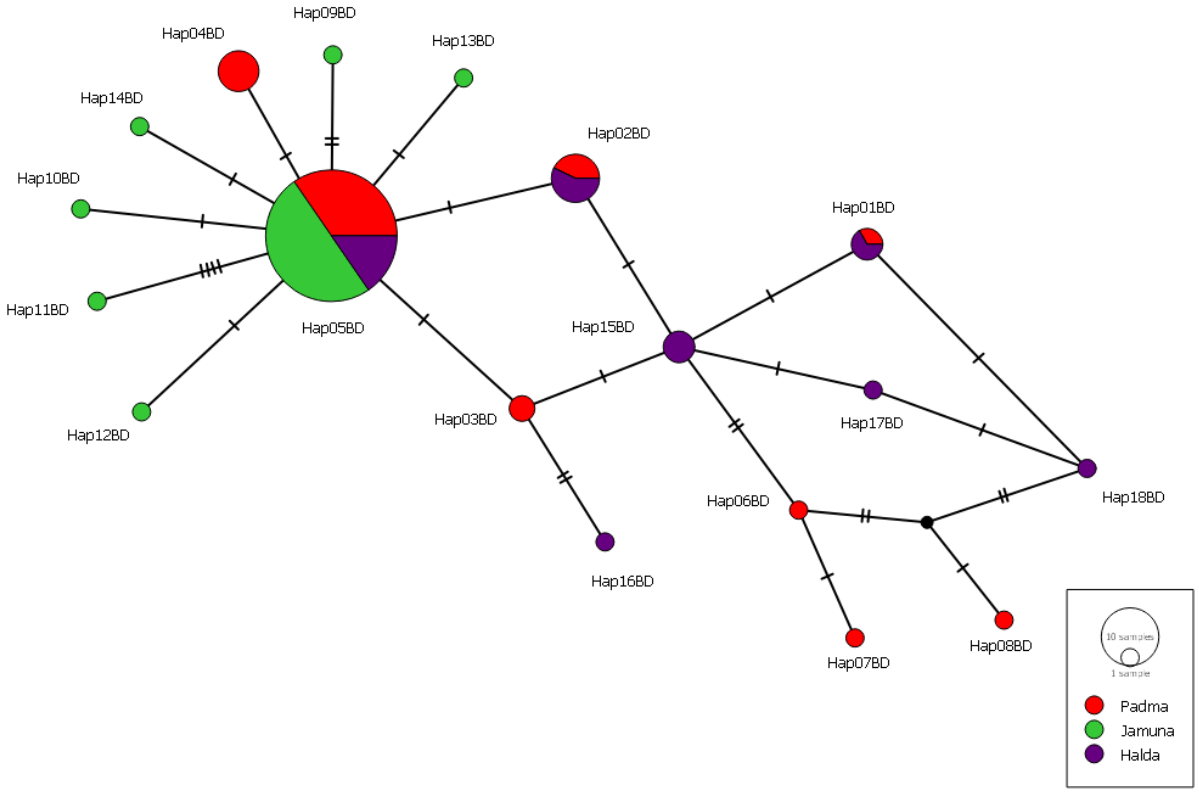

Figure 3.13: Haplotype network among Bangladesh's four *L. rohita* populations.

3.4.5 Phylogenetic analysis of *L. rohita mstn* gene

Figure 3.14 represents the phylogenetic relationships among the selected *mstn* haplotypes of *Labeo rohita* using the Maximum Likelihood (ML) method. The tree illustrates the genetic divergence and clustering patterns among the haplotypes, with branch lengths indicating evolutionary distances. Bootstrap support values are provided at key nodes, reflecting the reliability of the inferred relationships. The haplotypes are grouped into distinct clades, suggesting varying degrees of genetic differentiation within the studied populations. Notably, Hap07BD is positioned distantly from other haplotypes, indicating a potential unique lineage. Additionally, Hap05BD and Hap06BD form a closely related sub-cluster with strong bootstrap support (82), signifying a high level of genetic similarity. The presence of multiple haplotypes within certain clusters suggests genetic diversity within the *mstn* gene, which may be indicative of evolutionary forces shaping the population structure. Overall, the phylogenetic analysis highlights the genetic variation and evolutionary relationships among the *mstn* haplotypes, providing insights into the population genetics of *L. rohita*.

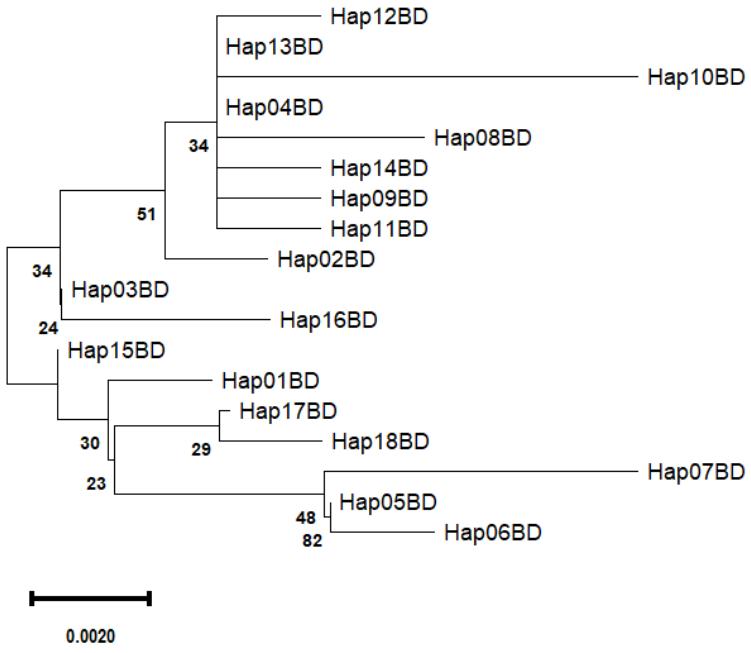

Figure 3.14 The Phylogenetic tree of the selected *mstn* haplotypes using the maximum likelihood method.

