Development and characterization of 49 novel microsatellite markers in the African catfish, *Clarias gariepinus* (Burchell, 1822)

Dóra Kánainé Sipos¹ · Katalin Bakos¹ · Ágnes Ősz¹ · Árpád Hegyi¹ · Tamás Müller¹ · Béla Urbányi¹ · Balázs Kovács¹

Received: 16 January 2019 / Accepted: 4 September 2019 / Published online: 18 September 2019
© The Author(s) 2019

Abstract

The African catfish or sharp tooth catfish (*Clarias gariepinus*) is one of the important species (due to its high environmental tolerance and easily controllable breeding habits) that can significantly contribute to reducing hunger in many countries. It is farmed in numerous African, Asian, and European countries. Moreover, during the last decades its production has grown significantly worldwide. Currently, following the carp, this species is produced in the second largest volume in Hungary. Despite its economic importance, the stocks have been maintained without genetic control or guided breeding. Molecular genetic data on bred populations or strains are very limited. In order to investigate the genetic structure of the stocks, 49 new microsatellite markers were characterized and tested on 32 individuals from a Hungarian farmed stock. All these markers were polymorph. The number of alleles per locus ranged from 2 to 11. The observed and expected overall heterozygosities were between 0.519 and 0.544 respectively and the overall inbreeding coefficient (Fis: 0.063) does not reveal the presence of inbreeding. However, 63% of the markers showed significant deviations from HWE. The results suggest that the maintenance of genetic variation within the stock require high attention in closed bred populations. These new markers provide a useful tool for population and conservation genetics of natural and bred African catfish populations.

Keywords African catfish · *Clarias gariepinus* · Microsatellite · Genetic diversity · Population genetics · Anthropogenic effect

Introduction

The African catfish, *Clarias gariepinus* (Burchell, 1822), belongs to the Siluriformes order and the *Clariidae* family. It is native in Africa and the Middle East, but it was introduced to Europe, Asia, and South-America for aquaculture purposes [1]. This species represents an important resource in many developing countries with growing capture and aquaculture production. The latter increased tenfold, to over 200,000 metric tons in the last decade [1]. In the same time, the uncontrolled stocking and capture fishing have impacts on natural populations (e.g. fluctuating population size, decreasing genetic diversity, adaptability to changing environment and resistance to disease) [2, 3].

For the analyses of these issues, species-specific molecular genetic markers were developed for many fish species, including microsatellites due to their efficiency in individual identification, parentage analyses and differentiation of populations, lines or stocks. These markers are also widely used to estimate the population genetic values (such as variability, heterozygosity, inbreeding or genetic structure) [3, 4]. Due to the economic importance of the African catfish, more and more genetic studies analyze the genetic variability, yield deterioration and the conservation genetic background of farmed [5, 19] and natural [2] productions. However, the availability of the species-specific microsatellite markers suitable for detailed analysis of genetic resources are limited in number. Until now, only seven microsatellites have been described from the African catfish genome [6] and 12 microsatellites from other *Clariidae* species were used to investigate *C. gariepinus* [7]. However, cross-amplifying loci can lead to obvious complications (i.e.: monomorphic or low allele number of the loci; non-orthologous loci; size homoplasy), in population genetics and evolutionary studies [8]. In this study, new microsatellite loci were characterized

✉ Balázs Kovács
Kovacs.Balazs@mkk.szie.hu

¹ Department of Aquaculture, Institute of Aquaculture and Environmental Safety, Faculty of Agricultural and Environmental Sciences, Szent István University, Páter K. u. 1, 2100 Gödöllő, Hungary

for *C. gariepinus* by using repeat-enriched genomic library sequencing approach. These new markers are characterized on a bred population to provide new useful tools for population, conservational, evolutionary, and structural genetic studies on this species.

