



Short Communication

Heterologous amplification and characterization of microsatellite markers in the Neotropical fish *Leporinus friderici*

A.M. Olivatti¹, T.A. Boni¹, N.J. Silva-Júnior², L.V. Resende¹,
F.O. Gouveia¹ and M.P.C. Telles¹

¹Laboratório de Genética e Biodiversidade,
Instituto de Ciências Biológicas I, Universidade Federal de Goiás,
Goiânia, GO, Brasil

²Mestrado em Ciências Ambientais e Saúde,
Pontifícia Universidade Católica de Goiás, Goiânia, GO, Brasil

Corresponding author: T.A. Boni
E-mail: talgeaix@gmail.com

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ABSTRACT. *Leporinus friderici*, native to the Amazon Basin and popularly known as “piau-três-pintas”, has great ecological and economic importance; it is widely fished and consumed throughout much of tropical South America. Knowledge of the genetic diversity of this native species is important to support management and conservation programs. We evaluated microsatellite loci amplification, using heterologous primers, in 31 individuals of *L. friderici*. These samples were collected from natural populations of the Araguaia River basin, in central Brazil, and the DNA was extracted from samples of muscle tissue. Eight loci were successfully analyzed. Six of them were polymorphic, and the number of alleles ranged from three to 10. Values of expected heterozygosities for these polymorphic loci ranged from 0.488 to 0.795. Exclusion probability (0.983), the identity probability

(0.000073), and the mean genetic diversity values were high, showing that these microsatellite markers are suitable for assessing the genetic variability of *L. friderici* populations. There is a growing interest in studies that evaluate the genetic variability of natural populations for various purposes, such as conservation. Here, we showed that a viable alternative to the costly development of specific primers for fish populations is simply testing for heterologous amplification of microsatellite markers available from research on other species.

Key words: SSR; Transferability; Genetic variability; Cross-amplification

INTRODUCTION

The fish *Leporinus friderici*, popularly known as “piau-três-pintas”, belongs to the family Anostomidae and is endemic to South America. This fish species is native to the Amazon and La Plata Basins, commonly inhabits rivers, lakes and flooded areas, and feeds on insects, fruits and seeds (Nomura, 1984). In addition to the large ecological importance, *L. friderici* has a reasonable economic value both for commercial and sport fishing. However, this species shortly migrates throughout rivers, and thus, their populations are suffering declines due to the intense construction of dams in Brazil.

The genetic diversity of native species is an important feature to be considered in conservation and management programs. The use of molecular techniques to assess patterns of genetic variation has supported conservation programs, by indicating which species require greater conservation efforts and also by selecting areas where natural populations are viable (Johnson et al., 2001).

Recently, molecular markers have been commonly used in population studies. Simple sequence repeat (SSR) markers are preferable because they are potentially codominant and highly polymorphic. In addition, microsatellites have a wide distribution in the genome and can be efficiently identified, which is essential in studies about the genetic variability of populations (Collevatti et al., 1999). The polymorphism obtained with microsatellite markers has provided powerful information to be considered in the management of fish stocks (Alam and Islam, 2005), population analysis and biodiversity conservation (Romana-Eguia et al., 2004).

However, the use of microsatellite markers requires the development of specific primers, i.e., the unique sequences of DNA flanking SSR loci of a species' genome. Given that the development of primers can be costly, several studies have tested for possible heterologous amplifications in a target species using primers already described for evolutionarily related species (Cipriani et al., 1999; Brondani et al., 2003) or even for evolutionarily related genera (Zhan et al., 2009). Many studies have successfully demonstrated heterologous amplification (Barbosa et al., 2006; Hatanaka et al., 2006; Morelli et al., 2007), thereby lowering the costs of future projects. However, the loci detected by successful heterologous amplification should also be tested for their ability to access the polymorphism in the genome of the target species. Therefore, the aim of this study was to investigate the heterologous amplification of microsatellite loci developed for different fish species in *L. friderici*.