Table 3.17 Nucleotide composition of 18 haplotypes. Letters denote one-letter abbreviations for standard nucleotides

Haplotypes	T%	C%	A%	G%
Hap01BD	28.5	22.5	28.5	20.6
Hap02BD	28.3	22.5	28.6	20.6
Hap03BD	28.3	22.5	28.6	20.6
Hap04BD	27.9	22.7	28.6	20.6
Hap05BD	28.8	22.5	28.6	20.6
Hap06BD	29.0	22.5	28.6	20.6
Hap07BD	29.0	22.5	28.5	20.6
Hap08BD	27.9	22.5	28.5	20.6
Hap09BD	28.1	22.7	28.6	20.6
Hap10BD	27.4	22.7	28.6	21.3
Hap11BD	27.9	22.7	28.6	20.7
Hap12BD	27.9	22.7	28.5	20.7
Hap13BD	28.1	22.7	28.5	20.7
Hap14BD	28.3	22.7	28.3	20.6
Hap15BD	28.5	22.3	28.6	20.6
Hap16BD	28.3	22.5	28.6	20.6
Hap17BD	28.5	22.5	28.5	20.6
Hap18BD	28.5	22.7	28.3	20.6
Avg.	28.3	22.5	28.5	20.7

3.4.6 Nucleotide composition analysis

The nucleotide composition of 18 haplotypes showed (**Table 3.17**) minor variations in the proportions of thymine (T), cytosine (C), adenine (A), and guanine (G). The average nucleotide composition across all haplotypes was 28.3% T, 22.5% C, 28.5% A, and 20.7% G**.** Among the haplotypes, T content ranged from 27.4% (Hap10BD) to 29.0% (Hap06BD, Hap07BD), while C content was relatively stable (22.3%–22.7%)**.** Adenine (A) showed minor fluctuations between 28.3% (Hap14BD, Hap18BD) and 28.6%**,** whereas G content remained the most conserved, varying only between 20.6% and 21.3%**.** Despite minor differences, the overall nucleotide composition remained balanced, with A and T being more abundant than C and G. This AT-rich composition is consistent with typical mitochondrial/nuclear DNA sequences observed in related species.

4 Discussion

The present study analyzes the genetic diversity and population structure of *L. rohita* populations in Bangladesh using mitochondrial *cytb*, control region, and *mstn* gene sequences. The analysis includes polymorphic sites, haplotype distribution, genetic distances, nucleotide composition, diversity metrics, and population-specific variations. Additionally, the study employs molecular variance analysis (AMOVA), pairwise Fst values, haplotype network analysis, phylogenetic analysis, and restriction fragment length polymorphism to provide a comprehensive understanding of the genetic diversity of these populations. In Bangladesh, *L. rohita* is a popular aquaculture species, and farmers used to harvest seeds from several rivers for aquaculture. The genetic structure of the *L. rohita* wild population is expected to have evolved, although relatively few studies have been done in this area. Previously, RAPD and allozyme markers were used to examine the genetic structure of *L. rohita* from the Halda, Jamuna, and Padma rivers (Islam and Alam, 2004; Khan *et al.,* 2006). Microsatellite markers were utilized to genetically characterize three wild populations of *L. rohita* taken from the Halda, Jamuna, and Padma rivers (Alam *et al.,* 2009).

4.1 *Cytb* gene

4.1.1 Haplotype distribution and genetic divergence in *L. rohita*

Among the four populations analyzed (three wild and one cultured), the cultured samples exhibited the highest haplotype and nucleotide diversity, followed by the Halda population. This elevated genetic diversity in the cultured group may be attributed to the mixed origin of broodstock commonly used in aquaculture practices, where individuals from different wild populations are often combined to enhance productivity. Such mixing can introduce and maintain a broader range of alleles, thereby increasing both haplotype and nucleotide diversity. The haplotype diversity (0.385) and nucleotide diversity (0.00118) observed in this study fall within the range previously reported for freshwater fish populations in Bangladesh and India (Grant and Bowen, 1998; Das et al., 2018; Joshi et al., 2019; Sah et al., 2011; Das et al., 2014; Pakrashi et al., 2015; Behera et al., 2016; Habib and Vindhya, 2011; Luhariya et al., 2012).

The sequencing of a 616 bp fragment of the *cytb* gene identified 11 haplotypes, characterized by 10 polymorphic sites, reflecting relatively low sequence variation (1.6%). Hap1BD was the most

prevalent haplotype, found across all four populations, comprising 78% of the individuals. This suggests that Hap1BD is likely the ancestral lineage, with the other haplotypes having diverged through mutations. Haplotypes Hap3BD, Hap6BD, and Hap7BD were shared between two populations, while the remaining haplotypes (Hap2BD, Hap4BD, Hap5BD, Hap8BD, Hap9BD, Hap10BD, and Hap11BD) were unique to specific populations. The widespread distribution of Hap1BD further supports its role as the earliest mitochondrial lineage from which other haplotypes emerged (Charlesworth, 2009).

Among the studied river populations, the Jamuna River exhibited the highest haplotype richness, with six haplotypes, although at low frequencies. This suggests that increasing the sample size could reveal additional haplotypes, providing a more comprehensive understanding of the genetic variation within this population. The conservation of these unique haplotypes should be a priority, as they contribute to the species' genetic diversity and may be vital for its long-term adaptability. Pairwise genetic distance analysis indicated varying levels of genetic differentiation among haplotypes, with Hap1BD showing the greatest genetic distance from Hap9BD, signifying substantial genetic divergence. These findings offer valuable insights into the genetic structure and evolutionary history of *L. rohita*, highlighting the importance of targeted conservation strategies to preserve genetic diversity within natural populations.

