Biologia Futura (2021) 72:385–393
https://doi.org/10.1007/s42977-021-00087-z

ORIGINAL PAPER

Twelve new microsatellite loci of Eurasian perch *Perca fluviatilis* Linnaeus, 1758

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Received: 16 December 2020 / Accepted: 7 May 2021 / Published online: 24 May 2021

Abstract

The Eurasian perch (*Perca fluviatilis* Linnaeus, 1758) is native to almost entire Eurasia. For over the last two decades, this species became an important candidate for intensive freshwater aquaculture due to its high consumer's acceptance and overall market value. Hence, the intensive production of Eurasian perch has increased considerably allowing effective domestication; there is still a need for the development of effective selective breeding programmes allowing its further expansion. This process, in turn, can be significantly facilitated by molecular genetics. The genetic information of Eurasian perch and its populations is limited. Up to date information of regarding genetic diversity of many populations is still missing, including microsatellites for Eurasian perch, which could be useful during the selective breeding programmes allowing parental assignment and/or to follow heritability of desired traits. In this study, we have developed and characterized new polymorphic microsatellites. Subsequently, those 12 markers have been used further to compare two Hungarian and one Polish Eurasian perch populations. The Hungarian stocks had high genetic similarity (with low diversity), as we assumed, while the Polish population differed significantly. All populations deviated significantly from the Hardy–Weinberg equilibrium, and heterozygote deficiency was detected in all, showing the presence of an anthropogenic effect.

Keywords Eurasian perch · *Perca fluviatilis* · Microsatellites · Genetic diversity · Population genetics

Introduction

The Eurasian perch (*Perca fluviatilis*) is a freshwater species native to northern Eurasia. It is popular among the consumers due to its delicious, high-quality meat. High market value and consumer's acceptance made it an important candidate for intensive fish farming (FAO 2014) and its production technology has been developing for over 25 years (Fontaine and Teletchea 2019). However, despite the huge progress in the development of numerous technological elements, including artificial feeding, rearing systems and the intensive breeding technologies (Härkönen et al. 2017), the aquaculture of Eurasian perch is still in its developmental phase. This is related to the lack of effective selective breeding programmes being the indispensable element of further expansion of commercial production of this species. Molecular biology can offer some possibilities in this process. Although the revolutionary technological development provides several effective solutions based on new generation sequencing (NGS) and genome projects, these are still costly and require complicated analytical pipelines before it can be potentially applied in the practice. Therefore, depending on the question to be answered (e.g. parentage, population genetics), it is still highly beneficial and easier to use microsatellite markers. It is mainly advisable for species with limited genetic information (such as Eurasian perch). Genetic microsatellite markers can provide information about targeted parts of the genome and thus provide information

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about the origin of the individuals, the genetic difference or diversity of the populations and the level of inbreeding. These can be used in genetic mapping, controlling selection programmes and verification of the effectiveness of andro- and gynogenesis (O'Conell and Wright 1997).

In the 1990s of twentieth century, the genetic variability of Eurasian perch was examined by allozymes (Heldstab and Katoh 1995), later by mitochondrial DNA (Nesbo et al. 1998) and Random Amplified Polymorphic DNA markers (RAPD) (Nesbo et al. 1999). Nevertheless, Lecrec et al. (2000) wrote that allozymes and mitochondrial DNA markers, as genetic markers, are inappropriate for a detailed description of the population genetic structure and deep phylogeographic characterization of species. First 10 (Lecrec et al. 2000), later 32 (Li et al. 2007) microsatellite markers were isolated from yellow perch, *P. flavescens*, and some of these markers were adapted to closely related species, including Eurasian perch. Gerlach et al. (2001) isolated and used *Sander vitreus* microsatellites for genetic diversity analyses of natural Eurasian perch population from Bodensee. Subpopulations were found that consist of closer relatives, moreover, in this case, microsatellites were used for ethological studies, as well. Because the gene flow among the subpopulations was limited or was completely absent, they could detect genetic differentiation on subpopulation level using microsatellites (Bergek and Björklund 2007, 2009; Bergek et al. 2010; Olsson et al. 2011). At first, Yang et al. (2009) isolated microsatellites from *P. fluviatilis*, and they experienced that genetic diversity of Eurasian perch is low in China—at the eastern border of the native habitat of the Eurasian perch (Yang et al. 2012). Later, Pukk et al. (2014) developed another polymorphic microsatellites for the species, using NGS method. From these works, only a few tested, efficient species-specific and interspecifically adapted microsatellites are available for analysing Eurasian perch (many of these have limited usability, because of size homoplasy and low allele number, Yue et al. 2010) and those did not provide the sufficient number of polymorph markers for detailed analyses such as intrafamily kinship investigation and population ecology or conservation biology studies.

