

Microsatellite variation and population genetic structure of a neotropical endangered Bryconinae species *Brycon insignis* Steindachner, 1877: implications for its conservation and sustainable management

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Piabanha (*Brycon insignis*) is a freshwater fish species from the drainages in Southeastern Brazil. During the 1950s, it was an important economic and food resource for local populations, but dramatic and continuous environmental degradation seriously jeopardized the *B. insignis* populations in the region. Microsatellite markers were used to assess the genetic structure of wild populations of *B. insignis* and compare the genetic variability and integrity of the wild populations with a captive population. Samples of DNA from 208 specimens from geographically isolated populations were analyzed. Population genetic structure was investigated using F_{ST} , R_{ST} estimates as well as AMOVA. All five *loci* used in this study were polymorphic with observed heterozygosity ranging from 0.77 (± 0.15) to 0.88 (± 0.07) in the wild population and 0.90 (± 0.09) in the captive population and the allelic richness average were 7.56 (± 0.27) and 5.80 (± 1.02), respectively. Overall genetic differences were significantly partitioned among populations ($F_{ST} = 0.072$, $p = 0.034$). Evidence of a genetic bottleneck was found in some of the wild populations, but especially in the captive population. The results showed that genetic variability still can be found in *B. insignis* populations which are currently structured possibly due to anthropic actions. The implications of these findings for the management and conservation of *B. insignis* populations are discussed.

Piabanha (*Brycon insignis*) é uma espécie de peixe de água doce oriunda de drenagens da região sudeste do Brasil. Durante os anos de 1950, esta espécie foi um importante recurso econômico para populações locais. Contudo, a intensa e contínua degradação ambiental afetou seriamente as populações de *B. insignis* na região. Marcadores microssatélites foram usados para avaliar a estrutura genética de populações selvagens de *B. insignis* e comparar a variabilidade genética e integridade das populações selvagens com uma população de cativo. Amostras de DNA de 208 espécimes de populações geograficamente isoladas foram analisadas. A estrutura populacional foi investigada usando-se estimadores de F_{ST} e R_{ST} bem como AMOVA. Todos os *loci* usados neste estudo foram polimórficos com heterozigosidades observadas variando de 0.77 (± 0.15) a 0.88 (± 0.07) em populações selvagens e 0.90 (± 0.09) na população de cativo e a riqueza alélica média foi de 7.56 (± 0.27) e 5.80 (± 1.02), respectivamente. A maioria das diferenças genéticas foi significativa entre populações ($F_{ST} = 0.072$, $p = 0.034$). Evidências de efeito gargalo foram observadas em algumas populações selvagens e especialmente também na população de cativo. Os resultados do presente estudo mostraram que as populações de *B. insignis* ainda apresentam variabilidade genética e que estas populações estão atualmente estruturadas geneticamente provavelmente devido a ações antrópicas. As implicações destes achados para o manejo e conservação das populações de *B. insignis* são discutidas.

Key words: Piabanha, Paraíba do Sul basin, Conservation, STR, Fish.

Introduction

Neotropical freshwater ecosystems support a very high fish diversity that has been estimated at over 6,000 species (Reis *et al.*, 2003). The Brazilian ichthyofauna has been regarded as being an invaluable asset in the global context of biodiversity, as well as an important food source for several

human populations through fisheries and aquaculture (Lowe-McConnell, 1984). Different *Brycon* species are important as food fish in almost all Brazilian drainages. They are present in commercial fisheries and even in sport fishing. Some of them have been used in the aquaculture industry (Bittencourt & Cox-Fernandes, 1990; Gomes *et al.*, 2000).

The Bryconinae subfamily includes 74 nominal species,

42 of which are valid for the genus *Brycon* (Lima, 2003). *Brycon* species are a widespread group that ranges from southern Mexico to the La Plata River in Argentina (Hiltsdorf *et al.*, 2008).

Some authors concur that different anthropogenic impacts have jeopardized not only species in general, but also genetically different populations within species (Lande, 1988; Allan & Flecker, 1993). The presence of several fish species in the Paraíba do Sul River and its tributaries has been jeopardized by industrial and agricultural occupation of the region over the last century. Pollution, river damming, sand extraction from the riverbed and floodplains, overfishing, and the introduction of alien species are regarded as the main causes of the loss in fish diversity and fisheries declines in the Paraíba do Sul River (Hiltsdorf & Petrere, 2002).

