



Genetic characterization of native and invasive *Plagioscion squamosissimus* (Perciformes, Sciaenidae) populations in Brazilian hydrographic basins

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ABSTRACT. The genetic diversity of invasive and native populations of *Plagioscion squamosissimus* (Heckel, 1840) from the Paraná, Parnaíba and Araguaia-Tocantins river basins was assessed by using Random Amplified Polymorphic DNA (RAPD) markers. Genetic data confirmed the hypothesis of low genetic variability within and among *P. squamosissimus* populations introduced in the upper Paraná River basin, and indicated that they likely originated from a common ancestor. Moreover, the data demonstrated that, in agreement with available historical records, the *P. squamosissimus* populations established in the Paraná River basin were derived from a population native to the Parnaíba River basin. The genetic

data presented here are of potential future application for the management of the invasive *P. squamosissimus* populations and for the preservation of the genetic legacy of native fish.

Key words: Corvina; Introduced species; RAPD

INTRODUCTION

Aquatic environments have undergone great changes due to natural and anthropogenic stresses, and the introduction of exotic species has been one of the reasons for the loss of biodiversity in these environments. In fact, the impact of this phenomenon has been the theme of several studies (Pinto-Coelho et al., 2008; Matsuzaki et al., 2013).

The introduction of new fish species into rivers and lakes is attributable to several different causes including aquaculture, sport fishing, ornamentation, biological control, and accidents. The practice may cause a series of consequences such as the loss of biodiversity (Gherardi, 2010), trophic changes (Pinto-Coelho et al., 2008), the introduction of parasites (Vignon et al., 2009), genetic deterioration caused by hybridization (Salmenkova, 2008), and socioeconomic effects. Consequently, native species are greatly affected, with a possible loss of rare alleles and a reduction in heterozygosity (Carvalho and Cross, 1998).

The hydrographic basins of the neotropical region have the highest degree of fish biodiversity in the world (Langeani et al., 2007), and they have received the greatest amount of exotic species. In fact, Brazil is the country with the greatest number of introductions of exotic fish (Agostinho and Júlio-Júnior, 1996). The artificial introduction of fish in Brazilian waters is due to the development of aquaculture, since most introduced species derive from pisciculture stations (Vieira and Pompeu, 2001).

The reconstitution of the ichthyofauna of rivers dammed for the construction of hydroelectric plants is another motive for the introduction of exotic species in Brazil (Júlio-Júnior et al., 2009). An example of such a practice is the great transfer of native species from the Amazon basin to pisciculture tanks in northeastern Brazil in the 1960s, and from there to the southeastern and southern regions of Brazil (Agostinho and Júlio-Júnior, 1996). The introduction of exotic species in the northeastern region of the country caused an increase in fish production in the dams in which it occurred (Vieira and Pompeu, 2001). *Plagioscion squamosissimus* (Heckel, 1840), popularly known as *corvina*, was similarly introduced, and it reached the Paraná River basin. This species is native to the Parnaíba and Amazon basins. In 1949, specimens of *P. squamosissimus* were captured in lakes in Nazaré (Nazaré PI, Brazil) and in Feitoria (Oeiras PI, Brazil) and transported to the Pisciculture Station of Lima Campos, Ceará, Brazil (Fontenele and Peixoto, 1978). In 1952, the first fry were distributed into the dams of the state of Ceará by the Departamento Nacional de Obras Contra as Secas (DNOCS). Since results were satisfactory, the Electric Energy Company of São Paulo (CESP) brought specimens of *P. squamosissimus* from the northeastern dams to the state of São Paulo between 1966 and 1973 (Torloni et al., 1993), with the first successful introduction occurring in the Limoeiro Reservoir on the Pardo River, from where specimens of *P. squamosissimus* reached the Grande River and then colonized the Paraná River in 1972 (Machado, 1974).

P. squamosissimus is thus the best example of a highly successful introduction of a species in the Paraná River basin, especially in reservoirs (Agostinho et al., 2008). Although it is considered successful because of high fishing yields in the region, high risks may also be associated with such a practice.

