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Microsatellite Marker-Based Study of Genetic Diversity of Common Carp Strain Representatives Raised in Lithuanian Fish Farms

Master's Thesis

Genetics Study Programme

Supervisor

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LIST OF ABBREVIATIONS

AGE – Agarose Gel Electrophoresis

AMOVA – Analysis of Molecular Variance

EST – Expressed Sequence Tags

HWE – Hardy-Weinberg Equilibrium

MtDNA – Mitochondrial DNA

PCoA – Principal Coordinate Analysis

SNP – Single Nucleotide Polymorphism

WGD – Whole Genome Duplication

VILNIAUS UNIVERSITETAS
GYVYBĖS MOKSLŲ CENTRAS

Gabriela Liubartaitė

Lietuvos žuvininkystės ūkiuose auginamų paprastojo karpio veislių atstovų genetinės įvairovės tyrimas paremtas mikrosatelitinių žymenų analize

Magistro baigiamasis darbas

SANTRAUKA

Paprastasis karpis (*Cyprinus carpio* Linnaeus 1758) yra viena svarbiausių žuvų rūšių pasauliniu mastu ekonominiu bei moksliniu požiūriu. Siekiant palaikyti šios žuvies plačią morfologinę ir genetinę įvairovę, būtina sutelkti esamų veislių išsaugojimo pastangas. Šiuo darbu buvo siekiama įvertinti Lietuvos žuvininkystės ūkiuose auginamų paprastųjų karpių veislių genetinę įvairovę, populiacijų diferenciaciją ir struktūrinę sudėtį, siekiant sukurti molekulinių duomenų bazę padėsiančią išsaugoti Lietuvoje auginamas karpių veisles. Iš viso buvo tirtos 5 karpių populiacijos: 1 čekiškos kilmės (Kaplių populiacija) ir 4 lietuviškos kilmės (Arnionys, Simnas, Bartžuvė ir Šilavotas), kurių genetinė įvairovė buvo įvertinta genotipuojant mėginius panaudojus 10 mikrosatelitinių žymenų. Viso tirtose populiacijose buvo nustatyti 97 aleliai, vidutinis alelių skaičius populiacijoje svyravo nuo 3.222 (Šilavotas) iki 3.977 (Kapliai). Visose populiacijose pastebėtas mažesnis heterozigotiškumas nei tikėtasi pagal Hardy-Weinberg pusiausvyrą, tačiau Šilavote skirtumas buvo mažiausias, o Simne – didžiausias. Taigi, nustatyta, kad Šilavoto populiacija pasižymėjo mažiausia įvairove, Kaplių – didžiausia, o Simno populiacija pasižymėjo inbrydingo depresijos požymiais. Įvertinus diferenciaciją tarp populiacijų, buvo atrasta, kad Kapliai ir Šilavotas labiausiai diferencijavosi nuo likusių 3 populiacijų. Visų 5 populiacijų genetinės struktūros analizė parodė, kad Kapliai ir Šilavotas turėjo unikalią ir savitą genetinę sudėtį, būdingą grynaveislėms populiacijoms, o Arnionys, Simnas ir Bartžuvė pasižymėjo mišria, tačiau panašia struktūra, leidžiančia manyti, kad tarp šių populiacijų vykta genų mainai. Šios išvados leidžia įžvelgti dabartinę Lietuvoje auginamų paprastojo karpio veislių genetinę būklę.

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SUMMARY

The common carp (*Cyprinus carpio* Linnaeus 1758) is one of the most important fish species across the world, economically and scientifically. Given the wide morphological and genetic diversity of the species, it is imperative to maintain the measures of preservation of its many varieties. This work sought to assess the genetic diversity, population differentiation, and structural composition of common carp strains raised within Lithuanian fish farms, creating a molecular background to help with the current conservation efforts within the country. In total there were 5 carp populations, 1 of Czech origin strain (Kapliai population), and 4 of Lithuanian origin strains (Arnionys, Simnas, Bartžuvė, and Šilavotas), the genetic diversity of which were assessed by genotyping the samples with a set of 10 microsatellite markers. In total, 97 alleles were determined across all populations, with the mean allele count per population ranging from 3.222 (Šilavotas) to 3.977 (Kapliai). All populations had lower observed heterozygosities than what was expected within the Hardy-Weinberg equilibrium, however, Šilavotas had the lowest difference, while Simnas – the highest. It was found, thus, that the Šilavotas population had the lowest diversity, Kapliai – the highest, and that the Simnas population was the most at risk of imminent inbreeding depression. An insight into population differentiation found that Kapliai and Šilavotas had the highest differentiation from the remaining 3 populations. Insight into the genetic structural composition across all 5 populations has found that Kapliai and Šilavotas had unique and distinct genetic compositions reminiscent of purebred strains, while Arnionys, Simnas, and Bartžuvė had a shared structure which suggests that these are hybrid strains that experience a high degree of stock exchange between these fish farms. The current findings serve as an insight into the current genetic stature of strains raised in Lithuania.

INTRODUCTION

The common carp (*Cyprinus carpio* Linnaeus 1758) is one of the most important fish species around the world, being a highly lucrative fish sold within the aquaculture trade on a global scale (Svåsand et al., 2007). Its long domestication history within Europe has influenced the economic and cultural significance the common carp has grown across the globe, and this, in turn, had influenced the development of its many morphological and genetic varieties (Balon, 2004; Wang et al., 2010; Vilizzi, 2012). Furthermore, due to its wide geographical spread, broad genetic variability, and the complexity of its genome, the common carp is beheld as a nearly perfect model animal for the study of freshwater fish (Nedoluzhko et al., 2020; Chen et al., 2022). As such, the importance of this fish species can't be limited to its purpose as a food source alone, thus making the preservation of the species a pertinent objective.

A certain effort has been put into investigating the genetic diversity of the common carp strains raised within Lithuania, however, it has been decidedly limited. The most recent investigation was a Bachelor's work which has investigated the genetic diversity of Lithuanian common carp strains within the government-funded Šilavotas Subdivision of the Department of Inland Waters and Aquaculture, or otherwise the Šilavotas subdivision (Liubartaitė, 2022). In this work, it has been found that the national purebred strains (Bubiai and Šilavotas) raised within this fish farm had been characterized by a critically low degree of genetic diversity, thus raising worries over the state of the common carp strains raised within the country. Furthermore, with the closing of the Šilavotas subdivision, the fate of the aforementioned purebred strains has become uncertain. Put altogether, the risk of genetic diversity loss, that may eventually cause the extinction of national strains, and the lack of genetic testing within carp fisheries has become a driving factor in the rising need for a country-wide assessment of the current state of genetic composition of common carp strains raised within the country.

Aim of the work

To assess the genetic diversity and structural composition of the common carp strains raised within 5 Lithuanian fish farms.

Objectives

1. To determine the genotypes of 122 carp individuals, utilizing 10 microsatellite markers.
2. To determine the genetic diversity parameters within the 5 studied carp populations.

3. To determine the genetic differentiation and structural composition within and between the 5 studied populations.

1. LITERATURE REVIEW

1.1. The importance of *Cyprinus carpio*

1.1.1. The common carp and its long-lasting significance

The common carp (*Cyprinus carpio* Linnaeus 1758), of the large freshwater fish family *Cyprinidae*, is one of the most important fish species on a global scale, and it bears a long domestication history that spans thousands of years (Thai et al., 2007; Laloei et al., 2013; Fabrice, 2018). Found natively across multiple European and Asian regions (Figure 1.1.), this species has long since become widely introduced and distributed globally, and is known for its variation in body shape, scale coverage, skin colour, and size – all of which are a by-product of the species' extensive geographical spread and long farming history (Laloei et al., 2013; Fabrice, 2018, Tóth et al., 2020). Consequently, the common carp is a high value fish in aquaculture trade: not only does it hold cultural significance across multiple countries, it is also often used for leisure activities such as angling, and accounts for nearly 10% of all freshwater aquaculture trade (Balon, 2004; Svåsand et al., 2007; Khatei et al., 2021).

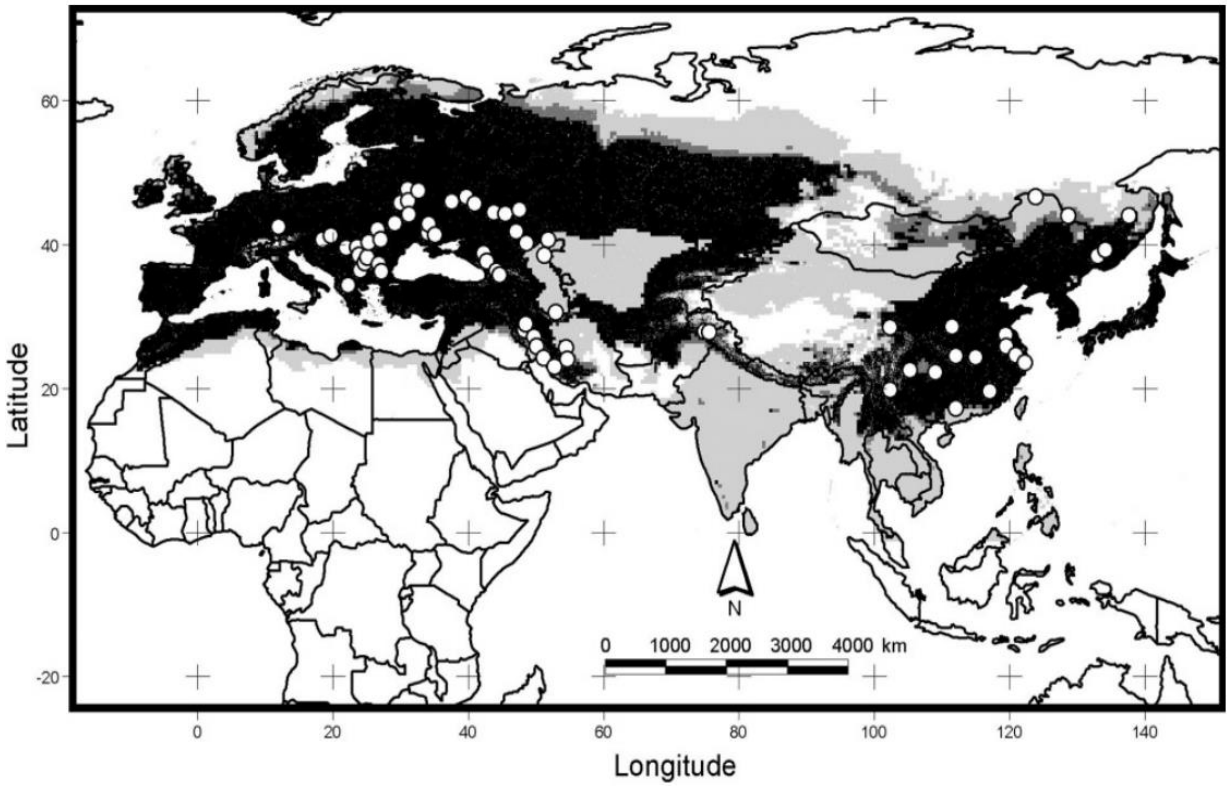

Figure 1.1. Common carp distribution natively through Eurasia. Potential distribution shown in coloured areas; grey indicating low likelihood of native distribution, black – high likelihood. Adapted from Zambrano et al., 2006.

The rise of this lucrative fish species in Europe dates back to the Roman Empire: nearly 2000 years ago, the ancient Romans have taken a liking to keeping certain fish species for food in self-fashioned reservoirs called ‘piscinae’; the common carp was among these fishes – in fact, it appears to have been the dominant fish species amongst all others (Balon, 2004). The growing popularity of these private reservoirs has thus influenced the active dissemination of carp across the Empire, and in the coming centuries the species was thereafter spread across the entire continent (Vandeputte, 2003; Balon, 2004).

In modern day, the common carp can be recognized from its tell-tale morphological traits (Figure 1.2.): an elongated, torpedo-shaped body, thick lips with two pairs of barbels, and with dense and large scales that are often yellow or grey in colour (Virbickas, 2000; Yaqoob, 2021). Besides their classic exterior traits, the common carp, as a fish species, is also known to be able to grow quite large: the common carp can grow to be upwards of 45 kilograms in weight and 150 centimetres in length (Virbickas, 2000). Although they are part of the same species, the wild and domesticated carp have significant differences, both morphological and biological, heralded by the long farming history of the species: the mouth gape and intestinal length are smaller in the domesticated carp as opposed to the wild fish; the body of the domesticated carp is deeper, less cylindrical, no doubt influenced by the lack of natural movement in unnatural bodies of water, which has likely also influenced the lower level of erythrocytes, a higher level of fat content, and worse vascularization in the organs within the domesticated form of the carp in comparison to the wild (Balon, 1995; Balon, 2004).

Figure 1.2. The common carp. Retrieved from *The Fishes of North Carolina*.

It is important to note, however, that the true wild form of the common carp is on the brink of extinction, and the feral colonies found within European waters are likely naturalized domesticated

carp stock that had escaped (or been released) into natural waters (Balon, 2004; Svåsand et al., 2007; *ClimeFish*, 2020).

1.1.2. The common carp: an ecological engineer

The common carp can survive and prosper in a variety of environments: lakes, water reservoirs, high- and low-stream rivers, flooded areas, and natural or man-made ponds (FAO, 2005). These fish are hardy and have high longevity, as well as high tolerance to environmental stressors; one of the few true requirements that the common carp needs for its environment is a proper place for spawning: these proportional spawners tend to release eggs at around 18°C, in spring or summer, preferably in inundated areas containing grass blades, and can spawn up to nearly 600,000 eggs per female fish (Balon, 1995; Kloskowski, 2011; Mutethya et al, 2020).

Generally, these fish are considered omnivores, with their primarily carnivorous diet often being supplemented by a variety of additions, such as plants, seeds, and stalks (FAO, 2005; Kloskowski, 2011). In addition to their varied diet and high tolerance to stress, the common carp is often dubbed as an "ecological engineer"; its way of bottom feeding via suction of the sediment tends to raise the turbidity of the water, thus lowering oxygen levels within the upper levels of the water and displacing nutrient availability within the water bed (Kloskowski, 2011; Rahman, 2015). This, in turn, tends to have adverse effects on biodiversity found within the ecological niche that the common carp makes a home in.

Altogether, these traits of high survivability, environment-disruptive feeding habits, and eager reproduction, culminate in the common carp being considered among the world's 100 worst invasive fish species (Mutethya et al., 2020).

1.1.3. The breeding practices of the common carp

Globally, multiple forms of carp production are utilized, ranging from pond cultures to commercial farms of a large scale; they can be raised in both, monocultural and polycultural systems, as well as either in stagnant natural waters, or in manmade water ponds (FAO, 2005; Vilizzi, 2012). The commercial breeding of these fish, most commonly, is done by either cross-breeding or selective breeding, although selective breeding, in particular, is important for the upkeep of the dozens of common carp strains which have developed over time (Vandeputte, 2003; Svåsand et al, 2007; Tóth et al., 2020).

It is believed that the common carp has been farmed in Lithuania from the the 17th century onwards, and that from the 19th century *C. carpio* has been among one of the most prevalent fish species in Lithuanian aquaculture (Pečiukėnas, 2006). In the years between the First and the Second World Wars, carps used to be raised in primarily monocultural man-made ponds, although polycultural stocks were also seldomly farmed. Today, that practice remains true in common carp fisheries: *C. carpio* are often raised in monocultural ponds (Figure 1.3.), sometimes introducing herbivore fish species alongside it (Virbickas, 2000).

Figure 1.3. Man-made common carp ponds in Lithuania. (A) Pond from the Šilavotas farm. (B) Pond from the Arnionys farm. Photographed by Dr. (HP) Dalius Butkauskas.

At the time, however, little is known about the breeding and spawning practices in current day fish farms. In 2022, the government-funded Šilavotas Subdivision of the Department of Inland Waters and Aquaculture has definitively closed down, leaving the remaining fisheries in Lithuania privately-funded and closed-practice. Before the closing of the Šilavotas subdivision, it was a common practice to share the Šilavotas carp stock with other carp fish farms in the country, thus allowing for a dissemination of genetic material between farms. Now, it is unclear whether an exchange of genetic material between the remaining farms occurs. Furthermore, the exact minutiae of carp breeding in Lithuania are currently not known: it isn't clear what metrics of spawner selection the farms utilize, or what the breeding schemes are, therefore making the stature of the current genetic structure of the common carp strains in Lithuania unknown.

1.2. The genetic intricacies of *Cyprinus carpio*

1.2.1. Genetic variation

The genetic composition of the common carp seems to be a dichotomy, separating its subspecies into the European common carp (*Cyprinus carpio carpio*) and the East Asian carp (*Cyprinus carpio haematopterus*), although the true phylogenetic differentiation of the species remains a topic of discussion (Wang et al., 2010; Dong et al., 2015). In 1967, Kirpitchnikov has suggested the existence of a third subtype, *Cyprinus carpio viridiviolaceus* (Figure 1.4.), however, this claim is yet to be substantiated on a genetic level and has later on been questioned by the author himself (Kirpitchnikov, 1967; Kirpitchnikov, 1999; Vilizzi, 2012). Instead, studies investigating the genetic composition of European and Asian carps have found that strains of each regional subtype tend to cluster with their own respective strains from either European or Asian regions, with some minor deviations (Vandeputte, 2003; Wang et al., 2010; Dong et al., 2015). Furthermore, direct genetic differences between the two subtypes have been noted, with up to 326 candidate genes determined to contain genetic differences between European and Asian carp strains (Xu et al., 2014).

