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MICROSATELLITE LETTERS

Development and characterization of 16 microsatellites for the Neotropical catfish *Pseudoplatystoma reticulatum* and cross species analysis

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Abstract Sixteen polymorphic microsatellite primers were developed for *Pseudoplatystoma reticulatum* and provided an efficient multiplex protocol to amplify these loci in four multiplex PCRs. Most microsatellites here obtained showed moderate to high polymorphism levels in *P. reticulatum* and *P. corruscans*, as well as interspecific allelic differences, and represents useful genetic markers for population studies in these fishes.

Keywords Fish genetics · 454 Pyrosequencing · Multiplex-PCR · Genetic diversity

Construction of dams, overfishing and the introduction of hybrids from aquaculture have placed populations of the catfishes *Pseudoplatystoma reticulatum* and *Pseudoplatystoma corruscans* at continuous risks of genetic extinction. These taxa have not been assessed for the IUCN, and the development of multiallelic markers, as microsatellites, may provide important genetic data for management and conservations plans.

Genomic DNA was extracted from fin clips following the "Wizard Genomic DNA Purification Kit" (Promega) protocols. The DNA from three individuals of *P. reticulatum* was pooled on a Roche 454 GS FLX sequencer using the Titanium platform "Genome sequencer 20 System". Primers were designed using Primer3 software (Rozen and Skaletsky 2000). We studied 22 individuals of *P. reticulatum* from the Cuiaba River, Paraguay basin, Brazil. Cross species amplification were conducted on 12 specimens of *P. corruscans* from four populations of Paraguay and Upper Parana basins, Brazil.

Multiplex PCRs were performed in a final volume of 10 µl containing 100 µM of each dNTP, 1.5 mM MgCl2, 1× Taq buffer, 0.5 units Taq polymerase (Quiagen Master mix), 0.4 µM reverse primer, 0.4 µM fluorescently-labeled forward primer (Applied Biosystems), 30 ng of genomic DNA; and performed in a thermal cycler Veriti™ (Applied Byosystems) for 15 min. at 95 °C followed by 30 cycles of 30 s at 94 °C, 1 min at 58 °C, 1 min at 72 °C and a final extension of 60 °C for 30 min. Each multiplex PCR was loaded on an automated DNA sequencer ABI prism_3730 (Applied Biosystems). Allele sizes were scored using Gene Mapper 3.7 (Applied Biosystems).

Genetic diversity estimations using Cervus 3.0 (Marshall et al. 1998) revealed a mean of 7.8 alleles per locus and an average expected heterozygosities (He) of 0.728 for *P. reticulatum* (Table 1). Genepop 3.4 (Raymond and Rousset 1995) demonstrated significant deviations from Hardy–Weinberg Equilibrium (HWE) ($P < 0.05$) for Prt3, 37 and 39. Microchecker 2.2.3 (Van Oosterhout et al. 2004) suggested the presence of null alleles for Prt3 and

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Table 1 Primer sequences and characterization of 16 microsatellites in *P. reticulatum* and *P. corruscans*

