

**COMPARATIVE CYTOGENETIC STUDY AMONG THREE FISH SPECIES:
AN ANALYSIS FROM A WATER DIVIDE REGION**

**ESTUDO CITOGÊNÉTICO COMPARATIVO EM TRÊS ESPÉCIES DE PEIXES:
UMA ANÁLISE DE UMA REGIÃO DE DIVISOR DE ÁGUAS**

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ABSTRACT

The aim of the present study was to characterize chromosomal differences between two populations separated in the past for a water divide. Processes of differentiation have been identified in the species studied, being justified by the separation of streams from past for a water divide. Karyotypic data were presented for *Astyanax altiparanae*, *Geophagus brasiliensis* and *Gymnotus carapo* populations of two different river systems of the upper Parana: ribeirão do Pântano (Tietê basin) and ribeirão do Feijão (Mogi Guaçu basin) in the São Carlos region, state of São Paulo. The *Astyanax altiparanae*, presented a diploid number of $2n=50$ chromosomes. The *Geophagus brasiliensis* diploid numbers was $2n=48$ chromosomes and *Gymnotus carapo* a diploid number of $2n=54$ chromosomes. An analysis was made of constitutive heterochromatin by C-banding, detection of nucleolar organizer regions and the technique of Hybridization *in situ* Fluorescent with probes 18S rDNA and 5S rDNA. These results indicate that the species analysed showed differences of karyotype, showing that each population follows its own evolution, as a result of a restrictive process of gene flow.

Keywords: Chromosome differentiation. Citotaxonomy. Fluorescent *in situ* hybridization. Biogeographic variation.

RESUMO

O principal objetivo deste estudo foi de caracterizar as diferenças cromossômicas entre duas populações isoladas por um divisor de águas. Processos de diferenciação

têm sido identificados nas espécies estudadas, os quais são justificados por processos de separação de bacias. Dados cariotípicos foram apresentados para populações de *Astyanax altiparanae*, *Geophagus brasiliensis* e *Gymnotus carapo* de dois diferentes rios do sistema da bacia do Alto Paraná: ribeirão do Pântano (bacia do Tietê) e ribeirão do Feijão (bacia do Mogi Guaçu) na região de São Carlos-SP. *Astyanax altiparanae* apresentou um $2n=50$ cromossomos. No *Geophagus brasiliensis* o número diplóide foi de $2n=48$ e em *Gymnotus carapo* o $2n$ foi de 54 cromossomos. Uma análise dos marcadores de heterocromatina constitutiva, detecção das regiões organizadoras de nucléolo e Hibridação *in situ* Fluorescente com sondas de rDNA 18S e 5S também foi realizada. Estes resultados indicam que as espécies analisadas mostraram diferenças cariotípicas, evidenciando que cada população segue seu curso evolutivo como consequência do processo de restrição do fluxo gênico.

Palavras-chave: Diferenciação cromossômica. Citotaxonomia. Hibridação *in situ* Fluorescente. Variação biogeográfica.

Introduction

Comparative cytogenetics studies among fish populations from the different river basins, situated in a closely region of the headwaters barriers has provided to generate hypotheses about karyotype evolution, species dispersal, biogeography, and has wide application in analysis cytotoxicomic (CENTOFANTE et al., 2003; VICARI et al., 2005; 2006; 2008; FERREIRA-NETO et al., 2009; ARTONI et al., 2008; 2009). This type of analysis, when well designed, has shown a greater sensitivity of karyotypic comparison among populations by restricting the study area and factually detecting the possible differences between populations that have evolved in different river basins, although in a limited geographical area.

The region of São Carlos (São Paulo, Brazil) represents a water divide, where two streams, ribeirão do Feijão e ribeirão do Pântano, were once united, were then separated by a barrier, which served as an impediment to gene flow between species were present, and therefore have been suffering processes of differentiation.

This study describes and compares cytogenetically three species (*Astyanax altiparanae*, *Geophagus brasiliensis* and *Gymnotus carapo*) from the Feijão and Pântano stream, pertaining to Alto Paraná basin, but isolated in adjacent headwaters barriers. The main objective was the

karyotype characterization, as well as a cytogenetic comparison among the different samples in order to establish possible evolutionary/biogeographical relationships among them.