Several factors, including genetic ones, must interact to permit the occupation of a region by an introduced species. According to Lockwood et al. (2005), an important step in the study of biological invasion is the identification of biological variables that are useful to predict which species will become invasive and successful in a specific environment. An important characteristic of *Plagioscion squamosissimus* is its wide feeding spectrum (Stefani and Rocha, 2009), which may overlap with the diet of native fish and bring this introduced species into competition with native species for space and spawning sites.

Further, the circumstances under which this species was introduced may not seem compatible with its high degree of success in colonization. Several authors suggest that a loss of genetic diversity decreases a species' adaptability to natural conditions and makes it more vulnerable to environmental disruptions. One would expect that the genetic base of *P. squamosissimus* would be narrow in the populations of the upper Paraná River as a result of its genetic derivation from a small sample of the natural population. Knowledge of the genetic variability of the species in the regions where it was introduced may contribute to an understanding of the mechanisms underlying its high adaptability.

Genetic variability of natural populations has been successfully studied using the tools of molecular biology. The Random Amplified Polymorphic DNA (RAPD) technique (Williams et al., 1990) has been successfully used to identify molecular polymorphisms suitable for genetic and phylogenetic studies. RAPD is based on simultaneous Polymerase Chain Reaction (PCR) amplification of anonymous loci in the genome, using primers with short and arbitrary sequences. Since these primers do not discriminate between coding and noncoding DNA sequences, the RAPD technique samples the genome in a highly randomized manner, and innumerable anonymous loci may be amplified (Williams et al., 1990). RAPD has been successfully used in fishes to establish phylogenetic relationships (Roy et al., 2014), to study interspecific hybridization (Oliveira et al., 2002; Sovic et al., 2012), and to study the genetics of populations and sub-populations (Muneer et al., 2011; Sivaraman et al., 2014).

In this study, RAPD was used to assess the genetic variability of *P. squamosissimus*, to verify the hypothesis of low genetic diversity within and among introduced *P. squamosissimus* populations in the upper Paraná River basin, and to contribute to an understanding of the genetic diversity of native *P. squamosissimus* populations.

MATERIAL AND METHODS

Fish sampling and DNA extraction

Specimens of four populations of *Plagioscion squamosissimus* were sampled from three hydrographic basins (Figure 1): the upper Paraná River floodplain (population PR), the Itaipu hydroelectric reservoir (population IT), the Tocantins River (population TO) within the Amazon River basin, and the Parnaíba River basin (population PI). The population native to the Parnaíba River basin was sampled at the following three localities: Lagoa de Nazaré, the natural lake from where *P. squamosissimus* specimens were originally captured and introduced into the Ceará hatchery; Mesa de Pedra reservoir, Poty River sub-basin; and Lagoa da Porta and Lagoa do Meio, two small natural lakes close to each other and connected to the Parnaíba River.

Samples of total genomic DNA were extracted according to the procedure of Panarari-Antunes et al. (2010). Approximately 100 mg of muscle tissue were macerated in liquid nitrogen

and then homogenized in 500 μ L TH buffer (10 mM Tris-HCl, 60 mM NaCl, 10 mM EDTA, 5% sucrose, 0.15 mM spermine, and 0.15 mM spermidine) plus 500 μ L PS buffer (0.2 M Tris-HCl, 30 mM EDTA, 2% SDS, and 5% sucrose) and 5 μ L proteinase K (20 μ g/ μ L). After shaking for 2 h at 37°C, DNA was phenol/chloroform extracted and then ethanol precipitated in an overnight incubation at -20°C. DNA samples were re-suspended in diluted TE buffer (0.1 mM Tris-HCl pH 8.0, 0.01 mM EDTA) containing RNase (20 μ g/mL) and quantified by comparison with known quantities of phage λ DNA in agar gel (0.8%) stained with ethidium bromide (20 μ g/100 mL).

