Doctoral (PhD) dissertation

Fatema Ali Al-Fatle

Gödöllő, Hungary

2022

Hungarian University of Agriculture and Life Sciences

Doctoral School of Biological Sciences

Ph.D. Dissertation

Population genetic structure and diversity of endangered Hungarian Tench (*Tinca tinca* L.1758) and Crucian carp (*Carassius carassius* L.1758) populations, with reference to the taxonomic status of two *Carassius* species—a foundation for conservation strategies

By

Fatema Ali Al-Fatle

Gödöllő, Hungary

2022

Title: Population genetic structure and diversity of endangered Hungarian Tench (*Tinca tinca* L.1758) and Crucian carp (*Carassius carassius* L.1758) populations, with reference to the taxonomic status of two *Carassius* species—a foundation for conservation strategies.

Discipline:	Biological Sciences
Name of Doctoral School:	Doctoral School of Biological Sciences
Head:	Prof. Dr. Zoltán Nagy Professor, DSc. Hungarian University of Agriculture and Life Sciences, Faculty of Agricultural and Environmental Sciences, Institute of Biological Sciences
Supervisor:	Dr. István Lehoczky National Centre for Biodiversity and Gene Conservation, Institute for Farm Animal Gene Conservation

....

Approval of Head of Doctoral School Approval of Supervisor

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

FAO	Food and Agriculture Organization
C.C	*Carassius carassius*
RAS	Recirculating Aquaculture Systems
IAA	Integrated Aquaculture–Agriculture
NBGK-	National Center for Biodiversity and Gene Conservation
HGI	Institute for Farm Animal Conservation
EPIC	Nuclear-encoded exon-Primed Intron-Crossing markers
IAS	Invasive Alien Species
IUCN	International Union for Conservation of Nature
AqGR	Aquatic Genetic Resources
RAS	Recirculating Aquaculture Systems
IAA	Integrated Aquaculture–Agriculture
CNV	Copy Number Variant
IUU	Unreported and Unregulated Fishing
Cytb	Cytochrome b gene
COI	Cytochrome C Oxidase I gene
Act	The second intron of the actin gene
RpS7	The first intron of the gene coding for the S7 ribosomal protein
NJ tree	Neighbour-Joining tree
Hap	Haplotype

1. INTRODUCTION AND OBJECTIVES

Crucian carp (*Carassius carassius* L. 1758) and tench (*Tinca tinca* L. 1758) are cyprinid fish native European waters (Brylinska et al., 1999; Copp et al., 2008). They are highly adaptable to a wide range of temperatures and low oxygen content in the water in both summer and winter. This ability to survive in anoxic conditions and at high densities in small bodies of water (Sollid, 2005; Kottelat & Freyhof, 2007) may allow tench and crucian carp to outcompete native species that are more sensitive to oxygen depletion and hence unable to thrive in such habitats when moved outside their native ranges. These characteristics make these species ideal candidates for adaptability to a wide range of environmental conditions. Because of their many advantages for aquaculture and also because of their taste and popularity among sport anglers (Szczerbowski & Szczerbowski, 2002; Ćirković et al., 2012), their production has increased in Europe. Nowadays, the entire economic value and contribution of these species to the fisheries sector are still limited. Nevertheless, climatic change may make these species ideal for future Hungarian aquaculture production. The numbers of these fishes are declining in Europe, and the main reasons for their decline are habitat loss and anthropogenic factors (Copp, 1991; Sayer et al., 2011; Simic et al., 2013). However, hybridization with closely related fish species that are not native, has also contributed to the decline in the number of crucian carp populations (Copp & Sayer, 2020). Well-documented data has shown that gibel carp (*Carassius gibelio* Bloch, 1782) competes with native fish and threatens the reproductive capacity of native crucian carp, causing species displacement (Smartt, 2007; Copp et al., 2010; Mezhzherin et al., 2012; Wouters et al., 2012). Furthermore, the identification of pure-bred *Carassius carassius* and hybrids using external morphological investigation is challenging, making these threats a concern throughout Europe (Hanfling et al., 2003). This significant morphological similarity between these species has hampered important insights into their taxonomic, biogeographic, and introduction histories (Rylková et al., 2013). However, despite the fact that these non-native species may cause the decline of native species or even their extinction (Pyšek et al., 2017; Dueñas et al., 2018), species invasion gives us the opportunity to consider the many different evolutionary processes that occur within species (Fitzpatrick et al., 2010).

According to Faulks et al. (2017) understanding the diversity, genetic structure, and high-quality habitat selection of endangered species is essential for participating in successful conservation and management programmes. Species' genetic diversity serves as a springboard for systematic action

planning aimed at preserving the species and reducing the risk of extinction (Souza-Shibatta et al., 2018). The structure of the population is the result of both current and historical processes. Therefore, in order to better understand how population diversity and species subdivision are constructed, it is also important to know their history. Pleistocene glacial cycles, in particular range shifts during the recolonization of glacial refugia in Europe, influenced the distributions and genetic diversity of present European species (HEWITT, 1999). Like most freshwater fish species in Eurasia, crucian carp and tench exhibit distinct phylogeographic subdivisions within their geographic ranges (Hewitt, 2004). Recent phylogeographic studies have found that the tench is separated into highly divergent Western and Eastern geographical clades based on analysis of nuclear and mitochondrial DNA sequence markers (Lajbner & Kotlík, 2011; Lujić et al., 2017; Karaiskou et al., 2020). On the other hand, human-aided transmissions may lead to introgressions across phylogroups, resulting in phylogeographic patterns that do not reflect natural historical processes (Lajbner et al., 2011). Likewise, there are two distinct European lineages of *Carassius carassius*, one found throughout the Northern, Central-Eastern drainages, the other nearly entirely limited to the Danubian catchment (Jeffries et al., 2016).

The availability of genome wide molecular markers, either protein or DNA (mitochondrial DNA or nuclear DNA), has already aided the identification of fish species, resolving taxonomic ambiguity and phylogenetic relationships and providing a deeper knowledge on their diversity, population, and genetic structure (Liu & Cordes, 2004; Tanya & Kumar, 2010; Hakim & Ahmad, 2017). Despite the fact that few studies on the population structure, genetic diversity, and phylogenetic relationships among populations of these species have been conducted, polymorphism of microsatellite markers (Kohlmann et al., 2010; Presti et al., 2010; Janson et al., 2015; Al Fatle et al., 2022), mitochondrial DNA (mtDNA) (Apalikova et al., 2011; Lajbner & Kotlík, 2011; Lo Presti et al., 2014; Knytl et al., 2018; Al Fatle et al., 2022), and a set of SNPs (Jeffries et al., 2016; Kumar et al., 2019) has been shown to be an excellent tool for detecting population structure, phylogeny, and biogeographic history in the above two species populations.

In the present study, a combination of microsatellite DNA markers, mtDNA Cytb gene sequences, mtDNA Cytochrome C Oxidase I (COI) gene sequences, and two nuclear markers (*Act*) and (*RpS7*) were used to meet the following objectives:

- To estimate the population structure and genetic diversity of seven wild populations of tench in Hungary, as well as eight wild populations and three stocks of crucian carp. Furthermore, we aimed to investigate the genetic differentiation between wild and cultured populations of crucian carp. Thus, providing essential knowledge for the development of successful selective breeding strategies on the one hand and sustainable conservation strategies on the other. In the case of crucian carp, the extent of hybridization with gibel carp, *Carassius gibelio* (Bloch, 1782), is also the focus of our research.
- To provide a systematic phylogeography of the wild tench populations as the basis for management and conservation efforts and for the purpose of determining population origins for potential translocations.
- To investigate the genetic divergence and phylogenetic relationship between *Carassius carassius* and the closely related *Carassius auratus gibelio,* and to estimate their taxonomic status in the genus *Carassius* using nucleotide sequence difference data from the mtDNA Cytochrome C Oxidase I (COI) region. Understanding the taxonomic status of these species in Hungarian waters can benefit conservation efforts.
- To achieve the stated goals, an ex-situ genetically described live GenBank of the two species must be established and developed. Identified hybrids should be excluded from GenBank activities.

2. LITERATURE REVIEW

2.1 Importance of aquaculture (global, Hungary), importance of genetic diversity in aquaculture

Early aquaculture goes back at least 2000 BC (Rabanal, 1988), and it wasn't until the last half of the twentieth century that it experienced rapid and systemic global growth (FAO, 2018). Advances in technology and development, widespread knowledge exchange at the national and international levels, and the need for sufficient supplies of protein food for human consumption have all contributed to the growth of aquaculture during this time span (Jones, 1987). Aquaculture is the fastest-growing food production sector in agriculture, with enormous potential to supply human protein demands in the future (Yue & Wang, 2017). It is growing, expanding in almost every part of the globe. Because the world's population is growing, so is the need for aquatic food items. Capture fisheries production has peaked, and most of the major fishing areas have achieved their maximum capacity. As a result, sustaining fish supplies from capture fisheries will not be able to fulfill the expanding worldwide demand for aquatic food, and aquaculture is now seen as a way to bridge the supply and demand gap in most parts of the globe (Subasinghe et al., 2009). Fisheries and aquaculture play an important role in providing healthy, low- carb meals to a growing global population. Alternative meals, such as edible seaweed, are also provided in this sector. Furthermore, this sector is vital to the lives of about 60 million people throughout the globe (FAO, 2018). Consumption of fish helps maintain a healthy lifestyle. Fish is a good source of several nutrients, all of which are important for human health, growth and development, cognition, and disease prevention. It is rich in long-chain omega-3 fatty acids, vital amino acids, vitamins (especially A, B, and D), and minerals including iron, iodine, calcium, zinc, and selenium. As a result of its distinct nutritional makeup, fish can be a great source of healthy dietary diversification, even in small quantities. Reduced risk of chronic diseases including depression or bipolar disorder and cardiovascular disease, as well as enhanced maternal health during pregnancy and lactation, enhanced physical and cognitive development throughout early childhood, reduction of health problems connected with anemia, stunting, and kid blindness are all advantages of fish consumption (Buscemi et al., 2014; Grosso et al., 2014; Troell et al., 2014; Nestel et al., 2015). Global apparent food fish consumption has risen at a pace much faster than world population growth for over 60 years. From 1961 to 2017, global food fish consumption grew at an annual rate of 3.1%, almost double the rate of annual world population growth (1.6 %) and more than all other

animal protein foods (meat, dairy, milk, etc.), which increased at a rate of 2.1 percent annually. Food fish consumption per capita increased by around 1.5 percent per year from 9.0 kg (live weight equivalent) in 1961 to 20.5 kg in 2018. The global consumption of food fish is expected to increase by 18% (28 million tonnes live weight equivalent) by 2030 compared to 2018. Fish consumption accounted for 17% of overall animal protein consumption and 7% of all protein consumption in 2017 (FAO, 2020c). Aquaculture, the farming of aquatic animals and plants, has developed faster than any other livestock sector in recent decades (Troell et al., 2014). The global production of fish in 2018 was about 179 million tons, with a total first selling value of 401 billion US dollars, of which aquaculture production was 82 million tons, with a value of 250 billion US dollars. (Figure 1).

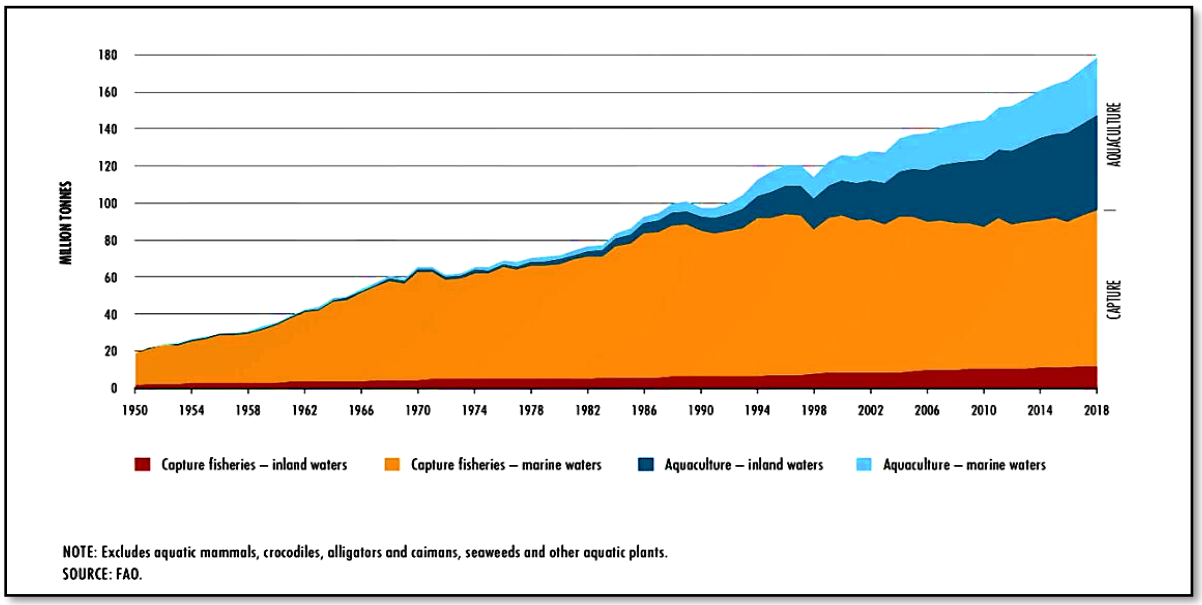


Figure 1. Global capture fisheries and aquaculture production (FAO, 2020c).

According to FAO's most recent global aquaculture statistics, world aquaculture production hit an all-time peak of 114.5 million tonnes in live weight in 2018. In 2018, world aquaculture contributed 46.0 percent to global fish production, up from 25.7 percent in 2000 and 29.7 percent in the rest of the world, excluding China, compared to 12.7 percent in 2000. In the period 2001–2018, global aquaculture productivity of farmed aquatic animals increased at a rate of 5.3 % per year on average, compared to just 4% in 2017 and 3.2% in 2018. The recent low growth rate was triggered by a recession in China, the world's largest producer, which saw aquaculture output rise

by only 2.2 percent in 2017 and 1.6 percent in 2018, although total production from the rest of the world grew by 6.7 percent and 5.5 percent, respectively in the same two years (FAO, 2020c). Despite the fact that marine or brackish waters cover more than 70% of our world, the production of freshwater fish species in the total fish output was higher compared to that for capture fisheries. Freshwater carps and cyprinids now dominate the aquaculture fish production, accounting for about 53.1 percent of overall fish output, followed by various freshwater fish species 19.5 percent, tilapia and other cichlids 11.0 percent, diadromous salmonids 6.5 percent, and coastal fishes 2.8 percent (Tacon, 2020). In 2018, Inland aquaculture, primarily in freshwater, produced the majority of farmed fish (51.3 million tonnes, or 62.5% of the global total), compared to 57.7% in 2000 (FAO, 2020c). Freshwater aquaculture has a significant ecological impact, as it contributes significantly to biodiversity and preservation of natural habitats as well as populations of wild animals, particularly bird populations. The EU has great potential: 68 thousand kilometers of coastline, 500 thousand natural lakes, and several long rivers: the Danube is 2,860 kilometers long (1,780 mi), the Tisza is 1,358 kilometers long (844 mi), the Rhine is 1,236 kilometers long (768 mi), and the Vistula is 1,047 kilometers long (651 mi) (Páczay & Páczay, 2018). 96 percent of Hungary's water comes from abroad. The Danube is Europe's second biggest river, and its second largest tributary, the Tisza, runs 417 and 597 kilometers through Hungary, respectively (Specziár & Erős, 2015). Hungary has long been a traditional producer of freshwater fish in Europe for decades. The key reason for this is that this nation has favorable hydrographic qualities as well as a long history of production. Despite the fact that Hungary is a small country, it may provide an excellent example of pond and intensive fish farming. It has traditional fish species that are in high demand in the global and EU markets; there are also several innovative technologies and developments (e.g. using geothermal water in intensive production, the latest feed) (Páczay & Páczay, 2018). Aside from saltwater aquaculture, there are several freshwater fish species that production might be significantly increased. *Cyprinus carpio* is the most common fish species produced in Hungarian aquaculture, accounting for 81% (15.080 t) of all breeding, while *Hypophthalmichthys molitrix* (1502 t) and *Ctenopharyngodon idella* (734 t) are the most important supplemental species *Sander lucioperca* (87 t), *Esox lucius* (92 t), *Silurus glanis* (19 t), *Tinca tinca* (12 t), *Hypophthalmichthys nobilis* (16 t), and a few other species (25 t) have modest contributions (Specziár & Erős, 2015). Although common carp is the main target species in pond aquaculture in Hungary, most farms also stock non-fed Chinese carp (up to 20% biomass) for improving water

quality and using trophic niches that are not used by common carp, as well as predatory species (1–3% of stocked biomass) to limit the occurrence of tiny trash fish that compete with common carp for feed (Gyalog et al., 2017). Hungary is one of the most active participants in the EU 6th and 7th Framework Program R & D projects, owing to the efforts of the Research Institute for Fisheries, Aquaculture, and Irrigation (HAKI) (Feledi et al., 2013). HAKI is now also part of MATE. The department of aquaculture at MATE focuses on the fishing and fish farming sectors, both of which have a long history in Hungary. This encompasses everything from the basics of pond fish farming to the most advanced molecular approaches. HAKI is an important participant in EU projects focused on improving pond fish farming technologies and developing disease-resistant common carp varieties, including Sustainaqua, Aquamax, and Eurocarp. When Hungary joined the European Union in 2004, aquaculture became a major focus of international development assistance (ODA) programmes, mainly in Laos and Vietnam. In 2004, HAKI established the Network of Aquaculture Centers in Central and Eastern Europe (NACEE), a non-governmental organization with 28 members from nine Central and Eastern European countries. HAKI is also a member of the Network of Aquaculture Centers in Asia -Pacific (NACA) and engages in a number of European projects aimed at fostering international cooperation. The European Aquaculture Technology and Innovation Platform (EATiP) has HAKI as a member (Feledi et al., 2013).

The wide range of climatic and environmental conditions present at sites around the world where aquaculture is practiced has resulted in a large number and variety of species used in different types of aquaculture production practices that use freshwater, brackish water, marine water, and inland saltwater. FAO documented aquaculture output for reporting countries and territories in 2018 under a total of 622 units, which are defined as “species items” for statistical purposes. These 622 species items correspond to 466 individual species, 7 interspecific finfish hybrids, 92 species groups at the genus level, 32 species groups at the family level, and 25 species groups at the order level or above. Finfish farming is the most diversified subsector, with 27 species and species groups accounting for more than 90% of total finfish output in 2018 (FAO, 2020c). According to Metian et al., 2020, studies show that a country's high species diversity is also correlated with increased productivity, although there are significant variances across countries. In 2017, Asia produced nine of the top ten countries rated highest by the Shannon Diversity Index, with China

providing the most varied collection of species. According to (Liao, 2000), the two means of diversification are the utilization of new cultured species and the introduction of alien species. On the other hand, the introduction of fish, which results in exotic species, is one of the most serious threats to the biodiversity of finfish (Moyle & Leidy, 1992). It is well known that the intentional or unintentional introduction of exotic aquatic species (alien species) may have a deleterious influence on local biodiversity. It has been reported that the introduction of fish has had negative effects on biodiversity in many parts of the world (Silva et al., 2009). And, globally, invasive alien species (IAS) are widely regarded as a major threat to native biodiversity, with the International Union for Conservation of Nature (IUCN) describing their influence as "immense, insidious, and often irreversible." (Clout & De Poorter, 2005). Furthermore, since high-impact invaders are more likely to belong to genera not already existing in the system, the potential influence of alien species on biodiversity cannot be overlooked (Ricciardi & Atkinson, 2004). Aquaculture relies heavily on genetic diversity. It enables organisms to grow, adapt to natural and human-caused changes like climate change, resist diseases and parasites, and continue to develop and adapt to farming systems. While the variety of farmed species is well understood, there is a scarcity of information on AqGR at the species level. Several factors are required for long-term growth, but one that is sometimes underestimated is the requirement to adequately manage aquatic genetic resources (AqGR). DNA, genes, chromosomes, tissues, gametes, embryos, and other early life-history stages, as well as individuals, strains, stocks, and communities of organisms with current or prospective value for food and agriculture, are all included in the AqGR (FAO, 2020c). The genetic profile of fish stocks is being used by catch fishery management to assist in determining catch, season, and trade policies (Martinsohn et al., 2011), as well as to prevent illegal fishing. Genetic information at the species level is employed in trade and consumer protection when fish are purposely mislabeled in order to avoid trade restrictions or fetch a higher market price. Moreover, with the rising relevance of aquaculture and the requirement to identify between farmed and wild fish of the same species, genetic information may aid in the differentiation of farmed and wild fish of the same species (Martinsohn & Ogden, 2009). DNA is the most fundamental level of biodiversity, it generates speciation and supports other levels of biodiversity such as functional features, species, and ecosystems. Trends in genetic diversity may now be analyzed more effectively, and they're ready to be included in biodiversity monitoring (Walters & Scholes, 2017).

2.2 Aquaculture obstacles and the impact of COVID-19 on its sector

Aquaculture has a variety of obstacles, including a scarcity of genetically improved species, a scarcity of species-specific feeds, high disease mortality, and ecosystem contamination. The rapid advancement of sequencing technologies has revolutionized biological sciences, providing the tools required to address these difficulties in aquaculture and assure its long-term viability and profitability. Draft genomes have been published for over 24 aquaculture species so far and have been utilized to solve key aquaculture concerns (Yue & Wang, 2017). Climate change is projected to have a considerable impact on fisheries as a consequence of changes in the abiotic and biotic conditions of aquatic habitats, which can influence aquatic species' distributional patterns, growth and size, catch potential, etc. (Barange, 2018). A combination of these climate factors, such as cyclones, droughts, and floods, as well as global warming, ocean acidification, rainfall variation, salinity, and sea level rise, often endangers aquaculture. However, climate change adaptation is required in order to produce more fish with minimal environmental impact. Integrated aquaculture, recirculating aquaculture systems (RAS), and expanding seafood farming are some of the adaptation approaches that might boost aquaculture production, environmental sustainability, and climate change adaptability. Integrated rice-fish farming, pond-based IAA (Integrated aquaculture–agriculture), and polyculture may all help to boost fish productivity while reducing environmental effects. Integrated mangrove-shrimp cultivation combined with mangrove restoration might boost blue carbon sequestration while also mitigating and adapting to climate change (Ahmed et al., 2019). Moreover, COVID-19's worldwide spread and the actions taken to prevent or delay its spread are having an impact on aquaculture; these effects are happening alongside other anthropogenic-driven threats like global climate change (Sarà et al., 2022). Despite the fact that COVID-19 does not infect aquatic food animals (Bondad-Reantaso et al., 2020), it has had a profound impact on fisheries and aquaculture food systems. The consequences have varied according to geography, species, markets, and farm financial capability. Many farmers who have been unable to sell their catch due to disruptions have had to keep significant amounts of live fish on hand. Others have failed to fulfill all the required seasonal activities, such as fish breeding. Aquaculture farms that supply the live-fish market or high-end food services (such as restaurants, tourism, and hotels) have been badly impacted. Moreover, the disturbance in international transportation has also had a significant impact on the species being bred for export. Restrictions on cargo movements, precautionary measures, and border closures have all had an influence on

the availability of labor and aquaculture inputs (e.g., medications, fingerlings, and feed) required for production (FAO, 2020a, 2020b). During the pandemic, both artisanal and industrial fishing activities have declined. Global industrial fishing activity has decreased by roughly 6.5 percent as of 04/28/2020 compared to prior years as a consequence of COVID-19-related limitations and closures, according to Global Fishing Watch (Clavelle, 2020). However, early forecasts from a few enterprises suggest that after the crisis has passed, they may be able to recover (Bondad-Reantaso et al., 2020). According to FAO, (2020c) aquaculture will continue to be the primary driver of global fish output increases, continuing a decades-long trend. In 2030, aquaculture output is expected to reach 109 million tonnes, up 32% (26 million tonnes) from 2018. In terms of species, freshwater species such as carp and pangas catfish (including Pangasius spp.) will account for 62% of global aquaculture output in 2030, compared to 60% in 2018. Increased production of high-value species such as shrimp, salmon, and trout is also expected.

2.3 Introduction of Tench

Tench (*Tinca tinca* L. 1758) is a freshwater fish that is considered one of the native cyprinid species in Europe and Asia (Brylinska et al., 1999; Kottelat & Freyhof, 2007) (Figure 2). It is now found on all continents except Antarctica, making it a sub-cosmopolitan species (Presti et al., 2010).

Figure 2. *Tinca tinca*; Lake Fertő, Hungary.

Tench, on the other hand, are now abundant in many temperate freshwater regions throughout the globe as a result of human influence (Lajbner et al., 2011; Lajbner & Kotlík, 2011; Leathwick et al., 2016). Recent genetic and phylogeographic analyses have revealed that human-mediated dispersal played a role in determining the current range of tench in Europe, including its introduction between the British Isles and the Iberian Peninsula at least as early as the 16th century (Lajbner et al., 2011). However, in certain countries it is considered as a foreign exotic species due to competition with the other domestic fish (Stokes et al., 2004; Hesthagen & Sandlund, 2007; Rowe, 2007; DeVaney et al., 2009; Avlijaš et al., 2018; Clavero, 2019).

Tench has great potential for aquaculture (Gela et al., 2006; Celada et al., 2007), and has great commercial interest (Wang et al., 2006). It contributes significantly to the sustainability of its ecosystems. As they stir up the muddy bottoms of lakes and streams in their search for food, tench help reduce eutrophication by avoiding algae blooms and returning minerals and nutrients that have accumulated on the bottom to the surface (Dulski et al., 2020). This species has traditionally been farmed in Great Britain and Central Europe, where it is valued as a sport fish (Wright & Giles, 1991). In European countries, the tench has been utilized as food as well as for leisure purposes, such as angling or as an ornamental fish, and it was also used as a water quality indicator for fish assemblages (Billard & Flajshans, 1995). Tench is employed as a model species in studies of manipulating chromosomes to improve performance characteristics (Buchtová et al., 2003a, 2003b). It is also used to study the biological and physiological differences between populations in which genomes have been manipulated and those produced through conventional breeding methods (Svobodová et al., 1998, 2001; Svobodova & Kolarova, 2004). Economically, tench is farmed in most parts of Europe (Ablak Gurbuz, 2011). The main problem with tench rearing is their slower growth rate compared to other fish (Kohlmann et al., 2007), and due to a number of environmental and biological constraints, natural propagation of this species is difficult (Lelek & Council of Europe, 1987). Tench have been reared almost semi-proportionately as supplemental fish in pond polyculture alongside other cyprinid species, primarily for export on the European market (Gela et al., 2006). Traditionally, it was co-cultured with common carp (*Cyprinus carpio* Linnaeus, 1758). Recently and after the political shifts that occurred in the former socialist countries in the 1990s, tench has gained great popularity, due to the growing interest in diversifying production (Kohlmann et al., 2007). The tench was first domesticated and selectively bred in the former Czechoslovakia, and in addition to the original five breeds, other breeds were introduced

from Hungary, France and Romania (Svobodova & Kolarova, 2004). It has been intensively domesticated in recent years, similar to how common carp were centuries ago (Dulski et al., 2020). Although it is one of the most widespread and least concerned species by the IUCN, tench is facing significant population decline in Hungarian waters and it is quite possible that tench may be an endangered species not only in Hungary but throughout Europe. The exact reason of the decline is not clearly understood. Italy (Pompei et al., 2012), Poland (Wołos et al., 2009) and the Balkans (Simic et al., 2013) have also shown declines of this species. However, the reasons for these declines may include invasion of the alien fish species, degradation of spawning areas and loss of habitat, eutrophication and other anthropogenic factors (Lujić et al., 2017). In the Italian red list, tench is mainly listed as "near threatened" (Zerunian, 2007; Pompei et al., 2012). The tench is also considered easily vulnerable to disease similar to those reported in the common carp. Losses of tench due to bacterial, viral, and fungal diseases have been reported during long-term conservation in ponds along with deaths resulting from alterations in environmental quality (Svobodova & Kolarova, 2004; Pękala-Safińska, 2018).

2.3.1 Description of the species (taxonomic status, distinctive taxonomic features, genetics and reproductive biology, distribution and habitat, history of introduction and invasion)

Single species of genus belong to the kingdom *Animalia*, phylum *Chordata*, subphylum *Vertebrata*, superclass *Osteichthyes*, class *Actinopterygii*, subclass *Neopterygii*, infraclass *Teleostei*, superorder *Ostariophysi*, order *Cypriniformes*, superfamily *Cyprinoidea*, and family *Cyprinidae*. The subfamily *Tinciane* only consists of *Tinca tinca* and the relationships of this species are not so clear and explicit (Kottelat & Freyhof, 2007). Tench has a number of unique characteristics that distinguish it from other members of the *Cyprinidae* family and have helped it become a popular experimental model (Flajšhans et al., 2010). An unmistakable body color, usually green to brown-green, with golden, blue, albinotic phenotypes also prevalent (Kvasnicka et al., 1998) and one pair of the maxillary barbells. There are 96-115 total lateral line scales, small and deeply embedded in the dermis (Kottelat & Freyhof, 2007) with visible sexual dimorphism in ventral fins (Kocour et al., 2010). 6 - 9.5 branched anal rays and 8 - 9.5 branched dorsal rays (Kottelat & Freyhof, 2007). The head is triangular with orange –red eyes. The mouth is a terminal, with thick lips and a pair of sophisticated barbells present in each corner of the mouth. The snout

is long and rounded (Spillman, 1961). Fish length and weight ranged from 10.9 cm to 41.5 cm and 24.0 g to 1127.2 g, respectively, at the age of 1 to 9 (KILIÇ & BECER, 2013). Female tench average 39.4 cm and 955g at 7 years of age in rich alkaline waters, while female tench of the same age in less productive waters may only average 27.5cm and 310g. Males grow at a slower rate than females after their first two years of life (Kennedy & Fitzmaurice, 1970). It is common for males to reach sexual maturity at the age of three and females at the age of four, when they are about 19.6 cm and 22 cm in length, respectively. Fecundity was associated with gonad weight, length, weight, and age of fish, where older and larger fish showed a higher fecundity (Gurbuz, 2011). They live up to 20 years. The females outlive the males by about a year more. The pelvic rays of females are strong, while those of males are much stronger and longer, extending beyond the anus (Vainikka et al., 2005; Kottelat & Freyhof, 2007). Tench belongs to the *Cyprinidae* family that spawns in May - October, generally in June and July in Central Europe, and grows at water temperatures from 20 to 29 ° C (Linhart et al., 2006; Kottelat & Freyhof, 2007; Ablak Gurbuz, 2011; Wolnicki et al., 2017). Females may spawn up to nine times a year, about every 11 to 15 days if the weather remains warm enough. However, when the temperature changes a lot, it causes a lot of embryonic mortality. They spawn amongst dense vegetation in still water. Eggs are released in several areas of the vegetation (Kottelat & Freyhof, 2007). The larvae and the juveniles live in dense vegetation and are highly tolerant of low oxygen levels, salt concentrations up to 12%. They usually feed on detritus (animal and plant) (Kottelat and Freyhof, 2007). Tench are omnivores (Gray & Dauble, 2001) that feed on a diverse diet, mainly macrophytes and algae (Alaş et al., 2010). It is a diploid species (2n = 48) according to genetic research (Arslan & Taki, 2012), which is beneficial for certain genetic investigations compared to numerous polyploid cyprinid species (Leggatt & Iwama, 2003). This species is typically found in shallow and densely vegetated lakes and backwaters. During the winter, it is often buried in mud (Kottelat & Freyhof, 2007).

Tench can be found in all major freshwater systems in Europe, but it is absent in Ireland. It stretches from the British Isles and the Iberian Peninsula in the west to Central Siberia in the east (Figure 3) (Lajbner et al., 2011). It has also recently been introduced into the eastern Adriatic basin, western and southern Greece, and northern Scandinavia. Native to Asia, eastward to the western Yenisei drainage south of 60°N. It has also been introduced to South and North Africa, as well as Tasmania, Australia, New Zealand, and India, as well as North America, Chile, and perhaps other parts of the world (Kottelat & Freyhof, 2007). In 1877, tench was first introduced from Europe to the USA.

By 1896, their progeny had spread to at least 36 states, and they were subsequently brought to North America and Canada, both of which were introduced from Germany (Lajbner et al., 2011). Although it has been introduced to water systems in central and western Turkey (Innal & Erk'akan, 2006), tench is most likely native to certain Black Sea river drainages in Turkey (Brylinska et al., 1999). However, since it has been cultivated for so long in Europe, pinpointing its exact native range might be difficult in certain regions. It could be native or introduced, although there isn't much evidence to support either view (Lajbner et al., 2011).

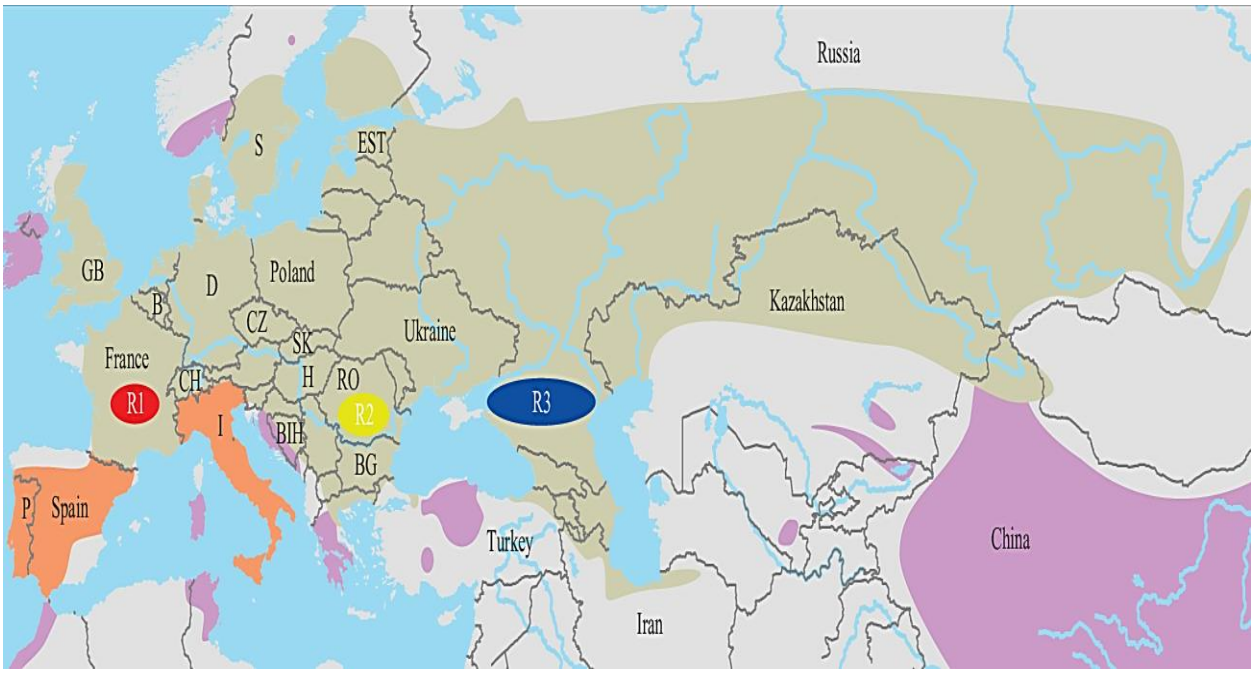


Figure 3. Tench's distribution range includes both native (olive) and non-native (violet) species. Orange highlights large regions where the origin is ambiguous. The main freshwater glacial refugia in Europe are shown here, including the Western/Atlantic (R1), Danubian (R2), and Ponto-Caspian (R3) regions (Lajbner et al., 2011).

As a consequence of recurrent isolation in glacial refugia during the Pleistocene, most freshwater fish species in Eurasia exhibit phylogeographic subdivisions of their geographic ranges (Hewitt, 2004). Recent phylogeographic research on the tench indicated that the species' Eurasian distribution is split into two highly divergent Western and Eastern phylogroups based on phylogenetic analyses of nuclear and mitochondrial DNA sequence markers (Lajbner et al., 2007, 2010). This divergence most likely occurred during the Pleistocene Ice Age, when two distinct refugia, the Ponto-Caspian refugium and the Western European refugium, evolved independently (Hewitt, 2004). Various intron-primed crossing-over (EPIC) and mitochondrial DNA (mtDNA) gene markers have recently been used to differentiate the newly discovered Western and Eastern

phylogroups of this species that are morphologically indistinguishable (Lajbner & Kotlík, 2011; Lujić et al., 2017). A Western phylogroup (clade W) found in Europe from the British Isles to Poland, while an Eastern phylogroup (clade E) found from Europe throughout Asia to China (Lajbner & Kotlík, 2011). Although Lajbner et al. (2010) indicated that the two phylogroups may represent distinct species, the authors demonstrated that tench from both phylogroups may readily interbreed in wild populations. They also found that, in Central and Western Europe, a wide zone of overlap persists, indicating a post-glacial connection with the occurrence of hybridization. However, human introductions of tench for aquaculture activities, on the other hand, may cause introgressions between these phylogroups, resulting in phylogeographic patterns that do not represent natural historical processes (Lajbner et al., 2011).

2.4 Introduction of Crucian carp

Crucian carp *Carassius carassius* (L. 1758) is a cyprinid fish species native to European waters. It is a freshwater fish that may be found in marshes, ponds, small well-vegetated lakes, and oxbows of lowland rivers (Figure 4). Its native range extends across most of northern and central Europe, from the freshwaters of the North Sea and Baltic Sea basins to the Alps and the Danube basin, and then east to Siberia (Lelek & Council of Europe, 1987; Kottelat & Freyhof, 2007). Due to its ability to tolerate anoxic conditions and thrive at high stocking densities, *Carassius carassius* has been maintained in ponds and small lakes (Szczerbowski & Szczerbowski, 2002).

Figure 4. *Carassius carassius*; Lake Fertő, Hungary.

The populations of this species in Europe are rapidly declining. This is mostly because of habitat loss caused by drought, draining of wetland pools and ponds (Copp, 1991; Wheeler, 2000; Sayer et al., 2011), poor water quality in the Danube watershed (Navodaru et al., 2002), and the invasion of other closely related fish species that are not-native to their original habitat, such as gibel carp (*Carassius gibelio* Bloch, 1782) (Lelek & Council of Europe, 1987; Copp et al., 2010; Mezhzherin et al., 2012; Wouters et al., 2012; Copp & Sayer, 2020) which has been regarded as one of the most successful invaders of native fish communities (Kırankaya, 2013; Ribeiro et al., 2015; van der Veer & Nentwig, 2015; FLORESCU et al., 2018). This phenomenon endangers the genetic integrity of *Carassius carassius* throughout Europe, as well as posing a big challenge in recognizing and differentiating pure-bred crucian carp from hybrids based on external morphological characteristics (Hänfling et al., 2005). Silver crucian carp *Carassius auratus gibelio*, on the other hand, was brought to Hungary in 1954 by a pisciculturist from Bulgaria (300 individuals) with the main goal of re-filling an empty ecological niche alongside the common carp (Tóth et al., 2005). Additionally, small population size may exacerbate the genetic isolation and bottlenecks of *Carassius carassius* (Hänfling et al., 2005).

Crucian carp is also a popular culinary item. However, its popularity has faded in Sweden and other Western European nations, yet it remains a delicacy in Eastern Europe (Janson et al., 2015). Studies on crucian carp are limited to growth, age and length of maturity, reproduction, egg size and fecundity (Copp et al., 2008; Tarkan et al., 2009, 2011). The conservation of crucian carp is a crucial matter right now (Sayer et al., 2011, 2020; Copp & Sayer, 2020) since there has been a recent reduction in the size and quantity of populations, resulting in a drop in the local populations (Copp et al., 2010; Savini et al., 2010; Rylková et al., 2013). The extinction of the crucian carp is becoming more widely recognized, and it is a red-listed species in several European countries, including the Czech Republic (Lusk et al., 2004), Austria (Wolfram & Mikschi, 2007), Serbia (Simic et al., 2009), and Croatia (Mrakovčić et al., 2007). However, there are few conservation practices in Europe, most notably in Norfolk, Eastern Europe (Copp & Sayer, 2010; Sayer et al., 2011, 2020).

2.4.1 Description of the species (taxonomic status, distinctive taxonomic features, genetics and reproductive biology, distribution and habitat, history of introduction and invasion)

Four species of the genus *Carassius* can be found in Europe, each with its own unique characteristics. The crucian carp *Carassius carassius* (L. 1758) is native to Europe, whereas the introduced exotic species include; goldfish *Carassius auratus auratus* (Linnaeus, 1758), gibel carp *Carassius auratus gibelio* (Bloch 1782), and *Carassius auratus langsdorfii* (Kalous et al., 2007; Kottelat & Freyhof, 2007; Vetešník et al., 2007; Lorenzoni et al., 2010; Savini et al., 2010).

The crucian carp is one of the four species found in the genus *Carassius*, belonging to the kingdom of *Animalia*, phylum *Chordata*, subphylum *Vertebrata*, superclass *Osteichthyes*, class *Actinopterygii*, subclass *Neopterygii*, infraclass *Teleostei*, superorder *Ostariophysi*, order *Cypriniformes*, superfamily *Cyprinoidea*, and family *Cyprinidae*. *Carassius carassius* is the only species that can be readily distinguished morphologically (Kottelat & Freyhof, 2007), and *Carassius* auratus complex refers to the remaining species that differ slightly in terms of morphological features (Rylková et al., 2013).

Crucian carp typically range in length from 20 to 45 cm, with a maximum of 50 cm (Maitland, 2004). It is distinguished by a body that is quite deep and compressed on the lateral sides, with an olive grey color on the back, grading to brassy green on the sides, and a dull brown color on the body. There are about 23 to 33 gill rakers and about 31 to 36 total lateral scales. The dorsal convex has a free edge. The peritoneum is whitish with a simple anal design, weakly serrated dorsal rays, and roughly 6,5 branched anal rays (Maitland, 2004; Kottelat & Freyhof, 2007). However, the fish can show phenotypic plasticity under variable environmental conditions (Robinson & Parsons, 2011). A variety of different-looking fish with different physiological features have been found in the crucian carp's natural habitat, which varies from small ponds with harsh abiotic environments to large lakes with many predatory species (Holopainen et al., 1997). In lakes, crucian carp may take on a number of body morphologies, including being deeper and more streamlined, having a smaller head size, as well as a wider color range (Poléo et al., 1995). According to Brönmark & Miner (1992), populations of *Carassius carassius* in northern Europe exposed to the northern pike fish (Esox lucius), a large ambush predator of the littoral habitat, developed a deeper body shape as a consequence of protective phenotypic variation.

Carassius carassius has 2n = 100 chromosomes (Knytl et al., 2013), whereas triploid individuals have chromosome numbers (3n = 150) ranging from 141 to 166 (Knytl et al., 2018). The longevity of this species is about 10 years (Tarkan et al., 2009). *Carassius carassius* spawns in batches. Typically, spawning occurs when the water temperature reaches 17-20°C. The reproductive period of a batch spawning species may last between 30 and 60 days. Reproductive period length and the number of batches produced each season are determined by water temperatures in the spring and early summer (Aho & Holopainen, 2000). The average age at maturity is 3.5 years for females and 3.3 years for males. Maturity onset begins at about 88 mm in males and in females at 98 mm or more. Crucian carp may reach maturity without spawning for a year or more (Copp et al., 2008). In England, the age range of the youngest mature crucian carp females and males was about age +1 with the mean age range of around 1.5 years (Tarkan et al., 2009). Males begin their reproductive period at 3 years of age, while females begin at 4 years of age in Central and Eastern Europe and about 2 years in Southern Europe. During the season, the females spawn three to five times. The eggs are sticky and are found attached to the water plants (Kottelat & Freyhof, 2007). It is an omnivore fish (Olsén & Lundh, 2016) that feeds on plankton, benthic invertebrates, plant materials, and detritus during the day and often at night. Crucian carp individuals, on the other hand, don't seem to be good competitors. Even though ichthyo and predatory species-rich waters don't support the presence of crucian carp, they may be found in abundance where they don't have much competition (Kottelat & Freyhof, 2007).

The crucian carp (*Carassius carassius*) is a freshwater fish that inhabits many Asian and European nations' lakes, rivers, and reservoirs. The fish is mostly found in densely vegetated lakes, small shallow oxbows of lowland rivers, and channels. This species is characterized by its extreme resistance to a wide range of temperatures (up to 35 ° (Holopainen et al.,1997; Sollid, 2005)), and very low oxygen levels in the water in both summer and winter (Szczerbowski & Szczerbowski, 2002; Kottelat & Freyhof, 2007; Janson et al., 2015). To survive in completely frozen ponds or environments that are almost completely dry, it may bury itself in the mud (Kottelat & Freyhof, 2007). North European crucian carp commonly live in small ponds that are hypoxic and subsequently anoxic for many months each winter because of ice cover (Holopainen et al., 1986). Thus, It is the only species of fish in this habitat that can survive in conditions of extreme hypoxia and anoxia (Sollid, 2003).

Carassius carassius (L. 1758) has a long and distinguished history. Subfossil bone fragments discovered at a prehistoric coastal site in present-day Estonia suggest that the crucian carp has been present in the Baltic Sea area for at least 3000 years (Wouters et al., 2012). It is distributed in Eurasia in the following basins: the North, Baltic, White, Barents, Black, and Caspian Sea basins; the Aegean Sea basin only in the Maritza drainage; eastward to the Kolyma drainage (Siberia); and westward to the Rhine and eastern drainages of England. Absent from the North Sea basin in Sweden and Norway, north to approximately 66°N in the Baltic basin. Even though it has been widely spread in Italy and France, it may often be confused with *Carassius gibelio* (Kottelat & Freyhof, 2007). In Sweden, the crucian carp is regarded as a native species (Kullander & Delling, 2012; Wouters et al., 2012). It is also a British native, as evidenced by zoogeographical and archaeological findings (Wheeler, 2000; Copp et al., 2005). In England, perhaps the first in Europe, evidence-based conservation programs were put in place to help protect this species and its habitat as part of a larger European plan. This country provides an ideal environment for the growth and reproduction of crucian carp. Also, the crucian carp is a species that is a priority for biodiversity action in the county of Norfolk (Copp & Sayer, 2020). The crucian carp has a long history of translocations across Europe, with no evidence of adverse effects in the receiving waters. It has been translocated for fishing amenity throughout its native range, including Slovenia (Povž et al., 1990) and the UK (Sayer et al., 2011). Although Crivelli, 1995 presented some information for the Mediterranean area, there is very little data on the introduction of crucian carp outside of its native range. Since there is relatively little evidence known about the negative effects of *Carassius carassius*, it has not been classified as an invasive species. However, other cyprinids, such as *Carassius auratus* (goldfish), *Carassius gibelio* (gibel carp) and *Cyprinus carpio* (common carp), are often confused with crucian carp (Hänfling et al., 2005; Innal, 2011). Morphometric testing methodologies commonly misidentify brown goldfish as crucian carps (Wheeler, 2000; Hickley & Chare, 2004). So, molecular genetic methods were developed to detect pure-bred crucian carp from its hybrids and backcrosses of at least the first two generations (Hanfling et al., 2003; Hänfling et al., 2005; Papoušek et al., 2008).

