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Research Article

Allozyme variation among three populations of the armored catfish *Hypostomus regani* (Ihering, 1905) (Siluriformes, Loricariidae) from the Paraná and Paraguay river basins, Brazil

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Abstract

Three Brazilian populations of the armored catfish *Hypostomus regani* (Ihering, 1905) were sampled, one from the Corumbá Reservoir in Goiás state, another from the Itaipu Reservoir in Paraná state and a third from the Manso Reservoir in Mato Grosso state. Allozyme electrophoresis was used to establish the genetic structure of the species, with the analysis of liver, heart and muscles tissues allowing the scoring of 25 loci from 14 enzymatic systems. Although no diagnostic loci were found, some exclusive rare alleles were recorded for the three populations. The genetically most similar populations were those from Corumbá and Itaipu, and the most distant were the populations from Manso and Corumbá. The allozyme data showed three structured populations belonging to the same species *H. regani* (F_{ST} = 0.173).

Key words: allozymes, genetic variability, *Hypostomus regani,* Loricariidae, Paraguay and Paraná Rivers.

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Introduction

Species of the armored catfish genus *Hypostomus* (Siluriformes, Loricariidae) feed mainly on rock bottoms by scraping the substratum (Delariva and Agostinho, 2001) and present the characteristics of benthonic and sedentary fish (Garavello and Garavello, 2004). These properties seem to be among the factors which have made *Hypostomus* one of the most speciose of the Neotropical genera, containing about 120 nominal species (Weber, 2003). The catfish *Hypostomus regani* (Ihering, 1905) is one of the most widespread species of the genus, ranging from the headwaters of the upper Paraná River basin in the Brazilian state of Goiás to the La Plata basin covering parts of Argentina, Brazil and Paraguay (Reis *et al.*, 1990; Weber, 2003), also occurring in the Paraguay River basin. The wide geographical range of *H. regani* not only contrasts with the narrow distribution of most *Hypostomus* species but can also raise doubts about the conspecificity of *H. regani* populations. Studies of the genetic population structure of Neotropical fish have mainly focused on known migratory species such as *Prochilodus lineatus* (Characiformes, Prochilodontidae) (Revaldaves *et al.*, 1997) and *Pseudoplatystoma corruscans* (Siluriformes, Pimelodidae) (Sekine *et al.*, 2002), with studies on non-migratory fish having been restricted to *Hoplias malabaricus* (Characiformes, Erythrinidae) (Dergam *et al.*, 1998; Peres *et al.*, 2002). We have no data about migratory behavior of *H. regani*, whether it is resident, short, medium or long distance migratory.

During the study described in this paper we used allozyme electrophoresis to investigate the genetic structuring of three geographically isolated Brazilian *H. regani* populations. The aim of the study was to estimate the genetic differentiation among the populations and verify if the fish in these populations belonged to the same species based on the presence of diagnostic loci that are fixed, or nearly fixed, for different alleles in two or more populations (Allendorf and Luikart, 2007) or a Nei's genetic identity below 0.80 (Thorpe, 1982).

Material and Methods

One of the Brazilian *H. regani* populations was collected in the Corumbá reservoir on the northern stretches of the Upper Paraná River basin in Goiás state (Figure 1), another was in the Itaipu reservoir in Paraná state, this reservoir constituting an ichthyofaunistic barrier splitting the Upper from the Medium Paraná River, while the third pop-

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ulation was from the Manso reservoir on the Manso River in the Paraguay River basin in Mato Grosso state. Hence these three populations were geographically isolated. Between April 1996 and February 2001 we collected 25 specimens from the Corumbá population (sampled at 17°59' S, 48°31' W; altitude 571 m), 32 from the Itaipu population (25°21' S, 54°32' W; altitude 218 m) and 33 from the Manso population (14°52' S, 55°47' W; altitude 277 m). Voucher specimens of the *H. regani* populations sampled are deposited in the ichthyological collection at the Paraná State University at Maringá (Address: Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia), Universidade Estadual de Maringá, PR, Brazil). The Accession numbers of the voucher specimens are NUP 2286 for the Corumbá material, NUP 2557 for the Itaipu material and NUP 3188 for the Manso material. This study was approved by the animal ethics committee of our institution and satisfied all requirements under Brazilian environmental laws. Sampling was carried out under permission of the Brazilian environmental agency (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA), protocol number 036/98 to Corumbá, 004/2001 to Itaipu, and 097 DIFAP/IBAMA to Manso samplings.