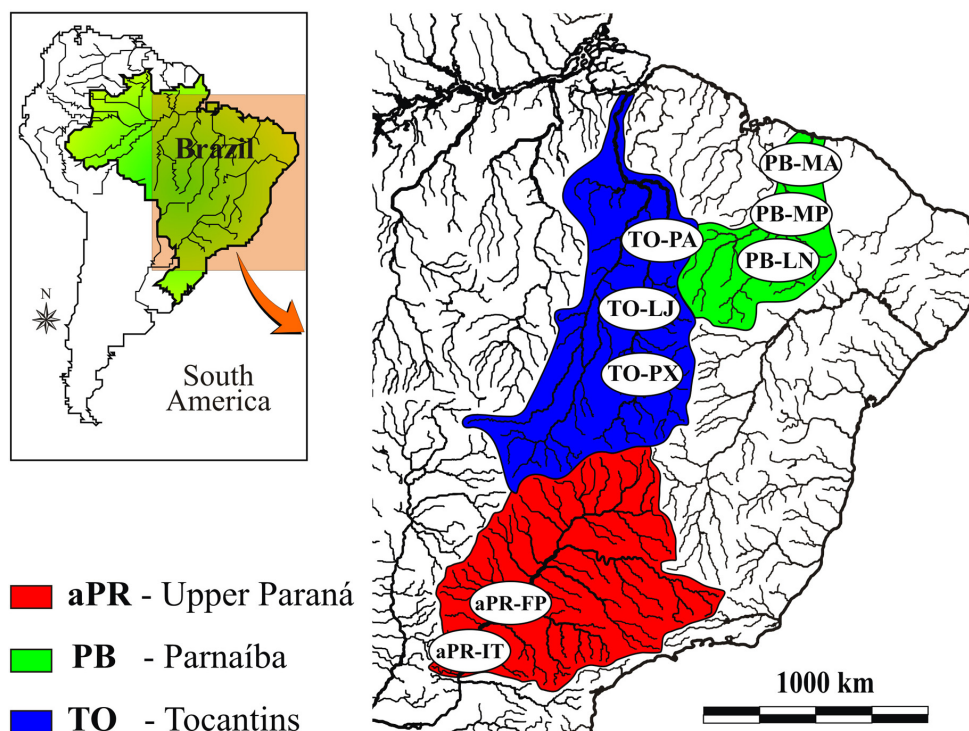


Figure 1. Sampling localities of *Plagioscion squamosissimus* native populations of Parnaíba (PB) and Tocantins (TO) river basins, and introduced populations of upper Paraná River basin (aPR). PB-MA: Lagoa da Porta and Lagoa do Meio, near Miguel Alves township (04°02'S, 42°49'W). PB-MP: Mesa de Pedra Reservoir in the Poty River (06°10'S, 41°58'W). PB-LN: Lagoa do Nazaré, near Nazaré do Piauí township (07°00'S, 42°39'W). TO-PA: Tocantins River, near Pedro Afonso township (08°58'S, 48°10'W). TO-PN: Lajeado Reservoir in the Tocantins River (10°37'S, 48°24'W). TO-PX: Tocantins River, near Peixe township (11°52'S, 48°35'W). aPR-FP: upper Paraná River floodplain (22°47'S, 53°19'W). aPR-IT: Itaipu Reservoir in the Paraná River (24°42'S, 54°18'W).

Amplification and analysis of RAPD loci

P. squamosissimus populations were evaluated by using RAPD markers. Analyses were performed on the invasive populations PR (25 specimens) and IT (30 specimens), and the native populations TO (25 specimens) and PI (31 specimens). Primers OPX-01, OPX-03, OPX-07, OPX-19, OPX-18, OPW-02, OPW-09, and OPW-17, from Operon Technologies (Alameda, CA, USA),