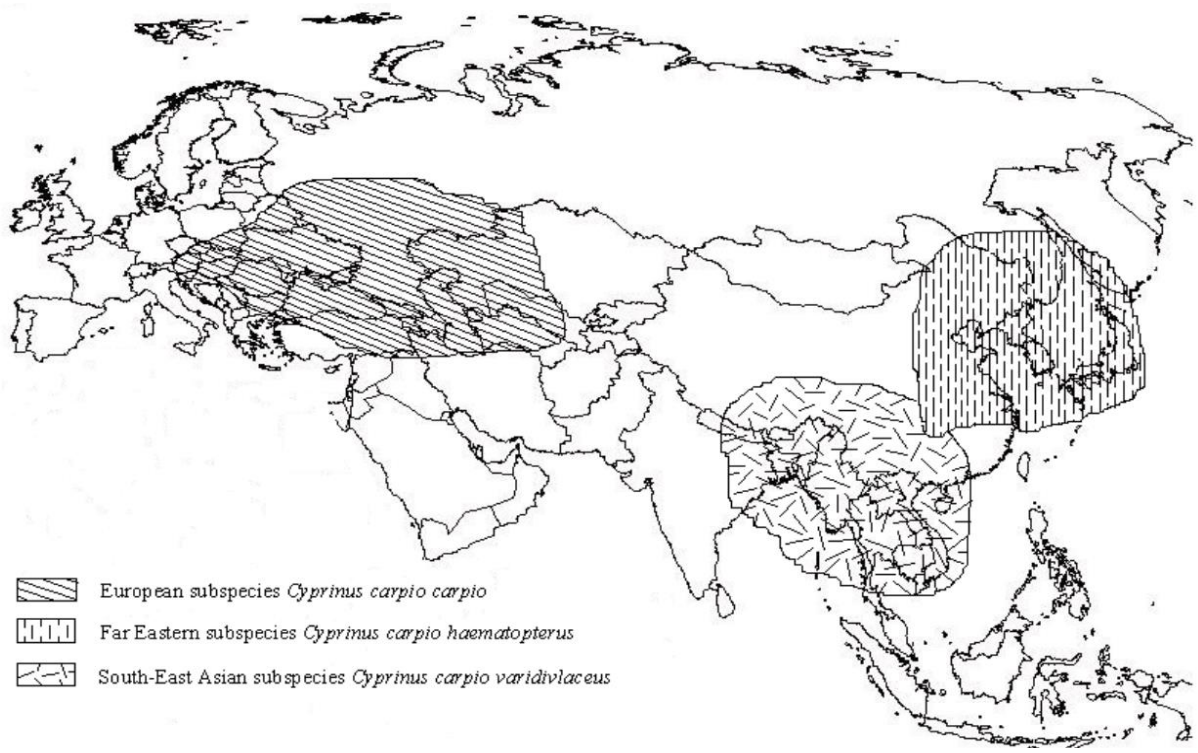

Figure 1.4. Suggested native distributions of common carp subspecies within Eurasia. Adapted from Chistiakov & Voronova, 2009.

On a morphological level, centuries of domestication, geographic dispersion, and adaptation, have influenced a range of variations across different carp populations and strains, with one of the most notable being scalation (Wang et al., 2010; Vilizzi, 2012). The four most common variations of scale cover are as follows (Figure 1.5.): (a) the fully scaled variation, with a full cover of scales across the body of the fish, resembling that of its wild ancestor; (b) the mirror variation, which bears large scales that are scattered across the body in an irregular pattern; (c) the line variation, which bears a lateral line of large scales; and lastly, (d) the leather variation, which has no scales and is considered a "nude" carp (Svåsand et al., 2007; Vilizzi, 2012). In full, a total of two alleles are responsible for the variation of scale cover: the S and N alleles, localized within two unlinked autosomal loci; the combinations of these alleles are responsible for nine possible genotypes, six of which are viable and responsible for the four described phenotypes: the scaled phenotype bears the genotype of SSnn or Ssnn; the mirror carp is coded by the genotype ssnn; the line carp has the genotypes of either SSNn or SsNn; and lastly, the nude carp has the genotype of ssNn. The genotypes ssNN, SsNN, and SSNN are unviable, suggesting to the lethal effects of the N allele (Kirpitchnikov, 1999). It is also important to note that not all viable phenotypes are equal: Kirpitchnikov himself has stated that the mirror carp and the fully scaled carp have a higher viability and disease resistance than the line and leather carp, which bear a far smaller scale cover than the former phenotypes.

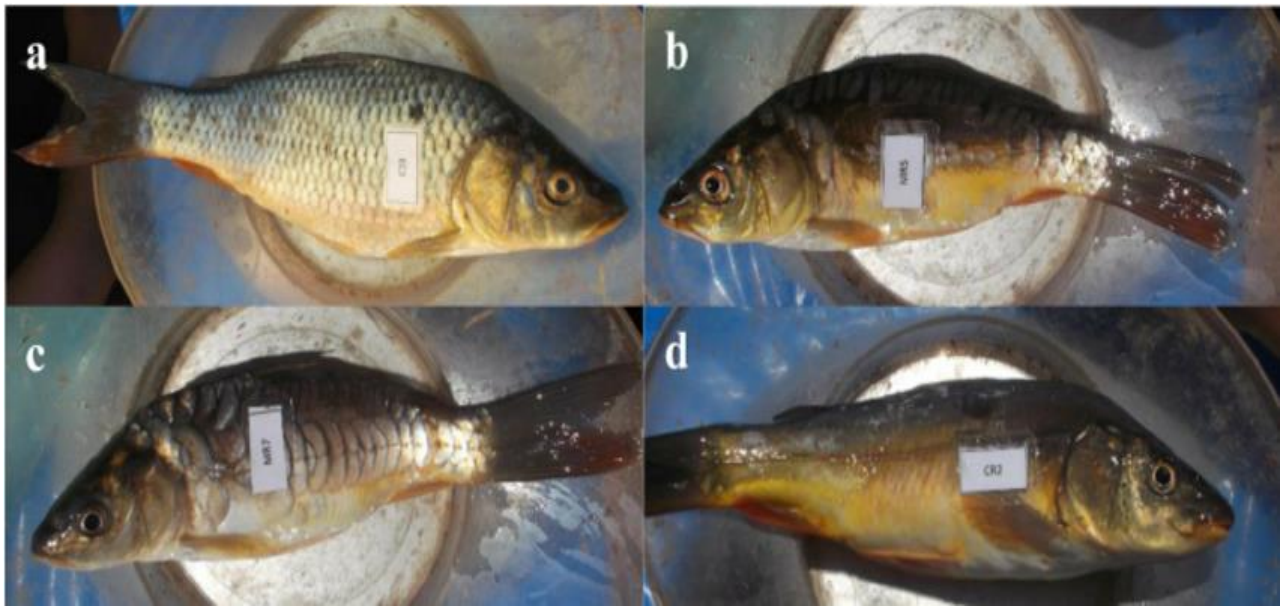

Figure 1.5. The four different scale cover types of the common carp. (a) Scaled carp, (b) mirror carp, (c) line carp, (d) nude carp. Adapted from Andria Mananjara et al., 2016.

Nishigoi, or the Asian koi fish, as it has come to be called in this day, is yet another genetic variation of the common carp (Dani et al., 2012). Besides the structural differences between *C. c. carpio* and *C. c. haematopterus*, the Koi carp bears a genetic colour aberration that provides it with its tell-tale variety of vibrant colours across the body, ranging from yellow, blue, silver, gold, red, black, and more (Balon, 1995). There are currently over 15 recognized colour aberrants of the koi fish (Balon, 2004). These colour aberrations are coded by complex interactions between several genes, including non-coding RNAs, as opposed to being coded by a singular or pair of genes (Dani et al., 2012; Luo et al., 2018). Furthermore, genetics determine only the localization of the pigmentation, meanwhile the diet of the fish determines the pigmentation itself (Khatei et al., 2021).

1.2.2. The tetraploid genome

The common carp genome comes up to a total of 1.7 Gb in size and 50 pairs of chromosomes, nearly twice the amount commonly found within the *Cyprinidae* fish family (Figure 1.6.; Xu et al., 2016; Li et al, 2021). The first common carp genome assembly was published in 2014, in which Xu et al. have combined sequencing data from several next-generation sequencing platforms to produce an assembly of *Cyprinus carpio* var. Songpu. This assembly characterized the common carp genome as containing nearly 53 thousand protein-coding genes (91.4% of which having been proven to be expressed), GC content of 37%, and 31% of the genome having been found to consist of transposable elements. Owing to the technical limitations at the time, however, the assembly is not without its faults: only half of the scaffolds have successfully anchored onto the chromosomes, leaving the remaining half ambiguous when it came to homoeologous relationships. Therefore, in 2019, the researchers have followed up on this study, conducting a more in-depth common carp genome analysis and assembly (Xu et al., 2019). In this study, the researchers have assembled the genomes of 3 different common carp strains: the Yellow River carp, the Hebao red carp, and the German Mirror carp. In comparison to the previous study, the new assemblies had improved coverage, 82-92% scaffolds anchored, and was characterized by 44 thousand annotated protein-coding genes, around 29% of transposable elements within the genomes, and with a GC content of 37%. Furthermore, in 2016 and 2021, respectively, the full transcriptome and full mitochondrial genome of the common carp have also been assembled (Kolder et al., 2016; Wang et al., 2021).

One of the more recent common carp genome assemblies was published in 2021: *Cyprinus carpio* var. Songpu was sequenced, assembled, and annotated with a much higher coverage than the

previous created assemblies (Li et al, 2021). Overall, this genome assembly was characterized by nearly 48 thousand protein-coding genes, 37% GC content, and had higher genome completeness, more anchored sequences, and more aligned RNA-seq reads than all the previous assemblies, ensuring that the current reference assembly is up to par with today's sequencing technologies.

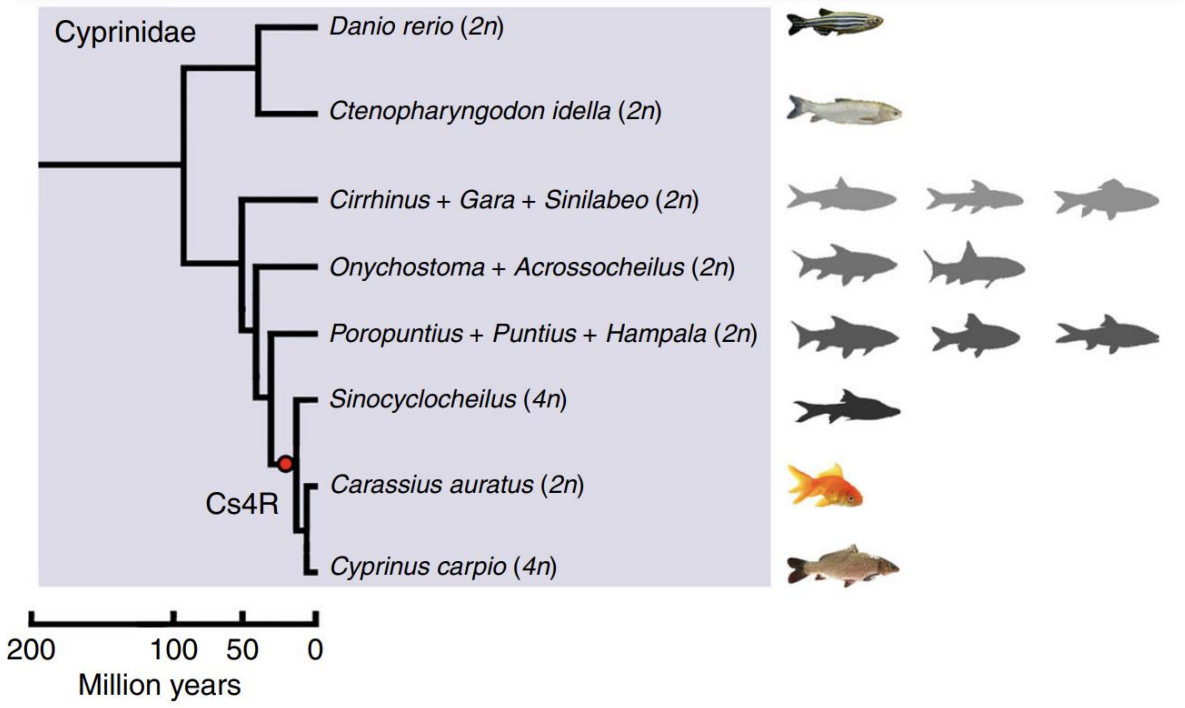

Figure 1.6. The phylogenetic relationships and genome ploidy distributions of fish species within the *Cyprinidae* family. A red dot represents the placement of the fourth (carp-specific) WGD (Cs4R). Adapted from Xu et al., 2019.

As mentioned before, the common carp is one of the few fish species that have double the amount of chromosomes commonly found within the *Cyprinidae* family (Xu et al., 2016). The hypothesis stating that the common carp genome is a tetraploid one has been raised nearly 60 years ago (Ohno et al., 1967). Over the years, research has been accruing proof of the possibility that the common carp, as a teleost fish, has undergone several whole-genome duplications (WGD); all teleost fishes have been theorized to have undergone WGD a total of 3 times, while the common carp, among a few other cyprinid fishes, has had a fourth WGD event after, thus making the genome tetraploid (Ohno et al., 1967; Chistiakov & Voronova, 2009; Xu et al., 2016). Molecular evidence implies that the most recent duplication occurred via an allopolyploidization event, as opposed to autopolyploidization (Xu et al., 2014).

Given the differing origins of the two subgenomes that make up the tetraploid carp genome, a considerable effort has been made to determine the origin of them both (Xu et al., 2019; Li et al., 2021; Chen et al., 2023). It is said that one of the subgenome progenitors must have originated from the subfamily lineage of *Barbinae* (subgenome B); the remaining subgenome (subgenome A), however, has no detected direct ancestors, suggesting that perhaps subgenome A may have belonged to a progenitor fish species that has gone extinct. Though quite a bit of effort has been made to determine the time of the latest *C. carpio* WGD (Larhammar & Risinger, 1994; Wang et al., 2012; Xu et al., 2019; Li et al., 2021), the exact time of it occurring remains elusive. In 2019, Xu et al. claimed that the tetraploidization may have occurred 12.4 million years ago, and more recently, Li et al. have claimed that the range of possible tetraploidization occurrence sits between 13.5 and 25.6 million years ago (Li et al., 2021).

Sizeable differences have been detected between the two subgenomes of the common carp. Xu et al. have noticed that while the subgenomes show no significant difference in gene structure, repeating element distribution, or GC content, they are differentially expressed: subgenome B has been found to be expressed dominantly, as well as containing a higher gene content than subgenome A (Xu et al., 2019). Similarly, Chen et al. have found subgenome differences that indicated the dominance and stability of subgenome B: the researchers have found that not only is subgenome B more conserved and stable than subgenome A, but it also contributes more to immune-related, developmental, and temperature tolerance functions (Chen et al., 2023). Tetraploid genomes, on a whole, have been found to often be evolutionarily advantageous for a species: higher heterozygosity, double the amount of chromosomes, and the hybridization of two different species may result in a permanent heterosis effect, which may have contributed to the high stress-resilience, adaptability, and phenotypic variability of the common carp (Chen et al., 2022; Chen et al., 2023).

Its status as a polyploid hybrid species, intense genetic and phenotypic variability, as well as its cultural importance and wide, global spread, has accumulated into the belief that the common carp is an ideal model for genetic fish studies (Nedoluzhko et al., 2020; Chen et al., 2022). In modern day, the common carp has been and is continuously studied in terms of vertebrate evolution, immunology and disease resistance, hybridization and selective breeding, phenotypic plasticity, and more (Xu et al., 2014; Xu et al., 2019; Chen et al., 2022; Chen et al., 2023).

1.2.3. An abundance of strains

Through differing environmental pressures, such as geographic isolation, mutations, adaptation, and human-driven selective breeding, the common carp as a species has been segmented into a large variety of different strains; to this day, there are over 35 established domesticated common carp strains globally, and a majority of them is maintained here in Europe, within the Research Institute for Fisheries, Aquaculture and Irrigation in the city of Szarvas, Hungary (FAO, 2005; Wang et al., 2010). These distinct strains bear differences in a range of characteristics, such as skin colour and scale cover, growth rate, stress-tolerance, body shape, and more (Chen et al., 2022). In 2019, Xu et al. have conducted a thorough investigation into the genetic differences lurking behind these phenotypic variations. For example, upon investigating the variation of body shape between strains, hundreds of candidate genes have been found, with a particular few being of interest: gene *trhr*, which has been previously associated with the development of a lean body shape (Liu et al., 2009), and gene *bmpr1b*, which is associated with regulating skeletal development (Qi et al., 2014). Furthermore, the researchers have also discovered several distinct gene candidates which may be responsible for the scale cover pattern and skin colouration. Similarly, in a study conducted last year, the ties between skin colour, strain differentiation, and a large list of morphological features have been explored (He et al., 2023). In this study, the researchers have found that between the differently-coloured Asian strains of the common carp, distinct differences exist, with variations spanning between growth rates, predatory ability, survival rate, and nutritional quality.

