

Genetic differentiation among populations of *Pseudoplatystoma corruscans* (Agassiz, 1829) (Osteichthyes, Pimelodidae) isolated by the Guaíra Falls in the Paraná River

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ABSTRACT. Until 1982, the Guaíra Falls, also named the Seven Falls, constituted a barrier to the dispersion of migratory fish in the Paraná River. The objective of this work was to verify if populations of *Pseudoplatystoma corruscans* (Osteichthyes, Pimelodidae) were isolated by the Guaíra Falls. Samples from the Upper Paraná River floodplain (PL), the Itaipu reservoir (IT), and downstream Yacyretá reservoir (YA) were compared by RAPD markers. Lynch's F_{ST} was significant between PL and IT (0.090), and PL and YA (0.112). Estimated gene flow (Nm) varied from 2.0 between PL and YA to 8.1 between IT and YA. Nei's genetic distance varied from $D = 0.0638$ between PL and YA to $D = 0.0174$ between IT and YA. These results indicate the existence of genetic differentiation and that, possibly, the Guaíra Falls isolated the populations reproductively. They also suggest the possibility of different spawning areas, partially avoiding the genetic homogenization of the IT and PL populations.

Key words: *Pseudoplatystoma corruscans*, pintado fish, RAPD, genetic differentiation, the Guaíra Falls, the Seven Falls.

RESUMO. Diferenciação genética entre populações de *Pseudoplatystoma corruscans* (Agassiz, 1829) (Osteichthyes, Pimelodidae) isoladas pelos saltos de Guaíra do rio Paraná. Os saltos de Guaíra, também denominados Sete Quedas, constituíam até 1982 uma barreira para a dispersão de peixes migradores. Este trabalho teve por objetivo verificar se populações de *Pseudoplatystoma corruscans* (Osteichthyes, Pimelodidae) eram isoladas pelos saltos de Guaíra. Amostras provenientes da planície de inundação do alto rio Paraná (PL), do reservatório Itaipu (IT) e de jusante de Yacyretá (YA), foram comparadas por RAPD. O F_{ST} de Lynch foi significativo entre PL e IT (0,090) e PL e YA (0,112). O fluxo gênico estimado (Nm) variou de 2,0 entre PL e YA a 8,1 entre IT e YA, e a distância genética de Nei de $D = 0,0638$ entre PL e YA a $D = 0,0174$ entre IT e YA. Estes resultados indicam existência de diferenciação genética e que, possivelmente, Sete Quedas isolavam reprodutivamente as populações. Sugerem, também, a possibilidade de áreas diferentes de desova, impedindo parcialmente a homogeneização genética das populações IT e PL.

Palavras-chave: *Pseudoplatystoma corruscans*, pintado, RAPD, diferenciação genética, saltos de Sete Quedas, saltos de Guaíra.

Introduction

Until the impounding of Itaipu hydroelectric reservoir in 1982, the Guaíra Falls, also named the Seven Falls, had been considered a geographic barrier for the dispersion of migratory fishes in the Paraná River. The Guaíra Falls separated two distinct ichthyofaunistic provinces - the Upper

Paraná and the Parano-Platense (Bonetto, 1986). This barrier was relocated 150 km downstream after the reservoir impounding. The area previously located between the Guaíra Falls and the Itaipu dam became continuous with Upper Paraná River. As a consequence, at least 17 species that inhabited the Parano-Platense province, downstream Guaíra Falls, colonized the Upper Paraná (Agostinho *et al.*, 1994).