Loci	Primer sequence (5′–3′)	Gen Bank no.	M-PCR	Repeat motif	*P. reticulatum*				*P. corruscans*			
					A	Size range (bp)	Ho	He	A	Size range (bp)	Ho	He
Prt3	F: AGTGGCGTTAGGTCTGTGTG^{NED}	KF701044		$(TG)_{13}$	5	185–197	0.409	0.660*	1	159	–	–
	R: CTCTGCCATCAATACGCTCA											
Prt4	F: AGGCGACAGCACTAACCAGT^{VIC}	KF701045		$(TA)_{10}$	7	177–191	0.727	0.799	5	176–180	0.333	0.714*
	R: GATGGAATGTGGCTGGATCT		A									
Prt25	F: CAAGGCGCTGTGTATCTTCTT^{PET}	KF701050		$(CA)_{15}$	10	171–207	0.818	0.798	1	169	–	–
	R: GATCATGCTTGGCTCAGACTT											
Prt30	F: CACCTGAGACACCACACGTT$^{6\text{-}FAM}$	KF701052		$(TC)_{8}$	8	105–133	0.455	0.605	7	69–91	0.917	0.786
	R: CGGAGGTAGGAGAGAAAGAGAG											
Prt5	F: GTGCTTCCTGCTGTGAGGTA^{PET}	KF701046		$(TG)_{15}$	10	246–270	0.909	0.885	11	225–279	1.000	0.909
	R: TGGCAACTGAGGCTTACTGA											
Prt6	F: CAGATTGCTGATGTGCTGTG^{NED}	KF701047		$(CA)_{15}$	3	210–218	0.455	0.553	4	210–228	0.417	0.562
	R: CTGCGTGATAACTTGCCAGA		B									
Prt27	F: TGTCTCGCATCAAACTACGC^{VIC}	KF701051		$(AC)_{16}$	7	211–233	0.591	0.498	12	251–285	0.917	0.928
	R: GTCGAAACCGGGACCTTC											
Prt34	F: GGTAGACCGCAAGACAGAACA$^{6\text{-}FAM}$	KF701053		$(TAGA)_{14}$	11	172–232	0.818	0.862	12	188–248	0.917	0.928
	R: GGAACTCCTGACCTCCTATGAA											
Prt11	F: TAGCAGCAGGCGATGAGAT$^{6\text{-}FAM}$	KF701048		$(GT)_{12}$	7	254–272	0.682	0.717	7	260–290	0.750	0.797
	R: CCTAATGTCCAGGGATTTGC											
Prt36	F: ACCGAGCACAGCACAGAAC^{NED}	KF701055		$(AGAT)_{14}$	6	232–252	0.682	0.799	12	329–405	0.750	0.902*
	R: AAGGCAATGGTTGGAAGAA		C									
Prt37	F: GGATTAGGATCGAGGTGATCTG^{PET}	KF701056		$(TATC)_{17}$	10	218–256	0.318	0.829*	11	196–252	0.667	0.920*
	R: AATTCCTCCTTCGAGACTTGG											
Prt40	F: AGACCGTTCACACGTCCTCT^{VIC}	KF701059		$(ACAT)_{7}$	4	252–260	0.500	0.500	4	248–256	0.500	0.656
	R: TGCAGTTGGTGGAGTTGATG											
Prt12	F: AGAGCCATGCTGTTGTTGTG^{PET}	KF701049		$(CA)_{13}$	5	272–282	0.636	0.675	7	296–310	0.667	0.822
	R: GTTTGTGGGACTCGGTGACT											
Prt35	F: TTCCACACAACCACCAGAAA^{NED}	KF701054		$(TTC)_{9}$	12	333–384	0.864	0.875	2	354–357	0.083	0.083
	R: GAAACCACAGAATGCCCTCA		D									
Prt38	F: CACACACCGCAACTTCTCAC^{VIC}	KF701057		$(ATA)_{11}$	8	316–343	0.636	0.783	2	307–310	0.083	0.083
	R: TTGCTCTCACCACACTGCTT											
Prt39	F: GCCGCCATATTGGATCAAG$^{6\text{-}FAM}$	KF701058		$(ATA)_{11}$	11	275–306	0.727	0.818*	8	260–287	0.917	0.855
	R: GCGACTCATTATACCACCTCGT											

F forward, *R* reverse, *bp* base pairs, *A* allele number, *Ho* observed heterozygosity, *He* expected heterozygosity, *M-PCR* multiplex-PCR, bold: loci with non overlapping allelic sizes between species

* $P<0.05$

Prt37, reflected by a homozygote excess. *P. corruscans* presented 14 polymorphic microsatellites (medium He of 0.710 for polymorphic loci) (Table 1) and Prt4, 37 and 39 were not in HWE, what can be explained by Wahlund effect. Moreover, six loci presented non-overlapping allelic sizes between species and can be future tested as diagnostic markers. These results indicated that the microsatellites here developed exhibited sufficient allelic variation to be successfully applied in genetic studies of these species, including population genetics and hybridization studies.

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