Based on these studies, it is clear that there appears to be a morphological and genetic variation between the strains of the common carp, however, morphology alone does not cover the plethora of differences found between the strains. In 2011, Jeney et al. have found that different strains have different resistance rates to bacterial infection with *Aeromonas hydrophila*, while Piačková et al. (2013) have similarly found different resistance rates to a viral infection with the cyprinid herpesvirus 3. In 2020, Nedoluzhko et al. have found over 700 candidate genes which could explain the differences in cold resistance between European and Northern-Russian carp strains.

These tangible differences suggest that strains from different regions and bearing different breeding histories are characterized by their own distinctive genetic structural compositions, which therefore must be investigated and described to achieve a full view of the distinction between the established strains. To achieve this, scientists have been abundantly interested in investigating the

genetic diversity of the common carp on a global scale (Ludanny et al., 2010; Tomljanović et al., 2013; Napora-Rutkowski et al., 2017; Tóth et al., 2020). The characterization of genetic diversity of a species is incredibly important for the sake of conservation of said species; although the common carp is a hardy and tenacious fish, factors such as over-fishing, habitat pollution, climate change, and over-exploitation of specific phenotypic traits still pose a risk of possible genetic diversity loss (Tóth et al., 2020; He et al., 2023). In fact, multiple studies have already established that genetic diversity found within fishing farms of domesticated common carp is commonly lower than in wild carp populations (Vandeputte, 2003; Svåsand et al., 2007; Matsuzaki et al., 2009). The degradation of genetic material within and between strains may lead to not just difficulties in further breeding programs and upkeep of healthy fish stock, but in loss of cultural significance that the different carp strains provide as well (Tóth et al., 2020; Ramya & Behera, 2023). In addition to this, an imperative point is food security: loss in genetic diversity inadvertently leads to loss in fitness and adaptability, therefore threatening the food security that the abundant common carp fish trade provides globally (Tóth et al., 2020; Khatei et al., 2021).

In Lithuania, several hybrid and purebred strains are kept across multiple farms; most notably, the national strain of Bubiai, which has been bred and maintained for many decades, and Šilavotas, which has been established as an official, selectively-bred strain in 2010 (Samuilovienė et al., 2018). Despite the cultural significance of these strains, little is known about the conservation efforts being put forth within our country. The Šilavotas strain has been bred and primarily maintained within the Šilavotas subdivision; with its closing, the fate of this strain became largely unknown to the public. Additionally, with the remaining fish farms in Lithuania being closed-practice, little is known about the current state of our national strains from a genetic perspective. To this degree, it is imperative to investigate the current carp stock raised within the country, and raise, alongside it, questions regarding the possible future conservation efforts pertaining these national strains.

1.3. The genetic diversity of *Cyprinus carpio*

1.3.1. Genetic markers

The primary tool for assessing the genetic diversity of a population is genetic markers. In aquaculture, particularly in the assessment of *C. carpio* genetic diversity, the most commonly used molecular markers have been allozymes, Random Amplified Polymorphic DNA, Restriction Fragment Length Polymorphisms, Amplified Fragment Length Polymorphisms, microsatellites,

mitochondrial DNA (mtDNA), Expressed Sequence Tags (EST), and Single Nucleotide Polymorphisms (SNP) (Xu et al., 2016; Khatei et al., 2021). Besides genetic diversity investigations, genetic markers can be applied in a variety of ways: manage aquaculture farm stock, determine evolutionary events such as population fluctuations, genetic drift, and mutation rates, as well as aid in determining the phylogeny of a species (Ramya & Behera, 2023). Within fisheries, in particular, genetic markers help with genetic marking of individuals, conservation efforts, pedigree determination, disease prevention, and the characterization of strains on a molecular level.

Despite the abundance of viable markers, with the development of genetic technologies, several markers have taken the spotlight when it came to assessing the genetic diversity of fishes: mtDNA, microsatellite markers, and SNPs (Chistiakov & Voronova, 2009; Khatei et al., 2021). However, not all genetic markers are created equal: in fact, it seems that in regards to *C. carpio* research, mtDNA and SNPs fall behind in comparison to microsatellite markers. MtDNA has a higher evolutionary rate than the nuclear genome, thus allowing for an informative phylogenetic and genetic diversity investigation; however, it poses several limitations, such as non-Mendelian inheritance, inconsistent evolutionary rates, and a financial burden necessitated by sequencing (Chistiakov & Voronova, 2009; Dong et al., 2015; Khatei et al., 2021). Likewise, although a SNP genotyping array has been developed, as well as naturally providing a high resolution investigation into genetic diversity, SNP studies within the common carp diversity field are not abundant (Xu et al., 2014; Xu et al., 2019; Li et al., 2021; Ji et al., 2022). The reasons for this might be the abundance of drawbacks that comes with using SNPs as a genetic diversity marker: SNPs require more DNA material than microsatellite markers, have lower polymorphic information content than microsatellite markers, and are far more costly (Ramya & Behera, 2023). In all, microsatellite markers appear to be used most often within the genetic diversity study field when it concerns the common carp, and the reasons for that are quite simple: they are abundant in information, are cost-effective, and show variability within and between populations (Biba et al., 2014; Singh et al., 2015; Tóth et al., 2020; Khatei et al., 2021).

1.3.2. Microsatellite markers

Microsatellites are 2-6 bp long tandem repeat sequences evenly distributed across the genome (Khatei et al., 2021). On average, in fish genomes, microsatellites are found roughly every 10 kb, however, due to the common carp's tetraploidy, microsatellite counts have been found to be increased (Liu & Cordes, 2004; Xu et al., 2016). Within the common carp genome, over 79

thousand microsatellites have been distinguished, with an occurrence rate of at least one microsatellite per 3.88 kb (Ji et al., 2012). These repeating sequences can be found in protein-coding, regulatory, and non-coding parts of the genome, however, they are primarily distributed across non-coding regions (Liu & Cordes, 2004; Khatei et al., 2021; Lei et al., 2021).

As was mentioned above, the usage of microsatellite sequences as genetic markers is quite wide and abundant. These co-dominant, neutral markers have high evolutionary rates, often resulting in high variability of allele lengths per microsatellite locus (Liu & Cordes, 2004; Khatei et al., 2021). However, their high polymorphism and mutagenicity are not the only upsides that make microsatellite markers so popular; in addition to these features, these markers are also primarily polymerase chain reaction-based (PCR), ensuring that products can be amplified from a small amount of DNA (Khatei et al., 2021).

Besides genetic diversity, these markers are often applied in pedigree analyses, genetic fluctuation monitoring, breeding strategy creation, and evolution studies (Thai et al., 2007, Ramya & Behera, 2023).

A wide range of microsatellite marker sets have been created for the common carp. The first set of markers was created in 1997 (Crooijmans et al., 1997). In this study, the researchers have isolated 32 polymorphic microsatellite loci of poly-CA repeats, several of which were capable of reflecting the tetraploid nature of the common carp genome. In the original study, the number of alleles within the amplified loci ranged from 2 to 7, and had mixed ranges of heterozygosity.

Two years later, Aliah et al. created a small set of microsatellite markers to measure the genetic diversity of the Koi carp (Aliah et al., 1999). This set of 3 markers was created on the isolation of GT microsatellite sequences, and the loci have shown significant polymorphism, with alleles ranging from 5 to 9 and a wide range of heterozygosity values. Similarly, in 2001, a new set of microsatellite markers was developed for the Koi carp (David et al., 2001). After screening the carp genome for microsatellites with CA and CT motifs, the researchers have isolated a full set of 47 new microsatellite markers, all of which averaged 4.7 alleles per locus and 44.2% mean heterozygosity. It is important to note, additionally, that although the markers Aliah et al. and David et al. have created were designed for the Koi carp, these markers can be used on other strains of the common carp, as well.

A more recent set of microsatellite markers for the common carp was created in 2004 (Yue et al., 2004). In this study, the researchers have attempted to isolate CA-motif microsatellite sequences

located within ESTs, in an attempt to create type I microsatellite markers. As such, they have created 36 microsatellite markers, the majority of which were located within the 5' or 3' untranslated regions. During the study it was found that 34 out of 36 isolated markers were polymorphic, with the average allele count per locus being 7.3.

Altogether, with over a hundred available microsatellite markers for the investigation of genetic diversity within the common carp, the abundance may be overwhelming when preparing for such an investigation. In their study, Yue et al. emphasize the importance of using a varied and standardized set of markers across all diversity investigations, however, the reality is quite different: over the past few decades, while the most commonly used set of markers belongs to Crooijmans et al. (1997), the selection of markers from this particular set rarely overlaps (Ludanny et al., 2010; Tomljanović et al., 2013; Napora-Rutkowski et al., 2017; Tóth et al., 2020).

1.3.3. Research of carp genetic diversity in Lithuania

The degree to which the diversity of the common carp has been assessed within Lithuania is limited. In 2017, an investigation of common carp strains raised within the Šilavotas subdivision was reported (Samuilovienė et al., 2018). In this report, the researchers have assessed the genetic differences between 5 strains (2 Lithuanian strains and 3 foreign) utilizing 4 microsatellite markers: MFW1, MFW6, MFW7, and MFW28. The number of alleles per population ranged between 3.25 and 5.25, with Lithuanian strains being characterized by a lower allele diversity than most foreign strains. This investigation has also reported that the mean heterozygosity across the populations was lower than what was statistically expected. This investigation was further supplied by a Bachelor's work in 2021, conducted within the Nature Research Centre laboratory of molecular ecology (Lentinaitė, 2021). In this work, a total of 9 strains (5 purebred and 4 hybrid) raised within the Šilavotas subdivision were assessed, utilizing 11 microsatellite markers (MFW1, MFW2, MFW3, MFW6, MFW7, MFW9, MFW11, MFW13, MFW17, MFW20, MFW28). The thorough research of these strains has yielded an interesting observation that the highest genetic diversity amongst all strains was detected within one Lithuanian carp strains (Bubiai strain), while the lowest – within another (Šilavotas strain).

The following year, these Lithuanians strains – Bubiai and Šilavotas – were investigated further within another Bachelor's work (Liubartaitė, 2022). In this work, 15 individuals per strain were investigated utilizing 11 microsatellite markers (MFW1, MFW2, MFW3, MFW6, MFW7, MFW9, MFW11, MFW13, MFW17, MFW20, and MFW28). The results of this investigation have

shown that the strains, at the time raised within the Šilavotas subdivision, were characterized by a worryingly low genetic diversity: the per locus allele count ranged from 2 to 4. This finding has raised some alarm for the state of the strains raised within Lithuanian fish farms, as a low genetic diversity among strains may lead to potential loss of the unique genetic makeup that can only be detected in national strains. As such, it had become an objective of this work to conduct a more thorough investigation into the genetic diversity and composition found within national carp fishery populations, in the hopes that the in-depth characterization of the strains raised within the country would provide aid in maintaining these carp stocks within a healthy capacity.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Sample collection

The common carp fish individual samples were collected within 5 different farmlands across Lithuania (Table 2.1.).

Table 2.1. Information on the collected samples

Population code	Strain	Location	Sample size
Šilavotas (Š)	Lithuanian (Bubiai and Šilavotas) (purebred, known origin)	Šilavotas, Šilavotas Subdivision of the Department of Inland Waters and Aquaculture*	30
Arnionys (A)	Lithuanian (mixed origin)	Arnionys	48
Simnas (S)	Lithuanian (mixed origin)	Simnas	15
Bartžuvė (B)	Lithuanian (mixed origin)	Elektrėnai, LLC "Bartžuvė"	15
Kapliai (K)	Czech (purebred, known origin)	Aukštieji Kapliai	44

*The Šilavotas population samples were collected two years prior to this investigation and were analysed in-depth within a previous work (Liubartaitė, 2022).

In total there were 152 samples, 122 of which were genotyped during this work, 30 – during a previous work (Liubartaitė, 2022). 2-3 cm of terminal fin clips were collected from each individual, thereafter stored in ethanol solution until DNA extraction.

2.1.2. DNA extraction reagents

1. 20% sodium dodecyl sulphate ("SERVA Electrophoresis GmbH", Germany).
2. 96% ethanol ("Thermo Fisher Scientific Baltics", Lithuania).
3. Homogenizing buffer: 10 mM Tris-HCl, pH 8,0; 0,4 M NaCl; 2 mM EDTA, pH 8,0 ("Reachim", Russia).
4. Isopropanol ("Chempur", Poland).
5. NaCl 6 M ("Artiomsol", Ukraine).
6. Proteinase K, 20 mg/mL ("Thermo Fisher Scientific Baltics", Lithuania).

2.1.3. Polymerase chain reaction reagents

1. Microsatellite locus primers (MFW1, MFW2, MFW3, MFW6, MFW7, MFW9, MFW11, MFW13, MFW17, MFW20, MFW28; "Applied Biosystems", United Kingdom).
2. DNA polymerase *DreamTaq* ("Thermo Fisher Scientific Baltics", Lithuania).
3. Nuclease-free water ("Thermo Fisher Scientific Baltics", Lithuania).

2.1.4. Agarose gel electrophoresis reagents

1. 50X TAE buffer ("Thermo Fisher Scientific Baltics", Lithuania).
2. Agarose *TopVision* ("Fermentas", Lithuania).
3. 6X DNA Loading Dye ("Thermo Fisher Scientific Baltics", Lithuania).
4. Ethidium bromide 10 mg/mL ("Invitrogen", United States).
5. *GeneRuler* 100 bp Plus DNA Ladder ("Thermo Fisher Scientific Baltics", Lithuania).

2.1.5. Fragment analysis reagents

1. *GeneScan* 600 LIZ dye Size Standard v2.0 ("Thermo Fisher Scientific Baltics", Lithuania).
2. *Hi-Di* formamide ("Thermo Fisher Scientific Baltics", Lithuania).

2.2. Methods

2.2.1. DNA extraction

The DNA extraction was performed according to the protocol developed by Aljanabi & Martinez (1997), with some minor adjustments. 0.1 g of a fin clip is cut into small, 1-2 mm strips and incubated in 200 µL of homogenizing buffer, 40 µL of 20% sodium dodecyl sulphate, and 8 µL of 20 mg/mL proteinase K at 55 ℃ in a thermostat that's set to continuously shake the samples. The samples are incubated for 1.5 h. Thereafter, 300 µL of 6 M NaCl is added, the samples are vortexed for 10 s and centrifuged for 15 min at 10 000 g. After centrifugation, the supernatant is collected into a new sterile tube and 500 µL of isopropanol is added. The sample is left to freeze at -20 ℃ for at least 24 hours to allow for the DNA to precipitate. Afterwards, the samples are centrifuged once again at the same conditions, the supernatant is discarded, and the precipitate is washed with 200 µL of 70% ethanol, then left to dry. Once dry, the samples are dissolved in 100 µL of nuclease-free water. The DNA concentrations of the samples are then measured via a spectrophotometer *NanoPhotometer* ("Implen", Germany).

2.2.2. Microsatellite loci amplification

Microsatellite loci were amplified via PCR utilizing 10 microsatellite loci primers that are marked with fluorescent dyes (Table 2.2.). The total volume of the PCR mix is 25 µL, which consists of: 5 µL of 50 ng/µL genomic DNA, 12.5 µL *DreamTaq* DNA polymerase mix, 1 µL of 5pmol/µL forward and reverse primer solutions, and 5.5 µL of nuclease-free water. A certain number of primers have been partnered together in a multiplex PCR (Table 2.2.; up to 2 primers per reaction) – in which case, the volume of added nuclease-free water is reduced to 3.5 µL. The unpartnered primers have been found to be difficult to amplify in a multiplexed reaction.

Table 2.2. Information on the microsatellite loci used for the investigation of genetic diversity. Microsatellite markers were created by Crooijmans et al., 1997. Adapted from Lentinaitė, 2021.

Locus	Primer sequence	T_m, °C	Fluorescent dye	Multiplex group	Fragment range, bp
MFW1	F: GTCCAGACTGTCATCAGGAG R: GAGGTGTACACTGAGTCACGC	62,3 65,4	6-FAM	1	172-225
MFW2	F: CACACCGGGCTACTGCAGAG R: GTGCAGTGCAGGCAGTTTGC	67,7 68,0	VIC	–	160-264
MFW6	F: ACCTGATCAATCCCTGGCT R: TTGGGACTTTTAAATCACGTTG	57,6 56,8	6-FAM	2	122-180
MFW7	F: TACTTTGCTCAGGACGGATGC R: ATCACCTGCACATGGCCACTC	61,6 64,1	VIC	2	186-286
MFW9	F: GATCTGCAAGCATATCTGTCG R: ATCTGAACCTGCAGCTCCTC	61,8 64,7	6-FAM	–	106-134
MFW11	F: CATTTGCCTTGATGGTTGTG R: TCGTCTGGTTTAGAGTGCTGC	54,5 61,4	VIC	–	152-206
MFW13	F: TGATGAGAACATTGTTTACAG R: TGAGAGAACAATGTGGATGAC	52,8 57,2	6-FAM	3	158-216
MFW17	F:CTCAACTACAGAGAAATTTCATC R: GAAATGGTACATGACCTCAAG	53,8 55,8	VIC	3	237-284
MFW20	F: CAGTGAGACGATTACCTTGG R: GTGAGCAGCCCACATTGAAC	55,0 58,6	NED	1	199-251
MFW28	F: GATCCCTTTTGAATTTTTCTAG R: ACAGTGAGGTCCAGAAGTCG	52,6 58,7	HEX	–	270-307

The reaction was done across all primers with the same conditions: 1 cycle of initial denaturation at 95 ℃ for 2 min; 35 cycles of denaturation at 94 ℃ for 30 s, primer annealing at 55 ℃ for 45 s, and extension at 72 ℃ for 45 s; lastly, 1 cycle of final extension at 72 ℃ for 5 min.