4.1.2 Nucleotide composition and molecular characteristics of the *cytb* gene in *L. rohita*

The nucleotide composition analysis of the *cytb* gene sequences provided crucial insights into the molecular characteristics of *L. rohita* populations. The observed nucleotide distribution 28.9% adenine, 27.5% thymine, 14.0% guanine, and 29.7% cytosine—falls within the range reported for freshwater fishes in previous studies (Esposti *et al.,* 1993; Cantatore *et al.,* 1994; Nei & Kumar, 2000; Yang *et al.,* 2006; Sarmasik *et al.,* 2008; Devi *et al.,* 2014; Li *et al.,* 2016; Alam *et al.,* 2021; Wei *et al.,* 2021; Choudhury & Das, 2021; Sarmah *et al.,* 2022). Specifically, our findings align with the reported range of adenine (25.5%–31.5%), thymine (24.5%–29.5%), and guanine (14.0%–17.5%), suggesting a degree of evolutionary conservation in mitochondrial nucleotide composition within the species. The relatively high AT content observed in *L. rohita* mitochondrial DNA is consistent with trends observed in other teleost fishes, where AT-rich sequences are

thought to enhance genome stability and replication efficiency. This pattern may also be influenced by selective constraints on mitochondrial genes, which are essential for cellular energy metabolism. The variations in nucleotide composition across haplotypes could be indicative of historical mutational processes, genetic drift, or adaptive selection, reflecting population-specific evolutionary trajectories.

These findings contribute to a broader understanding of mitochondrial genetic variation in *L. rohita* and establish a foundation for further investigations into population genetics, phylogenetics, and evolutionary biology. Future studies incorporating whole mitochondrial genome sequencing and comparative analyses with related cyprinid species may provide deeper insights into the functional and adaptive significance of these nucleotide variations.

4.1.3 Phylogenetic and population structure analysis of *L. rohita*

The phylogenetic analysis of the *cytb* gene in *L. rohita* provided valuable insights into the genetic relationships among haplotypes, revealing both population-specific and shared haplotypes. The haplotype network analysis highlighted unique genetic characteristics within and between populations, reflecting historical and contemporary evolutionary processes. The UPGMA dendrogram identified two distinct genetic clusters among the four studied populations, with one cluster consisting of the Halda River population and the other comprising the Jamuna and Padma River populations. This clustering pattern indicates significant genetic differentiation among populations, which is consistent with earlier studies on *L. rohita* from the Halda, Padma, and Jamuna rivers (Islam & Alam, 2004).

Our findings align with previous research on population genetic structures in *L. rohita* using different molecular markers. Alam et al. (2009) also reported two distinct clusters in major river populations of Rohu based on microsatellite DNA markers, demonstrating congruence between mitochondrial and nuclear DNA analyses. Similar clustering patterns have been observed in related species, such as *Catla catla*, where microsatellite marker studies revealed distinct population groupings (Rahman *et al.,* 2009; Ali *et al.,* 2015). The consistency of these findings across different

genetic markers suggests that these genetic clusters represent stable population subdivisions, likely maintained by geographic barriers, restricted gene flow, or local adaptations.

Comparative studies in other cyprinid species further support the role of genetic clustering in population differentiation. In *Cirrhinus mrigala,* morphometric analyses identified four major clusters, highlighting substantial intraspecific variation across geographical regions (Rasool *et al.,* 2013). Similarly, studies on *Labeo ariza* revealed a distinct genetic structure, with the Jamuna and Brahmaputra populations forming a single cluster, separate from the Atrai population (Ak *et al.,* 2015). These findings reinforce the importance of phylogenetic and population structure analyses in understanding genetic diversity and guiding conservation strategies.

The identification of genetically distinct clusters in *L. rohita* underscores the necessity of conservation efforts, particularly for wild populations, to maintain genetic diversity and ensure the sustainability of natural stocks. Given that genetic diversity plays a crucial role in adaptability and resilience to environmental changes, conservation strategies should prioritize the protection of unique haplotypes and genetic lineages. Future studies incorporating genome-wide markers and expanded sampling across broader geographical ranges may provide deeper insights into the evolutionary history and genetic structure of *L. rohita* populations, thereby informing more effective management and stock enhancement programs.

4.1.4 Genetic structure and variation in *L. rohita* populations based on *cytb* gene

The present study examined the genetic structure of three riverine and one hatchery population of *L. rohita*, revealing a higher within-population variation (90.35%) compared to among-population variation (9.65%), as determined by AMOVA. These findings are consistent with previous studies on *L. rohita* populations from India (Hamilton *et al.,* 2019; Sahoo *et al.,* 2018; Das *et al.,* 2012, 2018; Joshi *et al.,* 2019) and suggest a significant level of gene flow among populations (Slatkin & Barton, 1989; Allendorf *et al.,* 2012). A similar pattern was reported by Barman et al. (2003), who observed high within-species genetic similarity (97.5%) in farmed stocks of major carp in India, indicating relatively low levels of genetic differentiation.

Gene flow plays a crucial role in shaping genetic structure by reducing differentiation among populations. As suggested by Ferguso et al. (1995), extensive gene flow can contribute to genetic homogeneity, which may explain the relatively low fixation index (Fst = 0.0965; p = 0.0) observed in this study. This finding aligns with previous studies (Weir & Cockerham, 1984; Excoffier *et al.,* 1992) that also reported minimal genetic variation among populations of *L. rohita*.