Materials and methods

The sampled African catfish individuals were originated from the stock of NAIK-HAKI Szarvas, Hungary (GPS: 46°52'0.01" N 20°33'0.00" E). The stock was established in the mid-80 s and has been maintained in an intensive RAS system with closed breeding, however, some breeders were added to the broodstock unsystematically. For the genetic analyses and library preparation fin clips were collected from the tailfins (0.5 cm² tissue) of 32 individuals and the genomic DNA was isolated using E.Z.N.A. DNA Tissue Kit (Omega BioTek). DNAs were diluted to 50 ng/μl concentration for the microsatellite analyses and stored in Elution Buffer at –20 °C. Repeat enriched genomic DNA libraries were produced from pooled samples of three male specimens following the protocol of Glenn and Schable [9]. Rsa I, Hae III, Alu I, (Fermentas) and HpyCH4 V, (New-England BioLabs) endonucleases were used for DNA fragmentations. The Box I linker (F: 5'-Phos-ATG TCT GAA GGT ACC ACT GCT GTC CGA AA-3'; R: 5'-CGG ACA GCA GTG GTA CCT TCA GAC AT-3') was ligated to the ends of 300–1000 bp long isolated fragments. Biotinylated (CA)₁₀ probe, linker specific oligo, and pGEM-T Easy Vector System I (Promega) were used for library preparations. The sequences of the inserts were determined by BigDye terminator sequencing (3130 Genetic Analyser, Applied Biosystems). The sequences were deposited in the NCBI GeneBank (see Table 1). Primer3Plus [10] software was used for primer design on the flanking regions of repeats (Table 1). Tailed and tail specific fluorescent primers were used to reduce the costs of detection, as it was described by Shimizu et al. [11]. A 17 base pair long tail (5'ATTACC GCGGCTGCTGG- microsatellite specific sequence-3') were added to the forward primers' 5' ends to provide an attachment site for fluorescent dye labelled (FAM or VIC or NED or PET) tail-specific oligos. An additional labelled oligo was added to the reaction mixtures for fluorescent labelling of amplicons. Based on practical experiences the concentration of the labelled oligo was lower than the forward and reverse primers' to avoid the amplification of non specific fragments. The microsatellite PCRs were performed in 25 μL volumes including 1× *Taq* DNA polymerase buffer containing (NH₄)₂SO₄ or, buffer with KCl depending on which produced more abundant PCR products. The KCl containing buffer was used in case of the following markers Cg02, Cg03, Cg10, Cg40, Cg122 and Cg132 markers—Fermentas,

264 nM forward and reverse and 132 nM tail-specific-labelled primers, 1.5–3.0 mM MgCl₂ (Fermentas), 0.2 mM dNTP (Fermentas), 0.04 U/μL *Taq* DNA polymerase (Fermentas) and 150 ng template DNA. The thermocycling profile consisted of: preliminary denaturation at 95 °C for 2 min, followed by two cycles of pre-amplification (95 °C/15 s; ann.temp./1 min; 72 °C/2 min) and 35 (Cg002, Cg003, Cg010, Cg040, Cg122 and Cg132, Cg367, Cg652, Cg665) or 45 cycles of amplification (95 °C/15 s; ann. temp./20 s; 72 °C/40 s) in case of the remaining ones. The reactions were closed by a final extension (72 °C for 5 min). The genotypes were determined by 3130 Genetic Analyser (Applied Biosystems) using GeneScan LIZ 500 size standard (Applied Biosystems). The alleles were identified and scored using GENEMAPPER 4.0 software (Applied Biosystems). MICROSATellite TOOLKIT VER. 3.1.1 [12] was used to estimate the number of alleles per loci, mean number of alleles, polymorphic information content (PIC). The expected and observed heterozygosities and significance of deviation from Hardy–Weinberg equilibrium (HWE) were estimated by GENALEX VER. 6.5 [13]. The linkage disequilibrium was analyzed by Genetix 4.05 [14]. The minimum set of markers which provides a high resolution of parentage allocation and the probability of exclusion in paternity were tested by PARFEX v1.0 [15]. ML-Relate was used to maximum likelihood estimating of relatedness between individuals [16]. MICRO-CHECKER VER. 2.2.3 [17] was used to detect possible genotyping errors, allele dropout, and non-amplified alleles (null alleles), while the inbreeding coefficient (*F*_{is}) were calculated by FSTAT 2.9.3. [18].

