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Two genetic stocks of *Steindachneridion melanodermatum* living in sympatry in nature and genetic variability of wild parents and F_1 generation

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ABSTRACT. *Steindachneridion melanodermatum* is a large Brazilian catfish, highly prized for sport fishing and for its meat. Specimens of this species, both caught in nature from Iguacu River and F_1 fish born in captivity, were analyzed with regard to patterns of RAPD molecular markers. Genetic similarity ranged from 0.57 to 0.95; two groups were determined for the wild specimens. The results suggest different genetic lineages in sympatry in nature. Heterozygosity and percentage of polymorphic loci were 0.31 and 79% and 0.23 and 62%, respectively, for the two populations of wild specimens and 0.26 and 66%, respectively, for those born in captivity.

Key words: RAPD; Genetic conservation; Fish; Surubim

INTRODUCTION

Genetic conservation is a strategy of considerable value for whoever works with biodiversity (Solé-Cava, 2001). The determination of the degree of genetic variability in a local reproductive unit is of fundamental importance in this field of research, whether for conservation purposes or for breeding in captivity (Leuzzi et al., 2004).

The construction of dams for hydroelectric plants is one of the most serious problems regarding environmental changes for terrestrial and aquatic species. For fish, the greatest impact is perhaps the change in dispersion patterns for migratory fish, which, under certain conditions, are unable to adapt to these changes. This situation ends up reducing gene flow and altering diversity components (Agostinho et al., 1992; Vrijenhoek, 1998).

Steindachneridion melanodermatum is a species of catfish that migrates in its initial developmental phase and is endemic to the Iguaçu River basin (Brazil). Specimens from nature have been collected and kept in captivity for breeding at the Salto Segredo Experimental Station for Ichthyological Studies in the State of Paraná (Brazil). In the present study, random amplified polymorphic DNA (RAPD) (Williams et al., 1990) molecular markers were used in specimens from nature and specimens born in captivity in the genetic conservation and repopulation program developed by the Paraná Electric Company (COPEL) of the Nei Braga Hydroelectric Plant in Salto Segredo, Paraná. The aim of this study was to determine the degree of genetic variability in these specimens in order to generate information that may optimize their cultivation in captivity and better guide the conservation of the species, thereby providing assistance to the implementation of adequate management strategies for the *in situ* conservation of genetic variability.

MATERIAL AND METHODS

Steindachneridion melanodermatum specimens captured in the wild (N = 11) and others born in captivity (N = 19) were submitted to genetic analysis using RAPD markers. DNA extraction followed the protocol described by Sambrook and Russell (2001).

The data were included in a binary matrix depending on the presence or absence of fragments observed on the gel, assuming that alleles from different loci migrate to different positions on the gel and that each band represents a Mendelian locus of a dominant nature that is in Hardy-Weinberg equilibrium, with the recessive null allele not visible (Lynch and Milligan, 1994). The Ready-To-Go RAPD Analysis Bead kit (GE Healthcare) was used to identify the RAPD markers, which contains six 10-mer primers of arbitrary sequence (2.5 nmol each). The following primer sequences were used in the amplification: primer 1 (5' d[GGTGCGGGAA] 3'); primer 2 (5' d[GTTTCGCTCC] 3'); primer 3 (5' d[GTAGACCCGT] 3'); primer 4 (5' d[AAGAGCCCGT] 3'); primer 5 (5' d[AACGCGCAAC] 3'), and primer 6 (5' d[CCCGTCAGCA] 3'). Primer 2 was excluded from the analysis because it provided few markers, nearly all of which were monomorphic. The following reaction profile was used for all primers: one cycle at 95°C for 5 min and 45 cycles at 95°C for 1 min, 36°C for 1 min and 72°C for 2 min. Polymerase chain reactions (PCR) were carried out with 5 µL of each primer (25 pM), 2 µL template DNA (5 ng/µL) and 18 µL autoclaved ultra-pure water. All reactions were performed with control DNA provided by the kit, and the dilutions for the desired concentrations were carried out following manufacturer instructions. For each primer,

a second PCR was performed with randomly selected specimens to check for reproducibility of the electrophoretic pattern obtained. PCRs were repeated for individuals with band profiles that were not clear for inclusion in the statistical analysis or were excluded due to failing to maintain consistency in the band profile obtained. This was the case for wild specimens 1248, 1251 and 1255, which had flawed PCRs in the amplification of primers 1, 3, 5, and 6, although primer 4 was amplified well (Figure 1).

