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PERMANENT GENETIC RESOURCES NOTE

Microsatellite markers for the Amazon peacock bass (*Cichla piquiti*)

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Abstract

A set of primers to amplify 10 microsatellite DNA loci was developed for the Neotropical fish *Cichla piquiti*, one of the largest sized cichlids in the Amazon Basin. These loci were used to genotype individuals from two populations, one native population from the Tocantins River, the other an introduced population in southeast Brazil, Upper Paraná River. Cross-amplification was also successful for another species of peacock bass, *C. kelberi*. An average of 4.4 alleles per locus (2–9 alleles) was detected. These markers will be useful for the characterization of genetic structure of native populations, and also for invasive biology studies since *Cichla* species have been introduced in many river basins outside their native ranges.

Keywords: Cichlidae, conservation genetics, introduced populations, microsatellites, Peacock bass, Tucunaré

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The peacock bass (*Cichla piquiti*), also commonly known as tucunaré or pavon, is one of the largest sized cichlid fishes of the Amazonian basin. It is a very aggressive fish, being highly appreciated for sport fishing as well as a food resource (Nelson 1994). This species has been introduced into many river basins outside its native range, both within Brazil and in other countries where it has often become invasive and caused the local extinction of at least nine indigenous fish species (Zaret & Paine 1973). *C. piquiti* and the closely related *C. kelberi*, a species endemic to the Tocantins-Araguaia basin that is often found in sympatry with *C. piquiti*, are the most translocated species of *Cichla* in Brazil (Carvalho *et al*. unpublished). Such recently introduced populations can provide a unique opportunity for studying evolution, ecology and biogeography in the Neotropics (Vellend *et al*. 2005).

Here we report the isolation and characterization of 10 microsatellite DNA markers for *C. piquiti* and their cross-amplification with *C. kelberi*. Loci were isolated using an enrichment technique (Fischer & Bachmann 1998) modified as in Beheregaray *et al*. (2004). Genomic DNA was digested with *Rsa*I and *Hae*III and fragments ligated to two oligo adaptors. Two biotinylated oligo probes (dGA_{10} and dCA_{10}) were hybridized to the digested DNA and selectively retained using streptavidin magnetic particles (Promega). Polymerase chain reactions (PCRs) were performed on the microsatellite-enriched eluate using one of the oligo adaptors as a primer. The enriched library was purified using a gene clean kit (Qbiogene), ligated into pcR 2.1-TOPO vector (Invitrogen) and transformed into TOP10 cells. The plasmid DNA was PCR-amplified directly from the colonies using M13 forward (–20) and reverse (–40) primers, and DNA sequences were obtained from purified (Qbiogene) PCR products. Out of 120 putative positive clones sequenced, 20 had microsatellite sequences with enough flanking sequences to design primers. Primers were designed using Primer 3 (Rozen & Skaletsky 1997) and an M13 universal sequence (5′-TGTAAAACGACGGCCAGT) was appended to the 5′ end of each forward primer to facilitate subsequent fluorescent labelling.

Individual microsatellite loci were amplified for 34 samples of *C. piquiti*. Twenty of those originated from a native

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Table 1 Primers sequences and characteristics of 10 microsatellite loci isolated for *Cichla piquiti*. Number of alleles (N_A) and allelic size range are based on 34 individuals from two populations. Values of observed (H_O) and expected (H_E) heterozygosities were calculated based on 20 fish sampled from a native population