Material and Methods

Cytogenetic data were analyzed in two populations, Feijão and Pântano stream, São Carlos, São Paulo state-Brazil. The species collected were *A. altiparanae*, *Geophagus brasiliensis* and *Gymnotus carapo*. All specimens are currently deposited in the ichthyological collection of the Zoology Museum of the Universidade Federal de São Carlos (vouchers numbers 17497, 17521, 17537).

Were analyzed 17 specimens of *A. altiparanae*, eight from the Feijão stream (5 males and 3 females) and nine from the Pântano stream (5 males and 4 females). Were collected eight specimens of *Geophagus brasiliensis* (3 males and 5 females) from the Feijão stream and ten individuals (4 males and 6 females) from the Pântano stream and; *Gymnotus carapo* were obtained four specimens collected from the Feijão stream (2 males and 2 females) and 16 specimens from the Pântano stream (9 males and 7 females). The procedures were in compliance with the Ethics Committee on Animal Experimentation of the Universidade Estadual de Ponta Grossa (process n°: 04741/08).

Chromosome preparations were obtained from anterior kidney cells using *in vivo* colchicine treatment (BERTOLLO et al., 1978). Constitutive heterochromatin was visualized by C-banding (SUMNER, 1972). Nucleolar organizing regions (NORs) were detected using silver nitrate staining (Ag-NORs), according to Howell and Black (1980), and fluorescent *in situ* hybridization (FISH) to locate the 18S rDNA sites on the chromosomes. An 18S rDNA probe (nearly 1,800 bp) generated by PCR of nuclear DNA from the fish *Prochilodus argenteus* (HATANAKA; GALETTI Jr., 2004) was used. A 5S rDNA probe from the fish *Leporinus elongatus* (MARTINS; GALETTI, 1999) was employed to map the 5S rDNA sites on the chromosomes. Both probes were labelled with 14-dATP biotin by nick translation following the manufacturer's instructions (Bionick Labelling System - Invitrogen). The FISH signals were visualized according to Pinkel et al. (1986) and analyzed in an Olympus BX50 epifluorescence microscope. Chromosome images were captured using the software CoolSNAP-Pro (Media Cybernetics).

Nearly 30 metaphases per specimen were analyzed to determine the diploid chromosome number and the karyotype structure. Chromosomes were classified as m (metacentric), sm (submetacentric), st (subtelocentric), and a (acrocentric), according to Levan et al. (1964).

Results

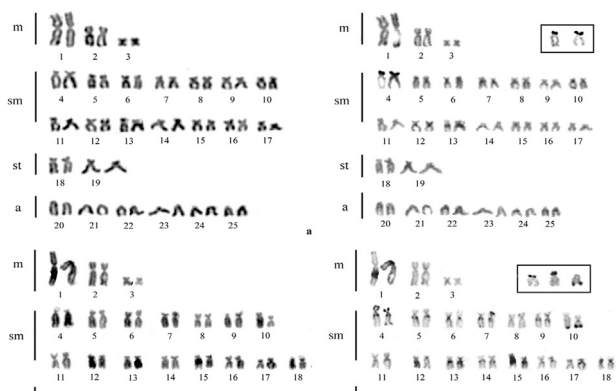
The both *A. altiparanae* populations analysed (Feijão and Pântano streams) had a karyotype structure consisting of $2n=50$ chromosomes (Fig. 1). These karyotypes were homomorphic, with an absence of morphologically differentiated sex chromosomes. Specimens of the Pântano stream had a fundamental number $FN=88$, organized in $6m+28sm+4st+12a$. The population of the Feijão stream showed $FN=94$ and karyotypic formulae $6m+30sm+8st+6a$ (Fig. 1). After C-banding, heterochromatic blocks were observed in the centromeric or pericentromeric regions of all chromosome pairs and in the telomeric regions

of some chromosomes with small differences among the analyzed populations (Fig. 1b and d, respectively).

The Ag-NORs were located terminally on the short arm of a submetacentric chromosome pair in Pântano stream specimens (figure 1b, box), and up to three chromosomes in specimens of ribeirão do Pântano (figure 1d, box). The 18S ribosomal sites were located by FISH in the terminal region of the short arm in one subtelocentric pair in Pântano stream specimens (Figure 2a), and in four terminal sites (one pair st in the terminal position of short arm, one submetacentric chromosome position on the short arm and one chromosome in the terminal region of the long arm) in the Feijão stream specimens (Figure 2b). In both *A. altiparanae* populations, the 5S rDNA sites were located in the proximal region of the long arm of the submetacentric pair (Figure 2c and 2d).