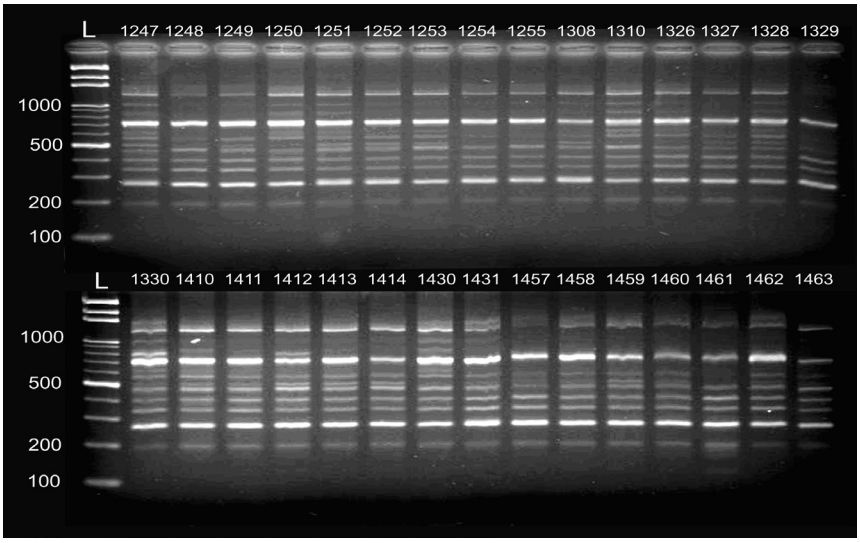

Figure 1. Band pattern obtained from RAPD markers for primer 4 in *Steindachneridion melanodermatum*. L = DNA ladder.

The PCR products were submitted to electrophoresis on a 3% agarose gel at 100 V (≈80 mA) for 4 h and 45 min. The gels were stained with 1% ethidium bromide (10 mg/mL). The molecular mass of each band was determined using a 100-bp marker (Biotec Ludwig) as the parameter. PCR was performed in a PTC-100 thermocycler (MJ Research).

The Numerical Taxonomy and Multivariate Analysis System (NTSYS-PC version 2.1) (Rohlf, 1998) was used to estimate the degree of intra-population genetic similarity, using the Jaccard coefficient (1901). Grouping analysis was performed using the unweighted pair group method with arithmetic average (UPGMA) (Sneath and Sokal, 1973). Values for heterozygosity, polymorphic loci percentage, Nei distance and identity (1978), structuring analysis and population differentiation exact test were determined using the TFPGA program (version 1.3) (Miller, 1997).

RESULTS

The five primers used in the present study produced 53 fragments on gels, 15.1% of which were in monomorphic bands and 84.9% were in polymorphic bands. Fragment size ranged from 200 to 2000 bp, and the number of bands ranged from one to 16. Genetic similarity ranged from 0.57 to 0.95. A study carried out by Ramella et al. (2006) with *S. scriptum* specimens from the Uruguai River found genetic similarity ranging from 0.0 to 1.00, indi-

cating a greater degree of diversity in the 13 specimens examined when compared to the 27 specimens examined in the present study. This seems to indicate a loss of genetic diversity in *S. melanodermatum*, although no other study has been carried out for this species as a comparative parameter for the data presented here.

The UPGMA-generated phenogram grouped the individuals in a fragmented manner, with the formation of three clades and variable genetic similarity values (Figure 2). Two distinct groupings were obtained for wild specimens, indicating that either there may be different genetically differentiated population stocks living in sympatry in nature or there may be more than one taxonomic unit. Likewise, the stocks kept as breeders and the specimens born in captivity also exhibited genetic differentiation from each other.

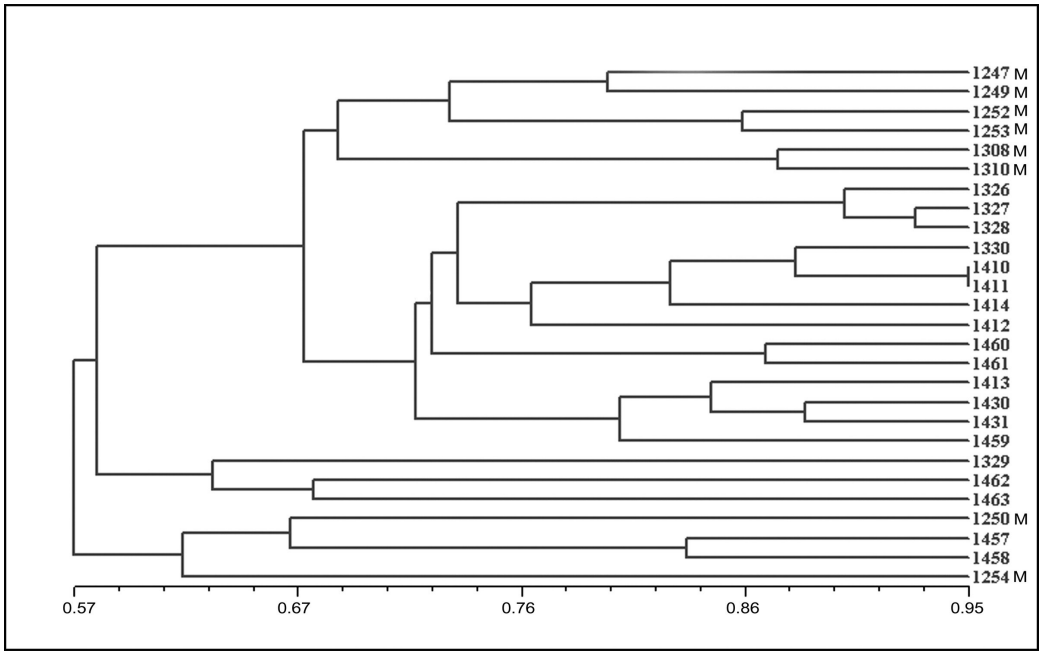

Figure 2. Phenogram obtained from Jaccard similarity and the UPGMA grouping method for *Steindachneridion melanodermatum* specimens. M = breeding specimens captured in nature.