Brycon insignis Steindachner, 1877, commonly named piabanha, is present in drainages in Southeastern Brazil, mainly in tributary rivers of the Paraíba do Sul River Basin (Bizerril, 1999). Reproduction period ranges from December to February and induced spawning of piabanha is currently applied to produce fingerlings for farming and stocking (Andrade-Talmelli *et al.*, 2002).

Brycon insignis was an important fishery resource in the Paraíba do Sul River Basin. In 1951, commercial fisheries caught 24 tons/year of *B. insignis*, which was sold in the São Paulo area of the Paraíba do Sul basin; this represented the main living income for several artisanal fishermen (Machado & Abreu, 1952). The present situation of *B. insignis* populations is drastic. The wild populations in the State of São Paulo have become extinct and only one single stock has been maintained as a broodstock for restocking purposes since the beginning of the 1980's at a local hydroelectric power plant hatchery. Wild populations can scarcely be found. As a result, presently

B. insignis is listed on the Brazilian red list of threatened species as critically endangered (Hiltsdorf *et al.*, 2008).

Diverse molecular techniques have been used in studies of genetic diversity and conservation biology (O'Brien, 1994; Beaumont & Bruford, 1999). Microsatellite markers have proven to be useful indicators of population structure in a variety of eukaryotic taxa, including fishes (Tautz & Schlötterer, 1994; Jarne & Lagoda, 1996; Menezes *et al.*, 2008).

This study aimed: (i) to assess the genetic composition within and between samples of *B. insignis*, currently isolated by anthropic actions, through microsatellite polymorphism, (ii) to compare the genetic variability and integrity of the wild populations with a captive population, and (iii) to discuss how these findings can be used to provide genetic baseline for conservation programs of *B. insignis*.

Material and Methods

Sample collection and DNA extraction

Samples were collected between 2001 and 2005 after an initial survey to identify the occurrence of *B. insignis* in drainages in Southeastern Brazil. Locations of wild populations were identified by local fishermen, who indicated possible sites for *B. insignis* sampling.

Fin clips were obtained from 208 adult fish and the individuals were returned to the water without being killed. Sampling locations were as follows: Paraíba do Sul River (PSR), Imbé River (IMR), Muriaé River (MUR), Itabapoana River (ITR) and the São João River (SJR) (Fig. 1, Table 1). In addition, the *B. insignis* broodstock (PHC) kept in the power plant hatchery was sampled.