Results

In total, 127 selected clones were sequenced from four CA-repeat enriched genomic libraries. One hundred and eighteen clones contained microsatellite motifs. Based on the length (the PCR product would be 100–500 bp) and nucleotide composition (the PCR product would be specific and unique) of the flanking sequences 55 microsatellite sequences were selected and primers were synthesized for later PCR analyses. Forty-nine out of 55 markers were used to genotype the 32 individuals and amplified polymorphic alleles successfully. None of the 49 markers was monomorphic. The number of alleles per locus ranged from 2 to 11, with a mean of 4.818 (SE: 1.973) alleles. The highest number (11) of amplified alleles was found in case of the Cg175 marker while Cg003 and Cg010 were also found to be highly polymorphic. The average polymorphic information content (PIC) value was 0.499 (from 0.142 to 0.763), 27 loci were highly polymorphic (PIC > 0.5), 18 were moderately polymorphic (0.2 < PIC < 0.5), and four showed low polymorphism (PIC < 0.2) in our bred population (Table 1).

Table 1 Characteristics of the 49 newly isolated *Clarias gariepinus* microsatellite DNA markers (microsatellites were tested on 32 individuals)

Locus	Primer sequence (5'–3')	Repeat motif	Ta (°C)	Allele size range (bp)	Nr of alleles	H _E	H _O	Deviation from HWE	PIC	GenBank accession Nr.	Null allele present	Linkage disequilibrium	F _{IS}
Cg002	F: AGCAGGAAAACGGGT CAC R: ATGGTTCAGCTGTAG TGTGG	(CA) ₂₆	59	86–112	6	0.679	0.833	ns	0.624	KX834137	No	Yes	–0.211
Cg003	F: CGCCCTACCTGTAAAC CTGT R: CGAGTTTCCAGGTAG AGCAG	(CA) ₁₇	59	132–150	10	0.737	1	ns	0.711	KX834138	No	no	–0.342
Cg010	F: GTCATGCTGGGAGAA CAAGA R: TGCATGATAGGTGAA AGGT	(GT) ₁₃	59	98–136	10	0.787	0.906	***	0.761	KX834139	No	no	–0.136
Cg040	F: AGAAGTTGTACTCGG GTGCA R: CAAACGGCACCTCAA ACA	(GT) ₈	59	149–160	3	0.528	0.906	***	0.418	KX834140	No	Yes	–0.709
Cg122	F: ACCTTCAGACATACT GAGGCTT R: GAGGATCAGATTATT GGATTGAT	(AC) ₁₅	56	154–166	6	0.468	0.552	ns	0.435	KX834141	No	Yes	–0.162
Cg123	F: GACCTTCACCTTCCT CCTTCT R: TGCTAATGTGATGCC AATGT	(TC) ₇	55	241–257	2	0.32	0.4	ns	0.269	KX834141	No	Yes	–0.234
Cg132	F: CAGAAATTCGCGAGA ATTGTGT R: CGAGTTACCCCTGGCA GATC	(GT) ₂₁	55	102–135	6	0.649	0.645	**	0.618	KX834142	No	No	0.023
Cg175	F: CCTGAAGAAGAAATC TCAGATAAGT R: ATCAGACTGAGGATG AGGTTC	(AC) ₁₅	55	153–200	11	0.793	0.875	***	0.763	KX834143	No	No	–0.088
Cg214	F: CCTGCATTATTCCT TTGAC R: AATAACAGACTATGT TGAATTAATGG	(CA) ₁₅	55	142–161	5	0.688	0.844	***	0.639	KX834144	No	No	–0.211

Table 1 (continued)