Both *Geophagus brasiliensis* populations showed $2n=48$ chromosomes, $FN=54$ and similar karyotypic formulae organised in $6sm+42st/a$ (Figure 3). C-positive bands located preferentially in centromeric/pericentromeric regions were evidenced in both *G. brasiliensis* populations, with minor differences among them (Figure 3 b and 3d).

Figure 1. Karyotypes of the *Astyanax altiparanae*: (a, b) from the ribeirão do Feijão and, (c, d) from the ribeirão do Pântano. (a, c) Conventionally- Giemsa stained; (b, d) sequentially C-banded chromosomes and Ag-NORs (in box). Bar = 5 μ m.



The Ag-NORs and FISH with an 18S rDNA probe, were located in the telomeric region of the short arm of st/a pair 6 for the both *G. brasiliensis* samples (Figure 3 – box and Figure 4 a, b). Already, the 5S rDNA sites were located in the interstitial region of the long arm of st/a pair (Figure 4 c, d).

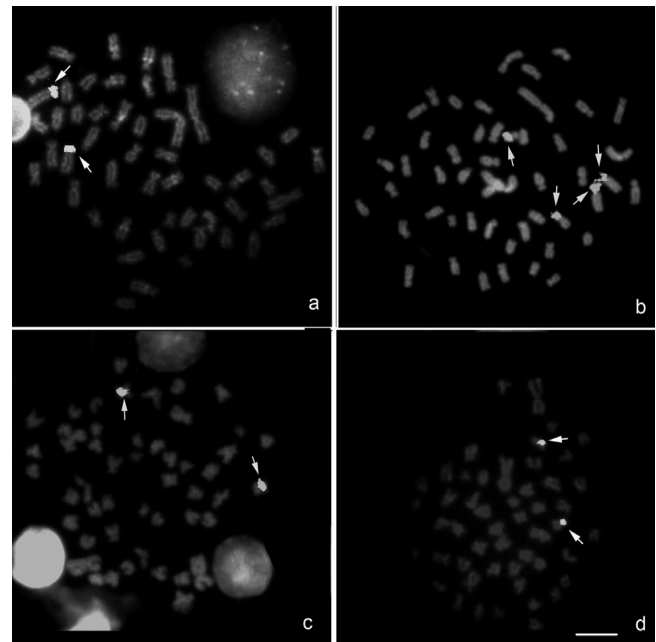
The *Gymnotus carapo* showed karyotypic structure composed of the $2n=54$ chromosomes, $FN=106$ and, karyotypic formulae organized in $44m+8sm+2st/a$ in both populations (Fig. 5a and 5c). The C-bands showed all chromosomes contained C-positive heterochromatic segments in the centromeric/pericentromeric region and, no or negligible inter-population differences were observed in both populations (Fig. 5b and 5d).

The NORs were situated in the interstitial region of the short arm in the first metacentric pair evidenced by silver nitrate impregnation (Fig. 5 – in boxes) and by FISH with 18S rDNA probing (Fig. 6 a and 6 b). The 5S rDNA-positive FISH sites were located in pericentromeric region of the almost all chromosomes of the complement (Fig. 6 c and 6 d).

