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First polymorphic microsatellite markers for the migratory goliath catfish *Pseudoplatystoma tigrinum* (Siluriformes: Pimelodidae), a commercially important resource in the Amazon Basin

Primeiros marcadores microssatélites polimórficos para o bagre migratório *Pseudoplatystoma tigrinum* (Siluriformes: Pimelodidae), um recurso comercialmente importante na Bacia Amazônica

DOI:10.34117/bjdv6n11-194

Recebimento dos originais: 11/10/2020
Aceitação para publicação: 11/11/2020

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ABSTRACT
The caparari (*Pseudoplatystoma tigrinum*) is a widely distributed species in the Amazon Basin. It has a high market value and it is commonly consumed by the local population. In order to protect the species from possible overfishing, there is a need for a better understanding of the population diversity, since knowledge regarding its biology is scarce, which makes it difficult to monitor. Seven microsatellite *loci* (simple sequence repeats – SSR) for the species were isolated and characterized in 46 individuals which were sampled in four locations from the Madeira River, in the Brazilian Amazon. The number of alleles per *locus* ranged from three to eleven. The observed and expected heterozygosity ranged from 0,326 to 0,705 and 0,322 to 0,758, respectively. No linkage disequilibrium between pairs of *loci* was detected. The seven microsatellite loci were additionally used for inter-specific amplification in other four species of *Pseudoplatystoma*. Therefore, this study contributes to the first molecular species-specific SSR markers, which can be used as a new tool for estimating the genetic variability of *P. tigrinum* and has potential for application in population-related studies.

Keywords: microsatellites, caparari, inter-specific amplification, *Pseudoplatystoma*, conservation.

RESUMO
O caparari (*Pseudoplatystoma tigrinum*) é uma espécie amplamente distribuída na bacia amazônica. Possui alto valor de mercado e é comumente consumido pela população local. Com o intuito de proteger a espécie de uma possível sobrepesca, é necessário um melhor entendimento da diversidade populacional, uma vez que o conhecimento sobre sua biologia é escasso, o que dificulta o monitoramento. Sendo assim, sete locos microssatélites (repetição simples de sequência - SSR) para a espécie foram isolados e caracterizados em 46 indivíduos que foram amostrados em quatro localidades do rio Madeira, na Amazônia brasileira. O número de alelos por loco variou de três a onze. A heterozigosidade observada e esperada variou de 0,326 a 0,705 e 0,322 a 0,758, respectivamente. Nenhum desequilíbrio de ligação entre pares de loci foi detectado. Os sete locos microssatélites foram usados adicionalmente para amplificação interespecífica em outras quatro espécies de *Pseudoplatystoma*. Portanto, este estudo contribui para os primeiros marcadores SSR espécie-específicos, que podem ser usados como uma nova ferramenta para estimar a variabilidade genética de *P. tigrinum* e tem potencial para aplicação em estudos populacionais relacionados.

Palavras-chave: microssatélites, caparari, amplificação heteróloga, Pseudoplatystoma, conservação.

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1 INTRODUCTION

Pseudoplatystoma tigrinum (Valenciennes in Cuvier & Valenciennes, 1840), a migratory goliath catfish, is popularly known as “caparari”, “surubim-tigre” in Brazil; “bagre tigre”, “pintadillo tigre” in Colombia; “bagre rayado” in Ecuador and “tigre zungaro” in Peru (BARTHEM & GOULDING, 2007). This species is widely distributed in the Amazon Basin and inhabits river channels, floodplains and streams, and feeds mainly on Characiformes (*Hoplias malabaricus*, *Mylossoma spp*., etc.) and Gymnotiformes (GOULDING, 1980; SOARES et al. 2008).

Pseudoplatystoma tigrinum has a high market value and is consumed daily in large quantities by the local population (CERDEIRA et al. 1997). It is one of the most common fish sold in the markets around the region (SATO et al. 1998), and its quality highly appreciated in regional cuisine (FARIA-JÚNIOR et al. 2020). It was also one of the most exploited species in the last few decades In the Mamoré and Guaporé rivers, before the turn of the century (GOULDING, 1979). In 2004, the annual production averaged 17.57 tonnes in the area of Guajará-Mirim and Porto Velho, in the state of Rondônia (DORIA et al. 2012), while during the years 2009 and 2011, the harvest in the area between Humaitá, Amazonas state and Costa Marques, Rondônia state was 16.432,0 tonnes of fishes (DORIA et al. 2015). The commercialization of this fish contributed significantly to fisheries production in communities located in the states of Pará (MARINHO & FARIA-JÚNIOR, 2020) and Roraima (RAPOSO & FERREIRA, 2020) in the period 2018/2019.