Locus	Primer sequence (5'–3')	Repeat motif	Ta (°C)	Allele size range (bp)	Nr of alleles	H _E	H _O	Deviation from HWE	PIC	GenBank accession Nr.	Null allele present	Linkage disequilibrium	F _{IS}
Cg236	F: CATCCTTTGTTTATGGCT TGTT R: TCATGCTTTAGAGTC TCATCTCAC	(GT) ₉	55	250–265	5	0.478	0.387	ns	0.437	KX834145	No	No	0.205
Cg243	F: TGTGTGAAGGTTTAT GTAACGAT R: TCAATCCTGCCCTAG ACCTA	(TG) ₁₅	55	273–298	5	0.625	0.323	***	0.587	KX834146	Yes	Yes	0.496
Cg287	F: GGGAATGTTTGATGA ATGTGA R: TGCCTTGGATATTGT TTGCT	(CA) ₅	55	107–119	6	0.607	0.969	***	0.532	KX834148	No	Yes	–0.585
Cg288	F: GCTGTCGCTATGACT CACCT R: GTCCTGTGGATGGCA CTTC	(GT) ₂₁	55	162–186	5	0.556	0.548	***	0.503	KX834149	No	Yes	0.03
Cg289	F: TACAGCATCTCTGAG GGACG R: CTGTCACCTTTAGTG TAATTCACAC	(GT) ₁₃	55	100–113	4	0.643	0.813	ns	0.571	KX834150	No	No	–0.232
Cg290	F: ACATACATCCATGGT GACTGC R: AACTTCACACATTTG AAGGAGAT	(AC) ₁₇	55	222–236	5	0.523	0.469	***	0.468	KX834151	No	No	0.119
Cg294	F: CAGCTGTCGATGTAT GACCTG R: GACCTGAAGGAGCCT GTCTG	(TG) ₁₈	55	308–338	6	0.649	0.469	**	0.585	KX834152	Yes	No	0.292
Cg298	F: CCGACCATCAGGAGA CAGA R: CGAGACTCGCCGTC CTC	(TG) ₉	55	168–202	6	0.655	0.621	***	0.605	KX834153	No	No	0.07
Cg299	F: ATGTGTCGAAGCTTA CAGCTC R: GCTATATCGCTCACT GATACCA	(AC) ₂₁	55	250–271	5	0.475	0.406	***	0.443	KX834154	No	No	0.16

Table 1 (continued)

Locus	Primer sequence (5'–3')	Repeat motif	Ta (°C)	Allele size range (bp)	Nr of alleles	H _E	H _O	Deviation from HWE	PIC	GenBank accession Nr.	Null allele present	Linkage disequilibrium	F _{IS}
Cg307	F: TACATCTCTGTTTAG CAAAAGTGG R: TCGGTGCGTAAAGCA TCT	(GT) ₇	55	96–100	2	0.441	0.281	*	0.344	KX834156	No	Yes	0.376
Cg312	F: TTGTGCACTGCAAGC GAT R: AGTCAATGCATTGG ACAGC	(CA) ₁₄	55	207–227	7	0.669	0.563	***	0.64	KX834158	No	No	0.175
Cg316	F: GTGTGTGCGCTTGTG TGG R: CGGAGAAAGTGCCGTA GCT	(AG) ₁₈	55	86–112	5	0.674	0.774	ns	0.613	KX834159	No	No	–0.133
Cg330	F: TACCTAAGAGGAAC GCAATGC R: TCTGAGGGACATTGT CTGAATAA	(CA) ₁₃	55	173–177	2	0.324	0.281	ns	0.271	KX834161	No	No	0.147
Cg332	F: CTCCTCTCTCTTTGTCA CTC R: ATGGTGCTCAGCAGT CATCT	(CA) ₁₄	55	140–157	5	0.644	0.594	ns	0.587	KX834162	No	No	0.094
Cg336	F: GCAGATAATCCGTTT CAGC R: CCAAGTCTTGCAATAC TGTGTGT	(CA) ₁₀	55	293–300	4	0.547	0.258	***	0.488	KX834163	Yes	No	0.54
Cg337	F: TGGTCAGACGAGGAA CACTT R: CACACTAACACAACC ACATATTCA	(GT) ₇	55	98–107	3	0.443	0.484	ns	0.369	KX834164	No	Yes	–0.077
Cg339	F: GCTCGATTCTTGATT TACAGG R: GCTGCTCCTCAGTCC AAGT	(TGTGTGAGAG) ₈	55	134–196	6	0.745	0.935	**	0.709	KX834165	No	No	–0.241
Cg341	F: GAGTCGTGCCGTAT CTCC R: GCTCAACCTGCTGTT ACTCAA	(TG) ₁₉	55	150–169	7	0.508	0.313	***	0.49	KX834166	Yes	No	0.399

