Short Communication

Microsatellite markers for Amazon pellona *Pellona castelnaeana* (Clupeiformes: Pristigasteridae)

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ABSTRACT. The Amazon pellona is one of the few species of Pristigasteridae with recognized commercial value in the Amazon. We isolated 24 and characterized 8 microsatellite loci for this species. The number of alleles ranges from 2-8 per locus. Observed heterozygosities ranged from 0.052-0.823, while expected heterozygosities from 0.052-0.836. These 8 microsatellites are potentially valuable tools for characterizing the levels and distribution of genetic diversity, population structure, and gene flow. They may also be important parameters for the genetic conservation of this species, as well as for its sister taxon *Pellona flavipinnis*.

Key words: Apapa; Amazon Basin; Enriched genomic library; Microsatellite loci; Population analysis

INTRODUCTION

Pellona castelnaeana Valenciennes 1847 feeds mainly on fish (Santos et al., 2006). Similar to other piscivorous animals, they reproduce during the dry season. Anticipation of the period of reproduction of other species allows the offspring to reach a size large enough to feed on larvae and the young of other Amazonian fish species that reproduce during the rainy season (Ikeziri et al., 2008). This species inhabits rivers and apparently does not enter the sea, although presumably it tolerates at least some degree of salinity at the Amazon mouth. The species is distributed in the Amazon River system from Iquitos in the Peruvian Amazon and the Ambyiacos River in Ecuador to the mouth of the Amazon in Pará and in the Guianas (Whitehead, 1985).

According to Ikeziri et al. (2008), the Amazon pellona is one of the few species of Pristigasteridae with recognized commercial value in the freshwater fisheries of the Amazon. Barthem and Fabré (2004) reported that the 2 species of *Pellona* Valenciennes 1837 were intermediate between the main species of fishing landing at the principal fish market of Amazonia. Santos et al. (2006) reported that the economic importance of Amazon pellona is approximately 20% and Yellowfin river pellona [*Pellona flavipinnis* (Valenciennes 1837)] is 5-20%; these numbers correspond to the fish marketed in Manaus.

Is important to note that these percentages correspond to consumption within the family and how family is not representative in the Amazon, so the percentages are high. Almeida (2006) reported that the Amazon pellona is among 1 of 30 new species marketed by the industrial fishing fleet. These data show that these species are important components of fishery resources of the Amazon basin, and as such must be studied in order to implement sustainable fishing practices.

MATERIAL AND METHODS

To assess the genetic parameters of *Pellona* in the Amazon Basin, we developed a partial genomic library of *P. castelnaeana* enriched for microsatellites using selective hybridization with biotinylated probe types $(CT)_8$ and $(GT)_8$, conjugated to streptavidin-coated magnetic beads (Refseth et al., 1997). After hybridization, the microsatellite-enriched sequences were amplified by polymerase chain reaction (PCR), ligated in to the pGEM-T Easy Vector, and transformed into *Escherichia coli* XL1-BLUE electroporated competent bacteria. Transformed bacteria were plated on solid medium containing 100 mg/mL LB-ampicillin + 2% X-gal. White colonies containing inserts were transferred and grown on 96-well plates in a liquid Tartoff-Hobbs Broth/ampicillin medium. A total of 96 positive clones were sequenced in both directions using the BigDye® Terminator v3.1 Cycle Sequencing Kit according to manufacturer instructions (Applied Biosystems, Foster City, CA, USA). The sequences were edited and aligned using the BioEdit software (Hall, 2007).

A total of 96 clones were sequenced, of which 24 were selected for primer design using the Primer3 software (Rozen and Skaletsky, 2000). In all primer pairs, the forward primers contained an M13(-21) tail added to its 5' end (Schuelke, 2000). An optimal annealing temperature was inferred using a gradient PCR program with temperatures set at 50-60°C. All primers were amplified at 60°C, and thus this temperature was used for characterization of 8 highly polymorphic loci from the original set of 24. Genotyping reactions were carried out in the Veriti™ Thermal Cycler (Life Technologies, Carlsbad, CA, USA) in a final volume of 10 μL. Each reaction contained 3.8 μL MilliQ water, 1.2 μL 50 mM $MgCl_2$, 1.0 μL 10 mM