Once the reaction is complete, its success is evaluated via qualitative agarose gel electrophoresis (AGE). The AGE is done on a horizontal 1.5% gel, using the ladder *GeneRuler* 100 bp Plus DNA Ladder, for up to 30 min at 90 V. Once the AGE is complete, the gel is analyzed visually under UV light (*BioDocAnalyze*; "Biometra", Germany). The gel is evaluated for:

unidentifiable bands (possible contamination) and respective microsatellite locus bands (if said bands are not present, the PCR is repeated).

2.2.3. Fragment analysis

The exact length of the amplified microsatellite loci are determined via Sanger's capillary electrophoresis by a laboratory technician. The samples are first prepared with a series of steps: first, the sample is mixed with formamide at a ratio of 1:9; thereafter, the mix is denatured (*Veriti 96 Well Thermal Cycler*; "Applied Biosystems", United States) at 95 ℃ for 5 min, after which the denatured mix is quickly cooled to 4 ℃. The prepared samples are analysed with the *3500 Genetic Analyzer* ("Applied Biosystems"/ "Hitachi", United States). The ladder used for the analysis is *GeneScan* 600 LIZ dye Size Standard v2.0.

2.2.4. Genotype identification

Once the samples have undergone capillary electrophoresis, the generated files are analysed with GeneMapper v4.1. The resulting file is an electropherogram – a graph in which one can observe the length of the detected DNA fragments, and the fluorescence of each fragment, the intensity of which indicates the volume of fragments detected per estimated length. These peaks – usually of a large fluorescent intensity – are identified among those of lesser intensity as the amplified microsatellite loci alleles. The length of the allele is determined by taking into account the original exemplary allele length per each locus, as was noted in the article describing the created alleles (Crooijmans et al., 1997), and the alleles determined in previous works within the laboratory (Lentinaitė, 2021; Liubartaitė, 2022), so as to not misidentify a non-allelic signal as an allele. Therefore, each locus has an approximate range of possible allele lengths per each locus (Table 2.2.).

The alleles are identified by evaluating the highest peaks within the given range of the locus; the presence of only one highermost allele denotes a homozygote genotype, meanwhile the identification of a heterozygote genotype can be more complicated. When observing the presence of two (or more) high peaks, the size difference both in fluorescence and fragment length are determined. First and foremost, due to the nature of the polymerase, there must be at least 3 nucleotides between each peak for them to be considered as separate alleles. Furthermore, the fluorescence intensity of the smaller peak (if they are not of the same intensity) must reach at least 10% of the larger peak.

Lastly, when identifying alleles, patterns are taken into consideration: each genotype is profiled visually and saved for comparison between each separate genotype; if a signal looks different and unique to other profiles, the sample is run through PCR and capillary electrophoresis a second time to ensure its validity.

2.2.5. Statistical analysis

The mean number of alleles (N_a), effective alleles (N_e), private alleles (P), allele frequencies, and observed and expected heterozygosities (H_o and H_e, respectively) within each population were calculated with GenAlEx v6.51 (Peakall & Smouse, 2006; Peakall & Smouse 2012). Principal coordinate analysis was also conducted with GenAlEx v6.51 on a standardized dataset. Estimated deviation from the Hardy-Weinberg equilibrium and heterozygosity deficit presence across the populations was calculated with the web edition of GenePop v4.7 (Raymond M. & Rousset F, 1995; Rousset, F., 2008), with Bonferroni corrections applied with 1000 dememorization steps, 100 batches, and 1000 iterations per batch. The within-population inbreeding coefficient values (F_{is}) were calculated with the software FSTAT v2.9.4 (Goudet, 1995).

The estimation for null allele frequencies within the populations were calculated with the software FreeNA (Chapuis & Estoup, 2007, Chapuis et al., 2008). This software was subsequently used to calculate the population differentiation values (F_{st}) and the Cavalli-Sforza and Edwards distances (D_c; Cavalli-Sforza & Edwards, 1967), adjusted for the estimated presence of null alleles. The calculations with FreeNA were conducted with 95% confidence and 1000 bootstrap iterations. The D_c distances were further used to create a dendrogram of all 5 populations with Mega v11.0.13 (Tamura et al., 2021) using the Neighbour-Joining method (Saitou & Nei, 1987).

The analysis of molecular variance (AMOVA) within and between the populations was done with Arlequin v3.5.2.2 (Excoffier & Lischer, 2010), based on allele frequency values with 1000 permutations.

Lastly, the software Structure v2.3.4 (Pritchard et al., 2000) was used to determine the admixture patterns of genetic structure between the populations. Analyses were run for K values 1-10, with 10 iterations for each K value, each with a burnin period of 10 000 and 50 000 Markov chain Monte Carlo repetitions. Afterwards, to ascertain the true K value (ΔK), the results were ran through STRUCTURE HARVESTER v0.7 (Earl & vonHoldt, 2012) using the Evanno method (Evanno et al., 2005).

3. RESULTS

The presented work is a continuation of a previous research conducted within the laboratory. It aims to assess the genetic diversity of one previously assessed and 4 newly assessed common carp populations raised in Lithuanian fish farms. To fully assess the genetic diversity and composition of said populations, the work sought to determine the genetic diversity parameters, genetic differentiation, and structural composition between and within the populations.

3.1. Genotyping results

In total, 122 carp individuals were genotyped during this work, utilizing 10 microsatellite loci markers. The full table of all determined genotypes can be found in appendices (Appendix 1). 44 samples had instances of null genotypes (0/0), whereas the alleles within the given loci had not amplified in their original and repeat PCR runs, assumedly due to possible mutations within the primer hybridization zone in DNA sequence. The presence of null alleles is assumed in loci MFW1 (in 5 samples), MFW2 (in 1 sample), and MFW9 (in 41 samples).

The analysis of molecular data was conducted on the genotypes assessed within this work, as well as including genotypes of 30 additional carp individuals that have been genotyped in a previous work (Liubartaitė, 2022; Šilavotas population), that have not been analysed on a genetic structural level. The full range of genotypes determined within this sample pool can be found in Liubartaitė, 2022.

All loci across all of the populations were assessed for the estimated frequency of other possible null alleles (Table 3.1.). Largely, all of the studied loci had some degree of estimated null allele presence, but the estimated frequencies varied from locus to locus. Loci MFW2, MFW6, and MFW20 were estimated to have null alleles present in up to 2 populations, with negligible frequencies that do not reach 10%. On the other hand, several loci exhibited large null allele estimates: MFW1, MFW9, and MFW28 were estimated to have null alleles across almost all populations (with the exception of MFW28, which had estimated null alleles in 4 of the 5 populations). These estimated frequencies ranged from 0.034 (MFW28 population Arnionys) to 0.707 (MFW9 population Šilavotas). The calculated presence of a null allele that was estimated to reach above 30% frequency was considered to be concerning and was exhibited by two loci: MFW1 and MFW9. The locus MFW1 exhibited a frequency above 30% within one population (0.420, population Simnas), while MFW9 exhibited such frequencies within all assessed populations.

Table 3.1. Null allele estimated frequencies across all studied loci and populations

Population	MFW1	MFW2	MFW6	MFW7	MFW9	MFW11	MFW13	MFW17	MFW20	MFW28
Kapliai	0.079	0.000	0.000	0.126	0.459	0.038	0.083	0.034	0.000	0.213
Arnionys	0.257	0.052	0.023	0.000	0.636	0.020	0.000	0.062	0.000	0.034
Simnas	0.420	0.076	0.000	0.074	0.605	0.143	0.122	0.000	0.066	0.172
Bartžuvė	0.071	0.000	0.000	0.000	0.691	0.000	0.067	0.139	0.000	0.242
Šilavotas	0.186	0.000	0.000	0.000	0.707	0.186	0.096	0.086	0.000	0.000

As such, MFW9 was discarded from further analyses.

3.2. Genetic diversity

In total, 97 alleles were determined across all populations. The mean allele count per population (Table 3.2.) ranged from 3.222 (Šilavotas) to 6.778 (Simnas and Kapliai), meanwhile the mean effective allele count ranged from 2.560 (Šilavotas) to 3.977 (Kapliai), indicating that the Šilavotas population had the lowest genetic diversity, while Kapliai – the highest. While the Lithuanian strain populations had a handful of private alleles (up to 7), the Czech strain population (Kapliai) had 20 private alleles. Across the populations, 3 to 5 loci significantly deviated from the Hardy-Weinberg equilibrium (HWE; Table 3.2.), with the highest count of deviating loci being within the Šilavotas population, while the lowest – within the Bartžuvė population. The observed heterozygosities ranged from 0.570 (Šilavotas) to 0.639 (Kapliai), and all of the populations had lower observed heterozygosities than it was expected within the HWE (expected heterozygosities ranged from 0.575 to 0.713). As such, all populations had a certain degree of inbreeding, with the highest being within Simnas, which had an inbreeding coefficient of 0.241. Conversely, Šilavotas and Arnionys had the lowest signs of inbreeding (0.025). Upon further investigation, a global HWE test for significant heterozygote deficit has found that 4 out of 5 populations (Kapliai, Arnionys, Simnas, and Bartžuvė) had a significant heterozygote deficit (P-value < 0.01).

Table 3.2. Genetic diversity metrics within each population. N_a – mean number of alleles within a population. N_e – mean number of effective alleles within a population. P – number of private alleles. dHWE – number of loci that significantly deviate from the Hardy-Weinberg equilibrium. H_e – expected mean heterozygosity. H_o – observed mean heterozygosity. F_{is} – inbreeding coefficient.

Strain	N_a	N_e	P	dHWE	H_e	H_o	F_{is}
Kapliai	6.778	3.977	20	4	0.710	0.639	0.112
Arnionys	6.111	3.026	3	4	0.643	0.608	0.064
Simnas	6.778	3.925	7	4	0.713	0.565	0.241
Bartžuvė	5.556	3.368	4	3	0.685	0.637	0.104
Šilavotas	3.222	2.560	–	5	0.575	0.570	0.025

Each locus within the populations had between 1 and 3 alleles dominating the allele pool by frequency in one or several populations (Table 3.3.). Alleles that are considered to dominate among the allele pool must take up at least 40% of all alleles within a given population; the full table of allele frequencies across all populations can be found in appendices (Appendix 2). Nearly all loci have had alleles that dominated over half of the allele pool, the exceptions being MFW7 and MFW13. The highest frequency of a common allele was 0.818 (MFW28, population Kapliai). Interestingly, alleles that have been found to dominate the allele pool within a Lithuanian strain population could also be commonly found in other Lithuanian strain populations, such as alleles 206 (MFW1), 142 (MFW6), 196 (MFW11), 237 (MFW17), 251 (MFW20), 285 (MFW28), and 301 (MFW28). Similarly, there have been alleles that had higher rates of frequency across the Lithuanian strain populations, but had an exceptionally low frequency within the Czech strain population, such as 206 (MFW1), 186 (MFW7), 182 (MFW13), 241 (MFW17), 242 (MFW20), 251 (MFW20), 285 (MFW28), and 301 (MFW28). Conversely, an instance of a high frequency in the Czech strain, but a low frequency in the Lithuanian strain populations, has been found within the allele 290 (MFW28).

Table 3.3. Common allele frequencies (≥ 0.4) within the given populations

Locus	Allele	Kapliai	Arnionys	Simnas	Bartžuvė	Šilavotas
MFW1	**206**	0.057	0.544	0.500	0.367	0.100
	212	0.386	0.222	0.385	0.233	0.550
MFW2	**250**	0.261	0.362	0.300	0.567	0.217
MFW6	**142**	0.307	0.802	0.633	0.600	0.600
MFW7	**186**	0.000	0.427	0.300	0.267	0.317
MFW11	**196**	0.352	0.406	0.333	0.433	0.750
MFW13	**182**	0.000	0.177	0.100	0.133	0.400
	190	0.205	0.427	0.367	0.367	0.217
MFW17	**237**	0.250	0.427	0.567	0.300	0.550
	241	0.057	0.208	0.100	0.433	0.000
	274	0.477	0.281	0.200	0.133	0.450
MFW20	**242**	0.159	0.396	0.267	0.267	0.717
	251	0.057	0.302	0.400	0.500	0.267
MFW28	**285**	0.068	0.240	0.200	0.400	0.517
	290	0.818	0.052	0.267	0.100	0.000
	301	0.057	0.563	0.400	0.400	0.100

3.3. Genetic differentiation and structure

A molecular variance analysis (AMOVA; Table 3.4.) has found that the majority of the

variation (86.16%) comes from within the populations, rather than between. The result has been found significant (P-value < 0.01).

Table 3.4. Analysis of molecular variance of 5 populations

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage variation, %	P-value
Among populations	4	122.821	0.481	13.840	0.000
Within populations	299	895.321	2.994	86.160	
Total	303	1018.141	3.475		

A principal coordinate analysis (PCoA; Figure 3.1.) has suggested the formation of 3 different clusters: a) a cluster consisting largely of the representatives of Šilavotas population; b) a cluster consisting largely of the Kapliai population; and c) a cluster consisting of the carps representing Arnionys, Simnas, and Bartžuvė populations. Distribution patterns of individual specimens in the PCoA plot discriminate Lithuanian (Šilavotas) and Czech (Kapliai) carp strains, with the Kapliai population forming a large, insular cluster that overlaps very little with the Lithuanian strains. In particular, some individuals representing the Simnas population seemed to, in part, mix with the representatives of the Kapliai population. Conversely, the Šilavotas population formed a distinct cluster that is comparably smaller in size. It should be noted that some representatives of the Šilavotas carp strain mixed with the populations of Arnionys, Simnas, and Bartžuvė. Lastly, the latter 3 populations formed a large cluster representing a highly intermixed genetic pool.

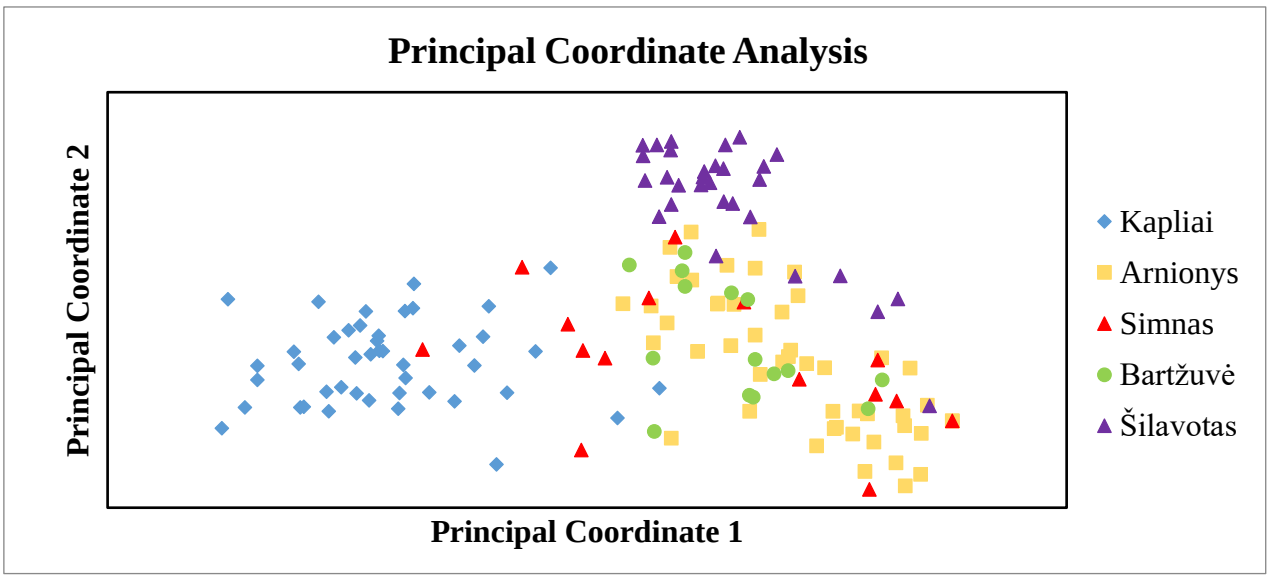

Figure 3.1. Results of the principal coordinate analysis depicting the genetic similarities and variabilities between the carp individuals attributed to five studied populations.

The F_{st} values have indicated a substantial differentiation between some of the populations (Table 3.5.). The smallest F_{st} value was found between the populations of Bartžuvė and Simnas (0.014), while the largest – between Simnas and Šilavotas (0.200). The largest differentiation values were found between the Kapliai population and the Lithuanian strain populations, and, interestingly, between Šilavotas and the remaining 3 Lithuanian strain populations. Arnionys, Simnas, and Bartžuvė had comparably low differentiation values between each other. The D_c values largely corresponded to the differentiation inferred from the F_{st} values: the largest distances were found to be between the Kapliai population and the rest of the Lithuanian carp populations, as well as between Šilavotas and the remaining Lithuanian carp populations. Likewise, the smallest distance was found to be between Bartžuvė and Arnionys (0.214), while the largest – between Kapliai and Šilavotas (0.529).