Maintaining genetic variability is essential for population fitness and resilience. Carvalho et al. (1993) emphasized that individuals with higher genetic diversity exhibit improved growth rates, developmental stability, reproductive success, and resistance to environmental stress and diseases. Given the relatively low genetic differentiation observed, conservation and breeding programs should focus on strategies that preserve genetic diversity, such as maintaining wild populations and implementing responsible aquaculture practices. Future research integrating genome-wide markers and a broader geographic sampling range could provide a more comprehensive understanding of gene flow dynamics and their implications for *L. rohita* conservation and management

4.1.5 Genetic connectivity and population management based on *cytb* gene

Among the four populations studied, we observed low-to-moderate levels of genetic differentiation, with pairwise Fst values ranging from 0.00376 to 0.17764. This aligns with the findings of Das et al. (2012), who reported similar low differentiation using mtDNA markers, and Hamilton et al. (2019), who noted low SNP marker diversity and a lack of genetic differentiation. The greatest genetic difference (Fst = 0.17764) was observed between the Padma and Culture populations, while the smallest difference (Fst = 0.00376) occurred between the Padma and Jamuna populations, suggesting relatively high gene flow between the Padma and Jamuna rivers. This is consistent with previous studies (Alam *et al.,* 2009), which also noted high gene flow between these two rivers. The reduced genetic differentiation between Padma and Jamuna populations may be attributed to smaller genetic distances and more gene flow.

However, the Padma and Jamuna populations showed significant divergence from the Halda and Culture populations at the 5% level. A similar pattern was found by Islam et al. (2004) in a RAPD-based study, where significant genetic differences were observed between the Halda and Padma

populations, and between the Padma and Culture populations, though these differences were not statistically significant. These findings are also supported by Alam et al. (2009), who reported significant population differentiation (Fst) between the Halda and Jamuna populations. It should be noted, however, that the Fst values in the present study may not be directly comparable to those from past studies due to differences in the DNA markers used. Despite this, the relative genetic distances among the populations remain consistent with previous research.

Our study also identified a notable genetic distance between the Halda and Jamuna populations (Fst = 0.05632, $p < 0.01$), as well as between the Halda and Padma populations (Fst = 0.11987, $p < 0.01$). Geographically, Padma and Jamuna are connected near Goalanda Upazila in the Rajbari district, while the Halda River is located further southeast (Akter *et al.,* 2012), which may account for the higher genetic distances observed between Halda and the other two populations. The presence of geographical barriers between the Halda and both the Padma and Jamuna rivers could further explain the observed genetic isolation.

Regarding the Culture population, no significant genetic differentiation was found between it and the Halda population (Fst = 0.05701, $p = 0.063$). This suggests that Culture stocks may have predominantly originated from the Halda River, though the presence of unique haplotypes in the Culture population implies the involvement of other sources, such as different rivers, haors, or beels. The higher haplotype and nucleotide diversity in the Culture population compared to wild populations further supports the idea that Culture stocks represent a mixture of various sources.

Geographically, Padma is closer to Jamuna than to Halda, with the two rivers being interconnected near Goalanda. This proximity likely facilitates the mixing of the Padma and Jamuna populations, which is reflected in the relatively low Fst values between them. In contrast, the Halda population is more genetically distinct, possibly due to its geographical isolation. These results echo previous findings by Alam et al. (2009), who observed low Fst values and strong gene flow between Padma and Jamuna, while noting greater differentiation between the Halda and the other rivers.

The genetic similarities between the Halda and Culture populations, as indicated by the low Fst values, suggest that Culture stocks may primarily derive from the Halda River, although additional sources may contribute to the genetic makeup of the Culture population. Halda is renowned as the largest natural breeding ground for carp species in Bangladesh (Saleh, 2020), and as a tidal river, it is the only river in the world where fertilized eggs can be directly collected (Kibria *et al.,* 2009).

Consequently, gene flow from the Halda River to hatcheries and culture systems may have contributed to the reduced genetic distance between the Halda and Culture populations in this study. Moreover, the mixed genetic composition of Culture samples, which likely originate from multiple sources, such as different rivers, lakes, and haors, could explain the significant differences observed between the Culture population and both the Padma and Jamuna populations.

4.1.6 Conservation Policies and their impact on genetic diversity

The historical timeline of fish conservation and protection regulations indicates that the Halda River, a crucial location for carp fish spawning, has been under a year-round fishing ban since 2007. In contrast, the Jamuna and Padma Rivers did not have such a strict ban until the current research began in 2021. The Halda has been the best breeding ground for carp fishes in Bangladesh, evidenced by higher genetic diversity in almost all previous and present reports (Islam *et al.,* 2004; Alam *et al.,* 2009; Tonny *et al.,* 2014). There might be many causes for these differences among the rivers, starting from river basin structure, water quality, water flow, river management, and conservation policies to the societal and economic status of the local peoples. However, the analysis of the genetic diversity studies published after 2007, including the present one, reveals that the genetic distances between Halda and Padma and Halda and Jamuna have increased over time, regardless of the overall decline of genetic variability in the *L. rohita* populations of Bangladesh (Islam *et al.,* 2004; Alam *et al.,* 2009; Tonny *et al.,* 2014).

In 2004, genetic diversity measures revealed low genetic differentiation among the three river populations of *L. rohita* (Islam *et al.,* 2004). Subsequent studies conducted after 2007 reported increased differences among the populations, with the Halda River gaining stricter policy measures compared to two other rivers (Alam *et al.,* 2009, Tonny *et al.,* 2014). The lack of stricter and specific conservation policy intervention for *L. rohita* in the Padma River may have contributed to a notable increase in genetic distance between the Halda and Padma River populations in 2014 compared to 2009. In the present study, the genetic distance between Halda and Padma rivers, as indicated by the pairwise Fst value, is the highest (0.119, significant) among the river pairs, which may reflect the conservation policy gaps between these two rivers. Similarly, the Halda and Jamuna populations also differentiate significantly from each other. Thus, the population genetic

data corresponds to the intensity of previous fishing interventions. However, policy efforts alone are insufficient for conserving biodiversity and genetic diversity.

