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TECHNICAL NOTE

Isolation of microsatellite loci in the Amazon sailfin catfish *Pterygoplichlhys pardalis* (Castelneau, 1855) (Teleostei: Loricariidae)

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Abstract *Pterygoplichlhys pardalis* is an important freshwater ornamental fish in the Amazon Basin. Studies involving populations of *P. pardalis* are of great importance for the conservation and management of this species. We developed nine microsatellite loci and applied them to investigate the genetic variation of 20 wild individuals from floodplain lakes of the Solimões river. The number of alleles per locus ranged from 2 to 12, with an average of 6.6. The observed and expected heterozygosity values ranged from 0.400 to 0.923 (average 0.706) and from 0.358 to 0.895 (average 0.692), respectively. The value of *f* ranged from −0.532 to 0.467 (average 0.032). One locus (Pp07) significantly deviated from Hardy–Weinberg equilibrium after Bonferroni correction (*P*: (5 %) < 0.006). No significant linkage disequilibrium was detected. These microsatellite loci will contribute towards studies of genetic diversity and conservation of *P. pardalis*.

Keywords *Pterygoplichlhys pardalis* · Genetic diversity · Wildlife conservation · Microsatellite markers

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More than 700 species of the Amazon sailfin catfish (Siluriformes; Loricariidae) occur in Central and South America (Wakida-Kusunoki et al. 2007; Ferraris 2007). The members of the *Pterygoplichthys* genus occur in the Orinoco and Amazon river basins (Weber 1992), and are popular among aquarists due to their algivorous habits and ornamental features (Levin et al. 2008). The *P. pardalis* is a species widely consumed and commercially exploited by fisherman of the Amazon basin. Expansion of exploitation of loricariid catfishes all over the world began in the mid-twentieth century owing to the development of an excessive pet fish trade (Hoover et al. 2004). Species of *Pterygoplichthys* are known as widespread invaders since they occur in many areas outside their native range, and the presumed mechanism of introduction is aquarium release or escape from aquaculture farms (Page and Robins 2006). We isolated and characterized microsatellite loci for the Amazon sailfin catfish to contribute to the management, conservation and control of this species.

Microsatellite loci were developed from a genomic enriched library following the protocol described by Billotte et al. (1999). The protocol used for total genomic DNA extraction was described by Sambrook et al. (1989). The purified total DNA was digested with *RsaI* and enriched for $(CT)_8$ and $(GT)_8$ repeats. Enriched fragments were amplified by polymerase chain reaction (PCR), ligated into a pGem-T Easy vector (Promega) and then transformed into competent XL1-Blue *Escherichia coli* cells. The positive clones were selected using the β-galactosidade gene and grown overnight in an HM/FM medium with ampicillin. Plasmid DNA was purified and 96 positive clones were sequenced using T7 and SP6 primers as well as the v3.1 Big Dye terminator kit (PerkinElmer Applied Biosystems) with an ABI 377 automated DNA sequencer (Applied Biosystems). A total of 12 primer pairs were

Table 1 Characteristics of the nine *P. pardalis* microsatellite loci: locus name, Genbank accession number, primer sequences (F: forward primer, R: reverse primer), repeat motif from a sequenced clone, annealing temperature (T_a), number of individuals (N), number of alleles (A), product size range in base pairs, expected (H_E) and observed heterozygosity (H_O), fixation index (f), polymorphism information content (PIC), P values for the HWE test, significance threshold adjusted using Bonferroni correction (P-HWE): (P: 5 % $\leq$ 0.006), ^{NS} not significant and * significant

SSR locus	GenBank accession no.	Primer sequences (5′–3′)	Repeat motif	T_a (°C)	N	A	Allele size (pb)	H_E	H_O	f	PIC	P value* HWE
Pp01	JN255187	F: ^{FAM}ACCACCTGCTGCTGCTTG	$(GT)_{15}$	47	15	3	216–244	0.640	0.400	0.467	0.563	0.108^{NS}
		R: CTACATGGTCCCAAACCACT										
Pp02	JN255188	F: ^{FAM}GCGTGGAAAACGAGGAGC	$(GT)_5GA(GT)_{18}$	55	19	5	171–179	0.684	0.800	−0.135	0.628	0.009^{NS}
		R: GGTGAAGACTTACAGGAAACG										
Pp03	JN255189	F: ^{FAM}GTGATTCTCTTGCTTACGC	$(AC)_{19}$	49	19	2	185–223	0.358	0.467	−0.273	0.294	0.537^{NS}
		R: ACCTTCCCATACAGATCC										
Pp04	JN255190	F: ^{FAM}GAATTTCAGCAGGTCGTC	$(GA)_{26}$	47	20	9	200–242	0.804	0.800	0.040	0.785	0.086^{NS}
		R: CATCGGCTTTCTCAGGTG										
Pp06	JN255191	F: ^{FAM}GGCACACACAAGGTTACAT	$(AC)_{21}$	55	18	3	153–159	0.592	0.923	−0.532	0.519	0.034^{NS}
		R: CGAGGGGTTCTAATCTGG										
Pp07	JN255192	F: ^{FAM}CCAAGACATCAGTATGAC	$(AG)_{26}$	49	20	12	200–276	0.879	0.583	0.374	0.867	0.000*
		R: CTCCTTCTTTGCATATCC										
Pp09	JN255193	F: ^{FAM}GGCAAGATTCCAAAGGGC	$(GT)_9$	55	18	4	133–151	0.589	0.733	−0.213	0.503	0.254^{NS}
		R: GCCTCCATGAATGAACGG										
Pp10	JN255194	F: ^{FAM}GTTGATTCTCTTGCTTACGC	$(AC)_{18}$	55	20	12	143–243	0.895	0.857	0.059	0.880	0.053^{NS}
		R: CCGATGCCATGCTTGATG										
Pp13	JN255195	F: ^{FAM}CTCATCTCACTATTTACAGG	$(TG)_{24}$	53	19	7	234–256	0.796	0.778	0.082	0.768	0.028^{NS}
		R: TGGACTAGCACAAGTGTG										

designed using the program Oligo Explorer v1.2 (Gene Link, Inc.) and a M13 sequence tail was added in the 5′ end of each forward primer in order to permit the fluorescent labeling protocol suggested by Schuelke (2000).