Discussion

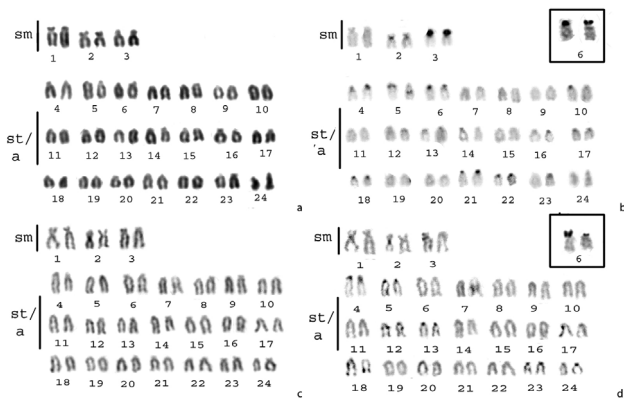
This study associated cytogenetic data, biogeographic aspects, and evolutive biology of fishes species located in the headwaters of two isolated rivers basins (Pântano and Feijão streams) in São Paulo State, Brazil. A great diversity in chromosome number has been found in the genus *Astyanax*, ranging from $2n=36$ in *A. schubarti* to $2n=50$ in *A. scabripinnis*, *A. bimaculatus*, *A. altiparanae* (FERREIRA-NETO et al., 2009; KANTEK et al., 2009) and in *Gymnotus* genus (MARGARIDO et al., 2007; LACERDA; MAISTRO, 2007; MILHOMEN et al., 2007). In turn, the Cichlidae species like *Geophagus* and others genus showed a minor chromosomal diversification, usually restrict to micro alterations in the chromosomes (VICARI et al., 2006; PERAZZO et al., 2010). Thus, the detection of diploid and fundamental number and the implementation of procedures generating molecular markers have been important to evolutionary analysis and cytotaxonomy these fishes.

Figure 2 - Fluorescent *in situ* hybridization in *Astyanax altiparanae*: (a, b) 18S rDNA probing and (c, d) 5S rDNA probing in the populations ribeirão do Feijão and Pântano, respectively. Bar = 5 μ m.



The presence of $2n=50$ chromosomes is considered the modal number for *Astyanax altiparanae* (FERNANDES; MARTINS-SANTOS, 2004; DOMINGUES et al., 2007; FERREIRA-NETO et al., 2009), as observed in this study too. Studies in *A. altiparanae* populations of the different basins present small karyotype diversification. Inter-population karyotypic differences related to chromosomal morphology, especially concerning submetacentric and acrocentric chromosomes, could be observed in the populations from the upper Paraná basin and Iguaçú river (FERREIRA-NETO et al., 2009). Non-robertsonianos events, such as pericentric inversions could be hypotetized in the karyotypic evolution of this species (DOMINGUES et al., 2007). Thus, the karyotypic variability found in this study and among populations may be related to the occupation of different habitats that were geographically isolated, and once occurring genetic variation, these can hardly be shared.

Figure 3 - Karyotypes of the *Geophagus brasiliensis*: (a, b) from the ribeirão do Feijão and, (c, d) from the ribeirão do Pântano. (a, c) Conventionally-Giemsa stained; (b, d) sequentially C-banded chromosomes and Ag-NORs (in box). Bar = 5 μ m.

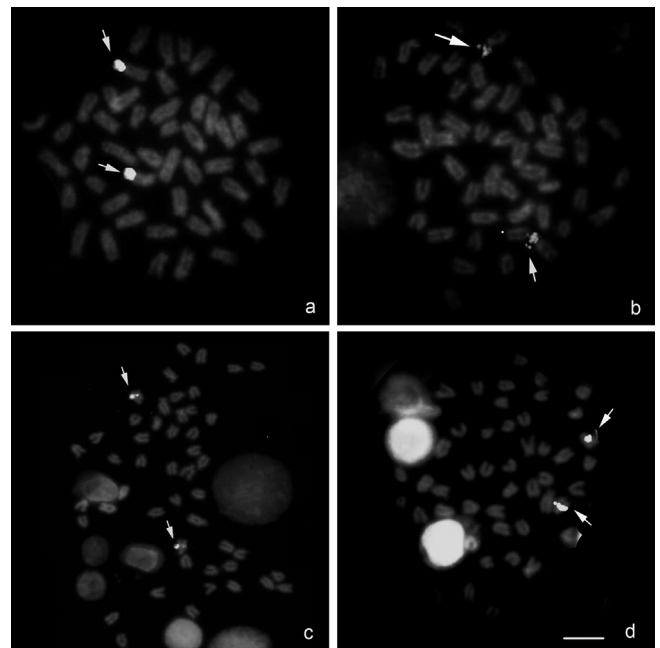


The heterochromatin distribution in *A. altiparanae* shows small differences among populations, usually found in pericentromeric or centromeric regions of all chromosome pairs and a few chromosomes in the telomeric region similar to that observed in other populations (FERNANDES; MARTINS-SANTOS, 2004; DOMINGUES et al., 2007; FERREIRA-NETO et al., 2009). The result differs from other species of the genus as *A. scabripinnis*, *A. fasciatus* and *A. janeiroensis*, which have an intense diversification of the karyotype in pairs submetacentric and acrocentric provided by amplification of repetitive DNA sequences AS51 (MANTOVANI et al. 2004; VICARI et al., 2008b; KANTEK et al., 2009). The maintenance of the standard distribution of constitutive heterochromatin in *A. altiparanae*, along with the great diversity of FN found for the species, support the hypothesis that structural variation could not be explained by amplification of heterochromatic sequences.

