Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Short Communication

Development of microsatellite markers for *Hoplias malabaricus* (Erythrinidae)

S.G.C.A. Gondim[1], L.V. Resende[1], R.P.V. Brondani[2], R.G. Collevatti[1], N.J. Silva-Júnior[3], R.R. Pereira[4] and M.P.C. Telles[1]

[1]Laboratório de Genética e Biodiversidade, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiânia, GO, Brasil
[2]Centro Nacional em Pesquisa em Arroz e Feijão, Embrapa, Goiânia, GO, Brasil
[3]Systema Naturae Consultoria Ambiental Ltda., Goiânia, GO, Brasil
[4]Programa de Pós-graduação em Biologia, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiânia, GO, Brasil

Corresponding author: M.P.C. Telles
E-mail: tellesmpc@gmail.com

Genet. Mol. Res. 9 (3): 1513-1517 (2010)
Received April 13, 2010
Accepted May 26, 2010
Published August 3, 2010
DOI 10.4238/vol9-3gmr877

ABSTRACT. We identified 14 microsatellite loci for the wolf fish, *Hoplias malabaricus* (Erythrinidae), from a genomic shotgun library. Twenty-five primers were designed, and 48 individuals of *H. malabaricus* from four localities of northwest Goiás, in central Brazil, were genotyped to characterize the polymorphism at each locus. Fourteen primers amplified clearly interpretable products using a single PCR protocol; six loci were polymorphic, but with a low number of alleles per locus (2 or 3). Expected heterozygosities for polymorphic loci ranged from 0.136 to 0.505. Combined paternity exclusion probability (0.638) was low and combined genetic identity (0.056) was high in studies of parentage. The low polymorphism may be due to the small microsatellite size and the large size of the motifs.

Key words: Cerrado; Erythrinidae; Microsatellite; Shotgun library

*Hoplias malabaricus (*Erythrinidae) is a widely distributed Brazilian wolf fish from lentic environments. Karyotypic analyses show high diversity in the number and type of chromosomes (e.g., Bertollo et al., 2000; Born and Bertollo, 2000), suggesting that *H. malabaricus* comprises a species complex. However, no information on population genetics using molecular markers is available for understanding population differentiation and clarifying species circumscription. In the last decades, the number of hydroelectric power plants have increased in Brazil, isolating and threatening fish populations due to habitat loss and population size reduction. Thus, the availability of molecular tools for population genetic studies is highly important to evaluate the impact of this process in the long-term population genetic viability.

Microsatellites are one of the most powerful molecular markers to estimate population genetic parameters and perform detailed parentage and gene flow analyses because of the high genetic information content (Morgante and Olivieri, 1993). Despite the advantages of microsatellite makers, reports on the development and use of microsatellite loci in Neotropical fish species are still scarce (but, see Revaldaves et al., 2005; Carvalho-Costa et al., 2006; Morelli et al., 2007; Batista et al., 2009). In this study, we report on the development and genetic characterization of 14 microsatellite loci for *H. malabaricus.*

Microsatellite isolation for primer design was based on a genomic shotgun library, coupled with bioinformatics tools. First, genomic DNA from 48 individuals of *H. malabaricus* was extracted from muscle strips using the Genomic Prep™ Cells and Tissue DNA Isolation Kit (GE HealthCare, Sweden). These individuals were sampled in four localities in Northwest Goiás, Central Brazil: 18 individuals from Caiapó River (16°18'53.25"S, 51°28'03.63"W); 21 individuals from Mucunã River (17°00'32.29"S, 49°21'24.40"W); 8 individuals from Anicuns Lake (16°28'09.37"S, 49°56'32.84"W), and 5 individuals from Serra da Mesa Lake (14°01'31.30"S, 48°18'09.37"W). Total genomic DNA (2.0 µg) from one individual was sheared using a sonicator, to obtain fragments of 200 bp to 1.0 kb. Fragments were recovered and cloned into pMOSBlue dephosphorylated blunt vector using the Blunt-ended polymerase chain reaction (PCR) Cloning Kit® (GE HealthCare). Cloned fragments were transformed into chemically pMOSBlue® competent cells (GE HealthCare) and plated onto Luria-Bertani (LB) plates containing ampicillin and X-Gal. Recombinant clones were grown overnight in liquid ampicillin LB medium, and plasmid DNA was extracted using a standard protocol (Sambrook and Russell, 2001). DNA inserts were sequenced using U19 primer in a 3100 automated DNA sequencer (Applied Biosystems, USA) using the DYEnamicET terminator kit (GE Healthcare), according to manufacturer instructions. The reads were filtered by their quality and length (phred value ≥20; length ≥150) and analyzed for their nucleotide content. The reads were filtered by their quality and length (phred value ≥20; length ≥150) and screened for microsatellites, and primers were designed using the web service primer designer (Martins et al., 2009).