These loci were characterized in 20 individuals of *P. pardalis* collected in lakes along Solimões River between the cities of Coari and Manaus (PIATAM Project). The microsatellite fragments were amplified by PCR in 10 µl containing 60 ng of genomic DNA template, each forward and M13 Label primer (FAM) at 0.4 µM, reverse primer at 0.8 µM, PCR Master Mix (2×) (Fermentas)—0.05 U/µl Taq DNA Polymerase in reaction buffer (10 mM Tris–HCl, pH 8.5 and 50 mM KCl), 4 mM $MgCl_2$, 0.4 mM dATP, 0.4 mM dCTP, 0.4 mM dGTP and 0.4 mM dTTP. PCR was carried out with two steps: denaturation (94 °C, 1 min) followed by 25 cycles of 20 s at 94 °C, 20 s at 56 °C (specific annealing temperature, Table 1), and 30 s at 68 °C; the second step consisted of 20 cycles with the following time and temperature profile: 20 s at 94 °C, 20 s at 53 °C, 30 s at 68 °C, and a final extension at 72 °C for 3 min. Amplifying products were checked by electrophoresis on 1 % agarose gels containing 0.1 mg ethidium bromide/ml in 1× TBE buffer (pH 8.0). The products from each PCR (1.0 µl) were analyzed on an ABI 3130*xl* Sequencer and sized using the GeneScan-500 LIZ internal size standard and scored using GeneMapper version 4.0 (Applied Biosystems) software.

Out of 12 microsatellite loci developed for *P. pardalis*, nine were polymorphic (Table 1) and three monomorphic. The number of alleles, the observed (H_O) and expected (H_E) heterozygosity and fixation index (f) were calculated using Genetix v4.05.2 (Belkhir et al. 2004). The test for conformity to the Hardy–Weinberg expectation (HWE) and linkage disequilibrium (LD) between all pairs of loci were calculated using GDA v1.1 (Lewis and Zaykin 2000). The polymorphism information content (PIC) was calculated using Cervus v3.0.3 (Kalinowski et al. 2007). Significance levels were adjusted to the number of simultaneous tests using sequential Bonferroni correction (Rice 1989). The number of alleles per locus ranged from 2 to 12, with an average of 6.6 alleles per locus (Table 1). Polymorphism information content (PIC) ranged from 0.294 to 0.880. Observed heterozygosity (H_O) and expected heterozygosity (H_E) ranged from 0.400 to 0.923 (average 0.706) and 0.358 to 0.895 (average 0.692), respectively. Values of f ranged from −0.532 to 0.467 with average of 0.032. Only one loci (Pp07) showed significant deviation from the Hardy–Weinberg equilibrium (HWE) after Bonferroni correction (P: (5 %) < 0.006), and probably this deviation occurred due to the presence of null alleles as suggested by the program Micro-Checker (van Oosterhout et al. 2004). Other possible reasons for the deviation from HWE might be the Wahlund effect or the small sample size. No significant linkage disequilibrium (LD) was detected among all pairs of loci. These nine polymorphic microsatellite loci are potential tools for genetic studies of wild populations of *P. pardalis*.

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References

Belkhir KP, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire du Génome et Populations, Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier, France

Billotte N, Lagoda PJR, Risterucci AM, Baurens FC (1999) Microsatellite-enriched libraries: applied methodology for the development of SSR markers in tropical crops. Fruits 54:277–288

Ferraris CJ (2007) Checklist of catfishes, recent and fossil (Osteichthys: Siluriformes), and catalogue of siluriform primary types. Zootaxa 1418:1–628

Hoover J, Killgore J, Cofrancesco A (2004) Suckermouth catfishes: threats to aquatic ecosystems of the United States? In: Aquatic Nuisance Species Research Bulletin 4–1. U.S. Army Corp of Engineers Research and Development Center, Vicksburg, Mississippi

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16:1099–1106

Levin BA, Phuong PH, Pavlov DS (2008) Discovery of the Amazon sailfin catfish *Pterygoplichthys pardalis* (Castelnau, 1855) (Teleostei: Loricariidae) in Vietnam. J Appl Ichthyol 24:715–717

Lewis PO, Zaykin D (2000) Genetic data analysis: computer program for the analysis of allelic Data, Version 11 (d15). University of Connecticut, Storrs, CT

Page LM, Robins RH (2006) Identification of sailfin catfishes (Teleostei: Loricariidae) in Southeastern Asia. Raffles Bull Zool 54:455–457

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43: 223–225

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, New York

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotech 18:233–234

van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) Microchecker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Wakida-Kusunoki AT, Ruiz-Carus R, Amador-Del-Angel E (2007) Amazon sailfin catfish, *Pterygoplichthys pardalis* (Castelnau, 1855) (Locariidae), another exotic species established in southeastern Mexico. Southwest Nat 52:141–144

Weber C (1992) Révision du genre *Pterygoplichthys* sensu latu (Pisces, Siluriformes, Loricariidae). Revue Francaise du Aquariologie 19:1–36