The impact of legal frameworks on biodiversity depends on how effectively they are implemented. There are challenges in implementing a total fishing ban on a river associated with the livelihood of local people (Azadi and Alam, 2011). It is important to apply strict conservation rules where anthropogenic pressures are high. Factors such as habitat degradation and destruction due to water pollution, encroachment, riverine transport and siltation, unauthorized fishing during the prohibited period, lack of awareness among stakeholders, absence of alternative livelihood options, and natural disasters may also have their impact. Research data on these factors concerning the three major rivers of the present study is largely unavailable. Still, the chronological analysis of the previous and present population genetic structure of *L. rohita* and policy efforts in the respective rivers suggest that the appropriate conservation policies with proper implementation might positively impact the conservation of population diversity.

In our discussion about the differences in conservation strategies among rivers, we have observed a potential relationship between conservation policy efforts and variations in genetic diversity. While this correlation does not necessarily prove a cause-and-effect relationship, it stresses the importance of implementing stricter conservation strategies to protect fish and other species. Furthermore, our work suggests the need to enhance the genetic diversity of less diverse populations, such as those in the Padma and Jamuna rivers, through controlled fishing practices, reintroduction, and other measures. Additionally, there is a need to develop commercial, allele-specific molecular markers for measuring population differences and supporting selective breeding programs. Conducting further studies on the genetic status and diversity of *L. rohita* populations across more areas will provide crucial data for improving conservation efforts and biodiversity management. This study can be extended to other fish species as well.

4.2 Control region sequence

4.2.1 Haplotype variation and genetic structure of *L. rohita* from control region analysis

The control region sequences from *L. rohita* populations revealed a total of 50 distinct haplotypes. However, our analysis focused on 18 haplotypes, each represented by at least two individuals. Among these, haplotype HP04 was found in all populations, suggesting it may be an ancestral haplotype. The presence of this haplotype across multiple populations indicates a shared genetic background and ongoing gene flow among them (Das *et al.,* 2017). In contrast, some haplotypes were unique to specific populations, implying restricted gene flow and independent haplotype emergence, likely due to mutation events (Sahoo *et al.,* 2018).

The identification of population-specific haplotypes suggests that certain populations have undergone localized genetic differentiation. Seven out of the 18 haplotypes were unique to specific populations, highlighting restricted genetic exchange. This finding aligns with the hypothesis that limited migration or reproductive isolation can lead to the independent emergence of haplotypes. These private haplotypes could serve as valuable genetic markers for future stock identification and management (Chenoweth *et al.,* 1998). Their presence further supports the role of mutation in shaping genetic diversity within *L. rohita* populations (Sahoo *et al.,* 2018).

The genetic analysis of control region identified 574 monomorphic sites, 24 polymorphic sites, 10 singleton sites, and 14 parsimony-informative sites, indicating a moderate level of genetic variation within the populations. The number of individuals per haplotype ranged from 2 to 48, further reflecting genetic diversity and population structure. The relatively high number of private haplotypes suggests that mutation-driven diversity plays a crucial role in shaping the genetic landscape of *L. rohita* populations. The presence of a widespread haplotype (HP04) alongside several population-specific haplotypes suggests a balance between historical gene flow and localized genetic differentiation. These findings provide valuable insights into the genetic structuring of *L. rohita* and underscore the need for population-specific conservation strategies.

The results emphasize the importance of genetic monitoring in managing *L. rohita* populations. The presence of shared and unique haplotypes highlights the need for tailored conservation strategies that consider both genetic connectivity and localized adaptations. The identification of private haplotypes could assist in the delineation of distinct genetic stocks, facilitating targeted breeding and conservation programs. Moreover, the observed genetic diversity underscores the significance of maintaining diverse genetic resources to ensure the sustainability and resilience of *L. rohita* populations.

The control region analysis of *L. rohita* populations revealed a complex genetic structure characterized by both widespread and unique haplotypes. The presence of haplotype HP04 in all populations suggests a common ancestral lineage, whereas the identification of population-specific haplotypes highlights restricted gene flow and independent evolutionary trajectories. These findings provide valuable genetic insights into *L. rohita* populations and have important implications for their conservation and management.

4.2.2 Nucleotide composition patterns in mitochondrial control region

The control region sequences of *L. rohita* populations in this study revealed notable genetic diversity and population structure. The nucleotide composition of 18 haplotypes exhibited a consistent pattern, with an average composition of 31.8% thymine (T), 18.6% cytosine (C), 36.2% adenine (A), and 13.4% guanine (G). This composition mirrors the general structure seen in other fish species, where the A+T content is higher than G+C. This characteristic is in line with previous studies that show similar nucleotide distribution patterns in mitochondrial genomes across various species, including red crucian carp, common carp, and zebrafish (Sbisa *et al.,* 1997; Pesole *et al.,* 1999).

The control region, being a non-coding region of the mitochondrial genome, evolves at a faster rate than the protein-coding genes and rRNA genes, making it a useful marker for genetic diversity studies (Guo *et al.,* 2003). The observed A>T>C>G nucleotide composition is consistent with findings from other fish species (Sahoo *et al.,* 2019; Johns and Avise, 1998; Bej *et al.,* 2012; Swain *et al.,* 2014). This pattern of nucleotide distribution is common in the mitochondrial genomes of

animals and is thought to reflect a bias in mitochondrial DNA replication and repair mechanisms (Brown, 1985).

The higher A+T content in *L. rohita*'s control region aligns with trends observed in other species within the family Cyprinidae. This suggests that mitochondrial control regions of fish might share some common evolutionary pressures, leading to similar nucleotide biases across diverse species. These findings offer further insight into the genetic structure of *L. rohita* populations, and the control region's variability could potentially be useful in delineating population boundaries and understanding evolutionary relationships within and between populations.

4.2.3 Genetic divergence in *L. rohita* population based on control region

In the present study, *L. rohita* populations displayed notable genetic diversity, as indicated by the high haplotype diversity (Hd = 0.876) and moderate nucleotide diversity (π = 0.00706). These values are consistent with similar studies on freshwater fish populations (Habib *et al.,* 2012) and suggest a healthy level of genetic variation within these populations. The combination of high haplotype and moderate nucleotide diversity may indicate a historical population bottleneck, which could have been caused by environmental factors such as habitat reduction and limited resources (Craul *et al.,* 2009; Goossens *et al.,* 2006). Such bottlenecks are common in populations that have experienced significant fluctuations in size due to changes in habitat or other ecological pressures.