were selected for RAPD analysis. The PCR reaction mixture consisted of buffer Tris-KCl (20 mM Tris-HCl pH 8.4 and 50 mM KCl), 2 mM $MgCl_2$, 0.46 μM primer, 0.19 mM dNTP, 1 U/reaction of *Taq* DNA Polymerase (Invitrogen Life Technologies, Grand Island, NY, USA), DNA (10 ng), and sterile deionized water to a total volume of 13 μL . The reaction mixture was heated at 92°C for 4 min, followed by 40 cycles of 1 min at 92°C, 1 min 30 s at 40°C, and 2 min at 72°C. Immediately after the last amplification cycle, the reaction mixture was maintained for 5 min at 72°C, cooled at 20°C for 20 min, and then kept at 4°C. In each experiment, a sample without template DNA was included as a negative control. Aliquots (10 μL) of PCR amplification products were applied to 1.4% agar gels stained with ethidium bromide (20 $\mu g/100$ mL) and separated by electrophoresis in TBE buffer (Tris-borate) at 5 V/cm for 3-4 h. Amplified fragments were visualized under UV radiation and photographed with a Kodak EDAS-290 (Rochester, NY, USA). Sizes of fragments were estimated by comparison with a standard 100-bp Ladder (Invitrogen Life Technologies).

The molecular fixation index F_{ST} , based on RAPD data, was estimated with Arlequin 3.0 (Excoffier et al., 2005). The significance level of the F_{ST} index was determined with a non-parametric permutation approach. The Shannon diversity index and the percentage of polymorphic loci within populations were estimated with PopGene 1.31 (Yeh et al., 1999). Genetic differentiation among populations was evaluated by applying the Mantel test to the matrix of Jaccard similarity, using Miller's (1999) software. Cluster analysis, based on the matrix of Jaccard similarity complements, was performed using the Unweighted Pair Group Methods of Arithmetic Means (UPGMA) algorithm of the FreeTree program (Pavlicek et al., 1999). The final presentation was produced with MEGA 6.

RESULTS

The number of sharp reproducible bands generated by each of the eight selected primers in the studied populations ranged between 5 and 10, corresponding to a total of 57 loci. Amplified fragments varied from 480 to 2070 bp in size. Figure 2 shows the electrophoretic profiles obtained with the RAPD OPW-09 primer. Four monomorphic loci were exclusive to both the non-native populations PR and IT from the Upper Paraná River basin, and to the native population PI from the Parnaíba River basin. The population TO, native to the Amazon basin, was characterized by four exclusive monomorphic loci. Comparative analysis revealed 38 monomorphic loci shared by all studied populations.

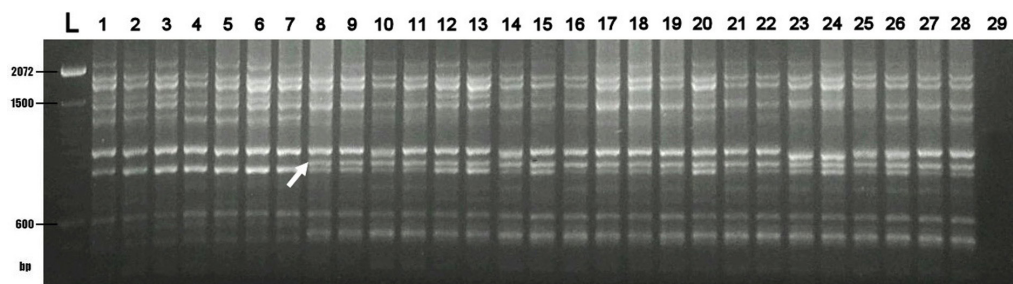


Figure 2. RAPD electrophoretic profiles using primer OPW-09 for invasive (*lanes 8-21*) and native (*lanes 1-7 and 22-28*) populations of *Plagioscion squamosissimus*. Lane L = molecular weight markers (100-bp Ladder). Lanes 1-7 = Tocantins River basin. Lanes 8-14 = upper Paraná River floodplain. Lanes 15-21 = Itaipu reservoir, upper Paraná River. Lanes 22-28 = Parnaíba River basin. Note DNA fragment exclusive to Paraná and Parnaíba basins (arrow). Lane 29 = negative control, without DNA.