Table 1 (continued)

Locus	Primer sequence (5'–3')	Repeat motif	Ta (°C)	Allele size range (bp)	Nr of alleles	H _E	H _O	Deviation from HWE	PIC	GenBank accession Nr.	Null allele present	Linkage disequilibrium	F _{IS}
Cg344	F: ACAGACATGAGACTA CGGTATGTAT R: CATGTGTTGTCTCC AGTCAATA	(TG) ₁₁	55	202–220	5	0.41	0.281	***	0.378	KX834167	Yes	No	0.328
Cg346	F: CAGGGCTACACAGCG TCG R: GATTTCATCAACACACA TTGCTCTC	(TG) ₁₁	55	149–160	3	0.147	0.156	ns	0.142	KX834168	No	Yes	–0.047
Cg350	F: GTCGTCGTATGCTGA ATAATGT R: TCCACTTCTAGTAGC ACTCTTC	(GT) ₁₇	53	81–111	7	0.606	0.406	**	0.557	KX834169	Yes	No	0.344
Cg352	F: TGGTTGAGATCGAGG TTGG R: TGACTCAGACTCGGA GACCTAC	(GT) ₁₉	55	204–246	6	0.554	0.613	ns	0.528	KX834170	No	No	–0.091
Cg354	F: GCAGACGGGAGCGTGA R: GCCGCTCCTTTATCA CAIT	(GT) ₁₇	55	199–219	5	0.6	0.552	***	0.559	KX834171	No	No	0.098
Cg355	F: CTCAGGGCACTCGGA ACA R: CCTTAAACCACCACC CTACTG	(AC) ₂₀	55	125–183	4	0.176	0.063	***	0.171	KX834172	Yes	Yes	0.654
Cg357	F: GCGTGCCAAACGGAGA R: CATTGCCAGAACTCTC TTAAGTTC	(TG) ₂₆	55	328–343	4	0.477	0.5	ns	0.434	KX834173	No	Yes	–0.028
Cg367	F: TGCCAAACCATCTCCGA R: CTCGGTTTCTCCAC	(CA) ₂₉	58	199–247	4	0.174	0.063	***	0.167	KX834174	Yes	Yes	0.651
Cg370	F: AGGTCCACAGGTCAC ATCAG R: TCTATCCCTACCGCC AAGA	(AC) ₁₉	55	275–287	5	0.506	0.484	ns	0.474	KX834175	No	No	0.061
Cg638	F: CCAGTGAGTGGCAGG AGTG R: TCATTACTACACACC ACATCACA	(AC) ₁₆	55	239–250	4	0.206	0.226	ns	0.195	KX834176	No	No	–0.08

Table 1 (continued)