Table 3.5. F_{st} values and Cavalli-Sforza and Edwards distance (D_c) values across all populations. F_{st} values are below the diagonal, and the D_c values are above the diagonal.

	Kapliai	**Arnionys**	**Simnas**	**Bartžuvė**	**Šilavotas**
Kapliai		0.469	0.423	0.467	0.529
Arnionys	0.163		0.276	0.214	0.357
Simnas	0.101	0.017		0.280	0.378
Bartžuvė	0.134	0.020	0.014		0.414
Šilavotas	0.195	0.114	0.200	0.119	

The phylogenetic relationships among the studied carp populations were reconstructed in the Neighbour-Joining tree (Figure 3.2.), revealing closer genetic distances between the Lithuanian carp populations of Arnionys, Bartžuvė, and Simnas, compared to the distances between the Lithuanian and Czech carp strains represented by Šilavotas and Kapliai populations, respectively. Furthermore, within the cluster of Lithuanian strain populations, it was found that the populations of Arnionys and Bartžuvė were the most genetically close. The distance between the Arnionys-Bartžuvė clade and the Simnas population was much smaller than between the 3 populations and Šilavotas.

To ascertain the distribution of genetic structure within the populations, a Bayesian admixture model was applied (Figure 3.3.). The ΔK value was determined to be 2 (Figure 3.3A.), however, for the purpose of illustrating the genetic composition distribution across all 5 populations, an analysis of K = 5 was selected as well (Figure 3.3B.). ΔK value of 2 suggests that the most probable genetic composition is differentiated into 2 distinct populations: one is represented by the Czech strain, and the other is represented by the pool of Lithuanian carp strain populations raised within the country.

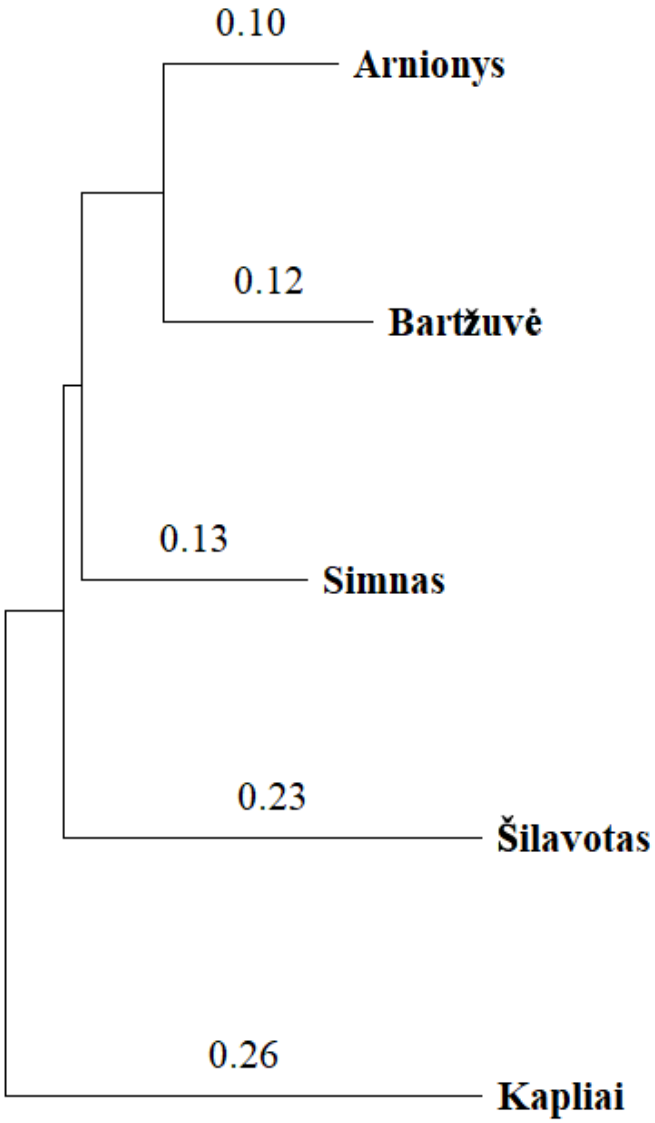

Figure 3.2. Neighbour-Joining dendrogram of 5 common carp populations based on the data of 9 microsatellite loci. Tree generated by Mega v11.0.13 with the Cavalli-Sforza and Edwards distance (D_c) values. Bootstrap value generated with 1000 replicates.

Within the K = 2 assessment (Figure 3.3A.), it was found that the Czech and Lithuanian carps shared some of the genetic structure: the Kapliai population had a small portion of specimens that shared a certain amount of microsatellite alleles characteristic of the studied Lithuanian carp populations. Likewise, all of the Lithuanian carp populations shared at least marginal levels of genetic similarity to the Czech strain – most of all, the population of Simnas had a visible and distinct shared genetic structure, which reflects what was gleaned from the principal coordinate analysis (Figure 3.1.).

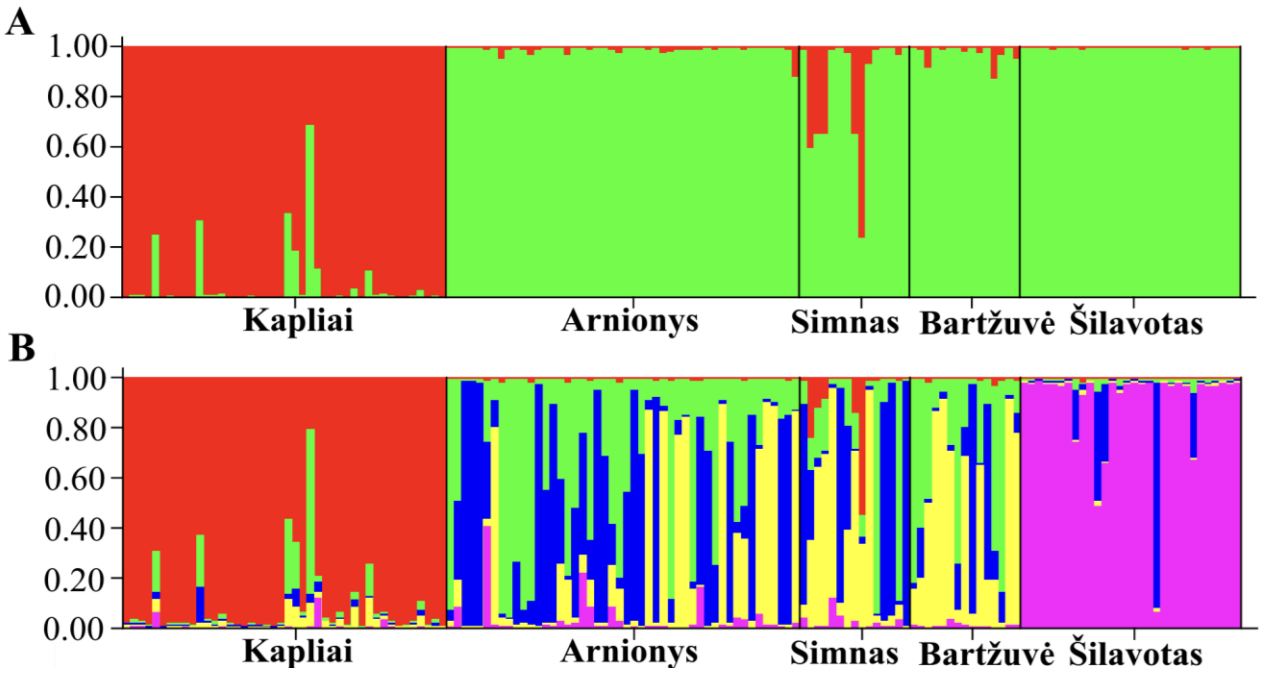

Figure 3.3. Assignment of individual fish to one of the studied carp populations estimated based on multi-locus genotyping data using Structure software, v2.3.4. The Y axis represents the estimated membership coefficient Q indicating individual proportion of membership to each of two (A) or five (B) clusters, respectively.

An analysis of K = 5 portrayed a much more in-depth look at the genetic distribution across the populations (Figure 3.3B.). The results of this analysis were threefold. First of all, the Kapliai population, like in the K = 2 analysis, showed a distinct genetic structure which was unique comparing to the other 4 populations. Second of all, and similarly, the Šilavotas population, despite being a representative of Lithuanian strain populations, showcased a unique genetic structure that had a fairly limited overlap with the other 3 Lithuanian carp populations. In addition to this, it was found that the genetic influence of the Šilavotas population was present within the remaining 3 Lithuanian carp populations. And third, while the genetic structure within the Simnas, Arnionys, and Bartžuvė populations varied from population to population, a clear common genetic composition was found. These results reflect what was ascertained within the principal coordinate analysis (Figure 3.1.), F_{st} value distribution (Table 3.5.), and the constructed dendrogram (Figure 3.2.).

4. DISCUSSION OF RESULTS

In this work, the genetic diversity and structural composition of 5 different carp populations – 4 of Lithuanian-strain representatives, and 1 of Czech-strain representatives – were assessed, utilizing 9 microsatellite markers.

4.1. Genetic diversity

4.1.1. Allelic distribution

It was found that the genetic diversity in terms of allelic distribution is quite comparable within most populations, with the exception of Šilavotas. The highest mean allele number was found within the Simnas population, and it was on par with the allelic diversity found within the Czech strain population (Kapliai), while also being comparably similar to two other Lithuanian strain populations (Arnionys and Bartžuvė). Similar allelic diversity values can also be found within other studies: for example, a 2017 study of common carp strains in Poland has found that the allelic diversity between 20 strains raised in Poland ranged from 2.182 to 9.273, with the mean value being 4.473 (Napora-Rutkowski et al., 2017). Likewise, an investigation of 8 Russian common carp strains has found the mean allele values ranging from 4.0 to 7.3 (mean value 5.7), (Ludanny et al., 2010); while an investigation of 5 farm-raised Croatian carp strains found the values ranging from 2.2 to 5.1 (Tomljanović et al., 2013). Furthermore, investigations of Czech strains by Kohlmann et al. (2005) and Hulak et al. (2010) have found that mean allele values per Czech strain to be 5.00 within both studies. This shows that, largely, the allelic diversity within carp strains investigated in Lithuanian fish farms is similar to values found in other European carp populations, which, in turn, must reflect the stock management practices within the currently operating Lithuanian fish farms that are on par with those in other European countries.

A most interesting reflection on the state of the Lithuanian carp stock management was found while investigating the strains previously raised within Šilavotas. At the time of its operation, the Šilavotas subdivision cultivated a wide array of different strains (Samuilovienė et al., 2018, Lentinaitė, 2021), among which were the aforementioned Bubiai and Šilavotas strains. Both of these strains composed the sample pool of the previous investigation (Liubartaitė, 2022). In it, it was found that the allelic diversity of these two strains was strictly limited (from 2 to 4 allele per locus), and these concerning results were found to be on par with a 2017 investigation conducted within the country (Samuilovienė et al., 2018). In this study, upon investigating 5 strains raised within the Šilavotas subdivision, the mean allele values for the Bubiai and Šilavotas strains were

found to be 3.50 and 3.25, respectively. On the other hand, in an aforementioned study conducted within Poland (Napora-Rutkowski et al., 2017), the mean allele value of the strain Bubiai was found to be much higher (5.091). Thus, the discovered low genetic diversity within these Lithuanian purebred carp strains that was revealed during the current study indicates a drop of allelic richness, possibly related with decreased numbers of spawning individuals during the previous breeding attempts.

Another point of interest in the investigation of diversity is the amount of private alleles within a given population (Szpiech & Rosenberg, 2011). While no private alleles were found among carps representing the Šilavotas population, which is reflective of its low genetic diversity, the remaining Lithuanian carp populations possessed from 3 to 7 private alleles. In terms of private allele distribution among different populations, this number is quite common: Napora-Rutkowski et al. (2017) have found private allele ranges between 1 and 7, Hulak et al. (2010) – between 0 and 5, and Kohlmann et al. (2005) – between 1 and 3. Rather than reflect a shared genetic structure, private alleles are indicative of differentiation that might occur between populations (Szpiech & Rosenberg, 2011) – this is quite visible in the dichotomy of private allele distribution between the Lithuanian strain populations and the Czech strain population. The determined number of private alleles within the Kapliai carp population (20) is nearly three times as much as the largest number of private alleles found between the other studied Lithuanian carp populations. This indicates that there must be a structural differentiation between the Czech and Lithuanian carp strains, as the Lithuanian carp strains bear comparably small amounts of private alleles, and yet their mean number of alleles is comparable to that found within the Czech strain population. These findings suggest that there is a larger amount of shared inter-population alleles within the Lithuanian carp populations, while maintaining that the large amount of private alleles observed within the Czech strain population must infer the distant genetic origin of the Czech strain on the contrary to the rest of the studied carp populations. An additional layer of this observation can be gleaned from the distribution of allele frequencies among the populations – in particular, the most common alleles, taking up over 40% of the gene pool within a given locus. Alleles common exclusively to the Lithuanian carp populations have been found in 6 out of 9 analysed loci, indicating that the Lithuanian strains and the Czech strain must have a degree of distinction between their genetic structural compositions.

4.1.2. Genotype distribution

Each population had a similar number of loci deviating from the HWE; simply put, around 40% of loci had deviated across the populations. A number of reasons, in particular within aquaculture farms, could influence deviation from the equilibrium: an unequal sex ratio among spawners or a limited number of spawners, deviations from the random mating model, presence of null allele mutations, or even Wahlund's effect (Napora-Rutkowski et al., 2017, Hulak et al., 2010). Largely, across European farms, it has been found that the common reason for deviation from the equilibrium was an uneven distribution of genotypes, both in terms of homozygote, and heterozygote excess (Tóth et al., 2020). In this study, it was found that 4 out of 5 populations had a significant heterozygote deficit, which is further reflected in the observed heterozygosity distributions: all of the populations had, while not always overtly concerning, lower rates of observed heterozygosities than what was expected based on the Hardy-Weinberg model.

High- and low-ends of the heterozygosity value spectrum were apparent. The Simnas population had a difference between expected and observed heterozygosities of 0.148 (nearly 15% less heterozygotes than there should have been), and an inbreeding coefficient of 0.241, suggesting that the current genotype distribution poses a risk of possible genetic loss and stock health complications due to inbreeding depression. In Europe, such occurrences appear to be highly dependent on the region: for example, in the Czech Republic, the majority of the studied 13 carp strains had indications of population inbreeding (F_{is} values ranged between 0.050 and 0.265), while the 13 strains assessed in Hungary by a wide majority displayed an excess of heterozygotes (F_{is} values ranged from -0.250 to 0.083; Hulak et al., 2010; Tóth et al., 2020).

On the other hand, the Šilavotas population displayed a nearly-perfect state of equilibrium in terms of genotype distribution, with a total difference between expected and observed heterozygosities being 0.05, and a F_{is} value of 0.025. This result is quite peculiar, considering the limited number of alleles across the investigated loci, large number of loci deviating from HWE, and comparably small frequencies of estimated null alleles. As such, the healthy genotype distribution and a comparably low occurrence of estimated null allele presence both suggest that the 5 deviating loci within this population must have deviated due to stock management.

Within the remaining populations, it is more plausible that the deviating loci were due to the presence of null alleles and a skewed distribution of genotypes, the latter of which can often arise if

the breeding occurs with a limited number of spawners, or if the breeding scheme is done with a particular focus on a specific phenotype.

4.2. Genetic composition

4.2.1. Molecular variance

The AMOVA test showed that the majority of variance was found within populations, rather than between, which indicates that high levels of diversity lie within individuals themselves and don't reflect a strict distinction between populations.

4.2.2. Strain differentiation

Two populations have been found to be differentiated from the remaining populations: Kapliai, which has shown a clear distinction between it and the Lithuanian strain populations, and Šilavotas, which has additionally showed a clear differentiation between it and the remaining 3 Lithuanian strain populations. These two populations have shown F_{st} values between 0.101 and 0.200; in theory these differentiation values vary from 0 to 1, with 0 indicating zero differentiation between populations, while 1 – complete differentiation between populations (Luo et al., 2019). Generally, a value greater than 0.15 is considered to reflect a significant differentiation between populations, suggesting that the genetic variance found within and between these populations is diverse enough to discern two (or more) different populations (Holsinger & Weir, 2009; Luo et al., 2019). In light of this, the results suggest several things: a) Arnionys, Simnas, and Bartžuvė may be considered as populations with a shared genetic structure; b) the Šilavotas population, representing Lithuanian carp strains, possessed a specific genetic structure, which discriminates it from the other studied carp populations; c) the Kapliai population, likewise, has a unique genetic structure that is differentiated from the rest of the studied Lithuanian carp populations. In a natural environment, this would be reflective of gene flow and migration; speaking in terms of aquaculture practices, these results suggest that there is a degree of stock exchange and integration that occurs more intensely between Arnionys, Simnas, and Bartžuvė – and far less or even restricted within the Kapliai and Šilavotas farms. In addition, the genetic differentiation of the representatives of the Czech carp strain could be predicted due to the strain being previously raised in Czech Republic prior to its import into Lithuania, suggesting its reproductive isolation from Lithuanian carp strains.