MATERIAL AND METHODS

First, 46 SSR loci developed for several Neotropical ichthyofauna species were tested (*Prochilodus argenteus*, *P. costatus*, *Leporinus macrocephalus*, *Astyanax fasciatus*, and *Poecilia reticulata*). Of these, eight loci indicated successful heterologous amplification for the *L. friderici* genome. In the present study, we characterized these eight loci in 31 samples of *L. friderici*. These loci were originally developed for the following fish species: *A. fasciatus* (Ast09), *L. macrocephalus* (Lmac03, Lmac05, Lmac08, Lmac09), *P. costatus* (Pcos14, Pcos18), and *P. argenteus* (Par03) (Table 1).

DNA was extracted from samples of muscle tissue using the phenol-chloroform extraction protocol (Monesi et al., 1998). Total DNA of each individual was quantified and diluted to a final concentration of 5 ng/ μ L. Each polymerase chain reaction (PCR) mixture consisted of 25 ng DNA and 10 μ L of a solution containing autoclaved MilliQ water, 3.69 μ M primer, 13 mg bovine serum albumin, 15X enzyme buffer, 12.5 mM MgCl₂, 250 μ M of each dNTP, and 1 U Taq-DNA polymerase (Phoneutria Biotechnology and Services Ltda.) in a final volume of 15 μ L.

The amplification products were analyzed by electrophoresis on vertical 6%, denaturing polyacrylamide gels using a molecular weight marker of 10 bp (Invitrogen™). Gels were stained with silver nitrate for the visualization of alleles, following the method described by Crete et al. (2001).

Descriptive analyses of genetic variability of the loci were done using the genotypes obtained. The number of alleles per locus and observed and expected heterozygosities under Hardy-Weinberg equilibrium were estimated. These analyses and the test for deviation from Hardy-Weinberg expectations were performed with TFPGA 1.3 (Tools for Population Genetic Analyses) (Miller, 1997). We also estimated the probability of genetic identity (I) and paternity exclusion probability (Q) for each polymorphic locus and overall loci, using the Identity 1.0 software (Wagner and Sefc, 1999).

RESULTS AND DISCUSSION

Two of the eight transferred loci were monomorphic (loci Pcos18 and Ast09), whereas the others were polymorphic. Considering the polymorphic loci, four were developed for *L. macrocephalus* (Lmac03, Lmac05, Lmac08, and Lmac09), one was developed for *P. argentus*

Table 1. Characteristics of the eight microsatellite loci successfully amplified for *Leporinus friderici*.

Locus	AT	Size range (bp)	NA	Ho	He	Q	I	Reference
Ast09	47	318	1	-	-	-	-	Strecker, 2003
Lmac03	60	220-242	9	0.433	0.680	0.491	0.166	Morelli et al., 2007
Lmac05	66	228-252	10	0.857	0.795	0.634	0.094	Morelli et al., 2007
Lmac08	58	150-166	8	0.541	0.751	0.534	0.175	Morelli et al., 2007
Lmac09	60	173-182	5	0.764	0.740	0.514	0.188	Morelli et al., 2007
Pcos14	62	127-136	3	0.225	0.488	0.213	0.537	Carvalho-Costa et al., 2006
Pcos18	68	200	1	-	-	-	-	Carvalho-Costa et al., 2006
Par03	62	290-320	6	0.411	0.603	0.385	0.265	Hatanaka et al., 2006

AT = annealing temperature (°C); NA = number of observed alleles; Ho = observed heterozygosity; He = expected heterozygosity; Q = paternity exclusion probability; I = identity probability.

(Par03), and one was developed for *P. costatus* (Pcos14). It is interesting that polymorphism was observed for the loci developed for the genera *Prochilodus*. However, polymorphism was more consistently observed for the loci developed for all species belonging to the *Leporinus* genus, emphasizing that heterologous amplifications are successfully observed for evolutionarily related species (Cipriani et al., 1999; Brondani et al., 2003).

Annealing temperatures of amplified polymorphic loci ranged between 58° and 66°C, whereas size variation of alleles ranged between 127 and 320 bp. Allelic diversity across the six microsatellite loci ranged from three to 10 alleles/locus, totaling 41 alleles (Figure 1).