Total genomic DNA extraction was performed according

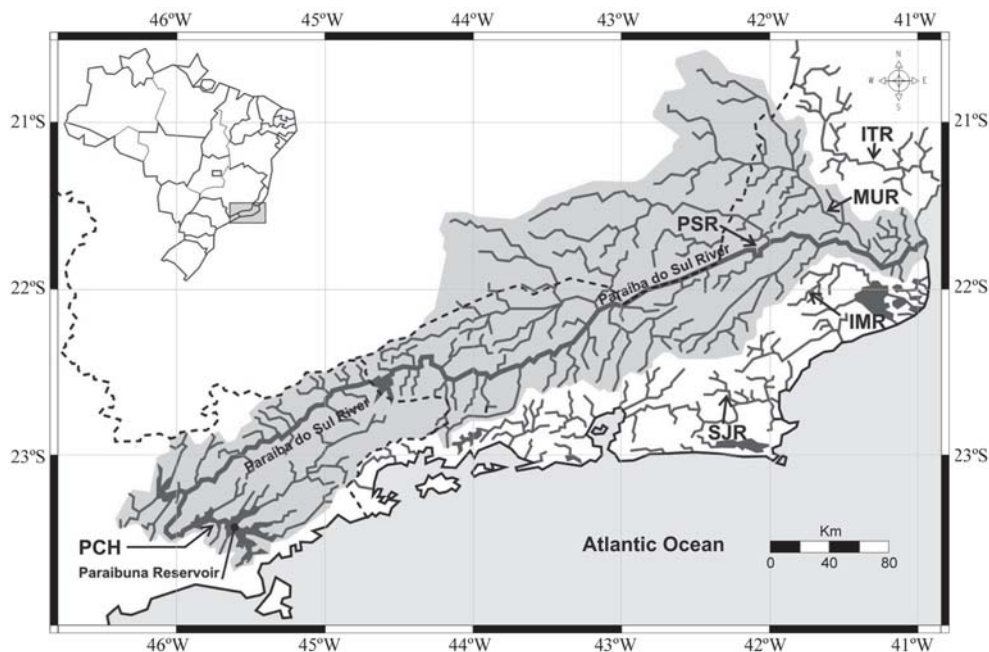


Fig. 1. Location of sampling sites of *Brycon insignis* in drainages in Southeastern Brazil. Power Company Hatchery (PCH), São João River (SJR), Paraíba do Sul River (PSR), Imbé River (IMR), Muriaé River (MUR) and Itabapoana River (ITR).

Table 1. Sampling sites and respective drainages, sampling years and number of samples.

Sampling site	Drainage	Sampling year	Sample size
Power Company Hatchery (PCH)	Paraíba do Sul	2001	40
Imbé River (IMR)	Paraíba do Sul	2003	40
Muriaé River (MUR)	Paraíba do Sul	2005	18
Paraíba do Sul River (PSR)	Paraíba do Sul	2001/2002	40
Itabapoana River (ITR)	Itabapoana	2002	40
São João River (SJR)	São João	2004/2005	30

to the methods of Taggart *et al.* (1992), except for the use of STE (0.1 M NaCl; 0.05 M Tris-HCl; 0.01 M EDTA) with a lower EDTA in the buffer concentration.

Microsatellite marker amplification and genotyping

Four microsatellite primers designed for *B. opalinus* (Barroso *et al.*, 2003) and three *B. cephalus* (Barroso, 2003) were tested in *B. insignis*. After optimizing PCR conditions, five dinucleotide motif primers (Table 2) were selected for the genetic analysis because they generated reproducible amplification within the size range expected and could be scored on a polyacrylamide gel.

The PCR reactions were performed in a 10 µl reaction volume containing 50 ng of the template DNA, 2 µM of each primer, 0.25 mM each of dNTPs, 0.5 U of *Taq* DNA polymerase, 1 µl 10X reaction buffer, and MgCl₂ concentrations as indicated in Table 2. For all *loci*, samples were subjected to an initial denaturation step at 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, at annealing temperature for 25 seconds, 72°C for 1 min, and a final extension at 72°C for 10 min (Table 2). Negative and positive controls were implemented to verify the consistency and reproducibility of the amplification products.

The PCR products were electrophoresed on 7.5% polyacrylamide gel at 6,000 W for 3 hours. The DNA fragments were revealed by standard silver staining techniques. Microsatellite allele sizing for every individual was estimated manually by two independent people comparing the samples to molecular weight standards (10 bp ladder; Invitrogen) using the “Alpha Index 6.5” program (AlphaImager™ - Alpha Innotech Corporation).

Analysis of genetic variability within and among populations

Genetic variability within populations was estimated by

calculating the mean number of alleles per *locus* (*A*), allelic richness per *locus* per sample (*A_r*), considering the alleles per *locus* independent of sample size (El Mousadik & Petit, 1996), gene diversity per *locus* and sample using an unbiased estimator (*h_s*) (Nei, 1987). *F_{IS}* values per *locus* per sampling site were calculated using FSTAT version 2.9.3.2 (Goudet, 2002). Deviations from Hardy-Weinberg expectations (HWE) were performed by an exact test using the Markov-Chain randomization approach (Guo & Thompson, 1992) implemented by Arlequin, version 3.1 (Excoffier *et al.*, 2005). The software Micro-Checker (Van Oosterhout *et al.*, 2004) was employed to infer the causes of possible departure from HWE produced for null alleles (mutation in flanking region), misscoring (stuttering), and large-allele dropout (short allele dominance).

Statistical comparisons were carried out to verify differences of *A_r*, *h_s* and *H_o* between the sites using a one-way analysis of variance (ANOVA) followed by Student Newman Keuls test (*p* > 0.05) implemented by the software Sigma Stat for Windows version 2.0 (Jandel Corporation).