Locus	Primer sequence (5'–3')	Repeat motif	Ta (°C)	Allele size range (bp)	Nr of alleles	H _E	H _O	Deviation from HWE	PIC	GenBank accession Nr.	Null allele present	Linkage disequilibrium	F _{IS}
Cg639	F: CAGCTTTGGCTCGGTCA R: AACACGTTTCAAGCGG TAGTC	(AC) ₁₀	55	177–220	5	0.71	0.344	***	0.665	KX834177	Yes	Yes	0.528
Cg644	F: GATGTAATACGGAAC AACACCA R: CACAGCCGTGTTCTC TTGC	(GT) ₁₆	55	127–134	3	0.557	0.563	ns	0.457	KX834179	No	No	0.005
Cg645	F: CTGTTTACTCTAACA CTGTGACCAC R: CACGGTGTCCAATGA TGTG	(AC) ₄₁	55	186–191	2	0.479	0.094	***	0.401	KX834180	Yes	No	0.81
Cg647	F: GGTCAGCACACCCTG AGAA R: TCCAGCTTTTAGCAA GTGTTAG	(GT) ₁₀	55	116–130	6	0.732	0.793	ns	0.688	KX834181	No	Yes	–0.065
Cg649	F: ATGATTGACCAGTCA AATCGA R: AGTTCGTCCCAATAAT GGCA	(CA) ₁₆	55	157–170	5	0.329	0.281	ns	0.315	KX834182	No	Yes	0.161
Cg651	F: AGAATAGCTGAGGAG TGAGGAG R: CATTGTGCTTCGGCT GAT	(GT) ₁₅	55	145–211	5	0.598	0.313	***	0.561	KX834183	Yes	No	0.489
Cg652	F: ATCAACATCACCCGC TCAC R: ACCTCATCAATGCCA CTGTC	(TG) ₂₁	58	112–161	5	0.574	0.531	**	0.546	KX834184	No	No	0.091
Cg653	F: TCTATTGATTAGG AAGCAGACT R: GATCGGTTTTCGGTGAT	(AC) ₈	55	180–192	4	0.654	0.71	*	0.595	KX834185	No	No	–0.069
Cg657	F: AGGAAGTAGAGTTGT TATGGCAG R: GACATGCCACAGGAC TGAAG	(GT) ₁₀	54	150–164	5	0.559	0.531	***	0.506	KX834186	No	No	0.066
Cg661	F: TTGGGTTTATCCGTG GTTC R: GAGATGCTGGTGATG GTGAG	(AC) ₁₃	55	106–145	7	0.765	0.906	*	0.728	KX834187	No	No	–0.169

Table 1 (continued)

Locus	Primer sequence (5'–3')	Repeat motif	Ta (°C)	Allele size range (bp)	Nr of alleles	H _E	H _O	Deviation from HWE	PIC	GenBank accession Nr.	Null allele present	Linkage disequilibrium	F _{IS}
Cg663	F: TCAATCTCAGAGAGG AACGACT R: TGATGGGACGATGT ACG	(GT) ₂₁	55	195–201	2	0.299	0.031	***	0.279	KX834188	Yes	No	0.899
Cg665	F: TGGACAGATGGGGTT AGGTA R: GGTCTCTTTGCTGCT GCTTA	(AC) ₁₀	58	119–181	5	0.694	0.548	*	0.641	KX834189	No	No	0.226

Ta annealing temperature, H_E expected heterozygosity, H_O observed heterozygosity, HWE Hardy–Weinberg equilibrium, F_{IS} inbreeding coefficient; levels of significance: non-significant (ns) or significant *P<0.05; **P<0.01; ***P<0.001

The bold markers are the members of the recommended minimum set of markers for parentage allocation

The expected heterozygosity (H_E) among loci ranged from 0.147 (Cg346) to 0.793 (Cg175) with a mean of 0.544 (SE: 0.024), whereas the observed heterozygosity (H_O) ranged from 0.031 (Cg663) to 1.000 (Cg003) with a mean of 0.519 (SE: 0.037). Thirty-one loci showed significant deviation from HWE. The deviation is partly due to the heterozygous deficiency at 24 loci, on the other hand, is due to heterozygosity excess at seven loci. [2], probably due to the interference of the different management practices adopted for the population studied. Selection, artificial mating system, fluctuations in population size or stocking may also explain deviations from HWE [19–21]. Probability of null-alleles was detected in the case of 12 markers (Table 1).

No parental relationships were identified by the exclusion or the maximum likelihood methods. However, the maximum likelihood analyses of relatedness showed a high probability of full-sib or half-sib relationships between individuals. All the individuals showed relationships with at least two specimens, while three of them had ten probable relatives (data not shown). In addition, linkage disequilibrium was assumed in the case of 16 markers. Nevertheless, the high number of related individuals can lead to spurious associations in the estimation of random distribution. The overall F_{IS} values were 0.063, while 29 markers had positive and 20 negative F_{IS} values (Table 1). The calculation of the minimum set of markers for parentage allocation or for identification over 99% probability resulted in a marker set containing 14 markers (Table 1).