An intriguing finding in this study was the observation that the cultured *L. rohita* population exhibited both the highest haplotype and nucleotide diversity among the populations analyzed. This increased diversity can likely be attributed to artificial breeding practices, which often incorporate genetic material from multiple sources. These practices can help maintain genetic variation and prevent inbreeding depression, a crucial factor in the long-term sustainability of cultured populations.

Genetic diversity is essential for species' survival and adaptability, as it allows populations to respond effectively to changing environmental conditions. The measures of haplotype and

nucleotide diversity serve as important indicators of genetic health (Keller and Waller, 2002; Frankham, 2005). In this study, the genetic differentiation index indicated minimal differentiation between populations within the study area. A low differentiation value (close to 0) suggests that gene flow among populations is high, and there is limited genetic isolation. On the other hand, higher values would indicate more substantial genetic divergence. The observed genetic variation within and among populations underscores the ability of *L. rohita* to adapt to various ecological conditions in different river systems.

Among the wild populations, the Jamuna River population stood out with the highest genetic diversity. The Jamuna River, one of Bangladesh's largest and most dynamic river systems, is characterized by an extensive network of rivers and tributaries. This interconnected system facilitates greater gene flow, which likely contributes to the observed higher diversity. In contrast, the Halda River population exhibited the lowest genetic diversity. The Halda River is relatively isolated compared to the Jamuna and Padma Rivers, which may limit gene flow and contribute to reduced genetic variation. These findings highlight the importance of habitat connectivity in maintaining genetic diversity and the potential risks posed by geographical isolation.

The observed levels of genetic diversity in *L. rohita* are within the expected range for freshwater fish populations in Bangladesh and India (Grant and Bowen, 1998; Das *et al.,* 2018; Joshi *et al.,* 2019). These findings support the idea that *L. rohita* populations in the region maintain a relatively high level of genetic variation, which is crucial for the species' long-term adaptability. Furthermore, the faster evolutionary rate observed in the mitochondrial control region of *L. rohita*, compared to other mitochondrial genes, is consistent with previous studies on related fish species (Sbisa *et al.,* 1997; Pesole *et al.,* 1999). The higher mutation rate in the control region allows it to serve as a useful marker for population genetic studies, providing insights into the evolutionary history and population structure of *L. rohita*.

Our present results of this study underscore the significance of habitat connectivity, artificial breeding practices, and evolutionary dynamics in shaping the genetic diversity of *L. rohita*. The findings contribute to a deeper understanding of the genetic structure of this species, with implications for its conservation and management.

4.2.4 Genetic structure and variation in *L. rohita* populations based on control region sequence

The analysis of molecular variance (AMOVA) revealed that the majority of genetic variation (95.302%) in *L. rohita* populations exists within populations, with only 4.697% attributed to differences among populations. This pattern is consistent with previous studies (Hamilton *et al.,* 2019; Sahoo *et al.,* 2018; Das *et al.,* 2012; Das *et al.,* 2018; Joshi *et al.,* 2019), suggesting that substantial gene flow among populations plays a critical role in shaping genetic structure (Slatkin and Barton, 1989; Allendorf *et al.,* 2012). High within-population genetic variation, coupled with low among-population differentiation, indicates the presence of effective gene flow, which homogenizes genetic differences and maintains diversity within populations.

The observed genetic homogeneity among *L. rohita* populations aligns with findings from Barman et al. (2003), who reported a high degree of genetic similarity (97.5%) in farmed stocks of major carp in India. This suggests that gene flow, facilitated by both natural and anthropogenic factors such as river connectivity and aquaculture practice, plays a crucial role in reducing genetic differentiation while preserving within-population genetic diversity (Ferguson *et al.,* 1995). The fixation index (Fst = 0.04698, $p < 0.05$) further supports this observation, indicating minimal genetic differentiation among populations, consistent with previous reports (Weir and Cockerham, 1984; Excoffier *et al.,* 1992).

Genetic variability is essential for species resilience, as it enhances adaptability to environmental changes, reproductive success, and resistance to diseases (Carvalho *et al.,* 1993). The population diversity analysis in this study showed variable haplotype and nucleotide diversity among riverine and cultured populations, reinforcing the role of geographic and ecological factors in shaping genetic structure. The presence of significant genetic differentiation among *L. rohita* populations, as reflected in pairwise Fst values (0.00677 to 0.14804), suggests that while gene flow is generally high, certain populations experience restricted genetic exchange due to geographic separation and unique haplotype compositions (Behera *et al.,* 2017).

The highest genetic differentiation (Fst = 0.14804) was observed between the Padma and Halda populations, likely due to their geographical distance and limited connectivity, which impedes gene flow. Conversely, the lowest genetic differentiation (Fst = 0.00677) between the Padma and cultured populations indicates a high level of genetic exchange, likely facilitated by the use of wild Padma stock in aquaculture, contributing to genetic homogenization. The significant divergence of the Padma population from the Halda and Jamuna populations at the 5% level underscores the impact of localized environmental factors and restricted migration patterns on genetic differentiation.

Previous studies using different molecular markers have reported similar patterns of genetic differentiation. Islam et al. (2004) observed comparable results in a RAPD-based study, where the highest genetic difference was noted between the Halda and Padma populations, while the Padma and cultured populations exhibited the least differentiation. Likewise, a microsatellite DNA marker-based study by Alam et al. (2009) reported significant genetic differentiation between the Halda and Jamuna populations. However, it is important to note that direct comparisons between studies using different genetic markers should be made with caution, as methodological differences may influence the estimated levels of genetic differentiation.