2.4.2 Hybridization with gibel carp (*Carassius gibelio* (Bloch, 1782))

As a phenomenon, hybridization is considered as the exchange of alleles among closely related groups of species (Baack & Rieseberg, 2007). It is more abundant in fishes than in other vertebrate

groups (Scribner et al., 2000). Hybridization has long been regarded as a critical step in the evolution of species (Payseur & Knyt, 2016), since it often leads to incomplete reproductive isolation. This is particularly true for species that rely on external fertilization and have spawning habitats that overlap spatially or temporally, such as fish (Knytl et al., 2018). Interspecific hybridization is a frequent occurrence in freshwater fishes. It is found in a wide range of fish species, regardless of taxonomy (Scribner et al., 2000). The anthropogenic activities linked to the breakdown of species isolating mechanisms, along with the introduction of closely related species, result in a high number of inter-specific hybrids. Other factors, such as aquaculture activities and habitat loss or alteration, may also influence the occurrence of hybridization (Scribner et al., 2000). The worldwide number of non-native fish species in freshwater environments is rising (Herborg et al., 2007; Mandrak & Cudmore, 2010; Gubiani et al., 2018). The introduction of these non-native species has been cited as one of the primary causes of aquatic fauna decline throughout the world (Cambray, 2003; Helfman, 2007; Toussaint et al., 2018). Thus, hybridization between native and non-native taxa is an indication of significant biodiversity loss (Savini et al., 2010; Toussaint et al., 2016) and can produce vigorous hybrids capable of outrunning the native species (Facon et al., 2005). The *Cyprinidae* family has the most hybridization of any freshwater fish group (Scribner et al., 2000; Higgins et al., 2015). Hybridization between closely related species is well- known to occur in this family (Kottelat & Freyhof, 2007; Hayden et al., 2010; Haynes et al., 2012; Higgins et al., 2015). Furthermore, hybridization and unisexual reproduction are associated with polyploidy (Schultz, 1969). *Cyprinidae* is the fish family with the most polyploid fish species (Leggatt & Iwama, 2003; Yang et al., 2015). However, polyploids in *Cyprinidae* may contribute to their diversification and adaptability to environmental changes (Li & Guo, 2020). Hybridization and subsequent introgression with three non-native species, gibel carp *Carassius auratus gibelio* (Bloch), goldfish *Carassius auratus auratus* (L.), and common carp *Cyprinus carpio* (L.), are two of the most commonly stated reasons for the decline of the *Carassius Carassius* (Hänfling et al., 2005; Smartt, 2007; Papoušek et al., 2008; Copp et al., 2010; Sayer et al., 2011; Mezhzherin et al., 2012; Wouters et al., 2012; Rylková et al., 2013). All of them are among Europe's top 25 non-native freshwater fish, and all of them have been introduced or are already invasive over most of *Carassius carassius'* native range (Savini et al., 2010). This hybridization threatens to displace the *Carassius carassius* genome with a hybrid genome, which could compromise the survival of this species (Spoz et al., 2014). Using genetic marker analyzes, many examples of hybridization

between *Carassius carassius*, *Carassius gibelio* and *Carassius auratus* have been identified in many countries, such as the Czech Republic (Papoušek et al., 2008), Sweden (Wouters et al., 2012), and Ukraine (Mezhzherin et al., 2012). These hybrids were diploid, triploid, and tetraploid in nature. *Carassius carassius* and *Carassius gibelio* hybrids are typically tetraploid, while hybrids between *Carassius carassius* and *Carassius auratus* are either diploid or triploid (Mezhzherin et al., 2012). According to Hänfling et al. (2005); and Smartt (2007), when there is hybridisation, there is also a chance of introgression, which impacts the genetic pool of *Carassius carassius.*

Carassius gibelio (Bloch, 1782) is considered one of the most successful invasive species in aquatic habitats, having been discovered throughout Europe with the exception of Scandinavia (Copp et al., 2009; Lusková et al., 2010; Yerli et al., 2014). By inducing quantitative changes in community structure and altering the physical and chemical properties of the habitat (Tarkan et al., 2012), *Carassius gibelio* has the potential to harm the native ichthyofauna and degrade the water quality. Moreover, the method of reproduction of *Carassius gibelio* is a key biological feature that contributes to its invasiveness (Başkurt et al., 2020). Triploid silver crucian carp can use diploid relatives' sperm to induce gynogenesis, according to research published (Yigui & Shixin, 1993; Teng et al., 1999). Due to the morphological similarities between *Carassius gibelio* and the native crucian carp, this species is often misidentified as the native crucian carp, *Carassius carassius* (L. 1758) (Vetemaa et al., 2005). Until the Flemish government outlawed both species in the 1990s, many legal stockings on Flemish public waterways were made from Prussian carp rather than crucian carp due to this misunderstanding. Then, in 2000, native crucian carp of known provenance were reintroduced to the stocking program (Verreycken et al., 2007). In the Amur basin, silver crucian carp and crucian carp share the same water, making phenotypic distinction almost impossible. It is possible to identify them by using their chromosomal numbers and reproductive styles (Zheng et al., 2010). In UK waters, there are also numerous populations of fish that look like both crucian and gibel carp, and conventional morphometric analysis has failed to determine their origins (Hanfling et al., 2003).

Prokes & Barus, 1996 reported hybridization between *Carassius carassius* and *Carassius gibelio*. Molecular (Papoušek et al., 2008; Wouters et al., 2012) and cytogenetic methods (Knytl et al., 2013, 2018) were subsequently used to validate this kind of hybridization. Using mitochondrial and microsatellite analyses, Papoušek et al. (2008) revealed the presence of hybrids between

Carassius gibelio and *Carassius carassius* in the Czech Republic. Similarly, Wouters et al. (2012) used both mitochondrial DNA sequence and nuclear microsatellite analysis to report the presence of such hybrids in the Western (Swedish) Baltic Sea region. Papoušek et al. (2008) also stated that the crucian carp had a green body with a yellow abdomen tinged with gold. The *Carassius auratus gibelio* had a dark green back and silvery sides. While the color of the hybrids' bodies was similar to that of *Carassius auratus gibelio*, with a touch of dark grey and black on the head, torso, and fins. The upper line of the dorsal fin in *Carassius carassius* was convex and the upper edge was rounded, while the upper line of the dorsal fin in *Carassius auratus gibelio* was ventrally somewhat concave. The hybrid individuals' dorsal fins included characteristics from both parental species. The cranial half of the dorsal fin was convex, similar to that of *Carassius carassius*, but the caudal half was somewhat concave, similar to that of *Carassius auratus gibelio*. *Carassius carassius* had a light, unpigmented peritoneum, while *Carassius auratus gibelio* had a black peritoneum with a pearly shine. The hybrids' peritoneal colors varied, with some having deeper colors and others having lighter ones. In diploid *Carassius auratus gibelio*, the average number of gill rakers was 48.3, in *Carassius carassius* 28.6, and in hybrids 39.4. The authors indicated that when other meristic features are compared without gill rakers, *Carassius auratus gibelio* can be told apart from both *Carassius carassius* and hybrids, but *Carassius carassius* and hybrids can't be told apart using the same criteria.

Furthermore, phylogenetic analysis of a crucian carp population from Helsinki, Finland (Knytl et al., 2018) showed that the nuclear genome of a triploid *Carassius* female (3n = 156) that had an obvious external appearance similar to that of the European crucian carp (*Carassius carassius*) contained three haplotype sets; two *Carassius gibelio* and one *Carassius carassius*. However, its mitochondrial DNA matched that of *Carassius gibelio*, proving its hybrid origin. According to Sun et al. (2009), silver crucian carp originated from normal crucian carp, which suggests that genetic material has been exchanged between triploid and diploid crucian carp on a regular basis. Zheng et al. (2010) showed that triploid and diploid crucian carp have comparable allelic sizes and distributions, confirming their close connection. Moreover, hybrids of these species were detected in natural lakes in Kazakhstan by (Goryunova & Skakun, 2002), and tetraploid hybrids of *Carassius gibelio* and *Carassius carassius* were also reported in Ukraine by (Mezhzherin et al., 2012). Thus, genetic studies are required to distinguish pure *Carassius carassius* individuals from other *Carassius* hybrids, especially when external morphological characteristics are similar.

2.5 Molecular markers and their applications in fisheries and aquaculture

Every living organism undergoes mutations that occur in their bodies due to cellular processes or interactions with the environment, which lead to genetic variation (polymorphism). This genetic variation increases the adoption rate, i.e., potential of the organism to survive under environmental changes. Along with other natural forces of evolution such as selection and genetic drift, variation between individuals leads to genetic differentiation in populations, species, and higher taxonomic levels (Tanya & Kumar, 2010; Askari et al., 2013). To be useful to geneticists, this variance must be (1) heritable and (2) discernible to the researcher, whether as a recognizable phenotypic variation or as a genetic mutation distinguishable via molecular techniques (Liu & Cordes, 2004). When the genomes of individuals within a population are compared to the species' reference genome sequence, many general types of genetic variants can be discovered: a deletion caused by the loss of one or more bases; an insertion caused by the gain of one or more bases; base substitution at different sites, often known as single nucleotide polymorphisms (SNPs); inversion of a DNA segment in its direction within a locus; rearrangements of multiple DNA segments within a small and larger scope of the genome; In addition to the copy number variant (CNV) due to insertion, deletion, and replication or multiplication of a DNA segments (Liu, 2011). Many distinct instances of each form of mutation could occur in any given species as a result of long evolutionary accumulation, and the number and degree of the multiple forms of mutations determine the genetic variance within a species. These mutations can be detected using DNA marker technologies (Liu & Cordes, 2004). The following chapters provide an overview of DNA marker technologies and their applications in aquaculture.

2.5.1 Types of molecular markers and their concepts

Aquaculture genetics makes extensive use of a number of marker types. It has been common practice in aquaculture genetics research to use allozyme and mtDNA markers. Recent marker types that are being used in this field also include restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellite, single nucleotide polymorphism (SNP), and expressed sequence tag (EST) (Liu & Cordes, 2004). There are two types of molecular markers: type I and type II. The type I markers are associated with gene function, while the type II markers are associated with anonymous genomic regions (Liu & Cordes, 2004; Askari et al., 2013) Table1.

However, researchers must decide what sort of marker is ideal for answering their scientific questions and the species they're studying. Most RFLP markers are classified as type I markers according to known classification criteria since they were identified during the analysis of known genes. Allozyme markers are also type I markers because they encode functional proteins. AFLP and RAPD markers are type II markers since they are amplified from unknown regions of the genome using polymerase chain reaction (PCR). Microsatellite markers are also type II markers unless they are associated with genes of known function. EST markers are type I markers since they reflect gene transcripts, unless they are developed from expressed sequences. SNP markers are predominantly type II markers (Liu & Cordes, 2004; Tanya & Kumar, 2010; Askari et al., 2013).

In aquaculture, molecular markers are used for a variety of purposes, including genetic identification and discrimination of hatchery stocks; detecting inbreeding events; marker - aided selection for selective breeding trials; and evaluating the impact of polyploidy induction and gynogenesis. Moreover, molecular markers can be used to detect genetic diversity between and within populations. They can also be used to find out what role potential parents play in mass spawning events. Another important field where molecular markers can be successfully utilized is disease detection (Sukumaran, 2018; Sukumaran & Gopalakrishnan, 2019). In addition to being used as markers in studies of populations, type I markers are often useful in studies of genetic linkage and QTL mapping. They are also useful in studies of comparative genomics and genome evolution. Type II markers are also great for making comparisons between different species, although the comparisons are limited to closely related species. This type of marker has also been shown to be useful in aquaculture genetics for species, strain, and hybrid detection, breeding studies, and more recently as QTL-related markers (Liu & Cordes, 2004).

Table 1. DNA marker types, their characteristics, and possible applications (Liu & Cordes, 2004)

Marker type	Acronym or alias	Requires prior molecular information?	Mode of inheritance	Type	Locus under investigation	Likely allele numbers	Polymorphism or power	Major applications
Allozyme		Yes	Mendelian, inheritance	Type I	Single	2 –6	Low	Linkage mapping, population studies
Mitochondrial DNA	mtDNA	No	Maternal codominant	_		Multiple haplotypes		Maternal lineage
Restriction fragment length polymorphism	RFLP	Yes	Mendelian, codominant	Type I or type II	Single	2	Low	Linkage mapping
Random amplified polymorphic DNA	RAPD, AP-PCR	No	Mendelian, dominant	Type II	Multiple	2	Intermediate	Fingerprinting for population studies, hybrid identification
Amplified fragment length polymorphism	AFLP	No	Mendelian, dominant	Type II	Multiple	2	High	Linkage mapping, population studies
Microsatellites	SSR	Yes	Mendelian, codominant	Mostly Type II	Single	Multiple	High	Linkage mapping, population studies, paternity analysis
Expressed sequence tags	EST	Yes	Mendelian, codominant	Type I	Single	2	Low	Linkage mapping, physical mapping, comparative mapping
Single nucleotide polymorphism	SNP	Yes	Mendelian, codominant	Type I or type II	Single	2, but up to 4	High	Linkage mapping, population studies?
Insertions/deletions	Indels	Yes	Mendelian, codominant	Type I or type II	Single	2	Low	Linkage mapping

2.5.1.1 Allozyme markers

Allozymes are allelic variations of proteins generated by a single gene locus, as they include polymorphism and represent gene protein products, making them type I markers (Liu & Cordes, 2004). Fishery geneticists have relied on starch gel electrophoresis of allozymes since the 1960s (Allendorf et al., 1987; Hillis et al., 1996). Analysis of allozyme loci remained one of the most common methods for studying population genetics and stock structure issues in fishes (Suneetha, 2000) until the begining of 2000s. It is an easy and rapid approach, inexpensive in nature and

studies variation without extensive morphological and quantitative surveys (Menezes et al., 1993). Variations in the presence or absence and relative frequencies of alleles are used to detect genetic diversity amongst species, populations and higher taxonomic classifications. Although their potency as codominant type I markers has been acknowledged, their use in aquaculture genetics has been restricted (Liu & Cordes, 2004). Allozymes have a number of drawbacks, including occasional heterozygote deficiencies caused by null alleles and sensitivity to the quantity and quality of tissue samples, as well as their association with low polymorphisms and low abundance. Some DNA sequence changes are also concealed at the protein level, which reduces the degree of variation that may be detected (Okumuş & Çiftci, 2003; Liu & Cordes, 2004). Despite the fact that allozyme analysis is restricted, it was employed in fisheries, particularly in fish systematics (Sodsuk & McAndrew, 1991; Van Der Bank, 1994; Kassler et al., 2002) and phylogenetic analysis (Unmack et al., 2017), phylogeographic analysis (Hammer et al., 2010; Chaturvedi et al., 2011), genetic diversity and species identification (Irnazarow, 1995; P. Ivanova et al., 2017). Allozymes have also been used in population genetic studies and genetic structure (Bechev, 2015; Morgan et al., 2019), stock identification (DeCovich et al., 2012), and conservation genetics (Ferguson et al., 1995). The use of allozyme loci in linkage mapping has been shown in studies of salmonids (Gharbi et al., 2006) and poeciliids (Morizot et al., 1991) , but the limited number of allozyme loci available prevents them from being used in large-scale genome mapping (Liu & Cordes, 2004).

2.5.1.2 Restriction fragment length polymorphism (RFLP)

RFLP markers were widely viewed as the first shot in the genome revolution (Dodgson et al., 1997), representing the beginning of a new era in biology. The discovery and application of restriction enzymes in 1973, as well as the introduction of DNA hybridization techniques in 1975, laid the groundwork for the development of the first type of DNA markers, RFLPs. Restriction endonucleases cleave DNA wherever they come across their recognition sequences. A restriction site can be gained, lost, or relocated owing to changes in the DNA sequence resulting from insertions or deletions (indels), base substitutions, or rearrangements of the restriction sites. Thus, the number and size of fragments generated by restriction enzyme digestion of DNA can vary across individuals, populations, and species. For RFLP analysis, two methods are often used. The first approach makes use of Southern blot hybridization, whereas the second makes use of PCR. However, the PCR approach may be used to replace time-consuming Southern blot analyses. Whether the length polymorphism is caused by a deletion or an insertion, gel electrophoresis of

the digested PCR products should reveal the size difference (Liu, 2011). The main advantage of RFLP markers is that they are codominant markers, which means that both alleles in an individual are detected in the analysis. The main drawback of RFLP is its poor level of polymorphism (Liu & Cordes, 2004; Liu, 2011). RFLP markers are among the most commonly used markers in genetic research. In early 2006, a PubMed search utilizing the key word RFLP yielded 30,000 citations. Despite their popularity, the application of RFLP markers in aquaculture genetics research remains somewhat restricted (Liu, 2008). These markers have typically been used to distinguish between species (Klinbunga et al., 2005; Chow et al., 2006; Clusa et al., 2017), strains, or populations (Apte et al., 2003; Lehoczky et al., 2005; Q. Zhang et al., 2005; Papakostas et al., 2006; Lajbner & Kotlík, 2011; Presti et al., 2012; Lujić et al., 2017; Alam et al., 2021). Many of these RFLP marker studies used mitochondrial DNA, or 16S rDNA (Klinbunga et al., 2001; Mamuris et al., 2001; de los Angeles Barriga-Sosa et al., 2005; Klinbunga et al., 2005; Presti et al., 2012; Lo Presti et al., 2014; Galal-Khallaf et al., 2017; Alam et al., 2021; Al Fatle et al., 2022).

2.5.1.3 Arbitrary nuclear DNA markers

When a fragment of DNA of unknown function is targeted, arbitrary markers are used. RAPD (Random Amplified Polymorphic DNA) and AFLP (Amplified Fragment Length Polymorphism) are two commonly used methods for amplifying unknown regions. Polymerase chain reaction-based RAPD uses an arbitrary primer to amplify unknown loci (Sukumaran & Gopalakrishnan, 2019). RAPD loci are inherited as dominant Mendelian markers and are scored as present or absent (Askari et al., 2013). The main advantages of RAPD markers include their applicability to all species regardless of knowledge of the organism's genetic makeup, relatively high polymorphic frequencies, ease of use, and low equipment and technical skill requirements (Liu, 2011).

RAPD markers have been used in taxonomic research and stock discrimination in marine and freshwater fishes and invertebrates (Steven X. Cadrin et al., 2005). They have also been used for species identification in fishes (Bhat et al., 2012) and mollusks (Klinbunga et al., 2001) marine algae (Van Oppen et al., 1996; Risjani & Abidin, 2020), genetic effect of environmental stressors analysis (Qu et al., 2019), effects of hybridization (Diamante et al., 2021), and analysis of genetic diversity and population structure (Pereira et al., 2010; Muneer et al., 2011; Hasan & Goswami, 2015; Ikpeme et al., 2015). The use of RAPD markers to identify species, strains, lines, and populations in model fish species such as zebrafish has been investigated and reported on (Johnson et al., 1994; Azad et al., 2022). Many linkage mapping studies in fish species have also used RAPD

markers (Borowsky & Wilkens, 2002; Khoo et al., 2003; Sun & Liang, 2004). The main limitation of this approach is its poor repeatability owing to the use of low annealing temperatures. Furthermore, since RAPD is a dominant marker, homozygous and heterozygous states cannot be distinguished, and these patterns are sensitive to even minor changes in amplification conditions. However, as a result of these shortcomings and the advent of more reliable and effective marker systems like AFLP, the usage of RAPD markers in fisheries research started to quickly drop (Liu, 2008).

The AFLP method relies on a series of extended primers to selectively amplify digested genomic DNA. Following the digestion of genomic DNA using two restriction enzymes, double-stranded oligonucleotide adaptors are ligated to the ends of DNA fragments, acting as primer binding sites for PCR amplification. A subset of the ligated fragments can be amplified selectively using primers that are complementary to the adapter and restriction site sequence with additional nucleotides at the 3' end (Vos et al., 1995). Polymorphisms and the presence or absence of DNA fragments can be detected on polyacrylamide gels. AFLPs have a high degree of polymorphism, require no prior knowledge, and can provide the most efficient, reliable, and economical analysis of fish population genetics. However, one of the major weaknesses of AFLP markers is their dominant nature of inheritance. Furthermore, the need for special equipment such as sequencers may restrict its widespread use (Steven X. Cadrin et al., 2005). It has been commonly used in aquaculture for a number of purposes, including molecular systematics and population structure analysis, strain identification, parentage identification, genetic diversity, reproduction contribution, and endangered species protection (Young et al., 2001; Whitehead et al., 2003; Campbell & Bernatchez, 2004; Zhu et al., 2013; Miyake et al., 2016; Napora-Rutkowski et al., 2017; Xiao et al., 2018; Panicz et al., 2019; Elameen et al., 2021). It has also been extensively used in genetic linkage analysis (Liu et al., 2003; Li et al., 2005; Vos et al., 2013), as well as analysis of parental genetic contributions involving interspecific hybridization (Firmat et al., 2013) and meiogynogenesis (Pan et al., 2017).

2.5.1.4 Specific nuclear DNA markers

Tandem repeats are portions of DNA that are repeated tens, hundreds, or thousands of times throughout the nuclear genome of eukaryotes. They are repeated in tandem at different loci in the genome and in different individuals, with the number of repeats varying. Minisatellite and microsatellite DNA are forms of repetitive DNA. Minisatellite DNA loci have repeat lengths

ranging from 9 to 65 bp. Compared to minisatellites, microsatellites are more abundant in the genome. When it comes to fish genome mapping and population genetics research, microsatellite loci are more useful than minisatellite loci since they occur once every 10 kbp, whereas the minisatellite occurs once every 1500 kbp. Minisatellite loci are primarily used in parentage analysis, although they are less useful in population genetic analysis unless large sampling sizes are used. They are also restricted by the complexity of the mutational processes that they undergo (Sukumaran, 2018).

Microsatellites are short, tandemly arrayed di-, tri-, or tetranucleotide repeat sequences with repeat sizes ranging from 1–6 bp that are repeated multiple times and flanked by nonrepetitive special DNA sequences (Tautz, 1989). (Tautz, 1989)and (Weber & May, 1989) were the first to show polymorphism at microsatellite loci. (Tautz, 1989) refers to them as "simple sequence repeat" (SSR) DNA, although (Edwards et al., 1991) refer to them as "short tandem repeat" (STR) DNA. In general, the dinucleotide microsatellites are the most abundant, followed by tri- or tetra-nucleotide repeats, though the tetranucleotide repeats can be more frequent than the trinucleotide repeats in some cases (Edwards et al., 1998). Microsatellites are found in the genome in all chromosome regions. Despite their wide distribution in genomes, microsatellites are mainly present in noncoding areas (Metzgar et al., 2000). Using the polymerase chain reaction, alleles at microsatellite loci can be amplified from small samples of genomic DNA (Saiki et al., 1988) (preferably using PCR amplicons within 200 bp (Liu, 2011), and then separated and effectively sized on a polyacrylamide gel as one or two bands, which are used to measure genetic differences within and between populations of species (O'Connell et al., 1997). Specific primers flanking the repeat units are necessary for single locus microsatellite analysis, and their sequences can be obtained from (1) genomic DNA libraries or (2) accessible sequences in gene banks. To save time and money, different primers are often tested in the same multiplex PCR reaction (Abdul-Muneer, 2014). Microsatellites are codominant markers that are inherited in a Mendelian fashion, with mutation rates as high as 10^{-2}-10^{-3} per generation estimated. Because of their hypermutability, microsatellites are highly polymorphic, resulting in the collection of different forms in a population of a given species. Microsatellite polymorphism is dependent on size differences induced by alleles having different numbers of repeat units at a given locus (Liu, 2011). Cross-amplification with designed primers in closely related species is also viable, which reduces the cost of developing microsatellite sequences in different species. These high levels of polymorphism demonstrated by

microsatellites, as well as their short size range, locus-specific in nature, uninterrupted stretches of identical repeat units, and ease of sample preparation, have all made them one of the most commonly used genetic markers in fisheries research (Abdul-Muneer, 2014). The presence of null alleles (existing alleles not detected by normal assays) and the presence of stutter bands are two limitations of using microsatellite markers. When mutations occur at the primer binding sites of a microsatellite locus, null alleles are observed (Okumuş & Çiftci, 2003). Parentage or relatedness analysis and assignment tests can be less precise because of null alleles, so discarding loci with null alleles is the best option (Hansen, 2003). Microsatellites have become a common marker in a wide range of genetic studies. They have been widely used in fisheries research over the last two decades. Because of their high level of polymorphism, microsatellite markers can be particularly useful for stock discrimination, population genetics analysis, and conservation aquaculture programs (Wright & Bentzen, 1995; Chauhan et al., 2007; Kohlmann et al., 2007; Mandal et al., 2009; Kohlmann et al., 2010; Xu et al., 2010; Abdul Muneer et al., 2012; Praebel et al., 2013; Li et al., 2017; Tiknaik et al., 2020; Coimbra et al., 2020; Al Fatle et al., 2022). In fisheries and aquaculture, microsatellite markers are also used for kinship and parentage determination (Hansen et al., 2001; Zhang et al., 2016; Wang et al., 2021), genomic mapping (Sanetra et al., 2009), and addressing taxonomic ambiguities in several other animals besides fishes (Presti et al., 2010; X. Lu et al., 2014; Kariuki et al., 2021) , as well as stocking and hybridization impacts (Hänfling et al., 2005; Papoušek et al., 2008; Wouters et al., 2012; Praebel et al., 2013; Shechonge et al., 2018; Solarte-Murillo et al., 2020; Blackwell et al., 2021), phylogenetic and phylogeographic studies (Jeffries et al., 2016; Ruzzante et al., 2020).

Single nucleotide polymorphisms (**SNPs**) are polymorphisms caused by single nucleotide substitutions (transitions or transversions) or single nucleotide insertions or deletions. It's a variant of scnDNA polymorphism that detects individual nucleotides (Okumuş & Çiftci, 2003). These point mutations result in different alleles with alternative bases at the given nucleotide site within a locus (Liu & Cordes, 2004). SNP markers are inherited as co-dominant markers. Several methods have been proposed for SNP genotyping. Direct sequencing, single base sequencing, allele-specific oligonucleotide (ASO), heteroduplex analysis, denaturing gradient gel electrophoresis (DGGE), SSCP assays, and ligation chain reaction (LCR) are all traditional approaches for SNP genotyping. Direct sequencing is clearly the most precise method for genotyping SNPs, but it is expensive and time-consuming (Liu, 2008). As SNPs are the most abundant polymorphism in any

organism and are adaptable to automation, exposing hidden polymorphism not found by other markers, they have become a focal point in the growth of molecular markers. Theoretically, a SNP within a locus can produce as many as two alleles, each containing one of two possible base pairs at the SNP site. Therefore, SNPs have been regarded as bi-allelic (Liu, 2011; Liu & Cordes, 2004). They can be used for population genetics research (Jeffries et al., 2016; Kumar et al., 2019), genomics, and disease detection (Palti et al., 2015; Jin et al., 2021; Sakseepipad et al., 2021).

2.5.1.5 Mitochondrial DNA markers

Mitochondrial DNA is a type of non-nuclear DNA found in mitochondria, which are cytoplasmic organelles. Mitochondrial DNA has a haploid genome that is inherited maternally. The whole genome is transcribed as a single unit. They are homologous markers, and they are not subjected to recombination. They exist in several copies in each cell and are selectively neutral. No editing or repair mechanisms are visible throughout their continuous unidirectional and symmetrical replication (Billington, 2003). Sequence divergence accumulates more rapidly in mitochondrial DNA than in nuclear DNA (Brown, 1985). This is due to the faster mutation rate in mtDNA because of the lack of repair mechanisms during replication (Jeffreys et al., 1985). Furthermore, since mitochondrial DNA is inherited maternally, the effective population size is smaller than nuclear DNA, making mitochondrial DNA variation more sensitive to population bottlenecks and hybridizations. Thus, the phylogenies and population structures obtained from mtDNA data may not provide a comprehensive picture of the nuclear genome, whether there is gender-biased migration, selection (Birky Jr et al., 1989), or introgression (Chow & Kishino, 1995). Technically, mtDNA markers are RFLP markers, except that the reference molecule is mtDNA rather than nuclear genomic DNA (Liu & Cordes, 2004). In terms of mtDNA variation, there are two primary ways to analyze it: RFLP analysis of entire purified mtDNA digested with restriction endonucleases or by DNA sequencing of small fragments of the mtDNA molecule produced by PCR amplification (Billington, 2003). Due to recent advances in sequencing technologies, direct sequencing of mitochondrial genes is now highly feasible.

Non-coding segments, such as the D-loop, which is mostly used for population comparisons, have higher levels of variation than coding sequences, such as the cytochrome b gene, possibly because of the reduced functional constraints and the lower selection pressure (Askari et al., 2013). However, the Cytochrome b region is also commonly used for intra-specific comparisons. Slowly evolving gene regions, such as CO I, II, III, and Cytochrome b, may be used for interspecific

comparisons, whereas rapidly evolving gene regions can be used for intraspecific comparisons (Steven X. Cadrin et al., 2005). In addition, since it is a slow-evolving region and because of its conserved nature over a wide range of taxa, the Mitochondrial Cytochrome C Oxidase I gene sequences have been identified as taxon 'barcodes' that can serve as the core of a global bioidentification system. DNA barcodes are mitochondrial COI gene fragments of around 600 base pairs that are a reliable and low-cost tool for species identification and diversification (Hebert et al., 2003). To achieve a species identity, this signature sequence can be compared to an existing database of "known" sequences from reference specimens. The Barcode of Life Data System (BOLD) database, an online interactive workbench used to build and curate a global barcode reference sequence library, stores and makes specimen and sequence data accessible. Global efforts to barcode the world's biodiversity are ongoing within the regulatory framework of the International Barcode of Life (iBOL) program, including Fish Barcode of Life (FISH-BOL) (Hanner et al., 2011). Over the past two decades, mitochondrial DNA genes have been widely used in fish taxonomy, and genetic studies. Analyses of mtDNA markers have been widely used to study stock structure in a host of fishes, including eels (Syazni et al., 2017; Marini et al., 2021), bluefish (Miralles et al., 2014), snappers (Souza et al., 2019; Andrews et al., 2020), and sharks (Lim et al., 2021; Canfield et al., 2022). Mitochondrial markers are widely used in genetic diversity and population structure studies in fisheries and aquaculture (Qi et al., 2015; Li & Guo, 2020; Freitas et al., 2021; Tóth et al., 2022). They are also used for species identification (Wu & Yang, 2012), resolving taxonomic ambiguities (Biswal et al., 2018), phylogenetic and phylogeographic studies (Lajbner & Kotlík, 2011; Jeffries et al., 2016; Lujić, Kohlmann, et al., 2017; Fatsi et al., 2020; Al Fatle et al., 2022) as well as hybrid detection (Hashimoto et al., 2016).

2.5.1.6 Expressed sequence tags (ESTs)

ESTs are single-pass sequences generated from the random sequencing of cDNA clones. Using various means of expression analysis, ESTs may be used to identify genes and interpret their expression. The genes expressed in certain tissue types under specific physiological conditions or developmental stages can be quickly and accurately analyzed (Liu & Cordes, 2004; Sukumaran & Gopalakrishnan, 2019). ESTs are useful for linkage mapping. Zheng et al. (2014) presented the first study to develop EST–SSRs markers from the transcriptome of *Carassius auratus*, which may be useful in linkage map studies as well as marker-assisted selection (MAS) in this species' breeding programs.

2.5.2 Application of molecular markers in phylogenetic and phylogeographic studies

Quantitative analyses for genetic stock identification have as their primary goal the provision of objective classifications of samples into groups. Phylogeographic studies are concerned with processes that affect geographical distribution, while phylogenetic studies are concerned with historical processes that influence species relationships. Phylogenetic studies could also provide information on conservation units and ecological patterns (Avise, 1994; Okumuş & Çiftci, 2003). For species conservation, a thorough knowledge of a species' native geographical range and the distribution of its diversity within that range is essential (Reed & Frankham, 2003; Gaston & Fuller, 2009; Rissler et al., 2015). The phylogeographic divisions of Europe's freshwater fish developed as a result of isolation in Pleistocene glacial refugia, as the rivers of Europe were frequently colonized from the Black Sea to rivers such as the Danube and the Dnieper (Hewitt, 2004). These Pleistocene cycles are considered to be a "speciation pump," and thus geographic speciation has contributed greatly to the diversification of freshwater fish (April et al., 2013). Human-mediated translocations, on the other hand, have had a significant effect on the present distributions of European freshwater fish. For many aquatic species, particularly freshwater fishes, international trade and human-assisted transportation provide an effective dispersal mechanism. Thus, phylogeographic patterns that don't represent natural historical processes can be developed by this human-aided dispersal, particularly in species that are vulnerable to deliberate human translocations. As a consequence, the effects of such translocations are more complicated to identify. That's where molecular phylogeography comes in handy, as it may be used to determine if a species is indigenous or introduced to a given region (Lajbner et al., 2011). However, molecular genetic data might well be used to study the evolutionary history and connections between species (Avise, 1994). Molecular marker technologies help to elucidate historical range-wide and demographic changes and determine linkages to previous climate changes (Scoble & Lowe, 2010). In phylogenetic and phylogeographic studies, mitochondrial DNA analysis has been shown to be an effective marker (Avise et al., 2016; Avise & Ph.D, 2000). mtDNA variation can determine relationships between species that have diverged for as long as 8–10 million years (Peacock et al., 2001). Because of its high mutation rate, small effective population size, and predominance of maternal inheritance, mtDNA provides great potential for identifying population structure, and due to the absence of recombination and the low efficiency of repair mechanisms, mtDNA evolves at

a rapid rate, making this molecule very valuable in phylogenetic analysis. Besides mtDNA analysis, nuclear DNA analysis has also been used as a powerful tool for phylogeographic analysis (Pauquet et al., 2018; Jang-Liaw et al., 2019; Gu et al., 2022).

2.5.3 Species and hybrid identification

The correct identification of the studied species is the most critical phase in any research concerned with biological material. Otherwise, research on biodiversity and distribution cannot be conducted, and these animals cannot be used as models in any form of investigation (Rylková et al., 2013). The use of meristic characters or a limited number of molecular markers to identify hybridization in its initial F1 stage is well known and can be achieved. However, identifying hybrid groups beyond the initial F1 generation and detecting introgression may be difficult and necessitates the use of a wide number of genomic markers (Boecklen & Howard, 1997; Smartt, 2007). DNA-based species identification is valuable where the specimen lacks the morphological characters required for regular taxonomic recognition (such as fish fillets), where morphological characters are poorly described, when no diagnostic morphological characters are identified (e.g. cryptic species), or when specimen conservation status precludes morphological analysis (Ovenden et al., 2015). DNA-based species identification is also used primarily to detect illegal, unreported, and unregulated (IUU) fishing (Martinsohn et al., 2019). In an aquaculture system, genetic identification of species or strains is often needed. Since most species have significant genetic variations, identifying them using DNA markers is reasonably simple. RFLP, RAPD, AFLP, and microsatellite markers are all useful in this field (Liu & Cordes, 2004), but prior molecular knowledge is essential.

2.5.4 Application of molecular markers in population structure; between and within population variations

Individuals vary genetically, resulting in differentiation at the population, species, and higher taxonomic levels. Genetic variation is an important factor in the survival of various species. Furthermore, natural populations are perhaps the greatest gene banks, providing a valuable resource for genetic diversity for existing and potential uses of genetic improvement for farmed species and advanced sport-fish applications (Dunham, 2011). A measure of genetic diversity is one that takes into account variation in the form of genes and noncoding loci within a population (Luikart et al., 2003; Hughes et al., 2008). Heterozygosity and the total number of alleles in a

population can both affect how well a population can adapt and grow (Allendorf & Luikart, 2009). However, several evolutionary factors (such as mutations, random genetic drift, gene flow, and natural selection) may change allele frequencies in a population. These evolutionary factors influence the amount and distribution of genetic variation among populations, and consequently population differentiation (Black IV et al., 2001; Allendorf et al., 2014; Allendorf, 2017). Applications of genetic diversity data have varied in research related to development, conservation, and management of natural resources and genetic improvement programmes. Genetic diversity may be detected to a great extent thanks to the development of molecular genetic markers. The use of molecular markers in conjunction with new statistical developments has enhanced the analytical capacity required to investigate genetic diversity (Tanya & Kumar, 2010). Molecular markers are often used in aquaculture to measure the loss of genetic diversity in hatcheries by comparing estimates of variation between hatchery stocks and their wild counterparts. The data can be used to keep track of farmed stocks in order to avoid inbreeding and to plan genetic improvement programs (Tanya & Kumar, 2010). As mentioned in the sections concerned with marker types, allozymes and mtDNA have traditionally been the most commonly used markers in fish, and they are useful in this regard as a tool for genetic diversity characterization and population structure studies. While microsatellites, RAPDs, AFLPs, SNPs, and next-generation DNA sequencing are the more recent markers. In terms of newer marker approaches, RAPIDs have the least ability to discriminate (Liu & Cordes, 2004; Liu, 2011). Because of their high rate of evolution, microsatellite DNA markers and the non-coding region of mtDNA (D-loop) are the most preferred DNA markers for stock structure studies.

2.5.5 Genetic markers used in the target species

Genetic markers for aquaculture species such as crucian carp (*Carassius carassius* L. 1758) and tench (*Tinca tinca* L. 1758) have made significant progress. Genetic diversity within and between tench populations has so far been based on allozymes (Kohlmann & Kersten, 1998), PCR-RFLP (Lajbner & Kotlík, 2011; Presti et al., 2012) microsatellite markers (Kohlmann & Kersten, 2006; Kohlmann et al., 2007, 2010), nuclear and mitochondrial DNA (mtDNA) (Lajbner et al., 2010, 2011; Lajbner & Kotlík, 2011; Presti et al., 2012; Lo Presti et al., 2014; Lujić et al., 2017) as well as genes of growth hormones (Kocour & Kohlmann, 2011, 2014). The tench, like many other widely distributed freshwater species, shows deep geographic divisions, as discussed in earlier chapters. Lajbner & Kotlík (2011) came up with a new way to distinguish two recently discovered

Eurasian clades that are morphologically indistinguishable. PCR-RFLP assays of two nuclear-encoded exon-primed intron-crossing (EPIC) markers and one mitochondrial marker were used to identify the Western and Eastern phylogroups as well as three different geographical mtDNA clades within the main Eastern phylogroup. Similarly, recent literature distinguishes tench into two phylogroups: Western (W) and Eastern (E) (Lajbner et al., 2010; Presti et al., 2012; Lujić et al., 2017; Al Fatle et al., 2022). Furthermore, a multiple-gene sequencing approach was used by Lajbner et al. (2011) to investigate the consequences of human-aided dispersion across the two phylogroups. By analyzing three mtDNA segments, D-loop, COI, and Cytb, from five wild European tench populations, Karaiskou et al. (2020) reported two phylogroups with greater variability than previously recorded, as well as a hybridization zone in the Danube river region. Kumar et al. (2019) developed a transcriptome-derived SNP array in tench, which was utilized to detangle the genetic structure of two cultured tench breeds (Tabor and Hungarian).

So far, genetic diversity and phylogenetic relationships in crucian carp have been based on nuclear and mitochondrial DNA markers (Zhang & An, 2007; Papoušek et al., 2008; Knytl et al., 2018). The development of polymorphic microsatellite markers to investigate the population structure, genetic diversity, and kindship of triploid crucian carp and its diploid relatives was described by Jia et al. (2006) and Zheng et al. (2010). Similarly, using nuclear microsatellite markers and mitochondrial DNA sequences, several authors reported cases of hybridization between crucian carp (*Carassius carassius*) and non-indigenous carp species such as gibel carp (*Carassius auratus gibelio*) and common carp (*Cyprinus carpio*) (Hanfling et al., 2003; Hänfling et al., 2005; Papoušek et al., 2008; Wouters et al., 2012). Furthermore, Random amplified DNA polymorphism (RAPD) analysis was used to assess genetic similarities and diversity between two wild and farmed populations of crucian carp (*Carassius carassius*) (Yoon & Park, 2002). Janson et al. (2015) conducted the first research on the genetic comparison of crucian carp from historic man-made ponds in the Scandinavian Peninsula with the goal of identifying historical unmixed populations and analyzing the relationships of pond populations in different provinces in Sweden using nine microsatellites developed for gibel carp (*Carassius gibelio*) and goldfish (*Carassius auratus*).

According to Jeffries et al. (2016), datasets from a combination of mitochondrial cytochrome b DNA, microsatellites, and genome wide SNPs obtained from RADseq agreed on broad phylogeographic patterns, revealing the presence of two lineages of *Carassius carassius* previously unknown in Europe, one of which is found throughout northern and central-eastern

European drainages, and the other almost entirely confined to the Danubian catchment, which should be included in prospective broad-scale monitoring and conservation strategies.

2.6 Fish GenBank (in vivo and in vitro gene banks, cryopreservation, fish gene banks in the world and in Hungary)

With the significantly increased pace of species extinction in recent decades, it is imperative to explore all avenues available to stop biodiversity loss and to find methods to preserve gametes, embryos, and somatic cells from all species on Earth (Saragusty, 2012). Conservation can be defined as an endeavor to preserve the genetic variety created by evolution during our planet's past 3.5 billion years (Eisner et al., 1995). Along with species and ecosystem diversity, genetic diversity is one of three categories of biodiversity recognized by the IUCN as worthy of protection (McNeely et al., 1990). Procedures to preserve live animals outside of their production or natural habitat (ex situ "in vivo") or via cryopreservation of germplasm (ex situ "in vitro") are being developed to conserve rare breeds and genetic diversity in addition to in situ conservation (Hiemstra, 2005). Wild fish, especially freshwater finfish and mollusks, are among the most endangered species exploited by humans, owing to habitat destruction or loss, as well as over-exploitation (Millennium Ecosystem Assessment (Program), 2005; Pullin, 2005). The list of threats to fish populations is extensive and well-documented (Harvey, 1998). One of the main causes of species extinction is habitat loss in main river channels and floodplains, as well as reduced hydrological connectivity between them (Aarts et al., 2004). Siltation and dam construction have the potential to destroy aquatic habitats. Siltation affects water flow and depth, limiting the ability of riverine fish to feed, navigate, migrate, and reproduce. The flood control embankments and dams obstruct fish migration, reproduction, and eventually species survival. Also, domestication, conventional genetic manipulation, sex reversal, hybridization, and crossbreeding, as well as releasing fry into natural water bodies, all contribute to the extinction of native species (Bart, 2002).The introduction of invasive and competitive species, as well as pollution, are also major threats to fish species (Millennium Ecosystem Assessment (Program), 2005; Reed & Czech, 2005).

The optimum approach for preserving threatened and endangered species is to preserve and restore the species' natural environment in situ. However, since habitat restoration is obviously a slow process, it is costly and time-consuming. Therefore, ex situ (conservation outside their native habitat) live or cryopreserved gene banks are one alternative (Bart, 2002). Fish live gene banks are

vital, yet they are costly to maintain, necessitating the construction of special facilities and are difficult to manage due to their labor-intensive nature (Bartley, 1998). While the development of long-term ex situ frozen gene banks, which are considered to be an excellent complement to habitat conservation and in situ gene banks, has fewer restrictions. In comparison to living gene banks, they are less expensive. Cryogenic gene banking eliminates the risk of contamination while requiring little space and equipment (Bart, 2002). By far, cryopreservation is the most effective and widely used method. It has led to the establishment of a number of genetic resource banks. Spermatozoa are relatively straightforward to cryopreserve due to their small size, condensed DNA, and lack of cytoplasm, and this has already been done in hundreds of species (Saragusty, 2012). Cryopreservation of fish sperm offers a technique for optimizing reproduction throughout the reproductive phase. It may be used as a genetic bank or germplasm, which can aid population management practices by ensuring genetic variety and reproductive success (Viveiros et al., 2011). Cryopreservation research on aquatic species' spermatozoa has advanced significantly in recent decades (Paniagua-Chavez et al., 2000; Adams et al., 2011; Yang et al., 2012; Zhu et al., 2014; Liu et al., 2015). A number of protocols for the preservation of fish spermatozoa in various fish species have been developed, with the majority of them focusing on salmonids, tilapia, and carp (Babiak et al., 1997, 1998; Akçay et al., 2004; Irawan et al., 2010; Viveiros et al., 2011; Nascimento et al., 2012; Judycka et al., 2016; Nynca et al., 2016; Boryshpolets et al., 2017; Kása et al., 2018; Bozkurt et al., 2019; Pereira et al., 2020; Judycka et al., 2021; Lee et al., 2021). However, many of Hungary's native fish species are endangered and need protection (Keresztessy, 1996). Past and recent human activities in Hungarian natural waters have had direct or indirect effects on fish populations, and habitat destruction, the introduction of non-native fish species, and overfishing are among the most serious concerns. The management of many natural water fisheries has lately moved toward stocking native fish species and the use of genuine strains over domesticated ones (e.g., common carp). Because of this trend, breeding of native fish species such as *Tinca tinca, Carassius carassius,* and *Leuciscus aspius* has improved (Specziár & Erős, 2015). In order to save native fish and commercially important fish species from extinction, several gene conservation initiatives have been developed in vivo and in vitro using genetics and molecular biology methods (István, 2017). Since Hungary is one of Europe's biggest producers of common carp, the country now has a diverse range of indigenous varieties and strains (Tóth et al., 2020). The establishment of the live gene bank of domesticated carp began in 1963 with the

collection of common carp strains from various areas of Hungary at the Fish Culture Research Institute (recently renamed the Research Institute for Fisheries, Aquaculture and Irrigation (HAKI)) in Szarvas (Gorda et al., 1995). Currently, the institution has 18 Hungarian strains (landraces) and 13 foreign strains, collected from Central and Eastern Europe as well as Asia (Bakos et al., 2001). The main goal of maintaining the gene bank was to improve the genetics of common carp by increasing the hybrid yield for production purposes (Bakos, 1976). Three outstanding hybrids (Szarvasi 215, P-31, and P-34) were produced as a result of this breeding line. From 1972 to 1994, HAKI supplied about 12,000 mature female and male brood fish to Hungarian commercial fish farms in order to establish parental lines (Lehoczky et al., 2005). The live gene bank of common carp not only fulfilled the industry's requirements, but it also helped to conserve the biological variety of Hungarian carp landraces (Gorda et al., 1995; Lehoczky et al., 2005). Furthermore, activities related to the sturgeon gene bank have also been developed. The study on sturgeon species in HAKI began in the late 1960s and is currently ongoing. The work is based on a live sturgeon gene bank that has been kept at the institution since the 1980s. Collaborations with local farmers and world-wide sturgeon experts have been enhanced as a result of this effort. In addition, new species, such as the paddlefish (*Polyodon spathula*), are also preserved and maintained in the live gene bank in order to improve the profitability of extensive pond systems. It's a prized species for its roe as well as its tasty flesh (Feledi et al., 2013). Several cryopreservation techniques are also being used to develop a cryopreserved gene bank at the Research Institute for Fisheries, Aquaculture, and Irrigation (HAKI) in Szarvas, Hungary (Horv√°th & Urb√°nyi, 2008). Successful procedures for sperm cryopreservation have already been reported (Urbányi et al., 1999; Horvath & Urbanyi, 2000; Horváth et al., 2003; Urbany et al., 2008; Bernáth et al., 2016; Bokor et al., 2019; Franěk et al., 2019; J. Molnár et al., 2020). Although they are considered critically endangered in a number of European countries (as mentioned in previous chapters), few conservation efforts have been made for the crucian carp (*Carassius carassius* (L. 1758)) and the tench (*Tinca tinca* (L. 1758)) so far (Rodina et al., 2007; Sayer et al., 2011; Linhartova et al., 2014; Lujić et al., 2017a). As such, for these species, the National Centre for Biodiversity and Gene Conservation has started working on ex-situ live GenBank and Cryobank.

3. MATERIALS AND METHODS

3.1 Target fish species

This study focused on two cyprinid fish species: crucian carp (*Carassius carassius* L. 1758) and tench (*Tinca tinca* L. 1758).

3.2 Study sites and samples collection

320 crucian carp fin tissue samples were collected from eleven different fish stocks in Hungary between 2017 and 2019, representing eight wild populations and three farmed stocks, as well as 175 tench samples from seven different sites in the country's eastern, central, and western regions. The collection data of sampled fishes are given in Table 2 and 3. The morphological identification of the species was carried out by experts using simple external investigation. Figure 5 and 6 depict the sample sites.

The fish were handled with care, and following sampling, they were released to their natural habitat. Prior to sample taking, fish were anesthetized with clove oil. Some preparations had to be made before the DNA isolation and molecular analysis could begin. The fresh fin tissue samples were kept in 2 ml Eppendorf tubes containing 70% ethanol, then subsequently labelled and transported to the molecular laboratory located at the National Centre for Biodiversity and Gene Conservation (NBGK-HGI). At the institute, these Eppendorf tubes were kept in a conventional freezer at a temperature of -20 C.

Table 2. Data for crucian carp sampled from different sites in Hungary.