It is possible that Guaíra Falls did not represent an absolutely uncrossable barrier to fish migration. Agostinho *et al.* (1997) considered that in periods of exceptional floods some species would be able to cross it. Agile species such as *Prochilodus lineatus* (curimba), *Salminus maxillosus* (dourado), and *Leporinus elongatus* (piapara) perhaps could cross it in situations in which the difference in level decreased markedly. However, even on these occasions, it would be unlikely that species of Siluriformes, such as *Pseudoplatystoma corruscans*, could climb the Guaíra Falls. The difficulty of Siluriformes in crossing barriers had been observed by Godinho *et al.* (1991), on a ladder located at the dam of Salto Morais hydroelectric power station, the Tijuco River, the Paraná River basins. Such difficulty in crossing barriers is a characteristic of *P. corruscans* also, as observed by Fernandez (2000). Even if Guaíra Falls were an impediment to the climb of Siluriformes, it could not be discarded the possibility of unidirectional migration and mixture of populations through the descent of adults, eggs and larvae at these Falls.

The species *P. corruscans*, object of this study, popularly known as "pintado" or "surubim", is a migratory species of great commercial interest, widely distributed in the Paraná and São Francisco River basins (Marques, 1993; Godinho *et al.*, 1997). Given the circumstances created by the Itaipu dam, populations of this species in the Paraná River have been subjected to environmental changes that are able to cause significant gene flow alterations. The existence of geographic barriers isolates fish populations, causing intraspecific polymorphisms and genetic divergences between populations over time. The alteration or removal of these barriers could initiate or intensify the gene flow between populations. Environmental changes such as these of the Paraná River allow analyses of genetic variability and inference on gene flow and genetic divergence between populations.

A quick and efficient method for conducting genetic analysis of population studies is RAPD (Random Amplified Polymorphic DNA), proposed by Williams *et al.* (1990). Many reports have confirmed the efficiency of this methodology in the detection of genetic variation in species and populations of diverse organisms, including fish (Bielawski and Pumo, 1997; Caccone *et al.*, 1997; Almeida, 1998; Dergan *et al.*, 1998; Kuusipalo, 1999; Liu *et al.*, 1999; Chiari and Sodr , 2001; Prioli, 2001).

In this paper, the genetic differentiation of *P. corruscans* populations of the Paraná River, from the

Parano-Platense and the Upper Paraná provinces, previously separated by the Guaíra Falls, was evaluated by the RAPD technique.

Material and methods

Collection and processing of the samples

P. corruscans populations from three regions of the Paraná River, which had been influenced by the Guaíra Falls before the closure of the dam at the Itaipu hydroelectric power station, were studied. The population of floodplain region, in Porto Rico, state of Paraná, Brazil, located approximately 200 km upstream the Guaíra Falls, was sampled as representative of the Upper Paraná province, and named PL. The population downstream the Yacyret  dam-Paraguay/Argentina, named YA, was sampled as a representative of the Parano-Platense province. Samples were collected also from the upper portion of the Itaipu reservoir, representing the area of the Parano-Platense province that starts continuity with the Upper Paraná, and they were named IT. The location of the sampling sites is shown in Figure 1.

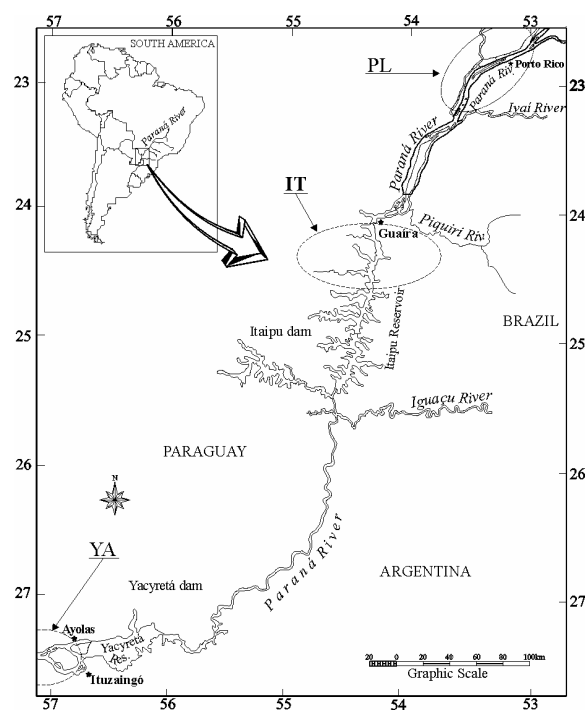


Figure 1. Location of the sampling areas of *P. corruscans* in the Paraná River: Upper Paraná River floodplain (PL), upper portion of the Itaipu hydroelectric power station reservoir (IT), downstream from the dam at the Yacyret  hydroelectric power station (YA)