The overall population subdivision was estimated using Wright’s *F* statistic (Weir & Cockerham, 1984) grounded in the assumptions of the infinite allele model by the HIERFSTAT package (Goudet, 2005). *R_{ST}* was calculated taking into account the stepwise mutation model (SMM) (Slatkin, 1995) by RST CALC (Goodman, 1997). An exact test for population differentiation was performed by testing the hypothesis of random distribution of the individuals between pairs of populations (Goudet *et al.*, 1996). A sequential Bonferroni adjustment was implemented to verify the significance levels for all the above simultaneous comparisons (Rice, 1989). Results are presented as mean ± *SE*.

Additionally, a Φ_{ST} (Weir & Cockerham, 1984) was calculated by the analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) with 10,000 permutations to test the significance of hierarchical population analysis using ARLEQUIN, version 3.1 (Excoffier *et al.*, 2005).

The graphical representation based on genetic distance data (Nei, 1972) was constructed by UPGMA algorithm (Felsenstein, 1985), implemented in TFGA “Tools for Population Genetic Analyses”, version 1.3 (Miller, 1997).

BOTTLENECK 1.2.02 (Cornuet & Luikart, 1996) test results were used for detecting a recent reduction in the effective

Table 2. Primer sequence, microsatellite motif, conditions for amplification and accession number on Genebank used for microsatellite genotyping of *Brycon insignis*.

<i>Locus</i>	Primer sequence (5'-3')	Motif	Annealing temp. (°C)	[MgCl ₂] (mM)	GeneBank accession number
BoM1	F: CCATCTCTACTTTTTGGTTCC R: TGCCCAGATACAGCCC	(CA)	60	2.5	AF513629
BoM2	F: CTGGGCAGCGGAAGAG R: CCCACATCTCTCCCCTTCG	(CA)	60	2.0	AF513630
Bom13	F: CATTTCCTCAGTCCTTTTCAGC R: CCCACTTAGGGTCGCAC	(CA)	62	3.0	AF513621
BC48-6	F: GGAGGACAGTGAGTGGACG R: GTTTGCTTGGACATCTGC	(CA)	62	3.0	AF513622
BC48-10	F: GTTCCACCTATTGCTGACAC R: GAAAATACTACTCTACGGGGAC	(CA)	65	2.0	AF513628

population size, to assess each population according to differences in heterozygosity excess or deficiency and to differences in allele number in a given population. Heterozygosity excess was measured under the two-phase mutation model (TPM, with 90% SMMs, 5,000 iterations), which is the intermediate model between the IAM and the SMM and is recommended for microsatellite *loci* evolution calculations (Di Rienzo *et al.*, 1994). The Wilcoxon test option was chosen to evaluate the significance level of heterozygosity excess or deficiency. This test provides relatively high statistical power and can be applied to few *loci* with low sample sizes.

Results

Efficiency of cross-amplification in microsatellite primers

This is the first study to examine genetic variability in *B. insignis*. No information on the genomic sequence of this species is available for the purpose of designing specific primer pairs for microsatellite *loci*. The use of microsatellite *loci* characterized from other *Brycon* species has proven to be suitable for microsatellite amplification of *B. insignis*. Similar conclusions were reached by Abila *et al.* (2004) for cichlid fish, and Salgueiro *et al.* (2003) for cyprinid fish. All five primer pairs used in the present study were polymorphic and amplified consistent and reproducible microsatellite alleles with the allele size ranging from 72 (BC48-10) to 194 bp (B0M13) (Table 3).

Allelic diversity and Hardy-Weinberg equilibrium

Sixty-one alleles were observed and the average number of alleles per *locus* per sample location ranged from 5.8 (± 1.02) in PHC to 9.4 (± 1.40) in PSR. Nei's gene diversity (*h_s*) was high among wild populations and also in the hatchery broodstock. It was found that this measurement of genetic variation ranged from 0.70 (± 0.11) to 0.83 (± 0.05) (Table 3). Allelic richness (*Ar*) was lower in the hatchery fish when compared to the other populations although ANOVA analysis did not show significant differences among populations for *Ar*, *h_s* and *H_o* parameters (Bonferroni adjusted alpha).