For the characterization of loci, DNA of the 48 sampled individuals of *H. malabaricus* were amplified using the designed primers in a total reaction volume of 15 µL containing 25 ng template DNA, 1.8 µM of each primer, 1 U Taq DNA polymerase (Phoneutria), 325 µM of each dNTP, 0.13 µg BSA and 1X reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$). PCR was carried out in a thermal cycler with the following conditions: 94°C for 5 min (one cycle), 94°C for 1 min, 54 to 68°C (according to the primer annealing temperature, see Table 1) for 1 min, and 72°C for 1 min (30 cycles), and 72°C for 7 min (one cycle). Individuals were genotyped on 6% denaturing polyacrylamide gels stained with silver nitrate (Creste et al., 2001) and sized by comparison to a 10-bp DNA ladder standard (Invitrogen).

Table 1. Microsatellite loci developed for *Hoplias malabaricus*.

Locus	Motif	Primer sequence	T_A	Range	N_A	H_O	H_E	*f*	*I*	*Q*	Accession No.
Hmal 9	$(GA)_6$	F-CAACACCTGTCCTGAGAGCA R-TGGCAGTCATACACCACAGA	58°C	226-232	2	0.170	0.158	-0.081	0.749	0.072	HM447127
Hmal 11	$(TTCA)_3$	F-TCACTCTAACCCAGGGCAAC R-ACATTCCGAGTAGGCTCCG	68°C	137	1	-	-	-	-	-	HM447128
Hmal 15	$(AG)_{10}$	F-CTTGTCAGCAGAAAGTGCCC R-GCTACCATTCATCACCAGCA	62°C	226	1	-	-	-	-	-	HM447129
Hmal 23	$(TAA)_4$	F-CACAAGAGCAGAGGCAGTTG R-TGGCAAGAATTTTCCACCTT	66°C	224-252	2	0.000	0.497	1.000	0.620	0.185	HM447130
Hmal 26	$(TGTT)_6$	F-GGGCTAAGCGCTATTCCTCT R-GCACTGCAAGCAAGCAAATA	68°C	204	1	-	-	-	-	-	HM447131
Hmal 33	$(AG)_6$	F-AGGGGATTTCTTCAAGTCCG R-GGTGGAGCGAAAAATTCAAA	66°C	184-190	2	0.000	0.456	1.000	0.545	0.202	HM447132
Hmal 34	$(AAAC)_4$	F-ACGCGCTCCTACAACAGTTT R-TGGTGAAAGGGTGGATTTTC	66°C	136-156	2	0.020	0.136	0.848	0.775	0.063	HM447133
Hmal 39	$(GGA)_4$	F-CCGATGTTTGTGGTATTTGC R-GATGTTGTGGTTGGAGTGGA	66°C	209	1	-	-	-	-	-	HM447134
Hmal 46	$(AT)_7$	F-GGGGCAGTGAGGATCTTTCT R-TCACCAACCATTCCCATTTT	54°C	189-196	2	0.000	0.505	1.000	0.624	0.187	HM447135
Hmal 59	$(TTTA)_4$	F-AGAAAACTGAATTGGCCGTG R-TGAGGATTAGCATCAAGGGC	68°C	154	1	-	-	-	-	-	HM447136
Hmal 60	$(AAAC)_3$	F-TTTAATTGAACCCGTGAGCC R-AGCATAACATTCCAGGGAGC	66°C	174-186	3	0.000	0.406	1.000	0.460	0.213	HM447137
Hmal 69	$(GA)_5$	F-AGCCTTTCAGCCAAGGACAG R-GGTGCCATTACTTGTTAGCCA	66°C	229	1	-	-	-	-	-	HM447138
Hmal 71	$(ACTC)_3$	F-ATCAATGGGTGGTTCCTCAG R-CACCTGTGACCATGGAATTG	66°C	236	1	-	-	-	-	-	HM447139
Hmal 72	$(ATT)_4$	F-TGCAGTTTGCTTTGAGGTTG R-CCCCTTAAACTCAGCTCCAA	58°C	155	1	-	-	-	-	-	HM447140
Overall loci						0.032	0.360	0.795	0.056	0.638	