Location	Label	GPS coordinate		Region	Sample size
Cún-Szaporca Holt-Dráva	Hd	N45,799126	E18,1481455	central	31
Dunafalva	Df	N46,1053107	E18,7950262	central	20
Kölked Misányi-fok	K	N45,9369798	E18,7264802	central	29
NBGK-HGI -genebank	Gb	N 47.594084	E19.376320	central	33
Lake Fertő	F	N47,6235593	E16,7551112	west	25
Lake Kolon	Kt	N46,7604735	E19,3395996	central	33
Belső-telep HAKI 24.	Ha	N46,856674	E20,515996	east	30
Rétimajor 21.	Rm	N46,8079938	E18,5906441	central	36
Baja 24.	B	N46,1951603	E18,93514	central	28
Alag Éger	A	N47,6465392	E19,1501648	central	30
Mura Vízgyűjtő	M	N46,3806524	E16,7710282	west	25

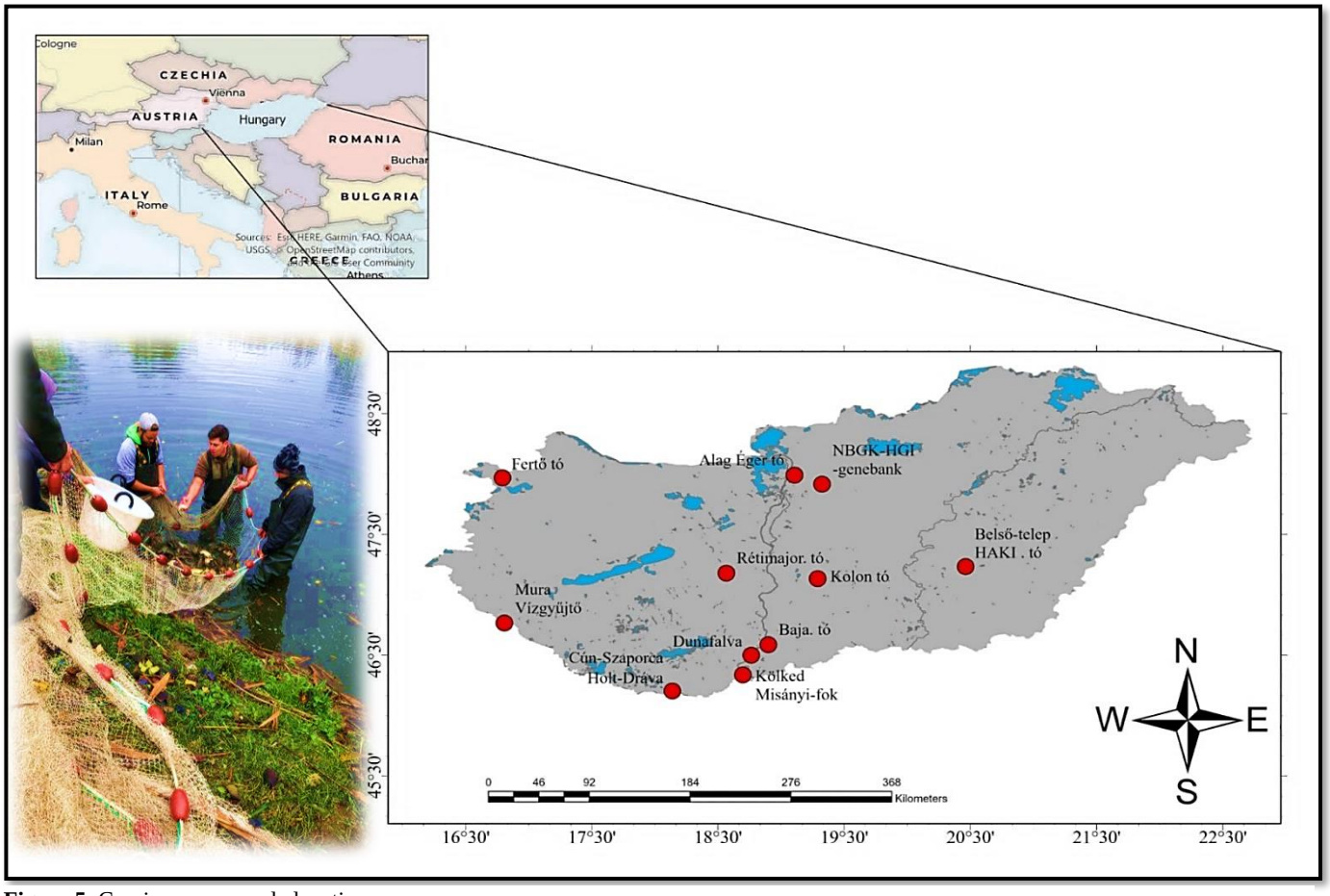


Figure 5. Crucian carp sample locations map.

Table 3. Data for tench sampled from different sites in Hungary.

Location	Label	GPS coordinate		Region	Sample size
Derecske	d	N47° 20' 20.48"	E21° 34' 25.13"	eastern	25
Lake Tisza	t	N47°39' 31.10"	E20° 41' 54.25"	eastern	10
Cibakházi Tisza	cht	N46° 59' 03.53"	E20° 10' 21.77"	eastern	39
Lake Kolon	k	N46° 45' 35.05"	E19° 20' 26.80"	central	32
Cun-Szaporca	csz	N45° 46' 55.10"	E18° 06' 09.31"	central	10
Csörnöc-Herpenyő	csh	N46° 59' 37.83"	E16° 36' 55.11"	western	25
Lake Fertő	f	N47° 38' 08.30"	E16° 44' 39.28"	western	34

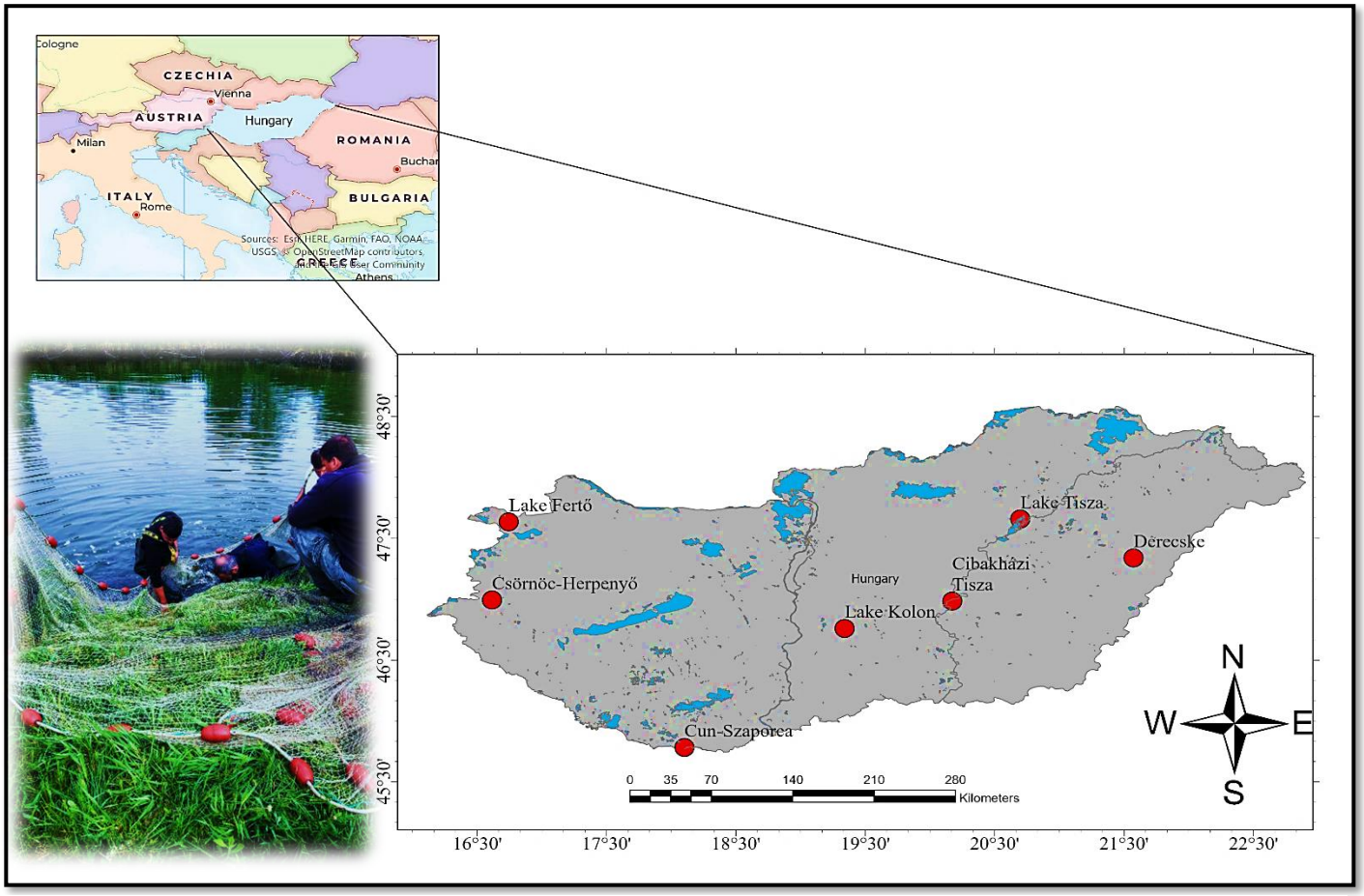


Figure 6. Tench sample locations map.

3.3 DNA extraction and quantification

The whole genomic DNA was extracted from the fin clips using a modified salting out protocol based on Miller et al. (1988). The procedure was modified in the NBGK-HGI molecular genetic laboratory in order to carry out the research work as detailed below. The protocol was performed over three days. On day one, the frozen fin tissue samples were thawed at room temperature. For the preparation of the solution, Nuclei lysis buffer (300 µl/sample, Table 4), sodium dodecyl sulfate ($NaC_{12}H_{25}SO_4$), SDS 10% (20 µl/sample), and proteinase K enzyme (5 µl/sample) were used. The amounts of the above solutions can also be changed depending on how many samples are being used.

325 µl of the above-prepared solution was pipetted into newly labeled eppendorf tubes. Following that, the thawed edges of the fish tails were cut and placed in eppendorf tubes containing 325 µl of solution. These samples, which contain total genomic DNA, were allowed to incubate and digest at 37 °C overnight. On the second day, 100 µl of 6 M NaCl was prepared and added to the previously incubated eppendorf tubes, which were then vortexed for about 20 sec. After the vortex was made, the tubes were centrifuged at 13,000 rpm for 10 min at room temperature. The DNA precipitate was washed with 300 µl of 2-propanol before being centrifuged for 10 minutes at 4 °C at 13,000 rpm. If the DNA could not be seen, it would be kept in the freezer again for another hour before centrifuging. The DNA precipitate was then washed twice with 70% ethanol (500 µl/wash) and centrifuged for 1 min. The eppendorf tubes holding the isolated DNA were left to dry for 1 hour at room temperature in a sterile cabinet before being mixed with TrisEDTA solution and incubated again at 37 °C overnight (Table 4). The tubes were then vortexed for 5-10 sec after incubation on the third day of isolation, and the DNA concentration was measured using a spectrophotometer (NanoDrop 2000c, Thermo-Scientific, and Waltham, NJ, USA). The purity of the extracted DNA was assessed at 260 and 280 nm wavelengths. The quality of the extracted DNA was tested on a 2% agarose gel.

Table 4. The composition of nuclei lysis buffer and TrisEDTA solution.

Composition	Nuclei lysis Buffer	TrisEDTA (TE) solution
Distilled water	100ml	100ml
2-Amino-2-(hydroxymethyl)-1,3-propanediol (TRIS base)	0.001 mol	0.001 mol
NaCl	0.04 mol	0.0001 mol
Ethylenediaminetetraacetic acid (EDTA) 0.5M	0.0002 mol	
pH	8.2	7.5

3.4 Molecular genetic methods and data analysis

To determine the distinct lineages and estimate the difference between them using phylogenetic analysis, as well as to identify the phylogeographic identity, mitochondrial DNA sequencing along with two independent nuclear-encoded exon-primed intron-crossing (EPIC) markers were employed in the study. In a population genetics approach, a collection of microsatellites was utilized to study the genetic structure of populations.

3.4.1 Microsatellite amplification and analysis

Polymerase chain reaction (PCR) was used to amplify DNA fragments from all specimens, using 13 different primer pairs for crucian carp and a set of 12 primer pairs for tench. Prior to actual amplification, the protocol for each marker was changed and repeated several times.

3.4.1.1 Microsatellite amplification in Crucian carp

13 microsatellite markers originally developed for other cyprinid species as described by Zheng et al. (1995), Crooijmans et al. (1997), Yue & Orban (2002), Baerwald & May (2004), Guo & Gui (2008), and Zheng et al. (2010) were cross-amplified in *Carassius carassius*. The description of the markers is shown in (Table 5). The PCR reaction was performed for (MFW7, GF1, GF29, YJ0010, YJ0022, HLJYJ017, HLJYJ028, HLJYJ029, HLJYJ041, HLJYJ046, HLJYJ082, J62, and CypG24) in a 15 µl reaction volume of PCR mixture comprising 30 ng/µl of genomic DNA containing the target region to amplify, 8.46 µl DW, 2 µl Mg free Dream Taq buffer (Thermo Fisher Scientific, Waltham, NJ, USA), 1.44 µl (25 mM) $MgCl_2$, 1 µl (10 mM) nucleotides, 0.4 µl

of each specific primer (5 µM), 0.20 µl tail primer of each pair (5 µM), and 0.1µl (DreamTaq DNA polymerase 5 Unit/µl (Thermo Scientific, Waltham, NJ, USA). The PCRs were carried out by the Kyratec PCR thermal cycler (Applied Biosystems, Foster City, CA, USA) using the following protocol; 3 min of initial denaturation at 94 °C, followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing temperature at 53-60 °C for 30 sec, and extension at 72 °C for 30 sec. The final extension lasted 5 min at 72 °C. The PCR mixture for the CypG24 marker was comprised of 7 µl of Multiplex mix (QIAGEN Multiplex kit), 30 ng/µl DNA, 0.3 µl each primer (5 µM) of the pair, 0.3 µl tail primer (5 µM), 2.5 µl Q solution, and 3 µl RNase-free water in a final volume of 13.4 µl. The PCR reactions were programmed as follows: initial denaturation at 95°C for 10 min, followed by 30 cycles of 94°C for 1 min, annealing temperature at 57°C for 1 min, and 72°C for 1 min. At 60 °C, the final extension was performed for 45 min.

As mentioned, for PCR amplification, universal tailed primers were utilized. Thus, three oligos were used in the reaction: a tailed specific forward oligo, a reverse oligo, and a tail primer. The tailed primer for five microsatellite markers, MFW7, GF1, GF29, YJ0010, and YJ0022, included a tag sequence (5'- ATTACCGCGGCTGCTGG-3) at the 5 end of the sequence specific primer, while (5'-CAGGACCAGGCTACCGTG-3) was used for the rest of the microsatellite loci (Schuelke, 2000; Blacket et al., 2012). After running at 90 V for 1:20 min on a 2% agarose gel, the approximate sizes of the PCR products were estimated using a 100 bp ladder.

For the analysis of microsatellite bands and allele sizes using capillary electrophoresis and a laser detection system, four pairs of primers were included in each multiplex PCR panel. The 5' end of each pair's tailed forward primer was labeled with one of the fluorescent labels (Table 5). The fluorescent dyes (NED, VIC, 6-FAM, and PET), were used for the ABI Prism 3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA).

Table 5. List of microsatellite markers and primer sequences used in crucian carp.

Microsatellite locus (reference)	Repeat motif	The sequences of the used primer pairs with the tags (5→3)	Annealig T (°C)
MFW7 **(Crooijmans et al., 1997)**	$(CA)_{13}$	F:ATTACCGCGGCTGCTGGTACTTTGCTCAG GACGGATGC R: ATCACCTGCACATGGCCACTC	55
GF1 **(Zheng et al., 1995)**	$(TG)_{14}$	F:ATTACCGCGGCTGCTGGATGAAGGGTAGG AAAAGTGTG R:ACAGGTTAGGGAGAAGAAGGAAT	58
GF29 **(Zheng et al., 1995)**	$(TG)_4CA(TG)_8$	F:ATTACCGCGGCTGCTGGATGCTAGGTGAC TGTTTGT R:CACCTCCACTCCTAATAAT	58
YJ0010 **(Guo & Gui, 2008)**	$(TG)_{11}$	F:ATTACCGCGGCTGCTGGGATGGTTGTGCT GTGAGCT R:GAGTTCGTTTACATCTGGAC	53
YJ0022 **(Guo & Gui, 2008)**	$(AC)_3T(CA)_2TACAA(AC)_4$	F:ATTACCGCGGCTGCTGGCACCAACTTTAG GCACATTTG R:CCAGACTCCCACGTCATG	53
HLJYJ017 **(Zheng et al., 2010)**	(ATAG)15	F:CAGGACCAGGCTACCGTGCAAGAAAATT GGCACAGGATG R: ACCCTTGACCTGTGGACCAT	60
HLJYJ028 **(Zheng et al., 2010)**	(ATCT)21(TCTG)20	F:CAGGACCAGGCTACCGTGGCTGCTTTTAG GAAAACTAGCTG R: TTTCCTCCACCAACAAAACT	60
HLJYJ029 **(Zheng et al., 2010)**	(AGAT)7(AGAC)10	F:CAGGACCAGGCTACCGTGCACCGAAATA CTGAGACAGACAG R: GCGCTTTCTTGGACTGAGAC	60
HLJYJ041 **(X. H. Zheng et al., 2010)**	(ATCT)15(TCTG)21	F:CAGGACCAGGCTACCGTGCAGTTCTCCCC TCTCGACAC R: ATAACCTCGGGGCTGATTCT	60
HLJYJ046 **(X. H. Zheng et al., 2010)**	(TAGA)10(AGAC)14	F:CAGGACCAGGCTACCGTGGGAGCAACAG ACAAATAGATAGGC R: TTCGGACGCTGGTATAAACA	60
HLJYJ082 **(Zheng et al., 2010)**	(TTG)5	F:CAGGACCAGGCTACCGTGGTTGCTGTTGT GGCTGTGAC R: GCAACAAATGCAGATGCAGA	59
J62 **(Yue & Orban, 2002)**	(TG)12	F:CAGGACCAGGCTACCGTGCTGGAGGTTAC TAGGGAAGAA R: TAATCAAATAAAGGGGAGACA	55
CypG24 **(Baerwald & May, 2004)**	(CAGA)19	F:CAGGACCAGGCTACCGTGCTGCCGCATC AGAGATAAACACTT R: TGGCGGTAAGGGTAGACCAC	57

3.4.1.2 Microsatellite amplification in Tench

Tench's PCR amplification was carried out with the use of 12 microsatellite markers (MTT-1, MTT-2, MTT-3, MTT-5, MTT-6, MTT-8, MTT-9, MT-3, MT-6, MT-8, CypG24, and MFW1). The primer sequences were described in Table 6. The PCR mixtures for loci (MTT-1, MTT-2,

MTT-3, MTT-5, and MTT-6) were prepared in a 15 µl reaction volume containing 30 ng/µl genomic DNA, 10.6 µl DW, 1.5 µl 10X Dream Taq buffer with 20 mM $MgCl_2$, 1 µl (10 mM) dNTPmix, 0.4 µl of each fluorescently labelled locus-specific primer (5 µM) (each forward primer was labeled with a fluorescence marker), and 0.1 µl DreamTaq DNA polymerase 5 Unit/µl (Thermo Fisher Scientific, Waltham, NJ, USA). In the case of loci (MTT-8 and MTT-9), amplification was done in a multiplex PCR reaction using the QIAGEN Multiplex kit with the manufacturer's indicated reagent concentrations, including 6.15 µl of Multiplex mix, 40 ng/µl genomic DNA, 0.3 µl fluorescently labelled forward primer (5 µM), 0.3 µl reverse primer (5 µM), 2.40 µl Q solution, and 1.55 µl RNase-free water in a final reaction volume of 12 µl. PCR reactions were programmed as follows; initial denaturation at 95°C for 5 min, followed by 5 cycles of 95°C for 1 min, annealing temperature at 55°C for 1 min, and 72°C for 1 min, then another 35 cycles of 1 min at 90°C, 1 min at 55°C, and 1 min at 72°C. Finally, a 7-min extension was performed at 72°C (Kohlmann & Kersten, 2006). The PCR mixtures for (MT-3, MT-6, and MT-8) were prepared in a 15 µl reaction volume comprising 30 ng/µl genomic DNA, 9.6 µl DW, 1.5 µl 10xDreamTaq Buffer with 20 mM $MgCl_2$, 1.5 µl (10 mM) dNTPmix, 0.5 µl of each primer (5 µM), 0.3 µl fluorescently-labelled tail sequence of each pair (5 µM), and 0.1µl DreamTaq DNA polymerase 5 Unit/µl (Thermo Fisher Scientific, Waltham, NJ, USA). Initial denaturation at 95°C for 4 min was followed by 30 cycles of 95°C for 30 sec, annealing temperature at 58°C for 30 sec, and 72°C for 1 min in the PCR reactions for these markers. The final 9 min extension was performed at 72 °C. Similarly, the MFW1 microsatellite marker was amplified in a 15 µl reaction volume that included 30 ng/µl genomic DNA, 10.8µl DW, 1.5 µl 10X DreamTaq Buffer with 20 mM $MgCl_2$, 1.0 µl (10 mM) dNTP mix, 0.2µl of each primer pair (5 µM), 0.2 µl fluorescently-labelled tail sequence (5 µM), and 0.1µl DreamTaq DNA polymerase 5 Unit/µl (Thermo Fisher Scientific, Waltham, NJ, USA). Initial denaturation at 95°C for 5 min was followed by 35 cycles of 95°C for 30 sec, annealing temperature at 55°C for 45 sec, and 72°C for 90 sec. Then, a 10 min extension at 72°C was performed.

The CypG24 marker was also amplified in a multiplex PCR reaction using the (QIAGEN Multiplex kit) with the manufacturer's indicated reagent concentrations, including 6 µl of Multiplex mix, 40 ng/µl genomic DNA, 2.0 µl Q solution, 0.3 µl fluorescently labelled forward primer (5 µM), 0,3 µl reverse primer (5 µM), 1.1 µl RNase-free water. Initial denaturation at 95°C for 10 min was followed by 35 cycles of 94°C for 1 min, annealing temperature of 57°C for 1 min,

and 72°C for 1 min in the PCR reactions. Final extension was performed at 72°C for 45 min. The PCR reaction products were then run on a 2% agarose gel at 90 Volts for 1:20 min and their approximate size was determined using a 100bp ladder. Similarly, fluorescent dyes (NED, VIC, 6-FAM and PET) were utilized for the ABI Prism 3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA).

Table 6. List of microsatellite markers and primer sequences used in tench.

Microsatellite locus (reference)	Repeat motif	The sequences of the used primer pairs with the tags (5→3)	Annealig T (ºC)
MTT-1 **(Kohlmann & Kersten, 2006)**	(CA)11	F:GTCCTCGCAATGCAAGAAAT R: TTGGCTCATATTGGGTGTGA	55
MTT-2 **(Kohlmann & Kersten, 2006)**	(AC)8	F:CTGGTCTCCTCCTTGTGCTC R:TGGGTGAAGGATTGGTTGTT	55
MTT-3 **(Kohlmann & Kersten, 2006)**	(AGAC)3(AG)12	F:CCAGCAGAGCCCTACACTTC R: AGGACGTGACCATCAACACA	55
MTT-5 **(Kohlmann & Kersten, 2006)**	(GA)4GG(GA)13	F:GGGAGCCAGTTCACACTCAT R: GACATGAAAACGGTGCTGTG	55
MTT-6 **(Kohlmann & Kersten, 2006)**	(CA)16	F:TGTGTGAGGTGGCACAGAAT R: ATGTGAGCAATGGCTGTGAG	55
MTT-8 **(Kohlmann & Kersten, 2006)**	(CA)12N6(CA)3 (GACA)2 (CA)8	F:GAAATGTCCCCACAAACCAC R: GACACCGCTATCACCATCAG	55
MTT-9 **(Kohlmann & Kersten, 2006)**	(AC)28	F:CAATCTGGTGGAAGTGAGCA R: ACGCGTCAGTGACAGAGAGA	55
MT-3 **(developed in the present study)**	(GTT)10	F:TTTCCCCTCACCTCAATCCT R:TTTCCAAACGCTGCCAACTC	58
MT-6 **(developed in the present study)**	(CTT)8	F:ACACCAACCATCAAACAAACCA R:ATAGAAGGCGAAGGAGGGATG	58
MT-8 **(developed in the present study)**	(AC)11	F:GTCCACCCCTGCCATATCAC R:TGTAACTGGGTGCATGACTG	58
MFW1 **(Crooijmans et al., 1997)**	(CA)7	F:GTCCAGACTGTCATCAGGAG R:GAGGTGTACACTGAGTCACGC	55
CypG24 **(Baerwald & May, 2004)**	(CAGA)19	F:CTGCCGCATCAGAGATAAACACTT R: TGGCGGTAAGGGTAGACCAC	57

3.4.1.3 Fragment analysis

The Automated ABI Prism 3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA) was used for sizing the fluorescently labeled PCR products. For the purpose of analysis, the amplified PCR products were denatured into single strands. As a first step prior to using the ABI Prism 3130 genetic analyzer, the PCR products labeled with different fluorescent dyes (FAM, VIC, NED, and PET) were pipetted into a single tube at a maximum volume of 0.5 to 1 µl, followed by the addition of 9.9 µl HiDi Formamide and 0.1 µl Gene Scan-1200 molecular weight marker labeled with LIZ. The denaturation process of the diluted mixtures lasted for 6 minutes at 94 °C before separation was carried out by capillary electrophoresis. The Pop7 polymer with 50 cm long capillary arrays with G5 dye set was used in the ABI 3130 (Applied Biosystems, Foster City, CA, USA) genetic analyzer to automatically size the different sets of fluorescently labeled samples in a single capillary. Separation of alleles was carried out at 60° C for 2220 sec at 15 KV.

3.4.2 Restriction fragment length polymorphism (RFLP) analysis in Tench

The RFLP analysis was carried out on a total of 175 tench individuals utilizing two nuclear-encoded Exon-Primed Intron-Crossing (EPIC) makers: the second intron of the actin gene (*ACT*) and the first intron of the gene encoding the S7 ribosomal protein (*RpS7*). The EPIC primers were chosen to amplify a 335 bp nuclear DNA sequence encoding the second intron of the actin gene (*Act*) as designed by Touriya et al. (2003). Likewise, the EPIC primers described by (Chow & Hazama, 1998) were used to amplify another part of nuclear DNA, a 923–927-bp amplicon containing the ribosomal protein S7 (*RpS7*) second intron (Table 7) (Lajbner & Kotlík, 2011).

Table 7. EPIC primers and their sequences (Lajbner & Kotlík, 2011).

Marker (amplicon size) (reference)	Primer	Sequence (5' to 3')
RpS7 (923–927 bp)	S7RPEX1F	TGGCCTCTTCCTTGGCCGTC
(Chow & Hazama, 1998)	S7RPEX2R	AACTCGTCTGGCTTTTCGCC
Act (335 bp)	Act-2-F	GCATAACCCTCGTAGATGGGCAC
(Touriya et al., 2003)	Act-2-F	ATCTGGCACCACACCTTCTACAA

Each PCR reaction was conducted in a 15 µl PCR mixture containing 30 ng/µl of genomic DNA, 10.7 µl DW, 1.2 µl 10X DreamTaq Buffer with 20 mM $MgCl_2$, 1µl (10 mM) nucleotides, 0.5 µl of each specific primer (5 µM), and 0.1 µl Taq DNA polymerase (5 U/ µl) (Thermo Scientific,

Waltham, NJ, USA). The PCR conditions were carried out using two different PCR programs. The amplification protocol for the *Act* marker included 3 min of predenaturation at 94°C, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min 30 sec, and 1 min 30 sec for the final extension at 72°C. While for the *RpS7* marker, the predenaturation temperature was 95°C for 1 min, followed by a denaturation temperature of 95°C for 30 sec, and all other parameters remained the same.

Two different restriction enzymes were employed to digest the amplified PCR products, which were selected based on previous findings (Lajbner & Kotlík, 2011). The *Act* gene was digested by Eco521 endonuclease, while the *RpS7* gene was broken down by NdeI endonuclease.

At 37°C for 10 hours, 5 µl of the PCR products were digested in 11 µl volumes containing 9 µl of nuclease-free water, 1 µl of PCR buffer, and 1µl (10u/µl) of restriction enzyme Eco52I or NdeI (Thermo Scientific, Waltham, NJ, USA). They were then inactivated by incubation for 20 min at 65 °C. The products digested by the aforementioned enzymes were useful in predicting the haplotype patterns between western and eastern haplotypes based on the known DNA sequences of the amplified PCR products (Lajbner et al., 2010).

Restriction fragments were separated electrophoretically on a 2% agarose gel containing GelGreen Nucleic Acid Stain (Biotium Inc, Landing Parkway Fremont, CA, USA), and assessed using a 1000 bp DNA ladder (Thermo Scientific). Subsequently, using VisionWorksLS analysis software (LTF Labortechnik GmbH & Co. KG., Wasserburg, Germany), the DNA fragment patterns were verified and photographed under UV light by a gel documentation system (Analytik Jena).

3.4.3 Mitochondrial DNA amplification and sequencing

3.4.3.1 Amplification and sequencing of the Mitochondrial Cytb gene in Tench

The universal primer pairs Glu-F (5′-AACCACCGTTGTATTCAACTACAA-3′) and Thr-R (5′ ACCTCCGATCTTCG-GATTACAAGACCG-3′) were used to amplify 175 tench Cytb (615-bp) gene sequences, which were designed by using the flanking tRNA sequences according to (Machordom & Doadrio, 2001).

The PCR reaction of each sample was carried out in a 15 µl PCR mixture containing 30 ng/µl of genomic DNA, 10.7 µl DW, 1.2 µl 10X DreamTaq Buffer with 20 mM $MgCl_2$, 1 µl (10 mM) nucleotides, 0.5µl of each specific primer (5 µM), and 0.1µl (5 U/ µl) Taq DNA polymerase (Thermo Fisher Scientific, Waltham, NJ, USA). The following protocol was used to conduct PCR

reactions in the Kyratec PCR thermal cycler (Applied Biosystems, Foster City, CA, USA): Initial denaturing at 95°C for 5 min, then 2 cycles at 94°C for 1 min, annealing temperature at 60°C for 1 min 30 s, 72°C for 2 min, then another 30 cycles at 94°C for 1 min, 1 min 30 s at 54°C, and 2 min at 72°C, then final extension for 10 min at 72°C. Successful PCR products were purified by NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel, Düren, Germany) before being sequenced. On a 2% agarose gel conducted in TBE buffer, the quality of the purified products was assessed. The purified products were then sequenced using an automated genetic analyzer, ABI 3130 (Applied Biosystems, Foster City, CA, USA), with a POP7 polymer and a 50 cm long capillary array, according to the manufacturer's instructions for the Big Dye terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA).

3.4.3.2 Amplification and sequencing of the Mitochondrial COI region in Crucian carp

For a total of 104 out of 320 crucian carp sampled from six wild populations and three farmed stocks in Hungary's eastern, central, and western regions, the Cytochrome Oxidase C subunit I gene (COI) was amplified for sequencing analysis using universal primer pairs CO1-FF2d-F (5′-TTCTCCACCAACCACAARGAYATYGG-3′) and CO1-FR1d-R (5′-CACCTCAGGGTGTCCGAARAAYCARAA -3′) (Ivanova et al., 2007). With the exception of two populations (Lake Fertő and Lake Kolon), the samples analyzed were obtained from all of the locations listed in chapter 3.2. The amplification process was as follows: 94°C for 2 min, 30 cycles at 94°C for 40 s, 52°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 7 min. 15 µl of PCR mixture contained 30 ng/µl of genomic DNA, 10.9 µl of DW, 1.2 µl 10X DreamTaq Buffer with 20 mM $MgCl_2$, 1 µl dNTP (10 mM), 0.4 µl of each primer (10 µM), and 0.1 µl (5 U/ µl) of Taq DNA polymerase (Thermo Fisher Scientific, Waltham, NJ, USA). NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel, Düren, Germany) were used to purify the PCR products. Purified products were quality tested on a 2% agarose gel, and then sequenced with the Big Dye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The ABI 3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA) was used to reveal the sequences.

3.4.4 Statistical analysis, population structure, and phylogenetic relationship estimation

The Genotyper 4.0 software package from Applied Biosystems was used to estimate fragment length. The parameters of genetic variance, including observed heterozygosity (Ho), expected heterozygosity (He), number of alleles (Na), and effective number of alleles (Neff), genetic variance (F_{st}), and heterozygote deficit, were calculated using GenAlEx6.5 (The Australian National University, Canberra, Australia). The data obtained from the Genotyper 4.0. software was encoded into the Excel file to calculate the aforementioned values (Peakall & Smouse, 2006). MICRO-CHECKER version 2.2.3 (The University of Hull, Hull, UK) (number of randomizations: 1000, 95% CI) was used to detect possible genotyping errors, allele dropout and non-amplified alleles (Oosterhout et al., 2004).

In cross-population comparisons, the Ho and F_{is} values were standardized for population sizes using weighted means. To compare indices of genetic variance, a Mann-Whitney U-test with Bonferroni correction (a significance threshold of 0.01) (SPSS for Windows 11.5 (SPSS Inc.: Chicago, IL, USA)) was utilized. GENEPOP software (Raymond & Rousset, 1995; Rousset, 2008) was used to assess deviations from the Hardy–Weinberg equilibrium (HWE) for each locus in each population using a Markov chain (5000 dememorizations, 500 batches, 5000 iterations per batch) (S. W. Guo & Thompson, 1992). Genetic divergence between populations was assessed by estimating the pairwise F_{st} of Weir (1996) (Weir, 1996) as well as Cavalli-Sforza and Edwards (1967) (Cavalli-Sforza & Edwards, 1967) genetic distance using FreeNA software (INRA, Montpellier, France) (Chapuis & Estoup, 2007). The ENA correction was used for the F_{st}, while the INA correction was used for genetic distance. For the computation of the bootstrap 95 % confidence intervals, 10,000 replicates were used. Using 9999 permutations in the GenAlEx 6.5 software, a paired Mantel test between F_{st} values and geographic distances of the populations was calculated. Based on linkage disequilibrium and allele frequencies, NeEstimator 2.1 software (Do et al., 2014) was used to estimate the effective population size (Ne) of all natural populations. The bottleneck effect was evaluated using BOTTLENECK 1.2.02 (INRA, Montpellier, France) (Piry et al., 1999). The Wilcoxon signed-rank test was conducted to assess significance under the two-phase mutation model (TPM).

The genetic relationship between populations and individual assignments of fish was inferred via a Bayesian clustering analysis using the statistical program STRUCTURE v2.3.3 (University of Chicago, Chicago, IL, USA) (Pritchard et al., 2000; Falush et al., 2003; Pritcharda et al., 2010).

The admixture model was used to assume the structure analysis parameters, and the analysis was run 10 times per K with a burn-in of 10^4, followed by 10^5 Markov chain-Monte Carlo (MCMC) repetitions. STRUCTURE HARVESTER software (University of California, Santa Cruz, CA, USA) (Earl & vonHoldt, 2012) was used to calculate the most likely cluster number K, the posterior probability (highest LnP (D)), and the ΔK (Evanno et al., 2005). The genetic relatedness and population structure were investigated further using Principal Coordinates Analysis (PCoA) (Reeves & Richards, 2009). MT-3, M-6, and M-8 markers were developed with Primer 3 software run on tench transcriptome sequences containing repeat motifs. Sequences were filtered using the BLAST function on the SRX3291387, SRX3291388, SRX3291389, SRX3291390, SRX3291491, SRX3291492, SRX3291493, SRX3291494 SRA data of the PRJNA414567 BIOProject.

Different software packages were used to analyze the mtDNA sequencing data. The haplotype diversity (Hd), nucleotide diversity (π), the number of segregating sites, and the total number of mutations for all populations and regions were estimated by DnaSP 5.10 software (Librado & Rozas, 2009). Using Mega-X 10.1 software (The Pennsylvania State University, University Park, TX, USA), DNA sequences were edited and aligned. The Cytb gene's revised alignment was 615 bp. NETWORK 10.0 software (Fluxus Technology Ltd., Colchester, England) (Bandelt et al., 1999) was used to generate haplotype network analyses for all populations in this study as well as GenBank sequencing. All tench haplotypes were linked to the NCBI-BLAST nucleotide database using the Megablast program (The Pennsylvania State University, University Park, TX, USA) (Z. Zhang et al., 2000). The COI haplotype sequences identified in *Carassius carassius* were compared and analyzed with the reference sequences of *Carassius carassius* and *Carassius gibelio* provided in Genbank to help understand the phylogenetic relationships between the two species in the genus *Carassius*. The phylogenetic trees were made using MegaX-11 software with Neighbour Joining fitting using the Bootstrap method with 1,000 bootstrap replicates, Tamura 3 parameter model Gamma-Distributed (G) (Tamura et al., 2021).

4. RESULTS

4.1 Microsatellite data analysis in Tench

4.1.1 Allele polymorphism and population size

All twelve microsatellite loci were successfully amplified, and all showed moderate to high levels of polymorphism, with a total of 64 alleles in all populations, 11 of which were private alleles. At polymorphic loci, the number of distinct alleles ranged from 2 (locus MTT-2) to 12 (locus MTT-9). The highest number of alleles was found in the population of Lake-Fertő at MTT-9, the most diverse site. Consequently, across all populations, the mean number of alleles per population was 3.22. Derecske (2.41 ± 1.16) and Lake-Fertő (4.16 ± 2.24) had the lowest and highest mean number of alleles, with allelic richness ranging from 2.02 ± 0.69 (in Derecske) to 3.27 ± 1.36 (in Lake-Fertő). The mean observed heterozygosity across all loci within a population ranged from 0.17 (in Derecske) to 0.47 (in Cun-Szaporca). In terms of both Ho and He, for all populations, the mean values were 0.37–0.40, respectively.

The Microchecker revealed the presence of null alleles in the cases of locus MTT8 in the Lake-Fertő, Lake Kolon, and Derecske populations; MTT1 in the Lake Tisza population; and MT3 in the Lake-Fertő population since there was no evidence of significant allelic dropout. The following loci were monomorphic: MT3 in the Derecske population, MT6 in the Derecske, Lake Tisza, and Cun-Szaporca populations, and MTT8 in the Cun-Szaporca population. Probability tests of Hardy-Wienberg showed that the MTT6, MTT8, MT3 in the Lake-Fertő population, MTT8, MTT2 in the Derecske population, MT3, MTT9 in the Lake Kolon population, MTT6, MTT8 in the Cibakházi Holt-Tisza population, and MT3 in the Cun-Szaporca population, were all found to be out of the HW equilibrium ($P<0.05$).

Thus, Lake-Fertő had the highest diversity. While the Derecske population had significantly lower genetic diversity. The effective number of alleles, heterozygosity (uHe and Ho), and private allelic richness were significantly lower compared to the Lake-Fertő population, and the F_{is} value was highest in the Derecske population. Table 8 lists the basic population genetic characteristics of the stocks.

Table 8. Genetic diversity data of the seven tench populations studied.

	Lake Fertő	Lake Kolon	Csörnöc-Herpenyő	Derecske	Cibakházi Holt-Tisza	Lake Tisza	Cun-Szaporca
Na	4.16 ± 2.24	3.83 ± 1.69	3.16 ± 1.40	2.41 ± 1.16	3.25 ± 1.42	2.91 ± 1.16	2.83 ± 1.19
Neff	2.22 ± 0.78 [a]	1.81 ± 0.57 [ab]	1.92 ± 0.61 [ab]	1.38 ± 0.29 [b]	1.80 ± 0.63 [ab]	1.92 ± 0.50 [ab]	1.92 ± 0.69 [ab]
Ho	0.43 ± 0.20 [b]	0.32 ± 0.16 [ab]	0.44 ± 0.22 [b]	0.17 ± 0.13 [a]	0.37 ± 0.20 [ab]	0.36 ± 0.18 [ab]	0.47 ± 0.31 [b]
uHe	0.49 ± 0.18 [a]	0.39 ± 0.20 [ab]	0.43 ± 0.19 [ab]	0.25 ± 0.16 [b]	0.40 ± 0.18 [ab]	0.47 ± 0.17 [ab]	0.43 ± 0.25 [ab]
F	0.11 ± 0.19 [ab]	0.15 ± 0.18 [ab]	-0.03 ± 0.15 [a]	0.28 ± 0.31 [b]	0.04 ± 0.19 [ab]	0.16 ± 0.30 [ab]	-0.12 ± 0.32 [a]
AR	3.27 ± 1.36	2.74 ± 1.02	2.59 ± 1.05	2.02 ± 0.69	2.50 ± 0.93	2.78 ± 1.03	2.72 ± 1.11
AR_p	0.40 ± 0.42 [a]	0.12 ± 0.22 [ab]	0.16 ± 0.21 [ab]	0.02 ± 0.06 [b]	0.12 ± 0.24 [ab]	0.12 ± 0.31 [ab]	0.10 ± 0.29 [ab]

Na: number of alleles per population, *Neff*: effective number of alleles, *uHe*: unbiased expected heterozygosity, *Ho*: observed heterozygosity values, *F*: inbreeding coefficient, A_R: allelic richness, A_{Rp}: private allelic richness. If indicated, superscript letters (a, b) indicate significant differences ($p < 0.05$) between the groups.

Application of the linkage disequilibrium approach to Ne estimation yielded the following effective population sizes (Ne) in the seven tench populations: Lake Fertő-infinite (CI 95%: 108.8–infinite), Lake Kolon- 25.9 (CI 95%: 15.7-47.9), Csörnöc-Herpenyő – 59.8 (CI 95%: 19.5-21.3), Derecske- infinite (CI 95%: 20.9-infinite), Cibakházi Tisza- 49.5 (CI 95%: 23.8-233.3), LakeTisza-infinite (CI 95%: 14.6-infinite) and Cun-Szaporca 9.8 (CI 95%: 2.4–infinite) individuals. However, BOTTLENECK's Wilcoxon signed-rank test for heterozygosity excess showed no evidence of a recent population bottleneck in any of the populations.

4.1.2 Population structure

Pairwise F_{ST} analysis of the microsatellite data also revealed a robust structure. The global F_{st} was 0.080 (95% CI: 0.057–0.108) with ENA correction, showing relatively modest genetic distances. Table 9 shows the pairwise F_{st} and Cavalli-Sforza and Edwards genetic distance values between stock pairs. Table S1 displays the Bootstrap 95% intervals.

Table 9. Pairwise F_{st} with the ENA correction (below the diagonal) and the Cavalli-Sforza and Edwards genetic distances with the INA correction (above the diagonal).

F_{st}/genetic Distance	Lake Fertő	Lake Kolon	Csörnöc-Herpenyő	Derecske	Cibakházi Tisza	Tisza-tó	Cun-Szaporca
Lake Fertő		0.228	0.209	0.299	0.256	0.243	0.286
Lake Kolon	0.065		0.246	0.232	0.237	0.195	0.291
Csörnöc-Herpenyő	0.032	0.104		0.255	0.236	0.245	0.250
Derecske	0.130	0.059	0.138		0.227	0.271	0.332
Cibakházi Tisza	0.077	0.069	0.101	0.061		0.232	0.266
Lake Tisza	0.008	0.029	0.043	0.120	0.042		0.258
Cun-Szaporca	0.054	0.138	0.071	0.219	0.127	0.051	

The Derecske population showed the highest separation (F_{st} ranged between 0.120 and 0.219) from all other populations except the Lake Kolon population, where the value was moderate (0.059). The mixed genetic background of the Lake Tisza population is well detectable in the F_{st} and genetic distance values: F_{st} ranges between 0.008 and 0.051 except for the Derecske population (0.120). The regional genetic separation of the populations was moderate. Considering all the seven populations, the Mantel test resulted in a non-significant association between the geographical distance (GeoD) and the F_{st} (GeoD = 617.8xF_{st} + 158.93, R^2 = 0.0983, p = 0.105). However, if the mixed Lake Tisza population was excluded, the association (GeoD = 1212.5xF_{st} + 96.969, R^2 = 0.3737; Figure 7) became significant (p = 0.032). Thus, the greatest distances exist between the geographically most distant populations.

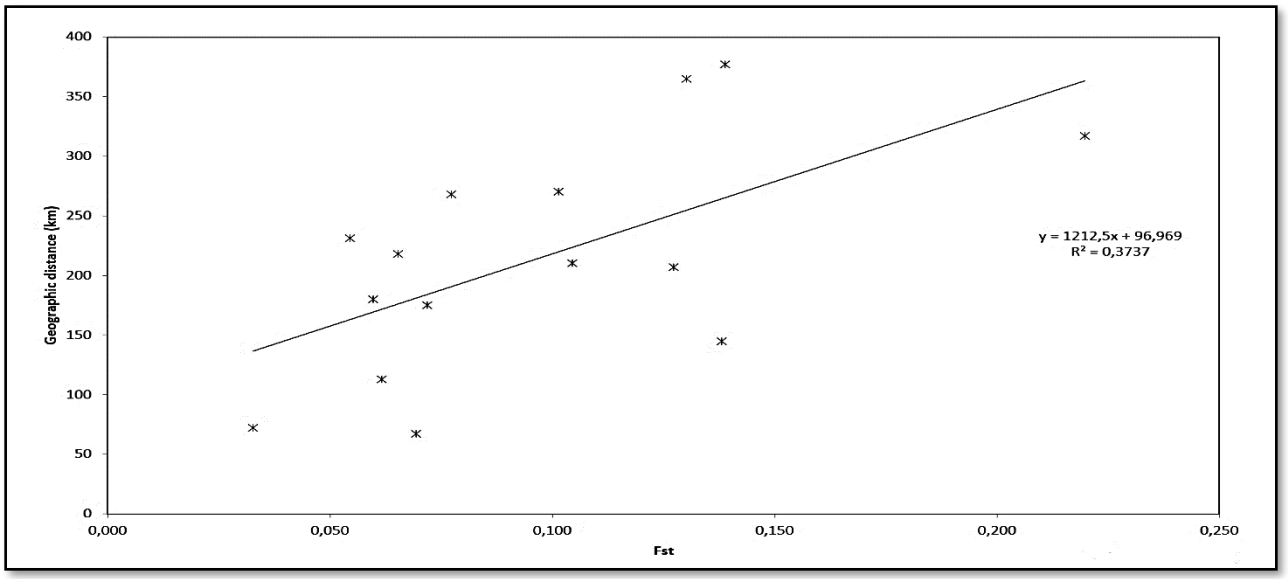


Figure 7. Regression between Fst and geographic distance calculated on the six population pairs (Mantel test, P = 0.028)

By clustering 175 individuals using the STRUCTURE software (Figure 8), the most probable number of K = 4 clusters was identified. The mean log likelihood data and Evanno's delta K of the hierarchical STRUCTURE analysis are shown in Table S2. Contrary to expectations, none of the four clusters were closely related to localization within or between watersheds; only their proportions were different. The first cluster (red color) is frequent in the North-Western region (Lake-Fertő); the second (green color) in the Central-Eastern region (Lake Kolon and Derecske); the third cluster (blue color) in the Central and South-Western regions (Csörnöc-Herpenyő, Cun-Szaporca); and the fourth (yellow color) in the Eastern region (Cibakházi Tisza). The population of Tisza Lake was a mixed population with an equal frequency for all clusters (most likely due to human intervention—tench are frequently stocked in this water body). This pattern of distribution was also seen in the Principal Coordinate Analysis (PCoA). Analysis of molecular variance (AMOVA) showed that genetic variance was predominantly within the population.