Our aim was to develop further, usable, polymorph microsatellites followed by validating their applicability by estimating genetic diversity of two Hungarian stocks and a Polish natural population. To do so, the developed markers were used to define the structure of these populations in order to gain new knowledge on the genetic background of the Central European Eurasian perch populations. Moreover, in a long-term perspective, the data generated in this study is expected to be used to establish a broodstock with an appropriate level of heterogeneity and possibility of further control over the genetic structure of the cultured population.

Materials and methods

Sampling and DNA isolation

Fin clips were collected from altogether 182 Eurasian perch individuals. These were from 3 different locations in Central Europe: two Hungarian stocks (Biatorbágy: Hu-B, $N = 80$; Dunaföldvár: Hu-D, $N = 43$) and one Polish natural population (Olsztyn: Po-O, $N = 59$). Along with sampling, each specimen was checked for sex (with the use of catheterization; as described by Żarski et al. 2011). Isolated tissues were stored at − 20 °C in cc. ethanol (Reanal, Hungary) until further processing. Standard phenol/chloroform (Rothi-Phenol, Carl Roth, Germany; Chloroform, Reanal, Hungary) extraction method was used to isolate DNA (Sambrook and Russell 2001). DNA of 20 µg was separated for genomic library construction while the remaining samples were diluted to 50 ng/µl concentration for microsatellite analysis.

Genomic library construction

The modified method of Glenn and Schable (2005) was used to construct a genomic library enriched with CA-repeats. The DNA used for library construction was isolated from male individuals since in the examined species the males carry the sex-determining region (however, morphologically different sex chromosomes are not identified, and therefore, they carry both sex chromosomes (Rougeot et al. 2002). The 20 µg of genomic DNA was digested by restriction endonuclease enzymes (*Rsa I*, Thermo Fisher Scientific, USA/*HpyCH4 V*, New England BioLabs, USA) resulting in blunt ends. All products within the length of 300–1000 base pairs were re-isolated using a NucleoSpin Extract II kit (Macherey-Nagel, Germany). After the phosphatase treatment (Shrimp Alkaline Phosphatase, Thermo Fisher Scientific, USA) of 10 µg of fragments, Box I linker was ligated to them according to following 200 µl reaction mixture: 10 µg DNA-fragment, 5 mM BoxI linker, 5% PEG 4000 (Thermo Fisher Scientific, USA), 0.03 U/µl T4 DNA ligase (Thermo Fisher Scientific, USA), 0.2 U/µl BoxI enzyme (Thermo Fisher Scientific, USA), 1× Tango buffer (Thermo Fisher Scientific, USA), 10 mM ATP (Thermo Fisher Scientific, USA). The reaction mixture was kept on 4 °C overnight. The nucleotide sequence of the oligonucleotide used for the Box I linker was F: 5′-Phos-ATGTCT GAAGGTACCACTGCTGTCCGAAA-3′; R: 5′-CGGACA GCAGTGGTACCTTCAGACAT-3′). The linkage of the adapter was proved by a PCR where primers were bound to the adapter sequence. The PCR was run in a final reaction volume of 25 µl with the following ingredients: 1× Taq