Fragments of adipose fin or muscle tissue of 12 specimens from each population were sampled between March 1998 and May 2000. Eight of the twelve samples from the Itaipu reservoir were

muscle tissue. Samples from the two other populations consisted of only adipose fins. The tissue samples originating from the floodplain were obtained directly from live specimens. In the other localities, they were obtained in local fish shops or directly from fishermen. Samples were preserved in commercial ethanol (97%) and stored at -20 °C.

The methodology proposed by Whitmore *et al.* (1992), with few modifications, was used to extract total genomic DNA. Tissue samples of approximately 30 mg were incubated at 42 °C in microcentrifuge tubes containing 500 µL of extraction buffer (100 mM Tris-HCl pH 8.0, 10 mM EDTA, 0.1% SDS, 50 mM dithiothreitol, and 50 µg proteinase K) for approximately 24 hours. DNA was purified with one extraction of phenol/Tris pH 8.0 and two of chloroform/isoamyl alcohol (24:1), precipitated with 100% ethanol, and resuspended in 30 µL TE buffer 1/10 (10 mM Tris pH 8.0; 1 mM EDTA) + RNase (20 µg/mL). The suspension was then incubated at 37 °C for 1 hour. Quantity of DNA of each sample was estimated by comparison with a known concentration of λ DNA in 0.8% agarose gel electrophoresis in TBE buffer (89 mM Tris-base, 89 mM boric acid, 2 mM EDTA pH 8.0). All samples were diluted to 5 ng/µL, and quantified again in order to reduce experimental error.

DNA samples of the three populations were compared through the RAPD technique. PCR amplification was done in a reaction mixture with a total volume of 13 µL, containing 10 ng DNA, 0.46 µM of each primer, 1 U *Taq*-DNA polymerase (Gibco BRL), 200 µM of each dNTP, 2 mM MgCl₂, and buffer (20 mM Tris-HCl pH 8.0, 50 mM KCl). Conditions for PCR amplifications were based on Almeida (1998), and they were performed in a Peltier thermal cycler (PTC-100HB-60, MJ Research Inc.), programmed for an initial stage of 4 min. at 92 °C, followed by 40 cycles of 1 min. at 92 °C, 1 min. 30 sec. at 40 °C, and 2 min. at 72 °C, followed by a final stage of 5 min. at 72 °C. Primers used in this study were selected from OPA, OPX and OPW kits (Operon Technologies, Inc., Alameda, California, USA). For the selection of primers, the DNA of one individual was used for the amplification of all the primers of the three kits used. After the amplification, the oligonucleotides that produced a large quantity of sharp bands were chosen.

PCR amplified samples were applied in a 1.4% agarose gel for electrophoresis in TBE buffer (0.09 M Tris-Borate, EDTA 1 mM), stained with ethidium bromide (0.02%). The gels were submitted

to a 3 V cm⁻¹ electric field. After a running of approximately 10 cm, they were visualized under UV radiation, and photographed for later analysis. Sizes of the amplified DNA fragments, in base pairs, were estimated visually, in agarose gel, by comparison with a standard (Ladder 100 bp, Gibco BRL).