Due to the morphological similarity of *Pseudoplatystoma tigrinum* and other species of the genus, all of them are commercialized under the same name in the Amazon. The “caparari/surubim” harvest was over 8.3 tonnes in the three years from 2009 to 2011 in Brazil, and the Amazonas state presented the highest indexes for fishery production, thus demonstrating the importance of *Pseudoplatystoma tigrinum* in the region (MPA, 2013). Despite its commercial importance, knowledge regarding this catfish is scarce and is limited to genetic studies that are based on mitochondrial DNA markers (mtDNA) (TORRICO et al. 2009) and heterologous microsatellite markers, which are designed particularly for *Pseudoplatystoma corruscans* (GARCIA-DAVILLA et al. 2013).

In this context, studies of population and genetic variability of *Pseudoplatystoma tigrinum* are important, since the population structures of caparari stocks have a high level of vulnerability (CHEUNG et al. 2005), as well as the fact that since the mid-1990s this fish has been being exploited above the limits of sustainability (RUFFINO & ISAAC, 1999). Polymorphic microsatellite DNA *loci* were developed and characterized for the catfish *P.*

tigrinum. Additionally, the heterologous amplification of the *loci* characterized was also verified in four other species of *Pseudoplatystoma* (*P. punctifer*, *P. corruscans*, *P. reticulatum*, and *P. magdaleniatum*). Therefore, it is hoped that the availability of the molecular markers for these species will have the potential to generate data in order to subsidize management initiatives and the conservation of this fishery resource.

2 MATERIALS AND METHODS

Microsatellite markers were developed from the construction of an enriched genomic library (BILLOTTE et al. 1999) in microsatellite DNA, using the genomic DNA of an individual obtained near the fishing ports and quays in Santarém (Pará, Brazil). Genomic DNA was extracted according to the protocol described by Sambrook et al (1989). The DNA was digested with the enzyme RsaI (Thermo Scientific) (10U/µL) and ligated to the Rsa21 10U/µL (5'-CTCTTGCTTACGCGTGGACTA-3') and Rsa25 10U/µL (5'-TAGTCCACGCGTAAGCAAGAGCACA-3 ') adapters.

Fragments containing microsatellite regions were hybridized with biotin-linked probes $(CT)_8$ and $(GT)_8$, amplified using polymerase chain reaction (PCR), linked to the pGEM-T cloning vector (Promega) and electrotransformed into XL1-Blue competent cells of *Escherichia coli*. Competent cells were incubated on a plate containing X-Gal/IPTG, Luria-Bertani (LB) solid medium and ampicillin (100 mg/mL) for 22 hours at 37 °C. Positive clones were selected and cultured on ELISA plates containing 2YT-HMFM culture medium and ampicillin (100 mg/mL).

Plasmid DNA from the clones was extracted (SAMBROOK et al. 1989), amplified with T7 (forward) and SP6 (reverse) primers, and bi-directionally sequenced with the Big Dye Terminator v3.1 Kit (Thermo Fisher) in an ABI 3130xl analyzer (Thermo Fisher). The DNA sequences were aligned and edited with CHROMAS 2.24 (Technelisyum Pty Ltd) and BIOEDIT 7.0.9.0 programs (HALL et al. 1999). The primers were designed using WEBSAT (MARTINS, 2009) and PRIMER3 (ROZEN et al. 2000), and at the beginning of the 5' end of each forward primer, an M13 tail was added in order to allow fluorescence labeling according to the Schuelke (2000) protocol.

Of the 188 clones obtained in the construction of the genomic library, 114 presented inserts (76%). The nucleotide sequences were conducted for the formation of the 113 contigs with a mean size of 250 bp. Primers were designed from the observed microsatellites and classified as perfect (52.0%), imperfect (22.0%), interrupted (15.0%) and compound (11.0%).

The fragments containing microsatellites were amplified by PCR following the parameters described by Batista et al. (2010), in a final volume of 10 µl. The reaction was carried out in two steps: 1) denaturation (68 °C for 1 min, and 92 °C for 30s), followed by 30 cycles of 30s at 92 °C, 35s with an annealing temperature for each specific primer (Table 1), and 35s at 68 °C of extension; 2) 20 cycles with the following time and temperature profile: 20s at 92 °C, 30s at 53 °C, 30s at 72 °C, and a final extension at 72 °C for 15 min followed by 68 °C for 15 min. The amplicons were analyzed by electrophoresis on 1.5% agarose gel, stained with GelRed (Biotium) and visualized on a UVP Biodoc (Imaging System) photo documentation system.

Three multiplex systems were developed in order to genotype the amplicons: 1st system (Pt07, Pt14 and Pt21), 2nd system (Pt20 and Pt25) and the 3rd system (Pt13 and Pt16). The GeneScan 500 ROX marker (Thermo Fisher) was added to each system, in order to estimate the size and standard of the alleles of the genotyped individuals and subjected to capillary electrophoresis in an ABI 3130xl Automatic Analyzer (Thermo Fisher). Genotypes were analyzed with the GeneMarker 1.97 program (Soft Genetics).