DNA polymerase buffer (Thermo Fisher Scientific, USA), 0.4 µM Box I reverse primer, 2 mM $MgCl_2$ (Thermo Fisher Scientific, USA), 0.2 mM dNTP (Thermo Fisher Scientific, USA), 1 U Taq DNA polymerase (Thermo Fisher Scientific, USA) and 4 µl template (adapter connected DNA-fragment). The PCR profile of the reaction was the following: initial denaturation for two minutes at 94 °C, 35 repeated cycles of 94 °C denaturation for 20 s, 60 °C annealing for 30 s, 72 °C elongation for 3 min and finally 72 °C for 5 min for the final elongation. (Mastercycler 5341, Eppendorf, Germany). The PCR was followed by the collection of tandem repeats containing DNA fragments. The fragments were hybridized with a $(CA)_{10}$ oligonucleotide (3′ biotinylated) using the following protocol: the initial denaturation (92 °C for 5 min) was followed by the cooling of the reaction mixture from 70 to 50 °C with the decreasing of the temperature by 0.2 °C/s. The mixture was kept on 50 °C for ten minutes and then it was cooled to 15 °C with the decreasing of the temperature by 0.1 °C/s. The hybridization complexes were bound to the surface of streptavidin-covered magnetic beads (Dynabeads M-270 Streptavidin, Thermo Fisher Scientific, USA) by the method described by Glenn and Schable (2005). The resulting complexes were removed from the solution by a magnet. The repeat-containing DNA fragments were finally eluted by TLE solution (pH = 8.00) on 95 °C. The eluted single-stranded DNA was transformed to double-stranded DNA by the previously described PCR (Mastercycler 5341, Eppendorf, Germany) using adapter-specific primers. The resulting product was ligated to T-vector (pGEM-T Easy Vector System I, Promega, USA) and was transformed into a competent *Escherichia coli* cell (XL10 GOLD, Stratagene, USA) following the protocol described by the manufacturer. Colonies were filtered by blue-white screening (Ullmann et al. 1967). The size of inserts was determined by agarose gel electrophoresis (1.5%, 1×TBE buffer) following a colony PCR initiated at the T-vector coded M13 primer binding sites. The PCR was carried out in a final volume of 25 µl with the following ingredients: 1×Taq DNA polymerase buffer (Thermo Fisher Scientific, USA), 0.26–0.26 µM M13 forward and reverse primers (F: 5′ TGTAAAACGACGGCCAGT 3′; R: 5′ CAG GAAACAGCTATGACC 3′), 2 mM $MgCl_2$ (Thermo Fisher Scientific, USA), 0.2 mM dNTP (Thermo Fisher Scientific, USA), 1 U Taq DNA Polymerase (Thermo Fisher Scientific, USA) and a few cells of bacteria colonies as a template. The PCR profile of the reaction was the following: 3 times 95 °C for 2 min, 55 °C for 1 min, 72 °C for 2 min, then 41 times 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s and finally 72 °C for 5 min. After the evaluation of colony PCR products that showed their ligation to a vector by their large size (> 300 bp) were cleaned up by PCR Advanced Clean Up System (Viogene, USA). The sequences of the inserts were determined (3130 Genetic Analyzer, Applied Biosystems) using the SP6-(5′ CAT ACGATTTAGGTGACACTATAG 3′) and T7-(5′ TAA TACGACTCACTATAGGG 3′) primers and 3.1 BigDye kit (Applied Biosystems, USA) on the cleaned PCR products. Sequences were evaluated by MEGA5 software (Tamura et al. 2011). The sequences containing at least 5 dinucleotide repeats were selected and primers were designed to their flanking regions using Primer3Plus software (Untergasser et al. 2007). The optimal reaction parameters were determined (Table 1) for all markers.

Microsatellite analysis

Microsatellite analysis was carried out by using tailed oligonucleotides for universal fluorescent labelling of the fragments (Shimizu et al. 2002) and capillary electrophoresis for fragment length determination with single nucleotide accuracy. The forward primers were 5′ elongated with a 17 bp long tail sequence (tail; 5′ ATTACCGCGGCT GCTGG-microsatellite-specific oligo-3′) which was non-specific for the examined region. PCRs were run in 25 µl final volume with adding fluorescently labelled tail primer (dye: FAM, VIC, NED, PET; 5′dye-ATTACCGCGGCT GCTGG-3′): 1×Taq DNA polymerase buffer (containing $(NH_4)_2SO_4$, Thermo Fisher Scientific, USA), 132 nM forward and reverse primers, 132 nM labelled tail primer, 1.5–3.5 mM $MgCl_2$ (Thermo Fisher Scientific, USA), 0.2 mM dNTP (Thermo Fisher Scientific, USA), 0.04 U/µl Taq DNA polymerase (Thermo Fisher Scientific, USA) and 150 ng template DNA. The temperature profile was the following: 95 °C/2 min preliminary denaturation—(95 °C/15 s; annealing temperatures/1 min; 72 °C/2 min)×2 cycles—(95 °C/15 s; annealing temperatures/20 s; 72 °C/40 s)×45 cycles; 72 °C/5 min final extension. Table 1 summarizes primer sequences, concentration of $MgCl_2$ and annealing temperature during PCR, average allelic richness, numbers and size ranges of detected alleles and GenBank accession numbers. Length of the amplified products was determined using GeneScan 500 LIZ (Applied Biosystems, USA) molecular weight marker in Pop7 polymer (Applied Biosystems USA) on 3130 Genetic Analyzer (Applied Biosystems, USA). Twelve markers were identified (*MS 426 Pf, MS 427 Pf, MS 428 Pf, MS 439 Pf, MS 464 Pf, MS 467 Pf, MS 500 Pf, MS 719 Pf, MS 725 Pf, MS 726 Pf, MS 732 Pf, MS 739 Pf;* in Table 1) and were further used to describe the genetic structure, variability and relatedness of the 2 stocks originating from Hungary and one population from Poland.