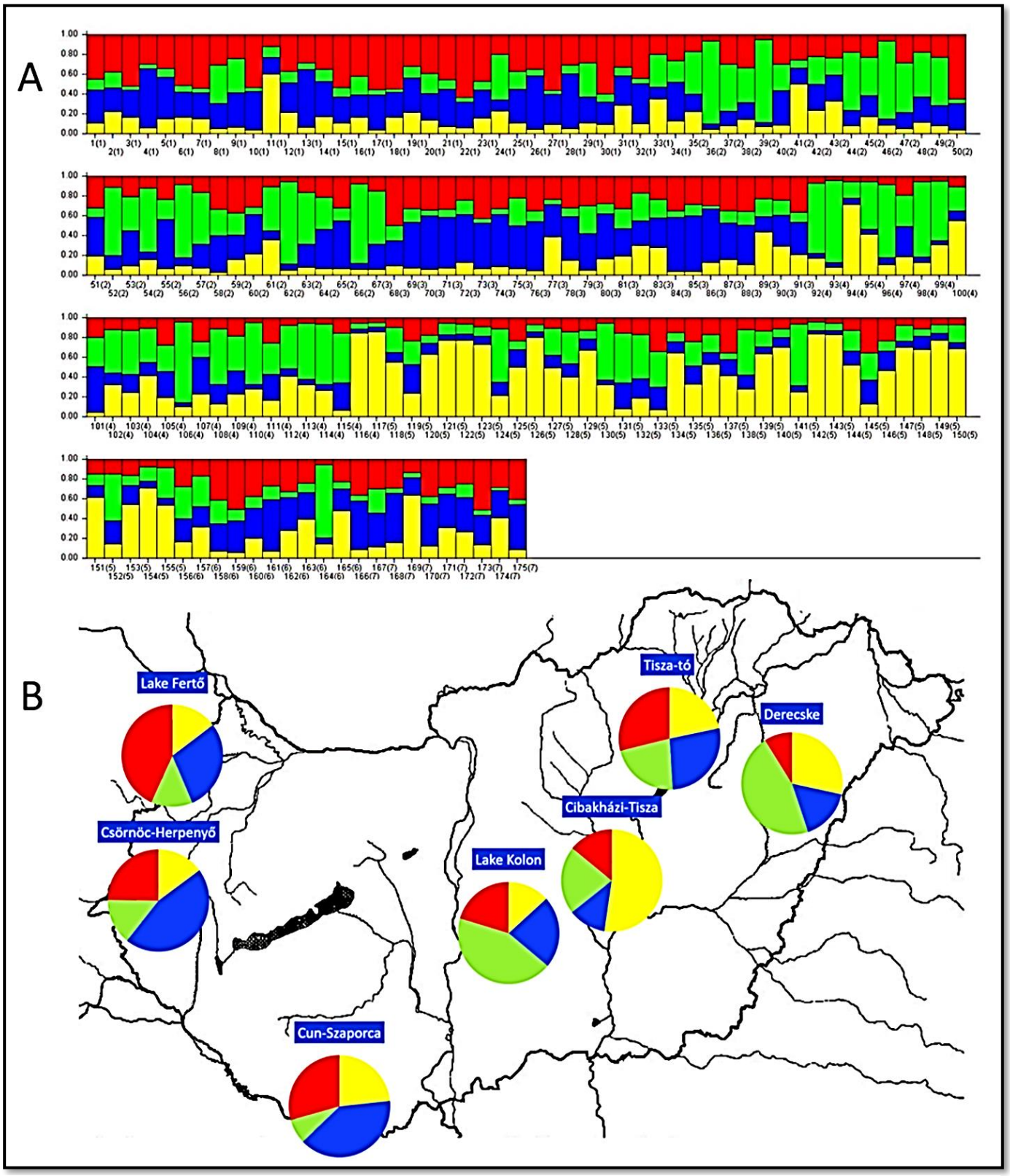


Figure 8. A Structure of the seven tench populations for K = 4, based on the microsatellite data. The populations are the following: 1st Lake Fertő, 2nd Lake Kolon, 3rd Csörnöc-Herpenyő, 4th Derecske, 5th Cibakházi Tisza, 6th Tisza-tó, 7th Cun-Szaporca. B The average contribution of the four genetic clusters in the seven tench populations.

4.2 Mitochondrial DNA analysis in Tench.

The seven tench populations yielded a total of 20 new mitochondrial Cytb haplotypes (Figure 7), two of which were identical to sequences in GenBank (hap6- HM560230.1, HM167941.1, HM167943.1, HM167945.1, HM167946.1, HM167949.1, JX974523.1, JX974524.1, JX974525.1, and hap11-NC_008648.1:14394-15, HM167950.1, HM167952.1, JX974520.1, and JX974521.1). The samples showed a predominance of four haplotypes (haplotypes 3, 4, 6, and 11). Most of the haplotypes with small numbers of samples were found in the Lake Fertő population, which had 14 of the 20 different haplotypes (Figure 9, Table 10).

The number of polymorphic sites within the haplotype groups was 14, with 13 of them being parsimony informative. The haplotype diversity in the polymorphic populations varied from 0.222 (Lake-Tisza) to 0.909 (Lake-Fertő), while the nucleotide diversity ranged from 0.0003 (Lake-Tisza) to 0.0072 (Lake-Fertő). The highest values for both indices were found in Lake-Fertő, which matched the high variance identified by microsatellite markers, suggesting its relevance as a genetic diversity reservoir. The diversity metrics for the Cytb gene sequences within the seven populations are presented in Table 10.

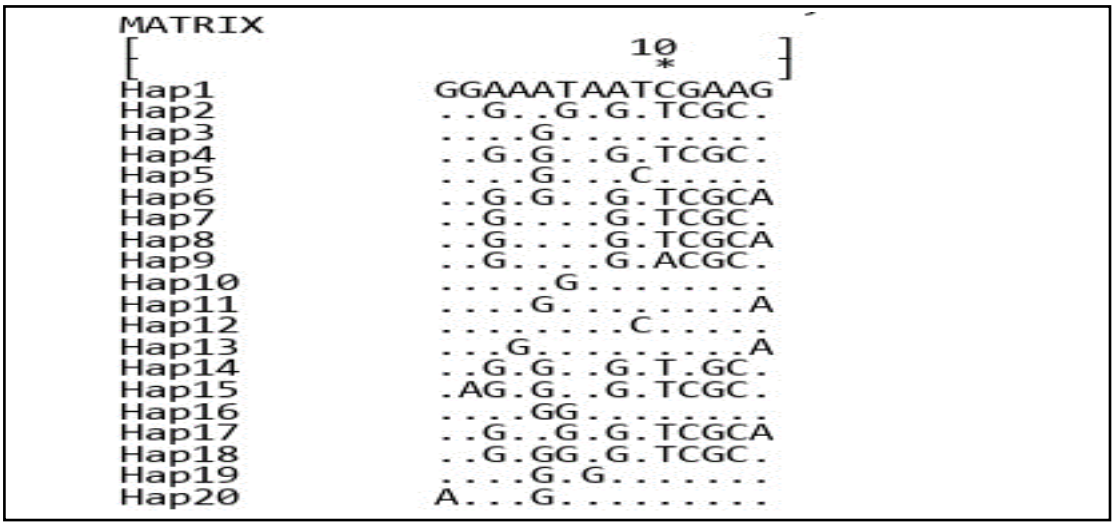


Figure 9. Hungarian tench polymorphic sites in Cytochrome b haplotypes generated by DnaSP 5.10 software (Librado et al., 2009).

Table 10. Diversity data of the mtDNA sequences in the seven tench populations.

Stock	Nh	S	Eta	Hd (mean ± SD)	Pi (mean ± SD)
Lake Fertő	14	11	12	0.909 ± 0.026	0.00728 ± 0.00035
Lake Kolon	5	9	9	0.556 ± 0.130	0.00290 ± 0.00123
Csörnöc-Herpenyő	9	9	9	0.813 ± 0.081	0.00456 ± 0.00111
Derecske	5	8	8	0.789 ± 0.057	0.00597 ± 0.00076
Cibakházi Tisza	6	10	10	0.655 ± 0.060	0.00552 ± 0.00037
Lake Tisza	2	1	1	0.222 ± 0.166	0.00036 ± 0.00027
Cun-Szaporca	4	7	7	0.778 ± 0.091	0.00596 ± 0.00094

Nh—Number of haplotypes; S—Number of polymorphic (segregating) sites, Eta—total number of mutations, Hd—haplotype diversity, Pi—nucleotide diversity, SD-Standard Deviation.

The twenty Cytb haplotypes described in the seven populations were divided into two major haplogroups defined by Lajbner et al. (2007) and Lajbner & Kotlík (2011). An average of 28% of individuals belonged to the Western haplotype group, while 72% belonged to the Eastern haplotype group. Interestingly, 100% of the Lake Tisza individuals belonged to the Eastern haplotype. However, since the lake is stocked with tench of unknown Cytb haplotype, this information must be regarded critically, and it is not advisable to draw far-reaching conclusions from this. When we excluded the Tisza Lake population, the highest proportion of Eastern haplotypes was found in Lake Kolon (92%). Lake-Fertő had the highest proportion of Western haplotypes, with 47% of individuals belonging to this group (Figure 10, Table 11).

Table 11. Proportion of Western and Eastern haplotypes of the mitochondrial Cytb gene in seven tench populations.

	Cytb	
	Western (%)	Eastern (%)
Derecske	37	63
Lake Tisza	0	100
Cibakházi Tisza	43	57
Lake Kolon	8	92
Cun-Szaporca	40	60
Csörnöc-Herpenyő	23	77
Lake Fertő	47	53
Average	28	72

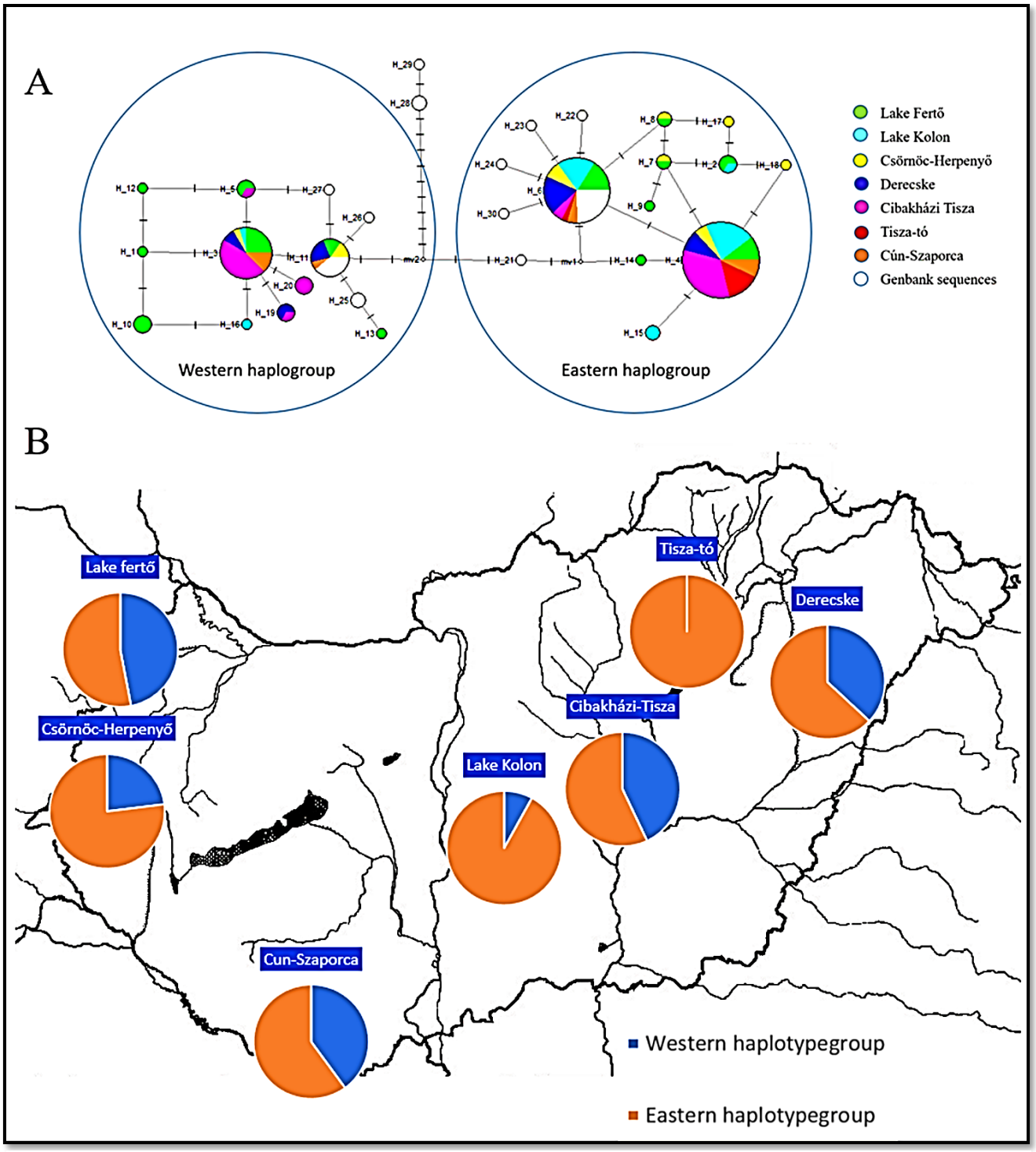


Figure 10. A. MtDNA haplotype networks for Cyt b mitochondrial DNA sequences. The size of the circles represents the number of observations of particular haplotypes. The GenBank identifiers of haplotypes described in the network figure but not found in the Hungarian samples are the following: H21: HM167942.1, H22: HM167944.1, H23: HM167947.1, H24: HM167948.1, H25: HM167951.1, JX974522.1, H26: HM167953.1, H27: HM167954.1, H28: HM167955.1, H29: HM167957.1, H30- MT605881.1. B. The relative contributions of the two haplogroups to the seven tench populations.

The NJ phylogenetic tree showed that the 20 haplotypes fell into two different phylogenetic lineages, the Eastern lineage (E) and the Western lineage (W). The first lineage was identified throughout the central and eastern Hungarian watersheds studied, while the second lineage was found predominantly in the western Hungarian catchments, mainly in Lake Fertő (Figure 11).

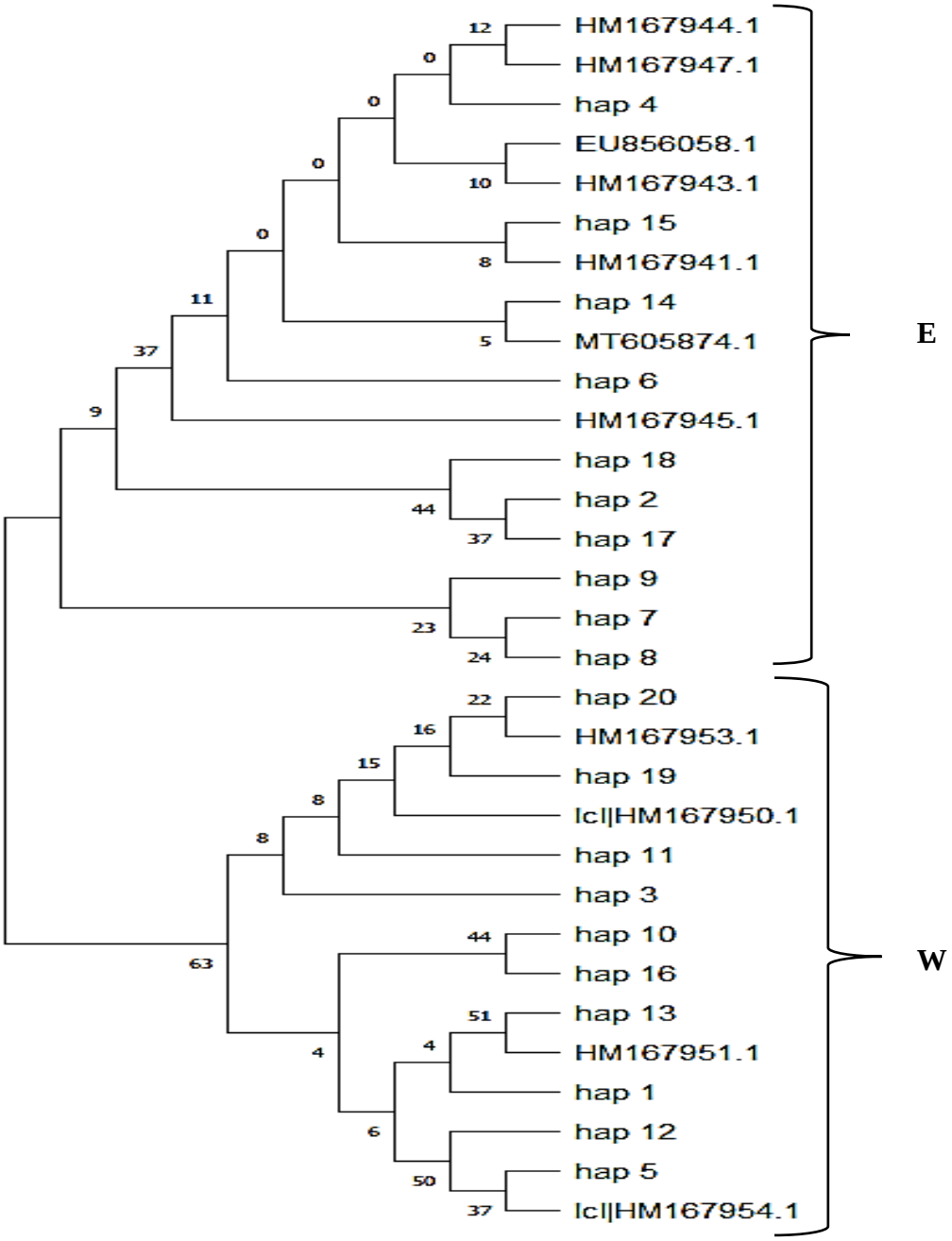


Figure 11. Neighbour-Joining tree of seven wild tench populations based on cytochrome b haplotypes. The NJ tree was constructed with MEGA-11 software using the Bootstrap method with 1,000 bootstrap replicates, Tamura 3 parameter model, Gamma-Distributed (G) (Tamura et al., 2021).

4.3 PCR-RFLP analysis of *Act* and *RpS7* nuclear genes for phylogeography

The PCR-amplified *Act* and *RpS7* genes were employed in the RFLP analysis. RFLP patterns were generated by digesting the amplicons of individuals possessing the known haplotypes of each of the two nuclear genes, using each endonuclease described in materials and methods. *Act* amplicons digested with Eco52I endonuclease and *RpS7* amplicons digested with NdeI endonuclease yielded three different patterns: eastern, western, and hybrids. The Eastern and Western phylogroups were found to have two and three-band patterns, respectively. Fragment sizes at certain cleavage sites were compared to those of (Lajbner & Kotlík, 2011) Figure 12.

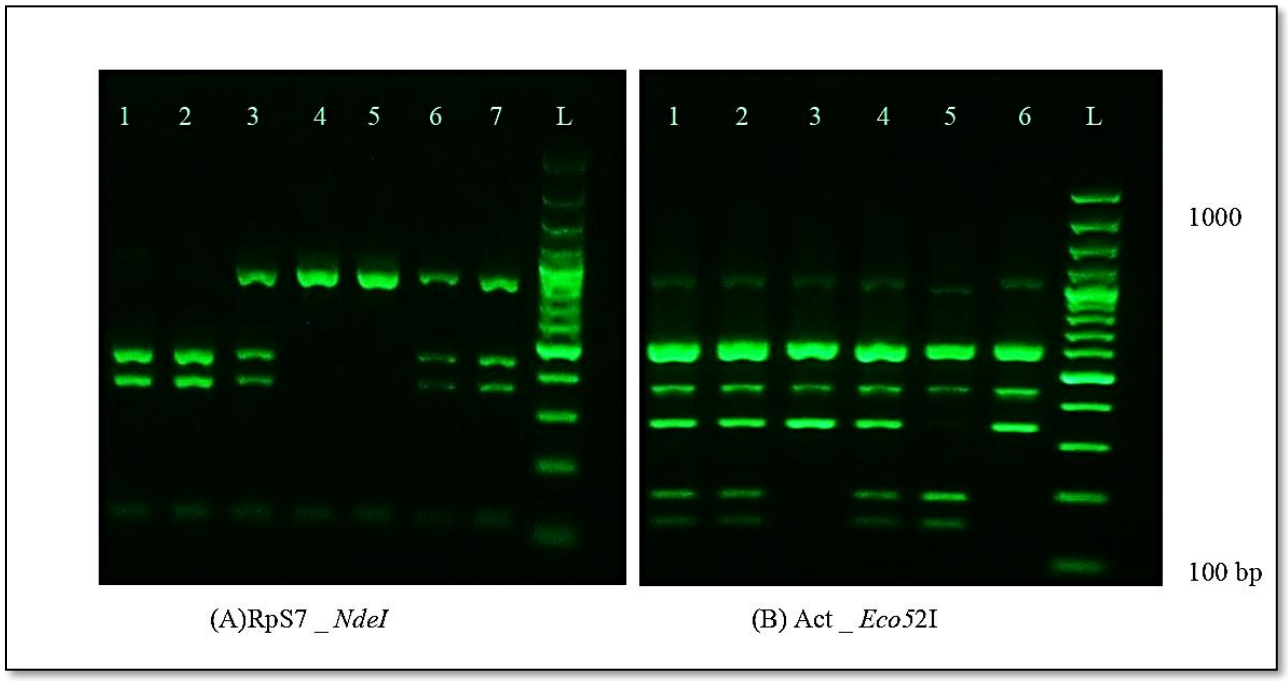


Figure 12. Restriction fragment patterns on agarose gels. Image-A shows the *RpS7* amplicons digested with NdeI (Western phylogroups were represented in lanes 1 and 2, while Eastern phylogroups were represented in lanes 4 and 5, and hybrids with Eastern-Western phylogroups were represented in lanes 3, 6, and 7). Image-B shows the *Act* amplicons digested with Eco52I (lanes 1, 2, and 4 represented Eastern-Western phylogroups; lanes 3 and 6 represented Eastern phylogroups; and lane 5 represented Western phylogroups). L; 1000-bp ladder.

The results indicated that the proportion of heterozygote individuals was close to 50%, 41% in the case of Rps7 and 50% in the case of *Act*, respectively (Table 12). Based on both nuclear genes, an average of 33% of individuals belonged to the Western lineage, whereas 17% (*Act*) and 26% (*Rps7*) belonged to the Eastern lineage. Interestingly, in the Cun-Szaporca population, 60% of individuals belonged to the Western lineage in the case of both nuclear genes, with no individuals

from the Eastern lineage present, while 40% of individuals were heterozygous. The Lake Tisza population showed the opposite picture for the two genes. In the case of the Act gene, 90% of the individuals were heterozygotes, while in the case of the *Rps7* gene, only 10% of the fish were heterozygous, and the Western lineage was dominant. The proportions belonging to each lineage were more equal in the rest of the populations studied.

Table 12. *Act* and *Rps7* haplotypes of the seven tench populations.

	Act (Eco52l)			*Rps7* (Ndel)		
	W (%)	E (%)	WE-het (%)	W (%)	E (%)	WE-het (%)
Derecske	8	36	56	4	56	40
Lake Tisza	10	0	90	60	30	10
Cibakházi Tisza	50	16	34	37	3	60
Lake Kolon	16	31	53	12	47	41
Cun-Szaporca	60	0	40	60	0	40
Csörnöc-Herpenyő	54	13	33	12	32	56
Lake Fertő	32	24	44	44	15	41
avarage	33	17	50	33	26	41

W - West; E - East; WE-het – Western-Eastern Heterozygote.

4.4 Microsatellite data analysis in Crucian carp

4.4.1 Genetic diversity and population size

All 13 microsatellite loci were found to be polymorphic in 320 individuals' genotypes. A total of 245 microsatellite alleles were described throughout the eleven natural populations and stocks of crucian carp. The lowest number of alleles was found on locus GF1 (3), while the highest number of alleles (75) was detected on HLJYJ041. Thus, the HLJYJ041 locus had higher polymorphism than the remaining loci. Wild populations had an average of 20 to 32 alleles, while cultured populations had an average of 14 to 25 alleles at this locus (HLJYJ041) (Table 13).

The number of alleles ranged from 61 to 133 in different populations, along with a total of 49 private alleles with frequencies ranging from 0.016 to 0.250, which are mostly found in wild populations. The private allele frequencies are shown in Figure 13.

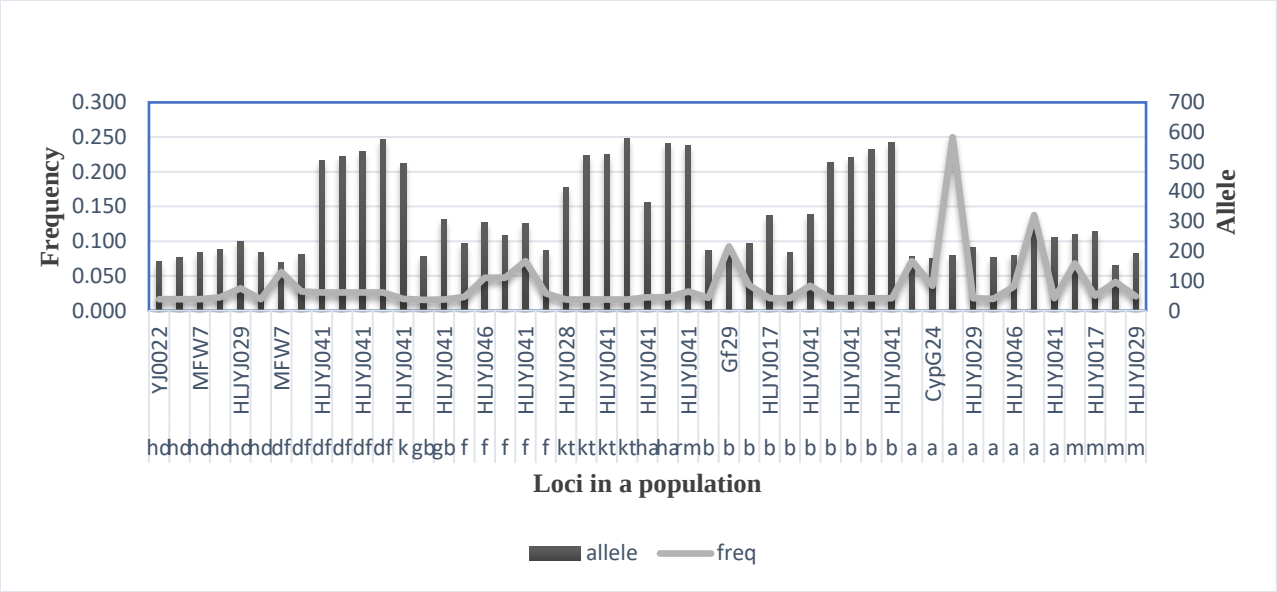


Figure 13. Frequencies of private alleles by population. Cún-Szaporca Holt-Dráva (hd), Kölked (k), Dunafalva (df), NBGK-HGI – genebank (gb), Belső-telep HAKI (ha), Lake-Fertő (f), Lake-Kolon (kt), Alag Éger (a), Baja (b), Rétimajor (rm) and Mura Vízgyűjtő (m).

The total number of alleles was inferred to be the lowest for the farmed Rétimajor population (61), while the wild Cún-Szaporca Holt-Dráva population had the largest total number of alleles (133), followed by the Mura Vízgyűjtő population (123) alleles and the Lake-Fertő population with (117) alleles, both of which are wild populations. The mean number of alleles for each population ranged from 4.69 ± 4.35 in the farmed population (Rétimajor) to 10.23 ± 9.19 in the wild population (Cún-Szaporca Holt-Dráva), with allelic richness ranging from 4.00 ± 3.34 in the farmed (Rétimajor) population to 7.98 ± 6.12 in the wild (Mura Vízgyűjtő) population. The mean genetic parameters for eight natural populations and three stocks are shown in Table 14. Although wild populations had a higher allelic richness (7.25 ± 5.48) than farmed populations (5.04 ± 3.50), the difference was not statistically significant (P = 0.007) (Table 15).

The mean observed heterozygosity (Ho) and expected heterozygosity (He) for all populations was 0.509 and 0.551, respectively, and the mean observed heterozygosity across all loci within a population ranged from 0.39 ± 0.35 (HAKI) to 0.62 ± 0.24 (Alag Éger) (Table 14). The mean values of observed heterozygosity and expected heterozygosity for the eight wild populations were (0.570 ± 0.283) and (0.616 ± 0.277), respectively, whereas for the cultivated populations they were lower (0.450 ± 0.330) and (0.531 ± 0.299). In general, wild crucian carp populations had a somewhat greater level of polymorphism than cultured populations when genetic parameters were

compared, including allelic richness, observed heterozygosity, and expected heterozygosity (Table 15). He and Ho values indicated that in most natural populations, these two values were close to each other. But, the Chi-square test for Hardy-Weinberg equilibrium showed a significant heterozygote deficit in six of the thirteen loci in two farmed stocks (the NBGK-HGI stock and the HAKI stock) (Table 13). In the eleven crucian carp populations, the linkage disequilibrium approach to Ne estimation revealed the following effective population sizes (Ne): Cún-Szaporca Holt-Dráva-59.7 (CI 95%: 44.8-86.7), Dunafalva-Infinite (CI 95%: 140.9-Infinite), Kölked Misányi-fok-192.9 (CI 95%: 79.4-Infinite), NBGK-HGI-genebank-10.1 (CI 95%: 8.6-12.0), Lake-Fertő-200.6 (CI 95%: 73.5-Infinite), Lake-Kolon-138.9 (CI 95%: 76.0- 563.7), Belső-telep HAKI-5.7 (CI 95%: 3.7- 7.6), Rétimajor-105.5 (CI 95%: 38.1-Infinite), Baja-Infinite (CI 95%: 322.7-Infinite), Alag Éger-26.4 (CI 95%: 20.9-34.5), Mura Vízgyűjtő-485.6 (CI 95%: 112.8-Infinite). According to BOTTLENECK's Wilcoxon signed-rank test for heterozygosity excess, there was not a recent population bottleneck in any of these populations.

Table 13. Genetic variability in eleven crucian carp populations based on thirteen polymorphic microsatellite loci.

Locus	Parameter	Cún-Szaporca Holt-Dráva	Dunafalva	Kölked Misányi-fok	NBGK-HGI -genebank	Lake-Fertő	Lake-Kolon	Belső telep HAKI 24.	Rétimajor 21.	Baja 24.	Alag Éger	Mura - Vízgyűjtő	Number of distinct alleles
GF1	N	30	20	29	29	20	28	30	36	27	28	23	3
	N_a	2	2	1	2	2	2	2	1	1	3	3	
	H_o	0.167	0.000	0.000	0.000	0.200	0.000	0.000	0.000	0.000	0.357	0.261	
	H_e	0.153	0.095	0.000	0.328	0.480	0.500	0.278	0.000	0.000	0.349	0.507	
	N_p	_	_	_	_	_	_	_	_	_	_	_	
	P_{HW}	0.619	0.000^{***}	_	0.000^{***}	0.009^{**}	0.000^{***}	0.000^{***}	_	_	0.912	0.028^{*}	
YJ0010	N	31	19	29	29	20	31	30	35	28	28	22	4
	N_a	3	2	3	4	2	3	2	2	3	4	3	
	H_o	0.323	0.474	0.345	0.103	0.350	0.548	0.000	0.029	0.500	0.714	1.000	
	H_e	0.476	0.494	0.485	0.295	0.489	0.541	0.064	0.028	0.488	0.672	0.561	
	N_p	_	_	_	_	_	_	_	_	_	_	_	
	P_{HW}	0.192	0.855	0.357	0.001^{***}	0.204	0.233	0.000^{***}	0.932	0.576	0.076	0.000^{***}	
GF29	N	30	19	29	29	23	31	30	36	27	28	22	26
	N_a	10	4	5	10	16	10	5	4	14	14	12	
	H_o	0.800	0.632	0.793	0.345	0.696	0.645	0.200	0.472	0.889	0.893	0.864	
	H_e	0.769	0.632	0.754	0.795	0.892	0.835	0.553	0.601	0.855	0.850	0.900	
	N_p	_	_	_	_	1	_	_	_	3	_	1	
	P_{HW}	0.097	0.045^{*}	0.654	0.000^{***}	0.000^{***}	0.002^{**}	0.000^{***}	0.000^{***}	0.004^{**}	0.018^{*}	0.041^{*}	
YJ0022	N	30	19	29	29	22	31	30	36	28	28	21	7
	N_a	3	3	2	3	4	3	3	2	3	4	3	
	H_o	0.400	0.421	0.103	0.448	0.409	0.419	0.333	0.667	0.214	0.321	0.667	
	H_e	0.476	0.342	0.098	0.530	0.402	0.370	0.531	0.498	0.195	0.337	0.649	
	N_p	1	_	_	_	_	_	_	_	_	1	_	
	P_{HW}	0.677	0.717	0.769	0.006^{**}	0.985	0.716	0.000^{***}	0.043^{*}	0.94	0.86	0.001^{***}	

Table 13. (Continued)

Locus	Parameter	Cún-Szaporca Holt-Dráva	Dunafalva	Kölked Misányi-fok	NBGK-HGI -genebank	Lake Fertő	Lake Kolon	Belső telep HAKI 24.	Rétimajor 21.	Baja 24.	Alag Éger	Mura Vízgyűjtő	Number of distinct alleles
MFW7	N	30	18	29	29	22	31	30	33	28	28	22	8
	N_a	5	4	3	2	3	2	2	1	2	2	2	
	H_o	0.333	0.167	0.345	0.103	0.818	0.806	0.033	0	0.893	0.714	0.182	
	H_e	0.496	0.252	0.347	0.098	0.501	0.481	0.033	0	0.494	0.459	0.165	
	N_p	2	2	_	_	_	_	_	_	_	_	_	
	P_{HW}	0.000***	0.006**	0.731	0.769	0.014*	0.000***	0.926	_	0.000***	0.003**	0.639	
CypG24	N	30	19	29	33	24	33	29	35	28	28	21	8
	N_a	3	3	2	4	5	5	4	3	4	7	5	
	H_o	0.167	0.316	0.207	0.303	0.375	0.273	0.621	0.371	0.214	0.786	0.381	
	H_e	0.212	0.277	0.185	0.487	0.33	0.315	0.539	0.465	0.198	0.735	0.404	
	N_p	_	_	_	_	_	_	_	_	_	2	_	
	P_{HW}	0.295	0.881	0.534	0.001**	0.999	0.556	0.000***	0.044*	0.999	0.745	0.569	
HLJYJ017	N	31	19	29	33	23	33	27	35	28	28	23	20
	N_a	13	11	9	11	12	11	7	9	9	11	14	
	H_o	0.935	0.895	0.828	0.818	0.870	0.848	0.778	0.657	0.857	0.929	0.783	
	H_e	0.882	0.889	0.844	0.803	0.868	0.854	0.748	0.802	0.840	0.878	0.905	
	N_p	_	_	_	_	_	_	_	_	1	_	1	
	P_{HW}	0.969	0.258	0.205	0.038*	0.652	0.509	0.530	0.205	0.832	0.602	0.209	
J62	N	26	19	29	32	16	32	29	35	28	16	15	13
	N_a	8	6	7	8	4	7	5	4	6	5	4	
	H_o	0.769	0.737	0.655	0.688	0.313	0.688	0.655	0.629	0.571	0.438	0.600	
	H_e	0.727	0.673	0.676	0.645	0.512	0.697	0.561	0.619	0.674	0.645	0.620	
	N_p	1	_	_	1	_	_	_	_	_	_	_	
	P_{HW}	0.455	0.010**	0.039*	0.000***	0.057	0.258	0.013*	0.559	0.004**	0.023*	0.794	

Table 13. (Continued)

Locus	Parameter	Cún-Szaporca Holt-Dráva	Dunafalva	Kölked Misányi-fok	NBGK-HGI -genebank	Lake Fertő	Lake Kolon	Belső telep HAKI 24.	Rétimajor 21.	Baja 24.	Alag Éger	Mura Vízgyűjtő	Number of distinct alleles
HLJYJ029	N	31	19	29	33	25	33	29	35	27	28	24	17
	N_a	8	3	4	3	8	4	2	1	3	9	9	
	H_o	0.419	0.316	0.31	0.091	0.4	0.485	0	0	0.444	0.571	0.458	
	H_e	0.492	0.417	0.349	0.286	0.353	0.575	0.067	0	0.46	0.659	0.484	
	N_p	1	_	_	_	_	_	_	_	_	1	2	
	P_{HW}	0.848	0.046*	0.929	0.000***	1	0.848	0.000***	_	0.695	0.014*	0.004**	
HLJYJ028	N	30	17	29	32	22	31	26	35	28	26	25	28
	N_a	21	18	19	14	14	16	8	12	16	12	18	
	H_o	0.733	1.000	0.897	0.906	0.636	0.903	0.769	1.000	0.964	0.500	0.560	
	H_e	0.936	0.926	0.922	0.891	0.895	0.901	0.834	0.882	0.891	0.875	0.928	
	N_p	_	_	_	_	_	1	_	_	_	_	_	
	P_{HW}	0.000***	0.485	0.728	0.122	0.003**	0.015*	0.833	0.986	0.959	0.000***	0.000***	
HLJYJ046	N	30	19	29	30	21	31	27	36	28	29	25	29
	N_a	21	15	16	11	16	15	7	6	18	14	17	
	H_o	0.833	0.842	1	0.733	0.619	0.677	0.778	0.667	0.857	0.759	0.36	
	H_e	0.933	0.914	0.891	0.793	0.907	0.821	0.726	0.756	0.925	0.895	0.85	
	N_p	_	_	_	_	1	_	_	_	1	3	_	
	P_{HW}	0.030*	0.403	0.383	0.005**	0.000***	0.263	0.356	0.216	0.617	0.001**	0.000***	
HLJYJ041	N	29	19	29	31	21	31	26	36	28	28	25	75
	N_a	32	22	22	21	26	26	15	14	25	20	29	
	H_o	0.966	0.895	0.931	0.871	0.952	0.903	0.808	0.778	0.964	0.893	0.840	
	H_e	0.954	0.940	0.939	0.905	0.948	0.933	0.894	0.838	0.938	0.936	0.954	
	N_p	_	4	1	1	2	3	2	1	5	1	_	
	P_{HW}	0.758	0.178	0.242	0.369	0.083	0.997	0.047*	0.017*	0.887	0.193	0.207	

Table 13. (Continued)

Locus	Parameter	Cún-Szaporca Holt-Dráva	Dunafalva	Kölked Misányi-fok	NBGK-HGI -genebank	Lake-Fertő	Lake-Kolon	Belső telep HAKI 24.	Rétimajor 21.	Baja 24.	Alag Éger	Mura Vízgyűjtő	Number of distinct alleles
HLJYJ082	N	30	19	28	32	20	31	28	36	28	23	25	7
	N_a	4	1	1	1	5	2	2	2	2	3	4	
	H_o	0.100	0.000	0.000	0.000	0.350	0.032	0.036	0.083	0.107	0.174	0.32	
	H_e	0.097	0.000	0.000	0.000	0.310	0.032	0.035	0.080	0.101	0.163	0.342	
	N_p	1	_	_	_	1	_	_	_	_	_	_	
	P_{HW}	1.000	_	_	_	1.000	0.927	0.923	0.794	0.765	0.976	0.909	

N = sample size; *Na* = number of alleles per locus; *Ne* = number of effective alleles; *I* = Shannon's index; *Ho* = observed heterozygosity; *He* = expected heterozygosity; N_p= number of private alleles; P_{HW}; *P*-value of Hardy–Weinberg probability test

*Represents deviation from Hardy–Weinberg equilibrium at $p < 0.05$

**Represents deviation from Hardy–Weinberg equilibrium at $p < 0.01$

**Represents deviation from Hardy–Weinberg equilibrium at $p < 0.001$

Table 14. Mean genetic parameters for eleven crucian carp populations.

Parameter	Cún-Szaporca Holt-Dráva	Dunafalva	Kölked Misányi-fok	NBGK-HGI -genebank	Lake Fertő	Lake-Kolon	Belső telep HAKI 24.	Rétimajor 21.	Baja 24.	Alag Éger	Mura-Vízgyűjtő
Na	10.23 ± 9.19	7.23 ± 6.94	7.23 ± 7.19	7.23 ± 5.93	9.00 ± 7.29	8.15 ± 7.27	4.92 ± 3.73	4.69 ± 4.35	8.15 ± 7.67	8.31 ± 5.54	9.46 ± 8.16
Ne	6.15 ± 6.85	5.13 ± 5.55	4.73 ± 5.09	3.67 ± 3.11	5.41 ± 5.49	4.58 ± 4.14	2.88 ± 2.45	2.91 ± 2.37	5.01 ± 5.03	5.12 ± 4.25	6.08 ± 6.33
I	1.47 ± 1.06	1.25 ± 1.03	1.20 ± 1.04	1.19 ± 0.86	1.45 ± 0.93	1.37 ± 0.88	0.94 ± 0.75	0.90 ± 0.84	1.31 ± 1.03	1.51 ± 0.81	1.54 ± 0.95
Ho	0.53 ± 0.31	0.52 ± 0.35	0.49 ± 0.37	0.42 ± 0.35	0.54 ± 0.24	0.56 ± 0.30	0.39 ± 0.35	0.41 ± 0.35	0.57 ± 0.35	0.62 ± 0.24	0.56 ± 0.26
He	0.58 ± 0.31	0.53 ± 0.33	0.50 ± 0.36	0.53 ± 0.31	0.61 ± 0.25	0.60 ± 0.27	0.45 ± 0.32	0.43 ± 0.36	0.54 ± 0.34	0.65 ± 0.25	0.64 ± 0.26
uHe	0.59 ± 0.31	0.54 ± 0.34	0.51 ± 0.37	0.54 ± 0.31	0.62 ± 0.26	0.61 ± 0.27	0.46 ± 0.32	0.43 ± 0.36	0.55 ± 0.34	0.66 ± 0.26	0.65 ± 0.26
F	0.09 ± 0.15	0.10 ± 0.32	0.01 ± 0.11	0.28 ± 0.36	0.08 ± 0.31	0.07 ± 0.36	0.29 ± 0.46	0.02 ± 0.17	-0.08 ± 0.24	0.02 ± 0.23	0.08 ± 0.33
A_R	7.74 ± 6.43	6.75 ± 6.24	6.01 ± 5.41	5.68 ± 4.21	7.69 ± 5.94	6.34 ± 4.92	4.21 ± 3.00	4.00 ± 3.34	6.64 ± 5.65	6.99 ± 4.34	7.98 ± 6.12
A_{Rp}	0.46 ± 0.50	0.46 ± 0.99	0.16 ± 0.42	0.15 ± 0.31	0.59 ± 0.91	0.22 ± 0.45	0.11 ± 0.39	0.15 ± 0.38	0.60 ± 1.20	0.75 ± 0.84	0.55 ± 0.82

Na = mean number of alleles per population; *Ne* = mean number of effective alleles; *I* = Shannon's index; *Ho* = observed heterozygosity; *He* = expected heterozygosity; *uHe* = unbiased expected heterozygosity; *F* = inbreeding coefficient, A_R = allelic richness; A_{Rp} = private allelic richness.

As mentioned, MFW7, HLJYJ028, and HLJYJ046 in the Cún-Szaporca Holt-Dráva population, Gf1, Gf29, MFW7, J62, and HLJYJ029 in the Dunafalva population, J62 in the Kölked Misányi-fok population, Gf1, Gf29, MFW7, HLJYJ028, HLJYJ046 in the Lake-Fertő, Gf1, Gf29, MFW7, and HLJYJ028 in the Lake-Kolon, Gf29, YJ0022, CypG24, and HLJYJ041 in the Rétimajor population, Gf29, MFW7, J62, in the Baja, Gf29, MFW7, J62, HLJYJ029, and HLJYJ028, HLJYJ046 in the Lake Alag Éger, Gf1, Gf29, YJ0010, HLJYJ028, HLJYJ029, and HLJYJ046 in the Mura Vízgyűjtő, Gf1, Gf29, YJ0010, YJ0022, CypG24, J62, HLJYJ029, and HLJYJ041 in the HAKI population, Gf1, Gf29, YJ0010, YJ0022, CypG24, J62, HLJYJ017, HLJYJ029, and HLJYJ046 in the NBGK-HGI, were all found to be out of the HW equilibrium ($P<0.05$). Gf1 in the Rétimajor population, Kölked Misányi-fok, and Baja, HLJYJ082 in the Dunafalva population, NBGK-HGI and Kölked Misányi-fok, MFW7 and HLJYJ029 in the Lake Rétimajor, were all monomorphic (Table 13).

Table 15. Genetic differentiation of wild and cultivated crucian carp populations.

Stocks	H_O (mean ± SD)	H_E (mean ± SD)	AR (mean ± SD)	AR_p (mean ± SD)	F_{IS} (mean ± SD)
Wild	0.570 ± 0.283	0.616 ± 0.277	7.25 ± 5.48	0.49 ± 0.81	0.047 ± 0.269
Cultivated	0.450 ± 0.330	0.531 ± 0.299	5.04 ± 3.50	0.15 ± 0.36	0.209 ± 0.373
P-values	0.041	0.126	0.007	0.001	0.022

Ho = observed heterozygosity; H_E = expected heterozygosity; A_R = Allelic Richness; A_{rp} = Private Allelic Richness; F_{IS} = inbreeding coefficient; *SD* = standard deviation.

4.4.2 Population structure and genetic differentiation

Pairwise F_{st} analyses were used to illustrate the patterns of genetic divergence among crucian carp populations across all loci. Without ENA correction and with it, the global Fst was 0.226 (95% CI: 0.137-0.340) and 0.217 (95% CI: 0.131-0.326) respectively, showing that the eleven populations studied had moderate to high genetic distances. Tables 16 and 17 provide the pairwise F_{st}, Cavalli-Sforza, and Edwards's genetic distance values between population pairs. The Kölked Misányi-fok population showed the greatest separation from the other populations, with an F_{st} ranged between 0.100 and 0.467 (Tables 17).

Table 16. Pairwise F_{st} (below diagonal) and Cavalli-Sforza and Edwards genetic distance (above diagonal).

Fst/genetic Distance	Cún-Szaporca Holt-Dráva	Dunafalva	Kölked Misányi-fok	NBGK-HGI - genebank	Lake-Fertő	Lake-Kolon	Belső-telep HAKI 24.	Rétimajor 21.	Baja 24.	Alag Éger	Mura Vízgyűjtő
Cún-Szaporca Holt-Dráva		0.284	0.303	0.587	0.428	0.394	0.644	0.692	0.454	0.496	0.435
Dunafalva	0.040		0.265	0.638	0.407	0.357	0.691	0.744	0.399	0.496	0 .487
Kölked Misányi-fok	0.058	0.013		0.618	0.423	0.368	0.674	0.716	0.419	0.505	0.500
NBGK-HGI –genebank	0.312	0.364	0.381		0.567	0.544	0.204	0.256	0.550	0.561	0.522
Lake Fertő	0.109	0.101	0.109	0.271		0.354	0.620	0.675	0.407	0.415	0.360
Lake Kolon	0.106	0.091	0.096	0.265	0.041		0.598	0.646	0.304	0.382	0.389
Belső-telep HAKI 24.	0.372	0.431	0.442	0.018	0.335	0.322		0.214	0.592	0.619	0.578
Rétimajor 21.	0.403	0.463	0.470	0.027	0.363	0.348	0.022		0.608	0.645	0.616
Baja 24.	0.195	0.176	0.191	0.279	0.055	0.066	0.340	0.359		0.381	0.436
Alag Éger	0.179	0.169	0.190	0.215	0.058	0.066	0.275	0.293	0.055		0.400
Mura Vízgyűjtő	0.135	0.174	0.190	0.195	0.051	0.070	0.253	0.277	0.110	0.068	

Table 17. (With null allele corrections), pairwise F_{st} with the ENA correction (below the diagonal) and the Cavalli-Sforza and Edwards genetic distances with the INA correction (above the diagonal).

Fst/genetic Distance	Cún-Szaporca Holt-Dráva	Dunafalva	Kölked Misányi-fok	NBGK-HGI - genebank	Lake-Fertő	Lake-Kolon	Belső-telep HAKI 24.	Rétimajor 21.	Baja 24.	Alag Éger	Mura Vízgyűjtő
Cún-Szaporca Holt-Dráva		0.318	0.318	0.593	0.444	0.423	0.647	0.693	0.470	0.504	0.451
Dunafalva	0.039		0.286	0.636	0.421	0.360	0.689	0.743	0.417	0.511	0.492
Kölked Misányi-fok	0.059	0.015		0.628	0.440	0.391	0.682	0.718	0.424	0.520	0.513
NBGK-HGI –genebank	0.288	0.337	0.364		0.570	0.543	0.216	0.290	0.569	0.583	0.540
Lake Fertő	0.105	0.090	0.108	0.253		0.368	0.620	0.678	0.415	0.419	0.361
Lake Kolon	0.106	0.082	0.100	0.246	0.038		0.598	0.649	0.321	0.401	0.398
Belső-telep HAKI 24.	0.344	0.397	0.420	0.019	0.311	0.298		0.256	0.610	0.633	0.587
Rétimajor 21.	0.395	0.451	0.467	0.042	0.361	0.347	0.031		0.610	0.649	0.619
Baja 24.	0.192	0.163	0.190	0.269	0.055	0.069	0.323	0.357		0.391	0.449
Alag Éger	0.174	0.157	0.190	0.197	0.055	0.063	0.252	0.288	0.055		0.406
Mura Vízgyűjtő	0.132	0.165	0.191	0.183	0.052	0.071	0.236	0.280	0.114	0.069	

The most significant genetic difference was found between populations Kölked Misányi-fok and Rétimajor (0.467), where these populations are geographically distant from each other (127km), while the least significant genetic difference was found between populations Dunafalva and Kölked Misányi-fok (0.015), which are geographically close (Table 17). In the same way, all of the other populations had moderate to high genetic differences, and most of them had no association with geographic distance (GeoD), as indicated by the mantel test (Figure 14).