The determined F_{st} values were found to be reflective of the differentiation determined within other studies. Samuilovienė et al. (2018) have found that the differentiation between Lithuanian

strains (Bubiai and BVP) was low (0.050), while the differentiation between Lithuanian strains and strains from other regions was comparably as high as it was found within this work, ranging from 0.068 to 0.289. Similarly, Hulak et al. (2010) have found that the F_{st} values between strains of different origins ranged between 0.064 and 0.189, while Kohlmann et al. (2005) have found the ranges between the European strains to be between 0.018 and 0.319.

4.2.3. Structural composition

Concerning the structural composition of the investigated populations, the result of the PCoA has separated the populations into 3 distinct clusters: separating the Czech strain (Kapliai), the Lithuanian strains raised within Šilavotas, and the remaining 3 Lithuanian carp populations (Arnionys, Simnas, and Bartžuvė). As such, the populations of Kapliai and Šilavotas harbour an insular nature that suggests a unique genetic structure found within these populations. In addition, the Kapliai population formed a large-sized cluster that suggests a high genetic variability within the population, meanwhile the comparably small size of the Šilavotas population suggests a low genetic variability and a genetic structure characteristic of an isolated population with a restricted gene pool, thus discriminating it from other Lithuanian carp populations. In addition, though largely insular, both of these populations were found to have had a certain degree of mixing with other populations. Some individuals from the Kapliai population were found to mix with a portion of the Simnas population, which suggests a possible strain representative exchange between Kapliai and Simnas. Šilavotas, on the other hand, was found to mix with the populations of Arnionys, Simnas, and Bartžuvė, directly indicating that certain shared genetic characteristics exist between the Lithuanian strain populations. Lastly, the large cluster of Arnionys, Simnas, and Bartžuvė indicates that these populations must be highly intermixed, or, in other words, the representatives of these farms must have a largely shared genetic background. Because of the fact that these results can’t be explained by natural migration and gene flow, it must be supposed that the clustering of these populations must be due to stock exchange. The distribution of the samples suggests what has been reflected within the previous results: Arnionys, Simnas, and Bartžuvė appear to be highly “panmitic”, suggesting a high level of stock exchange between the 3 farms. On another note, the isolated nature of the populations of Kapliai and Šilavotas indicates that these populations were kept isolated and had little to none new stock integration.

The differentiation and distance found between these populations was further visualized within the Neighbour-Joining tree. Here, the results of the F_{st} values and PCoA were therefore

reflected in the findings that Arnionys, Simnas, and Bartžuvė clustered together on the basis of distance, thus indicating that these populations are genetically close. Furthermore, the distant and unique genetic nature of both, Kapliai and Šilavotas, was reflected in the larger genetic distances of these two populations comparing to the remaining 3 Lithuanian carp populations.

Lastly, the genetic structural compositions inferred from the previous findings were confirmed once the genetic structure was investigated with the Structure software. The collective findings of this study had indicated that there is a clear divide between the investigated strains of two different regions: Lithuania and the Czech Republic. This is something that has been visibly reflected in the Structure results where K value was 2, which has separated the sample pool into two subpopulations: the Czech strain, and the Lithuanian strains. Furthermore, the structural analysis had further validated what was visible from the PCoA: to a certain degree, despite its unique gene set, the Kapliai population had a small-scale influence on the Lithuanian strains – in particular, the Simnas population, which appeared to share a good portion of its gene set with the Czech strain, indicating that a stock exchange must have occurred at some point between these two fish farms.

An expansion of these results was elucidated with a K = 5 analysis. Here a number of revelations were made. First of all, it had become clear that the populations of Arnionys, Simnas, and Bartžuvė indeed share a common genetic basis: although structural composition varied from population to population, clear trends of shared structures were visible. This had been suggested by the low genetic distance and F_{st} values between these populations. Furthermore, it had become clear that the fish stock that was held in Šilavotas had a degree of influence on the structural composition of Arnionys, Simnas, and Bartžuvė, as portions of Šilavotas-characteristic alleles were found within these populations, which suggests that a dissemination of Šilavotas fish stock did indeed occur at some point within other Lithuanian fish farms. This integration of fish stock raised in Šilavotas was also apparent from the lack of private alleles within the population. However, it isn't clear to what degree this stock exchange occurred: while it is clear that the genetic structure found within Šilavotas influenced the genetic structure of the remaining Lithuanian strain populations, from the K = 5 analysis it was evident that the influence was not large. In fact, the structural composition found within Arnionys, Simnas, and Bartžuvė was quite different to the composition found within Šilavotas. The Šilavotas population was found to have a composition that was mostly unified and unique among the Lithuanian strains populations. This suggests that while this population is not unique in terms of gene contents, it is unique in its particular composition. Similarly, like Šilavotas, the Kapliai population distinguished itself with a unique structural composition which corresponds

to the large genetic distances and F_{st} values found between the Czech strain and the remaining populations.

Altogether, the distinction of Šilavotas and Kapliai shows that these populations were most likely kept as purebred stocks, while Arnionys, Simnas, and Bartžuvė are likely hybrid strain populations that experience a high turnover of stock exchange.

4.3. Concluding thoughts

4.3.1. Study limitations

Several statistical factors exist which may have influenced the gathered results. First is sample size. Previously, it has been suggested that, for a microsatellite analysis of 5 to 10 studied markers, an appropriate number of samples per investigated population should be a minimum of 50 (O'Connell & Wright, 1997). Recent studies, however, have suggested that a practical number of samples for a microsatellite marker analysis could be between 25 and 35 per population (Hulak et al., 2010; Tóth et al., 2020). This idea comes from the fact that a larger amount of samples might detect private and less frequent alleles, however, the statistical power of these uncommon alleles is questionable (Tóth et al., 2020). With that said, 2 out of 5 of the studied populations don't meet this requirement: Simnas and Bartžuvė each had 15 samples, while the remaining populations had at least 30 samples within them. As such, the results garnered from these populations may be questionable; an unequal distribution of samples between each population, moreover, may have influenced the results to have a particular bias towards certain populations (such as Kapliai and Arnionys, which had the largest amounts of samples per population).

Yet another limitation is the panel of microsatellite markers selected for this work. Out of the 10 selected markers, 2 have shown estimates of null alleles present in all populations; 4 have shown null allele estimates in 4 out of 5 populations. Besides locus MFW9, the majority of determined estimates were low in the studied populations – as such, a large portion of the statistical analyses have been conducted by adjusting the panel of markers (reducing the panel from 10 markers to 9) and by utilizing calculation models adjusted for the probable presence of null alleles. However, the high likelihood of null allele presence within 6 of the 10 studied loci shines light onto the possible bias that could have been introduced into the statistical analyses. While null alleles are a statistical inevitability, given the high mutation rate of microsatellite regions (Chapuis & Estoup, 2007), it is important to review the gathered results critically and apply changes in future research, either by utilizing additional model adjustments or adjusting the panel of microsatellite markers used.

Furthermore, the differences between microsatellite markers pose a limitation in terms of data comparison. Genetic structure depends on the parameters determined within a locus, such as allele frequency and diversity, divergence of alleles between the investigated populations, and overall locus polymorphism (Hulak et al., 2010). As such, directly comparing the results gathered from different panels of microsatellite markers across genetic diversity studies can become challenging. For example, Napora-Rutkowski et al. (2017) have used 11 microsatellite markers, 8 of which were created by Crooijmans et al., (1997), 6 of which aligned with the markers chosen for this study. Similarly, Tóth et al., (2020) have investigated 12 loci, out of which only 5 aligned with the loci within this study. Bearing the diversity differences that occur from locus to locus, it becomes clear that certain biases or incorrect conclusions can arise when comparing inter-study findings. Moreover, the exact process of allele identification, as well as direct allele identities found within specific loci, are often omitted in published studies, thus creating a gap in the possibility of comparing results. As such, it would be recommendable to establish a wide-use microsatellite marker panel, first taking into consideration the easily comparable parameters of the chosen markers, such as commonly-found diversity, tendency to deviate from the HWE, and frequently found rates of estimated null allele frequencies.

4.3.2. Conservation efforts

Altogether, the picture gleaned from these results is multifaceted. On the one hand, the diversity parameters within our investigated populations have been reflected within studies on a global scale, all populations have high rates of heterozygosity, and all populations have varying degrees of genetic diversity, which suggests that the currently-raised carp stocks are in relatively good health. On the other hand, certain populations exhibited warning signs of possible future diversity loss due to inbreeding. Outbreeding heterozygotes out of the genepool may indeed lead to the extinction not just of the genetic variety within a strain, but, eventually, the strain itself as well (Tóth et al., 2020). Improperly conducted selection and a limited number of high-yield spawners eventually lead to a loss of fitness within a population, with the potential to introduce recessive diseases and a lowered resistance, leading to further loss of stock and subsequent diversity (Kohlmann et al., 2003, Tomljanović et al., 2013). Studies of carps raised within fish farms have observed that farm-raised carps were characterized by a significantly lower diversity than carps in wild populations (Tomljanović et al., 2013, Hulak et al., 2010, Napora-Rutkowski et al., 2017), facilitated by the unnatural bottleneck and founder effects raised by the selection schemes within fisheries. To this effect, it does not go without mentioning the recent events which have put the

national strains raised within the Šilavotas subdivision in jeopardy. These national strains, raised and cared for within the country for decades, now lay under the question of to whom and to where the individuals of these strains were disseminated. The potential loss of these strains is astounding.

As such, it is imperative to maintain the measures of preservation of these strains, the basis of which should be the constant genetic characterization and observation of the strains raised within the country. The results of this work have successfully described the genetic composition of common carp populations from 5 Lithuanian fish farms, with the hope of assisting with a more in-depth and controlled strain maintenance, conservation efforts, and the improvement of carp stocks within the country.

CONCLUSIONS

1. 122 carp individuals have been successfully genotyped utilizing 10 microsatellite markers.
2. The mean allele values per population ranged from 3.222 (Šilavotas population) to 6.778 (Simnas and Kapliai populations), the private alleles found within the populations ranged from 0 (Šilavotas population) to 20 (Kapliai population), and the inbreeding coefficient (F_{is}) values were found to range between 0.025 (Šilavotas population) and 0.241 (Simnas population). The determined genetic diversity metrics suggest that the Šilavotas population had the lowest genetic diversity among all populations, Kapliai – the largest, and that the Simnas population is the most at risk of diversity loss due to inbreeding depression.
3. 2 populations had higher degrees of differentiation from the remaining 3: the carp population maintained in Kapliai had the highest differentiation, discriminating it from other studied Lithuanian strains, and the Šilavotas population had, additionally, the highest differentiation between it and the remaining 3 Lithuanian carp populations (Arnionys, Simnas, and Bartžuvė), thus suggesting that these 2 populations harbour a distinct genetic composition represented by purebred strains.
4. The structural composition distribution across all 5 populations had been found to be distinct within the Kapliai and Šilavotas populations, thus confirming their unique genetic structure and a likely state of purebred strains. Arnionys, Simnas, and Bartžuvė, on the other hand, had a distinct and yet shared genetic structure, suggesting that these populations are kept as hybrid strains and have a high degree of stock exchange between these fish farms.

DESCRIPTION OF PERSONAL INPUT

Personal input of the author included the organization and execution of data collection, analysis of the raw data, data processing, and statistical data analysis.

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LIST OF REFERENCES

Aliah, R., Takagi, M., Dong, S., Teoh, C., & Taniguchi, N. (1999). Isolation and Inheritance of Microsatellite Markers in the Common Carp Cyprinus carpio. *Fisheries Science*, *65*, 235–239. https://doi.org/10.2331/fishsci.65.235

Aljanabi, S. M., & Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, *25*(22), 4692–4693. https://doi.org/10.1093/nar/25.22.4692

Andria Mananjara, D., Andriamanivo, H., & Vandeputte, M. (2016). Comparison of rearing performances and intermuscular bone number in the mirror and nude genotypes of common carp (Cyprinus carpio L.) in a controlled field test in Madagascar. *Aquaculture Reports*, *3*, 77–81. https://doi.org/10.1016/j.aqrep.2015.12.005

Balon, E. K. (1995). Origin and domestication of the wild carp, *Cyprinus carpio*: From Roman gourmets to the swimming flowers. *Aquaculture*, *129*(1), 3–48. https://doi.org/10.1016/0044-8486(94)00227-F

Balon, E. K. (2004). About the oldest domesticates among fishes. *Journal of Fish Biology*, *65*(s1), 1–27. https://doi.org/10.1111/j.0022-1112.2004.00563.x

Biba, A., Hoda, A., & Vardhami, E. (2014, April 23). *Genetic diversity of Cyprinus carpio from Ohrid Lake, estimated by four microsatellite loci*.

Cavalli-Sforza, L. L., & Edwards, A. W. F. (1967). Phylogenetic analysis. Models and estimation procedures. *American Journal of Human Genetics*, *19*(3 Pt 1), 233–257.

Chapuis, M.-P., & Estoup, A. (2007). Microsatellite Null Alleles and Estimation of Population Differentiation. *Molecular Biology and Evolution*, *24*(3), 621–631. https://doi.org/10.1093/molbev/msl191

Chapuis, M.-P., Lecoq, M., Michalakis, Y., Loiseau, A., Sword, G. A., Piry, S., & Estoup, A. (2008). Do outbreaks affect genetic population structure? A worldwide survey in Locusta migratoria, a pest plagued by microsatellite null alleles. *Molecular Ecology*, *17*(16), 3640–3653. https://doi.org/10.1111/j.1365-294X.2008.03869.x

Chen, L., Li, C., Li, B., Zhou, X., Bai, Y., Zou, X., Zhou, Z., He, Q., Chen, B., Wang, M., Xue, Y., Jiang, Z., Feng, J., Zhou, T., Liu, Z., & Xu, P. (2023). Evolutionary divergence of subgenomes in common carp provides insights into speciation and allopolyploid success. *Fundamental Research*. https://doi.org/10.1016/j.fmre.2023.06.011

Chen, L., Xu, J., Sun, X., & Xu, P. (2022). Research advances and future perspectives of genomics and genetic improvement in allotetraploid common carp. *Reviews in Aquaculture*, *14*(2), 957–978. https://doi.org/10.1111/raq.12636

Chistiakov, D. A., & Voronova, N. V. (2009). Genetic evolution and diversity of common carp Cyprinus carpio L. *Central European Journal of Biology*, *4*(3), 304–312. https://doi.org/10.2478/s11535-009-0024-2

ClimeFish. (2020). *Common Carp (Cyprinus carpio)*. Retrieved April 3, 2024, from https://climefish.eu/common-carp/

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

Dani, B., Sigal, K., Lior, S., & Gad, D. (2012). Genetic diversity of color phenotypes in the koi (*Cyprinus carpio* L.) as identified by molecular markers. *Journal of Biophysical Chemistry*, *2012*. https://doi.org/10.4236/jbpc.2012.33029

David, L., Rajasekaran, P., Fang, J., Hillel, J., & Lavi, U. (2001). Polymorphism in ornamental and common carp strains (Cyprinus carpio L.) as revealed by AFLP analysis and a new set of microsatellite markers. *Molecular Genetics and Genomics: MGG*, *266*(3), 353–362. https://doi.org/10.1007/s004380100569

Dong, C., Xu, J., Wang, B., Feng, J., Jeney, Z., Sun, X., & Xu, P. (2015). Phylogeny and Evolution of Multiple Common Carp (Cyprinus carpio L.) Populations Clarified by Phylogenetic Analysis Based on Complete Mitochondrial Genomes. *Marine Biotechnology (New York, N.Y.)*, *17*(5), 565–575. https://doi.org/10.1007/s10126-015-9639-7

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

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

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*(3), 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Fabrice, T. (2018). Fish Domestication: An Overview. In *Animal Domestication*. IntechOpen. https://doi.org/10.5772/intechopen.79628

FAO. (2005). Cyprinus carpio. Cultured Aquatic Species Information Programme. Text by Peteri, A. *Fisheries and Aquaculture.* Retrieved March 24, 2024, from https://www.fao.org/fishery/en/culturedspecies/cyprinus_carpio.