Statistical analysis

Allele sizes were determined by GeneMapper 4.0 (Applied Biosystems, USA) as a basis to calculate population genetic

Table 1 Characterization of the 12 newly developed microsatellites

Locus	Forward primer (5′–3′)	Reverse primer (5′–3′)	$MgCl_2$ (mM)	At (°C)	Na	Ar	ASR (bp)	Acc. No.
MS 426 Pf	F: ACTGTATCTATGCTGAAA CGAAGT	R: TGCTATGAATATCTGTGC CTCTA	2.00	55	6	15,7	148–160	KX834191
MS 427 Pf	F: GCTGAGTTACAGTTTAAT GTATTTGA	R: ACAGCAGATTAGTATGGA GCAG	2.00	55	40	16,5	153–271	KX834192
MS 428 Pf	F: TATTAATCAAGTGTCCTG AAAGCT	R: GAGTTGTAATGTAGCATC ACGC	3.50	55	48	1,4	89–227	KX834193
MS 439 Pf	F: TCTGCCTGTCTCCCACCT	R: TTAAATCCATGCGACAAC TG	3.00	55	3	17,7	185–191	KX834195
MS 464 Pf	F: GCTCAACAAGGCTTTCAC AT	R: CACCAGGAAATGTTGCTT ATC	2.00	55	38	3,3	169–275	KX834200
MS 467 Pf	F: GTCCACCACAGCTTTACC AG	R: CCAACAGCCCACAATGC	2.00	55	5	2,2	136–144	KX834201
MS 500 Pf	F: AGACTATCGTGCTCCTGA GGT	R: CTAATTATAAATGACCTC CTCTGC	3.00	52	5	7,7	120–133	KX834203
MS 719 Pf	F: AACACATCTTCACAAGGA TTCC	R: AGCCTGTGGTTGATTGAT GA	2.00	55	13	5,8	125–155	KX834205
MS 725 Pf	F: TCAGTTCGTCAACACTAA TGGT	R: AGCAACCAACAATCACAA TAAG	2.00	55	15	10,2	218–327	KX834207
MS 726 Pf	F: TACTCATGCTACTAATGC TCATGT	R: TATGTATTGGCCTGTGTA TTGTAT	3.00	55	26	4,8	137–214	KX834208
MS 732 Pf	F: AGAACTGGGCTCAAGTGT CA	R: TCAACATCTTGTCAAACA GGTC	2.00	55	10	7,0	201–230	KX834212
MS 739 Pf	F: AGATCAATCGGTCAATGA GG	R: GAGAGACTCCATCTTCCA CAAC	2.00	55	13	15,7	173–209	KX834214

Primer sequences, $MgCl_2$ concentration and annealing temperature (At) during PCR reaction, number of detected alleles (Na), average allelic richness (Ar) allele size range (ASR) and accession numbers (Acc. no.)