Table 1 shows the genetic variability estimates within each population based on the percentage of polymorphic RAPD loci and the Shannon diversity index. The percentage of polymorphic loci in the populations ranged from 5.26 to 15.79%. The Tocantins population (TO) had the highest percentage of polymorphic loci. The data revealed low genetic variability within the non-native populations PR and IT and within the native population PI. Genetic variability within the *P. squamosissimus* population native to the Amazon River basin (population TO) was approximately two to three times higher than in the other three populations. Specimens were grouped into two major clusters in the UPGMA dendrogram, which was based on Jaccard similarity. The two non-native populations and the population native to Parnaíba basin were clustered together and distinguished from population TO, which was grouped into a distinct cluster. This clustering bifurcation was supported by a bootstrap value of 100% (Figure 3).

Table 1. Percentage of polymorphic loci (PL) and Shannon diversity index (SI) of *Plagioscion squamosissimus* populations from upper Paraná River floodplain (FP), Itaipu reservoir (IT), Tocantins River (TO), and Parnaíba River basin (PB).

Populations	% PL	SI
FP	5.26	0.0264
IT	8.77	0.0473
TO	15.79	0.0778
PB	7.02	0.0380

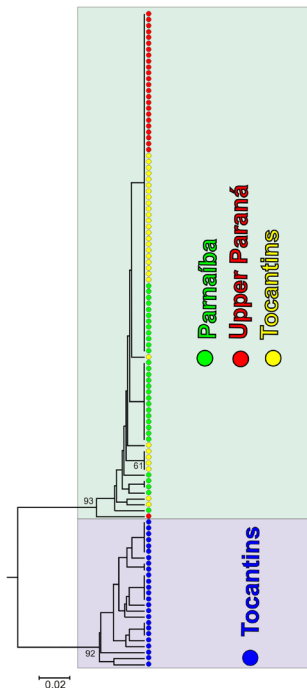


Figure 3. Dendrogram based on Jaccard similarity coefficients of native and introduced populations of *Plagioscion squamosissimus*, obtained from RAPD markers and grouped by the UPGMA method. Numbers above branches indicate bootstrap values (only values above 50 are shown).

As shown in Table 2, the molecular fixation index F_{ST} and the Jaccard similarity data, based on the RAPD analysis, indicate that the *P. squamosissimus* native to the Amazon River basin (population TO) is genetically distant from both the invasive populations (PR and IT) and the Parnaíba native population. The fixation index F_{ST} was significant for all combinations of the four populations. However, the F_{ST} magnitude of the non-native populations (PR and IT) and the native Parnaíba population (PI) was low when compared with the native population TO. In addition, genetic distances were small among the PR, IT, and PI populations, demonstrating high genetic similarity between the introduced *P. squamosissimus* groups and the population native to the Parnaíba basin. Indications of minimal or undetectable genetic differentiation among these populations was corroborated by the Mantel test (Table 3). By contrast, population TO was genetically differentiated (above 0.94 in the Mantel test) from the invasive population PI and the non-native populations. Hence, all analyses confirmed the genetic differentiation between the two *P. squamosissimus* native populations sampled in the adjoining Amazon and Parnaíba hydrographic basins, and the high genetic similarity between the Parnaíba and invasive populations.

Table 2. Jaccard similarity (lower left) and molecular fixation index F_{ST} (upper right), based on RAPD markers, among *Plagioscion squamosissimus* populations introduced to the upper Paraná River floodplain (FP) and Itaipu reservoir (IT), and native either to the Tocantins River (TO) or to the Parnaíba River basin (PB).

Populations	FP	IT	TO	PI
FP	-	0.0523*	0.8825**	0.0564*
IT	1.00	-	0.8592**	0.0618**
TO	0.88	0.88	-	0.8796**
PB	1.00	1.00	0.88	-

*Significant at the 5% probability level. **Significant at the 1% probability level.