Goudet, J. (2003). Fstat (ver. 2.9.4), a program to estimate and test population genetics parameters. Available from http://www.unil.ch/izea/softwares/fstat.html Updated from Goudet [1995]

He, X., Wen, Y., Li, Z., Zhou, Y., Hu, W., Sun, J., & Liu, Q. (2023). Body color selection of domesticated carp (*Cyprinus carpio*) in traditional agricultural systems: Insight provided by growth performance, nutritional quality, and genetic diversity. *Aquaculture*, *572*, 739528. https://doi.org/10.1016/j.aquaculture.2023.739528

Holsinger, K. E., & Weir, B. S. (2009). Genetics in geographically structured populations: Defining, estimating and interpreting FST. *Nature Reviews. Genetics*, *10*(9), 639–650. https://doi.org/10.1038/nrg2611

Hulak, M., Kaspar, V., Kohlmann, K., Coward, K., Tešitel, J., Rodina, M., Gela, D., Kocour, M., & Linhart, O. (2010). Microsatellite-based genetic diversity and differentiation of foreign common carp (*Cyprinus carpio*) strains farmed in the Czech Republic. *Aquaculture*, *298*(3), 194–201. https://doi.org/10.1016/j.aquaculture.2009.10.021

Jeney, G., Ardó, L., Rónyai, A., Bercsényi, M., & Jeney, Z. (2011). Resistance of genetically different common carp, Cyprinus carpio L., families against experimental bacterial challenge with Aeromonas hydrophila. *Journal of Fish Diseases*, *34*(1), 65–70. https://doi.org/10.1111/j.1365-2761.2010.01211.x

Ji, D., Su, X., Yao, J., Zhang, W., Wang, R., & Zhang, S. (2022). Genetic Diversity and Genetic Differentiation of Populations of Golden-Backed Carp (Cyprinus carpio var. Jinbei) in Traditional Rice Fields in Guizhou, China. *Animals*, *12*(11), Article 11. https://doi.org/10.3390/ani12111377

Ji, P., Zhang, Y., Li, C., Zhao, Z., Wang, J., Li, J., Xu, P., & Sun, X. (2012). High throughput mining and characterization of microsatellites from common carp genome. *International Journal of Molecular Sciences*, *13*(8), 9798–9807. https://doi.org/10.3390/ijms13089798

Khatei, A., Tripathy, P. S., & Parhi, J. (2021). Molecular Markers in Aquaculture. In P. K. Pandey & J. Parhi (Eds.), *Advances in Fisheries Biotechnology* (pp. 165–174). Springer Nature. https://doi.org/10.1007/978-981-16-3215-0_11

Kirpitchnikov V.S. (1967). Homologous hereditary variation and evolution of wild common carp *Cyprinus carpio* L, *Genetika 8*, 65–72 (in. Russian).

Kirpitchnikov, V. S. (1999). *Genetics and Breeding of Common Carp*. Editions Quae.

Kloskowski, J. (2011). Impact of common carp Cyprinus carpio on aquatic communities: Direct trophic effects versus habitat deterioration. *Fundamental and Applied Limnology / Archiv Für Hydrobiologie*, *178*, 245–255. https://doi.org/10.1127/1863-9135/2011/0178-0245

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. https://doi.org/10.1016/S0990-7440(03)00082-2

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), 253–266. https://doi.org/10.1016/j.aquaculture.2005.02.024

Kolder, I. C. R. M., van der Plas-Duivesteijn, S. J., Tan, G., Wiegertjes, G. F., Forlenza, M., Guler, A. T., Travin, D. Y., Nakao, M., Moritomo, T., Irnazarow, I., den Dunnen, J. T., Anvar, S. Y., Jansen, H. J., Dirks, R. P., Palmblad, M., Lenhard, B., Henkel, C. V., & Spaink, H. P. (2016). A full-body transcriptome and proteome resource for the European common carp. *BMC Genomics*, *17*(1), 701. https://doi.org/10.1186/s12864-016-3038-y

Laloei, F., Gilkolaei, S. R., & Taghavi, M. J. (2013). Genetic Diversity and Differentiation of Common Carp(Cyprinus carpio L.) in the southern part of Caspian Sea by using microsatellite markers. *Asian Fisheries Science*, *26*(2), 115–127.

Larhammar, D., & Risinger, C. (1994). Molecular genetic aspects of tetraploidy in the common carp Cyprinus carpio. *Molecular Phylogenetics and Evolution*, *3*(1), 59–68. https://doi.org/10.1006/mpev.1994.1007

Lei, Y., Zhou, Y., Price, M., & Song, Z. (2021). Genome-wide characterization of microsatellite DNA in fishes: Survey and analysis of their abundance and frequency in genome-specific regions. *BMC Genomics*, *22*(1), 421. https://doi.org/10.1186/s12864-021-07752-6

Lentinaitė, I. (2021). Genetic Diversity of Common Carp *Cyprinus Carpio* L. Strains Found in Lithuania. [Bachelor's thesis, Vilnius University].

Li, J.-T., Wang, Q., Huang Yang, M.-D., Li, Q.-S., Cui, M.-S., Dong, Z.-J., Wang, H.-W., Yu, J.-H., Zhao, Y.-J., Yang, C.-R., Wang, Y.-X., Sun, X.-Q., Zhang, Y., Zhao, R., Jia, Z.-Y., & Wang, X.-Y. (2021). Parallel subgenome structure and divergent expression evolution of allo-tetraploid common carp and goldfish. *Nature Genetics*, *53*(10), 1493–1503. https://doi.org/10.1038/s41588-021-00933-9

Liu, X.-G., Tan, L.-J., Lei, S.-F., Liu, Y.-J., Shen, H., Wang, L., Yan, H., Guo, Y.-F., Xiong, D.-H., Chen, X.-D., Pan, F., Yang, T.-L., Zhang, Y.-P., Guo, Y., Tang, N. L., Zhu, X.-Z., Deng, H.-Y., Levy, S., Recker, R. R., … Deng, H.-W. (2009). Genome-wide association and replication studies identified TRHR as an important gene for lean body mass. *American Journal of Human Genetics*, *84*(3), 418–423. https://doi.org/10.1016/j.ajhg.2009.02.004

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

Liubartaitė, G. (2022). Genotyping of *Cyprinus carpio* in Offspring Generation of Lithuanian Strains Utilising Microsatellite Markers. [Bachelor's thesis, Vilnius University].

Ludanny, R. I., Chrisanfova, G. G., Prizenko, V. K., Bogeruk, A. K., & Semyenova, S. K. (2010). Polymorphism of microsatellite markers in Russian common carp (Cyprinus carpio L.) breeds. *Russian Journal of Genetics*, *46*(5), 572–577. https://doi.org/10.1134/S1022795410050108

Luo, M., Wang, L., Zhu, W., Fu, J., Song, F., Fang, M., Dong, J., & Dong, Z. (2018). Identification and characterization of skin color microRNAs in Koi carp (Cyprinus carpio L.) by Illumina sequencing. *BMC Genomics*, *19*(1), 779. https://doi.org/10.1186/s12864-018-5189-5

Luo, Z., Brock, J., Dyer, J. M., Kutchan, T., Schachtman, D., Augustin, M., Ge, Y., Fahlgren, N., & Abdel-Haleem, H. (2019). Genetic Diversity and Population Structure of a Camelina sativa Spring Panel. *Frontiers in Plant Science*, *10*. https://doi.org/10.3389/fpls.2019.00184

Matsuzaki, S. S., Mabuchi, K., Takamura, N., Nishida, M., & Washitani, I. (2009). Behavioural and morphological differences between feral and domesticated strains of common carp Cyprinus carpio. *Journal of Fish Biology*, *75*(6), 1206–1220. https://doi.org/10.1111/j.1095-8649.2009.02345.x

Mutethya, E., Yongo, E., Laurent, C., Waithaka, E., & Lomodei, E. (2020). Population biology of common carp, Cyprinus carpio (Linnaeus, 1758), in Lake Naivasha, Kenya. *Lakes & Reservoirs: Science, Policy and Management for Sustainable Use*, *25*(3), 326–333. https://doi.org/10.1111/lre.12322

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

Nedoluzhko, A. V., Slobodova, N. V., Sharko, F., Shalgimbayeva, G. M., Tsygankova, S. V., Boulygina, E. S., Jeney, Z., Nguyen, V. Q., Pham, T. T., Nguyen, Đ. T., Volkov, A. A.,

Fernandes, J. M. O., & Rastorguev, S. M. (2020). A new strain group of common carp: The genetic differences and admixture events between Cyprinus carpio breeds. *Ecology and Evolution*, *10*(12), 5431–5439. https://doi.org/10.1002/ece3.6286

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

Ohno, S., Muramoto, J., Christian, L., & Atkin, N. B. (1967). Diploid-tetraploid relationship among old-world members of the fish family Cyprinidae. *Chromosoma*, *23*(1), 1–9. https://doi.org/10.1007/BF00293307

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

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*, *28*(19), 2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Pečiukėnas, A. (2006). *Akvakultūra Lietuvoje: istorinė apžvalga*. Lietuvos hidrobiologų draugija.

Piačková, V., Flajšhans, M., Pokorová, D., Reschová, S., Gela, D., Čížek, A., & Veselý, T. (2013). Sensitivity of common carp, Cyprinus carpio L., strains and crossbreeds reared in the Czech Republic to infection by cyprinid herpesvirus 3 (CyHV-3; KHV). *Journal of Fish Diseases*, *36*(1), 75–80. https://doi.org/10.1111/jfd.12007

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959. https://doi.org/10.1093/genetics/155.2.945

Qi, H., Jin, M., Duan, Y., Du, X., Zhang, Y., Ren, F., Wang, Y., Tian, Q., Wang, X., Wang, Q., Zhu, Y., Xie, Y., Liu, C., Cao, X., Mishina, Y., Chen, D., Deng, C., Chang, Z., & Chen, L. (2014). FGFR3 induces degradation of BMP type I receptor to regulate skeletal development. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1843*(7), 1237–1247. https://doi.org/10.1016/j.bbamcr.2014.03.011

Rahman, M. M. (2015). Role of common carp (Cyprinus carpio) in aquaculture production systems. *Frontiers in Life Science*, *8*(4), 399–410. https://doi.org/10.1080/21553769.2015.1045629

Ramya, V. L., & Behera, B. K. (2023). Molecular Markers and Their Application in Fisheries and Aquaculture. In B. K. Behera (Ed.), *Biotechnological Tools in Fisheries and Aquatic Health Management* (pp. 115–150). Springer Nature. https://doi.org/10.1007/978-981-99-2981-8_7

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

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

Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*(4), 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Samuilovienė, A., Staponkus, R., Kontautas, A., Skersonas, A., & Bertašiūtė, V. (2018). Genetinis karpio veislės/ių šilavoto poskyryje identifikavimas.

Singh, E., Sharma, O., Hk, J., Sharma, A., Ojha, M., & Saini, V. P. (2015). Microsatellite Based Genetic Diversity and Differentiation of Common Carp, Cyprinus carpio in Rajasthan (India). *National Academy Science Letters*, *38*. https://doi.org/10.1007/s40009-014-0340-6

Svåsand, T., Crosetti, D., García-Vázquez, E., & Verspoor, E. (2007). Genetic impact of aqua culture activities on native populations. Genimpact fi nal scientifi c report (EU contract n. RICA-CT-2005-022802). 176 p. http://genimpact.imr.no/

Szpiech, Z. A., & Rosenberg, N. A. (2011). On the size distribution of private microsatellite alleles. *Theoretical Population Biology*, *80*(2), 100–113. https://doi.org/10.1016/j.tpb.2011.03.006

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Thai, B. T., Burridge, C. P., & Austin, C. M. (2007). Genetic diversity of common carp (*Cyprinus carpio* L.) in Vietnam using four microsatellite loci. *Aquaculture*, *269*(1), 174–186. https://doi.org/10.1016/j.aquaculture.2007.05.017

The Fishes of North Carolina. (n.d.). Cyprinus carpio » NCFishes.com. *NCFishes.Com*. Retrieved May 20, 2024, from https://ncfishes.com/freshwater-fishes-of-north-carolina/cyprinus-carpio/

Tomljanović, T., Treer, T., Cubric Curik, V., Safner, T., Sprem, N., Piria, M., Matulić, D., Safner, R., & Aničić, I. (2013). Microsatellite-based genetic variability and differentiation of hatchery and feral common carp Cyprinus carpio L. (Cyprinidae, Cypriniformes) populations in Croatia. *Archives of Biological Sciences*, *65*, 577–584. https://doi.org/10.2298/ABS1302577T

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

Vandeputte, M. (2003). Selective breeding of quantitative traits in the common carp (*Cyprinus carpio*): A review. *Aquatic Living Resources*, *16*(5), 399–407. https://doi.org/10.1016/S0990-7440(03)00056-1

Vilizzi, L. (2012). The common carp, Cyprinus carpio, in the Mediterranean region: Origin, distribution, economic benefits, impacts and management. *Fisheries Management and Ecology*, *19*(2), 93–110. https://doi.org/10.1111/j.1365-2400.2011.00823.x

Virbickas, J. (2000). *Lietuvos žuvys*. Mokslas.

Wang, C., Li, S., Nagy, Z. T., Lehoczky, I., Huang, L., Zhao, Y., Song, X., & Jeney, Z. (2010). Molecular genetic structure and relationship of Chinese and Hungarian common carp (Cyprinus carpio L.) strains based on mitochondrial sequence. *Aquaculture Research*, *41*(9), 1339–1347. https://doi.org/10.1111/j.1365-2109.2009.02422.x

Wang, J.-T., Li, J.-T., Zhang, X.-F., & Sun, X.-W. (2012). Transcriptome analysis reveals the time of the fourth round of genome duplication in common carp (Cyprinus carpio). *BMC Genomics*, *13*, 96. https://doi.org/10.1186/1471-2164-13-96

Wang, P., Christiana, O., Yu, L., Zhang, C., Su, S., & Tang, Y. (2021). The complete mitochondrial genome of the common strain black carp (Cyprinus carpio var. Baisenensis). *Mitochondrial DNA. Part B, Resources*, *6*(2), 542–545. https://doi.org/10.1080/23802359.2020.1869607

Xu, J., Jiang, Y., Zhao, Z., Zhang, H., Peng, W., Feng, J., Dong, C., Chen, B., Tai, R., & Xu, P. (2019). Patterns of Geographical and Potential Adaptive Divergence in the Genome of the Common Carp (Cyprinus carpio). *Frontiers in Genetics*, *10*. https://doi.org/10.3389/fgene.2019.00660

Xu, P., Jiang, Y., Xu, J., Li, J., & Sun, X. (2016). 10—Genomics in the common carp. In S. MacKenzie & S. Jentoft (Eds.), *Genomics in Aquaculture* (pp. 247–274). Academic Press. https://doi.org/10.1016/B978-0-12-801418-9.00010-X

Xu, P., Xu, J., Liu, G., Chen, L., Zhou, Z., Peng, W., Jiang, Y., Zhao, Z., Jia, Z., Sun, Y., Wu, Y., Chen, B., Pu, F., Feng, J., Luo, J., Chai, J., Zhang, H., Wang, H., Dong, C., … Sun, X. (2019). The allotetraploid origin and asymmetrical genome evolution of the common carp Cyprinus carpio. *Nature Communications*, *10*(1), 4625. https://doi.org/10.1038/s41467-019-12644-1

Xu, P., Zhang, X., Wang, X., Li, J., Liu, G., Kuang, Y., Xu, J., Zheng, X., Ren, L., Wang, G., Zhang, Y., Huo, L., Zhao, Z., Cao, D., Lu, C., Li, C., Zhou, Y., Liu, Z., Fan, Z., … Sun, X. (2014). Genome sequence and genetic diversity of the common carp, Cyprinus carpio. *Nature Genetics*, *46*(11), 1212–1219. https://doi.org/10.1038/ng.3098

Yaqoob, S. (2021). A Review of Structure, Origin, Purpose & Impact of Common Carp (Cyprinuscarpio) in India. *Annals of the Romanian Society for Cell Biology*, *25*(6), Article 6.

Yue, G. H., Ho, M. Y., Orban, L., & Komen, J. (2004). Microsatellites within genes and ESTs of common carp and their applicability in silver crucian carp. *Aquaculture*, *234*(1), 85–98. https://doi.org/10.1016/j.aquaculture.2003.12.021

Zambrano, L., Martínez-Meyer, E., Mendes, N., & Peterson, A. (2006). Invasive potential of exotic aquaculture fish in American freshwater systems. *Canadian Journal of Fisheries & Aquatic Sciences*, *63*, 1903–1910.