properties. Excel Microsatellite Toolkit ver. 3.1.1 (Park 2001) was used to detect expected (H_E) and observed heterozygosity (H_O) values per populations and PIC-values (Polymorphic Information Content) in every single locus per population. F_{IS} and overall F_{ST} values were calculated using F_{STAT} ver. 2.9.3.2 (Goudet 1995). Deviation from Hardy–Weinberg equilibrium was determined using Genpop ver. 4.1.0 (Rousset 2008) software. Number of alleles per loci and per populations, gene diversity values per populations, mean number of alleles per populations and pairwise F_{ST} values were calculated by Arlequin ver. 3.5 (Excoffier et al. 2005) software while private alleles, number of effective alleles per populations, AMOVA analyses and PCoA (Principal Coordinate Analysis) were performed by GenAlEx ver. 6.502 (Peakall and Smouse 2012; Smouse et al. 2015). Populations ver. 1.2.32 (Langella 2002) was used for the definition of Nei's genetic distance. Based on the results for individual microsatellite genotyping the genetic structure of the examined stocks (without the usage of information on populations) was determined by Structure ver. 2.3.3 (Pritchard et al. 2000; Hubisz et al. 2009) software. This analysis was run with the following settings: Length of Burnin Period: 50,000; Number of MCMC Reps after Burnin: 200,000. The possible cluster distribution was analysed from $K=1$ to $K=8$ (Earl and vonHoldt 2012). The number of the most probable genetic cluster was determined based on likelihood analysis of each K (L' (K), L'' (K) and ΔK) values by Structure Harvester (Evanno et al. 2005; Earl and vonHoldt 2012). Micro-Checker version 2.2.3 (Van Oosterhout et al. 2004) was used to detect possible genotyping errors, allele dropout and non-amplified alleles (null alleles).

Results

Two CA-dinucleotide enriched genomic libraries were constructed with two restriction enzymes (*Rsa I*, *HpyCH4 V*). Overall, 88 unique sequences were identified among the 95 sequenced clones and 93% of unique sequences contained repeat regions that are typical for microsatellites showing the effectiveness of the enrichment method. Based on the above-mentioned sequences 12 fully functional microsatellite DNA markers were developed. The sequences were deposited in the GenBank (the accession numbers are in Table 1). The newly developed markers were tested to determine their functionality and characteristics and were used for genetic diversity analyses of stocks. All these markers are working well and polymorph. The number of detected alleles ranged between 3 and 48. The highest number of amplified allele

(48) was found in case of *MS 428 Pf* marker. Moreover, other highly polymorphic markers (*MS 427 Pf, MS 464 Pf* and *MS 726 Pf*) were also identified. Based on the results, the isolated markers are suitable for further studies due to their high polymorphisms and easy usage, presumably in the case of closely related species, as well.

Two examined populations originated from Hungarian broodstocks (Dunaföldvár: Hu-D; Biatorbágy: Hu-B) and one from Polish natural population (Olsztyn: Po-O). The two Hungarian stocks are very similar based on the mean number of alleles (Hu-D 8.66; Hu-B 9.5) and effective allele numbers (4.3 and 4.0). The *MS 439 Pf* marker was monomorphic, while *MS 427 Pf, MS 428 Pf* and *MS 464 Pf* were highly polymorphic in both Hungarian stocks. The overall and the effective allele numbers (10.6 and 6.3) were higher in the Polish population. The most polymorph markers were the *MS 427 Pf, MS 464 Pf* and *MS 726 Pf*. Compared, the *MS 439 Pf* was polymorphic in Polish Eurasian perch population (low level, 3 alleles), while the polymorphism of the *MS 428 Pf* marker was only average (9 alleles).

The population genetic characteristics for the examined stocks are summarized in Table 2. The heterozygosity values were low in all three analysed stocks (indicating heterozygote deficiency); the populations were deviated from Hardy–Weinberg equilibrium significantly; however, the level of genetic differentiation was stayed on moderate (overall $F_{ST} = 0.247$) level.

The analysed stocks were divided according to the presence of private alleles, as well. The *MS 428 Pf* marker produced an extremely high number of private alleles (16 in Hu-B, 8 in Hu-D and 7 in Po-O). In addition, the Po-O population had a high number of private alleles (21, 18, 14 and 10) in case of *MS 427 Pf, MS 726 Pf, MS 464 Pf* and *MS 725 Pf* markers, respectively. The possibility of linking between the markers and the sex was also tested, but none of the loci showed linkage to sex or each other in none of the stocks.

The AMOVA analysis was found the largest variance (54%) within individuals and only 17% was among individuals. At the same time, 24% variance was found among populations indicating a moderate level of population differentiation. The genetic distance analyses reviled that the difference originates from the diversity of Polish–Hungarians populations (Table 3). The Nei's distance (0.149) was small between the two Hungarian populations, while the distances (> 0.6) were larger between the Polish population and Hungarian stocks. Based on the F_{ST} values and Nei's distances, there was remarkable genetic difference between Hungarian and Polish stocks.