The average of the observed and expected heterozygosities per location per sample ranged from 0.77 (± 0.15) to 0.90 (± 0.90), and from 0.72 (± 0.04) to 0.83 (± 0.10), respectively (Table 3). Significant departures from HWE were verified by genotype specific testing in most of the genotypes in all population samples of *B. insignis*, with significant heterozygote excess for *loci* BoM 1, BoM 13 and BC48-6 and heterozygote deficit for *loci* BoM2 for all populations and BC48-10 for wild populations (Table 3). All HWE population comparisons remained significant after sequential Bonferroni correction ($p < 0.001$), except *loci* Bc48-10 for IMR, BoM2 for MUR and BoM2 for SJR that showed an excess of heterozygosity in most *loci* and populations (Negative F_{IS} values) (Table 3). *Loci* BoM2 and BC48-10 showed deficit of heterozygosity across the wild population, but only in *locus* BoM2 the presence of null allele was detected through the MICRO-CHECKER software analysis.

Genetic bottleneck

Paraíba do Sul (PSR), Itabapoana (ITR) and Imbé (IMR) Rivers wild populations and the captive stock (PHC) showed a significant departure ($p < 0.05$) from drift-mutation equilibrium under the TPM (90-10% SMM-IAM), determined with the Wilcoxon test. Populations from the São João River (SJR) and Muriaé (MUR) Rivers did not demonstrate significant heterozygosity excess ($p > 0.05$). Despite Itabapoana (ITR) and Imbé (IMR) populations having shown significant heterozygosity excess under the TPM model, under the strict SMM model, these populations did not demonstrate a significant probability ($p = 0.11$) of heterozygosity excess. Therefore, it is not certain that the ITR and IMR populations went through a bottleneck.

Intrapopulation diversity and genetic structuring

Pairwise R_{ST} values (Table 4) showed significant differentiation between all population pairs. Lower levels of genetic structuring were detected between the IMR and PSR populations, as well as between those two and the ITR population. Moderate genetic differentiation was observed among the MUR and the PSR, IMR, and ITR populations. In average, the higher values of pairwise genetic differentiation were found when SJR and PHC were compared to all other sampling sites.

For the AMOVA analysis, considering all populations as a group, we found that 7.37% of the total genetic variance was due to differences among populations ($\Phi_{ST} = 0.07$, $p < 0.001$) (Table 5). If hierarchical AMOVA analysis were implemented with the PHC as one group, the SJR as another, and IMR, ITR, MUR, and PSR as third group, there would still be a significant separation between groups ($\Phi_{CT} = 0.068$, $p < 0.05$). This estimate corroborates the effect of location at the level of population differentiation estimated by the HIERFSTAT package ($F_{ST} = 0.072$, $p = 0.034$).

Discussion

Genetic diversity in wild populations

Despite the large biodiversity of Neotropical freshwater fishes, many of them important for fishery and aquaculture, there are a few studies of population genetics using microsatellites (Oliveira *et al.*, 2009). The findings herein revealed genetic differences between *B. insignis* populations, as was expected because the currently physical isolation among them.

The present study demonstrates that *Loci* BoM2 exhibited heterozygosity deficiency throughout the wild populations surveyed, which suggests that the presence of null alleles is the cause of the heterozygosity deficiency at this *locus*. The excess of homozygotes observed in the *loci* BC48-10 in the wild populations is possibly attributable to the Wahlund effect since no null allele was detected for this *locus*.

The presence of heterozygosity excess in the other *loci* suggests a possible reduction in the size of the populations of *B. insignis* in this study. Considering a significant departure

Table 3. Summary statistic for genetic diversity at five microsatellite *loci* of *Brycon insignis* in six sampling sites: number of alleles (A), allelic richness (A_R); Nei's genetic diversity (h_s), observed (H_o) and expected heterozygosity (H_e); and inbreeding coefficient (F_{IS}). ^{NS}- Not significant and ** - Highly significant ($p < 0.01$) after Bonferroni adjustment (nominal $\alpha = 0.05$). Power Company Hatchery (PCH), São João River (SJR), Paraíba do Sul River (PSR), Imbé River (IMR), Muriaé River (MUR) and Itabapoana River (ITR).