dNTPs, 1.0 μL PCR buffer (100 mM Tris-HCl, pH 8.5, 500 mM KCl), 0.4 μL 2 μM tailed forward primer, 0.4 μL 2 μM fluorescently labeled primer, 0.8 μL 2 μM reverse primer, 0.3 μL 2.5 U *Taq* DNA polymerase, and 1 μL DNA (50-100 ng/μL). Reactions were carried out under the following cycling profile: hot start at 94°C for 60 s, followed by 25 cycles of denaturing at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 68°C for 30 s; the labeling step consisted of 20 cycles of denaturing at 94°C for 20 s, annealing at 52°C for 30 s, and extension at 72°C for 60 s; final extension was performed at 72°C for 30 min. One microliter PCR product was combined with 1 μL ROX size standard (Dewoody et al., 2004) and 8 μL Hi-Di formamide (Life Technologies), and fragments were resolved on the ABI 3130xl automatic sequencer (Life Technologies). Genotypes were visualized and binned using the GeneMapper v4.0 software (Life Technologies). Microsatellite loci were characterized in individuals of *P. castelnaeana* from Santarém in the Amazon Basin. Using the Micro-Checker v2.2.3 program (Van Oosterhout et al., 2004), we verified that null alleles were not present in the data set. Genetic parameters were obtained using the Arlequin 3.5 program (Excoffier and Lischer, 2010).

RESULTS AND DISCUSSION

The *Pellona castelnaeana* population showed heterozygosities ranging from 0.052-0.823, expected heterozygosities from 0.052-0.836, and the number of alleles per locus varied from 2 (locus Pc63 and Pc66) to 8 (locus Pc77) (Table 1). No significant deviations from Hardy-Weinberg equilibrium were observed. All loci were unlinked. A test of transferability was conducted in individuals of *Pellona flavipinis* from Manaus (Amazonas, Brazil) and showed that most loci were amplified and were polymorphic (Table 2). The microsatellite loci developed for *P. castelnaeana* indicated that these 8 microsatellites can be used to characterize the levels and distribution of genetic diversity, population structure, and gene flow, among others important parameters for the genetic conservation of this species, as well as for its sister taxon *P. flavipinnis*.

Table 1. Characterization of 8 microsatellite loci in 9-19 individuals of *Pellona castelnaeana* from municipality of Santarém, Pará, Brazil.

GenBank accession		Primer (5'-3')	Repeat motif	T (°C)	Size range (bp)	N/A	H_O	H_E
KJ013303	Pc 3	F: CCCTGGTCTTGACGCACT	$(TGA)_{19}$	60	310-316	19/3	0.368	0.655
		R: CAGTCCCGAGGCACACTT						
KJ013304	Pc 11	F: AAAAATTCCTGACCTGAGCA	$(TG)_{19}$	60	201-213	19/5	0.473	0.627
		R: †GTTTCTTCCAATACCATACATAGAGCACAAA						
KJ013305	Pc 63	F: GAATAGTGCCTGACGCAACA	$(CA_5)TGCATAA_6$	60	212-214	19/2	0.263	0.308
		R: †GTTTCTTGTCGGACACAAGTTCTGCAA						
KJ013306	Pc 66	F: TGACATACGGTTAGCATGTAGCA	$(TG_3)TATGT_{10})$	60	182-186	19/2	0.052	0.052
		R: GTTTCTTCCATTTCGCGAGAGAAAATC						
KJ013307	Pc 77	F: GCCAGGATCCTAAACACCAA	$(AG)_5AA(AG)_{10}$	60	312-334	17/8	0.823	0.836
		R: GTTTCTTCCCAAACACACACGATCACA	$A(AG)_5A(TG)_{10}$					
KJ013308	Pc 78	F: GGAGACTCTGTGGGTGGAAA	$(GT)_{14}$	60	242-260	9/4	0.666	0.725
		R: TACCTTTCCCTGCACTTGCT						
KJ013309	Pc 81	F: TATTGGCCCTATTGCCTTTG	$(TG)_{15}$	60	312-316	17/3	0.470	0.383
		R: TGACACACATGCTGTCAAACTC						
KJ013310	Pc 93	F: TCTGGAACCATGGATTTGGT	$(TG)_{15}$	60	167-171	19/3	0.368	0.362
		R: †GTTTCTTCCCGTGCTAGCAGAGAGTGT						
	All loci					17/3.8	0.435	0.493

N/A = number of individuals/allele number; H_O = observed heterozygosity; H_E = expected heterozygosity. †Underlined bases were added to 5' end of the reverse primers to promote adenylation by *Taq* DNA polymerase (Brownstein et al., 1996).

Table 2. Cross-species amplification results of 8 microsatellite loci developed for *Pellona castelnaeana* tested in *P. flavipinnis* from Manaus (AM, Brazil).

Locus	*Pellona flavipinnis*			
	Size-range	N/A	H_O	H_E
Pc3	296-318	31/10	0.312	0.688
Pc11	181-193	31/6	0.593	0.563
Pc63	266-270	27/4	0.375	0.530
Pc66	166-184	27/5	0.172	0.384
Pc77	276-292	31/7	0.531	0.712
Pc78	266-276	27/7	0.571	0.671
Pc81		-		
Pc93	179-199	28/12	0.633	0.782
All loci		29/6.7	0.455	0.533

N/A = number of individuals/allele number; H_O = observed heterozygosity; H_E = expected heterozygosity; (-) = no amplification.

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