Table 3. Correlations (R), Type I errors (P), and determination coefficients (R^2) by the Mantel test, with 10,000 random permutations, comparing the complement matrix of Jaccard similarity with the model matrix built on basis of *Plagioscion squamosissimus* sampling localities: upper Paraná River floodplain (FP), Itaipu reservoir (IT), Tocantins River (TO), and Parnaíba River basin (PB).

Populations	R	P	R^2
FP x TO	0.9586	0.001	0.9189
FP x IT	-0.0123	0.7443	0.0001
FP x PB	0.1276	0.001	0.016
TO x IT	0.9409	0.001	0.8852
TO x PB	0.9576	0.001	0.9169
IT x PB	0.1134	0.001	0.012

DISCUSSION

According to our results, the *P. squamosissimus* invasive populations from the Upper Paraná River basin and the population native to the Parnaíba River basin demonstrated low polymorphism and high genetic similarity. In contrast, they were genetically differentiated from the *P. squamosissimus* native to the Araguaia-Tocantins River basin, the most polymorphic population. Since its introduction into hydroelectric reservoirs of the upper Paraná River basin about thirty-five years ago, *P. squamosissimus* has successfully spread and occupied a wide diversity of habitats (Torloni et al., 1993).

In the absence of gene flow, genetic differentiation between the population native to the Araguaia-Tocantins River basin and the population native to the Parnaíba River basin plus invasive populations, was revealed by the occurrence of exclusive monomorphic RAPD loci. In the Amazon River basin, previous genetic studies have been reported on native populations of *P. squamosissimus* (Teixeira et al., 2002; Torres, 2006), but intraspecies genetic data on these populations and on populations native to the Parnaíba River basin are recent and were obtained by Panarari-Antunes et al. (2012) for the first time. In the current study, the genetic variability between native and invasive populations of *P. squamosissimus* was verified by examining the mtDNA control region across populations. The results were consistent with those from RAPD, and they showed low genetic variability within and among the populations of the floodplain of the upper Paraná River, the Itaipu reservoir, and Parnaíba, and high genetic variability in the populations of the Tocantins River basin (Panarari-Antunes et al., 2012).

The level of genetic differentiation between the population native to the Parnaíba River basin and the population native to the Araguaia-Tocantins River basin is consistent with level of intraspecies genetic differentiation estimated for other fish species, including neotropical species (Silvia et al., 2008; Muneer et al., 2011). For instance, genetic differentiation as inferred from RAPD markers was relatively low between two *Pseudoplatystoma corruscans* populations isolated by the former Guaira Falls in the Paraná River basin (Sekine et al., 2002). Similar results were found among three *Schizothorax richardsonii* fish populations from Chirapani, Kosi, and Gola River of Himalaya, India (Sivaraman et al., 2014). In contrast, levels of genetic differentiation are typically high between species, including between closely related neotropical fish species that hybridize and produce offspring. For example, RAPD analyses were consistent in revealing high genetic distances between *Steindachnerina brevipinna* and *Steindachnerina insculpta* (Oliveira et al., 2002) and between *Cichla monoculus* and *Cichla* sp invasive to the Paraná River basin (Oliveira et al., 2006). The molecular data support our view that the two native populations existing in neighboring hydrographic basins both belong to the species *P. squamosissimus*.

In invasive fish species, maintenance of genetic variability in a recently established population depends upon the number of introduced individuals and upon multiple introductions from different populations (Salmenkova, 2008). The low genetic variability within and among the studied invasive *P. squamosissimus* populations strongly indicates that they originated from a single population. Furthermore, their high genetic similarity to the population native to the Parnaíba River basin and their genetic distinctiveness from the Amazonian native population demonstrates that these populations invasive to the Paraná River basin were indeed derived from a population native to the Parnaíba basin. These results agree with historical reports compiled by DNOCS and CESP which describe the first *P. squamosissimus* transfers (Torloniet al., 1993), registered in 1949 (Fontenele and Peixoto, 1978).