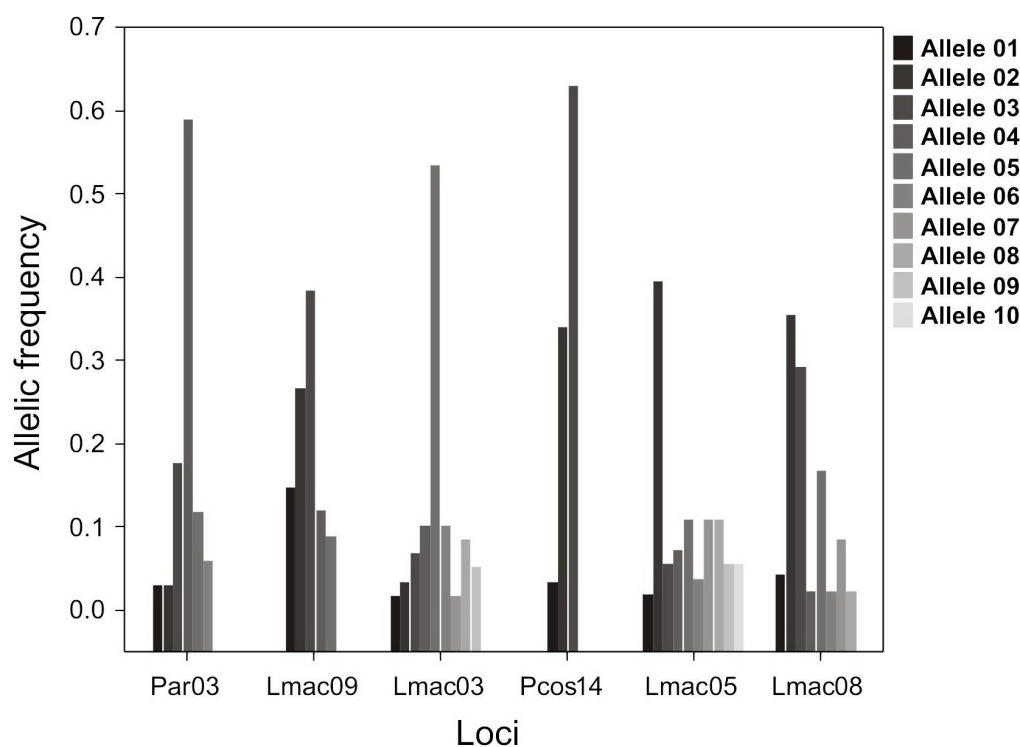


Figure 1. Allelic frequencies of the six polymorphic loci successfully transferred for the fish *Leporinus friderici*.

The average expected and observed heterozygosities across loci were 0.67 and 0.54, respectively, and all loci were in Hardy-Weinberg equilibrium ($P < 0.05$). As expected, higher values of heterozygosities were observed when primers developed for species of the genus *Leporinus* were tested, in comparison with those developed for other fish genera (Table 1). The mean genetic diversity value was 0.750. The high values of heterozygosity and mean genetic diversity showed considerable variability among individuals. Paternity exclusion (Q) and identity (I) probabilities were 0.983 and 0.000073, respectively. Therefore, the set of loci analyzed could be used for gene flow estimation in paternity analysis. The paternity exclusion probabilities across loci were NE-1P = 0.0133 and NE-2P = 0.0013 for all populations. The combined non-exclusion P(ID) was 3×10^{-8} .

Molecular markers are useful tools in studies aiming to assess the genetic variability of natural fish populations, which could generate, for instance, important conclusions about the consequences of threats to natural populations, such as habitat fragmentation due to the construction of dams in Brazilian rivers. In fact, populations of migratory fish such as *L. friderici* are suffering declines in floodplain systems (Alves, 2006). Particularly, microsatellite markers successfully transferred for *L. friderici* could be efficiently used in studies focusing on the genetic variability of populations. To the best of our knowledge, there is no record in GenBank (see, www.ncbi.nlm.nih.gov) or in the literature about specific microsatellite primers developed for *L. friderici*. In accordance, studies on the genetic structure of populations of Neotropical fish are still scarce (Renno et al., 1991; Telles et al., 2010).

The development of microsatellite primers is costly and sometimes ineffective to detect polymorphism in natural populations (e.g., Telles et al., 2010). Therefore, the successful heterologous amplification observed here suggests that further studies on other species should focus on transferability as an alternative to find suitable and inexpensive primers that could be used to evaluate genetic variability in natural populations. Indeed, the six markers that were amenable to heterologous amplification in the fish *L. friderici* may be used in future programs of the management, monitoring and conservation of this species.

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