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Preliminary Qualitative Analysis on mtDNA in *Astyanax fasciatus* Populations Cuvier, 1819 (Teleostei; Characidae) Indicate Population Distinctiveness

Daniele Aparecida Matoso[1], Cesar Martins[2], Roberto Ferreira Artoni[3*] and Pedro Manoel Galetti Jr[4]

[1]*Departamento de Genética Universidade; Federal do Paraná; 81531-990; Curitiba - PR - Brasil.* [2]*Departamento de Morfologia; Instituto de Biociências; Universidade Estadual Paulista Júlio de Mesquita Filho; Distrito de Rubião Junior s/nº; 18618-000; Botucatu - SP - Brasil.* [3]*Departamento de Biologia Estrutural, Molecular e Genética*; *Universidade Estadual de Ponta Grossa; Av. Carlos Cavalcanti, 4748; 84030-900; Ponta Grossa - PR - Brasil.* [4]*Departamento de Genética e Evolução; Universidade Federal de São Carlos; Via Washington Luís, km 235; C. P.: 676; 13565-905; São Carlos - SP - Brasil*

ABSTRACT

A preliminary qualitative analysis of genetic variability status in Astyanax fasciatus *(Cuvier, 1819) from upper Tibagi River headwaters and Vila Velha State Park (VVSP) was carried out by enzymatic digestion (RFLP) of D-Loop region from mtDNA. The results showed that Tibagi and VVSP populations were genetically different.*

Key words: Neotropical fish; RFLP; conservation genetics

INTRODUCTION

Fish from the genus *Astyanax* are small characid, characterised by an extensive phenotypic and genetic diversity, likely related to their biological characteristics or even to the non-monophyletic status of the group (Morelli et al., 1983, Moreira-Filho and Bertollo, 1991, Garutti and Britski, 2000). The Vila Velha State Park (VVSP), located in the Tibagi River basin, is characterised by the presence of natural rocky formations assigned sinkholes dated from Pleistocene. These sinkholes are very deep collapsed-like rocks covered by a nearly 50m freshwater column and populations of *Astyanax fasciatus* are abundantly found inside the sinkholes. The aim of this work was to analyse the D-Loop mtDNA region from *A. fasciatus* by Restriction Fragment Length Polymorphism (RFLP) to assess the genetic variation those populations.

MATERIAL AND METHODS

Samples of 38 individuals of *A. fasciatus*, a neotropical freshwater fish, were taken from three sites of Vila Velha State Park and from one site in the main channel of upper Tibagi River (Figure 1).