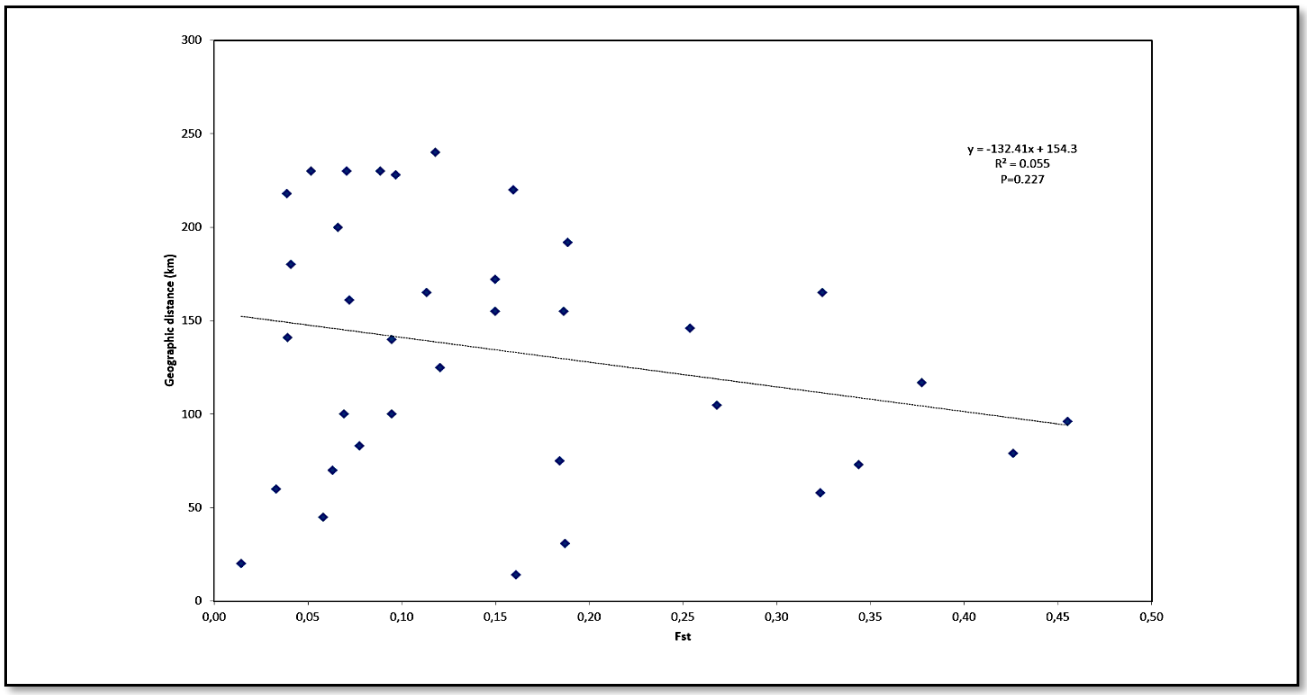


Figure 14. The figure shows the estimated regression between F_{st} and geographic distance (GeoD) for the eleven crucian carp populations (Mantel test, *P* =0.277).

The Bayesian model-based clustering (STRUCTURE) analysis of eleven crucian carp populations showed the highest value at K = 2 (Figure 15A). One distinct cluster was classified as "natural populations," while the other cluster was classified as farmed stocks (Figure 15B). Three of the eleven populations, NBGK-HGI, HAKI, and Rétimajor, were grouped into the cluster of farmed stocks, suggesting that they had different origins, whereas the other eight wild populations were clustered together.

Further (STRUCTURE) analysis of the eight wild populations was performed to determine the most probable K (K = 3), which showed that populations are mainly clustered into three sub-populations within the eight native populations (Figure 16A). This revealed that three distinct groups existed within these eight populations. The uniformity or mixing of the colors defined the population's genetic structure. For each sample site, the presence of just one color implies a population with no admixture; the presence of two or more colors shows genetic admixture at that particular sample site (Figure 16B). This analysis confirmed the genetic variability of the crucian carp populations. The two natural populations, Dunafalva and Kölked (close to the Danube River), were genetically homogenous, with no genetic admixture. These are completely isolated pond populations with a small number of individuals, most likely as a result of a prior occurrence of a founder effect. The populations from the Danube region, Lake-Kolon, Baja, Alag, and Cún-Szaporca Holt-Dráva from the Drava region, showed relatively minimal genetic admixture, suggesting fewer contributions or mixing with other populations. Such ponds with little or no admixture have great potential to serve as future sources for native crucian carp populations. Although there was no significant association between the geographical distance of the wild populations of Lake-Fertő, Mura and some other populations, these populations showed a great deal of admixture in terms of population structure. It was found that these populations share some clusters, indicating gene flow or mixing across the populations, either naturally or by the introduction of fish from other populations where gene flow has occurred. The average log likelihood data and Evanno delta for hierarchical structure analysis are presented in (Table S3). Individual relationships within and between groups were also evident using Principal Coordinate Analysis (PCoA) (Figures 15C, 16C). The analysis of molecular variances (AMOVA) revealed that 20% of the total molecular variance was among populations, 16% was among individuals, and 64% was within individuals, with a significant level (P<0.001).

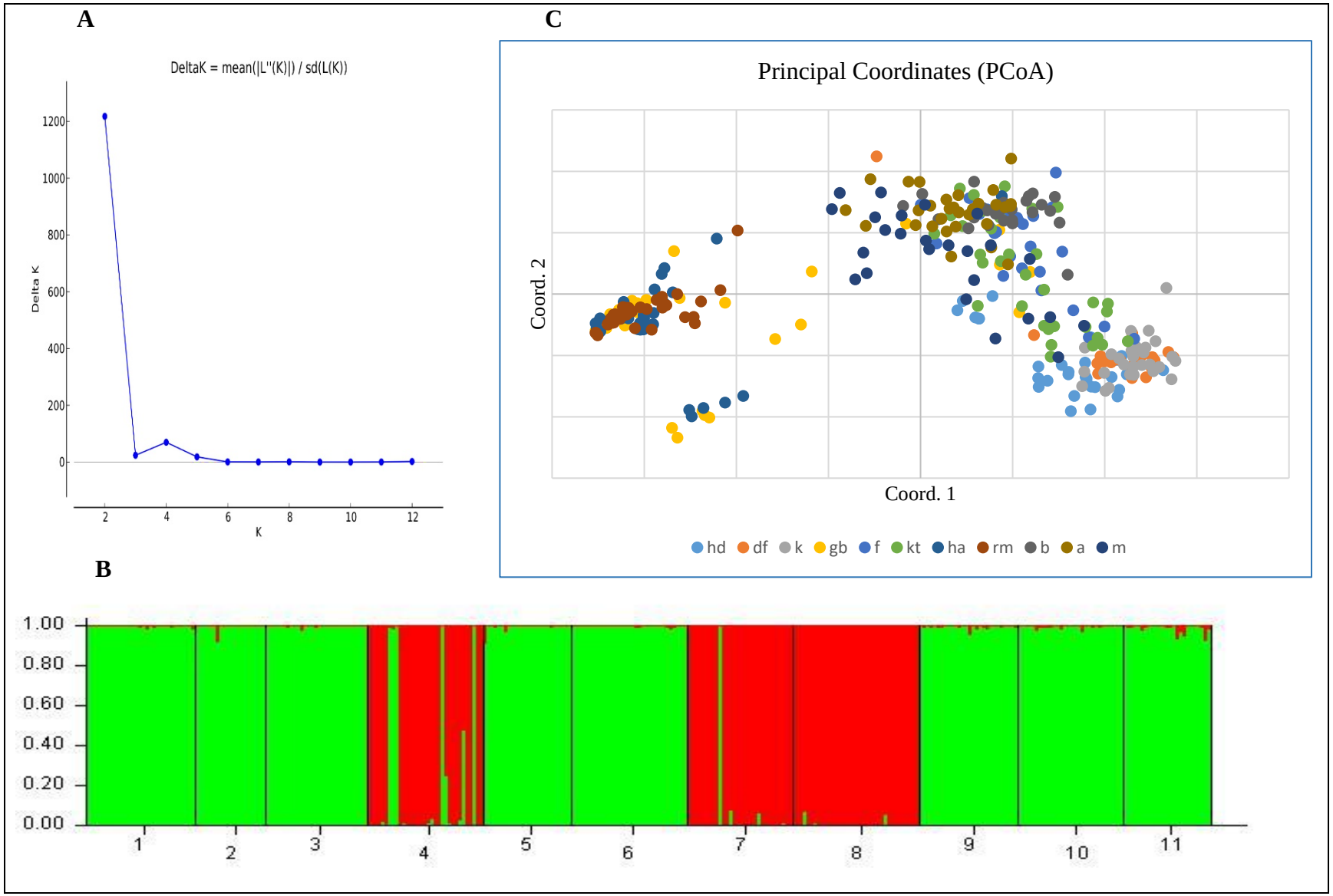


Figure 15. Population structure results for eleven crucian carp populations: A Estimation of the Cluster number (K). B Population Structure Cluster Analysis of the eleven crucian carp populations. The two clusters are indicated by the colors green (wild populations) and red (farmed stocks), respectively. C Principle Coordinate Analysis (PCoA) of allele frequencies in eleven crucian carp populations (eight wild populations and three farmed stocks).

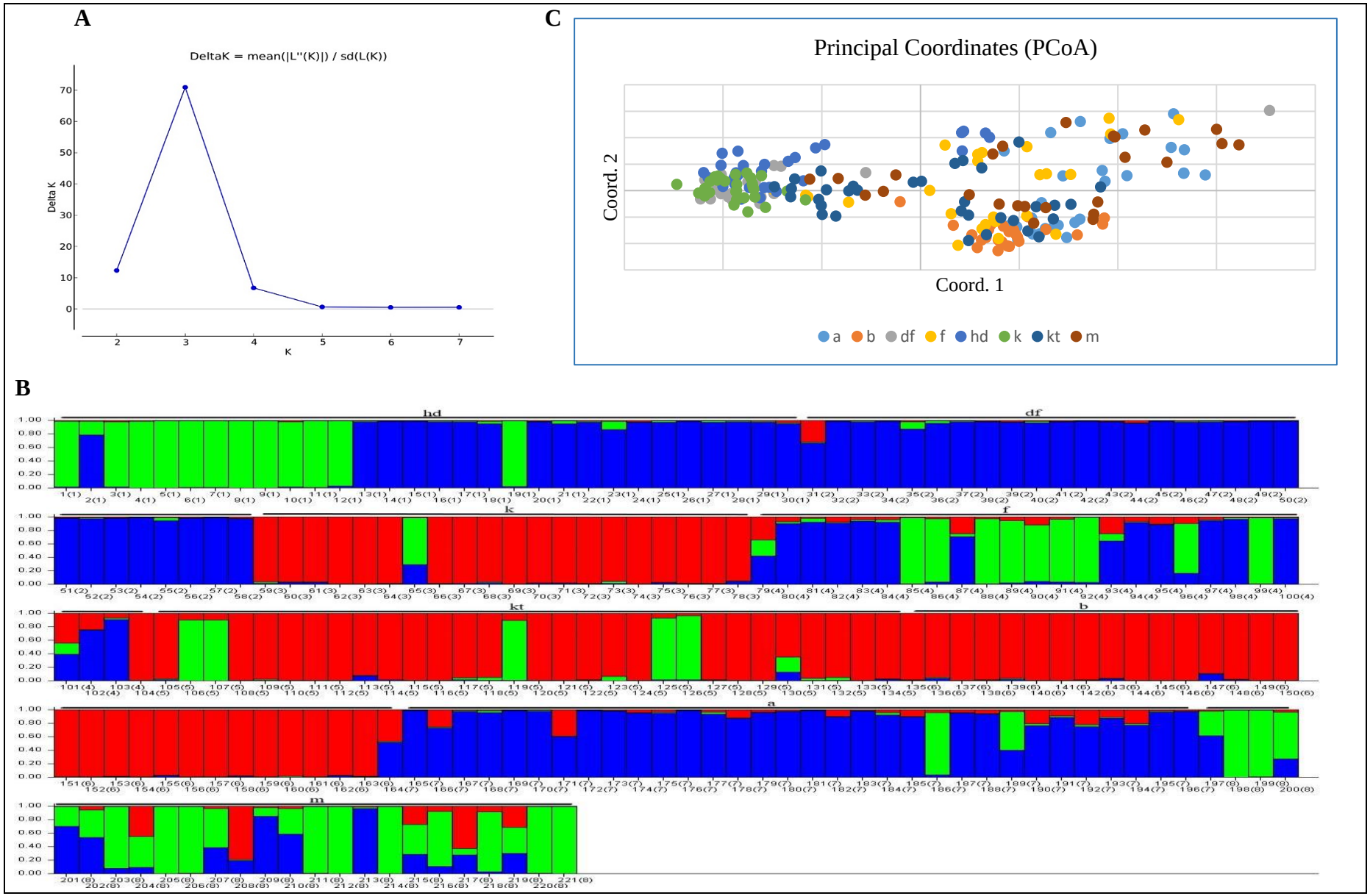


Figure 16. Population structure results for eight natural crucian carp populations: A Best delta K estimation. B Bayesian clustering analysis of eight wild crucian carp populations using STRUCTURE v2.3.3. Three clusters (k = 3) were inferred, indicating the presence of three distinct subpopulations of crucian carp in Hungary. In this plot, these sub-populations are represented by three colors: green, red, and blue. Each vertical line represents one individual. A population of uniform color (i.e. either green, red or blue) represents a population with little or no admixture. However, admixture is present in most populations in the current study. The only two exceptions are population 2 (df) and population 3 (k). C Principle Coordinate Analysis (PCoA) of allele frequencies in eight wild crucian carp populations.

4.5 Phylogenetic analysis of Crucian carp based on the COI gene sequence

The phylogenetic relationship between two fishes of the genus *Carassius*, the crucian carp (*Carassius carassius*) and the silver crucian carp (*Carassius auratus gibelio*), was assessed by comparing their mitochondrial DNA sequences. The COI haplotypes of *Carassius carassius* and *Carassius gibelio* available in GenBank were compared to the new COI haplotypes of *Carassius carassius* found in this study.

The primer pairs COI-FF2d-F (5′-TTCTCCACCAACCACAARGAYATYGG-3′) and COI-FR1d-R (5′- CACCTCAGGGTGTCCGAARAAYCARAA -3′) were used to successfully amplify 611-bp-long fragments of the Cytochrome C Oxidase I (COI) gene from 104 crucian carp samples. The 104 COI sequences yielded a total of 47 haplotypes, 43 of which were novel and four were previously described (Figure 17). The number of polymorphic sites within the haplotypes was 90 (Figure S1). The NCBI database was employed to verify the sequences' evolutionary origins. After being blasted, six haplotypes (hap6, hap20, hap21, hap22, hap23, hap24) were found to be those of silver Prussian carp (*Carassius auratus gibelio*), while the rest were identified as crucian carp (*Carassius carassius*). Thus, the haplotypes found showed two main groups in the network diagram (Figure 17).

One (hap6-MW564549.1) of the six haplotypes was dominant and was found to be identical to the gibel carp sequences provided by GenBank. An individual from NBGK-HGI-gene bank, two individuals from Mura Vízgyűjtő and two individuals from Cún-Szaporca Holt-Dráva were all carriers of this haplotype. The crucian carp group showed a predominance of five haplotypes: haplotypes 1, 2, 3, 43, and 49. GenBank sequences matched up with three of them (hap1-HQ961040.1, KJ128440.1; hap2-HQ960942.1; and hap3-HQ960610.1). Consequently, no common haplotypes were shared by the two species.

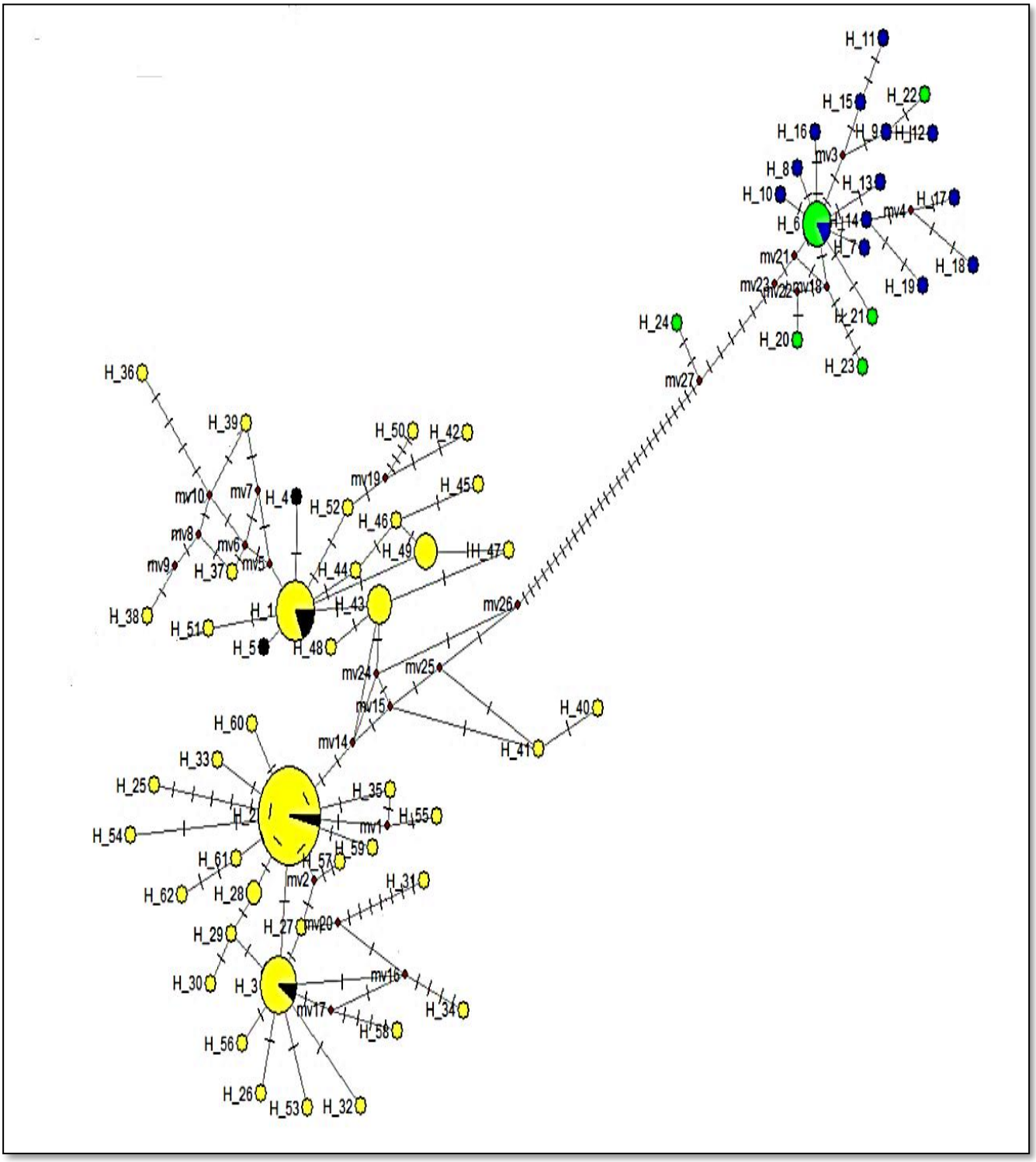
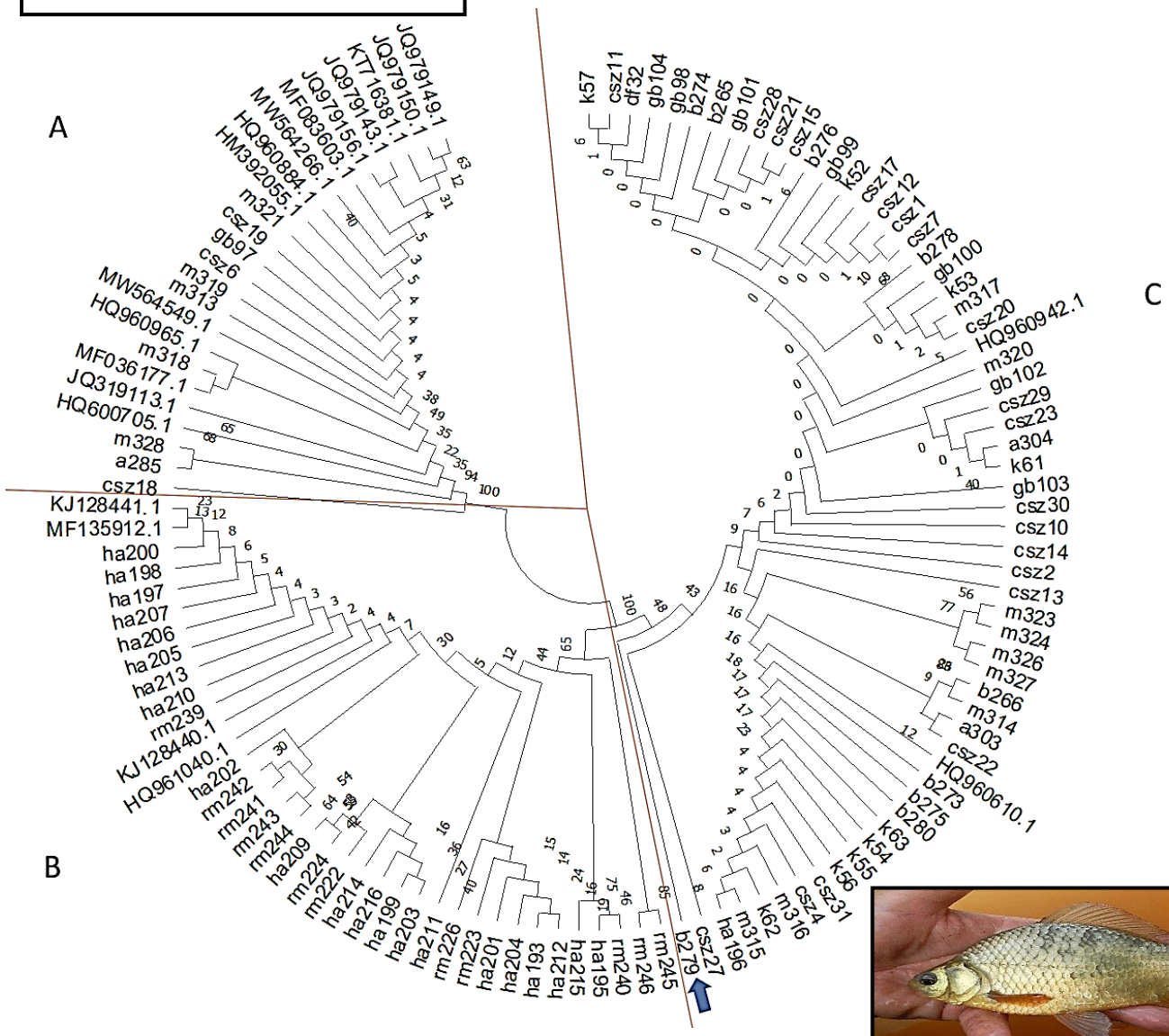


Figure 17. Haplotype networks of COI mitochondrial DNA sequences, showing the relationship between native crucian carp and closely related gibel carp species. Each circle represents a haplotype, and the sizes of the circles indicate the frequencies of each haplotype. Yellow, crucian carp; Green, gibel carp; Black, GenBank reference sequences. The median vector with red dots represents a predicted haplotype that was not identified.

In addition, the Neighbor-Joining phylogenetic tree revealed that the sequences were divided into three separate clusters, one of which included the *Carassius auratus gibelio* sequences, which were morphologically identified as crucian carps. The six gibel carp haplotype sequences identified among the new sequences, together with the 14 gibel carp haplotype sequences described in GenBank, formed the first cluster (A). The hap24, which was assigned to *Carassius auratus gibelio* in the network, was found to be separated from the cluster (A). The second cluster (B) included four *Carassius carassius* sequences from GenBank as well as individuals from rm, ha, and gb stocks, confirming the results of the microsatellite analysis and highlighting the different origins of the farmed stocks. The remaining haplotypes of individuals from all locations were represented in the biggest cluster (C), which was identified as a *Carassius carassius* species supported by two *Carassius carassius* sequences from the GenBank (Figure 18).

Carassius gibelio



Carassius carassius

Figure 18. Neighbour-Joining tree of genetic distances between *Carassius carassius* and *Carassius gibelio* based on COI mtDNA sequences. Cluster A represents *Carassius gibelio*, clusters B and C represent *Carassius carassius*. The blue arrow indicates the separation of haplotype 24 (see Network) from the three clusters. The NJ tree was constructed with MEGA-11 software using the Bootstrap method with 1,000 bootstrap replicates, Tamura 3 parameter model, Gamma-Distributed (G) (Tamura et al., 2021).

5. DISCUSSION

5.1 Characterization of the genetic variability and phylogenetic status of Tench (*Tinca tinca* L. 1758) populations in Hungary using nuclear and mitochondrial markers

The genetic diversity and phylogeographic identity of wild and cultured tench populations are still poorly understood. In this study, we used 12 microsatellite DNA markers, a mitochondrial Cytb gene, and two nuclear genes (*Act* and *Rps7*) to learn more about the genetic affinities of seven natural populations of the species inhabiting the Carpathian-basin. With some exceptions, the genetic variability findings obtained were mainly compatible with earlier investigations. Kohlmann et al. (2007) revealed that only seven of the microsatellites developed and studied were polymorphic in two wild populations (Döllnsee and Felchowsee) and four cultured strains (Königswartha, Germany; Tabor, Marianske Lazne, and Vodnany, Czech Republic). Throughout their research, they described a total of 49 alleles and found that the wild populations had more genetic diversity than cultured strains. The average number of alleles per loci in their study ranged from 2.57 (Ho: 0.273) to 5.86 (Ho: 0: 367), while we found values between 2.41 (Ho: 0.170) and 4.16 (Ho: 0: 43). The average allelic richness value was lower in Hungarian populations (2.66) than in German natural populations (3.73). In a subsequent study (Kohlmann et al., 2010), they found similar values in terms of the average number of alleles per locus, adding that they also found three populations with extremely low within-population variability. One Spanish farm population from near Badajoz was homozygous at all loci in 50 analyzed individuals. The total number of alleles observed in the 21 populations was 66. They also studied a Hungarian strain derived from the live gene bank of Vodnany (Czech Republic), but the original source of the stock was not mentioned. The average number of alleles per loci was 2.71, with an observed heterozygosity of 0.352. The number of private alleles was 13 in the study (Kohlmann et al., 2007) while working with 200 individuals originating from 6 populations using 7 microsatellites and 20 in the (Kohlmann et al., 2010) study when they used 9 species-specific microsatellite markers to characterize 792 individuals representing 21 wild and cultured populations. In the current study, the number of private alleles was lower (Kujawa et al., 2011). Three of them were observed in the Lake Fertő population. Interestingly, in the Neighbor-joining tree in Kohlmann et al. (2010), the

Hungarian stock is located separately from the other Central and Western European populations and much closer to the neighboring branch of the tree where the Chinese, Turkish, and Spanish (probably introduced from Central Europe) populations are located. According to the authors, the three populations may represent the species' Eastern lineage. The F_{is} values (inbreeding coefficient) in this research ranged from -0.03 to 0.28, which is close to the findings of Lajbner et al. (2010) but higher than those of Kohlmann et al. (2010). The Derecske population's high inbreeding coefficient may be explained by the fact that the two small ponds where the population inhabits are landlocked and isolated from all other surface water bodies.

The genetic variability found in nature is the existing basis of all future selective breeding programs. Artificial selection and selective breeding may have a negative effect on genetic diversity and effective population sizes (Molnár et al., 2020). When the goal of generating large genetic gain is combined with limited facilities and the necessity to breed exclusively genetically and phenotypically better individuals, it may result in the creation of small populations with a high probability of genetic drift and inbreeding. This phenomenon may compromise the sustainability of such programs (Ponzoni et al., 2010). Based on our results, one can say that the maintenance of the genetic variability of natural tench populations is not only important for conservation efforts but in order to be able to establish a selective breeding program for the species.

A total of 20 Cytb haplotypes were described from Hungarian samples in the present study. Of these, 18 (including the common H3 and H4) have not yet been observed before. Two out of the three haplotypes (C1, C2, and C3) described in 50 samples by Karaiskou et al. (2020) show a match with two haplotypes described in this study: C1 is identical to the H11 we describe, which characterized the Western phylogroup, while C2 is identical to the H6 described here and characterized the Eastern phylogroup. Haplotype C3 (MT605881.1, hap 30 in the network diagram of our study) was not present among Hungarian samples, but it was described in the Romanian samples and belongs to the Eastern phylogroup. Six Cytb haplotypes were described in a 2014 study (Lo Presti et al., 2014), five of which had previously been identified. Haplotype 6 (H6) of this study matched the sequences JX974523.1 (haplotypes H2a, H2b, H2d, H2e, H2f, H3 and H8), JX974524.1 (haplotype H2c) and JX974525.1 (haplotype H7) of the previously mentioned study, which belonged to the haplogroup B (Eastern) described by them. The Hap 11 haplotype (H11) of this study matched the sequences JX974520.1 (haplotype H1a) and JX974521.1 (haplotype H4), which belonged to the haplogroup A (Western) group in their study.

According to the haplotype network, 98 individuals (67.1%) belonged to the Eastern haplogroup, whereas 48 individuals (32.9%) belonged to the Western haplogroup (Table 9). Among the populations, the Tisza Lake population contained only individuals belonging to the Eastern haplogroup, while individuals from both haplogroups were found in different proportions in the other populations. The populations of Kolon Lake, Csörnöc Herpenyő, and Derecske contained a higher proportion of Eastern haplotypes, while in the other populations the haplogroups were largely equally present.

5.2 Tench phylogeography and postglacial recolonization in Europe

The two phylogeographical lineages were most likely split when Western Europe was colonized from the Black Sea Basin due to recurrent isolation in two refugia during the Pleistocene interglacial (Hewitt, 2004; Lajbner et al., 2007). This separation produced a high evolutionary divergence (1.3% for the cytochrome b gene) approaching the level between different fish species (Lajbner et al., 2010). The Ponto-Caspian region and the Danube basin were probably the glacial refugia of the Eastern phylogroup (Lajbner & Kotlík, 2011). The two refugia were responsible for the recolonization of Central Europe. However, the lineages did not show reproductive separation in the contact zone but a mixed ancestry hybrid zone was formed (Lajbner et al., 2010). The current study's findings support the use of PCR-RFLP analysis of nuclear markers in tench phylogeographic research. By combining mitochondrial and nuclear markers, this approach proved successful in detecting the main western and eastern phylogeographic clades of this species, as well as in identifying hybrids across clades.

Lujić et al. (2017) described the hypothesis that there is a characteristic pattern of Cytb haplotypes in the Balkan region and the Eastern haplotypes dominate the region south of the Danube River while the Western haplotypes dominate the regions north of the river. Based on the examination of the Rps7 and Cyt b genes, this hypothesis suggests a natural invasion of the western lineage; the main route of which is the Danube and Tisza rivers. Our results can support this hypothesis only partially since it assumes a clear change in the ratio of the two lineages in the Hungarian populations. On the contrary, we found that on both sides of the Danube River, the Eastern Cytb haplotypes were dominant, with the proportion ranging from 53% to 100%. Nevertheless, the PCR-RFLP analysis of the nuclear Act and Rps7 genes showed results not consistent with the Cytb results. The majority of the individuals belong to the hybrid group (50% in the case of Act and 41% in the case of the Rps7 gene) or to the Western haplotype (33% in the case of both genes),

showing the evidence of a long-term, repeated (hybrid) crossing of Western and Eastern clades. There are probably two main factors that alter the pattern of the natural invasion of the western lineage: the human-aided dispersal of the lineages (Lajbner et al., 2010; Clavero, 2019) and the dispersal ability of the species in the different habitats (Morissette et al., 2021; Bernos et al., 2022). The microsatellite analysis of the natural populations supports the human impact on the natural populations. None of the four genetic clusters were typical for locations. The admixture of the clusters showed only changes in the proportions according to the geographical differences.

5.3 Characterization of the genetic diversity of crucian carp (*Carassius carassius* (L. 1758)) populations in Hungary using microsatellite markers

The genetic variability of a species or a population plays an important role in survival. A population with higher genetic variability has a higher chance of adapting to a changing environment. For this reason, in the present work we described the genetic variability of eleven crucian carp stocks and populations. The comparison of results in this study with results on the genetic variability of other crucian carp populations is barely possible because there is no such data to be found in the literature. In the case of *Carassius* species, all authors focus on hybridization, even when they use nuclear markers such as microsatellite DNA markers (Hänfling et al., 2005; Papoušek et al., 2008), and overlook the importance of genetic variability. For the abovementioned reasons in this chapter, the genetic variability of Hungarian crucian carp populations is compared to closely related *Cyprinid* species such as common carp, gibel carp, and goldfish.

The microsatellite analysis of the eleven crucian carp stocks and populations using 13 microsatellite markers resulted in the description of the presence of 245 microsatellite alleles, which means an average of 18.64 alleles/locus. This number is considered very high, but it involves an extremely variable locus, HLJYJ041, which alone represents 75 alleles. Without this locus, the average number of alleles is 14.16. DeWoody & Avise (2000) reported that the average number of alleles per locus in wild freshwater fish species in general is 7.5. Kohlmann et al. (2003) described an average of 8.17 in common carp (*Cyprinus carpio).* In a subsequent study (Kohlmann et al., 2005), they found the mean number of alleles/locus to be between 2.5 and 14.25 in the case of 22 different common carp stocks. In the same study, the number of alleles per locus was found to be between 27 and 47, which is comparable to our results. In contrast, Chen et al. (2014) used 10 microsatellite markers to describe the genetic variability of 6 goldfish (*Carassius auratus*)

populations. They described the presence of 243 alleles in the 243 individuals, which means the average number of alleles per locus was 24.3. This value is even higher than that described in the present study. It is also worth noting that Zaijie et al. (2018) found 341 alleles using 12 microsatellite markers, with an average of 28.67 alleles per locus from four wild populations of common carp. Similarly, the genetic diversity parameters reported for 14 common carp strains in Hungary by (Tóth et al., 2020) showed greater values than those estimated for crucian carp populations in this study. The authors used 12 species-specific microsatellite markers and reported a higher mean value of heterozygosity (Ho = 0.840) than was found in the crucian carp populations in this study (Ho = 0.509). Moreover, the mean total number of alleles per strain was greater (11), compared to the 7.7 we found. The number of private alleles they found in 630 common carp individuals was also higher (117) than the private alleles we found. However, the number of alleles may be influenced by the sample size employed (O'Connell & Wright, 1997). The extremely variable locus HLJYJ041, which alone accounts more than 30% of the alleles, was also used in other studies. Zheng et al. (2010) developed 59 polymorphic trinucleotide and tetranucleotide markers (including HLJYJ041) for the silver crucian carp and tried them on a test panel of 34 crucian carp individuals. They described 12 alleles on this locus, while Lu et al. (2016) found 18 alleles when they analysed 10 gynogenetic clones of Fangzheng silver crucian carp (*Carassius auratus gibelio*).

In the present study, all of the farmed crucian carp populations analyzed had low genetic diversity parameters assessed. We found the lowest number of alleles in Rétimajor (61), while the population of Cún-Szaporca Holt-Dráva had the largest total number of alleles (133), followed by the Mura Vízgyűjtő population (123) alleles and the Lake-Fertő population with (117) alleles. Based on these data, one can conclude that natural populations of *Carassius carassius* in Hungary are more variable than farmed stocks of the species. When we look at the results of AMOVA, our results are different from the results of Chen et al. (2014), since in the present study, 20% of the total molecular variance was among populations, 16% was among individuals, and 64% was described within individuals, while they found that 98.65% of genetic variation contributed to differences within individuals and only 16.35% contributed to differences among populations. The pairwise F_{st} values across the populations were slightly lower in their study, ranging between 0.012 and 0.304, while we found pairwise F_{st} values ranging between 0.015 and 0. 467. In contrast, the

AMOVA results from Tóth et al. (2020) revealed a much lower level of genetic difference among populations (3.79%).

In the present study, the geographically closely located populations showed lower pairwise F_{st} values, but the association with distance (GeoD) was not statistically significant. All other population pairs showed moderate to high genetic differences. While studying common carp stocks and populations from all over the species' habitat range, Kohlmann et al. (2005) found that at the regional level, the highest differentiation was found among the East/South-East Asian populations (average F_{st} = 0.343), followed by the European (average F_{st} = 0.138) and Central Asian (average F_{st} = 0.002) stocks. Interestingly, the geographical distances in the mentioned study are really long, but pairwise F_{st} values are in the same range as in the present study. In all populations, they found negative F_{is}, which is interesting. On the other hand, we found both negative and positive F values.

5.4 Carassius carassius and Carassius auratus gibelio phylogenetic relationship

Non-native fish have taken over many of Hungary's lowland streams and rivers, including the Danube River. Fishing pond escapees such as gibel carp, topmouth gudgeon, and pumpkinseed, and the recent invasion of Ponto-Caspian gobies are of special concern (Takács et al., 2017). Outcompeting native species and altering their habitats are significant environmental consequences of *Carassius auratus gibelio* in Europe (Savini et al., 2010). Also, it is well documented that feral goldfish (*Carassius auratus*) compete with native crucian carp for resources, endangering native populations and reducing reproductive potential (Copp et al., 2010; Mezhzherin et al., 2012; Copp & Sayer, 2020). According to previous reports, Hungary and all countries of the Danube basin are at serious risk from these invasions (Ferincz et al., 2018). Therefore, it is necessary to take these catchment boundaries into account while conserving *Carassius carassius* in central Europe.

This is the first attempt to assess a phylogenetic relationship between crucian carp and gibel carp in Hungarian waters using mtDNA Cytochrome C Oxidase I (COI) gene sequences. The COI gene sequence variation of 104 Hungarian crucian carp individuals was investigated, with 47 COI haplotypes reported. Of which, 43 were novel, previously unidentified haplotypes. Among the sequences identified as crucian carp, five haplotypes were shown to be the most frequent, including individuals from Rétimajor, Kölked Misányi-fok, Belső-telep HAKI lake, NBGK-HGI-genebank, Baja, Mura Vízgyűjtő, and Cún-Szaporca Holt-Dráva (Figure 17). Furthermore, six of our own

sequences got integrated into the gibel carp group along with other sequences from GenBank identified in previous studies. Individuals identified as belonging to *Carassius auratus gibelio* were found to be morphologically indistinguishable from those of *Carassius carassius*. Therefore, our findings support the gibel carp invasion of crucian carp populations, which resulted in hybridization between females of the introduced *Carassius auratus gibelio* and males of the native *Carassius carassiuas* (gibel carp x crucian carp hybrids) as previously reported (Papoušek et al., 2008; Wouters et al., 2012).

Gibel carp's taxonomic status remains a mystery, as it's still unclear if it's a distinct species, a subspecies of *Carassius auratus*, or a hybrid species (Hanfling et al., 2003). Despite several studies illustrating the evolutionary relationships among the *Cyprinid* species in the genus *Carassius*, insufficient information was available on the phylogenetic relationships between *Carassius carassius* and its closely related species such as gibel carp. On the other hand, the taxonomy of the genus *Carassius* is still being questioned and, given the significant morphological resemblance amongst the species, however, has hindered valid judgments on their taxonomy, biogeography, and introduction history until now (Rylková et al., 2013). Moreover, the silver crucian carp (*Carassius auratus*) represents the *Carassius auratus* subspecies complex, known for its ambiguous taxonomic and evolutionary relationships (Podlesnykh et al., 2012). Using RFLP analysis of the ND3, ND4L, ND4 and 12S,16S rRNA regions, as well as examination of nucleotide sequence variation in the Cytochrome b gene, Apalikova et al. (2011) found that *Carassius carassius* had different mitochondrial DNA haplotypes than *Carassius auratus gibelio*. On the other hand, the authors support that the two *Carassius auratus* forms, *Carassius auratus gibelio* and *Carassius auratus cuvieri*, are genetically close to *Carassius carassius* and *Cyprinus carpio.* However, the authors indicated that after separating from the common carp lineage, the ancestral form diverged into silver crucian carp and crucian carp. Consequently, the results of this research are consistent with theirs. This study's preliminary phylogenetic investigation indicated taxonomic divergence predictions for the two species of the genus *Carassius*. The analysis of the mtDNA Cytochrome C Oxidase I (COI) gene showed distinct clusters on the phylogenetic tree. The haplotypes of *Carassius carassius* and *Carassius auratus gibelio* were well separated, indicating that the evolutionary paths of the two species diverged at some point (Figure 18). However, in order for these species' evolutionary relationships to be accurately established, further data with more samples will be included in the next stage of our studies.

6. NEW SCIENTIFIC RESULTS

1. The population genetic structure of tench (*Tinca tinca* L. 1758) and crucian carp (*Carassius carassius* L. 1758) reported in this study supported previous studies from different geographical regions in Europe. We were able to confirm the combined use of nuclear and mitochondrial markers as an effective way to better understand the genetic makeup of a population since each contributes unique features. For both species, we applied a larger number of microsatellite markers than had been used in most previous studies.

2. This study is the first to describe the genetic diversity and population structure of tench (*Tinca tinca* L. 1758) populations in the Carpathian Basin.

3. The current study revealed that, in addition to the sequencing of an mtDNA (Cytb) segment, the PCR-RFLP analysis of two independent nuclear-encoded exon-primed intron-crossing (EPIC) markers, (*Act*) and (*RpS7*), is an efficient method for assessing phylogeographic structure and identifying the Western and Eastern origins of Hungarian tench populations.

4. A total of twenty Cytb haplotypes from Hungarian tench samples were described in this study. Of these, 18 (including the common H3 and H4) had not been previously observed.

5. Based on COI gene data, we provide the first phylogenetic and taxonomic overview of native crucian carps and the closely related invasive gibel carps found in Hungarian waters. A total of 47 COI haplotypes were identified, with 43 of them being novel. Six of the new haplotype sequences were identified as belonging to *Carassius gibelio*, confirming previous reports of their invasion across different parts of Europe.

7. CONCLUSIONS AND PERSPECTIVES

In this thesis, the population structure and diversity of endangered crucian carp (*Carassius carassius* L. 1758) and tench (*Tinca tinca* L. 1758), as well as phylogenetic relationships and systematic phylogeography, are explained using genomic approaches, paving the way for the accurate identification of Hungary's conservation management units and selective breeding programmes. The following are the main conclusions:

- A key consideration in species conservation is to protect and maintain genetic variability, as described in the literature section above. This part of the thesis revealed that the wild living populations of tench in Hungary are genetically moderately diverse compared to other natural populations of the species living in Western Europe, and this is the first study in which genetic diversity in native Hungarian tench populations was described. However, they still represent significant aquatic genetic resources and could serve as a good basis for future selective breeding programmes. The genetically most variable Lake Fertő, Lake Kolon and Csörnöc-Herpenyő populations can be the most promising candidates for future breeding programs, while populations with considerably high private allelic richness (such as Cibakházi-Tisza and Lake-Tisza) should also be involved in order to start such a program with the highest genetic variability involved.

  This part of the thesis also showed that there are moderate to high genetic differences in the populations of Hungarian crucian carp, which is seen as a first initiative in assessing the genetic diversity and population structure of Hungarian aquaculture stocks. The natural populations, on the other hand, had higher variability. Two wild populations, Dunafalva and Kölked (close to the Danube River), were found to have minimal genetic diversity and a homogeneous genetic structure, perhaps owing to the presence of a founder effect in these isolated pond populations. The ponds that don't have much or any mixing have a good chance of becoming future stocks for the native crucian carp populations.

  To ensure the survival of the population and its ability to adapt to environmental change, a wide range of genetic variations is necessary. Wild populations are essential genetic resources, and therefore it is crucial to preserve them. We recommend monitoring the genetic diversity of the farmed stocks of these species in order to keep biodiversity from being lost. Genetic variation in stocks can be more effectively monitored with the use of

molecular approaches. This helps us understand how this diversity can be maintained through selective breeding. Appropriate breeding practices, as with crossbreeding, can be used to enhance genetic variety in farmed stocks having limited genetic variation. However, uncontrolled crossbreeding must be avoided. Additionally, increasing effective population sizes will help reduce inbreeding levels, which must be considered while developing breeding programs for these species. In the future, the research will be expanded to include more wild populations from a wider geographic area and more cultivated stocks so that the genetic profile of the populations found in this study can be tracked.

- Tench's natural range is also an important consideration. In this part, the phylogeographic analysis described the Western and Eastern phylogroups and was able to find hybrids between the two clades. Thus, Hungary is in the transition zone between the two lineages of the species, with a high level of hybridization, suggesting that these phylogroups were dispersed outside of their native range through human activities. This interrogation will help to distinguish the diverse geographic origins of populations and track the phylogroups spread across wild populations mediated by humans, which is important for breeding efforts. However, to learn more about this phenomenon, the natural and human-aided processes of hybridization in this zone need to be studied in more depth.
- We found indications of invasive *Carassius auratus gibelio* extension in Hungarian crucian carp populations and assessed their phylogenetic relationship. Mitochondrial data has provided useful insights in resolving taxonomic ambiguities among closely related species that are difficult to define morphologically. The Cytochrome C Oxidase I (COI) gene was shown to be useful in identifying the phylogenetic relationships between *Carassius carassius* and the closely related invasive gibel carp, *Carassius auratus gibelio*. The distribution of haplotypes indicated that these two species had diverged in their evolution. Therefore, although most of the hybrids exhibited morphology similar to *Carassius carassius*, *Carassius carassius* populations should be treated as separate management units for conservation activities. However, the current understanding of the taxonomic status of the two species provided by these data is still insufficient. Therefore, more specimens will be included in the next step of our studies to precisely define their taxonomic position in the *Cyprinidae* family. On the other hand, the invasion of species gives us the opportunity

to consider the many different evolutionary processes that occur within species. This result encourages further research into the impact of invasive species on the genetic diversity of Hungarian native species.

- The National Centre for Biodiversity and Gene Conservation has established an ex-situ live GenBank and a cryobank to help achieve the study's intended aims. Ex-situ conservation practices must emphasize the genetic structure and identity of translocated individuals.

8. SUMMARY

Tench (*Tinca tinca* L. 1758) and crucian carp (*Carassius carassius* L. 1758) are two cyprinid fish species with declining population numbers and sizes in Hungary and in the neighboring countries. The conservation of these species requires an understanding of their population structure and also phylogeographic knowledge. Due to a number of strengths, mitochondrial DNA (mtDNA) and microsatellite markers are increasingly being employed in such studies. The present study is a part of biosystematics studies that focus on the importance of population structure and diversity, biogeographic history, and phylogenetic relationships at the molecular level. During the 2018–2022 research period, the main objectives achieved are summarized below. However, the COVID-19 pandemic hampered the lab work and caused the research to fall behind schedule.

First, the genetic diversity and phylogeographic structure of seven wild tench populations were investigated to establish the genetic knowledge base for successful conservation efforts and selective breeding. Twelve microsatellite markers and the sequencing of a 615 bp section of mtDNA (Cytb) were used to analyze the genetic variation and structure among 175 individuals. Furthermore, phylogeographic identification of individuals from each site was assessed using PCR-RFLP analysis of two nuclear markers (*Act*) and (*RpS7*). All microsatellite loci were found to have moderate levels of polymorphism. The pairwise F_{st} values between population pairings were moderate; the populations were aligned into four clusters. The Cytb gene showed 20 haplotypes; 67.1% of individuals were categorized as Eastern, while 32.9% belonged to the Western haplogroup. On both sides of the Danube, Eastern origins were dominant. However, the results of the PCR-RFLP analysis of the nuclear *Act* and *Rps7* genes did not match those of the Cytb genes. The majority of the individuals belonged to the hybrid group (50% in the case of *Act* and 41% in the case of the *Rps7* gene) and to the Western haplotype (33% in the case of both genes), indicating a significant level of hybridization among the two geographic clades within the sampled populations. Thus, Hungarian tench populations are genetically less diverse compared to natural populations in Western Europe, but they still represent valuable genetic resources. Lake Fertő, Lake Kolon, and Csörnöc-Herpenyő populations can be optimal candidates for future selective breeding programs. Moreover, the PCR-RFLP assays of the two independent nuclear-encoded exon-primed intron-crossing (EPIC) markers were shown to be a reliable approach for

assessing phylogeographic structure and identifying the Western and Eastern origins of the Hungarian tench populations.