The location of Ag-NOR and 18S rDNA sites follows a common trend in the genus *Astyanax*, with multiple ribosomal sites on chromosomes distributed st/a (MANTOVANI et al., 2000; FERRO et al., 2001; FERNANDES; MARTINS-SANTOS, 2006; VICARI et al., 2008a; 2010). The Ag-NOR and FISH with 18S rDNA probe revealed a single pair marked in the Pântano stream population, while the population of ribeirão do Feijão presented

multiple telomeric bands in different chromosomes, like studies by other authors in the species (PACHECO et al., 2001; DOMINGUES et al., 2007). The Ag-NORs and 18S rDNA FISH data also revealed variations between both populations of this species probably resulting from the current gene flow restriction.

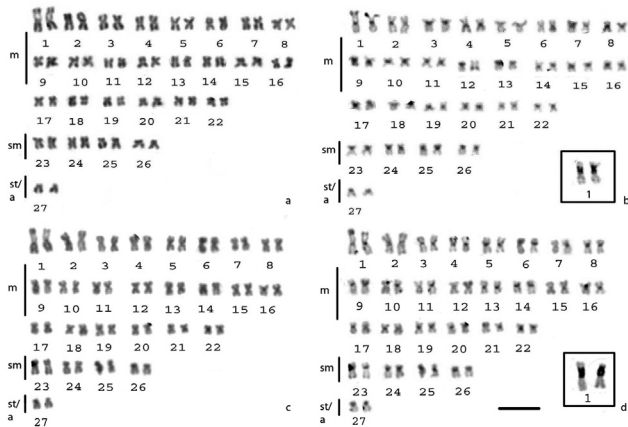
Figure 4 - Fluorescent *in situ* hybridization in *Geophagus brasiliensis*: (a, b) 18S rDNA probing and (c, d) 5S rDNA probing in the populations ribeirão do Feijão and Pântano, respectively. Bar = 5 μ m.



While interpopulation variations occur in the number of sites of the 18S rDNA in *A. altiparanae*, only one chromosome pair showed sm carrier site of 5S rDNA in the proximal long arm (FERNANDES; MARTINS-SANTOS, 2004, 2006; DOMINGUES et al., 2007; PERES et al., 2008; FERREIRA-NETO et al., 2009; this study). Studies in different species of the genus *Astyanax* had 5S rDNA sites preserved in two chromosome pairs, one metacentric and one acrocentric, marked in the proximal region (FERRO et al., 2001; ALMEIDA-TOLEDO et al., 2002; MANTOVANI et al., 2005), suggesting a possible synapomorphy for the genus. In this study, both populations show one pair submetacentric bearing 5S rDNA sites in proximal region. Studies in *Astyanax* sp. and *A. janeiroensis*, the 5S rDNA site was located in a single pair in the proximal

region of the long arm, comparable to that found in complex *A. scabripinnis* (KANTEK et al., 2007; VICARI et al., 2008b, respectively). Mantovani et al. (2000) proposed that sites of 5S rDNA in the proximal position of the long arm to indicate a pattern conserved for these genes in *Astyanax*. Thus, the chromosomes bearing the 5S rDNA sites are comparable between the populations of *A. altiparanae* studied, however, differ in chromosome morphology and number of sites of those found in other populations of the genus.

Figure 5 - Karyotypes of the *Gymnotus carapo*: (a, b) from the ribeirão do Feijão and, (c, d) from the ribeirão do Pântano. (a, c) Conventionally- Giemsa stained; (b, d) C-banded chromosomes and Ag-NORs (in box). Bar = 5 µm.