[*]Author for correspondence: rfartoni@pesquisador.cnpq.br

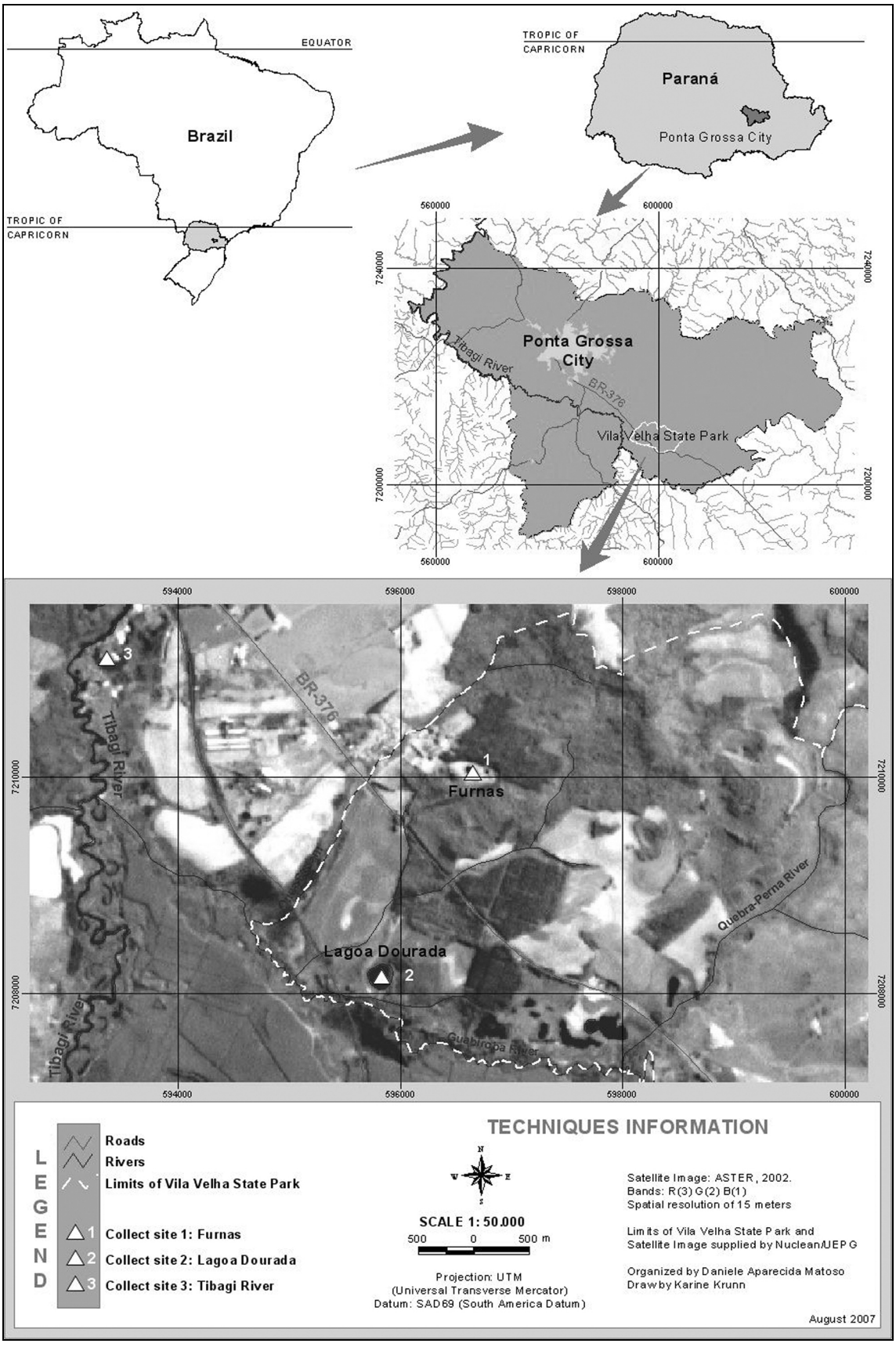

Figure 1 - Collect sites VVSP (Furna 1, 2 and Lagoa Dourada) and Tibagi River.

These samples were used for DNA extraction following Sambrook et al., (1989). The control region mtDNA was amplified using primers FTTF 5' GCC TAA GAG CAT CGG TCT TGT AA 3' (forward) and F12R 5' GTC AGG ACC ATG CCT TTG TG 3' (reverse) (Sivasundar et al., 2001). PCR amplifications were performed in a final volume of 50 µl containing 2mM $MgCl_2$, 2.5 U of *Taq* DNA Polymerase, 200 µM of each dNTPs, 4 µM each primer, 1x buffer and 200 ng of template DNA. Amplifications were carried out at the following conditions: pre-denaturation at 94ºC for 5 min followed by 35 cycles denaturation-annealing-elongation (92ºC, 1 min; 50ºC, 1 min; 70ºC, 1 min 30 s) and a final elongation step of 72ºC for 5 min. The enzymatic digestion from D-Loop PCR amplified region was performed for all the samples (data not shown) with seven restriction enzymes randomly chosen: *Eco*RI, *Eco*RV, *Alu*I, *Pvu*II, *Rsa*I, *Hae*III, and *Cla*I, incubated overnight at 37ºC in separated solutions in a final volume of 10 µl containing 7 µl of amplified DNA, 2 µl of ultra pure water, 0.5 µl of buffer solution, 0.5 µl enzyme 1x (10U/µl). The digestion products were run in agarosis gel 2%, 100 volts for 1 hour and stained ethidium bromide (10 mg/ml). The voucher specimens were deposited in the Museu de Zoologia of Universidade Estadual de Londrina (MZUEL 1792, 1794, 1795, 3735). The haplotypes were qualitatively organized according to the following phenotypes: A = absence of restriction site, B = presence of one restriction site, C = presence of two restriction sites, D = presence of three restriction sites (Fig. 2, Table I). Due to low number of individuals sampled (N= 38) and also the disproportionate cross-section (2 specimens from Furna 1, 20 specimens from Furna 2, 5 specimens from Lagoa Dourada and 11 specimens from Tibagi River), no statistical test was performed to avoid any bias interpretation or mistaken conclusions.

RESULTS AND DISCUSSION

A DNA fragment of approximately 1.3 Kb was obtained after PCR amplification (Fig. 2). Two haplotypes were observed in the Tibagi population showing an intrapopulational variation. On the other hand, individuals from VVSP (Furna 1, Furna 2 and Lagoa Dourada) presented only one haplotype. Two possibilities could be considered concerning this result: there was a gain of new haplotypes among individuals from Tibagi River, and/or, the VVSP populations showed genetic structure due to founder effect (Table 1).