Seven microsatellite *loci* were characterized in 44–46 individuals of *Pseudoplatystoma tigrinum* from four locations on the Madeira River located from Porto Velho, Rondônia state (N = 17) to Itacoatiara, Amazonas state (N = 19) including the locations of Manicoré, Amazonas state (N = 4) and Humaitá, Amazonas state (N = 9). All samples obtained from fishery landings and local commerce had specimens of muscle tissue deposited in the genetic resources collection at INPA.

The observed and expected heterozygosity of each *locus* were estimated with MSTOOLS v3 (PARK, 2001). The Hardy–Weinberg equilibrium (HWE) test was performed using GENEPOP v4 (RAYMOND & ROUSSET, 1995). The descriptive statistics, linkage disequilibrium (LD) and the number of alleles per *locus* were inferred using FSTAT v2.9.3.2 (GOUDET, 2002).

All seven *loci* were tested in a trans-specific amplification in four other species of *Pseudoplatystoma* (N= 4 specimens) (*P. punctifer*, *P. corruscans*, *P. reticulatum* and *P. magdaleniatum*).

3 RESULTS AND DISCUSSION

Seven polymorphic microsatellite *loci* were characterized for *Pseudoplatystoma tigrinum* (Table 1). The number of alleles per *locus* ranged from three to eleven, with an average of 5.67 alleles per *locus*. The observed heterozygosity ranged from 0,326 (Pt13) to

0,705 (Pt20 and Pt25), with a mean of 0,550 and expected heterozygosity ranged from 0,322 (Pt14) to 0,758 (Pt07) with a mean of 0,572 (Table 1). The polymorphism levels found in *P. tigrinum* corroborate those observed in other congeners, such as *P. punctifer* (SAULO-MACHADO et al. 2010), *P. reticulatum* (PRADO et al. 2014) and *P. corruscans* (REVALDAVES et al. 2005) (Table 3).

The polymorphism information content (PIC) (BOTSTEIN et al. 1980) ranged from 0,274 (Pt14) to 0,730 (Pt07), with a mean of 0,523 (Table 1). Three *loci* (Pt07, Pt20, and Pt21) showed deviations in the Hardy-Weinberg equilibrium (HWE) test after Bonferroni correction (P: (5%) ≤ 0.0071) (RICE, 1989). There was no linkage disequilibrium between *loci*, whereas F_{IS} values ranged from -0,465 (Pt20) to 0,299 (Pt21). The presence of null alleles was detected in one *locus* (Pt21) after checking with the MICRO-CHECKER 2.2.3 program (VAN OOSTERHOUT et al. 2004).

The seven developed microsatellite loci were amplified in all four individuals of *Pseudoplatystoma magdaleniatum* and *P. corruscans*, whereas, for *P. punctifer* and *P. reticulatum*, one of the *locus* was not amplified, loci Pt13 and Pt07, for each respectively and the number of alleles ranged from 1 to 6 per *locus* among the species of the genus. The highest total number of alleles (AT = 24) was observed in *P. reticulatum* and the lowest number of alleles (AT = 14) in *P. corruscans* (Table 2).

Considering the species-specific *loci* developed to date for three *Pseudoplatystoma* species, the heterologous amplification of the *loci*, as well as those obtained in the present study, *P. reticulatum* and *P. corruscans* have the highest number of *loci* with a total of 34 each of them, while *P. punctifer* has 21 *loci* respectively and *P. tigrinum* has the lowest number (19 *loci*) (Table 3).

The *loci* developed for *Pseudoplatystoma tigrinum* (Table 1) can be used in further studies of population genetics related to “caparari”, and should aid in the development of conservation and management policies for *P. tigrinum*. The microsatellite *loci* herein developed can also be applied to other species of *Pseudoplatystoma*, which will improve the genetic knowledge of this group of fish, and to contribute to conservation and management of this fishery resource.

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Table 1. Characterization of seven microsatellite loci for *Pseudoplatystoma tigrinum* in 44–46 individuals from four locations on the Madeira river.