Discussion

In the present study, 49 new *Clarias gariepinus* microsatellites were characterized. The applied method for isolation of microsatellite repeat containing sequences was highly efficient (more than 90% of the sequences contained repeats). It is a cost-effective and time-saving method when we need efficient, highly polymorphic markers in enough number for population genetic analysis and not for genome-wide screening. The “universal”, tailed primer based fluorescent labelling of amplicons [11] was also used successfully; therefore we could further reduce the cost of the analysis.

However the mean number of alleles was moderate (5.06 ± 1.9), compared to the average of freshwater fish (9.1 ± 6.1) and the bred and natural African catfish populations (ranging from 3.80 ± 0.84 to 10.83 ± 3.66) in Kenya [2, 22], 28 of the isolated markers had moderately high or high PIC values (> 0.5). These markers are recommended for population genetic analyses [23] even in closely related species.

The genetic effects of long-term intensive breeding were also investigated on the examined Hungarian African catfish population. The result of “closed” breeding (without

or rare population refreshing) is frequently the degradation of genetic variability and inbreeding. These effects were described in some Thai [5] and South Indian [24] populations of catfish. While the variability in “open” breeding system at African farms (they have collected males from wild habitats regularly to provide milt for egg fertilization) is higher than in the natural lakes [2]. Among the Hungarian population, the expected and observed heterozygosity differed significantly from each other in case of most markers. The heterozygote surplus and deficiency varied from marker to marker; the differences compensated each other in overall analyses. The inbreeding coefficient also showed the presence of heterozygote surplus and deficiency in a wide range (from −0.709 to 0.899) at different markers. Similar effects were observed in the Indian bred populations of African catfish [24]. All these can be the consequence of intra-population genetic structures (non random mating), or small founder group with rare and unsystematic crossbreeding with other strains. This phenomenon of structured populations was also observed in the bred populations of Thailand [5]. In addition, the high number of probable null-alleles and linkage disequilibrium (Table 1) can also be the consequence of intra-population genetic structures or the small numbers of analysed individuals and not the real physical linkage on a chromosome.

The presence of intra-population genetic structures was confirmed by the maximum likelihood estimation of relatedness and the parentage allocation. Although, none of these two methods identified parental relationships among the individuals. The maximum likelihood analyses highlighted the presence of a high number of half-sib and full-sib individuals in the analysed group. During the parentage analyses, the most efficient set of markers was also calculated. The marker set contains 14 markers (Table 1) and usable for parentage allocation or for identification of individuals over 99% probability in exclusion analyses.

In conclusion, 49 newly isolated *C. gariepinus* microsatellites were tested and characterised. Those can be an important genetic toolkit for the genetic diversity and population structure analysis of African catfish populations.

Based on a Hungarian population, an effective set of markers were identified for parentage analyses. In this population, the genetic variation is recommended to be increased and the relatedness should be decreased. The introduction of breeders from other “domesticated” and/or genetically improved populations are the most obvious solution. Furthermore, the breed stock size should be maximized and at least 50 breeding pairs are recommended to use from generation to generation in order to avoid inbreeding.

Acknowledgements Open access funding provided by Szent István University (SZIE). This study was supported by OTKA (Hungarian Scientific Research Fund) K105393 project and the Higher Education

Institutional Excellence Program FEKUT2019: TUDFO/47138/2019-ITM awarded by the Ministry of Human Capacities (Hungary).