In a previous RAPD analysis a greater genetic similarity between the three VVSP populations was also observed when compared to Tibagi population, and it was suggested that in the main channel of the Tibagi River a superposition population might exist, while genetic drift might have occurred in the isolated VVSP populations (Matoso et al., 2004). The conservation status of *A. fasciatus* in the VVSP region must be concerned. Some years ago, the Furna 1 fish population was totally extinct due to eutrophization by droppings during the reproductive period of birds, resulting in the depletion of dissolved oxygen in column water. Thus, the remaining Furna 2 population characterised by genetic isolation could be considered endangered since they were restricted to the smaller sinkholes (Artoni and Almeida, 2001, Artoni and Shibatta, 2006). Population differentiation has already been revealed in other species of the genus *Astyanax*.

Sofia et al., (2006) found low gene flow and genetic divergence between *A. scabripinnis* from Cambé River basin. Similarly, Leuzzi et al., (2004) reported genetic divergence between the populations of *A. altiparanae* of Paranapanema River and concluded that it was necessary to consider such biological units for a whole conservation of this species. In the present study, results obtained with the enzymatic digestion from D-Loop mtDNA allowed to identify only few haplotypes for each locality. At present the causes of reduced number of haplotypes remained unknown. However possible causes for this reduced number of haplotypes could be related with several environmental and evolutionary factors such as the genetic drift during the VVSP sinkholes occupation, endogamic depletion by absence of gene flow among the populations of Furnas, positive selection or hitchhiking effect. These propositions are hypothesis that need to be better investigated in future studies using other genetics tools and also larger samples. Because fish population extinction was already registered

in the VVSP area (Furna 1), and the Furna 2 and Lagoa Dourada populations also showed only one haplotype, it could be strongly suggested that these populations could be threatened and that genetic structure diagnoses should be helpful for needed conservation polices.

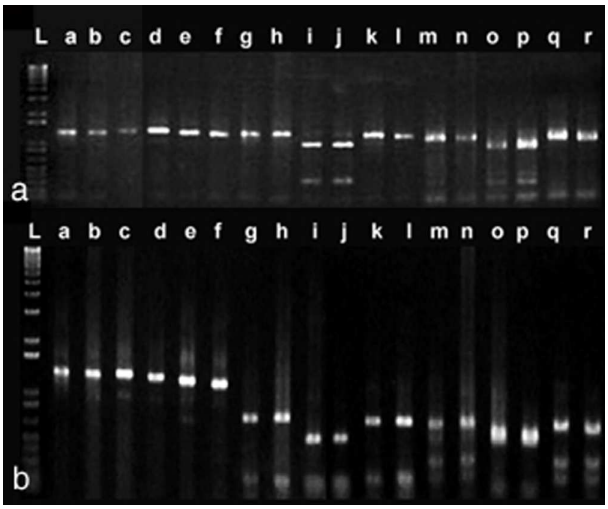

Figure 2 - Enzymatic digestion by RFLP of D-Loop mtDNA region of *Astyanax fasciatus* with approximately 1.3 Kb. a) endonucleases *Eco*RI (a – f), *Eco*RV (g – l), *Alu*I (m – r). b) endonucleases *Pvu*II (a – f), *Rsa*I (g – l), *Hae*III (m – r). Furna 1 (a, b, g, h, m, n); Tibagi River (c, d, i, j, o, p), Lagoa Dourada (e, f, k, l, q, r). L - ladder.

Table 1 - Haplotypes of D-loop mtDNA of *Astyanax fasciatus*. A) Absence of restriction site; B) Presence of one restriction site; C) Presence of two restriction sites, D) Presence of three restriction sites. ∗ Digestion not enforced. † Extinct population.

	Localities			
Enzyme	**Furna 1 †**	**Furna 2**	**Lagoa Dourada**	**Tibagi River**
*Eco*RI	A	∗	A	A
*Eco*RV	A	A	A	B
*Alu*I	A	∗	A	B
*Pvu*II	A	A	A	A
*Rsa*I	B	B	B	D
*Hae*III	C	C	C	D
*Cla*I	∗	∗	A	A/B

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RESUMO

Uma análise qualitativa da variabilidade genética em *Astyanax fasciatus* (Cuvier, 1819) do alto Rio Tibagi e Parque Estadual de Vila Velha (PEVV) foi conduzida por digestão enzimática (RFLP) da

região D-Loop do mtDNA. Os resultados evidenciaram que essas populações são geneticamente diferentes.

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