Heart, liver and white skeletal muscle samples were removed from the captured *H. regani* specimens and stored in liquid nitrogen until the moment of analysis. We analyzed the 14 enzymatic systems shown in Table S1. The electrophoretic procedures are detailed in Zawadzki *et al.* (1999). The nomenclature used for the loci and enzymes was proposed by Murphy *et al.* (1996). Alleles were designated with lower case letters in italics in ascending order of anodal mobility. The data were analyzed by the software Biosys 1 (Swofford and Selander, 1981). Genetic structuring was appraised using Wright's F-statistic (Wright, 1978) and the significance tested by the chi-square χ^2 test (Workman and Niswander, 1970). Genetic interpretation of the zymogram patterns was based on the quaternary structure of enzymes described by Ward *et al.* (1992). Genetic identity was assessed as the unbiased Nei's genetic identity (Nei, 1978).

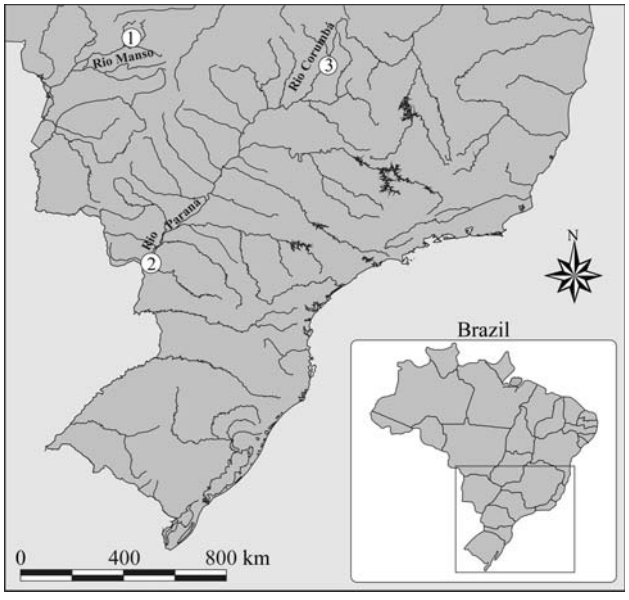

Figure 1 - Southern portion of South America showing the collection sites of *Hypostomus regani* from the Manso (1), Itaipu (2) and Corumbá reservoirs (3).