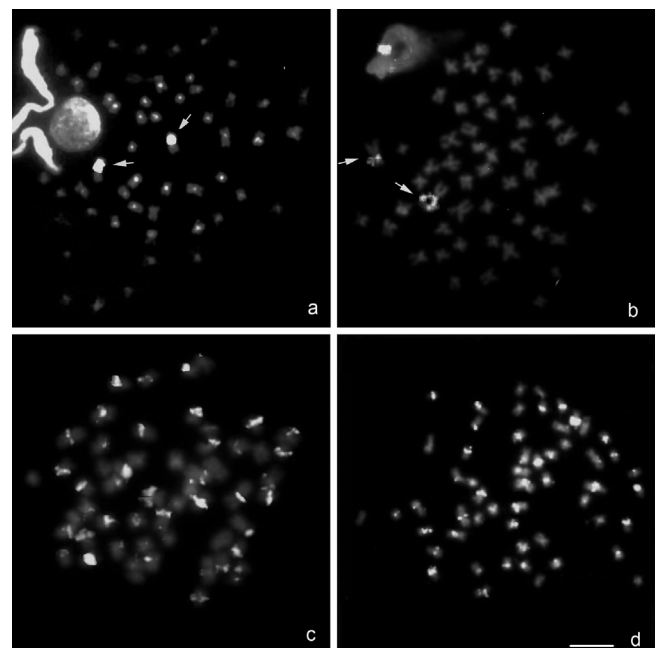
In the Cichlidae family, karyotypic analysis have demonstrated the maintenance of the diploid number in most species. As observed to the most of Neotropical cichlids, the diploid number of 48 chromosomes was constant in both *G. brasiliensis* here analyzed, corroborating with the ancestral condition for both Cichlidae and Perciformes (THOMPSON 1979; FELDBERG; BERTOLLO, 1985; MARTINS et al., 1995; VICARI et al., 2006; RONCATI et al., 2007; PERAZZO et al., 2010). However, this diploid number can vary between 32 and 60 chromosomes (FELDBERG et al., 2003) in some cichlids species.

Kullander (1998) based on morphological and molecular, proposed that “*Geophagus brasiliensis*” seems to be a complex of species. Cytogenetic studies reviewed by Feldberg et al. (2003) corroborate this

proposition. Both *G. brasiliensis* populations here analyzed had a diploid number of $2n=48$ and $FN=54$. At the same time, several studies in *G. brasiliensis* show a conserved $2n=48$ chromosomes and a variable NF (52 – 56) (PIRES et al., 2008).

Feldberg et al. (2003) demonstrated in Neotropical Cichlids that constitutive heterochromatin is in the pericentromeric region of most or all chromosomes of the complement. This ditribution pattern was observed to Feijão and Pântano streams populations. Vicari et al. (2006) showed a peculiar pattern of distribution of heterochromatin in *G. brasiliensis* river Jaguaiaíva presenting some pairs conspicuous heterochromatic blocks and intersticiais. So, in *G. brasiliensis* populations minors chromosomal divergences could be atributed to heterochromatin expansion or exchange how observed in both populations analyzed.

Figure 6 - Fluorescent *in situ* hybridization in *Gymnotus carapo*: (a, b) 18S rDNA probing and (c, d) 5S rDNA probing in the populations ribeirão do Feijão and Pântano, respectively. Bar = 5 µm.



Yet, *G. brasiliensis* has the characteristic of single NOR located on subtelocentric chromosomes in terminal regions of the short arm (BRUM et al., 1998; FELDBERG et al., 2003; VICARI et al.,

2006; LARISSA et al., 2008). Vicari et al. (2006) analyzed by fluorescence *in situ* hybridization three different populations of *G. brasiliensis* on the number and location of sites of 18S rDNA and 5S. They found that the chromosomes carrying the 18S ribosomal sites were homologous among the species and/or populations, always located on the short arm of chromosome pair st/a. Still, the ribosomal sites among different samples could present a length polymorphism. For the 5S rDNA site the two populations of *G. brasiliensis* in this study exhibited a subtelocentric chromosome pair with interstitial sites of the long arm. These results were the same as found by Vicari et al. (2006) in others populations of this species, being a characteristic well conserved in the group.

According to Oliveira et al. (1988), populations that have lower mobility and number of individuals are more stable in relation to their macro-structure, since the gene flow is lower, thus providing a higher rate of fixation of chromosomal abnormalities. The data obtained confirm that *Geophagus brasiliensis* has preserved cytogenetic features in relation to other studied populations of this species, and the differences are mainly based on karyotypic formulae, may be the result of chromosomal rearrangements such as pericentric inversions, indicating a possible initial process of speciation.