Locus name is followed by repeat motifs present in sequenced fragment, primer sequences, primer annealing temperature (T_A), size range of alleles in base pairs (Range), number of alleles (N_A), observed (H_O) and expected (H_E) heterozygosities, fixation index (*f*), probability of genetic identity (*I*), probability of paternity exclusion (*Q*), GenBank accession number (Accession No.).

The number of alleles per locus, observed and expected heterozygosities under Hardy-Weinberg (Nei, 1978), and inbreeding coefficient (*f*) were estimated (Weir and Cockerham, 1984). Analyses were performed with FSTAT 2.9.3.2 (Goudet, 2002) and randomization-based tests with Bonferroni's correction were performed to test for deviation from Hardy-Weinberg expectations and for linkage disequilibrium (Goudet et al., 1996). We also estimated the probability of genetic identity (*I*) (Paetkau et al., 1995) and paternity exclusion probability (*Q*) (Weir, 1996) for each polymorphic locus and overall loci, using the Identity 1.0 software (Wagner and Sefc, 1999).

Of the 864 clones sequenced, 78 (9.03%) showed nucleotide repeat motifs recognized as microsatellites. Flanking primers were designed for 25 fragments containing microsatellites, and 14 loci were successfully amplified using a single PCR protocol. Of the 14 microsatellite loci, five displayed no polymorphism and only one polymorphic locus showed more than 2 alleles (Hmal60). Expected heterozygosities ranged from 0.137 to 0.505, and all polymorphic loci deviated significantly from Hardy-Weinberg equilibrium (Table 1). Combined probability of identity was high (0.056), and probability of paternity exclusion was low (0.638), showing that further studies on parentage analysis and fine-scale genetic structure will require the search for a new set of polymorphic loci.

The low polymorphism found in this study is most likely due to the small microsatellite size found in *H. malabaricus*. Besides, most microsatellite loci are tri-, tetra- and pentanucleotides, which typically show lower levels of polymorphisms due to lower mutation rates when compared to dinucleotides (Chakraborty et al., 1997; Ellegren, 2000, 2004). The number of alleles per locus is positively correlated with the number of repeat motifs (Weber, 1990; Taramino and Tingey, 1996; Ellegren, 2004; Brandström and Ellegren, 2008). Although the number of sequences surveyed in this study was limited, our results show that the *H. malabaricus* genome has a low abundance of microsatellites (only 9.02% of the sequences showed microsatellites) and also that repeat length is short, which may limit polymorphism.

ACKNOWLEDGMENTS

Research supported by Systema Naturae Consultoria Ambiental. M.P.C. Telles and R.G. Collevatti, both included in the research program of Molecular Ecology, were recipients of CNPq fellowships.

REFERENCES

Batista JS, Farias IP, Formiga-Aquino K, Sousa ACB, et al. (2009). DNA microsatellite markers for "dourada" (*Brachyplatystoma rousseauxii*, Siluriformes: Pimelodidae), a migratory catfish of utmost importance for fisheries in the Amazon: development, characterization and inter-specific amplification. *Conserv. Genet. Res.* DOI: 10.1007/s12686-009-9117-5.