In order to determine the most conclusive parameters, the stock was subdivided into sample 1 (N = 8 wild specimens) and sample 2 (N = 19 cultivated specimens). Heterozygosity and percentage of polymorphic loci values were calculated for both samples (Table 1).

Table 1. Heterozygosity and percentage of polymorphic loci values in *Steindachneridion melanodermatum* samples.

	Sample 1 Wild (N = 8)	Sample 2 Cultivated (N = 19)	Entire sample (N = 27)
Heterozygosity	0.23	0.26	0.31
% Polymorphic loci	62.26	66.03	79.24

The corrected Nei genetic distance and genetic identity (1978) values were 0.1096 and 0.8962, respectively. The population differentiation exact test provided a high chi-square value and highly significant P value (χ^2 = 148, P = 0.0041), with differences between the samples tested, even when analyzed together. This test was congruent with the theta population structuring index (θst), which was 0.2313, corresponding to a moderate genetic structuring value, according to the F-statistic (Wright, 1978).

DISCUSSION

Based on the results presented here, the total genetic variation of *S. melanodermatum* is not compromised, as determined by the percentage of polymorphic loci and total heterozygosity index (Table 1). Nonetheless, the range of exiting variation is considerably low in comparison to genetic similarity values obtained for *S. scriptum* (Ramella et al., 2006).

Although the *S. melanodermatum* captivity program at the COPEL Salto Segredo Experimental Station may be considered well established with regard to ecological variables such as nutrition and induced breeding (Ludwig et al., 2005), the lack of genetic data on the stock kept as breeders in captivity as well as the number of specimens used in breeding may give rise to future genetic drift, thereby reducing diversity in future generations. According to Wasko et al. (2004), one way to minimize the harm caused by low genetic variability in cultivated stocks is to genotype potential breeders and breed those with the greatest divergence between one another or back breed cultivated specimens with wild specimens. This strategy is effective when interbreeders make up part of the same genetic group. Determining the genotype of the entire broodstock is important in avoiding gene introgression in populations that are already structured or genetically differentiated.

The genetic monitoring of fish is an extremely useful tool for conservation and repopulation programs, but there are recent approaches that use genetic techniques for the assessment of wild and cultivated stocks of Neotropical species. Analyzing four stocks [one wild and three cultivated of *Brycon cephalus* of the CEPTA/IBAMA (Center for Studies, Research and Applied Technology/Brazilian Environmental Protection Agency) genetic conservation program], Wasko et al. (2004) found significant differences in the genetic composition of the stocks with regard to the percentage of polymorphic loci and genetic similarity index, which were significantly higher in the wild stock. In the present study, the low heterozygosity and percentage of polymorphic loci in the broodstock may not reflect the natural situation of wild populations, given the small number of specimens analyzed. However, if we consider this result as representative of the scenario encountered in nature, it may be explained by the limited dispersion of *S. melanodermatum* specimens due to a compromised natural habitat or due to typical behavioral characteristics of the species in its adult phase. However, it was not the objective of the present study to carry out a broad-based investigation of these parameters of this species in nature, but rather to randomly investigate the composition of the specimens used in induced breeding trials with regard to F_1 segregant generations.

The RAPD method is used in fish studies aimed at the genetic monitoring of population stocks (Wasko et al., 2004), the identification of species and broodstocks (Partis and Wells, 1996; Prioli et al., 2002; Ali et al., 2004), structuring analysis of natural populations (Wasko and Galetti Jr., 2002; Almeida et al., 2003; Hatanaka and Galetti Jr., 2003; Leuzzi et al., 2004; Matoso et al., 2004; Sofia et al., 2006) and genetic variability in populations or species not yet studied from the genetic standpoint (Ramella et al., 2006). In other groups, RAPD

markers have been combined with methodologies of a less arbitrary nature in order to generate better defined DNA patterns, i.e., DNA fingerprinting, for the purpose of molecular characterization (Aggarwal et al., 2008; Demir et al., 2010), for analysis of genetic diversity (Brahmane et al., 2008; Rabello et al., 2008) to provide practical information for selection of parentals in planning breeding strategies (Ahmad et al., 2010), and to determine phylogenetic relationships (Marouelli et al., 2010) besides population differentiation (Klinbunga et al., 2010) between wild and cultivated stocks (Sesli and Yegenoglu, 2010). Although the RAPD method does not provide accurate information regarding the specific genotype of the specimens analyzed or the dominant character of the marker, it is a fast, inexpensive tool that allows access to information on general genetic variability within and between populations, even when considering that the observed variation may be underestimated.

Considering the need for genetically guided management for *S. melanodermatum* stocks in captivity, the results presented here point to the need for continuity of the program to enable the conservation of the species through programmed breeding and the broadening of information regarding existing genetic variability in natural populations.

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