Results and Discussion

For the 14 enzymatic systems analyzed in *H. regani* we detected 42 alleles in 25 loci, the tissue-specific expression patterns being similar to those found in *Hypostomus myersi* (Gosline, 1947) from the Iguaçu River, Brazil (Zawadzki *et al.*, 2001). Table S2 shows the allele frequencies of the three populations analyzed. Single locus allele frequency heterogeneity was found for the following enzyme loci: *sAta-A* (χ^2 = 13.20, p = 0.0013); *sAta-B* (χ^2 = 50.62, p = 0.0000); *Adh-A* (χ^2 = 74.80, p = 0.0000); *Gpi-B* (χ^2 = 19.19, p = 0.00007); *mIcdh-A* (χ^2 = 19.45, p = 0.00006); *sMdh-A* (χ^2 = 14.32, p = 0.0063); and *Pgm-A* (χ^2 = 7.41, p = 0.0245). The three populations showed allele frequency heterogeneity for all analyzed loci (χ^2 = 214.946 for 34 degrees of freedom (df), p = 0.0000). Almost all polymorphic loci analyzed were in Hardy-Weinberg equilibrium (HWE). Loci not in HWE were *Acp-A* for the Corumbá population, *sAat-A* and *sAat-B* for the Itaipu population and *Gcdh-A* and *Icdh-A* for the Manso population. No diagnostic locus was found for these three populations. The estimated genetic variability for the three populations is shown in Table 1, with significant heterozygote deficiency being found in the Corumbá population, for which the inbreeding coefficient (F_{IS}) was 0.180 (χ^2 = 4.50; p < 0.05.), and Manso populations (F_{IS} = 0.274, χ^2 = 9.05, p < 0.05), but not in Itaipu population (F_{IS} = 0.090; χ^2 = 2.89, p > 0.05). The unbiased Nei's genetic identity for the three populations is presented in Table S2. Genetic similarity was 0.989 between the Itaipu and Corumbá populations and 0.976 between the Corumbá and Manso populations. The Wright's F-statistics for all loci were different from zero, indicating an overall heterozygote deficiency. The estimated F_{IS} value was 0.1419 (χ^2 = 12.77, p < 0.001), the overall inbreeding coefficient (F_{IT}) was 0.2902 (χ^2 = 104.47, p < 0.001), indicating heterozygote deficiency, and the relative genetic differentiation (F_{ST}) was 0.1728 (χ^2 = 31.10, p < 0.001), which, taken together, indicates that the species was genetically structured in three populations. When the three populations were directly compared to each other the F_{ST} values were significant and also indicated that the three populations were structured with F_{ST} = 0.0827 for the Corumbá population *vs.* the Itaipu population, F_{ST} = 0.2139 for the Corumbá population *vs.*

Table 1 - Allelic frequencies and genetic variability measures for three populations of *Hypostomus regani* from the Manso, Itaipu and Corumbá reservoirs. Numbers in bold type indicate exclusive alleles. K = mean number of alleles per locus; $P_{0.99}$ = frequency of polymorphic loci; H_o = mean observed heterozygosity; H_e = mean expected heterozygosity. Numbers in parenthesis indicate the standard error.

Locus*	Allele	Population		
		Corumbá (n = 25)	Itaipu (n = 32)	Manso (n = 33)
sAta-A	*a*	0.040	0.016	0.015
	b	0.620	0.984	0.985
	c	0.340	-	0.985
sAta-B	*a*	1.000	0.891	1.000
	b	-	**0.109**	-
Acp-A	*a*	0.060	0.016	0.015
	b	0.940	0.984	0.955
	c	-	-	**0.030**
Adh-A	*a*	0.340	0.453	1.000
	b	0.660	0.547	-
Gcdh-A	*a*	1.000	1.000	0.970
	b	-	-	**0.030**
G3pdh-A	*a*	1.000	1.000	1.000
G3pdh-B	*a*	1.000	1.000	1.000
G6pdh-A	*a*	1.000	1.000	1.000
G6pdh-B	*a*	1.000	1.000	1.000
Gpi-B	*a*	0.040	0.063	-
	b	0.940	0.938	1.000
	c	**0.020**	-	-
Gpi-A	*a*	-	**0.156**	-
	b	1.000	0.844	1.000
mIcdh-A	*a*	0.960	0.625	0.818
	b	0.040	0.375	0.182
sIcdh-A	*a*	1.000	1.000	0.970
	b	-	-	**0.030**
Ldh-B	*a*	0.020	0.047	0.030
	b	0.980	0.953	0.970
Ldh-A	*a*	1.000	1.000	1.000
sMdh-A	*a*	-	-	**0.106**
	b	1.000	0.984	0.894
	c	-	**0.016**	-
mMdh-A	*a*	1.000	1.000	1.000
sMdh-B	*a*	1.000	1.000	1.000
sMdhp-A	*a*	1.000	1.000	1.000
mMdhp-B	*a*	1.000	1.000	1.000
Per-1	*a*	1.000	1.000	1.000
Per-2	*b*	1.000	1.000	1.000
Per-3	*c*	1.000	1.000	1.000
Pgm-A	*a*	-	**0.063**	-
	b	1.000	0.938	1.000
Sod-A	*a*	1.000	1.000	1.000
K		1.3 (0.1)	1.4 (0.1)	1.3 (0.1)
$P_{0.99}$		24.0	44.0	28.0
Ho		0.0432 (0.0224)	0.0712 (0.0238)	0.0230 (0.0110)
He		0.0527 (0.0269)	0.0784 (0.0251)	0.0317 (0.0124)