Amongst the Gymnotiformes are 33 valid species described of *Gymnotus* and many additional undescribed are known in collections (ALBERT et al., 2005; CRAMPTON et al., 2006). A large variability in chromosome number is found among species and populations of this fish group (ALMEIDA-TOLEDO et al., 2007), which may vary from $2n=24$ in *Apteronotus albifrons* (ALMEIDA-TOLEDO et al., 1981) to $2n=54$ for *Gymnotus carapo* (FORESTI et al., 1984). Data from other studies show that in Gymnotiformes, the karyotype is more divergent than conservative (SILVA; MARGARIDO, 2005).

The reduction in chromosome number among species is an evolutionary trend in the group, with *G. carapo* presenting $2n=54$ chromosomes appear to be basal (FERNANDES-MATIOLI et al., 1998). In this study, specimens of *Gymnotus carapo* collected

in the Feijão and Pântano streams presented the same $2n=54$ chromosomes. This possible variation in diploid number in *Gymnotus* genus and in Gymnotidae can be explained by chromosome fusion events.

The distribution of constitutive heterochromatin in Gymnotiformes is generally characterized by large blocks scattered over almost all chromosomes, with few interpopulational divergences (FERNANDES-MATIOLI; ALMEIDA-TOLEDO, 2001; LACERDA; MAISTRO, 2007; MARGARIDO et al., 2007, present study). The FISH with 5S rDNA probe shows the most of the chromosomes signals in the pericentromeric region in both *G. carapo* populations. These 5S rDNA sites were similar to pericentromeric heterochromatin location. The occurrence of multiple sites of the 5S rDNA in *G. carapo* are possibly due to a family of disperse repetitive DNA like SINE (short interspaced nuclear element) inserted in the NTS (non transcribed spacer) (that could be evaluated in pseudogenes derived from 5S rDNA, described in others species studies in the Gymnotidae (CLARO, 2008, SILVA, 2010) and in the Erythrinidae family (FERREIRA et al., 2007).

The NOR localization is a good taxonomic tool among Gymnotidae because different species showing $2n=54$ m/sm chromosomes can be distinguished by the NOR chromosome bearing and localization (GALETTI Jr. et al., 1984; FERNANDES-MATIOLI et al., 1998; PEREIRA et al., 2002; LACERDA; MAISTRO, 2007). The location of NORs present in one chromosome pair is a common feature found among the Gymnotiformes (FONTELES et al., 2008). However, in both *G. carapo* populations here analyzed, as well as other populations already studied, had a simple NOR system, located in the interstitial region of the first metacentric pair.

The data of the present study demonstrates the importance of cytogenetic studies, molecular and other morphological comparisons divisor headwaters areas to better understand the evolution of the group, especially when considering the complexity of the *Astyanax*, *Geophagus* and *Gymnotus* genus. Thus, this study compared three

species from two different headwaters basins (Pântano and Feijão streams), suggesting species maintenance identity, and the minor differences founded in some karyotypes species suggest each population follows its own evolutionary course with few or no one gene flow.