Second, in the case of crucian carp (*Carassius carassius* L. 1758), thirteen microsatellites were used to assess the genetic diversity and differentiation of 320 crucian carp individuals from eight wild populations and three farmed populations in Hungary. All microsatellite loci had high levels of polymorphism, with a total number of 245 alleles in all populations. The number of alleles in different populations ranged from 61 to 133, with a total of 49 private alleles with frequencies ranging from 0.016 to 0.250, mostly observed in wild populations. The lowest number of alleles was found on locus GF1 (3), while the highest number of alleles (75) was detected on HLJYJ041. The mean observed heterozygosity (Ho) and expected heterozygosity (He) for all populations was 0.509 and 0.551, respectively. From the He and Ho values, it was inferred that in most of the natural populations, these two values were close to each other, while in the case of two farmed stocks (NBGK-HGI stock and HAKI stock), the Chi-square test for Hardy-Weinberg equilibrium presented a significant heterozygote deficit in six of the thirteen loci. According to genetic parameters including allelic richness, observed heterozygosity, and expected heterozygosity, wild crucian carp populations showed a rather higher level of polymorphism than cultured populations. The global F_{st} was 0.226 (95% CI: 0.137-0.340). Pairwise F_{ST} from low to high values (0.015-0.467) showed a robust structure and a significant level of population differentiation. Bayesian model-based clustering (STRUCTURE) analysis has consistently confirmed the existence of three sub-populations within the eight populations that are classified as wild populations. AMOVA revealed that genetic variation was mostly found within populations. The above results revealed moderate to high genetic variation in the populations of Hungarian crucian carp, and that the farmed populations had lower genetic diversity compared to the wild populations. Thus, it is important to maintain the wild crucian carp populations since they are vital genetic resources.

Finally, in an effort to better understand the taxonomic status of two species in the genus *Carassius*, *Carassius carassius* and *Carassius gibelio*, a preliminary study of the phylogenetic relationship between the two species was conducted. Sequence variation patterns in the mtDNA Cytochrome C Oxidase I (COI) region from 104 crucian carp samples were compared to those available in GenBank for *Carassius carassius* and *Carassius gibelio*. Among the 104 new COI sequences, 47

haplotypes were found, six of which were found to belong to *Carassius gibelio*. The presence of gibel carp haplotypes in four crucian carp populations (Mura Vízgyűjtő, Cún-Szaporca Holt-Dráva, NBGK-HGI -genebank, and Alag Éger) suggests an invasion of this invasive species and a possible hybridization event. The Neighbor-Joining phylogenetic tree revealed that the samples' genetic profiles were clearly separated. At the same time, the distinct distribution of haplotypes makes it possible to presume that these two species are different from each other.

9. ÖSSZEFOGLALÓ

A compó (Tinca tinca L. 1758) és a széles kárász (Carassius carassius L. 1758) két őshonos pontyféle, melyek populációi hanyatló tendenciát mutatnak mind hazánkban, mind pedig a környező országokban. A fajok megőrzéséhez szükséges, hogy megértsük populációgenetikai struktúrájukat és információt nyerjünk filogeográfiájukról. Előnyös tulajdonságaik miatt az ilyen jellegű vizsgálatokhoz gyakran alkalmaznak mitokondriális DNS markereket, illetve mikroszatellit DNS markereket. Jelen vizsgálat egy olyan pályázat részeként valósult meg, amely a két faj megőrzését célozza a fajok hazai populációinak molekuláris genetikai vizsgálatával, a genetikai változatosságuk leírásával, filogenetikai kapcsolatrendszerük feltárásával. A 2018-2022-ig terjedő időszakban a projekt elérte fő célkitűzéseit, bár a Covid-19 világjárvány nagyban akadályozta munkánkat és komoly késéseket indukált a laboratóriumi vizsgálatok során.

A munka során először 7 vadon élő compó populáció 175 egyedének genetikai változatosságát és filogeográfiai szerkezetét vizsgáltuk annak érdekében, hogy a megőrzési munkához, illetve a szelekciós tenyésztés megkezdéséhez is megfelelő információkkal bírjunk. 12 mikroszatellit DNS marker (fragmensanalízissel vizsgálva), a mitokondriális Cytb gén egy 615 bázispár hosszúságú szakasza (szekvenálással vizsgálva), illetve az Act és RpS7 nukleáris gének (PCR-RFLP-vel vizsgálva) analízisével igyekeztünk a fent leírt céljainakt elérni. Minden mikroszetllit lókusz esetében közepes szintű polimorfizmust találtunk. Az egyes populációpárok közötti Fst értékek ugyancsak közepesek voltak, a populációk négy klaszterba sorolódtak. A Cytb gén esetében 20 haplotípust írtam le; az egyedek 67,1 %-a keleti, míg 32,9 %-a nyugati haplocsoportba tartozott. A Duna mindkét oldalán a keleti csoportba tartozó egyedek voltak túlsúlyban. Az Act és Rps7 nukleáris gének vizsgálatának eredményei nincsenek összhangban a Cytb gén vizsgálati eredményeivel. Az egyedek döntő többsége vagy a hibrid csoporthoz tartozik (50% az Act és 41% a Rps7gén esetében), vagy pedig a nyugati haplotípushoz (33% mindkét gén esetében. Ez a két csoport közötti hibridizáció magas szintjét mutatja a vizsgált területen és populációkban. A hazai compó populációk összességében kevésbé változatosak, mint a Nyugat-Európában élő természetes compó populációk, de így is jelentős genetikai erőforrásként tekinthetünk rájuk. A Fertő-tó, a Kolon-tó és a Csörnöc-herpenyő compó állományai jó alapul

szolgálhatnak jövőbeni szelekciós tenyésztési programokhoz. Ezen kívül elmondható, hogy a 2 fentebb említett EPIC (nuclear-encoded exon-primed intron-crossing) marker (Act és Rps7gén) megbízható eszköznek bizonyult a hazai compópopulációk filogeográfiai struktúrájának leírásához, a keleti és nyugati haplotípusok elkülönítéséhez.

A munka második fázisában 13 mikroszatellit DNS marker segítségével vizsgáltam 7 természetes vízi és négy farmokról származó széles kárász (Carassius carassius L. 1758) állomány 320 egyedének genetikai változatosságát. A vizsgált markerek nagyon változatosak voltak, összesen 245 allél jelenlétét írtam le a vizsgálat során. Az allélszámok az egyes populációkban 61 és 133 között változtak, míg az egyedi allélok száma 49 volt, frekvenciájuk pedig 0,016 és 0,250 között mozgott. Az egyedi allélek nagy többségét a természetes vízi populációkban találtam. A legalacsonyabb allélszámot (3) a GF1-es lókuszon találtam, míg a legmagassabat (75) a HLJYJ041 lókusz esetében írtam le. Az átlagos megfigyelt heterozigozitás és átlagos várt heterozigozitás az összes populációra vonatkoztatva 0,509 és 0,551 volt. A két érték a természetes vízi populációkban közel van egymáshoz, míg a farmokon tartott állományok esetében, a két érték jobban eltér egymástól, a Hardy-Weinberg egyensúly tesztelésére használt Khí-négyzet próba a 13-ból hat lókusz esetében mutatott ki szignifikánsan heterozigóta deficitet.

Összességében elmondható, hogy a különféle populációgenetikai paraméterek alapján, beleértve az allélgazdagságot, a várt és megfigyelt heterozigozitás értékeket a vadon élő, természets vízi széles kárász populációk magasabb genetikai változatossággal bírnak, mint a farmokon tartott állományok. A vizsgálat során az Fst érték 0,226 (95% CI: 0,137-0,340) volt. A populációpárok közötti Fst értékek az alacsonytól (0,014) a magas értékekig (0,455) változtak és jelentős struktúráltságot, valamint a populációk közöttis szignifikáns differenciálódást jeleztek.

A Bayesi modellen alapuló Structure analízis három klaszter jelenlétét valószínűsíti a nyolc vizsgált természetes vízi populáció esetében. Az AMOVA eredményei alapján kijelenthetjük, hogy a genetikai változatosság legnagyobb részét a populációkon belüli genetikai változatosság teszi ki. A fenti eredmények szerint a hazai természetes vízi széles kárász állományok közepes vagy erős genetikai változatossággal bírnak, míg a farm populációk genetikai változatossága alacsonyabb. Mindezek alapján kijelenthetjük, hogy fontos a természetes vízi széles kárász állományok védelme, mert fontos genetikai erőforrást jelentenek.

Végül munkám befejező részében előzetes vizsgálatokat végeztem két, a Carassius genusba tartozó faj filogenetikai kapcsolatainak felderítésének érdekében. A mitokondriális cytochrome c oxidase I (COI) gén szekvenciáit hasonlítottam össze 104 általam gyűjtött széles kárász és a GeneBank-ban talált széles kárász (Carassius carassius) és ezüstkárász (Carassius gibelio) esetében. A 104 COI szekvencia között 47 haplotípost találtam, melyek közül 43 haplotípus újnak bizonyult. Az új haplotípusok közül 5 az ezüstkárász fajhoz tartozik, míg a többit széles kárász haplotípusként azonosítottam. Az ezüstkárász haplotípusok jelenléte a széles kárász állományokban jelzi, hogy az invazív ezüstkárász változatlanul hibridizál a széles kárásszal, veszélyeztetve ezzel annak populációit. A Neighbor-Joining módszerrel készült filogenetikai fa reprezentálja, hogy a két fajból származó minták genetikai profilja jól elkülönül egymástól. Ugyanakkor a haplotípusok eltérő eloszlása lehetővé teszi, hogy a két fajt és hibridjeiket elkülönítsük egymástól.

10. APPENDICES

10.1 References

Aarts, B. G. W., Van Den Brink, F. W. B., & Nienhuis, P. H. (2004). Habitat loss as the main cause of the slow recovery of fish faunas of regulated large rivers in Europe: The transversal floodplain gradient: FLOODPLAIN FISH COMMUNITIES. *River Research and Applications*, *20*(1), 3–23. https://doi.org/10.1002/rra.720

Abdul Muneer, P. M., Gopalakrishnan, A., Musammilu, K. K., Basheer, V. S., Mohindra, V., Lal, K. K., Padmakumar, K. G., & Ponniah, A. G. (2012). Comparative Assessment of Genetic Variability in the Populations of Endemic and Endangered Yellow Catfish, Horabagrus brachysoma (Teleostei: Horabagridae), Based on Allozyme, RAPD, and Microsatellite Markers. *Biochemical Genetics*, *50*(3), 192–212. https://doi.org/10.1007/s10528-011-9462-4

Abdul-Muneer, P. M. (2014). Application of Microsatellite Markers in Conservation Genetics and Fisheries Management: Recent Advances in Population Structure Analysis and Conservation Strategies. *Genetics Research International*, *2014*, 1–11. https://doi.org/10.1155/2014/691759

Ablak Gurbuz, O. (2011). AGE AND REPRODUCTION FEATURES OF TENCH (Tinca tinca (L., 1758)) FROM HIRFANLI DAM LAKE, KIRŞEHIR, TURKEY. *Journal of FisheriesSciences.Com*. https://doi.org/10.3153/jfscom.2011019

Adams, S. L., Smith, J. F., Tervit, H. R., McGowan, L. T., Roberts, R. D., Janke, A. R., King, N. G., Gale, S. L., & Webb, S. C. (2011). Cryopreservation of molluscan sperm: Pacific oyster, green-lipped mussel, and Paua abalone. *Cryopreservation in Aquatic Species. Second. World Aquaculture Society*, 562–573.

Ahmed, N., Thompson, S., & Glaser, M. (2019). Global Aquaculture Productivity, Environmental Sustainability, and Climate Change Adaptability. *Environmental Management*, *63*(2), 159–172. https://doi.org/10.1007/s00267-018-1117-3

Aho, J., & Holopainen, I. J. (2000). Batch spawning of crucian carp (Carassius carassius (L.)) in mono- and multispecies communities. *Annales Zoologici Fennici*, *37*(2), 101–111.

Akçay, E., Bozkurt, Y., Seçer, S., & Tekin, N. (2004). Cryopreservation of mirror carp semen. *Turkish Journal of Veterinary and Animal Sciences*, *28*(5), 837–843.

Al Fatle, F. A., Meleg, E. E., Sallai, Z., Szabó, G., Várkonyi, E., Urbányi, B., Kovács, B., Molnár, T., & Lehoczky, I. (2022). Genetic Structure and Diversity of Native Tench (Tinca tinca L. 1758) Populations in Hungary—Establishment of Basic Knowledge Base for a Breeding Program. *Diversity*, *14*(5), 336. https://doi.org/10.3390/d14050336

Alam, M. S., Projna, F., Zafrin, Mst. S., Das, R., & Khan, M. G. Q. (2021). Assessment of genetic diversity, detection of strain-specific single nucleotide polymorphisms and identification of the Bangladesh and Vietnam strain of Channa striata by PCR-RFLP analysis of the mitochondrial COI gene fragment. *Aquaculture and Fisheries*. https://doi.org/10.1016/j.aaf.2020.12.006

Alaş, A., Altındağ, A., Yılmaz, M., Kırpık, M. A., & Ak, A. (2010). Feeding habits of tench (Tinca tinca L., 1758) in Beyşehir Lake (Turkey). *Turkish Journal of Fisheries and Aquatic Sciences*, *10*(2), 187–194.

Allendorf, F. W. (2017). Genetics and the conservation of natural populations: Allozymes to genomes. *Molecular Ecology*, *26*(2), 420–430. https://doi.org/10.1111/mec.13948

Allendorf, F. W., Berry, O., & Ryman, N. (2014). *So long to genetic diversity, and thanks for all the fish*. Wiley Online Library.

Allendorf, F. W., & Luikart, G. (2009). *Conservation and the Genetics of Populations*. John Wiley & Sons.

Allendorf, F. W., Ryman, N., & Utter, F. M. (1987). Genetics and fishery management. *Population Genetics and Fishery Management*, 1–19.

Andrews, K. R., Copus, J. M., Wilcox, C., Williams, A. J., Newman, S. J., Wakefield, C. B., & Bowen, B. W. (2020). Range-Wide Population Structure of 3 Deepwater Eteline Snappers Across the Indo-Pacific Basin. *Journal of Heredity*, *111*(5), 471–485. https://doi.org/10.1093/jhered/esaa029

Apalikova, O. V., Podlesnykh, A. V., Kukhlevsky, A. D., Guohua, S., & Brykov, V. A. (2011). Phylogenetic relationships of silver crusian carp Carassius auratus gibelio, C. auratus cuvieri, crucian carp Carassius carassius, and common carp Cyprinus carpio as inferred from mitochondrial DNA variation. *Russian Journal of Genetics*, *47*(3), 322–331.

April, J., Hanner, R. H., Dion-Côté, A.-M., & Bernatchez, L. (2013). Glacial cycles as an allopatric speciation pump in north-eastern American freshwater fishes. *Molecular Ecology*, *22*(2), 409–422. https://doi.org/10.1111/mec.12116

Apte, S., Star, B., & Gardner, J. P. (2003). A comparison of genetic diversity between cultured and wild populations, and a test for genetic introgression in the New Zealand greenshell mussel Perna canaliculus (Gmelin 1791). *Aquaculture*, *219*(1–4), 193–220.

Arslan, A., & Taki, F. N. (2012). C-banded karyotype and nucleolar organizer regions of Tinca tinca (Cyprinidae) from Turkey. *Caryologia*, *65*(3), 246–249. https://doi.org/10.1080/00087114.2012.740194

Askari, G., Shabani, A., & Paknejad -Previous Surname Kolangi Miandare, H. (2013). Application of molecular markers in fisheries and aquaculture A R T I C L E I N F O. *Animal Science*, *2*, 82–88.

Avise, J. C. (1994). *Molecular Markers, Natural History and Evolution*. Springer US. https://doi.org/10.1007/978-1-4615-2381-9

Avise, J. C., Bowen, B. W., & Ayala, F. J. (2016). In the light of evolution X: Comparative phylogeography. *Proceedings of the National Academy of Sciences*, *113*(29), 7957–7961.

Avise, J. C., & Ph.D, D. P. E. & E. B. J. C. A. (2000). *Phylogeography: The History and Formation of Species*. Harvard University Press.

Avlijaš, S., Ricciardi, A., & Mandrak, N. E. (2018). Eurasian tench (*Tinca tinca*): The next Great Lakes invader. *Canadian Journal of Fisheries and Aquatic Sciences*, *75*(2), 169–179. https://doi.org/10.1139/cjfas-2017-0171

Azad, M. A. K., Rahman, M. S., Rabbane, M. G., Kabir, M. A., Raknuzzaman, M., & Hossain, J. (2022). Genetic diversity of wild zebrafish Danio rerio populations available in Bangladesh. *Ecological Genetics and Genomics*, *23*, 100116.

Baack, E. J., & Rieseberg, L. H. (2007). A genomic view of introgression and hybrid speciation. *Current Opinion in Genetics & Development*, *17*(6), 513–518. https://doi.org/10.1016/j.gde.2007.09.001

Babiak, I., Glogowski, J., Brzuska, E., Szumiec, J., & Adamek, J. (1997). Cryopreservation of sperm of common carp, Cyprinus carpio L. *Aquaculture Research*, *28*(7), 567–571. https://doi.org/10.1046/j.1365-2109.1997.00897.x

Babiak, I., Glogowski, J., Kujawa, R., Kucharczyk, D., & Mamcarz, A. (1998). Cryopreservation of sperm from asp Aspius aspius. *The Progressive Fish-Culturist*, *60*(2), 146–148.

Baerwald, M. R., & May, B. (2004). Characterization of microsatellite loci for five members of the minnow family Cyprinidae found in the Sacramento-San Joaquin Delta and its tributaries. *Molecular Ecology Notes*, *4*(3), 385–390. https://doi.org/10.1111/j.1471-8286.2004.00661.x

Bakos, J. (1976). *Crossbreeding Hungarian races of common carp to develop more productive hybrids*. FAO Technical Conference on Aquaculture, Kyoto (Japan), 26 May 1976. https://agris.fao.org/agris-search/search.do?recordID=XF7602950

Bakos, J., Gorda, S., & Nations, F. and A. O. of the U. (2001). *Genetic Resources of Common Carp at the Fish Culture Research Institute, Szarvas, Hungary*. Food & Agriculture Org.

Bandelt, H. J., Forster, P., & Rohl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, *16*(1), 37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Barange, M. (2018). *Impacts of climate change on fisheries and aquaculture: Synthesis of current knowledge, adaptation and mitigation options*. http://www.fao.org/3/i9705en/i9705en.pdf

Bart, A. (2002). *Conservation of Fish Genetic Diversity: Need for Development of a Cryogenic Genebank in Bangladesh*.

Bartley, D. M. (1998). Ex Situ conservation, gene banks, and responsible fisheries. *BEFORE*, *16*, 45.

Başkurt, S., Vatan, P., emiroğlu, Ö., & Aksu, S. (2020). *Gynogenetic Reproduction Potential of Invasive Carassius gibelio in Ula Pond*. *14*, 8–10.

Bechev. (2015). *Two new species of Tipulidae (Diptera) to the fauna of Bulgaria*.

Bernáth, G., Żarski, D., Kása, E., Staszny, Á., Várkonyi, L., Kollár, T., Hegyi, Á., Bokor, Z., Urbányi, B., & Horváth, Á. (2016). Improvement of common carp (Cyprinus carpio) sperm cryopreservation using a programable freezer. *General and Comparative Endocrinology*, *237*, 78–88. https://doi.org/10.1016/j.ygcen.2016.08.013

Bernos, T. A., Avlijaš, S., Hill, J., Morissette, O., Ricciardi, A., Mandrak, N. E., & Jeffries, K. M. (2022). Spatiotemporal changes in genetic diversity and structure of a recent fish invasion in eastern North America. *BioRxiv*.

Bhat, A. A., Haniffa, M. A., Divya, P. R., Gopalakrishnan, A., Milton, M. J., Kumar, R., & Paray, B. A. (2012). Molecular characterization of eight Indian Snakehead species (Pisces: Perciformes Channidae) using RAPD markers. *Molecular Biology Reports*, *39*(4), 4267–4273. https://doi.org/10.1007/s11033-011-1213-4

Billard, R., & Flajshans, M. (1995). The current state of research and culture of tench, Tinca tinca Linnaeus [1758]. Concluding remarks on the workshop. *Polskie Archiwum Hydrobiologii*, *42*(1–2).

Billington, N. (2003). Population genetics: Principles and applications for fisheries scientists, Bethesda, Maryland. *American Fisheries Society*, 59–100.

Birky Jr, C. W., Fuerst, P., & Maruyama, T. (1989). Organelle gene diversity under migration, mutation, and drift: Equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. *Genetics*, *121*(3), 613–627.

Biswal, J. R., Singh, R. K., Lal, K. K., Mohindra, V., Kumar, R., Kumar, R. G., Basheer, V. S., & Jena, J. K. (2018). Molecular and morphological evidences resolve taxonomic ambiguity between Systomus sarana sarana (Hamilton, 1822) and S. sarana subnasutus (Valenciennes) and suggest elevating them into distinct species. *Mitochondrial DNA Part B*, *3*(2), 838–844. https://doi.org/10.1080/23802359.2018.1481775

Black IV, W. C., Baer, C. F., Antolin, M. F., & DuTeau, N. M. (2001). P OPULATION G ENOMICS: Genome-Wide Sampling of Insect Populations. *Annual Review of Entomology*, *46*(1), 441–469. https://doi.org/10.1146/annurev.ento.46.1.441

Blacket, M. J., Robin, C., Good, R. T., Lee, S. F., & Miller, A. D. (2012). Universal primers for fluorescent labelling of PCR fragments—An efficient and cost-effective approach to genotyping by fluorescence. *Molecular Ecology Resources*, *12*(3), 456–463. https://doi.org/10.1111/j.1755-0998.2011.03104.x

Blackwell, T., Ford, A. G. P., Ciezarek, A. G., Bradbeer, S. J., Juarez, C. A. G., Smith, A. M., Ngatunga, B. P., Shechonge, A., Tamatamah, R., Etherington, G., Haerty, W., Palma, F. D., Turner, G. F., & Genner, M. J. (2021). Newly discovered cichlid fish biodiversity threatened by hybridization with non-native species. *Molecular Ecology*, *30*(4), 895–911. https://doi.org/10.1111/mec.15638

Boecklen, W. J., & Howard, D. J. (1997). GENETIC ANALYSIS OF HYBRID ZONES: NUMBERS OF MARKERS AND POWER OF RESOLUTION. *Ecology*, *78*(8), 2611–2616. https://doi.org/10.1890/0012-9658 (1997)078[2611: GAOHZN] 2.0.CO; 2

Bokor, Z., Bernáth, G., Várkonyi, L., Molnár, J., Láng, Z. L., Tarnai-Király, Z., Solymosi, E., & Urbányi, B. (2019). The applicability of large-scale sperm cryopreservation in wels catfish (Silurus glanis) optimized for hatchery practice. *Aquaculture*, *506*, 337–340. https://doi.org/10.1016/j.aquaculture.2019.03.064

Bondad-Reantaso, M. G., Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, Rome, Italy, Mackinnon, B., Bin, H., Jie, H., Tang-Nelson, K., & Surachetpong, W. (2020). Viewpoint: SARS-CoV-2 (The Cause of COVID-

19 in Humans) is Not Known to Infect Aquatic Food Animals nor Contaminate Their Products. *Asian Fisheries Science*. https://doi.org/10.33997/j.afs.2020.33.1.009

Borowsky, R., & Wilkens, H. (2002). Mapping a cave fish genome: Polygenic systems and regressive evolution. *Journal of Heredity*, *93*(1), 19–21.

Boryshpolets, S., Sochorová, D., Rodina, M., Linhart, O., & Dzyuba, B. (2017). Cryopreservation of carp (Cyprinus carpio L.) sperm: Impact of seeding and freezing rates on post–thaw outputs. *Biopreservation and Biobanking*, *15*(3), 234–240.

Bozkurt, Y., Yavaş, İ., Bucak, M. N., & Yeni, D. (2019). Effect of different cryoprotectants (glycerol, methanol and dimethyl sulfoxide) on post-thaw quality, viability, fertilization ability and DNA damage of cryopreserved Nile tilapia (Oreochromis niloticus) spermatozoa. *CryoLetters*, *40*(1), 11–17.

Brönmark, C., & Miner, J. G. (1992). Predator-Induced Phenotypical Change in Body Morphology in Crucian Carp. *Science*, *258*(5086), 1348–1350. https://doi.org/10.1126/science.258.5086.1348

Brown, W. M. (1985). The mitochondrial genome of animals. In "Molecular Evolutionary Genetics"(RJ MacIntyre, Ed.). *Plenum Press, New York. Clary, DO, and Wolstenholme, DR (1985). The Mitochondrial DNA Molecule of Drosophila Yakuba: Nucleotide Sequence, Gene Organization, and Genetic Code. J. Mol. Evol*, *22*, 252–271.

Brylinska, M., Brylinski, E., & Bninska, M. (1999). Tinca tinca (Linnaeus, 1758). *The Freshwater Fishes of Europe*, *5*(1), 225–228.

Buchtová, H., Svobodová, Z., Flajšhans, M., & Vorlová, L. (2003a). Analysis of slaughtering value of diploid and triploid population of tench (Tinca tinca, Linnaeus 1758). *Czech Journal of Animal Science*, *48*(7), 285–294.

Buchtová, H., Svobodová, Z., Flajšhans, M., & Vorlová, L. (2003b). Analysis of growth, weight and relevant indices of diploid and triploid population of tench *Tinca tinca* (Linnaeus 1758): Differences between diploid and triploid populations of tench. *Aquaculture Research*, *34*(9), 719–726. https://doi.org/10.1046/j.1365-2109.2003.00874.x

Buscemi, S., Nicolucci, A., Lucisano, G., Galvano, F., Grosso, G., Belmonte, S., Sprini, D., Migliaccio, S., Cianferotti, L., Brandi, M. L., & Rini, G. B. (2014). Habitual fish intake and clinically silent carotid atherosclerosis. *Nutrition Journal*, *13*(1), 2. https://doi.org/10.1186/1475-2891-13-2

Cambray, J. A. (2003). Impact on indigenous species biodiversity caused by the globalisation of alien recreational freshwater fisheries. *Hydrobiologia*, *500*(1), 217–230. https://doi.org/10.1023/A:1024648719995

Campbell, D., & Bernatchez, L. (2004). Generic scan using AFLP markers as a means to assess the role of directional selection in the divergence of sympatric whitefish ecotypes. *Molecular Biology and Evolution*, *21*(5), 945–956.

Canfield, S. J., Galván-Magaña, F., & Bowen, B. W. (2022). Little Sharks in a Big World: Mitochondrial DNA Reveals Small-scale Population Structure in the California Horn

Shark (Heterodontus francisci). *Journal of Heredity*, esac008. https://doi.org/10.1093/jhered/esac008

Cavalli-Sforza, L. L., & Edwards, A. W. (1967). Phylogenetic analysis. Models and estimation procedures. *American Journal of Human Genetics*, *19*(3 Pt 1), 233.

Celada, J. D., Aguilera, A., Carral, J. M., Sáez-Royuela, M., Melendre, P. M., & Pérez, J. R. (2007). Effects of stocking density on survival and growth of juvenile tench (Tinca tinca L.). *Aquaculture International*, *15*(6), 461–465. https://doi.org/10.1007/s10499-007-9111-4

Chapuis, M.-P., & Estoup, A. (2007). Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, *24*(3), 621–631.

Chaturvedi, A., Mohindra, V., Singh, R. K., Lal, K. K., Punia, P., Bhaskar, R., Mandal, A., Narain, L., & Lakra, W. S. (2011). Population genetic structure and phylogeography of cyprinid fish, Labeo dero (Hamilton, 1822) inferred from allozyme and microsatellite DNA marker analysis. *Molecular Biology Reports*, *38*(5), 3513–3529. https://doi.org/10.1007/s11033-010-0462-y

Chauhan, T., Lal, K. K., Mohindra, V., Singh, R. K., Punia, P., Gopalakrishnan, A., Sharma, P. C., & Lakra, W. S. (2007). Evaluating genetic differentiation in wild populations of the Indian major carp, Cirrhinus mrigala (Hamilton–Buchanan, 1882): Evidence from allozyme and microsatellite markers. *Aquaculture*, *269*(1), 135–149. https://doi.org/10.1016/j.aquaculture.2007.05.007

Chen, Z., Hu, C., Wang, L., Chen, G., Wang, J., Huang, H., He, A., Wang, C., & Zhao, W. (2014). Microsatellite-based genetic structure and differentiation of goldfish (Carassius auratus) with sarcoma. *Open Journal of Animal Sciences*, *5*(01), 36.

Chow, S., & Hazama, K. (1998). Universal PCR primers for S7 ribosomal protein gene introns in fish. *Molecular Ecology*, *7*(9), 1255–1256.

Chow, S., & Kishino, H. (1995). Phylogenetic relationships between tuna species of the genus Thunnus (Scombridae: Teleostei): inconsistent implications from morphology, nuclear and mitochondrial genomes. *Journal of Molecular Evolution*, *41*(6), 741–748.

Chow, S., Suzuki, N., Imai, H., & Yoshimura, T. (2006). Molecular species identification of spiny lobster phyllosoma larvae of the genus Panulirus from the northwestern Pacific. *Marine Biotechnology*, *8*(3), 260–267.

Ćirković, M., Ljubojević, D., \DJor\djević, V., Novakov, N., Petronijević, R., Matekalo-Sverak, V., & Trbović, D. (2012). The breed effect on productivity and meat nutrient compsition of fish. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, *18*(5), 75–780.

Clavelle, T. (2020, May 12). Global Fisheries During COVID-19. *Global Fishing Watch*. https://globalfishingwatch.org/data/global-fisheries-during-covid-19/

Clavero, M. (2019). Beyond one bone: Interdisciplinarity to assess nativeness of the tench (Tinca tinca) in Spain. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *29*(11), 1863–1869. https://doi.org/10.1002/aqc.3184

Clout, M. N., & De Poorter, M. (2005). International initiatives against invasive alien species. *Weed Technology*, *19*(3), 523–527.

Clusa, L., Ardura, A., Fernández, S., Roca, A. A., & García-Vázquez, E. (2017). An extremely sensitive nested PCR-RFLP mitochondrial marker for detection and identification of salmonids in eDNA from water samples. *PeerJ*, *5*, e3045. https://doi.org/10.7717/peerj.3045

Coimbra, M. R. M., Dantas, H. L., Luna, M. M. S., Lima, M. A., Sales, M., Silva, B. C. N. R. da, & Lima, A. P. S. (2020). High gene flow in two migratory Neotropical fish species, Salminus franciscanus and Brycon orthotaenia, and implications for conservation aquaculture. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(6), 1063–1073. https://doi.org/10.1002/aqc.3336

Copp, G. H. (1991). Typology of aquatic habitats in the great ouse, a small regulated lowland river. *Regulated Rivers: Research & Management*, *6*(2), 125–134. https://doi.org/10.1002/rrr.3450060208

Copp, G. H., Černý, J., & Kováč, V. (2008). Growth and morphology of an endangered native freshwater fish, crucian carp Carassius carassius, in an English ornamental pond. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *18*(1), 32–43. https://doi.org/10.1002/aqc.820

Copp, G. H., & Sayer, C. D. (2010). Norfolk Biodiversity Action Plan–Local species action plan for crucian carp (Carassius carassius). Norfolk Biodiversity Partnership Reference: LS/3. Centre for Environment. *Fisheries & Aquaculture Science, Lowestoft*.

Copp, G. H., & Sayer, C. D. (2020). Demonstrating the practical impact of publications in Aquatic Conservation–The case of crucian carp Carassius carassius in the East of England. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(9), 1753–1757.

Copp, G. H., Vilizzi, L., Mumford, J., Fenwick, G. V., Godard, M. J., & Gozlan, R. E. (2009). Calibration of FISK, an Invasiveness Screening Tool for Nonnative Freshwater Fishes. *Risk Analysis*, *29*(3), 457–467. https://doi.org/10.1111/j.1539-6924.2008.01159.x

Copp, G. H., Wesley, K. J., & Vilizzi, L. (2005). Pathways of ornamental and aquarium fish introductions into urban ponds of Epping Forest (London, England): The human vector*. *Journal of Applied Ichthyology*, *21*(4), 263–274. https://doi.org/10.1111/j.1439-0426.2005.00673.x

Copp, G., Tarkan, S., Godard, M., Edmonds, N., & Wesley, K. (2010). Preliminary assessment of feral goldfish impacts on ponds, with particular reference to native crucian carp. *Aquatic Invasions*, *5*(4), 413–422. https://doi.org/10.3391/ai.2010.5.4.11

Crivelli, A. J. (1995). Are fish introductions a threat to endemic freshwater fishes in the northern Mediterranean region? *Biological Conservation*, *72*(2), 311–319. https://doi.org/10.1016/0006-3207 (94)00092-5

Crooijmans, R. P. M. A., Poel, J. J. V. der, Groenen, M. a. M., Bierbooms, V. a. F., & Komen, J. (1997). Microsatellite markers in common carp (Cyprinus carpio L.). *Animal Genetics*, *28*(2), 129–134. https://doi.org/10.1111/j.1365-2052.1997.00097.x

de los Angeles Barriga-Sosa, I., Pérez-Ramírez, M. Y., Soto-Aguirre, F., Castillo-Rivera, M., & Arredondo-Figueroa, J. L. (2005). Inter-specific variation of the mitochondrial r16S gene among silversides,“Peces Blancos”,(Atherinopsidae: Menidiinae) and its utilization for species identification. *Aquaculture*, *250*(3–4), 637–651.

DeCovich, N. A., Chenoweth, E. L., Dann, T. H., Fox, E. K., Habicht, C., Jasper, J. R., Liller, H. L., Olive, S. D. R., & Templin, W. D. (2012). *Chum salmon baseline for the Western Alaska Salmon Stock Identification Program*. Alaska Department of Fish and Game, Division of Sport Fish, Research and ….

DeVaney, S. C., McNyset, K. M., Williams, J. B., Peterson, A. T., & Wiley, E. O. (2009). A Tale of Four “Carp”: Invasion Potential and Ecological Niche Modeling. *PLoS ONE*, *4*(5), e5451. https://doi.org/10.1371/journal.pone.0005451

DeWoody, J. A., & Avise, J. C. (2000). Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *Journal of Fish Biology*, *56*(3), 461–473. https://doi.org/10.1111/j.1095-8649.2000.tb00748.x

Diamante, N. A., de Oliveira, A. V., Petry, A. C., Catelani, P. A., Pelicice, F. M., Mueller, L. P., Prioli, S. M., & Prioli, A. J. (2021). Genomic markers confirm introgressive hybridization in Cichla (Teleostei) in an invaded coastal system. *International Review of Hydrobiology*, *106*(1), 48–57.

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. (2014). NeEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular Ecology Resources*, *14*(1), 209–214. https://doi.org/10.1111/1755-0998.12157

Dodgson, J. B., Cheng, H. H., & Okimoto, R. (1997). DNA marker technology: A revolution in animal genetics. *Poultry Science*, *76*(8), 1108–1114.

Dueñas, M.-A., Ruffhead, H. J., Wakefield, N. H., Roberts, P. D., Hemming, D. J., & Diaz-Soltero, H. (2018). The role played by invasive species in interactions with endangered and threatened species in the United States: A systematic review. *Biodiversity and Conservation*, *27*(12), 3171–3183. https://doi.org/10.1007/s10531-018-1595-x

Dulski, T., Kozłowski, K., & Ciesielski, S. (2020). Habitat and seasonality shape the structure of tench (Tinca tinca L.) gut microbiome. *Scientific Reports*, *10*(1), 4460. https://doi.org/10.1038/s41598-020-61351-1

Dunham, R. A. (2011). *Aquaculture and fisheries biotechnology: Genetic approaches*. Cabi.

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359–361. https://doi.org/10.1007/s12686-011-9548-7

Edwards, A., Civitello, A., Hammond, H. A., & Caskey, C. T. (1991). DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *American Journal of Human Genetics*, *49*(4), 746.

Edwards, Y. J., Elgar, G., Clark, M. S., & Bishop, M. J. (1998). The identification and characterization of microsatellites in the compact genome of the Japanese pufferfish, Fugu

rubripes: Perspectives in functional and comparative genomic analyses. *Journal of Molecular Biology*, *278*(4), 843–854.

Eisner, T., Lubchenco, J., Wilson, E. O., Wilcove, D. S., & Bean, M. J. (1995). Building a Scientifically Sound Policy for Protecting Endangered Species. *Science*, *269*(5228), 1231–1232. https://doi.org/10.1126/science.269.5228.1231

Elameen, A., Stueland, S., Kristensen, R., Fristad, R. F., Vrålstad, T., & Skaar, I. (2021). Genetic analyses of saprolegnia strains isolated from salmonid fish of different geographic origin document the connection between pathogenicity and molecular diversity. *Journal of Fungi*, *7*(9), 713.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. *Molecular Ecology*, *14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Facon, B., Jarne, P., Pointier, J. P., & David, P. (2005). Hybridization and invasiveness in the freshwater snail Melanoides tuberculata: Hybrid vigour is more important than increase in genetic variance: Hybrid vigour in an invasive snail. *Journal of Evolutionary Biology*, *18*(3), 524–535. https://doi.org/10.1111/j.1420-9101.2005.00887.x

Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of Population Structure Using Multilocus Genotype Data: Linked Loci and Correlated Allele Frequencies. *Genetics*, *164*(4), 1567–1587.

FAO. (2018). *The State of World Fisheries and Aquaculture 2018: Meeting the sustainable development goals*. FAO. https://www.fao.org/documents/card/en/c/I9540EN/

FAO. (2020a). *How is COVID-19 affecting the fisheries and aquaculture food systems*. FAO. https://doi.org/10.4060/ca8637en

FAO. (2020b). *Summary of the impacts of the COVID-19 pandemic on the fisheries and aquaculture sector: Addendum to the State of World Fisheries and Aquaculture 2020*. FAO. https://doi.org/10.4060/ca9349en

FAO. (2020c). *The State of World Fisheries and Aquaculture 2020: Sustainability in action*. FAO. https://doi.org/10.4060/ca9229enAlso Available in: Arabic Russian French Spanish Chinese

Fatsi, P. S. K., Hashem, S., Kodama, A., Appiah, E. K., Saito, H., & Kawai, K. (2020). Population genetics and taxonomic signatures of wild Tilapia in Japan based on mitochondrial DNA control region analysis. *Hydrobiologia*, *847*(6), 1491–1504. https://doi.org/10.1007/s10750-020-04203-3

Faulks, L. K., Kerezsy, A., Unmack, P. J., Johnson, J. B., & Hughes, J. M. (2017). Going, going, gone? Loss of genetic diversity in two critically endangered Australian freshwater fishes, Scaturiginichthys vermeilipinnis and Chlamydogobius squamigenus, from Great Artesian Basin springs at Edgbaston, Queensland, Australia. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *27*(1), 39–50.

Feledi, T., Gyalog, G., Rónyai, A., & Varadi, L. (2013). Hungarian Aquaculture. *Fish Farmers Magazine*.

Ferguson, A., Taggart, J. B., Prodöhl, P. A., McMeel, O., Thompson, C., Stone, C., McGinnity, P., & Hynes, R. A. (1995). The application of molecular markers to the study and conservation of fish populations, with special reference to Salmo. *Journal of Fish Biology*, *47*(sA), 103–126. https://doi.org/10.1111/j.1095-8649.1995.tb06048.x

Ferincz, Á., Staszny, Á., Urbányi, B., Czeglédi, I., Erős, T., Specziár, A., Vitál, Z., Weiperth, A., Sály, P., & Takács, P. (2018, May 24). The distribution patterns, risk and potential effect of non-indigenous fish species of Hungarian waters. *Proceedings of the 5th European Congress of Conservation Biology*. 5th European Congress of Conservation Biology, Jyväskylä, Finland. https://doi.org/10.17011/conference/eccb2018/108097

Firmat, C., Alibert, P., Losseau, M., Baroiller, J.-F., & Schliewen, U. K. (2013). Successive Invasion-Mediated Interspecific Hybridizations and Population Structure in the Endangered Cichlid Oreochromis mossambicus. *PLoS ONE*, *8*(5), e63880. https://doi.org/10.1371/journal.pone.0063880

Fitzpatrick, B. M., Johnson, J. R., Kump, D. K., Smith, J. J., Voss, S. R., & Shaffer, H. B. (2010). Rapid spread of invasive genes into a threatened native species. *Proceedings of the National Academy of Sciences*, *107*(8), 3606–3610.

Flajšhans, M., Gela, D., Kocour, M., Buchtová, H., Rodina, M., Pšenička, M., Kašpar, V., Piačková, V., Sudová, E., & Linhart, O. (2010). A review on the potential of triploid tench for aquaculture. *Reviews in Fish Biology and Fisheries*, *20*(3), 317–329. https://doi.org/10.1007/s11160-009-9144-z

FLORESCU, L., CATANĂ, R., GAVRILIDIS, A.-A., & MOLDOVEANU, M. (2018). THE IMPACT OF INVASIVE SPECIES ON URBAN ECOSYSTEMS. *Oltenia Journal for Studies in Natural Sciences*, *34*(1), 212–217.

Franěk, R., Marinović, Z., Lujić, J., Urbányi, B., Fučíková, M., Kašpar, V., Pšenička, M., & Horváth, Á. (2019). Cryopreservation and transplantation of common carp spermatogonia. *PLOS ONE*, *14*(4), e0205481. https://doi.org/10.1371/journal.pone.0205481

Freitas, M. V. de, Ariede, R. B., Hata, M. E., Mastrochirico-Filho, V. A., Pazo, F. D., Villanova, G. V., Mendonça, F. F., Porto-Foresti, F., Hashimoto, D. T., Freitas, M. V. de, Ariede, R. B., Hata, M. E., Mastrochirico-Filho, V. A., Pazo, F. D., Villanova, G. V., Mendonça, F. F., Porto-Foresti, F., & Hashimoto, D. T. (2021). Haplotypes traceability and genetic variability of the breeding population of pacu (Piaractus mesopotamicus) revealed by mitochondrial DNA. *Genetics and Molecular Biology*, *44*(1). https://doi.org/10.1590/1678-4685-gmb-2020-0249

Galal-Khallaf, A., Mohammed-Geba, K., Osman, A. G. M., AbouelFadl, K. Y., Borrell, Y. J., & Garcia-Vazquez, E. (2017). SNP-based PCR-RFLP, T-RFLP and FINS methodologies for the identification of commercial fish species in Egypt. *Fisheries Research*, *185*, 34–42. https://doi.org/10.1016/j.fishres.2016.09.031

Gaston, K. J., & Fuller, R. A. (2009). The sizes of species' geographic ranges. *Journal of Applied Ecology*, *46*(1), 1–9. https://doi.org/10.1111/j.1365-2664.2008.01596.x

Gela, D., Flajšhans, M., Kocour, M., Rodina, M., & Linhart, O. (2006). Tench (Tinca tinca) broodstock management in breeding station under conditions of pond culture: A review. *Aquaculture International*, *14*(1–2), 195–203. https://doi.org/10.1007/s10499-005-9025-y

Gharbi, K., Gautier, A., Danzmann, R. G., Gharbi, S., Sakamoto, T., Høyheim, B., Taggart, J. B., Cairney, M., Powell, R., & Krieg, F. (2006). A linkage map for brown trout (Salmo trutta): Chromosome homeologies and comparative genome organization with other salmonid fish. *Genetics*, *172*(4), 2405–2419.

Gorda, S., Bakos, J., Liska, J., & Kakuk, Cs. (1995). Live gene bank of common carp strains at the Fish Culture Research Institute, Szarvas. *Aquaculture*, *129*(1–4), 199–202. https://doi.org/10.1016/0044-8486 (94)00248-M

Goryunova, A. I., & Skakun, D. (2002). Biological characterization on crucian carps. *Tethys Aqua Zoological Research*, *1*, 33–48.

Gray, R. H., & Dauble, D. D. (2001). *Some life history characteristics of cyprinids in the Hanford Reach, Mid-Columbia River*.

Grosso, G., Galvano, F., Marventano, S., Malaguarnera, M., Bucolo, C., Drago, F., & Caraci, F. (2014, March 18). *Omega-3 Fatty Acids and Depression: Scientific Evidence and Biological Mechanisms* [Review Article]. Oxidative Medicine and Cellular Longevity; Hindawi. https://doi.org/10.1155/2014/313570

Gu, Q., Wang, S., Zhong, H., Yuan, H., Yang, J., Yang, C., Huang, X., Xu, X., Wang, Y., & Wei, Z. (2022). Phylogeographic relationships and the evolutionary history of the Carassius auratus complex with a newly born homodiploid raw fish (2nNCRC). *BMC Genomics*, *23*(1), 1–19.

Gubiani, É. A., Ruaro, R., Ribeiro, V. R., Eichelberger, A. C. A., Bogoni, R. F., Lira, A. D., Cavalli, D., Piana, P. A., & da Graça, W. J. (2018). Non-native fish species in Neotropical freshwaters: How did they arrive, and where did they come from? *Hydrobiologia*, *817*(1), 57–69. https://doi.org/10.1007/s10750-018-3617-9

Guo, S. W., & Thompson, E. A. (1992). Performing the Exact Test of Hardy-Weinberg Proportion for Multiple Alleles. *Biometrics*, *48*(2), 361. https://doi.org/10.2307/2532296

Guo, W., & Gui, J.-F. (2008). Microsatellite marker isolation and cultured strain identification in Carassius auratus gibelio. *Aquaculture International*, *16*(6), 497–510. https://doi.org/10.1007/s10499-007-9161-7

Gyalog, G., Oláh, J., Békefi, E., Lukácsik, M., & Popp, J. (2017). Constraining Factors in Hungarian Carp Farming: An Econometric Perspective. *Sustainability*, *9*(11), 2111. https://doi.org/10.3390/su9112111

Hakim, M., & Ahmad, S. (2017). Advances in Molecular Markers and Their Applications in Aquaculture and Fisheries. *Genetics of Aquatic Organisms*, *17*. https://doi.org/10.4194/2459-1831-v1_1_05

Hammer, M. P., Unmack, P. J., Adams, M., Johnson, J. B., & Walker, K. F. (2010). Phylogeographic structure in the threatened Yarra pygmy perch Nannoperca obscura

(Teleostei: Percichthyidae) has major implications for declining populations. *Conservation Genetics*, *11*(1), 213–223.

Hänfling, B., Bolton, P., Harley, M., & Carvalho, G. R. (2005). A molecular approach to detect hybridisation between crucian carp (Carassius carassius) and non-indigenous carp species (Carassius spp. And Cyprinus carpio). *Freshwater Biology*, *50*(3), 403–417. https://doi.org/10.1111/j.1365-2427.2004.01330.x

Hanfling, B., Harley, M., & Environment Agency. (2003). *A molecular approach to detect hybridisation between crucian carp (Carassius carassius) and non indigenous carp species (Carassius auratus and Cyprinus carpio) in UK waters, including a consideration of the taxonomic status of gibel carp (Carassius spp.).*

Hanner, R., Floyd, R., Bernard, A., Collette, B. B., & Shivji, M. (2011). DNA barcoding of billfishes. *Mitochondrial DNA*, *22*(sup1), 27–36. https://doi.org/10.3109/19401736.2011.596833

Hansen, M. M. (2003). *Application of molecular markers in population and conservation genetics, with special emphasis on fishes*.

Hansen, M. M., Kenchington, E., & Nielsen, E. E. (2001). Assigning individual fish to populations using microsatellite DNA markers: Assignment methods using microsatellites. *Fish and Fisheries*, *2*(2), 93–112. https://doi.org/10.1046/j.1467-2960.2001.00043.x

Harvey, B. (1998). An overview of action before extinction. *BEFORE*, *16*, 1.

Hasan, I., & Goswami, M. M. (2015). Genetic variation among cat fish (Mystus vittatus) population assessed by randomly amplified polymorphic (RAPD) markers from Assam, India. *Journal of Aquaculture Research and Development*, *6*(4). https://www.cabdirect.org/cabdirect/abstract/20153219732

Hashimoto, D. T., Prado, F. D., Foresti, F., Porto-Foresti, F., Hashimoto, D. T., Prado, F. D., Foresti, F., & Porto-Foresti, F. (2016). Molecular identification of intergenus crosses involving catfish hybrids: Risks for aquaculture production. *Neotropical Ichthyology*, *14*(2). https://doi.org/10.1590/1982-0224-20150139

Hayden, B., Pulcini, D., Kelly-Quinn, M., O'Grady, M., Caffrey, J., McGrath, A., & Mariani, S. (2010). Hybridisation between two cyprinid fishes in a novel habitat: Genetics, morphology and life-history traits. *BMC Evolutionary Biology*, *10*(1), 169. https://doi.org/10.1186/1471-2148-10-169

Haynes, G. D., Gongora, J., Gilligan, D. M., Grewe, P., Moran, C., & Nicholas, F. W. (2012). Cryptic hybridization and introgression between invasive Cyprinid species Cyprinus carpio and Carassius auratus in Australia: Implications for invasive species management. *Animal Conservation*, *15*(1), 83–94. https://doi.org/10.1111/j.1469-1795.2011.00490.x

Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1512), 313–321. https://doi.org/10.1098/rspb.2002.2218

Helfman, G. S. (2007). *Fish Conservation: A Guide to Understanding and Restoring Global Aquatic Biodiversity and Fishery Resources*. Island Press.