Data analysis

The comparisons began with the determination of the presence (1) or absence (0) of RAPD fragments in each individual. Polymorphic loci at a 5% level were used for the analyses. Lynch and Milligan's (1994) F_{ST} statistic, analogous to Wright's F_{ST} statistic, was used to calculate gene flow (Nm) between populations. Chi-square analyses (χ^2) were used to test the F_{ST} significance (Workman and Niswander, 1970). Genetic distance D was calculated according to the unbiased distance of Nei (1978) with correction of gene frequencies using Lynch and Milligan's (1994) method. Genetic distances and gene flow were calculated with RAPDdist and RAPDF_{ST} software programs (Black, 1995).

Results

Sixty primers from OPA, OPX and OPW kits were tested. From these, nineteen were selected based on the number and the intensity of the bands they produced. Differences in the quality of the amplified fragments among samples collected from live specimens and fish shops were not observed. As shown in Table 1, the number of fragments produced by each primer varied from 5 to 14 and the size of the fragments varied from 400 to 2540 bp. A total of six primers, out of the nineteen selected ones, amplified only monomorphic bands (32 bands), and the remaining thirteen primers amplified 120 loci, totalling 152. From these, 41 bands (27%) were considered polymorphic at a 5% level.

F_{ST} values, using Lynch and Milligan's (1994) statistic, varied from 0.030 to 0.112 (Table 2). Through the χ^2 test, F_{ST} was significant between the PL and YA populations and between the PL and IT populations, and not significant between IT and YA. The gene flow was also high between the IT and YA populations, with average number of migrants $Nm = 8.1$. The genetic distance, as shown in Table 3, was smaller between IT and YA ($D = 0.0174$) than between PL and YA ($D = 0.0638$).

Table 1. RAPD primers selected from A, X and W Operon kits for the comparison of *P. corruscans* populations in the Paraná River, number of polymorphic loci and size, in base pairs, of the amplified fragments

Primers	Nucleotide Sequence (5' → 3')	No. of loci detected	No. of polymorphic loci at 5%	Fragment size (bp)
OPA-01	CAGGCCCTTC	13	2	620 - 2090
OPA-10	GTGATCGCAG	07	3	430 - 1220
OPA-11	CAATCGCCGT	07	4	550 - 1700
OPA-12*	TCGGCGATAG	05	0	670 - 2180
OPA-16	AGCCAGCGAA	14	7	600 - 2340
OPX-05*	CCTTTCCTC	06	0	1000 - 1800
OPX-07*	GAGCGAGGCT	05	0	800 - 1900
OPX-09*	GGTCTGGTTG	05	0	400 - 1200
OPX-17	GACACGGACC	06	1	630 - 1620
OPW-03	GTCCGGAGTG	06	5	470 - 1580
OPW-05	GGCGGATAAG	10	4	570 - 1640
OPW-06	AGGCCCGATG	13	3	410 - 2320
OPW-08*	GACTGCCTCT	05	0	400 - 1780
OPW-09	GTGACCGAGT	14	6	690 - 2160
OPW-10	TCGCATCCCT	06	3	880 - 1690
OPW-11	CTGATGCGTG	12	1	590 - 2540
OPW-15*	ACACCGGAAC	06	0	550 - 1900
OPW-17	GTCCTGGGTT	05	1	690 - 1930
OPW-19	CAAAGCGCTC	07	1	500 - 2260
Total		152	41	

* Primers that amplified only monomorphic loci

Table 2. Estimate of the population fixation index (F_{ST}), results of the F_{ST} significance test and average number of migrants per generation (Nm) between *P. corruscans* populations from the Paraná River. PL = upper Paraná River floodplain; IT = region upstream of the dam at the Itaipu hydroelectric power station; YA = region downstream of the dam at the Yacyretá power station

	F_{ST}	F_{ST} significance test		Nm
		χ^2	P (DF = 1)	
PL - YA	0.112 (0.128)	5.376	* < 0.05	2.0
PL - IT	0.090 (0.127)	4.320	* < 0.05	2.5
IT - YA	0.030 (0.074)	1.440	> 0.05	8.1

The numbers between parentheses represent standard deviation; * significant to 5%

Table 3. Nei's unbiased genetic distance (1978) among three *P. corruscans* populations. PL = upper Paraná River floodplain; IT = region upstream of the dam at the Itaipu hydroelectric power station; YA = region downstream of the dam at the Yacyretá hydroelectric power station

	PL	IT	YA
PL	0.0000		
IT	0.0438	0.0000	
YA	0.0638	0.0174	0.0000

Discussion

The results indicated low genetic divergence between the three *P. corruscans* populations studied. However, estimates showed that differences persist between the populations. They are differences of small magnitude, but reflect the existence of interpopulation genetic heterogeneity.