Locality	Locus	Allele size range (bp)	A	A_R	h_s	H_o	H_e	F_{IS}
PCH	BoM1	146-162	8	7.40	0.85	1.00**	0.86	-0.17
	BoM2	118-134	8	7.68	0.83	0.55**	0.84	0.34
	BoM13	156-176	6	5.10	0.68	1.00**	0.68	-0.46
	BC48-6	113-135	3	3.00	0.62	1.00**	0.70	-0.43
	BC48-10	74-90	4	3.45	0.62	0.97**	0.63	-0.56
	All <i>loci</i>		5.80 (1.02)	5.32 (0.97)	0.72 (0.05)	0.90 (0.09)	0.74 (0.04)	
IMR	BoM1	144-164	10	8.63	0.86	1.00**	0.86	-0.16
	BoM2	118-136	9	8.21	0.85	0.59**	0.84	0.30
	BoM13	154-186	11	9.34	0.87	1.00**	0.87	-0.14
	BC48-6	113-147	4	3.45	0.66	0.89**	0.66	-0.36
	BC48-10	72-94	12	10.20	0.86	0.77 ^{NS}	0.86	0.10
	All <i>loci</i>		9.20 (1.93)	7.97 (1.18)	0.82 (0.04)	0.85 (0.08)	0.82 (0.04)	
MUR	BoM1	146-164	6	6.00	0.80	1.00**	0.80	-0.25
	BoM2	120-136	6	6.00	0.64	0.50 ^{NS}	0.64	0.22
	BoM13	158-184	9	9.00	0.88	1.00**	0.88	-0.13
	BC48-6	113-141	5	5.00	0.69	1.00**	0.70	-0.44
	BC48-10	74-94	10	10.0	0.88	0.83**	0.88	0.06
	All <i>loci</i>		7.20 (0.97)	7.20 (0.97)	0.78 (0.05)	0.86 (0.09)	0.78 (0.05)	
PSR	BoM1	140-164	12	11.09	0.90	1.00**	0.90	-0.10
	BoM2	118-140	10	8.22	0.84	0.63**	0.85	0.29
	BoM13	158-194	11	9.60	0.88	1.00**	0.88	-0.13
	BC48-6	113-147	4	3.70	0.64	0.95**	0.65	-0.46
	BC48-10	72-92	10	9.13	0.87	0.82**	0.87	0.06
	All <i>loci</i>		9.40 (1.4)	8.34 (1.25)	0.83 (0.04)	0.88 (0.07)	0.83 (0.04)	
ITR	BoM1	144-164	11	10.28	0.89	1.00**	0.89	-0.11
	BoM2	118-138	11	9.60	0.86	0.60**	0.86	0.30
	BoM13	156-184	8	7.28	0.78	1.00**	0.78	-0.28
	BC48-6	113-135	3	3.00	0.63	1.00**	0.63	-0.59
	BC48-10	74-94	9	7.33	0.83	0.75**	0.83	0.10
	All <i>loci</i>		8.40 (1.47)	7.50 (1.76)	0.80 (0.05)	0.87 (0.08)	0.80 (0.04)	
SJR	BoM1	140-164	12	10.69	0.89	1.00**	0.90	-0.11
	BoM2	124-136	6	4.82	0.27	0.23 ^{NS}	0.27	0.16
	BoM13	160-180	8	7.79	0.85	1.00**	0.86	-0.17
	BC48-6	113-135	4	3.99	0.70	1.00**	0.70	-0.43
	BC48-10	72-94	8	7.02	0.81	0.63**	0.81	0.22
	All <i>loci</i>		7.60 (1.32)	6.80 (1.18)	0.70 (0.11)	0.77 (0.15)	0.71 (0.11)	

from mutation-drift equilibrium based on the differences in standardized test outcomes under the assumption that all *loci* fit the TPM mutation model, a possible bottleneck was detected in the IMR, ITR and PSR populations.

In fact, *B. insignis* wild populations are jeopardized by continuous anthropogenic habitat alteration. The species have vanished from the southern region of the Paraíba do Sul basin and just a few populations remain in tributaries in the northernmost area and nearby drainages of the Paraíba do Sul basin. These few *B. insignis* wild populations are isolated due to physical and environmental obstacles such as hydroelectric dams and industrial and urban effluents. Also, the difficulty in sampling specimens for the present study together with the lack of *B. insignis* in the commercial fisheries may be an indication of overall population decline. These results reinforce the listing of *B. insignis* on the Official List of Endangered Fish Species issued by the Brazilian government.