Herborg, L.-M., Mandrak, N. E., Cudmore, B. C., & MacIsaac, H. J. (2007). Comparative distribution and invasion risk of snakehead (Channidae) and Asian carp (Cyprinidae) species in North America. *Canadian Journal of Fisheries and Aquatic Sciences*, *64*(12), 1723–1735.

Hesthagen, T., & Sandlund, O. T. (2007). Non-native freshwater fishes in Norway: History, consequences and perspectives. *Journal of Fish Biology*, *71*(sd), 173–183. https://doi.org/10.1111/j.1095-8649.2007.01676.x

HEWITT, G. M. (1999). Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*, *68*(1–2), 87–112. https://doi.org/10.1111/j.1095-8312.1999.tb01160.x

Hewitt, G. M. (2004). Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *359*(1442), 183–195. https://doi.org/10.1098/rstb.2003.1388

Hickley, P., & Chare, S. (2004). Fisheries for non-native species in England and Wales: Angling or the environment? *Fisheries Management and Ecology*, *11*(3–4), 203–212. https://doi.org/10.1111/j.1365-2400.2004.00395.x

Hiemstra, S. J. (2005). Cryopreservation strategies for farm animals. *International Workshop "Options and Strategies for the Conservation of Farm Animal Genetic Resources,"* 78.

Higgins, C. L., Love-Snyder, A., Wiegreffe, W., & Pfau, R. S. (2015). Lack of hybridization between naturally sympatric populations of red and blacktail shiner (Cyprinella lutrensis and C. venusta) in Texas, but evidence of introgression among three lineages of the C. lutrensis species group. *Copeia*, *103*(2), 272–280.

Hillis, D. M., Moritz, C., Mable, B. K., & Olmstead, R. G. (1996). *Molecular systematics* (Vol. 23). Sinauer Associates Sunderland, MA.

Holopainen, I. J., Hyvärinen, H., & Piironen, J. (1986). Anaerobic wintering of crucian carp (Carassius carassius L.)—II. Metabolic products. *Comparative Biochemistry and Physiology Part A: Physiology*, *83*(2), 239–242. https://doi.org/10.1016/0300-9629(86)90568-2

Holopainen, I. J., Tonn, W. M., & Paszkowski, C. A. (1997). Tales of two fish: The dichotomous biology of crucian carp (Carassius carassius (L.)) in northern Europe. *Annales Zoologici Fennici*, 1–22.

Horv√°th, √Å, & Urb√°nyi, B. (2008). Cryopreservation of Common Carp Sperm. In E. Cabrita, V. Robles, & P. Herr√°ez (Eds.), *Methods in Reproductive Aquaculture* (Vol. 20083251, pp. 351–356). CRC Press. https://doi.org/10.1201/9780849380549.ch15

Horváth, Á., Miskolczi, E., & Urbányi, B. (2003). Cryopreservation of common carp sperm. *Aquatic Living Resources*, *16*(5), 457–460. https://doi.org/10.1016/S0990-7440(03)00084-6

Horvath, A. (Szent I. E., & Urbanyi, B. (Szent I. E. (2000). Preliminary results of Cyprinidae and Perciade sperm cryopreservation. *Fisheries Development Hungary*. XXIV. Halaszati Tudomanyos Tanacskozas, Szarvas (Hungary), May 2000. https://agris.fao.org/agris-search/search.do?recordID=HU2002000349

Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N., & Vellend, M. (2008). Ecological consequences of genetic diversity: Ecological effects of genetic diversity. *Ecology Letters*, *11*(6), 609–623. https://doi.org/10.1111/j.1461-0248.2008.01179.x

Ikpeme, E., Udensi, O., Ekaluo, U., Kooffreh, M., Okolo, C. M., Ekpo, P., & Ogbonna, N. C. (2015). Unveiling the Genetic Diversity in Clarias gariepinus (Burchell, 1822) Using Random Amplified Polymorphic DNA (RAPD) Fingerprinting Technique. *Asian Journal of Animal Sciences*, *9*, 187–197. https://doi.org/10.3923/ajas.2015.187.197

Innal, D. (2011). Distribution and impacts of Carassius species (Cyprinidae) in Turkey: A review. *Management of Biological Invasions*, *2*(1), 57–68. https://doi.org/10.3391/mbi.2011.2.1.06

Innal, D., & Erk'akan, F. (2006). Effects of exotic and translocated fish species in the inland waters of Turkey. *Reviews in Fish Biology and Fisheries*, *16*(1), 39–50. https://doi.org/10.1007/s11160-006-9005-y

Irawan, H., Vuthiphandchai, V., & Nimrat, S. (2010). The effect of extenders, cryoprotectants and cryopreservation methods on common carp (Cyprinus carpio) sperm. *Animal Reproduction Science*, *122*(3), 236–243. https://doi.org/10.1016/j.anireprosci.2010.08.017

Irnazarow, I. (1995). Genetic variability of Polish and Hungarian carp lines. *Aquaculture*, *129*(1–4). https://www.elibrary.ru/item.asp?id=682030

István, S. (2017). *Génbanki kutatások régi haszonállataink védelmében: M\Huhelytanulmányok a tudományos génmeg\Horzés tárgyköréb\Hol*. Budapest.

Ivanova, N. V., Zemlak, T. S., Hanner, R. H., & Hebert, P. D. (2007). Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, *7*(4), 544–548.

Ivanova, P., Raykov, V., Nikolov, V., & Nenciu, M. (2017). Allozyme identification of wrasses species (Osteichthyes: Perciformes: Labridae) along the bulgarian black sea coast. *Journal of Environmental Protection and Ecology*, *18*, 940–946.

Rissler, L., J. Hijmans, R., H. Graham, C., Moritz, C., & B. Wake, D. (2015). Phylogeographic Lineages and Species Comparisons in Conservation Analyses: A Case Study of California Herpetofauna. *The American Naturalist*. https://doi.org/10.1086/503332

Jang-Liaw, N.-H., Tominaga, K., Zhang, C., Zhao, Y., Nakajima, J., Onikura, N., & Watanabe, K. (2019). Phylogeography of the Chinese false gudgeon, Abbottina rivularis, in East Asia, with special reference to the origin and artificial disturbance of Japanese populations. *Ichthyological Research*, *66*(4), 460–478. https://doi.org/10.1007/s10228-019-00686-w

Janson, S., Wouters, J., Bonow, M., Svanberg, I., & Olsén, K. H. (2015). Population genetic structure of crucian carp (Carassius carassius) in man-made ponds and wild populations in Sweden. *Aquaculture International*, *23*(1), 359–368. https://doi.org/10.1007/s10499-014-9820-4

Jeffreys, A. J., Wilson, V., & Thein, S. L. (1985). Hypervariable 'minisatellite' regions in human DNA. *Nature*, *314*(6006), 67–73. https://doi.org/10.1038/314067a0

Jeffries, D. L., Copp, G. H., Handley, L. L., Olsén, K. H., Sayer, C. D., & Hänfling, B. (2016). Comparing RADseq and microsatellites to infer complex phylogeographic patterns, an

empirical perspective in the Crucian carp, Carassius carassius, L. *Molecular Ecology*, *25*(13), 2997–3018. https://doi.org/10.1111/mec.13613

Jia, Z.-Y., Sun, X.-W., Liang, L.-Q., Lu, C.-Y., & Lei, Q.-Q. (2006). Isolation and characterization of microsatellite markers from Fangzheng silver crucian carp, Carassius auratus gibelio (Bloch), and cross-amplification in the closely related species crucian carp, Carassius auratus auratus (Linnaeus). *Molecular Ecology Notes*, *6*(4), 1141–1143. https://doi.org/10.1111/j.1471-8286.2006.01464.x

Jin, R.-M., Huang, H.-Z., Zhou, Y., Wang, Y.-Y., Fu, H.-C., Li, Z., Fu, X.-Z., & Li, N.-Q. (2021). Characterization of mandarin fish (Siniperca chuatsi) IL-6 and IL-6 signal transducer and the association between their SNPs and resistance to ISKNV disease. *Fish & Shellfish Immunology*, *113*, 139–147.

Johnson, S. L., Midson, C. N., Ballinger, E. W., & Postlethwait, J. H. (1994). Identification of RAPD Primers That Reveal Extensive Polymorphisms between Laboratory Strains of Zebrafish. *Genomics*, *19*(1), 152–156. https://doi.org/10.1006/geno.1994.1026

Jones, A. (1987). Historical Background, Present Status, and Future Perspectives of the Aquaculture Industry on a Worldwide Basis. *IFAC Proceedings Volumes*, *20*(7), 1–9. https://doi.org/10.1016/S1474-6670 (17)59149-1

Judycka, S., Dietrich, M. A., Żarski, D., Karol, H., Palińska-Żarska, K., B\lażejewski, M., & Ciereszko, A. (2021). Towards standardization of the cryopreservation procedure of cultured pikeperch (Sander lucioperca) semen. *Aquaculture*, *538*, 736539.

Judycka, S., Nynca, J., Liszewska, E., Dobosz, S., Zalewski, T., & Ciereszko, A. (2016). Potassium ions in extender differentially influence the post-thaw sperm motility of salmonid fish. *Cryobiology*, *73*(2), 248–256.

Kalous, L., ŠLechtová, V., Bohlen, J., Petrtýl, M., & ŠVátora, M. (2007). First European record of Carassius langsdorfii from the Elbe basin. *Journal of Fish Biology*, *70*(sa), 132–138. https://doi.org/10.1111/j.1095-8649.2006.01290.x

Karaiskou, N., Gkagkavouzis, K., Minoudi, S., Botskaris, D., Markou, K., Kalafatakis, S., Antonopoulou, E., & Triantafyllidis, A. (2020). Genetic structure and divergence of tench TINCA *TINCA* EUROPEAN POPULATIONS. *Journal of Fish Biology*, *97*(3), 930–934. https://doi.org/10.1111/jfb.14448

Kariuki, J., Tibihika, P. D., Curto, M., Alemayehu, E., Winkler, G., & Meimberg, H. (2021). Application of microsatellite genotyping by amplicon sequencing for delimitation of African tilapiine species relevant for aquaculture. *Aquaculture*, *537*, 736501. https://doi.org/10.1016/j.aquaculture.2021.736501

Kása, E., Lujić, J., Marinović, Z., Kollár, T., Bernáth, G., Bokor, Z., Urbányi, B., Lefler, K. K., Jesenšek, D., & Horváth, Á. (2018). Development of sperm vitrification protocols for two endangered salmonid species: The Adriatic grayling, Thymallus thymallus, and the marble trout, Salmo marmoratus. *Fish Physiology and Biochemistry*, *44*(6), 1499–1507.

Kassler, T. W., Koppelman, J. B., Near, T. J., Dillman, C. B., Levengood, J., Swofford, D. L., VanOrman, J. L., Claussen, J. E., & Philipp, D. P. (2002). Molecular and morphological

analyses of the black basses: Implications for taxonomy and conservation. *American Fisheries Society Symposium*, *2002*(31), 291–322.

Kennedy, M., & Fitzmaurice, P. (1970). The Biology of the Tench Tinca tinca (L.) in Irish Waters. *Proceedings of the Royal Irish Academy. Section B: Biological, Geological, and Chemical Science*, *69*, 31–82.

Keresztessy, K. (1996). *Threatened freshwater fish in Hungary* (pp. 73–77). https://doi.org/10.1007/978-3-0348-9014-4_8

Khoo, G., Lim, M. H., Suresh, H., Gan, D. K., Lim, K. F., Chen, F., Chan, W.-K., Lim, T. M., & Phang, V. P. (2003). Genetic linkage maps of the guppy (Poecilia reticulata): Assignment of RAPD markers to multipoint linkage groups. *Marine Biotechnology*, *5*(3), 279–293.

KILIÇ, S., & BECER, Z. A. (2013). Some Growth Characters of Tench (Tinca tinca L., 1758) in Lake Yeniçağa, Bolu, Turkey. *Journal of Applied Biological Sciences*, *7*(3), 99–104.

Kırankaya, Ş. (2013). Life-history traits of the invasive population of prussian carp, Carassius gibelio (Actinopterigi: Cypriniformes: Cyprinidae), from Gelingüllü Reservoir, Yozgat, Turkey. *Acta Ichthyologica Et Piscatoria*, *43*, 31–40. https://doi.org/10.3750/AIP2013.43.1.05

Klinbunga, S., Khamnamtong, B., Puanglarp, N., Jarayabhand, P., Yoosukh, W., & Menasveta, P. (2005). Molecular Taxonomy of Cupped Oysters (Crassostrea, Saccostrea, and Striostrea) in Thailand Based on COI, 16S, and 18S rDNA Polymorphism. *Marine Biotechnology*, *7*(4), 306–317. https://doi.org/10.1007/s10126-004-0036-x

Klinbunga, S., Siludjai, D., Wudthijinda, W., Tassanakajon, A., Jarayabhand, P., & Menasveta, P. (2001). Genetic heterogeneity of the giant tiger shrimp (Penaeus monodon) in Thailand revealed by RAPD and mitochondrial DNA RFLP analyses. *Marine Biotechnology*, *3*(5), 428–438.

Knytl, M., Kalous, L., Rylková, K., Choleva, L., Merilä, J., & Ráb, P. (2018). Morphologically indistinguishable hybrid Carassius female with 156 chromosomes: A threat for the threatened crucian carp, C. carassius, L. *PLOS ONE*, *13*(1), e0190924. https://doi.org/10.1371/journal.pone.0190924

Knytl, M., Kalous, L., Symonová, R., Rylková, K., & Ráb, P. (2013). Chromosome Studies of European Cyprinid Fishes: Cross-Species Painting Reveals Natural Allotetraploid Origin of a ***Carassius*** Female with 206 Chromosomes. *Cytogenetic and Genome Research*, *139*(4), 276–283. https://doi.org/10.1159/000350689

Kocour, M., Gela, D., Rodina, M., & Flajšhans, M. (2010). Performance of different tench, Tinca tinca (L.), groups under semi-intensive pond conditions: It is worth establishing a coordinated breeding program. *Reviews in Fish Biology and Fisheries*, *20*(3), 345–355. https://doi.org/10.1007/s11160-009-9140-3

Kocour, M., & Kohlmann, K. (2011). Growth hormone gene polymorphisms in tench, Tinca tinca L. *Aquaculture*, *310*(3), 298–304. https://doi.org/10.1016/j.aquaculture.2010.10.006

Kocour, M., & Kohlmann, K. (2014). Distribution of five growth hormone gene haplogroups in wild and cultured tench, Tinca tinca L., populations. *Journal of Applied Ichthyology*, *30*(s1), 22–28. https://doi.org/10.1111/jai.12428

Kohlmann, K., Gross, R., Murakaeva, A., & Kersten, P. (2003). Genetic variability and structure of common carp (Cyprinus carpio) populations throughout the distribution range inferred from allozyme, microsatellite and mitochondrial DNA markers. *Aquatic Living Resources*, *16*(5), 421–431.

Kohlmann, K., & Kersten, P. (1998). Enzyme variability in a wild population of tench (Tinca tinca). *Polskie Archiwum Hydrobiologii*, *45*(3), 303–310.

Kohlmann, K., & Kersten, P. (2006). Microsatellite loci in tench: Isolation and variability in a test population. *Aquaculture International*, *14*(1), 3–7.

Kohlmann, K., Kersten, P., & Flajšhans, M. (2005). Microsatellite-based genetic variability and differentiation of domesticated, wild and feral common carp (Cyprinus carpio L.) populations. *Aquaculture*, *247*(1–4), 253–266.

Kohlmann, K., Kersten, P., & Flajšhans, M. (2007). Comparison of microsatellite variability in wild and cultured tench (Tinca tinca). *Aquaculture*, *272*, S147–S151. https://doi.org/10.1016/j.aquaculture.2007.08.003

Kohlmann, K., Kersten, P., Panicz, R., Memiş, D., & Flajšhans, M. (2010). Genetic variability and differentiation of wild and cultured tench populations inferred from microsatellite loci. *Reviews in Fish Biology and Fisheries*, *20*(3), 279–288. https://doi.org/10.1007/s11160-009-9138-x

Kottelat, M., & Freyhof, J. (2007). *Handbook of European Freshwater Fishes*. Publications Kottelat. https://books.google.iq/books?id=OZEWAQAAIAAJ

Kujawa, R., Kucharczyk, D., Mamcarz, A., Żarski, D., & Targońska, K. (2011). Artificial spawning of common tench Tinca tinca (Linnaeus, 1758), obtained from wild and domestic stocks. *Aquaculture International*, *19*(3), 513–521. https://doi.org/10.1007/s10499-010-9366-z

Kullander, S. O., & Delling, B. (2012). *Nationalnyckeln till Sveriges flora och fauna: Str\aalfeniga fiskar: Chordata: Actinopterygii. Ryggsträngsdjur. DZ 35-108*. ArtDatabanken, Sveriges lantbruksuniversitet.

Kumar, G., Langa, J., Montes, I., Conklin, D., Kocour, M., Kohlmann, K., & Estonba, A. (2019). A novel transcriptome-derived SNPs array for tench (Tinca tinca L.). *PLOS ONE*, *14*(3), e0213992. https://doi.org/10.1371/journal.pone.0213992

Kvasnicka, P., Flajshans, M., Ráb, P., & Linhart, O. (1998). Brief communication. Inheritance studies of blue and golden varieties of tench (Pisces: Tinca tinca L.). *Journal of Heredity*, *89*(6), 553–556. https://doi.org/10.1093/jhered/89.6.553

Lajbner, Z., Kohlmann, K., Linhart, O., & Kotlík, P. (2010). Lack of reproductive isolation between the Western and Eastern phylogroups of the tench. *Reviews in Fish Biology and Fisheries*, *20*(3), 289–300. https://doi.org/10.1007/s11160-009-9137-y

Lajbner, Z., & Kotlík, P. (2011). Pcr-Rflp Assays to Distinguish the Western and Eastern Phylogroups in Wild and Cultured Tench Tinca Tinca. *Molecular Ecology Resources*, *11*(2), 374–377. https://doi.org/10.1111/j.1755-0998.2010.02914.x

Lajbner, Z., Linhart, O., & Kotlik, P. (2007). Molecular phylogeography of the tench Tinca tinca (Linnaeus, 1758). *The 12th European Congress of Ichthyology, Book of Abstracts, Cavtat*, 35.

Lajbner, Z., Linhart, O., & Kotlík, P. (2011). Human-aided dispersal has altered but not erased the phylogeography of the tench. *Evolutionary Applications*, *4*(4), 545–561. https://doi.org/10.1111/j.1752-4571.2010.00174.x

Leathwick, J. R., Collier, K. J., Hicks, B. J., Ling, N., Stichbury, G., & Winton, M. (2016). Predictions of establishment risk highlight biosurveillance priorities for invasive fish in New Zealand lakes. *Freshwater Biology*, *61*(9), 1522–1535. https://doi.org/10.1111/fwb.12792

Lee, Y. H., Park, J. Y., Lee, I. Y., Zidni, I., & Lim, H. K. (2021). Effects of cryoprotective agents and treatment methods on sperm cryopreservation of stone flounder, Kareius bicoloratus. *Aquaculture*, *531*, 735969. https://doi.org/10.1016/j.aquaculture.2020.735969

Leggatt, R. A., & Iwama, G. K. (2003). Occurrence of polyploidy in the fishes. *Reviews in Fish Biology and Fisheries*, *13*(3), 237–246. https://doi.org/10.1023/B:RFBF.0000033049.00668.fe

Lehoczky, I., Jeney, Z., Magyary, I., Hancz, C., & Kohlmann, K. (2005). Preliminary data on genetic variability and purity of common carp (Cyprinus carpio L.) strains kept at the live gene bank at Research Institute for Fisheries, Aquaculture and Irrigation (HAKI) Szarvas, Hungary. *Aquaculture*, *247*(1), 45–49. https://doi.org/10.1016/j.aquaculture.2005.02.017

Lelek, A., & Council of Europe, S. (France) E. C. for the C. of N. and N. R. eng. (1987). *The freshwater fishes of Europe. v. 9: Threatened fishes of Europe*. https://agris.fao.org/agris-search/search.do?recordID=XF2015007071

Li, L., Lin, H., Tang, W., Liu, D., Bao, B., & Yang, J. (2017). Edwards et al. 1998. *Aquaculture and Fisheries*, *2*(2), 78–83. https://doi.org/10.1016/j.aaf.2017.03.004

Li, L., Xiang, J., Liu, X., Zhang, Y., Dong, B., & Zhang, X. (2005). Construction of AFLP-based genetic linkage map for Zhikong scallop, Chlamys farreri Jones et Preston and mapping of sex-linked markers. *Aquaculture*, *245*(1), 63–73. https://doi.org/10.1016/j.aquaculture.2004.12.015

Li, X., & Guo, B. (2020). Substantially adaptive potential in polyploid cyprinid fishes: Evidence from biogeographic, phylogenetic and genomic studies. *Proceedings of the Royal Society B*, *287*(1920), 20193008.

Liao, I. C. (Taiwan F. R. I. (2000). The state of finfish diversification in Asian aquaculture. *Cahiers Options Méditerranéennes (CIHEAM)*. Seminar of the CIHEAM Network on Technology of Aquaculture in the Mediterranean on Recent advances in Mediterranean aquaculture finfish species diversification, Zaragoza (Spain), 24-28 May 1999. https://agris.fao.org/agris-search/search.do?recordID=QC2000600610

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*(11), 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Lim, K. C., Then, A. Y.-H., Wee, A. K. S., Sade, A., Rumpet, R., & Loh, K.-H. (2021). Brown banded bamboo shark (Chiloscyllium punctatum) shows high genetic diversity and differentiation in Malaysian waters. *Scientific Reports*, *11*(1), 14874. https://doi.org/10.1038/s41598-021-94257-7

Linhart, O., Rodina, M., Kocour, M., & Gela, D. (2006). Insemination, fertilization and gamete management in tench, Tinca tinca (L.). *Aquaculture International*, *14*(1–2), 61–73. https://doi.org/10.1007/s10499-005-9014-1

Linhartova, Z., Rodina, M., Güralp, H., Gazo, I., Saito, T., & Psenicka, M. (2014). Isolation and cryopreservation of early stages of germ cells of tench (Tinca tinca). *Czech Journal of Animal Science*, *59*, 381–390. https://doi.org/10.17221/7589-CJAS

Liu, Y., Xu, T., Robinson, N., Qin, J., & Li, X. (2015). Cryopreservation of sperm in farmed blacklip abalone (Haliotis rubra Leach, 1814). *Aquaculture Research*, *46*(11), 2628–2636. https://doi.org/10.1111/are.12415

Liu, Z. J. (Ed.). (2011). *Next Generation Sequencing and Whole Genome Selection in Aquaculture: Liu/Next Generation Sequencing and Whole Genome Selection in Aquaculture*. Wiley-Blackwell. https://doi.org/10.1002/9780470958964

Liu, Z. J., & Cordes, J. F. (2004). DNA marker technologies and their applications in aquaculture genetics. *Aquaculture*, *238*(1), 1–37. https://doi.org/10.1016/j.aquaculture.2004.05.027

Liu, Z. (John). (2008). *Aquaculture Genome Technologies*. John Wiley & Sons.

Liu, Z., Karsi, A., Li, P., Cao, D., & Dunham, R. (2003). An AFLP-Based Genetic Linkage Map of Channel Catfish (*Ictalurus punctatus*) Constructed by Using an Interspecific Hybrid Resource Family. *Genetics*, *165*(2), 687–694. https://doi.org/10.1093/genetics/165.2.687

Lo Presti, R., Kohlmann, K., Kersten, P., Lisa, C., & Di Stasio, L. (2014). Sequence variability at the mitochondrial ND1, ND6, cyt *b* and D-loop segments in tench (*Tinca tinca* L.). *Journal of Applied Ichthyology*, *30*, 15–21. https://doi.org/10.1111/jai.12423

Lorenzoni, M., Ghetti, L., Pedicillo, G., & Carosi, A. (2010). Analysis of the biological features of the goldfish Carassius auratus auratus in Lake Trasimeno (Umbria, Italy) with a view to drawing up plans for population control. *Folia Zoologica*, *59*(2), 142–156.

Lu, C. Y., Cheng, L., Li, C., Zheng, X. H., Cao, D. C., & Sun, X. W. (2016). *Comparison of genetic diversity and growth traits among Fangzheng silver crucian carp (Carassius auratus gibelio) gynogenetic clones*. https://aquadocs.org/handle/1834/11967

Lu, X., Xu, H., Li, Z., Shang, H., Adams, R. P., & Mao, K. (2014). Genetic Diversity and Conservation Implications of Four Cupressus Species in China as Revealed by Microsatellite Markers. *Biochemical Genetics*, *52*(3), 181–202. https://doi.org/10.1007/s10528-013-9638-1

Luikart, G., England, P. R., Tallmon, D., Jordan, S., & Taberlet, P. (2003). The power and promise of population genomics: From genotyping to genome typing. *Nature Reviews Genetics*, *4*(12), 981–994. https://doi.org/10.1038/nrg1226

Lujić, J., Bernáth, G., Marinović, Z., Radojković, N., Simić, V., Ćirković, M., Urbányi, B., & Horváth, Á. (2017a). Fertilizing capacity and motility of tench Tinca tinca (L., 1758) sperm following cryopreservation. *Aquaculture Research*, *48*(1), 102–110. https://doi.org/10.1111/are.12865

Lujić, J., Kohlmann, K., Kersten, P., Marinović, Z., Ćirković, M., & Simić, V. (2017). Phylogeographic Identification of Tench *Tinca tinca* (L., 1758) (Actinopterygii: Cyprinidae) from the Northern Balkans and Adjacent Regions and its Implications for Conservation. *Zoological Studies*, *56*. https://doi.org/10.6620/ZS.2017.56-03

Lusk, S., Hanel, L., & Luskova, V. (2004). Red List of the ichthyofauna of the Czech Republic: Development and present status. *FOLIA ZOOLOGICA-PRAHA-*, *53*(2), 215–226.

Lusková, V., Lusk, S., Halačka, K., & Vetešník, L. (2010). Carassius auratus gibelio—The most successful invasive fish in waters of the Czech Republic. *Russian Journal of Biological Invasions*, *1*(3), 176–180. https://doi.org/10.1134/S2075111710030069

Machordom, A., & Doadrio, I. (2001). Evidence of a Cenozoic Betic–Kabilian Connection Based on Freshwater Fish Phylogeography (Luciobarbus, Cyprinidae). *Molecular Phylogenetics and Evolution*, *18*(2), 252–263. https://doi.org/10.1006/mpev.2000.0876

Maitland, P. S. (2004). Keys to the freshwater fish of Britain and Ireland, with notes on their distribution and ecology. *Keys to the Freshwater Fish of Britain and Ireland, with Notes on Their Distribution and Ecology*. https://www.cabdirect.org/cabdirect/abstract/20053011028

Mamuris, Z., Stamatis, C., Moutou, K. A., Apostolidis, A. P., & Triantaphyllidis, C. (2001). RFLP analysis of mitochondrial DNA to evaluate genetic variation in striped red mullet (Mullus surmuletus L.) and red mullet (Mullus barbatus L.) populations. *Marine Biotechnology*, *3*(3), 264–274.

Mandal, A., Lal, K. K., Mohindra, V., Singh, R. K., Punia, P., Chauhan, U. K., & Lakra, W. S. (2009). Evaluation of Genetic Variation in the Clown Knifefish, Chitala chitala, Using Allozymes, RAPD, and Microsatellites. *Biochemical Genetics*, *47*(3), 216–234. https://doi.org/10.1007/s10528-008-9219-x

Mandrak, N. E., & Cudmore, B. (2010). The fall of Native Fishes and the rise of Non-native Fishes in the Great Lakes Basin. *Aquatic Ecosystem Health & Management*, *13*(3), 255–268. https://doi.org/10.1080/14634988.2010.507150

Marini, M., Pedrosa-Gerasmio, I. R., Santos, M. D., Shibuno, T., Daryani, A., Romana-Eguia, M. R. R., & Wibowo, A. (2021). Genetic diversity, population structure and demographic history of the tropical eel Anguilla bicolor pacifica in Southeast Asia using mitochondrial DNA control region sequences. *Global Ecology and Conservation*, *26*, e01493. https://doi.org/10.1016/j.gecco.2021.e01493

Martinsohn, J. T., Geffen, A. J., Maes, G. E., Nielsen, E. E., Ogden, R., Waples, R. S., & Carvalho, G. R. (2011). Tracing fish and fish products from ocean to fork using advanced molecular technologies. In *Food Chain Integrity* (pp. 259–282). Elsevier. https://doi.org/10.1533/9780857092519.3.259

Martinsohn, J. Th., & Ogden, R. (2009). FishPopTrace—Developing SNP-based population genetic assignment methods to investigate illegal fishing. *Forensic Science International: Genetics Supplement Series*, *2*(1), 294–296. https://doi.org/10.1016/j.fsigss.2009.08.108

Martinsohn, J. Th., Raymond, P., Knott, T., Glover, K. A., Nielsen, E. E., Eriksen, L. B., Ogden, R., Casey, J., & Guillen, J. (2019). DNA -analysis to monitor fisheries and aquaculture: Too costly? *Fish and Fisheries*, *20*(2), 391–401. https://doi.org/10.1111/faf.12343

McNeely, J. A., Miller, K. R., Reid, W. V., Mittermeier, R. A., & Werner, T. B. (1990). Conserving the world's biological diversity. *Conserving the World's Biological Diversity.* https://www.cabdirect.org/cabdirect/abstract/19911620843

Menezes, M. R., Naik, S., & Martins, M. (1993). Genetic characterization in four sciaenid species from the Arabian Sea. *Journal of Fish Biology*, *43*(1), 61–67.

Metian, M., Troell, M., Christensen, V., Steenbeek, J., & Pouil, S. (2020). Mapping diversity of species in global aquaculture. *Reviews in Aquaculture*, *12*(2), 1090–1100. https://doi.org/10.1111/raq.12374

Metzgar, D., Bytof, J., & Wills, C. (2000). Selection against frameshift mutations limits microsatellite expansion in coding DNA. *Genome Research*, *10*(1), 72–80.

Mezhzherin, S. V., Kokodii, S. V., Kulish, A. V., Verlatii, D. B., & Fedorenko, L. V. (2012). Hybridization of crucian carp Carassius carassius (Linnaeus, 1758) in Ukrainian reservoirs and the genetic structure of hybrids. *Cytology and Genetics*, *46*(1), 28–35. https://doi.org/10.3103/S0095452712010069

Millennium Ecosystem Assessment (Program) (Ed.). (2005). *Ecosystems and human well-being: Synthesis*. Island Press.

Miller, S. A., Dykes, D. D., & Polesky, H. F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*, *16*(3), 1215. https://doi.org/10.1093/nar/16.3.1215

Miralles, L., Juanes, F., Pardiñas, A. F., & Garcia-Vazquez, E. (2014). Paleoclimate Shaped Bluefish Structure in the Northern Hemisphere. *Fisheries*, *39*(12), 578–586. https://doi.org/10.1080/03632415.2014.976701

Miyake, T., Isowa, K., Ishikawa, T., Komaru, A., & Kawamura, K. (2016). Evaluation of genetic characteristics of wild and cultured populations of the Japanese pearl oyster Pinctada fucata martensii by using AFLP markers. *Aquaculture International*, *24*(2), 537–548. https://doi.org/10.1007/s10499-015-9943-2

Molnár, J., Bokor, Z., Várkonyi, L., Izsák, T., Füzes-Solymosi, E., Láng, Z. L., Csorbai, B., Tarnai-Király, Zs., Urbányi, B., & Bernáth, G. (2020). The systematic development and optimization of large-scale sperm cryopreservation in northern pike (Esox lucius). *Cryobiology*, *94*, 26–31. https://doi.org/10.1016/j.cryobiol.2020.05.003

Molnár, T., Benedek, I., Kovács, B., Zsolnai, A., & Lehoczky, I. (2020). Genetic consequences of pond production of a pikeperch (Sander lucioperca L.) stock with natural origin: The effects of changed selection pressure and reduced population size. *PeerJ*, *8*, e8745.

Morgan, J. A. T., Sumpton, W. D., Jones, A. T., Campbell, A. B., Stewart, J., Hamer, P., & Ovenden, J. R. (2019). Assessment of genetic structure among Australian east coast populations of snapper Chrysophrys auratus (Sparidae). *Marine and Freshwater Research*, *70*(7), 964. https://doi.org/10.1071/MF18146

Morissette, O., Lecomte, F., Vachon, N., Drouin, A., & Sirois, P. (2021). Quantifying migratory capacity and dispersal of the invasive Tench (Tinca tinca) in the St. Lawrence River using otolith chemistry. *Canadian Journal of Fisheries and Aquatic Sciences*, *78*(11), 1628–1638.

Morizot, D. C., Slaugenhaupt, S. A., Kallman, K. D., & Chakravarti, A. (1991). Genetic linkage map of fishes of the genus Xiphophorus (Teleostei: Poeciliidae). *Genetics*, *127*(2), 399–410.

Moyle, P. B., & Leidy, R. A. (1992). Loss of Biodiversity in Aquatic Ecosystems: Evidence from Fish Faunas. In P. L. Fiedler & S. K. Jain (Eds.), *Conservation Biology* (pp. 127–169). Springer US. https://doi.org/10.1007/978-1-4684-6426-9_6

Mrakovčić, M., Buj, I., Mustafić, P., Ćaleta, M., & Zanella, D. (2007). Croatian red list: Freshwater fish. *Department of Zoology, Faculty of Science, Zagreb*.

Muneer, P. M. A., Sivanandan, R., Gopalakrishnan, A., Basheer, V. S., Musammilu, K. K., & Ponniah, A. G. (2011). Development and Characterization of RAPD and Microsatellite Markers for Genetic Variation Analysis in the Critically Endangered Yellow Catfish Horabagrus nigricollaris (Teleostei: Horabagridae). *Biochemical Genetics*, *49*(1), 83–95. https://doi.org/10.1007/s10528-010-9389-1

Napora-Rutkowski, Ł., Rakus, K., Nowak, Z., Szczygieł, J., Pilarczyk, A., Ostaszewska, T., & Irnazarow, I. (2017). Genetic diversity of common carp (Cyprinus carpio L.) strains breed in Poland based on microsatellite, AFLP, and mtDNA genotype data. *Aquaculture*, *473*, 433–442. https://doi.org/10.1016/j.aquaculture.2017.03.005

Nascimento, A. F., Gonçalves, A. C. S., Neto, R. V. R., Leal, M. C., & Viveiros, A. T. M. (2012). *Extender composition, osmolality, cryoprotectant and equilibration time effects on fresh sperm motility of two Characiformes fish: Piracanjuba (Brycon orbignyanus) and streaked prochilod (Prochilodus lineatus)*. 8.

Navodaru, I., Buijse, A. D., & Staras, M. (2002). Effects of Hydrology and Water Quality on the Fish Communityin Danube Delta Lakes. *International Review of Hydrobiology*, *87*(2–3), 329–348. https://doi.org/10.1002/1522-2632(200205)87:2/3<329::AID-IROH329>3.0.CO;2-J

Nestel, P., Clifton, P., Colquhoun, D., Noakes, M., Mori, T. A., Sullivan, D., & Thomas, B. (2015). Indications for Omega-3 Long Chain Polyunsaturated Fatty Acid in the Prevention and Treatment of Cardiovascular Disease. *Heart, Lung and Circulation*, *24*(8), 769–779. https://doi.org/10.1016/j.hlc.2015.03.020

Nynca, J., Judycka, S., Liszewska, E., Dobosz, S., Grudniewska, J., Arai, K., Fujimoto, T., & Ciereszko, A. (2016). Utility of different sugar extenders for cryopreservation and post-thaw storage of sperm from Salmonidae species. *Aquaculture*, *464*, 340–348.

O'Connell, M., Danzmann, R. G., Cornuet, J.-M., Wright, J. M., & Ferguson, M. M. (1997). Differentiation of rainbow trout (Oncorhynchus mykiss) populations in Lake Ontario and the evaluation of the stepwise mutation and infinite allele mutation models using microsatellite variability. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*(6), 1391–1399.

O'Connell, M., & Wright, J. M. (1997). Microsatellite DNA in fishes. *Reviews in Fish Biology and Fisheries*, *7*(3), 331–363. https://doi.org/10.1023/A:1018443912945

Okumuş, İ., & Çiftci, Y. (2003). Fish population genetics and molecular markers: II-molecular markers and their applications in fisheries and aquaculture. *Turkish Journal of Fisheries and Aquatic Sciences*, *3*(1).

Olsén, K. H., & Lundh, T. (2016). Feeding stimulants in an omnivorous species, crucian carp Carassius carassius (Linnaeus 1758). *Aquaculture Reports*, *4*, 66–73. https://doi.org/10.1016/j.aqrep.2016.06.005

Oosterhout, C. V., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). micro-checker: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, *4*(3), 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Ovenden, J. R., Berry, O., Welch, D. J., Buckworth, R. C., & Dichmont, C. M. (2015). Ocean's eleven: A critical evaluation of the role of population, evolutionary and molecular genetics in the management of wild fisheries. *Fish and Fisheries*, *16*(1), 125–159. https://doi.org/10.1111/faf.12052

Páczay, G., & Páczay, G. (2018). Sustainable freshwater aquaculture in the EU and in Hungary. *International Journal of Aquaculture and Fishery Sciences*, *4*(3), 039–044.

Palti, Y., Vallejo, R. L., Gao, G., Liu, S., Hernandez, A. G., Rexroad III, C. E., & Wiens, G. D. (2015). Detection and validation of QTL affecting bacterial cold water disease resistance in rainbow trout using restriction-site associated DNA sequencing. *PloS One*, *10*(9), e0138435.

Pan, Z.-J., Zhu, C.-K., Wang, H., Chang, G.-L., Ding, H.-Y., Qiang, X.-G., & Yu, X.-S. (2017). Induction of meiotic gynogenesis in bagrid catfish (*Pseudobagrus ussuriensis*) with homologous sperm and its confirmation for female homogamety. *Aquaculture Research*, *48*(11), 5659–5665. https://doi.org/10.1111/are.13388

Paniagua-Chavez, C. G., Buchanan, J. T., Supan, J. E., & Tiersch, T. R. (2000). Cryopreservation of sperm and larvae of the Eastern oyster. *Cryopreservation in Aquatic Species, Edited by TR Tiersch & PM Mazik. World Aquaculture Society, Baton Rouge, Louisiana, USA*, 230–239.

Panicz, R., Napora-Rutkowski, \Lukasz, Keszka, S., Skuza, L., Szenejko, M., & Śmietana, P. (2019). Genetic diversity in natural populations of noble crayfish (Astacus astacus L.) in north-western Poland on the basis of combined SSR and AFLP data. *PeerJ*, *7*, e7301.

Papakostas, S., Dooms, S., Triantafyllidis, A., Deloof, D., Kappas, I., Dierckens, K., De Wolf, T., Bossier, P., Vadstein, O., & Kui, S. (2006). Evaluation of DNA methodologies in identifying Brachionus species used in European hatcheries. *Aquaculture*, *255*(1–4), 557–564.

Papoušek, I., Vetešník, L., Halačka, K., Lusková, V., Humpl, M., & Mendel, J. (2008). Identification of natural hybrids of gibel carp Carassius auratus gibelio (Bloch) and crucian carp Carassius carassius (L.) from lower Dyje River floodplain (Czech Republic). *Journal of Fish Biology*, *72*(5), 1230–1235. https://doi.org/10.1111/j.1095-8649.2007.01783.x

Pauquet, G., Salzburger, W., & Egger, B. (2018). The puzzling phylogeography of the haplochromine cichlid fish *Astatotilapia burtoni*. *Ecology and Evolution*, *8*(11), 5637–5648. https://doi.org/10.1002/ece3.4092

Payseur, B. A., & Knyt, L. H. (2016). A genomic perspective on hybridization and speciation. *Molecular Ecology*, *25*(11), 2337–2360. https://doi.org/10.1111/mec.13557

Peacock, M. M., Dunham, J. B., & Ray, C. (2001). Recovery and Implementation Plan for Lahontan Cutthroat Trout in the Pyramid Lake, Truckee River and Lake Tahoe Ecosystem Genetics Section. *Draft Report. Biological Resources Research Center Department of Biology, University of Nevada, Reno*, *83*.

Peakall, R., & Smouse, P. E. (2006). genalex 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, *6*(1), 288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Pękala-Safińska, A. (2018). Contemporary threats of bacterial infections in freshwater fish. *Journal of Veterinary Research*, *62*(3), 261–267. https://doi.org/10.2478/jvetres-2018-0037

Pereira, J. C., Lino, P. G., Leitão, A., Joaquim, S., Chaves, R., Pousãao-Ferreira, P., Guedes-Pinto, H., & Santos, M. N. dos. (2010). Genetic differences between wild and hatchery populations ofDiplodus sargus andD. vulgaris inferred from RAPD markers: Implications for production and restocking programs design. *Journal of Applied Genetics*, *51*(1), 67–72. https://doi.org/10.1007/BF03195712

Pereira, V. A., de Alencar, D. B., de Araújo, I. W. F., Rodrigues, J. A. G., Lopes, J. T., Nunes, L. T., Ferreira, Y. M., Lobato, J. S., Montenegro, A. R., & Vanderley, C. S. B. S. (2020). Supplementation of cryodiluent media with seaweed or Nile tilapia skin sulfated polysaccharides for freezing of Colossoma macropomum (Characiformes: Serrasalmidae) semen. *Aquaculture*, *528*, 735553.

Piry, S., Luikart, G., & Cornuet, J.-M. (1999). Computer note. BOTTLENECK: A computer program for detecting recent reductions in the effective size using allele frequency data. *Journal of Heredity*, *90*(4), 502–503. https://doi.org/10.1093/jhered/90.4.502

Podlesnykh, A. V., Apalikova, O. V., & Brykov, V. A. (2012). Phylogenetic relationships of silver crucian carp in Carassius auratus complex based on mtDNA analysis. *Russian Journal of Genetics*, *48*(12), 1207–1217.

Poléo, A. B., Osxnevad, S. A., Österbye, K., Heibo, E., Andersen, R. A., & Vøllestad, L. A. (1995). Body morphology of crucian carp Carassius carassius in lakes with or without piscivorous fish. *Ecography*, *18*(3), 225–229.

Pompei, L., Franchi, E., Giannetto, D., & Lorenzoni, M. (2012). Growth and reproductive properties of Tench, *Tinca tinca* Linnaeus, 1758 in Trasimeno Lake (Umbria, Italy). *Knowledge and Management of Aquatic Ecosystems*, *406*, 07. https://doi.org/10.1051/kmae/2012024

Ponzoni, R. W., Khaw, H. L., Nguyen, N. H., & Hamzah, A. (2010). Inbreeding and effective population size in the Malaysian nucleus of the GIFT strain of Nile tilapia (Oreochromis niloticus). *Aquaculture*, *302*(1–2), 42–48. https://doi.org/10.1016/j.aquaculture.2010.02.009

Povž, M., Sket, B., Furlan, B., Červek, S., Raičkovič, B., & Sedmak, A. (1990). *Naše sladkovodne ribe*. Mladinska knjiga.

Praebel, K., Westgaard, J.-I., Amundsen, P.-A., Siwertsson, A., Knudsen, R., Kahilainen, K. K., & Fevolden, S.-E. (2013). A diagnostic tool for efficient analysis of the population structure, hybridization and conservation status of European whitefish (Coregonus lavaretus (L.)) and vendace (C. albula (L.)). *Advances in Limnology*, *64*, 247–255. https://doi.org/10.1127/1612-166X/2013/0064-0026

Presti, R. L., Kohlmann, K., Kersten, P., Gasco, L., Lisa, C., & Stasio, L. D. (2012). Genetic variability in tench (Tinca tinca L.) as revealed by PCR-RFLP analysis of mitochondrial DNA. *Italian Journal of Animal Science*, *11*(1), e19. https://doi.org/10.4081/ijas.2012.e19

Presti, R. L., Kohlmann, K., Kersten, P., Gasco, L., & Stasio, L. D. (2010). Tinca Gobba Dorata del Pianalto di Poirino: Genetic characterization by microsatellite markers. *Italian Journal of Animal Science*, *9*(4), e85. https://doi.org/10.4081/ijas.2010.e85

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of Population Structure Using Multilocus Genotype Data. *Genetics*, *155*(2), 945–959.

Pritcharda, J. K., Wena, X., & Falushb, D. (2010). Documentation for structure software: Version 2.3. *Chicago: University of Chicago*, *39*.

Prokes, M., & Barus, V. (1996). On the natural hybrid between common carp (Cyprinus carpio) and Prussian carp (Carassius auratus gibelio) in the Czech Republic. *Folia Zoologica (Czech Republic)*.

Pullin, R. S. (2005). Conservation of farmed fish genetic resources: Broad options, strategies, availability of methods, and comparisons with those for conservation of the genetic resources of other farmed animals. *International Workshop "Options and Strategies for the Conservation of Farm Animal Genetic Resources,"* 87.

Pyšek, P., Blackburn, T. M., García-Berthou, E., Perglová, I., & Rabitsch, W. (2017). Displacement and local extinction of native and endemic species. In *Impact of biological invasions on ecosystem services* (pp. 157–175). Springer.

Qi, P., Qin, J., & Xie, C. (2015). Determination of genetic diversity of wild and cultured topmouth culter (Culter alburnus) inhabiting China using mitochondrial DNA and microsatellites.

Biochemical Systematics and Ecology, *61*, 232–239. https://doi.org/10.1016/j.bse.2015.06.023

Qu, M., Ding, J., Wang, Y., Chen, S., Zhang, Y., & Di, Y. (2019). Genetic impacts induced by BaP and Pb in Mytilus coruscus: Can RAPD be a validated tool in genotoxicity evaluation both in vivo and in vitro? *Ecotoxicology and Environmental Safety*, *169*, 529–538.

Rabanal, H. r. (1988). *History of aquaculture*. https://agris.fao.org/agris-search/search.do?recordID=XF19890110195

Raymond, M., & Rousset, F. (1995). GENEPOP (Version 1.2): Population Genetics Software for Exact Tests and Ecumenicism. *Journal of Heredity*, *86*(3), 248–249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Reed, D. H., & Frankham, R. (2003). Correlation between Fitness and Genetic Diversity. *Conservation Biology*, *17*(1), 230–237. https://doi.org/10.1046/j.1523-1739.2003.01236.x

Reed, K. M., & Czech, B. (2005). *Causes of fish endangerment in the United States, or the structure of the American economy*. *30*, 36–38.

Reeves, P. A., & Richards, C. M. (2009). Accurate inference of subtle population structure (and other genetic discontinuities) using principal coordinates. *PLoS One*, *4*(1), e4269.

Ribeiro, F., Rylková, K., Moreno-Valcárcel, R., Carrapato, C., & Kalous, L. (2015). Prussian carp Carassius gibelio: A silent invader arriving to the Iberian Peninsula. *Aquatic Ecology*, *49*(1), 99–104. https://doi.org/10.1007/s10452-015-9508-5

Ricciardi, A., & Atkinson, S. K. (2004). Distinctiveness magnifies the impact of biological invaders in aquatic ecosystems: Distinctiveness magnifies impact of invaders. *Ecology Letters*, *7*(9), 781–784. https://doi.org/10.1111/j.1461-0248.2004.00642.x

Risjani, Y., & Abidin, G. (2020). Genetic diversity and similarity between green and brown morphotypes of Kappaphycus alvarezii using RAPD. *Journal of Applied Phycology*, *32*(4), 2253–2260.