Genetic diversity is considered one of the factors that may facilitate the adaptability of invasive species (Kawamura et al., 2010). In spite of the low genetic variability in introduced populations of *P. squamosissimus* in the Paraná River basin, this variability is nevertheless comparable to the genetic variability of the original population from the Parnaíba basin. This fact may account for the successful establishment of the species, since its genetic diversity is comparable to that of the native population. Furthermore, a combination of biological factors including food, reproduction, and "evolution trap" strategies, may overcome the effects of low genetic diversity (Schlaepfer et al., 2005).

Several studies have shown that invasive species have a superior capacity to exploit local resources when compared to native species (Byers, 2000). Reservoirs are fast-changing

environments and may receive species from other hydrographic basins, and the pre-existing characteristics and adaptations of native species may not be highly advantageous over the long term within a change-prone environment. In many instances, native organisms may be “cheated” by their evolutionary history and may be unable to adapt quickly and compete with invasive species. Survival may be reduced and extinction of native species may occur over the long term (Schlaepfer et al., 2005).

Recent surveys in the upper Paraná River basin recorded 310 fish species with seven (2.3%) exotic species. Among the allochthonous and exotic species, 37 species occurred in the upper Paraná River after the construction of the Itaipu dam (Langeani et al., 2007). The success of exotic species in the Paraná River basin suggests that it offers favorable conditions for the establishment of invasive species. According to research on the Paraná River basin, Amazon fish are the most successful colonizers (Agostinho et al., 2005), especially species of the genus *Cichla* and *P. squamosissimus*. The absence of other species of the family Scianidae (Langeani et al., 2007) and of predatory species that are phylogenetically close to *P. squamosissimus* in the upper Paraná River may also be a factor that favors the successful establishment of this species. Further, trophic habits that permit the occupation of greater varieties of habitats and reproductive strategies are another important aspect that may have contributed towards the success of the species (Oliveira et al., 2005). Food flexibility must have helped in the adaptation of *P. squamosissimus* to the Paraná River basin since juveniles have different feeding habits than adults (Stefani and Rocha, 2009). The piscivorous habit may also be related to the species' success since most exotic species are fish-eating species (Agostinho et al., 2008; Pinto-Coelho et al., 2008). The feeding preferences of *P. squamosissimus* are very wide and more than thirty species have been reported in its diet in the Itaipu reservoir (Hahn et al., 1997). With respect to habitat occupation, *P. squamosissimus* has been recorded in deep slow-water environments (Agostinho et al., 2008), which are typical of reservoirs, where only a few species survive due to the inhospitable characteristics of the habitat.

In spite of the colonization success of *P. squamosissimus* in the upper Paraná River, risks to native species are very great and the literature has already reported a decrease of certain species due to the invasion of *P. squamosissimus* (Torres, 2006; Barros et al., 2012).

Our results show that the hypothesis of low genetic diversity within introduced populations settled in the upper Paraná River basin has been confirmed. Since these populations are characterized by high genetic similarity homogeneity, it may be inferred that they had a common ancestor. We have also demonstrated that the invasive populations that settled in the Paraná River basin were derived from the *P. squamosissimus* population native to the Parnaíba River basin. Owing to geographic isolation, the two native populations have accumulated genetic polymorphisms and, as inferred by the RAPD markers, they have evolved independently, although the two populations still belong to the same species, *P. squamosissimus*. Low genetic variability and no genetic differentiation among the studied subpopulations from the Parnaíba River basin suggests that in the recent geological past this native *P. squamosissimus* population went through a bottleneck.

The molecular data obtained in current study may permit the identification of exclusive diagnostic loci suitable for distinguishing populations belonging to the two native *P. squamosissimus* groups. Therefore, efforts to rapidly identify suitable diagnostic nuclear loci are recommended to improve phylogeographical studies and for surveillance of future possible reintroductions of *P. squamosissimus* in the Paraná River basin, as well as introductions into other hydrographical basins. Further studies should be undertaken to sample a higher number of sites in the Araguaia-Tocantins and Parnaíba River basins and to identify new polymorphisms to preserve the genetic legacy of these native *P. squamosissimus* populations.

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