The overall F_{ST} , R_{ST} and Φ_{ST} estimates among wild

populations indicated that most of the *B. insignis* populations were significantly differentiated from each other. Pairwise genetic difference evaluations showed that SJR is the most genetically distinct population. SJR is a geographically isolated population located in a drainage basin near the Atlantic Ocean (Fig. 1). The presence of *B. insignis* species (verified by *16S* mitochondrial DNA sequencing, data not shown) was an interesting finding, since there are few records of *B. insignis* occurrence outside the Paraíba do Sul basin. Furthermore, the genetic differences found between SJR and the other *B. insignis* populations and its geographic isolation make SJR a unique genetic pool that should be preserved as a MU - Management Unit - (Moritz, 1994).

According to Wright (1978) F_{ST} or its analogous R_{ST} estimates below 0.05 indicates little genetic differentiation; an estimate between 0.05 and 0.15, moderate differentiation; an estimate between 0.15 and 0.25, great differentiation; and estimates above 0.25, very great genetic differentiation.

Table 4. Pairwise R_{ST} statistics (Slatkin, 1995) between *Brycon insignis* populations based on five microsatellite loci. All pairwise $p < 0.01$. Power Company Hatchery (PCH), São João River (SJR), Paraíba do Sul River (PSR), Imbé River (IMR), Muriaé River (MUR) and Itabapoana River (ITR).

Populations	PCH	IMR	MUR	PSR	ITR	SJR
PCH	-					
IMR	0.096	-				
MUR	0.221	0.108	-			
PSR	0.182	0.038	0.174	-		
ITR	0.092	0.027	0.078	0.097	-	
SJR	0.156	0.050	0.119	0.108	0.033	-

Table 5. Analysis of Molecular Variance (AMOVA) among populations of *Brycon insignis* (based in 1023 permutations). Structure tested: Group 1 (PCH); Group 2 (SFR); Group 3 (IMR, ITR, MUR and PSR).

Source of variation	Variance components	Percentage of variation	Φ -statistics	p-value
Among groups	0.1478	6.86	$\Phi_{CT} = 0.0686$	< 0.05
Among populations within groups	0.0645	3.00	$\Phi_{CS} = 0.03216$	< 0.001
Among individuals within populations	-0.1930	-8.96	$\Phi_{IS} = -0.0994$	> 0.05
Within individuals	2.1346	99.10	$\Phi_{IT} = 0.00895$	> 0.05

However it is important to point out that even low F_{ST} or R_{ST} estimates (< 0.05) may reflect an important genetic differentiation between populations (Balloux & Lugon-Moulin, 2002).

The populations located north of the Paraíba do Sul basin showed low to moderate levels of genetic structuring according to R_{ST} pairwise estimates (Table 4). IMR, PSR, and ITR were genetically more similar to each other, whereas MUR showed moderate genetic differences. ITR is located in the Itabapoana river basin without contact with Paraíba do Sul basin and the other populations IMR, PSR and MUR are separated in different levels by six hydroelectric power plant dams. Despite the current physical isolation, this low to moderate genetic differences between the IMR, PSR, ITR and MUR may still reflecting the past connection between the population from this rivers.

The same genetic structuring pattern was also observed in two other *Brycon* species: *B. hillari* (Valenciennes) (Sanches & Galetti Jr., 2007) and *B. opalinus* (Cuvier) (Barroso *et al.*, 2005).

UPGMA dendrogram (Fig. 2) based on Nei's genetic distance (1978) showed a pattern of population division that corroborates the R_{ST} pairwise population analysis, where the lowest distances were verified among IMR, PSR, ITR sites.