Locus	Primer sequences (5′–3′)†	Repeat structure	N_A	Size range (bp)‡	H_O/H_E	GenBank Accession no.
Tuc3	TTTCATGCGGAAAAATAGCAC CCCTCCAGACTTCAGCTTTC	$(GT)_{21\ imperfect}$	5	264–300	0.650/0.473	EU551726
Tuc4	ATACCCATCTCCCTCTGCAT CCCCCAGTTTGGGTTAGAAA	$(TG)_{23\ imperfect}$	2	194–196	—	EU551727
Tuc5	GCTTGTTGTGCTGGTGAGTG AACACTCTAAGTAGCCTTTGTTTTTG	$(CA)_9$	9	232–261	0.555/0.668	EU551728
Tuc9	TCGCTGTTGCAGCTTATCAC GACTGAAAGGGGGTGGAGAG	$(CA)_{10\ imperfect}$	3	206–218	0.250/0.333	EU551729
Tuc10	TCTCCCTGACCTTCAACCAG TGCTTAGATGAGCTGCAAGG	$(GT)_4$	2	186–188	0.000/0.105	EU551730
Tuc11	AAGAGGCAAAAACGGGAAAG CCATGCTCCTGCTTGTGTAG	$(GT)_{13}$	2	163–165	0.000/0.100	EU551731
Tuc12	CCTGCGCTAGATCATCATTC TCCTTTCGATCTCCCAAATG	$(GT)_7\ (GA)_1$	2	238–240	0.005/0.005	EU551732
Tuc13	CGAAAAGACAGCATGTCAGC CCCATATTGACCCACTGATTC	$(CA)_{17}$	5	235–257	0.312/0.521	EU551733
Tuc16	AGATCTTCTGTGGGGAGGAG AAAAACAACACCATGGCAAG	$(AC)_7$	4	171–183	0.389/0.559	EU551734
Tuc18	ATTCGTGACGGAGAGATAGA TCAATAATACTGCCCCACAAA	$(TC)_{23\ imperfect}$	9	96–142	0.526/0.863*	EU551735

*significant deviation from Hardy–Weinberg equilibrium, $P < 0.05$.
†forward primers were tagged with a 5′M13 universal sequence (5′-TGTAAAACGACGGCCAGT-3′).
‡size range excluding 5′M13 universal sequence.

population (Tucuruí reservoir, Tocantins River, Amazon Basin) and 14 from an introduced population in southeast Brazil (Itumbiara reservoir, Upper Paraná Basin). In addition, 20 samples of *C. kelberi* were used to test for cross-amplification. Amplification followed the method described by Schuelke (2000) in which PCR products are fluorescently labelled through the inclusion of a third (fluorescent M13) primer in each reaction. Reactions were performed in a final volume of 10 µL containing 1× Flexi Buffer Go*Taq* (Promega), 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 U Go*Taq* Flexi DNA polymerase (Promega), BSA (0.1%), 0.05 µM forward primer, 0.2 µM reverse primer and 0.2 µM fluorescent M13 primer. PCR amplifications consisted of 94 °C for 3 min, followed by a 32-cycle touchdown (94 °C for 20 s; 63 °C down to 55 °C until the fifth cycle for 45 s; 72 °C for 60 s), and 72 °C for 4 min. Of the 20 primer pairs tested, 10 amplified consistently and were selected for further genotyping.

Amplification products were detected on an ABI 3130 Sequencer (Applied Biosystems) at the DNA Sequencing Facility of Macquarie University. The resulting microsatellite profiles were examined using GeneMapper 4.0 (Applied Biosystems) and peaks were scored manually. GenePop version 3.3 (Raymond & Rousset 1995) was used to estimate expected and observed heterozygosities, number of alleles, linkage disequilibrium and Hardy-Weinberg (HW) proportions. Bonferroni corrections were applied when conducting multiple statistical tests (Rice 1989).

All loci were polymorphic for *C. piquiti* and showed an average of 4.4 alleles per locus (between 2 and 9 alleles per locus). Although some loci displayed low variation in our sample of 34 individuals (e.g. Tuc4, Tuc10, Tuc11 and Tuc12, all with 2 alleles per locus), some appeared to have species-specific alleles that could be useful for assessing hybridization in *Cichla*. Interestingly, Tuc4 was monomorphic for the native populations, but had a different allele in the introduced population. All loci were in HW equilibrium in the native populations, except for Tuc18 which showed an excess of homozygotes probably related to null alleles (Table 1). No evidence for linkage disequilibrium was detected in locus-pair/population comparisons. All primer sets also amplified in samples of *C. kelberi*, suggesting that these markers could be used for genetic characterization of its populations. Together with the genetic characterization of native populations of *C. piquiti* and *C. kelberi*, these microsatellites are expected to prove useful to better understand the colonization process of these species in southeastern Brazil and shed light on the processes that drive their successful invasions.

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