The statistically significant F_{ST} estimates show the existence of differentiation by genetic drift between PL and YA and between PL and IT. F_{ST}

between IT and YA was not significant, showing that these two populations are not differentiated by drift.

The genetic-population parameter Nm derived from F_{ST} and is equivalent to the number of migrants per generation. Nm values above 1 indicate that gene flow has been an active factor against genetic differentiation between the populations (Spieth, 1974). The gene flow estimates varied from $Nm = 2.0$ to $Nm = 8.1$. There is evidence, therefore, that *P. corruscans* populations from the regions studied have been, in fact, connected by gene flow in the period before the construction of the two dams. However, as it was demonstrated by F_{ST} , gene flow has imposed a limit, but not eliminated the differentiation between PL and YA and between PL and IT.

The subdivision of the populations, revealed by the F_{ST} statistic, was confirmed by the genetic distances estimated by Nei's methodology. The distance values varied inside of an interval similar to those that were found using the same method in populations of *Morone saxatilis* (Bielawski and Pumo, 1997). The genetic differences between PL and IT ($D = 0.0438$) and between PL and YA ($D = 0.0638$) are greater than the difference between IT and YA ($D = 0.0174$). Thus, the distancing found supports the interpretation that, although at low levels, there is genetic diversity between the *P. corruscans* populations of the Parano-Platense and the Upper Paraná ichthyofaunistic provinces.

These results provide indications to sustain the conclusion that the Guaíra Falls constitute a barrier that could have promoted, at least partially, the reproductive isolation between the *P. corruscans* populations upstream and downstream the falls. The obstacle presented by the Guaíra Falls was not absolute because gene flow equivalent to two migrants per generation was detected between the PL and YA populations. However, it should be considered that this number of migrants could represent an overestimate in relation to that which has been found before the closure of the Itaipu dam, and the consequent contact between the populations. The individuals from the Medium Paraná River flooded area, represented a sample of the population downstream. Any level of genetic exchange between the individuals withdrawn during the damming and the populations of the floodplain could have increased the estimated Nm between PL and YA.

It should be emphasised that all the estimated population parameters showed that the IT population was genetically closer to YA population than to PL. The F_{ST} index between these populations

was three times smaller than that between PL and IT. In agreement with these results, the estimated gene flow between IT and YA was three times greater than that estimated for PL and IT. Furthermore, the same pattern was found with Nei's genetic distance.

A plausible interpretation for the greater distancing between PL and IT in relation to the IT and YA combination would be the use of different sites for reproduction. This species could tend to use the closer tributaries for spawning. The Piquiri River, immediately upstream from the Itaipu reservoir, and the Ivinheima River, on the floodplain, are used by this species in times of reproduction (Vazzoler *et al.*, 1997). Thus, the Itaipu reservoir population (IT) could be reproducing in closer rivers, as the Piquiri River and the floodplain population (PL) in rivers upstream, such as the Ivinheima. As the sexual maturity of *P. corruscans* begins at about three years of age (Resende *et al.*, 1996; Sato *et al.*, 1997), the use of different spawning areas could have prevented intense genetic exchange between the two populations for at least six generations after the closure of the Itaipu dam. However, the genetic differentiation constitutes tenuous indication and there is a need of additional studies in order to obtain a direct proof of the use of different reproduction sites by the populations from the Paraná River floodplain and the Itaipu reservoir.

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