Genetic diversity in the captive broodstock (PCH)

The restocking program is one of the legal mitigation measures used by hydroelectric power companies in Brazil to reduce the impacts on the ichthyofauna caused by river impoundments. The *B. insignis* restocking started in the

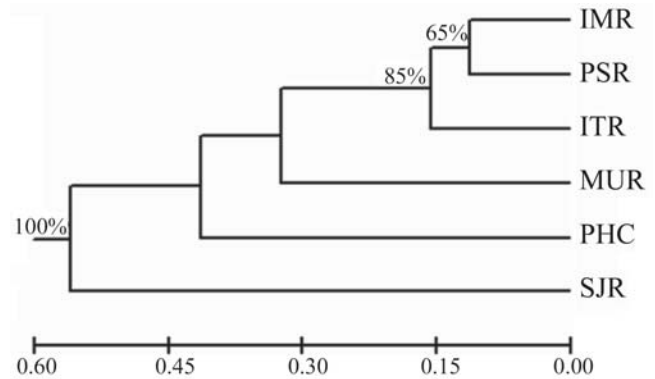


Fig. 2. UPGMA clustering of the Nei's genetic distance (1972) of the *Brycon insignis* sampling locations based on six microsatellite loci. Bootstrap values above 50% are shown above branches indicating percentage support in 5000 permutations. Power Company Hatchery (PCH), São João River (SJR), Paraíba do Sul River (PSR), Imbé River (IMR), Muriaé River (MUR) and Itabapoana River (ITR).

1980's. This species was selected because of its vulnerability and extremely reduced number of wild individuals in rivers located near hydroelectric reservoirs.

The results of the bottleneck analysis showed that the PCH broodstock went through some level of reduction during stock the formation. As a result, the heterozygosity values found in the hatchery broodstock sample were high and allelic richness was low (Table 3).

Little information about the formation of the PCH broodstock is available. A reduced effective population size during broodstock creation in breeding programs generally is the first problem found in hatcheries responsible for reintroductions. This, combined with poor breeding management, is the cause of the loss of genetic variability in captive stocks.

The R_{ST} values show significant differences when PCH is compared to other populations, which may have been caused by the bottleneck effect during the broodstock formation or may even be attributable to the fact that the individuals originally caught for the PCH breeding program in the early 1980's possibly originated from a distinct genetic population. Consequently, the broodstock kept at the hatchery has likely been maintaining the genetic diversity formerly present in some rivers, which currently no longer exist in natural populations of *B. insignis*.

Implications for conservation and management of *Brycon insignis*

There is no information available about the biological aspects of *B. insignis* migration pattern. Studies of *Brycon orbignyanus* (Valenciennes) showed that this species is regarded as a short-distance migrant, *i.e.*, fish that migrate less than 100 km (Agostinho *et al.*, 2003). If the migratory movement of *B. insignis* follows the same short-distance pattern, as found in *B. orbignyanus*, discrete populations

should be more genetically different from each other.

The depletion of *B. insignis* populations has been a continuous process attributable chiefly to habitat degradation of the main rivers of the Paraíba do Sul basin. For instance, the Piabanha River was named Piabanha due to the presence of *B. insignis* in its waters. Although the deterioration of its water quality during recent years caused the extinction of piabanha, the river still holds the *B. insignis* common name (Figueiredo & Rosas, 2003).

The present results showed that *B. insignis* populations are genetically different from each other, showing from the moderate to high level of genetic structure. Also, a clear bottleneck has been detected in the captive stock, possibly occurred during the broodstock formation. As a result, a 28-57% reduction in allelic richness was found when compared to wild population. Furthermore, PHC is genetically distinct from other wild populations and despite the low level of variability of this stock it is an important genetic resource of the species.

In the present situation, the recovery of *B. insignis* populations depends on two main implementation measures. The first measure is to conserve or even reverse habitat degradation of the rivers and riparian environments, where the species occurs so that natural recovery of wild populations can take place, as is the case of São João River (SJR). The second, is to promote stock enhancement into local areas where *B. insignis* populations no longer exist, as well as enhancement of populations in rivers where populations are extremely reduced. To implement such a measure, the genetic divergence still found among different populations of *B. insignis* must be taken into account establish the conservation strategies. This genetic variability can be maintained by keeping these populations in captivity under appropriate breeding management aimed at reducing the risks of genetic drift, inbreeding, and to minimizing the bottleneck effect present in the PHC (Duchesne & Bernatchez, 2002).

Since the Brazilian legislation for aquatic environmental protection covers a set of measures that consider hatchery fish release and fish-passage facilities as methods for mitigating the impacts of river damming on fish, the knowledge of genetic differences among populations assessed in the present study may serve as the initial guidelines for establishing an adequate number of unrelated and noninbred founders for the future successful reintroduction programs of *B. insignis*.

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