*Key: *Acp*, acid phosphatase; *Adh*, alcohol dehydrogenase; *Ata*, aspartate transaminase; *Gcdh*, glucose 1-dehydrogenase - NAD+; *G3pdh*, glycerol-3-phosphate dehydrogenase; *G6pdh*, glucose-6-phosphate dehydrogenase; *Gpi*, glucose-6-phosphate isomerase; *Icdh*, isocitrate dehydrogenase - NADP+; *Ldh*, L-lactate dehydrogenase; *Mdh*, malate dehydrogenase; *Mdhp*, malate dehydrogenase - NADP+; *Pgm*, phosphoglucomutase; *Per*, peroxidase; and *Sod*, superoxide dismutase.

the Manso population and F_{ST} = 0.1270 for the Itaipu population *vs.* the Manso population.

Our allozyme survey revealed high genetic differentiation in the three *H. regani* populations, with F_{ST} values ranging from 0.0039 for the *Ldh-B* locus to 0.3454 for the *Adh-A* and an average of F_{ST} = 0.1728 ($p < 0.001$). These results show that 17.28% of the total heterozygosity was due to the population subdivision. Furthermore, the χ^2 contingency test indicated that the three populations were very different (χ^2 = 214.946 for 34 df, $p < 0.0001$). According to Wright (1978), F_{ST} values below 0.05 indicate low genetic differentiation, values from 0.05 to 0.15 moderate differentiation, values from 0.15 to 0.25 high differentiation and values above 0.25 very high differentiation. Nevertheless, the unbiased Nei's genetic distances indicated that the differences between the three populations analyzed were within the limits of populations from the same species (Thorpe, 1982; Thorpe and Sole-Cava, 1994), indicating that they did indeed belong to the same species.

The genetic variability analysis revealed a variation in heterozygosity values among the three *H. regani* populations, but overall genetic variability (0.065 ± 0.012) was near the mean 0.051 for freshwater fish (Ward *et al.*, 1992). The estimated expected heterozygosity (*He*) for the Itaipu population was higher (*He* = 0.0784) than for the Manso population (*He* = 0.0527) or the Corumbá population (*He* = 0.0317). Similar findings were reported by Zawadzki *et al.* (2002) for other *Hypostomus* species common to the Itaipu and Corumbá reservoirs. According to Zawadzki *et al.* (2005) the populations of Itaipu reservoir may have heterozygosity values increased by secondary contacts with populations from previously isolated tributaries that are

now in contact due to the formation of Itaipu Lake in 1982, which flooded and covered the falls at the mouth of each tributary which probably used to act as a geographic barrier to free gene flow. On the other hand, the Corumbá River is a tributary of the Paranaíba, a river with a relative degree of fish endemism (Pavanelli and Britski, 1999), which may promote endogamy which in turn causes low heterozygosity.

Studies on fish population genetics have contributed to population management programs (Haig, 1998; Sole-Cava, 2001) and the detection of incipient ecological species (Dergam *et al.*, 1998; Beheregaray and Sunnucks, 2001). In our present paper we have shown that the genetic distances between the different *H. regani* populations correlated with the geographic distance and river flow. However, caution is needed in the interpretation of these results because a study involving the mitochondrial genes *ATPase 6* and *ATPase 8* of *Prochilodus lineatus* from 13 localities from rivers of the Paraná-Paraguay basin did not produce genetic distance results corresponding to the geographic distances separating the localities (Sivasundar *et al.*, 2001). McGlashan and Hughes (2000) also found no correlation between genetic and geographic distances when they used allozymes and the mitochondrial gene *ATPase 6* to study *Craterocephalus stercusmuscarum* (Atheriniformes, Atherinidae) from northeastern Australia. Genetic differentiation among freshwater fish populations can be created not only by headwater captures, waterfall uplift and population distance which results in reduced gene flow (McGlashan and Hughes, 2000) but also by, differences in altitude, temperature, water velocity, food resources and reproductive strategies of which can lead to natural selection for local adaptations.