Despite these methodological variations, the findings of the present study provide valuable insights into the genetic structure of *L. rohita* populations, which can be instrumental in future conservation and management strategies. The significant divergence observed in the Halda population suggests that it may be more vulnerable to environmental changes and anthropogenic pressures, necessitating targeted conservation efforts. Additionally, continuous genetic monitoring using high-resolution genomic tools could further elucidate population dynamics and inform sustainable fisheries management practices.

4.2.5 Genetic clustering of *L. rohita* populations based on control region

The UPGMA dendrogram constructed using control region sequences effectively illustrates the genetic relationships among four populations of *L. rohita*. The close clustering of the Jamuna and Halda populations, supported by a high bootstrap value of 100, indicates strong genetic similarity

between these two populations. In contrast, the Padma population exhibits a greater genetic distance from the others, forming a separate branch. This clear genetic differentiation among populations suggests distinct evolutionary lineages and potential habitat-specific adaptations.

Our findings are consistent with previous studies on *L. rohita* population genetics. Islam and Alam (2004) similarly identified significant genetic differentiation among populations from the Halda, Padma, and Jamuna rivers. Moreover, Alam et al. (2009) reported two distinct genetic clusters in riverine Rohu populations using microsatellite DNA markers, demonstrating congruence between mitochondrial and nuclear DNA analyses. The observed clustering pattern aligns with studies on related species, such as *Catla catla*, where microsatellite marker analyses revealed distinct genetic groupings (Rahman *et al.,* 2009; Ali *et al.,* 2015). These similarities reinforce the robustness of using mitochondrial and nuclear markers to assess genetic structure in freshwater fish populations.

The haplotype network further highlights genetic relationships and diversity among the four *L. rohita* populations. The presence of larger haplotypes shared among multiple populations suggests ancestral or widely distributed haplotypes, which may indicate historical gene flow or common ancestry. The distinct separation of certain populations in both the UPGMA tree and haplotype network underscores the role of geographical barriers and ecological factors in shaping genetic divergence.

The UPGMA analysis, along with haplotype network patterns, provides valuable insights into the genetic differentiation and connectivity of *L. rohita* populations. These findings contribute to a broader understanding of population structure and genetic diversity, which is essential for developing effective conservation and management strategies for this ecologically and economically significant species.

4.3 *Mstn* gene

4.3.1 Uncovering homozygous and heterozygous variability in *L. rohita* population based on mstn gene

The present study examines the genetic diversity and population structure of *L. rohita* populations from three major rivers in Bangladesh—Halda, Jamuna, and Padma—using single nucleotide polymorphisms (SNPs) in the myostatin*1* (*mstn*) gene. The observed differences in allele and genotype frequencies, alongside diversity indices, indicate significant genetic differentiation among the studied populations. Previous studies utilizing RAPD and allozyme markers (Islam and Alam, 2004; Khan *et al.,* 2006) have provided insights into the genetic structure of *L. rohita* in these rivers. However, the application of microsatellite DNA markers has been widely acknowledged as a more robust and reliable approach for detecting genetic variability, offering a more precise assessment of population differentiation.

Our findings reveal distinct allelic and haplotypic patterns among the populations, highlighting the role of genetic variation in shaping population structure. The predominance of the *A9* allele across all populations suggests that this allele may confer a selective advantage or be under stabilizing selection. Notably, the higher frequencies of the *A10* allele in the Jamuna (20%) and Padma (24%) populations imply potential differential selective pressures or environmental influences unique to these regions. Conversely, the *A8* allele exhibited the lowest frequency (2–4%), with no homozygous individuals detected, suggesting its rarity and possible selective disadvantage.

Genotypic distributions further reinforce these patterns. The dominance of the *A9/A9* genotype and the elevated heterozygosity levels in the Padma and Jamuna populations suggest higher genetic variability, potentially influenced by ecological or anthropogenic factors such as habitat fragmentation, fishing pressures, and environmental pollution. In contrast, the Halda population demonstrated the highest haplotype diversity (0.800) and nucleotide diversity (0.00296), which may be attributed to its relatively undisturbed habitat, enabling the retention of a rich genetic reservoir. Conversely, the Jamuna population exhibited the lowest haplotype diversity (0.345), indicating restricted genetic variability, which could be a consequence of genetic drift or habitat degradation.

Our present findings underscore the importance of conserving genetic diversity within *L. rohita* populations to ensure their long-term adaptability and resilience. The observed genetic differentiation among populations highlights the need for region-specific conservation strategies, particularly in the Jamuna and Padma rivers, where genetic variability appears to be relatively constrained. Further studies incorporating additional nuclear and mitochondrial markers, alongside environmental assessments, would provide a more comprehensive understanding of the evolutionary dynamics influencing *L. rohita* populations in Bangladesh.

4.3.2 Genetic Diversity of *L. rohita* based on *mstn* gene

The population genetic analysis of *L. rohita* from the wild population revealed moderate levels of genetic diversity, as evidenced by the observed haplotype diversity (0.609) and nucleotide diversity (0.00244). These values align with previous studies on freshwater fish populations in Bangladesh and India, which reported similar diversity indices based on mitochondrial and nuclear DNA markers (Islam & Alam, 2004; Islam *et al.,* 2005; Khan *et al.,* 2006; Mostafa *et al.,* 2009; Alam *et al.,* 2009; Tonny *et al.,* 2014; Nesa *et al.,* 2018; Grant & Bowen, 1998; Das *et al.,* 2018; Joshi *et al.,* 2019). This consistency suggests that the genetic diversity of *L. rohita* populations in these regions may be governed by similar ecological and evolutionary processes.

A total of 18 haplotypes were identified across 84 individuals, with haplotype Hap5BD being the most prevalent, comprising 61.9% of the sample. This dominant haplotype may represent an ancient mitochondrial lineage, from which other haplotypes could have emerged through mutations, as suggested by Charlesworth (2009). The predominance of this haplotype underscores the role of historical evolutionary events in shaping the current genetic structure of *L. rohita* populations. The observation that 12 of the 18 haplotypes were unique to specific populations further suggests that gene flow between populations is restricted, which may lead to the formation of population-specific genetic markers.