Bertollo LAC, Born GG, Dergam JA, Fenocchio AS, et al. (2000). A biodiversity approach in the Neotropical Erythrinidae fish, *Hoplias malabaricus*. Karyotypic survey, geographic distribution of cytotypes and cytotaxonomic considerations. *Chrom. Res.* 8: 603-613.

Born GG and Bertollo LA (2000). Comparative cytogenetics among allopatric populations of the fish, *Hoplias malabaricus*. Cytotypes with 2n = 42 chromosomes. *Genetica* 110: 1-9.

Brandström M and Ellegren H (2008). Genome-wide analysis of microsatellite polymorphism in chicken circumventing the ascertainment bias. *Genome Res.* 18: 881-887.

Carvalho-Costa LF, Hatanaka T and Galetti PM Jr (2006). Isolation and characterization of polymorphic microsatellite

markers in the migratory fish *Prochilodus costatus*. *Mol. Ecol. Res.* 8: 818-819.
Chakraborty R, Kimmel M, Stivers DN, Davison LJ, et al. (1997). Relative mutation rates at di-, tri-, and tetranucleotide microsatellite loci. *Proc. Natl. Acad. Sci. U. S. A.* 94: 1041-1046.
Creste S, Tulmann Neto A and Figueira A (2001). Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining. *Plant. Mol. Biol. Res.* 19: 299-306.
Ellegren H (2000). Heterogeneous mutation processes in human microsatellite DNA sequences. *Nat. Genet.* 24: 400-402.
Ellegren H (2004). Microsatellites: simple sequences with complex evolution. *Nat. Rev. Genet.* 5: 435-445.
Goudet J (2002). FSTAT: a program to estimate and test gene diversities and fixation indices.Version 2.9.3.2. Available at [http://www.unil.ch/izea/softwares/fstat.html]. Accessed February 16, 2010._
Goudet J, Raymond M, de Meeus T and Rousset F (1996). Testing differentiation in diploid populations. *Genetics* 144: 1933-1940.
Martins WS, Soares Lucas DC, de Souza Neves KF and Bertioli DJ (2009). WebSat - A web software for microsatellite marker development. *Bioinformation* 3: 282-283.
Morelli KA, Revaldaves E, Oliveira C and Foresti F (2007). Isolation and characterization of eight microsatellite loci in *Leporinus macrocephalus* (Characiformes: Anostomidae) and cross-species amplification. *Mol. Ecol. Notes Res.* 7: 32-34.
Morgante M and Olivieri AM (1993). PCR-amplified microsatellites as markers in plant genetics. *Plant J.* 3: 175-182.
Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.
Paetkau D, Calvert W, Stirling I and Strobeck C (1995). Microsatellite analysis of population structure in Canadian polar bears. *Mol. Ecol.* 4: 347-354.
Revaldaves E, Pereira LHG, Foresti F and Oliveira C (2005). Isolation and characterization of microsatellite loci in *Pseudoplatystoma corruscans* (Siluriformes: Pimelodidae) and cross-species amplification. *Mol. Ecol. Notes Res.* 5: 463-465.
Sambrook J and Russell DW (2001). Molecular Cloning: a Laboratory Manual. 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
Taramino G and Tingey S (1996). Simple sequence repeats for germplasm analysis and mapping in maize. *Genome* 39: 277-287.
Wagner HW and Sefc KM (1999). IDENTITY, 1.0. Centre for Applied Genetics. University of Agricultural Sciences, Vienna.
Weber JL (1990). Informativeness of human (dC-dA)n (dG-dT)n polymorphisms. *Genomics* 7: 524-530.
Weir BS (1996). Genetic Data Analysis II - Methods for Discrete Population Genetic Data. Sinauer Associates, Sunderland.
Weir BS and Cockerham CC (1984). Estimating F-statistics for the analysis of population structure. *Evolution Res.* 38: 1358-1370.