A highly important factor in the genetic differentiation of fish populations is the migratory capacity of a specific fish species, because the genetic structure of migratory fish exhibit less population differentiation than non-migratory fish. Marine fish populations have fewer barriers to gene flow and usually show less genetic differentiation than freshwater fishes (Gyllensten, 1985; Ward *et al.*, 1994). Some migratory freshwater fish species such as *P. lineatus* (Revaldaves *et al.*, 1997; Sivasundar *et al.*, 2001) and *P. corruscans* (Sekine *et al.*, 2002) show low genetic differentiation while poorly migratory or resident species such as *H. malabaricus* show high genetic differentiation (Dergam *et al.*, 1998).

The Corumbá reservoir is about 1,110 kilometers from the Itaipu reservoir by river and several waterfalls used to naturally separate these two localities but some of these waterfalls have been eliminated by manmade reservoirs such as the Jupiá (flooded in 1974), Ilha Solteira (flooded in 1978) and São Simão (flooded in 1978) reservoirs. Furthermore, the Manso reservoir is about 4,400 from the Corumbá reservoir and 3,300 kilometers from the Itaipu reservoir by river, and hence we feel that the main reason for the high population differentiation of *H. regani* must be the natural barriers to gene flow in the localities surveyed.

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Supplementary Material

The following online material is available for this article:

- Table S1. Enzyme systems.
- Table S2. Genetic identity.

This material is made available as part of the online article from http://www.scielo.br.gmb

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Table S1. Names, enzyme commission number (E.C.n^{o}.), tissues, buffers, quaternary structure (Q.S.) and number of loci for each enzyme detected in *Hypostomus regani* from the Corumbá, Itaipu and Manso reservoirs. L = liver; M = muscle; H = Heart; TBE = Tris/borate/EDTA (pH 8.7) (Boyer *et al.*, 1963); TC = Tris/citrate (pH 7.0) (Shaw and Prasad, 1970). +

Enzyme (Abbreviation)	**E.C. n^{o}.**	**Tissue**	**Buffer**	**Q. S.**	**Loci**
Acid phosphatase (Acp)	3.1.3.2	L	TC	Dimeric	1
Alcohol dehydrogenase (Adh)	1.1.1.1	L	TBE	Dimeric	1
Aspartate transaminase (Ata)	2.6.1.1	L, H, M	TBE	Dimeric	2
Glucose 1-dehydrogenase - NAD+ (Gcdh)	1.1.1.118	L	TBE	Dimeric	1
Glycerol-3-phosphate dehydrogenase (G3pdh)	1.1.1.8	L, H, M	TC	Dimeric	2
Glucose-6-phosphate dehydrogenase (G6pdh)	1.1.1.49	L	TBE	Tetrameric	2
Glucose-6-phosphate isomerase (Gpi)	5.3.1.9	L, H, M	TC	Dimeric	2
Isocitrate dehydrogenase - NADP+ (Icdh)	1.1.1.42	L, H, M	TC	Dimeric	2
L-Lactate dehydrogenase (Ldh)	1.1.1.27	H, M	TC	Tetrameric	2
Malate dehydrogenase (Mdh)	1.1.1.37	L, H, M	TC	Dimeric	3
Malate dehydrogenase - NADP+ (Mdhp)	1.1.1.40	L, H, M	TC	Tetrameric	2
Phosphoglucomutase (Pgm)	5.4.2.2	L, H, M	TC	Monomeric	1
Peroxidase (Per)	1.11.1.6	L, H	TC	Tetrameric	3
Superoxide dismutase (Sod)	1.15.1.1	L, H, M	TBE	Dimeric	1

Table S2. Nei's unbiased genetic identity (*I*) is shown in the upper diagonal and genetic distance (*D*) in the lower diagonal for the three *Hypostomus regani* populations from the Corumbá, Itaipu and Manso reservoirs.

Population	Corumbá	Itaipu	Manso
Corumbá	----	0.989	0.976
Itaipu	0.011	----	0.984
Manso	0.024	0.016	----