***Locus* name**	**Primer sequence 5'-3'**	**Accession nº**	**Dye**	**Repeat Motif**	**Ta (°C)**	**Allele size (bp)**	**N**	**Na**	**H_O**	**H_E**	***P*-HWE**	**F_{IS} (*f*)**	**PIC**
Pt07	F:[M13]CGAACCCTGTGAACTGTGTGT	MK905530	FAM	$(TG)_{16}$	59	207-243	44	11	0,6820	0,7580	0,000*	0,1020	0,7300
	R:CACATTAGTCAAACCCCGGA												
Pt13	F:[M13]GTGGACTATGTGTTTGTGCGT	MK905531	HEX	$(GT)_8$	60	144-162	46	3	0,3260	0,3720	0,4067	0,1260	0,3250
	R:AAACCTCGCGCTTATAGACAC												
Pt14	F:[M13]TTGCTCAAGGGCACCTC	MK905532	HEX	$(GT)_6$	60	213-233	46	3	0,3910	0,3220	0,4478	-0,2170	0,2740
	R:AGCATGTGAGTGCAGCGT												
Pt16	F:[M13]CCTTTAGGTTTGTAGGATCAAGT	MK905533	HEX	$(TG)_9$	60	216-232	44	6	0,6360	0,7250	0,0363	0,1240	0,6760
	R:GCAGACACTGAAAACAGCAAC												
Pt20	F:[M13] CGCGAGCTGTGTAAGTGATT	MK905534	HEX	$(TG)_6$ tata $(TG)_{10}$ ttttttt $(TG)_9$	60	171-187	44	3	0,7050	0,4840	0,0013*	-0,4650	0,3920
	R:ATAGTGCCATAGTGCCATACAT												
Pt21	F:[M13]CGGTGTGCAGTGTGTGT	MK905535	NED	$(GT)_{10}$	54	244-286	44	10[a]	0,4320	0,6140	0,0055*	0,2990	0,5750
	R:CGCGTGGACTAACTTCTG												
Pt25	F:[M13]ACATTGATTTGTCAGAGTGTGTTT	MK905536	NED	$(TG)_{13}$	59	168-220	44	8	0,7050	0,7320	0,0749	0,0390	0,6890
	R: TCATGTTGATCTCTCTGCCG												

Notes: F, primer forward; R, primer reverse; Ta, annealing temperature (°C); N, sample size; Na, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; *P*-HWE, Hardy-Weinberg equilibrium p-values; F_{IS}, inbreeding coefficient; *a, presence of null allele; PIC, Polymorphism Information Content. *Significant departure from HWE after the sequential Bonferroni correction (P < 0.0071); [M13], the M13 nucleotide sequence tail with 18bp.

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Table 2: Cross-species amplification of seven *Pseudoplatystoma tigrinum* microsatellite markers *loci* in four *Pseudoplatystoma* species (N=4 for each species).

Locus	***Pseudoplatystoma reticulatum***		***Pseudoplatystoma magdaleniatum***		***Pseudoplatystoma punctifer***		***Pseudoplatystoma corruscans***	
	Allele size (bp)	**Na**	**Alelle size (bp)**	**Na**	**Allele size (bp)**	**Na**	**Allele size (bp)**	**Na**
Pti07	x	x	190	1	192-208	3	190	1
Pti13	242-258	2	224-256	3	x	x	236-254	2
Pti14	207-279	6	201-213	2	203-229	4	201-225	3
Pti16	204-232	4	218-230	4	218-232	2	218-234	3
Pti20	175-205	4	181-209	3	177-187	4	177-181	2
Pti21	250-270	4	244-272	4	270-272	2	270-272	2
Pti25	172-200	4	170-182	2	186-200	2	174	1

Notes: PCR annealing temperature as in Table 1; Na, number of alleles and x no amplification.

Table 3: Species-specific *loci* developed to genus *Pseudoplatystoma*.

Species	**Specie-Specific SSR *loci***	**H_O**	**H_E**	**Polymorphic Heterologous SSR *loci***	**Total**
P. tigrinum	7[a]	0,326 - 0,705	0,322 - 0,758	12[b]	19
P. punctifer	15[b]	0,025 - 0,972	0,025 - 0,931	6[a]	21
P. reticulatum	16[c]	0.318 - 0.909	0.498 - 0.875	6[a]+12[b]	34
P. corruscans	8[d]	0.500 - 0,615	0.725 - 0,942	5[a]+7[b]+14[c]	34
P. fasciatum	0	_	_	7[d]	7
P. magdaleniatum	0	_	_	6[a]+10[b]	16

Notes: a, This study; b, SAULO-MACHADO et al. (2010); c, PRADO et al. (2014); d, REVALDAVES et al. (2005); H_O, observed heterozygosity; H_E, expected heterozygosity.

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ACKNOWLEDGEMENTS

This work was supported by grants FAPEAM/PPP (458/12) to JSB, FAPEAM/PIPT (514/2008) to J.A.A.G., FAPEAM/SEDECTI/Governo do Estado do Amazonas - PAPAC 005/2019 and INPA/MCTI/PPI. Permits for field collection and molecular analyses were given by IBAMA (N°. 130) and SISBIO (N°. 40844-2). The authors thank Nasser Hijazi and Ricardo Lopes for some sample collection and Claudio Oliveira (UNESP/Botucatu) for *P. corruscans* samples. FGO was funded by a Postgraduate/CAPES fellowship.