Table 3 Nei's genetic distance (Da, 1983) and F_{ST} (in brackets) between pairs of populations

Populations	Hu-D	Hu-B	Po-O
Hu-D	0.000 (0.000)		
Hu-B	0.149 (0.038)	0.000 (0.000)	
Po-O	0.616 (0.289)	0.691 (0.312)	0.000 (0.000)

Hu-D, Dunaföldvár; Hu-B, Biatorbágy; Po-O, Olsztyn

The STRUCTURE analyses of the populations based on microsatellite data are shown in Fig. 1. The relative structure of the 3 populations was presented in case of $K = 2$ (because the $K/\Delta K$ function was the maximum in this case), $K = 3$ (because we analysed 3 different stocks) and $K = 5$ (because STRUCTURE HARVESTER has detected a local maximum at $K = 5$ in $K/\Delta K$ function). According to the Evanno analyses, the most likely number of the cluster is $K = 2$; therefore, the population from 3 different places can be genetically classified into two major distinct groups. One group consists of the two Hungarian stocks (Hu-D and Hu-B) and the other one is the natural Polish population (Po-O).

The result of the genetic distance-based principal coordinate analysis (PCoA) is shown in Fig. 2. The figure reflects the overlapping structure of Hungarian stocks and the genetic separation of Hungarian and Polish populations.

Discussion

Over the last 20 years, development of biotechnological and molecular biology improvement has made species investigation feasible (and their populations or stocks) that previously have been scientifically peripheral (including *P. fluviatilis*), by the emergence of simpler and cheaper methods. There have been only a few studies on the genetic

Table 2 The population genetic characters of examined populations

Populations	*N*	MNa	Nea	H_E	H_O	Sign	Gd	F_{IS}
Dunaföldvár (Hu-D)	43	8.667	4.329	0.620	0.459	***	0.515	0.262
Biatorbágy (Hu-B)	80	9.500	4.090	0.608	0.533	***	0.605	0.123
Olsztyn (Po-O)	59	10.667	6.390	0.535	0.414	***	0.463	0.228
All	182	9.611		0.708	0.477	***		

Number of examined individuals (*N*); mean number of alleles (MNa); number of effective alleles (Nea), H_E: expected heterozygosity, H_O: observed heterozygosity, significance of deviation from Hardy–Weinberg equilibrium: *p* value ***< 0.001; gene diversity (Gd); F_{IS}: an index describing the variance within the population