APPENDICES

Appendix 1. All determined genotypes within the assessed populations of this work

Code	MFW1	MFW2	MFW6	MFW7	MFW9	MFW11	MFW13	MFW17	MFW20	MFW28
A1	206/221	238/258	142/142	191/195	119/134	196/202	190/194	241/274	242/251	285/301
A2	206/206	212/238	130/142	186/195	130/130	196/202	182/194	241/274	237/251	285/301
A3	206/206	222/250	142/142	186/286	0/0	196/202	178/190	237/237	242/251	301/301
A4	206/206	222/250	142/142	186/286	0/0	196/202	178/190	237/237	242/251	301/301
A5	206/206	222/238	142/142	195/286	130/130	202/202	178/190	237/237	247/251	301/301
A6	0/0	0/0	130/142	186/186	134/134	196/206	182/194	237/274	242/242	285/301
A7	212/212	222/264	142/142	199/280	119/119	196/202	190/190	237/237	247/247	281/301
A8	212/212	250/258	142/147	186/195	119/119	196/202	190/190	241/274	242/242	281/290
A9	212/221	250/258	142/142	186/195	111/111	196/206	190/190	241/274	242/251	281/285
A10	206/206	258/258	142/147	186/186	134/134	202/206	190/190	274/274	247/251	285/301
A11	206/221	238/250	142/147	186/195	119/119	202/206	190/194	241/274	247/251	285/301
A12	206/212	238/258	142/147	186/195	119/119	202/206	190/190	274/274	247/251	290/301
A13	206/206	250/250	142/142	186/230	134/134	202/206	182/194	237/237	242/251	301/301
A14	206/212	258/258	142/142	186/186	134/134	196/206	190/194	237/274	242/242	301/301
A15	206/212	212/258	142/142	186/261	0/0	202/202	182/190	237/237	247/247	301/301
A16	206/206	208/250	127/142	186/253	119/119	202/202	190/190	241/241	242/247	301/301
A17	212/212	250/250	142/147	186/230	0/0	196/196	194/194	241/270	242/251	285/301
A18	0/0	258/258	142/142	186/195	0/0	202/206	182/194	237/274	242/251	285/301
A19	202/206	258/258	142/147	237/261	0/0	196/202	190/190	237/237	242/247	301/307
A20	212/212	250/250	142/142	186/195	130/130	196/202	182/190	237/270	242/242	285/301
A21	206/206	212/250	142/142	186/261	0/0	196/202	182/190	237/274	242/247	301/301
A22	206/206	212/250	142/147	186/261	0/0	196/202	190/194	241/274	242/251	301/301
A23	0/0	212/250	142/147	186/261	0/0	202/206	190/194	274/274	247/251	285/285
A24	212/212	250/258	142/142	186/195	119/119	196/202	194/194	237/237	242/247	285/290
A25	206/206	258/258	142/142	186/261	0/0	196/206	194/194	241/274	247/251	301/301
A26	206/206	250/264	142/142	186/195	119/119	196/196	182/194	237/237	242/251	301/301
A27	206/206	258/258	142/142	186/195	119/119	202/206	194/194	237/274	251/251	301/301
A28	219/219	250/264	142/142	186/286	0/0	196/202	190/194	237/274	223/251	281/285
A29	206/206	258/258	142/142	186/286	119/119	196/202	182/182	237/274	223/251	301/301
A30	206/221	250/258	138/142	280/280	0/0	196/196	182/194	241/251	242/251	281/290
A31	206/212	250/258	142/142	186/186	134/134	196/206	190/194	241/274	242/247	290/301
A32	212/219	250/264	147/147	186/195	130/130	152/206	182/190	237/237	242/247	281/301
A33	212/221	250/264	142/142	195/199	119/119	152/206	190/194	241/247	247/247	281/285
A34	212/221	250/258	142/142	195/195	119/119	196/202	190/194	237/237	242/247	281/285
A35	206/206	202/238	130/142	195/261	134/134	196/206	194/194	237/274	242/242	301/301
A36	206/206	250/258	142/142	186/195	134/134	196/206	194/194	237/274	242/251	301/301
A37	206/206	238/250	142/147	186/230	119/119	196/206	190/194	241/241	242/247	301/301
A38	212/219	250/250	142/142	199/286	119/119	196/196	190/194	237/247	247/247	281/281
A39	206/206	238/264	142/142	195/195	119/119	196/206	190/194	237/274	242/251	301/301
A40	212/219	238/250	142/142	186/195	119/119	202/202	182/190	237/241	242/247	281/285
A41	219/221	238/250	142/142	191/230	0/0	196/202	190/194	237/237	242/242	285/301
A42	206/206	202/264	142/142	186/195	130/130	202/202	190/190	237/270	242/247	301/301
A43	183/183	202/258	142/142	186/253	0/0	196/196	190/194	241/241	242/251	285/285
A44	219/219	250/264	142/142	186/286	0/0	196/196	182/190	247/274	251/251	281/285
A45	219/219	250/264	147/147	186/186	0/0	196/196	182/182	237/247	242/247	281/285

Appendix 1. All determined genotypes within the assessed populations of this work. Continued.

A46	206/206	212/250	142/142	186/261	0/0	202/206	190/194	274/274	242/251	301/301
A47	206/206	250/258	142/147	195/230	134/134	196/206	182/194	237/237	242/251	301/301
A48	202/206	202/250	142/142	186/280	119/119	193/193	190/211	241/241	247/251	285/285
K1	212/212	178/232	142/147	230/230	0/0	196/206	194/208	237/264	245/249	290/290
K2	178/212	190/244	142/151	195/230	119/134	193/196	194/194	274/274	229/242	281/290
K3	172/212	170/170	147/151	195/230	119/119	196/196	158/202	237/264	242/251	290/290
K4	172/221	170/170	147/151	195/257	134/134	193/206	194/194	237/274	229/245	290/290
K5	206/212	190/244	130/151	195/257	0/0	196/206	194/194	237/274	203/242	285/285
K6	178/225	170/190	142/147	195/257	134/134	162/193	194/202	274/274	245/249	290/290
K7	212/221	170/250	130/142	195/195	134/134	196/206	194/194	274/274	223/245	290/290
K8	212/212	170/170	151/151	195/230	119/134	193/202	158/194	264/274	245/251	290/290
K9	172/212	232/244	130/142	230/230	0/0	162/196	194/202	237/274	203/242	290/290
K10	172/212	170/232	130/142	257/257	119/119	162/193	190/190	274/274	203/245	290/290
K11	206/212	178/250	142/151	195/247	134/134	193/196	158/190	241/274	223/245	301/301
K12	178/219	170/250	142/147	195/230	134/134	162/193	194/194	237/274	242/245	290/290
K13	212/212	190/250	142/142	195/230	134/134	162/193	190/190	270/274	245/249	290/290
K14	212/212	170/244	130/142	195/230	134/134	202/206	158/194	237/270	203/245	281/281
K15	172/212	170/190	142/151	230/247	0/0	196/202	158/194	237/274	223/245	290/290
K16	212/212	178/244	142/147	230/247	0/0	162/193	158/194	237/237	223/249	290/290
K17	178/192	170/232	130/142	230/230	0/0	193/196	158/194	274/274	203/245	290/290
K18	212/221	170/250	130/142	195/195	0/0	196/196	194/194	264/274	203/245	290/290
K19	172/212	190/244	151/151	230/257	134/134	196/206	158/194	264/274	245/251	290/290
K20	212/221	190/250	147/147	195/230	134/134	193/196	158/202	274/274	203/245	290/290
K21	172/178	170/244	130/151	195/230	119/134	193/206	158/194	274/274	229/245	290/290
K22	212/221	178/244	147/147	195/195	134/134	202/206	194/205	264/264	223/245	290/290
K23	206/212	178/250	151/151	195/247	0/0	202/202	158/158	241/274	223/251	285/285
K24	206/225	196/250	142/151	195/195	134/134	193/206	194/211	241/274	245/245	290/301
K25	221/221	250/250	151/155	230/247	134/134	202/206	194/202	237/280	214/245	290/290
K26	206/212	190/238	142/142	191/191	134/134	193/193	190/190	241/274	242/242	290/301
K27	178/192	232/250	130/151	199/199	134/134	193/196	158/194	274/274	242/242	290/301
K28	196/196	232/250	147/155	195/195	134/134	193/196	190/190	274/284	203/249	290/290
K29	178/221	244/244	147/151	230/230	134/134	196/196	190/211	264/274	242/249	290/290
K30	212/221	178/250	142/147	195/257	134/134	196/196	190/190	237/270	199/245	290/290
K31	172/212	170/190	130/147	195/257	134/134	193/196	158/158	237/270	203/242	290/290
K32	219/221	250/250	142/151	195/247	134/134	202/202	194/202	237/280	242/249	290/290
K33	225/225	170/178	147/151	195/230	119/134	193/193	158/202	270/274	229/245	290/290
K34	221/221	190/250	130/142	195/195	119/134	193/193	158/190	241/280	229/245	285/285
K35	212/212	190/264	130/151	195/195	119/134	193/206	158/202	270/270	229/245	281/281
K36	172/178	190/250	130/151	199/199	0/0	193/193	190/190	237/274	245/249	290/290
K37	212/212	178/244	142/147	195/247	134/134	196/196	202/202	237/274	242/249	290/290
K38	212/212	232/244	130/151	230/230	134/134	196/196	158/158	274/274	245/249	290/290
K39	225/225	190/250	147/155	230/247	134/134	196/196	158/202	237/237	223/245	290/290
K40	212/221	250/250	142/151	230/230	0/0	193/196	158/190	237/237	245/249	290/290
K41	219/225	250/250	142/155	195/195	119/134	193/206	194/202	274/274	242/251	290/290
K42	192/212	190/244	130/151	257/257	134/134	196/196	158/194	237/274	203/245	290/290
K43	225/225	178/250	142/142	195/247	0/0	196/206	190/190	274/274	249/249	290/290
K44	225/225	190/244	130/142	257/257	119/119	193/193	158/194	237/274	203/249	290/290

Appendix 1. All determined genotypes within the assessed populations of this work. Continued.

S1	206/206	202/250	127/142	195/230	130/130	196/206	186/194	237/274	242/242	301/301
S2	206/206	250/264	130/142	195/230	0/0	193/202	186/194	237/237	247/251	290/290
S3	0/0	208/244	142/142	230/265	119/119	202/202	194/194	237/274	247/251	285/290
S4	0/0	250/264	142/151	186/265	0/0	193/202	194/194	237/274	247/251	285/290
S5	202/202	250/250	142/142	253/253	106/106	196/196	190/216	237/247	214/247	270/301
S6	206/206	212/212	127/142	186/230	130/130	196/196	190/190	237/247	242/242	301/301
S7	206/206	160/212	142/142	186/225	0/0	206/206	190/190	241/280	214/251	301/301
S8	212/212	250/258	122/127	186/257	119/119	193/193	186/202	237/274	237/237	285/285
S9	212/212	170/264	142/151	230/230	119/119	196/202	186/190	274/284	247/251	290/290
S10	212/219	160/250	138/142	191/261	130/130	167/167	182/182	237/237	251/251	290/290
S11	212/212	258/258	130/142	186/195	134/134	196/206	190/190	241/274	251/251	281/285
S12	206/212	212/250	142/147	186/186	119/119	202/202	182/190	237/237	242/247	301/301
S13	206/206	225/225	142/142	286/286	0/0	196/202	178/190	237/237	242/251	281/301
S14	212/212	238/258	142/151	186/191	119/134	196/206	194/194	237/241	242/251	281/285
S15	206/206	222/250	142/142	186/286	0/0	196/202	178/190	237/237	242/251	301/301
B16	212/212	250/250	122/142	186/195	0/0	196/206	182/190	241/241	242/251	285/301
B17	206/212	250/264	142/147	186/195	0/0	196/202	190/190	237/274	247/247	281/301
B18	206/212	250/258	142/142	195/230	130/134	193/196	186/194	241/241	247/251	281/281
B19	219/219	222/250	122/142	191/261	0/0	196/206	182/194	241/241	251/251	285/285
B20	183/183	212/250	142/180	195/286	134/134	196/206	164/186	237/237	251/251	285/285
B21	183/219	202/250	142/147	186/195	0/0	196/206	182/194	241/274	242/251	285/285
B22	206/212	202/250	122/142	186/195	119/119	196/206	190/194	241/274	242/251	301/301
B23	206/219	184/202	142/147	186/253	130/130	196/202	190/190	237/241	242/251	290/301
B24	206/219	202/250	142/142	261/286	0/0	202/206	194/194	237/237	242/242	301/301
B25	206/221	250/250	147/147	230/253	119/119	196/196	186/194	241/241	242/251	285/285
B26	206/212	202/250	142/142	191/286	0/0	202/206	182/190	237/237	237/251	301/301
B27	206/212	212/250	142/142	186/191	0/0	202/202	190/190	270/270	247/251	290/290
B28	206/206	250/258	142/151	186/195	130/134	196/206	194/194	241/274	247/251	285/301
B29	219/219	202/250	142/142	186/191	0/0	202/202	194/194	237/247	237/251	285/285
B30	172/206	250/250	134/147	199/199	111/111	196/196	190/190	241/251	242/251	301/301

Appendix 2. All allele frequencies by population

Locus	Allele	Kapliai	Arnionys	Simnas	Bartžuvė	Šilavotas
MFW1	**172**	0.102	0.000	0.000	0.033	0.000
	178	0.091	0.000	0.000	0.000	0.000
	183	0.000	0.022	0.000	0.100	0.000
	192	0.034	0.000	0.000	0.000	0.000
	196	0.023	0.000	0.000	0.000	0.000
	202	0.000	0.022	0.077	0.000	0.350
	206	0.057	0.544	0.500	0.367	0.100
	212	0.386	0.222	0.385	0.233	0.550
	219	0.034	0.111	0.038	0.233	0.000
	221	0.148	0.078	0.000	0.033	0.000
	225	0.125	0.000	0.000	0.000	0.000
MFW2	**160**	0.000	0.000	0.067	0.000	0.000
	170	0.193	0.000	0.033	0.000	0.000
	178	0.102	0.000	0.000	0.000	0.000
	184	0.000	0.000	0.000	0.033	0.000
	190	0.170	0.000	0.000	0.000	0.000
	196	0.011	0.000	0.000	0.000	0.000
	202	0.000	0.043	0.033	0.200	0.317
	208	0.000	0.011	0.033	0.000	0.000
	212	0.000	0.064	0.133	0.067	0.167
	222	0.000	0.043	0.033	0.033	0.000
	225	0.000	0.000	0.067	0.000	0.000
	232	0.080	0.000	0.000	0.000	0.000
	238	0.011	0.106	0.033	0.000	0.300
	244	0.159	0.000	0.033	0.000	0.000
	250	0.261	0.362	0.300	0.567	0.217
	258	0.000	0.277	0.133	0.067	0.000
	264	0.011	0.096	0.100	0.033	0.000
MFW6	**122**	0.000	0.000	0.033	0.100	0.000
	127	0.000	0.010	0.100	0.000	0.283
	130	0.182	0.031	0.067	0.000	0.117
	134	0.000	0.000	0.000	0.033	0.000
	138	0.000	0.010	0.033	0.000	0.000
	142	0.307	0.802	0.633	0.600	0.600
	147	0.193	0.146	0.033	0.200	0.000

Appendix 2. All allele frequencies by population. Continued.

	151	0.273	0.000	0.100	0.033	0.000
	155	0.045	0.000	0.000	0.000	0.000
	180	0.000	0.000	0.000	0.033	0.000
MFW7	**186**	0.000	0.427	0.300	0.267	0.317
	191	0.023	0.021	0.067	0.133	0.000
	195	0.398	0.240	0.100	0.233	0.000
	199	0.045	0.031	0.000	0.067	0.300
	225	0.000	0.000	0.033	0.000	0.000
	230	0.295	0.052	0.200	0.067	0.000
	237	0.000	0.010	0.000	0.000	0.000
	247	0.102	0.000	0.000	0.000	0.000
	253	0.000	0.021	0.067	0.067	0.200
	257	0.136	0.000	0.033	0.000	0.000
	261	0.000	0.083	0.033	0.067	0.183
	265	0.000	0.000	0.067	0.000	0.000
	280	0.000	0.042	0.000	0.000	0.000
	286	0.000	0.073	0.100	0.100	0.000
MFW11	**152**	0.000	0.021	0.000	0.000	0.000
	162	0.068	0.000	0.000	0.000	0.000
	167	0.000	0.000	0.067	0.000	0.000
	193	0.330	0.021	0.133	0.033	0.000
	196	0.352	0.406	0.333	0.433	0.750
	202	0.102	0.344	0.300	0.267	0.217
	206	0.148	0.208	0.167	0.267	0.033
MFW13	**158**	0.273	0.000	0.000	0.000	0.000
	164	0.000	0.000	0.000	0.033	0.000
	178	0.000	0.031	0.067	0.000	0.000
	182	0.000	0.177	0.100	0.133	0.400
	186	0.000	0.000	0.133	0.100	0.000
	190	0.205	0.427	0.367	0.367	0.217
	194	0.341	0.354	0.267	0.367	0.383
	202	0.136	0.000	0.033	0.000	0.000
	205	0.011	0.000	0.000	0.000	0.000
	208	0.011	0.000	0.000	0.000	0.000
	211	0.023	0.010	0.000	0.000	0.000
	216	0.000	0.000	0.033	0.000	0.000

Appendix 2. All allele frequencies by population. Continued.

MFW17	**237**	0.250	0.427	0.567	0.300	0.550
	241	0.057	0.208	0.100	0.433	0.000
	247	0.000	0.042	0.067	0.033	0.000
	251	0.000	0.010	0.000	0.033	0.000
	264	0.091	0.000	0.000	0.000	0.000
	270	0.080	0.031	0.000	0.067	0.000
	274	0.477	0.281	0.200	0.133	0.450
	280	0.034	0.000	0.033	0.000	0.000
	284	0.011	0.000	0.033	0.000	0.000
MFW20	**199**	0.011	0.000	0.000	0.000	0.000
	203	0.125	0.000	0.000	0.000	0.000
	214	0.011	0.000	0.067	0.000	0.000
	223	0.080	0.021	0.000	0.000	0.017
	229	0.068	0.000	0.000	0.000	0.000
	237	0.000	0.010	0.067	0.067	0.000
	242	0.159	0.396	0.267	0.267	0.717
	245	0.330	0.000	0.000	0.000	0.000
	247	0.000	0.271	0.200	0.167	0.000
	249	0.159	0.000	0.000	0.000	0.000
	251	0.057	0.302	0.400	0.500	0.267
MFW28	**270**	0.000	0.000	0.033	0.000	0.000
	281	0.057	0.135	0.100	0.100	0.150
	285	0.068	0.240	0.200	0.400	0.517
	290	0.818	0.052	0.267	0.100	0.000
	301	0.057	0.563	0.400	0.400	0.100
	307	0.000	0.010	0.000	0.000	0.233