These findings highlight the importance of conserving unique haplotypes to preserve the genetic diversity of *L. rohita*, as the loss of such diversity could impair the species' adaptive potential and resilience to environmental changes. Furthermore, the discovery of private haplotypes specific to

certain populations points to the potential for using these markers in management strategies aimed at identifying distinct stocks and improving aquaculture practices. The identification of these private haplotypes supports the hypothesis of independent origins driven by mutations, which could contribute to the differentiation of these populations over time (Sahoo *et al.,* 2018).

The results of this study emphasize the need for targeted conservation efforts and the inclusion of additional samples to further enhance our understanding of the genetic structure of *L. rohita*. Preserving the genetic integrity of the species through careful management of its population diversity will be essential for ensuring the sustainability of both wild and farmed stocks.

4.3.3 Patterns of Nucleotide Distribution in *L. rohita* based on *mstn* gene

The nucleotide composition of the 18 haplotypes studied in this research reveals minor variations in the proportions of thymine (T), cytosine (C), adenine (A), and guanine (G). Across all haplotypes, the average nucleotide composition was found to be 28.3% T, 22.5% C, 28.5% A, and 20.7% G. This composition is characterized by a higher A+T content compared to G+C content, a pattern commonly observed in the mitochondrial genomes of fish species. This is consistent with findings from previous studies on freshwater fish, where mitochondrial DNA (mtDNA) shows a similar nucleotide distribution, with a tendency toward a higher proportion of adenine and thymine relative to guanine and cytosine (Sbisa *et al.,* 1997; Esposti *et al.,* 1993; Cantatore *et al.,* 1994; Nei & Kumar, 2000; Yang *et al.,* 2006).

The nucleotide composition of the *mstn* gene in *L. rohita* falls within the expected patterns for teleost fish, confirming evolutionary conservation in the structure of mitochondrial genomes. This study reinforces the significance of AT-rich regions in mitochondrial DNA, which may play an essential role in ensuring the stability and replication efficiency of the genome, supporting the long-term survival and adaptability of *L. rohita* in its natural habitat. Further studies focusing on the functional implications of this nucleotide composition could provide deeper insights into the role of mitochondrial DNA in the evolutionary success of freshwater fish species.

4.4 Conclusions

The present study provides comprehensive insights into the genetic diversity and population structure of *L. rohita* populations in Bangladesh using mitochondrial *cytb*, control region, and nuclear *mstn* gene sequences. The analysis of polymorphic sites, haplotype distribution, genetic distances, nucleotide composition, and diversity metrics revealed significant genetic variations among and within populations.

In our present investigation, the presence of unique haplotypes underscores the importance of conservation strategies to preserve genetic diversity. Haplotype and nucleotide diversity were observed to be higher in cultured populations compared to wild populations, suggesting that aquaculture practices may introduce genetic material from diverse sources. Among wild populations, the Halda River exhibited the highest genetic diversity, whereas the Padma and Jamuna populations showed moderate genetic differentiation. The control region sequences exhibited higher haplotype diversity (Hd = 0.876) and moderate nucleotide diversity ($\pi = 0.00706$), suggesting a historical population bottleneck likely caused by habitat reduction and resource limitations. The highest genetic diversity was recorded in the Jamuna River population, likely due to extensive river connectivity facilitating gene flow, whereas the Halda River population exhibited the lowest diversity, potentially due to geographic isolation. Pairwise Fst values indicated low to moderate genetic differentiation, with the highest genetic divergence observed between the Padma and cultured populations. Analysis of Molecular Variance (AMOVA) revealed that 90.35% of genetic variation was attributed to within-population differences, while 9.65% was due to variation among populations, aligning with previous studies that suggest substantial gene flow among riverine populations. Phylogenetic and haplotype network analyses identified two distinct genetic clusters, corroborating earlier findings based on microsatellite DNA markers. Notably, the genetic similarity between the cultured and Halda populations suggests that the Halda River serves as a primary source of broodstock for aquaculture.

4.5 Future recommendations

To ensure the long-term sustainability of *L. rohita* populations, targeted conservation and management strategies must be implemented. Protecting and conserving unique haplotypes, particularly in wild populations, is essential for maintaining genetic diversity. Establishing genetic conservation programs and protected breeding zones in key riverine habitats, such as the Halda and Jamuna Rivers, would help safeguard these genetic resources. Sustainable aquaculture practices should incorporate genetic monitoring of hatchery stocks to prevent genetic homogenization and inbreeding depression. Utilizing genetically diverse broodstock from multiple sources and implementing rigorous genetic screening protocols can help maintain healthy population structures. Regular genetic assessments using molecular markers should be conducted to track genetic variations and mitigate the risk of genetic bottlenecks in both natural and cultured populations. Additionally, controlled restocking programs using genetically diverse individuals from different rivers may help counteract the observed lower genetic diversity in Bangladesh compared to India. However, these programs must be carefully designed to avoid disrupting local genetic adaptations. Habitat protection and restoration efforts are also critical, particularly in major rivers such as the Halda, where preserving spawning and nursery grounds can facilitate natural gene flow and enhance population sustainability. Environmental policies should prioritize mitigating habitat degradation caused by pollution, overfishing, and dam construction. Finally, further research incorporating larger sample sizes and additional molecular markers, such as SNPs and microsatellites, is necessary to gain a more comprehensive understanding of the genetic diversity and evolutionary history of *L. rohita*. Comparative studies with populations from other South Asian regions can provide broader genetic insights and inform more effective conservation strategies.

This study highlights the importance of integrating molecular genetics into fisheries management to ensure the conservation and sustainable utilization of *L. rohita* populations in Bangladesh. Implementing these recommendations will help maintain genetic health and adaptability in both wild and cultured populations.

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