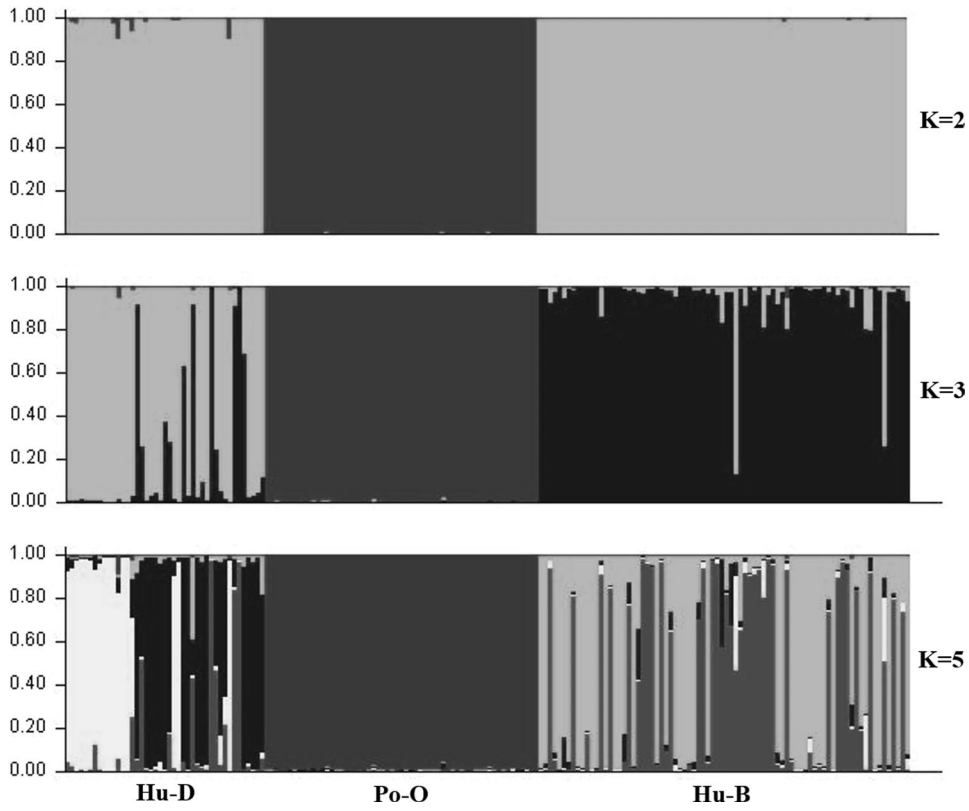

Fig. 1 Results of the STRUCTURE analysis: the three examined populations can be classified into two ($K=2$) genetically different groups according to the STRUCTURE HARVESTER analyses. The results were also presented in case of $K=3$, because we analysed three different stocks from three different places and in case of $K=5$, because the $K/\Delta K$ function at $K=5$ cluster number has detected a local maximum. Hu-D, Dunaföldvár; Po-O, Olsztyn; Hu-B, Biatorbágy

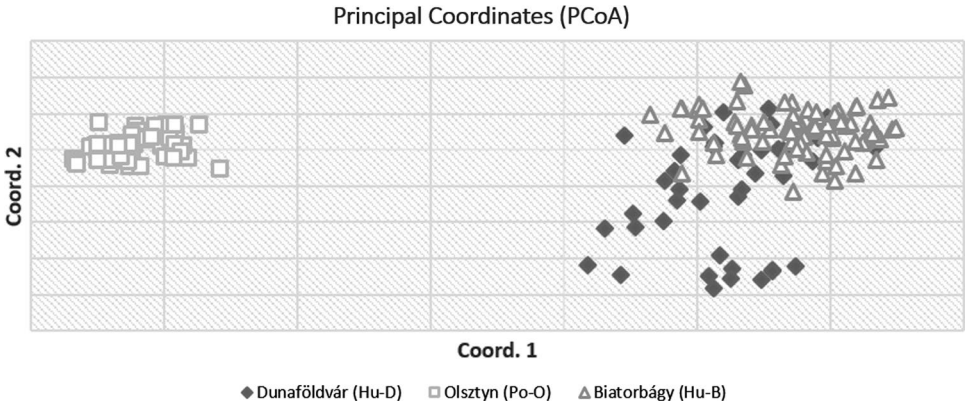

Fig. 2 Results of PCoA analysis based on genetic distances calculated by the GENALEX software. The illustration demonstrates the similarity between Hu-D (Dunaföldvár stock) and Hu-B (Biatorbágy stock) and the genetic difference between Hungarian and Polish populations (Hu-D, -B and Po-O)

diversity of natural Eurasian perch populations and farmed stocks and the monitoring of anthropogenic effects. Most of these studies investigated bred stocks and natural populations in Western (Gerlach et al. 2001; Khadher et al. 2015, 2016) or Northern Europe (Bergek and Björklund 2007; 2009; Bergek et al. 2010; Olsson et al. 2011) and only one examined Asian stock (Yang et al. 2012), while only one mitochondrial sequence-based population genetic analysis has been made on Central European populations of Eurasian perch (Vanina et al. 2019) and few individuals from more population took parts of spatial genetic variability pattern analyses in European water basins (Toomey et al. 2020). Microsatellite-based analyses from these regions have not been conducted until now.

The slightly modified method of microsatellite isolation proved to be very efficient. The results showed that the enrichment and library preparation was nearly optimal since the inserts contained more than 92% unique sequences, and 93% of the sequences carried microsatellite-specific repeating regions. It is a cost- and time-saving method for isolation of microsatellites if there is no possibility of NGS sequencing, and moreover, we need markers for population genetic analysis (not for genome-wide screening). The "universal tail"-based fluorescent labelling of amplicons

was also used successfully to further reduce the cost of the analysis. Based on the identified sequences 12 new polymorph markers were developed and tested. The conditions of their operation were determined, and the polymorphisms of the markers were tested on two Hungarian stocks (Hu-D: Dunaföldvár, Hu-B: Biatorbágy) and one natural population from Poland (Po-O: Olsztyn). However, the average allele numbers were similar or a bit higher than the average of freshwater fishes (Dewoody and Avise 2000); some of the developed markers (*MS 428 Pf, MS 427 Pf, MS 464 Pf, MS 726 Pf*) showed extremely high polymorphisms (number of the alleles: 26–48), which are markedly recommended for genetic analysis.