Compliance with ethical standards

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

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. FAO (2018) Fishery and Aquaculture Statistics Statistiques des pêches et de l'aquaculture Estadísticas de pesca y acuicultura. Fao. <https://doi.org/10.5860/CHOICE.50-5350>
2. Barasa J, Mdyogolo S, Abila R, Grobler PJ, Skilton R, Bindeman H, Njahira NM, Chemoiwa E, Dangasuk OG, Kaunda-Arara B, Verheyen E (2017) Genetic diversity and population structure of the African catfish, *Clarias gariepinus* (Burchell, 1822) in Kenya: implication for conservation and aquaculture. Belg J Zool. <https://doi.org/10.26496/bjz.2017.9>
3. Holmlund CM, Hammer M (2004) Effects of fish stocking on ecosystem services: an overview and case study using the Stockholm archipelago. Environ Manage. <https://doi.org/10.1007/s00267-004-0051-8>
4. Chauhan T, Rajiv K (2010) Molecular markers and their applications in fisheries and aquaculture. Adv Biosci Biotechnol. <https://doi.org/10.4236/abb.2010.14037>
5. Wachirachaikarn A, Rungsin W, Srisapoom P, Na-Nakorn U (2009) Crossing of African catfish, *Clarias gariepinus* (Burchell, 1822) strains based on strain selection using genetic diversity data. Aquaculture. <https://doi.org/10.1016/j.aquaculture.2009.01.036>
6. Galbusera P, Volckaert FA, Hellemans B, Ollevier F (1996) Isolation and characterization of microsatellite markers in the African catfish *Clarias gariepinus* (Burchell, 1822). Mol Ecol. <https://doi.org/10.1111/j.1365-294X.1996.tb00366.x>
7. Yue GH, Kovacs B, Orban L (2003) Microsatellites from *Clarias batrachus* and their polymorphism in seven additional catfish species. Mol Ecol Notes. <https://doi.org/10.1046/j.1471-8286.2003.00486>
8. Yue GH, Kovacs B, Orban L (2010) A new problem with cross-species amplification of microsatellites: generation of non-homologous products. Zool Res 31:131–140
9. Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. Methods Enzymol. [https://doi.org/10.1016/S0076-6879\(05\)95013-1](https://doi.org/10.1016/S0076-6879(05)95013-1)
10. Untergasser A, Nijveen H, Rao X et al (2007) Primer3Plus, an enhanced web interface to Primer3. Nucleic Acids Res. <https://doi.org/10.1093/nar/gkm306>
11. Shimizu M, Kosaka N, Shimada T et al (2002) Universal fluorescent labeling (UFL) method for automated microsatellite analysis. DNA Res. <https://doi.org/10.1093/dnares/9.5.173>
12. Park SDE (2001) Trypanotolerance in West African cattle and the population genetics effects of selection. Ph.D. thesis, University of Dublin

13. Peakall R, Smouse PE (2012) GenALEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*. <https://doi.org/10.1093/bioinformatics/bts460>
14. Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2004) 1996–2004 GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier (France)
15. Sekino M, Kakehi S (2012) PARFEX v1.0: an EXCEL TM based software package for parentage allocation. *Conserv Genet Res* 4(275):278. <https://doi.org/10.1007/s12686-011-9523-3>
16. Kalinowski ST, Wagner AP, Taper ML (2006) ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. *Mol Ecol Not*. <https://doi.org/10.1111/j.1471-8286.2006.01256.x>
17. Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Not*. <https://doi.org/10.1111/j.1471-8286.2004.00684.x>
18. Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices, ver. 2.9.3. AN UPDATE FOR Goudet J (1995). FSTAT (vers. 1.2): a computer program to calculate F-statistics. *J Hered*. 86:485–186. <https://en.bio-soft.net/tree/FSTAT.html>
19. Smouse PE, Whitehead MR, Peakall R (2015) An informational diversity framework, illustrated with sexually deceptive orchids in early stages of speciation. *Mol Ecol Resour*. <https://doi.org/10.1111/1755-0998.12422>
20. Allendorf F, Luikart G, Aitken S (2013) Conservation and the genetics of populations. *Livro*. <https://doi.org/10.1093/jhered/esl039>
21. Endo T, Sekino M, Fujiwara A, Sogabe A (2018) Development and characterization of 19 novel microsatellite markers in the Pacific seaweed pipefish *Syngnathus schlegeli* using next-generation sequencing. *Mol Biol Rep*. <https://doi.org/10.1007/s11033-018-4396-0>
22. De Woody JA, Avise JC (2000) Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *J Fish Biol*. <https://doi.org/10.1111/j.1095-8649.2000.tb00748.x>
23. Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet* 32:314–331
24. Ezilrani P, Marimuthu K, Godwin C (2016) Genetic diversity of African catfish *clarias gariepinus* in south India evaluated by microsatellite DNA. *Int J Zool Appl Biosci*. <https://doi.org/10.5281/zenodo.1310770>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.