Robinson, B. W., & Parsons, K. J. (2011). Changing times, spaces, and faces: Tests and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. *Canadian Journal of Fisheries and Aquatic Sciences*. https://doi.org/10.1139/f02-144

Rodina, M., Gela, D., Kocour, M., Alavi, S. M. H., Hulak, M., & Linhart, O. (2007). Cryopreservation of tench, Tinca tinca, sperm: Sperm motility and hatching success of embryos. *Theriogenology*, *67*(5), 931–940. https://doi.org/10.1016/j.theriogenology.2006.11.007

Rousset, F. (2008). genepop'007: A complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, *8*(1), 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Rowe, D. K. (2007). Exotic fish introductions and the decline of water clarity in small North Island, New Zealand lakes: A multi-species problem. *Hydrobiologia*, *583*(1), 345–358. https://doi.org/10.1007/s10750-007-0646-1

Ruzzante, D. E., Simons, A. P., McCracken, G. R., Habit, E., & Walde, S. J. (2020). Multiple drainage reversal episodes and glacial refugia in a Patagonian fish revealed by sequenced

microsatellites. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1928), 20200468. https://doi.org/10.1098/rspb.2020.0468

Rylková, K., Kalous, L., Bohlen, J., Lamatsch, D. K., & Petrtýl, M. (2013). Phylogeny and biogeographic history of the cyprinid fish genus Carassius (Teleostei: Cyprinidae) with focus on natural and anthropogenic arrivals in Europe. *Aquaculture*, *380–383*, 13–20. https://doi.org/10.1016/j.aquaculture.2012.11.027

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., & Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, *239*(4839), 487–491. https://doi.org/10.1126/science.239.4839.487

Sakseepipad, C., Nozaki, R., Yoshii, K., Fukuda, Y., Mizuno, K., Kawakami, H., Hirono, I., & Kondo, H. (2021). Development of single nucleotide polymorphism (SNP) application for detection and genotyping of RSIV-type megalocytiviruses. *Journal of Fish Diseases*, *44*(9), 1337–1342.

Sanetra, M., Henning, F., Fukamachi, S., & Meyer, A. (2009). A Microsatellite-Based Genetic Linkage Map of the Cichlid Fish, Astatotilapia burtoni (Teleostei): A Comparison of Genomic Architectures Among Rapidly Speciating Cichlids. *Genetics*, *182*(1), 387–397. https://doi.org/10.1534/genetics.108.089367

Sarà, G., Mangano, M. C., Berlino, M., Corbari, L., Lucchese, M., Milisenda, G., Terzo, S., Azaza, M. S., Babarro, J. M. F., Bakiu, R., Broitman, B. R., Buschmann, A. H., Christofoletti, R., Deidun, A., Dong, Y., Galdies, J., Glamuzina, B., Luthman, O., Makridis, P., … Helmuth, B. (2022). The Synergistic Impacts of Anthropogenic Stressors and COVID-19 on Aquaculture: A Current Global Perspective. *Reviews in Fisheries Science & Aquaculture*, *30*(1), 123–135. https://doi.org/10.1080/23308249.2021.1876633

Saragusty, J. (2012). Genome Banking for Vertebrates Wildlife Conservation. In I. Katkov (Ed.), *Current Frontiers in Cryobiology*. InTech. https://doi.org/10.5772/35012

Savini, D., Occhipinti–Ambrogi, A., Marchini, A., Tricarico, E., Gherardi, F., Olenin, S., & Gollasch, S. (2010). The top 27 animal alien species introduced into Europe for aquaculture and related activities. *Journal of Applied Ichthyology*, *26*(s2), 1–7. https://doi.org/10.1111/j.1439-0426.2010.01503.x

Sayer, C. D., Copp, G. H., Emson, D., Godard, M. J., Zięba, G., & Wesley, K. J. (2011). Towards the conservation of crucian carp Carassius carassius: Understanding the extent and causes of decline within part of its native English range. *Journal of Fish Biology*, *79*(6), 1608–1624. https://doi.org/10.1111/j.1095-8649.2011.03059.x

Sayer, C. D., Emson, D., Patmore, I. R., Greaves, H. M., West, W. P., Payne, J., Davies, G. D., Tarkan, A. S., Wiseman, G., Cooper, B., Grapes, T., Cooper, G., & Copp, G. H. (2020). Recovery of the crucian carp Carassius carassius (L.): Approach and early results of an English conservation project. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(12), 2240–2253. https://doi.org/10.1002/aqc.3422

Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology*, *18*(2), 233–234. https://doi.org/10.1038/72708

Schultz, R. J. (1969). Hybridization, unisexuality, and polyploidy in the teleost Poeciliopsis (Poeciliidae) and other vertebrates. *The American Naturalist*, *103*(934), 605–619.

Scoble, J., & Lowe, A. J. (2010). A case for incorporating phylogeography and landscape genetics into species distribution modelling approaches to improve climate adaptation and conservation planning: Incorporating molecular marker information into species modelling. *Diversity and Distributions*, *16*(3), 343–353. https://doi.org/10.1111/j.1472-4642.2010.00658.x

Scribner, K. T., Page, K. S., & Bartron, M. L. (2000). Hybridization in freshwater fishes: A review of case studies and cytonuclear methods of biological inference. *Reviews in Fish Biology and Fisheries*, *10*(3), 293–323. https://doi.org/10.1023/A:1016642723238

Shechonge, A., Ngatunga, B. P., Tamatamah, R., Bradbeer, S. J., Harrington, J., Ford, A. G. P., Turner, G. F., & Genner, M. J. (2018). Losing cichlid fish biodiversity: Genetic and morphological homogenization of tilapia following colonization by introduced species. *Conservation Genetics*, *19*(5), 1199–1209. https://doi.org/10.1007/s10592-018-1088-1

Silva, S. S. D., Nguyen, T. T. T., Turchini, G. M., Amarasinghe, U. S., & Abery, N. W. (2009). Alien Species in Aquaculture and Biodiversity: A Paradox in Food Production. *AMBIO: A Journal of the Human Environment*, *38*(1), 24–28. https://doi.org/10.1579/0044-7447-38.1.24

Simic, V., Lujic, J., Kostic, D., Cirkovic, M., Bjelic-Cabrilo, O., Simic, S., & Markovic, G. (2013). Diversity characteristics of the fish species important for fishery in the waters of Serbia. *Bulgarian Journal of Agricultural Science*, *19*(1), 77–87.

Simic, V., Simic, S., Cirkovic, M., & Pantovic, N. (2009). Preliminary red list of the fishes of Serbia. *COMBAFF-First Conference on Conservation and Management of Balkan Freshwater Fishes*.

Smartt, J. (2007). A possible genetic basis for species replacement: Preliminary results of interspecific hybridisation between native crucian carp Carassius carassius (L.) and introduced goldfish Carassius auratus (L.). *Aquatic Invasions*, *2*(1), 59–62.

Sodsuk, P., & McAndrew, B. J. (1991). Molecular systematics of three tilapüne genera Tilapia, Sarotherodon and Oreochromis using allozyme data. *Journal of Fish Biology*, *39*(sA), 301–308. https://doi.org/10.1111/j.1095-8649.1991.tb05093.x

Solarte-Murillo, L. V., Sandoval-Herrera, I. D., Marín-Colorado, J. A., & Rodríguez-Pulido, J. A. (2020). An economic method for the identification of catfish hybrids using microsatellites: A case study of Pseudoplatystoma metaense × Leiarius marmoratus hybrids. *Conservation Genetics Resources*, *12*(4), 701–707. https://doi.org/10.1007/s12686-020-01166-4

Sollid, J. (2003). Hypoxia induces adaptive and reversible gross morphological changes in crucian carp gills. *Journal of Experimental Biology*, *206*(20), 3667–3673. https://doi.org/10.1242/jeb.00594

Sollid, J. (2005). Temperature alters the respiratory surface area of crucian carp Carassius carassius and goldfish Carassius auratus. *Journal of Experimental Biology*, *208*(6), 1109–1116. https://doi.org/10.1242/jeb.01505

Souza, A. S. de, Dias Júnior, E. A., Perez, M. F., Cioffi, M. de B., Bertollo, L. A. C., Garcia-Machado, E., Vallinoto, M. N. S., Pedro Manoel, G., & Molina, W. F. (2019). Phylogeography and Historical Demography of Two Sympatric Atlantic Snappers: Lutjanus analis and L. jocu. *Frontiers in Marine Science*, *6*. https://www.frontiersin.org/article/10.3389/fmars.2019.00545

Souza-Shibatta, L., Kotelok-Diniz, T., Ferreira, D. G., Shibatta, O. A., Sofia, S. H., de Assumpção, L., Pini, S. F. R., Makrakis, S., & Makrakis, M. C. (2018). Genetic Diversity of the Endangered Neotropical Cichlid Fish (Gymnogeophagus setequedas) in Brazil. *Frontiers in Genetics*, *9*, 13. https://doi.org/10.3389/fgene.2018.00013

Specziár, A., & Ero˝s, T. (2015). Freshwater resources and fisheries in Hungary. In *Freshwater Fisheries Ecology* (pp. 196–200). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118394380.ch15

Spillman, C. J. (1961). Faune de France: Poissons d'eau douce. Paris. *Fédération Française*.

Spoz, A., Boron, A., Porycka, K., Karolewska, M., Ito, D., Abe, S., Kirtiklis, L., & Juchno, D. (2014). Molecular cytogenetic analysis of the crucian carp, Carassius carassius (Linnaeus, 1758)(Teleostei, Cyprinidae), using chromosome staining and fluorescence in situ hybridisation with rDNA probes. *Comparative Cytogenetics*, *8*(3), 233.

Steven X. Cadrin, Kevin D. Friedland, & John R. Waldman. (2005). *Stock Identification Methods*. Elsevier. https://doi.org/10.1016/B978-0-12-154351-8.X5000-X

Stokes, K., O'Neill, K., & McDonald, R. A. (2004). Invasive species in Ireland. *Unpublished Report to Environment & Heritage Service and National Parks & Wildlife Service. Quercus, Queens University Belfast, Belfast*.

Subasinghe, R., Soto, D., & Jia, J. (2009). Global aquaculture and its role in sustainable development. *Reviews in Aquaculture*, *1*(1), 2–9. https://doi.org/10.1111/j.1753-5131.2008.01002.x

Sukumaran, S. (2018). *Importance of Molecular Taxonomy in Fishery Biology Investigations*. 6.

Sukumaran, S., & Gopalakrishnan, A. (2019). *Applications of Molecular Markers in Fisheries and Aquaculture*. 190–200. http://eprints.cmfri.org.in/14036/

Sun, X., & Liang, L. (2004). A genetic linkage map of common carp (Cyprinus carpio L.) and mapping of a locus associated with cold tolerance. *Aquaculture*, *238*(1–4), 165–172.

Sun, X. W., Yang, Y. H., Lu, C. Y., Cao, D. C., & Lei, Q. Q. (2009). Comparative analysis of genetic composition of population between silver crucian carp and normal crucian carp. *Freshw Fish*, *39*, 34–39.

Suneetha, K. B. (2000). *Intraspecific and interspecific genetic variation in selected mesopelagic fishes with emphasis on microgeographic variation and species characterization*. University of Bergen.

Svobodová, Z., Flajšhans, M., Kolářová, J., Modrá, H., Svoboda, M., & Vajcová, V. (2001). Leukocyte profiles of diploid and triploid tench, Tinca tinca L. *Aquaculture*, *198*(1–2), 159–168.

Svobodova, Z., & Kolarova, J. (2004). A review of the diseases and contaminant related mortalities of tench (Tinca tinca L.). *Veterinární Medicína*, *49*(1), 19–34.

Svobodová, Z., Kolářová, J., & Flajšhans, M. (1998). The First Findings on the Differences in Complete Blood Count Between Diploid and Triploid Tench, Tinca tinca L. *Acta Veterinaria Brno*, *67*(4), 243–248.

Syazni, A., Khaleel, A., Ismail, N., Komilus, C., Nguang, S. I., & Ha, H.-C. (2017). Population Structure of Swamp Eel Monopterus albus in East Coast of Peninsular Malaysia Inferred from 16S Mitochondrial DNA. *World Applied Sciences Journal*, *35*, 1392–1399. https://doi.org/10.5829/idosi.wasj.2017.1392.1399

Szczerbowski, J. A., & Szczerbowski, A. J. (2002). Carassius carassius (Linnaeus, 1758). *The Freshwater Fishes of Europe*, *5*, 43–78.

Tacon, A. G. J. (2020). Trends in Global Aquaculture and Aquafeed Production: 2000–2017. *Reviews in Fisheries Science & Aquaculture*, *28*(1), 43–56. https://doi.org/10.1080/23308249.2019.1649634

Takács, P., Czeglédi, I., Ferincz, Á., Sály, P., Specziár, A., Vitál, Z., Weiperth, A., & Er\Hos, T. (2017). Non-native fish species in Hungarian waters: Historical overview, potential sources and recent trends in their distribution. *Hydrobiologia*, *795*(1), 1–22.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027.

Tanya, C., & Kumar, R. (2010). Molecular markers and their applications in fisheries and aquaculture. *Advances in Bioscience and Biotechnology*, *2010*. https://doi.org/10.4236/abb.2010.14037

Tarkan, A. S., Copp, G. H., Zięba, G., Godard, M. J., & Cucherousset, J. (2009). Growth and reproduction of threatened native crucian carp Carassius carassius in small ponds of Epping Forest, south-east England. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *19*(7), 797–805. https://doi.org/10.1002/aqc.1028

Tarkan, A. S., Gaygusuz, Ö., Godard, M. J., & Copp, G. H. (2011). Long-term growth patterns in a pond-dwelling population of crucian carp, Carassius carassius: Environmental and density-related factors. *Fisheries Management and Ecology*, *18*(5), 375–383. https://doi.org/10.1111/j.1365-2400.2011.00791.x

Tarkan, A. S., Gaygusuz, Ö., Gürsoy GAYGUSUZ, Ç., Saç, G., & Copp, G. H. (2012). Circumstantial evidence of gibel carp, *Carassius gibelio*, reproductive competition exerted on native fish species in a mesotrophic reservoir. *Fisheries Management and Ecology*, *19*(2), 167–177. https://doi.org/10.1111/j.1365-2400.2011.00839.x

Tautz, D. (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research*, *17*(16), 6463–6471. https://doi.org/10.1093/nar/17.16.6463

Teng, C. B., Sun, X. W., Shen, J. B., Yan, X. C., & Liang, L. Q. (1999). RAPD analysis of gynogenetic crucian carp activated by heterologous sperm and their parents. *J Fish China*, *23*(4), 420–423.

Tiknaik, A., Khedkar, C., Khedkar, G., Prakash, B., Mamatha, D. M., Sangale, D., & Kalyankar, A. (2020). Microsatellite Genotyping Corroborated Loss of Genetic Diversity in Clarias batrachus as a Result of Lack of Regulatory Reforms in Aquaculture. *Biochemical Genetics*, *58*(4), 595–616. https://doi.org/10.1007/s10528-020-09963-0

Tóth, B., Ashrafzadeh, M. R., Khosravi, R., Bagi, Z., Fehér, M., Bársony, P., Kovács, G., & Kusza, S. (2022). Insights into mitochondrial DNA variation of common carp Cyprinus carpio strains in the Centre of Carpathian Basin. *Aquaculture*, *554*, 738116.

Tóth, B., Khosravi, R., Ashrafzadeh, M. R., Bagi, Z., Fehér, M., Bársony, P., Kovács, G., & Kusza, S. (2020). Genetic Diversity and Structure of Common Carp (Cyprinus carpio L.) in the Centre of Carpathian Basin: Implications for Conservation. *Genes*, *11*(11), 1268. https://doi.org/10.3390/genes11111268

Tóth, B., Várkonyi, E., Hidas, A., Meleg, E. E., & Váradi, L. (2005). Genetic analysis of offspring from intra- and interspecific crosses of Carassius auratus gibelio by chromosome and RAPD analysis. *Journal of Fish Biology*, *66*(3), 784–797. https://doi.org/10.1111/j.0022-1112.2005.00644.x

Touriya, A., Rami, M., Cattaneo-Berrebi, G., Ibanez, C., Augros, S., Boissin, E., Dakkak, A., & Berrebi, P. (2003). Primers for EPIC amplification of intron sequences for fish and other vertebrate population genetic studies. *BioTechniques*, *35*(4), 676–682. https://doi.org/10.2144/03354bm02

Toussaint, A., Beauchard, O., Oberdorff, T., Brosse, S., & Villéger, S. (2016). Worldwide freshwater fish homogenization is driven by a few widespread non-native species. *Biological Invasions*, *18*(5), 1295–1304. https://doi.org/10.1007/s10530-016-1067-8

Toussaint, A., Charpin, N., Beauchard, O., Grenouillet, G., Oberdorff, T., Tedesco, P. A., Brosse, S., & Villéger, S. (2018). Non-native species led to marked shifts in functional diversity of the world freshwater fish faunas. *Ecology Letters*, *21*(11), 1649–1659. https://doi.org/10.1111/ele.13141

Troell, M., Naylor, R. L., Metian, M., Beveridge, M., Tyedmers, P. H., Folke, C., Arrow, K. J., Barrett, S., Crépin, A.-S., Ehrlich, P. R., Gren, Å., Kautsky, N., Levin, S. A., Nyborg, K., Österblom, H., Polasky, S., Scheffer, M., Walker, B. H., Xepapadeas, T., & de Zeeuw, A. (2014). Does aquaculture add resilience to the global food system? *Proceedings of the National Academy of Sciences*, *111*(37), 13257–13263. https://doi.org/10.1073/pnas.1404067111

Unmack, P. J., Sandoval-Castillo, J., Hammer, M. P., Adams, M., Raadik, T. A., & Beheregaray, L. B. (2017). Genome-wide SNPs resolve a key conflict between sequence and allozyme data to confirm another threatened candidate species of river blackfishes (Teleostei: Percichthyidae: Gadopsis). *Molecular Phylogenetics and Evolution*, *109*, 415–420. https://doi.org/10.1016/j.ympev.2017.02.013

Urbany, B., Horvath, L., Horvath, A., & Bokor, Z. (2008). Cryopreservation of Pike Perch Sperm in Hatchery Conditions. *Israeli Journal of Aquaculture-Bamidgeh*, *60*, 20493.

Urbányi, B., Horváth, Á., Varga, Z., Horváth, L., Magyary, I., & Radics, F. (1999). Effect of extenders on sperm cryopreservation of African catfish, *Clarias gariepinus* (Burchell). *Aquaculture Research*, *30*(2), 145–151. https://doi.org/10.1046/j.1365-2109.1999.00313.x

Vainikka, A., Kortet, R., Paukku, S., Rantala, M. J., & Pirhonen, J. (2005). What do male tench, Tinca tinca, advertise with morphological ornaments? *Acta Ethologica*, *8*(2), 70–78. https://doi.org/10.1007/s10211-005-0002-2

Van Der Bank, F. H. (1994). Molecular systematics of fifteen old world cichlid species using allozyme data. *Biochemical Systematics and Ecology*, *22*(8), 791–801. https://doi.org/10.1016/0305-1978 (94)90082-5

van der Veer, G., & Nentwig, W. (2015). Environmental and economic impact assessment of alien and invasive fish species in Europe using the generic impact scoring system. *Ecology of Freshwater Fish*, *24*(4), 646–656. https://doi.org/10.1111/eff.12181

Van Oppen, M. J., Klerk, H., Olsen, J. L., & Stam, W. T. (1996). Hidden diversity in marine algae: Some examples of genetic variation below the species level. *Journal of the Marine Biological Association of the United Kingdom*, *76*(1), 239–242.

Verreycken, H., Anseeuw, D., Thuyne, G. V., Quataert, P., & Belpaire, C. (2007). The non-indigenous freshwater fishes of Flanders (Belgium): Review, status and trends over the last decade. *Journal of Fish Biology*, *71*(sd), 160–172. https://doi.org/10.1111/j.1095-8649.2007.01679.x

Vetemaa, M., Eschbaum, R., Albert, A., & Saat, T. (2005). Distribution, sex ratio and growth of Carassius gibelio (Bloch) in coastal and inland waters of Estonia (north-eastern Baltic Sea). *Journal of Applied Ichthyology*, *21*(4), 287–291. https://doi.org/10.1111/j.1439-0426.2005.00680.x

Veteŝník, L., Papoušek, I., Halačka, K., Lusková, V., & Mendel, J. (2007). Morphometric and genetic analysis of Carassius auratus complex from an artificial wetland in Morava River floodplain, Czech Republic. *Fisheries Science*, *73*(4), 817–822.

Viveiros, A. T., Amaral, T. B., Orfão, L. H., Isau, Z. A., Caneppele, D., & Leal, M. C. (2011). Sperm cryopreservation of tiete tetra Brycon insignis (Characiformes): Effects of cryoprotectants, extenders, thawing temperatures and activating agents on motility features. *Aquaculture Research*, *42*(6), 858–865.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T. van de, Hornes, M., Friters, A., Pot, J., Paleman, J., Kuiper, M., & Zabeau, M. (1995). AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research*, *23*(21), 4407–4414. https://doi.org/10.1093/nar/23.21.4407

Vos, S. D., Bossier, P., Stappen, G. V., Vercauteren, I., Sorgeloos, P., & Vuylsteke, M. (2013). A first AFLP-Based Genetic Linkage Map for Brine Shrimp Artemia franciscana and Its Application in Mapping the Sex Locus. *PLOS ONE*, *8*(3), e57585. https://doi.org/10.1371/journal.pone.0057585

Walters, M., & Scholes, R. J. (Eds.). (2017). *The GEO Handbook on Biodiversity Observation Networks*. Springer International Publishing. https://doi.org/10.1007/978-3-319-27288-7

Wang, J., Min, W., Guan, M., Gong, L., Ren, J., Huang, Z., Zheng, H., Zhang, J., Liu, H., & Han, Y. (2006). Tench farming in China: Present status and future prospects. *Aquaculture International*, *14*(1), 205–208. https://doi.org/10.1007/s10499-005-9026-x

Wang, X., Hua, Q., Wu, L., Weng, Z., Huang, W., & Meng, Z. (2021). Development of a multiplex microsatellite assay for parentage assignment in orange-spotted groupers (Epinephelus coioides). *Journal of the World Aquaculture Society*, *52*(2), 435–444. https://doi.org/10.1111/jwas.12774

Weber, J. L., & May, P. E. (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics*, *44*(3), 388–396.

Weir, B. S. (1996). *Genetic data analysis II. Sunderland, MA*. Sinauer Associates, Inc.

Wheeler, A. (2000). Status of the crucian carp, Carassius carassius (L.), in the UK. *Fisheries Management and Ecology*, *7*(4), 315–322. https://doi.org/10.1046/j.1365-2400.2000.007004315.x

Whitehead, A., Anderson, S. L., Kuivila, K. M., L. Roach, J., & May, B. (2003). Genetic variation among interconnected populations of Catostomus occidentalis: Implications for distinguishing impacts of contaminants from biogeographical structuring. *Molecular Ecology*, *12*(10), 2817–2833.

Wolfram, G., & Mikschi, E. (2007). *Rote Liste der Fische (Pisces) Österreichs*. na.

Wolnicki, J., Kamiński, R., & Sikorska, J. (2017). Combined effects of water temperature and daily food availability period on the growth and survival of tench (*Tinca tinca*) larvae. *Aquaculture Research*, *48*(7), 3809–3816. https://doi.org/10.1111/are.13207

Wołos, A., Zdanowski, B., & Wierzchowska, M. (2009). Long-term changes in commercial fish catches in Lake Mamry Północne (northeastern Poland) on the background of physical, chemical, and biological data. *Fisheries & Aquatic Life*, *17*(4), 195–210. https://doi.org/10.2478/v10086-009-0011-2

Wouters, J., Janson, S., Lusková, V., & Olsén, K. H. (2012). Molecular identification of hybrids of the invasive gibel carp Carassius auratus gibelio and crucian carp Carassius carassius in Swedish waters. *Journal of Fish Biology*, *80*(7), 2595–2604. https://doi.org/10.1111/j.1095-8649.2012.03312.x

Wright, J. M., & Bentzen, P. (1995). Microsatellites: Genetic markers for the future. In G. R. Carvalho & T. J. Pitcher (Eds.), *Molecular Genetics in Fisheries* (pp. 117–121). Springer Netherlands. https://doi.org/10.1007/978-94-011-1218-5_7

Wright, R. M., & Giles, N. (1991). The population biology of tench, Tinea tinea (L.), in two gravel pit lakes. *Journal of Fish Biology*, *38*(1), 17–28. https://doi.org/10.1111/j.1095-8649.1991.tb03087.x

Wu, L., & Yang, J. (2012). Identifications of Captive and Wild Tilapia Species Existing in Hawaii by Mitochondrial DNA Control Region Sequence. *PLOS ONE*, *7*(12), e51731. https://doi.org/10.1371/journal.pone.0051731

Xiao, Y., Guan, S., Liu, Q., Liu, H., Yu, D., Ma, D., Xu, S., Liu, J., Dai, M., Xiao, Z., & Li, J. (2018). Genetic characteristics of broodstock and offspring of the seven-band grouper (*Hyporthodus septemfasciatus*) using fluorescent-AFLP markers. *Journal of the Marine Biological Association of the United Kingdom*, *98*(2), 261–267. https://doi.org/10.1017/S0025315416001260

Xu, T., Quan, X., Sun, Y., Zhao, K., & Wang, R. (2010). A First Set of Polymorphic Microsatellite Loci from the Marbled Rockfish, Sebastiscus marmoratus. *Biochemical Genetics*, *48*(7), 680–683. https://doi.org/10.1007/s10528-010-9349-9

Yang, H., Hu, E., Cuevas-Uribe, R., Supan, J., Guo, X., & Tiersch, T. R. (2012). High-throughput sperm cryopreservation of eastern oyster Crassostrea virginica. *Aquaculture*, *344*, 223–230.

Yang, L., Sado, T., Hirt, M. V., Pasco-Viel, E., Arunachalam, M., Li, J., Wang, X., Freyhof, J., Saitoh, K., & Simons, A. M. (2015). Phylogeny and polyploidy: Resolving the classification of cyprinine fishes (Teleostei: Cypriniformes). *Molecular Phylogenetics and Evolution*, *85*, 97–116.

Yerli, S., Mangıt, F., emiroğlu, Ö., Vedat, Y., Uysal, R., Unlu, E., Alp, A., Buhan, E., Yildirim, T., & Zengin, M. (2014). Distribution of Invasive Carassius gibelio (Bloch, 1782)(Teleostei: Cyprinidae) in Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*.

Yigui, D. J. X. Y. J., & Shixin, S. (1993). THE ANALYSIS OF HETEROLOGOUS GENETIC MATERIALS IN ALLOGYNOGENETIC CRUCIAN CARP AND ITS ARTIFICIAL HYBRIDS [J]. *Acta Hydrobiologica Sinica*, *1*.

Yoon, J.-M., & Park, H.-Y. (2002). Genetic Similarity and Variation in the Cultured and Wild Crucian Carp (Carassius carassius) Estimated with Random Amplified Polymorphic DNA. *Asian-Australasian Journal of Animal Sciences*, *15*(4), 470–476. https://doi.org/10.5713/ajas.2002.470

Young, W. P., Ostberg, C. O., Keim, P., & Thorgaard, G. H. (2001). Genetic characterization of hybridization and introgression between anadromous rainbow trout (Oncorhynchus mykiss irideus) and coastal cutthroat trout (O. clarki clarki). *Molecular Ecology*, *10*(4), 921–930.

Yue, G. H., & Orban, L. (2002). Polymorphic microsatellites from silver crucian carp (Carassius auratus gibelio Bloch) and cross-amplification in common carp (Cyprinus carpio L.). *Molecular Ecology Notes*, *2*(4), 534–536. https://doi.org/10.1046/j.1471-8286.2002.00308.x

Yue, G. H., & Wang, L. (2017). Current status of genome sequencing and its applications in aquaculture. *Aquaculture*, *468*, 337–347. https://doi.org/10.1016/j.aquaculture.2016.10.036

Zaijie, D., Nian, L. I. U., Jianjun, F. U., Wenbin, Z. H. U., Lanmei, W., & Shengyan, S. U. (2018). Genetic analysis for six wild and selection populations of common carp (Cyprinus carpio) using microsatellites. **南方水**产科学, *14*(4), 46–55.

Zerunian, S. (2007). Problematiche di conservazione dei Pesci d'acqua dolce italiani. *Biologia Ambientale*, *21*(2), 49–55.

Zhang, J., Ma, W., Wang, W., Gui, J.-F., & Mei, J. (2016). Parentage determination of yellow catfish (Pelteobagrus Fulvidraco) based on microsatellite DNA markers. *Aquaculture International*, *24*(2), 567–576. https://doi.org/10.1007/s10499-015-9947-y

Zhang, J.-M., & An, J. (2007). Cytokines, Inflammation and Pain. *International Anesthesiology Clinics*, *45*(2), 27–37. https://doi.org/10.1097/AIA.0b013e318034194e

Zhang, Q., Allen, S. K., & Reece, K. S. (2005). Genetic Variation in Wild and Hatchery Stocks of Suminoe Oyster (Crassostrea ariakensis) Assessed by PCR-RFLP and Microsatellite Markers. *Marine Biotechnology*, *7*(6), 588. https://doi.org/10.1007/s10126-004-5105-7

Zhang, Z., Schwartz, S., Wagner, L., & Miller, W. (2000). A Greedy Algorithm for Aligning DNA Sequences. *Journal of Computational Biology*, *7*(1–2), 203–214. https://doi.org/10.1089/10665270050081478

Zheng, W., Stacey, N. E., Coffin, J., & Strobeck, C. (1995). Isolation and characterization of microsatellite loci in the goldfish Carassius auratus. *Molecular Ecology*, *4*(6), 791–792. https://doi.org/10.1111/j.1365-294X.1995.tb00282.x

Zheng, X. H., Lu, C. Y., Zhao, Y. Y., Lee, C., Cao, D. C., Chang, Y. M., Liang, L. Q., & Sun, X. W. (2010). A Set of Polymorphic Trinucleotide and Tetranucleotide Microsatellite Markers for Silver Crucian Carp (Carassius auratus gibelio) and Cross-Amplification in Crucian Carp. *Biochemical Genetics*, *48*(7–8), 624–635. https://doi.org/10.1007/s10528-010-9344-1

Zheng, X., Kuang, Y., Lü, W., Cao, D., & Sun, X. (2014). Transcriptome-derived EST–SSR markers and their correlations with growth traits in crucian carp Carassius auratus. *Fisheries Science*, *80*(5), 977–984. https://doi.org/10.1007/s12562-014-0782-2

Zhu, B. F., Huang, Y., Dai, Y. G., Bi, C. W., & Hu, C. Y. (2013). Genetic diversity among red swamp crayfish (Procambarus clarkii) populations in the middle and lower reaches of the Yangtze River based on AFLP markers. *Genetics and Molecular Research*, *12*(1), 791–800. https://doi.org/10.4238/2013.March.13.8

Zhu, W., Li, X., Qu, D., Liu, Y., & Clarke, S. (2014). Cryopreservation of sperm from wild greenlip abalone (Haliotis laevigata) in Australia. *Aquaculture*, *432*, 60–66. https://doi.org/10.1016/j.aquaculture.2014.04.027

10.2 Supplemental figures and tables

Table S1. Shows the Bootstrap 95% intervals in (Tench).

Bootstrap resampling over loci
95% Confidence Interval
F_{st} using ENA
pop 1 2 3 4 5 6
2 0.026597
3 0.012056 0.039435
4 0.055988 0.034012 0.049325
5 0.035837 0.040072 0.035934 0.018674
6 -0.007836 -0.013096 0.008634 0.028294 0.005958
7 0.024281 0.061687 0.020606 0.125868 0.018682 -0.001042
1 2 3 4 5 6
2 0.112890
3 0.053829 0.187639
4 0.221342 0.085823 0.232698
5 0.133531 0.101153 0.166058 0.106995
6 0.030467 0.088785 0.081721 0.262879 0.087417
7 0.086011 0.216891 0.125099 0.301231 0.262631 0.115319
Bootstrap resampling over loci
95% Confidence Interval
Dc using INA
pop 1 2 3 4 5 6
2 0.182155
3 0.160872 0.188869
4 0.240800 0.187950 0.166871
5 0.201712 0.173565 0.158259 0.168431
6 0.174579 0.153780 0.173678 0.196591 0.159823
7 0.244113 0.228536 0.193688 0.243137 0.196719 0.180876
1 2 3 4 5 6
2 0.278428
3 0.258626 0.307429
4 0.359948 0.275390 0.349669
5 0.309089 0.296183 0.314396 0.285505
6 0.306838 0.237443 0.319085 0.338340 0.303587
7 0.330250 0.356527 0.301229 0.411157 0.334063 0.330196

Table S2. The mean log likelihood data and Evanno's delta K of the hierarchical in STRUCTURE analysis in (Tench).

# k	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Delta K
1	10	-3428.4700	0.0483	NA	NA	NA
2	10	-3390.0200	5.0376	38.45	1.38	0.27394
3	10	-3352.9500	16.4488	37.07	0.36	0.021886
4	10	-3316.2400	11.4254	36.71	46.47	4.067243
5	10	-3326.0000	17.2303	-9.76	41.42	2.403900
6	10	-3377.1800	72.2788	-51.18	87.78	1.214465
7	10	-3516.1400	53.9164	-138.96	71.51	1.326313
8	10	-3583.5900	80.2669	-67.45	23.04	0.287042
9	10	-3628.0000	77.1919	-44.41	46.15	0.59786
10	10	-3718.5600	151.2057	-90.56	NA	NA

Table S3. The mean log likelihood data and Evanno's delta K of the hierarchical in STRUCTURE analysis in (Crucian carp).

K	**Reps**	**Mean LnP(K)**	**Stedev LnP(K)**	**Ln'(K)**	**|Ln"(K)|**	**Delta K**
1	10	-14582.010000	0.882484	----	-----	-----
2	10	-12219.690000	1.503662	2362.320000	1830.750000	1217.52745
3	10	-11688.120000	6.999016	531.570000	168.510000	24.076242
4	10	-11325.060000	3.6485	363.060000	254.600000	69.782094
5	10	-11216.600000	6.717804	108.460000	124.470000	18.528377
6	10	-11232.610000	28.533117	-16.010000	17.780000	0.623136
7	10	-11230.840000	83.932184	1.770000	39.970000	0.476218
8	10	-11189.100000	104.807177	41.740000	96.530000	0.921025
9	10	-11243.890000	187.877593	-54.790000	25.130000	0.133757
10	10	-11273.550000	200.618772	-29.660000	24.050000	0.119879
11	10	-11327.260000	245.509503	-53.710000	90.910000	0.370291
12	10	-11290.060000	260.954497	37.200000	562.280000	2.154705
13	10	-11815.140000	538252486	-525.080000	-----	-----

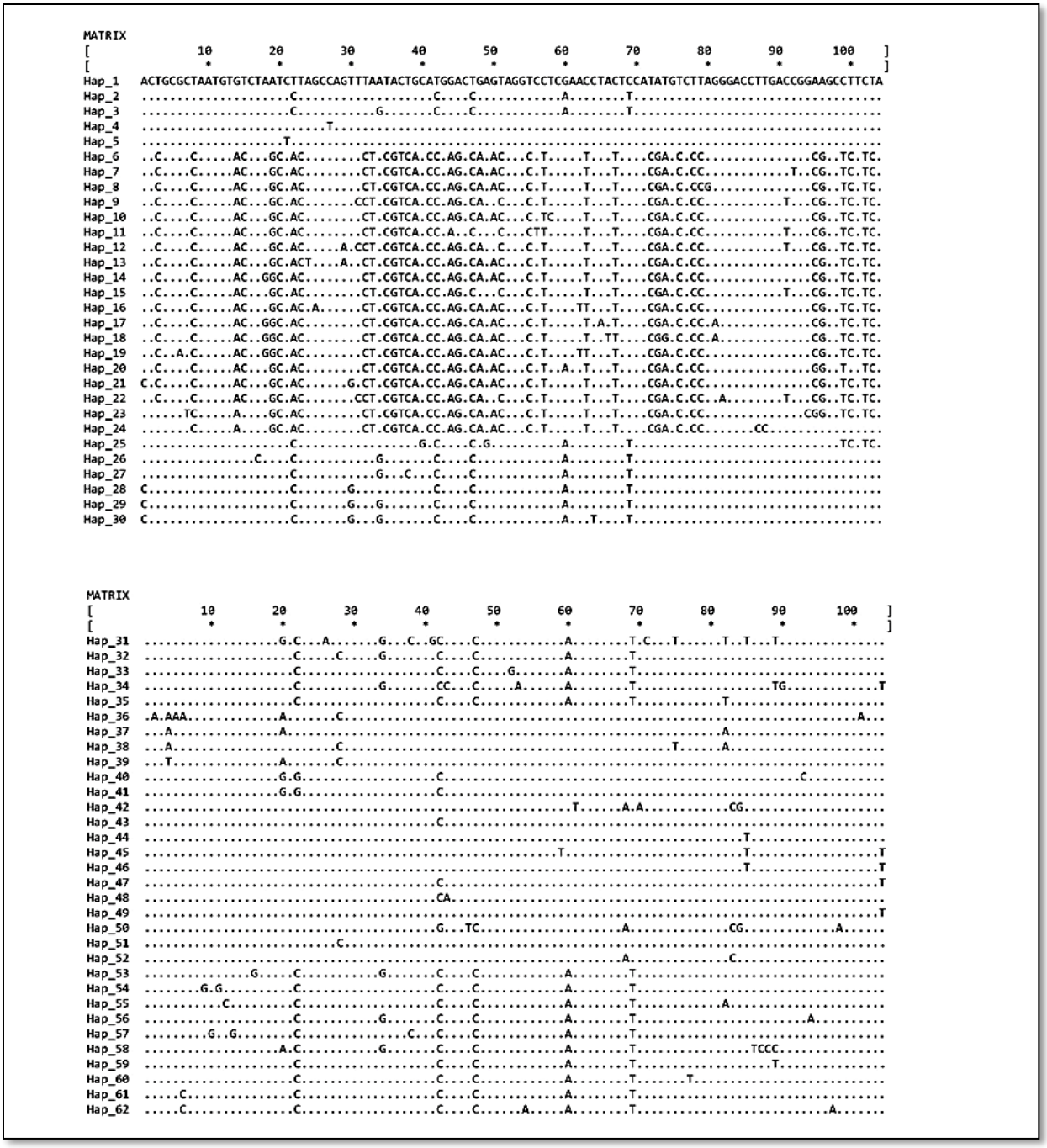


Figure S1. Polymorphic sites in Cytochrome C Oxidase 1 (COI) haplotypes of the Hungarian crucian carp, made by DnaSP 5.10 (Librado & Rozas, 2009).

11. LIST OF PUBLICATIONS

Peer-reviewed articles with impact factor

1-Q1; impact factor: 2.465

Al Fatle, F. A., Meleg, E. E., Sallai, Z., Szabó, G., Várkonyi, E., Urbányi, B., Kovács, B., Molnár, T., & Lehoczky, I. (2022). Genetic Structure and Diversity of Native Tench (*Tinca tinca* L. 1758) Populations in Hungary—Establishment of a Basic Knowledge Base for a Breeding Program. *Diversity*, *14*(5), 336. https://doi.org/10.3390/d14050336.

2-Q1; impact factor: 2.752

Molnár, T., Lehoczky, I., Edviné Meleg, E., Boros, G., Specziár, A., Mozsár, A., Vitál, Z., Józsa, V., Allele, W., & Urbányi, **Al Fatle, F.A.,** Kovács, B., B. (2021). Comparison of the Genetic Structure of Invasive Bigheaded Carp (Hypophthalmichthys spp.) Populations in Central-European Lacustrine and Riverine Habitats. *Animals*, *11*(7), 2018.

International conferences

Al-Fatle, F. A., Meleg, E. E., Molnár, T., & Lehoczky, I. (2019). A PRELIMINARY GENETIC STUDY FOR THE CONSERVATION OF HUNGARIAN CRUCIAN CARP (*CARASSIUS CARASSIUS*) POPULATIONS. *Живые Системы-2019*, 88–89.

F. Al-Fatle Ali Abdulhur, E. Edviné Meleg, K. Ihász, T. Molnár, B. Kovács, I. Lehoczky (2019) Conservation of the native Cyprinid species in Hungary – preliminary results on the genetic variability of Crucian carp (*Carassius carassius* L. 1758) and Tench (*Tinca tinca* L. 1758) populations and stocks. Aquaculture Europe 19. Berlin, Germany, 2019.10.07.-10. Book of Abstracts p: 54-55.

F. A. Al Fatle, E. Edviné Meleg, T. Molnár, B. Kovács, & I. Lehoczky. (2021). Genetic characterization of crucian carp (*Carassius carassius* L. 1758) populations in Hungary using microsatellite markers for conservation. CASEE Universities as Laboratories for New Paradigms in Life Sciences and Related Disciplines" June 7th – 8th, 2021, 30–31.

Al Fatle Fatema Ali, Tamás Molnár, Erika Edviné Meleg, Gergely Szabó, Gábor Fekete, Zoltán Sallai, Balázs Kovács and István Lehoczky (2021) Genetic variability of Hungarian Tench (*Tinca tinca* Linnaeus 1758) populations – preliminary results, 56th Croatian & 16th International Symposium on Agriculture, Vodice, Croatia, 2021.09.05.-10. Book of Abstracts pp: 211.

Al Fatle Fatema Ali, Tamás Molnár, Erika Edviné Meleg, Gergely Szabó, Gábor Fekete, Zoltán Sallai, Balázs Kovács and István Lehoczky (2021) Genetic Analysis of seven natural populations of Tench (*Tinca tinca L. 1758*) in Hungary – establishment the biological basis of selective breeding. Aquaculture Europe, Madeira, 2021.10.04.-07. Book of Abstracts p: 350-351.

Fatema Ali Al Fatle, Tamás Molnár, Erika Edviné Meleg, Gergely Szabó, Gábor Fekete, István Kópor, Zoltán Sallai, Gergely Bernáth, Zoltán Bokor, Balázs Kovács and István Lehoczky (2022) Establishment of novel cyprinid genebanks in the National Centre for Biodiversity and Gene Conservation. 57th Croatian & 17th International Symposium on Agriculture, Vodice, Croatia, 2022.06.19.-24. Book of Abstracts pp: 219.

Abstracts and posters in Hungary

Al Fatle Fatema Ali, Quynh Nguyen Thi, Erika Edviné Meleg, Tamás Molnár, Balázs Kovács, Dóra Kánainé S., Lehoczky István (2018) Genetic characterization of Hungarian Crucian carp populations using microsatellite markers for genetic conservation - preliminary results. XLII. Halászati Tudományos Tanácskozás, Szarvas. 2018.05.30.-31. Összefoglalók gyűjteménye p: 47.

Lehoczky István, **Al Fatle Fatema Ali**, Tóth-Ihász Katalin, Edviné Meleg Erika, Molnár Tamás, Kovács Balázs, Őshonos pontyfélék (széles kárász, compó) génmegőrzési rendszerének kialakítása – A biológiai alapok felmérése (előzetes eredmények). (2019) XLIII. Halászati Tudományos Tanácskozás, Szarvas. 2019.05.29.-30. Összefoglalók gyűjteménye p: 105-107. ISSN 0230-8312.

Al Fatle Fatema Ali, Molnár Tamás, Edviné Meleg Erika, Szabó Gergely, Fekete Gábor, Sallai Zoltán, Kovács Balázs és Lehoczky István (2021) Hét hazai compó (*Tinca tinca* L 1758) populáció genetikai változatosságának vizsgálata – a tenyésztőmunka biológiai alapjainak megteremtése. A Magyar Biológiai Társaság XXXII. Vándorgyűlése, Tápiószele 2021. November 25-26. Összefoglalók gyűjteménye p: 100.

12. ACKNOWLEDGEMENTS

Firstly, let me begin by saying how grateful I am to God for being by my side during this demanding journey and for giving me the strength to overcome obstacles in order to complete my PhD. Secondly, I must express my gratitude to the Iraqi Ministry of Higher Education for nominating me and the Hungarian government for awarding me the Stipendium Hungaricum Scholarship, which allowed me to pursue a Ph.D.

As a PhD student, I have been fortunate enough to have the support of some special people along the way. Yet, I would like to thank my supervisor, Dr. István Lehoczky, Head of the Department of Aquatic Genetic Resources Conservation, National Centre for Biodiversity and Gene Conservation, Institute for Farm Animal Gene Conservation, for his scientific support, patience, and guidance during my PhD study. Without his help and encouragement, this project would not have been achieved. I would also like to thank Dr. Balázs Kovács, from the Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, for his noble guidance and help with the analysis.

I am grateful to Dr. Tamás Molnár, from the National Centre for Biodiversity and Gene Conservation, Institute for Farm Animal Gene Conservation, for his useful remarks and suggestions, as well as his assistance with statistical analysis of the data. I am also thankful to all members of the staff at the Institute for Farm Animal Gene Conservation for their support and encouragement during my PhD study. Erika Edviné Meleg's guidance and advice during the experimental work, as well as her explanations of different techniques, are deeply appreciated.

I would also like to thank Dr. Hidas András and Dr. Pálinkás Nóra for reviewing my dissertation. Their wise suggestions and thought-provoking questions helped improve the quality of my PhD dissertation.

My great appreciation to Prof. Dr. Zoltán Nagy, Head of the Doctoral School of Biological Sciences at the Hungarian University of Agricultural and Life Sciences, for giving me the opportunity to become a PhD student in his school. I also wish to thank Associate Prof. Dr. János György Nagy, Secretary of the Biological Sciences School; Csilla Kánai, Head of the International Relations Centre; Judit Tallárom Czingili, International Relations Coordinator; Zsuzsanna Tassy,

International PhD Coordinator; and Mónika Törökné Hajdú, Head of the Doctoral and Habilitation Center, who have all been very helpful and supportive over the years.

Words cannot express my gratitude to my former supervisor, Prof. Dr. Péter Nagy, Head of the Department of Zoology and Animal Ecology at the Hungarian University of Agriculture and Life Sciences for making me feel so welcome and giving me advice when I first moved to Hungary. Even with his busy schedule, he was always willing to lend a hand, whether it was with a research problem or a worldly topic. I feel proud to have had the opportunity to work with him, even if it was for one year due to a change of major.

A big thanks to Prof. Dr. Hussein Daood, from the Doctoral School of Food Sciences, Hungarian University of Agriculture and Life Sciences, who has been a supportive father to me here, for his encouragement and guidance, and for giving me the confidence to finish my PhD.

Dr. Krisztina Liptói, Head of the Department of Gene Conservation Science and Animal Husbandry, deserves a special thanks for being a constant source of inspiration and moral support for me. Her warm words have always made me feel like I'm part of her family. She is an amazing leader and role model.

My heartfelt gratitude also goes to my officemate, Dr. Thieu Ngoc Lan Phuong, who was always willing to help with any issues I had.

I extend my sincere thanks and appreciation to my best friends for their help and support during my PhD journey. From the bottom of my heart, I would like to thank Sára Szarka. Her genuine love, care, and encouragement meant a lot to me, especially when things would get a little difficult or during moments of homesickness. Tamás Holló, Qigong expert and trainer, I am very thankful not only for your psychological and moral support but also for your encouragement and advice in my business endeavors. Some special words of gratitude go to my PhD colleagues; Dr. Mahek Anand, Dr. Imane Haddidi, Dr. Mutaz Al-Alawi, Dr. Osama Kanbar, Dr. Dhanashree Katekhaye, and Sama Huseynova, with whom I have shared bright and dark moments over the course of the study years. I am also very grateful to my friends, Associate Prof. Dr. Sura Fouad, Associate Prof. Dr. Lamia Abdel Reda, and Dr. Jwana Yassin, from the University of Baghdad, who, while being thousands of miles away, have been a constant source of moral support and friendship during this journey.

I gratefully acknowledge the generous financial support provided by the Stipendium Hungaricum Scholarship and the Tempus Public Foundation, as well as the VEKOP-2.3.2-15-2016-00012 (National Research, Development, and Innovation Office) and TKP2020-NKA-16 (National Research, Development, and Innovation Office) projects.

I would like to account my special appreciation and thanks to all of the fishermen and farmers who helped us in collecting samples from various geographical regions in Hungary.

A special gratitude goes to my father, Ali Al Fatle, for the loving support and optimism he has shown me throughout the course of this study. I wouldn't be where I am now without his encouragement, love, and blessings. My heartfelt gratitude also goes to my late mother, who was a driving force behind my career growth. I pray for her soul's eternal rest in heaven. My sisters, Azhar, Sumea, and Marwa, and my brothers deserve all the thanks in the world for being there for me and putting up with my fears and complaints during my study years.

Finally, I owe my sincere thanks to my husband for supporting and encouraging me whenever I needed him. The last word goes to my lovely kids, Ahmed and Mousa, who helped me reach my goal by being extremely patient. Much appreciation for the strength you gave me. ″ I dedicate this thesis to the angels (Ahmed and Mousa) ‟. Without their support and patience, this study would not be possible.