The heterozygosity of the Hu-D and Po-O stocks proved to be lower than the average of freshwater fish (H_O: 0.54) while the Hu-B corresponds to it (Dewoody and Avise 2000). Nonetheless, the heterozygous deficit was detected in all three populations and the examined populations overall differed significantly from the Hardy–Weinberg equilibrium. In the Hungarian populations, almost all the analysed markers showed the discrepancy, while in the Polish group only half of them. This is probably the consequence of anthropogenic effects of fishery management (lack of panmictic mating, high level of drift and/or the low number of the reproductive individuals, because of the regular fishing and angling) of all Hungarian and the Polish stocks. Based on Nei's genetic distances, F_{ST} values per population pairs, or the analysis of the STRUCTURE and PCoA, Dunaföldvár (Hu-D) and Biatorbágy (Hu-B) stocks (where the geographical distance is smaller) were genetically more similar to each other. One group of Dunaföldvár individuals was positioned to the Biatorbágy stock in a genetic point of view, while the Polish Eurasian perch formed a genetically distinct group (Fig. 2). This differentiation is also supported by the presence of remarkably high numbers of private alleles. The partly overlapping distribution of PCoA analyses revealed that a gene flow has happened between the two Hungarian stocks, what could be associated with the high probability of that the two locations are occasionally stocked from the same origin (Danube river) or that the two locations are physically connected through the Danube river (~ 82 km waterway distance) and both populations were having chance to cross.

Conclusions for future biology

We have developed a new genetic tool system that—in itself and combined with the earlier described genetic markers—can be used to analyse the genetic background of Eurasian perch populations. Our work is unique in that aspect that study deals with a microsatellite-based analysis in the Central European perch populations. It has been shown that the genetic differences and the appearance of unexpected anthropogenic effect in the analysed stocks could take place what should be a warning for the managers of those water bodies to potentially take action. The level of the heterozygosity is recommended to be increased in these populations by the reduction of anthropogenic effects, increased protective measures (protecting spawning grounds, increasing allowed size-at-catching or similar) or by targeted crossing. Our analysis draws attention to this species (which becomes economically more and more important and which, even despite of the expansion of its commercial aquaculture) seems to be already influenced by strong anthropogenic effects. It highlights the importance of genetic conservation and genetic monitoring of both farmed and natural populations of Eurasian perch, for which the results of our study bring novel potential tools.

Acknowledgements We would like to thank all those colleagues who participated in sample collection. The research was supported by OTKA (Hungarian Scientific Research Fund) PD 79177 Project and by the Ministry of Innovation and Technology within the framework of the Thematic Excellence Programme 2020, Institutional Excellence Subprogramme (TKP2020-IKA-12), and the EFOP-3.6.3-VEKOP-16-2017-00008 project co-financed by the European Union and the European Social Fund.

Author contributions DKS, DŻ, II, KCsB, LK, ZB, BU and BK conceived and designed the experiments. II, DŻ, LK and ZB collected the samples. DKS, KCsB, ÁŐ and BK performed the experiments. DKS, ÁŐ and BK analysed the data. DKS, KCsB, ÁŐ, BU and BK contributed reagents/materials/analysis tools. DKS, KCsB, ÁŐ, DŻ, II, LK, ZB, BU and BK wrote the paper.

Funding Open access funding provided by Hungarian University of Agriculture and Life Sciences. This work was supported by Project OTKA (Hungarian Scientific Research Fund) PD 79177 Project and the Ministry of Innovation and Technology within the framework of the Thematic Excellence Programme 2020, Institutional Excellence Subprogramme (TKP2020-IKA-12) and the EFOP-3.6.3-VEKOP-16-2017-00008 project.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval All procedures involving the handling and treatment of fish used during this study were approved by the Capital and Pest County Government Office for Food Chain Safety and Animal Health (permission number: XIV-I-001/2302–4/2012 and XIV-I-001/2304–4/2012).

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