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REFERENCES

- ALBERT, J. S.; THORSEN, D. H.; LOVEJOY, N. R. Phylogenetic systematics and historical biogeography of the Neotropical electric fish *Gymnotus* (Teleostei: Gymnotidae). **Systematics and Biodiversity**, v.2, p. 375-417, 2005.
- ALMEIDA-TOLEDO, L. F.; DANIEL-SILVA, M. F. Z.; MOYSÉS, C. B.; FORESTI, F. Chromosome variability in Gymnotiformes (Teleostei, Ostariophysi). In: PISANO, E, OZOUF-COSTAZ, C.; FORESTI, F.; KAPOOR, B. D. (Eds.). **Fishes cytogenetics** Science Publishers, Enfield, 2007, p.16-39.
- _____; DANIEL-SILVA, M. F. Z.; MOIZÉS, C. B.; FONTELES, S. A. B.; LOPES, C. E.; AKAMA, A.; FORESTI, F. Chromosome evolution in fish: sex chromosome variability in *Eigenmannia virescens* (Gymnotiformes: Sternopygidae). **Cytogenetics Genome Research**, v.99, p.164-169, 2002.
- _____; FORESTI, F.; TOLEDO-FILHO, A. S. Constitutive heterochromatins and nucleolus organizer regions in the Knifefish, *Apteronotus albifrons*. **Experientia**, v.37, p.953-954, 1981.
- ARTONI, R. F.; GROSS, M. C.; SCHNEIDER, C.; VICARI, M. R.; ALMEIDA, M. C.; MATOSO, D. A. Epifluorescence and light microscopy evidencing structural and functional polymorphism of ribosomal DNA in fish (Teleostei: *Astyanax fasciatus*). **Micron** (Oxford), v. 39, p. 1156-1159, 2008.
- ARTONI, R. F.; VICARI, M. R.; ALMEIDA, M. C.; MOREIRA-FILHO, O.; BERTOLLO, L. A. C. Karyotype diversity and fish conservation of southern field from South Brazil. **Reviews in fish biology and fisheries**, v.19, p.393-401 2009.
- BERTOLLO, L. A. C.; TAKAHASHI, C. S.; MOREIRA-FILHO, O. Cytotaxonomic consideration on *Hoplias lacerdae* (Pisces, Erythrinidae). **Brazilian Journal of Genetics**, v.1, p.103-120, 1978.
- BRUM, M. J. I.; MURATORI, C. F. M. L.; LOPES, P. R. D.; VIANNA, P. R. G. A ictiofauna do sistema lagunar de Maricá (RJ). **Acta Biologica Leopoldensia**, v.16, n.2, p. 45-55, 1998.
- CENTOFANTE, L.; BERTOLLO, L. A. C.; JUSTI, A. J.; MOREIRA-FILHO, O. Correlation of chromosomal and morphologic characters in two *Astyanax* species (Teleostei: Characidae). **Ichthyological Exploration of Freshwaters**, v.14, p.361-368, 2003.
- CLARO, F. L. ***Gymnotus carapo e Gymnotus sylvius* (Teleostei: Gymnotidae): uma abordagem citogenética-Molecular**. Dissertação de Mestrado (Universidade de São Paulo), 2008.
- CRAMPTON, W. G. R. Evolution of electric signal diversity in Gymnotiform fishes. II. Signal design. In: LADICH, F., COLLIN, S. P., MOLLER, P., KAPOOR B. G. **Communication in fishes**. Enfield-NH: Science Publishers 2006, p.697-731.
- DOMINGUES, M. S.; VICARI, M. R.; ABILHOA, V.; WAMSER, J. P.; CESTARI, M. M.; BERTOLLO, L. A. C.; ALMEIDA, M. C.; ARTONI, R. F. Cytogenetic and comparative morphology of two allopatric populations of *Astyanax altiparanae* Garutti & Britski, 2000 (Teleostei, Characidae) from upper rio Paraná basin. **Neotropical Ichthyology**, v.5, p.37-44, 2007.
- FELDBERG, E.; PORTO, J. I. R.; BERTOLLO, L. A. C. Chromosomal changes and adaptation of Cichlid fishes during evolution, 258-308. In: VAL, A. L.; KAPOOR, B. G. **Fish adaptations**. Science Publishers, INC. New Dehli & New York, 2003, p.418.
- _____; BERTOLLO, L. A. C. Karyotypes of 10 species of Neotropical Cichlids (Pisces, Perciformes). **Caryologia**, v.38, p.257-268, 1985.
- FERNANDES-MATIOLI, F. M. C.; MARCHETTO, M. C. N.; ALMEIDA-TOLEDO, L. A.; TOLEDO-FILHO, S. A. High intraspecific karyological conservation in four species of *Gymnotus* (Pisces: Gymnotiformes) from Southeastern Brazilian basins. **Caryologia**, v.51, p. 221-234, 1998.
- _____. M. C.; ALMEIDA-TOLEDO, L. F. A molecular phylogenetic analysis in *Gymnotus* species (Pisces:

- Gymnotiformes) with inferences on chromosome evolution. **Caryologia**, v.54, n.1, p.23-30, 2001.
- FERNANDES, C. A.; MARTINS-SANTOS, I. C. Cytogenetics studies in two populations of *Astyanax altiparanae* (Pisces, Characiformes). **Hereditas**, v.141, p.328-332, 2004.
- _____. Mapping of the 18S and 5S ribosomal RNA genes in *Astyanax altiparanae* Garutti & Britski, 2000 (Teleostei, Characidae) from the upper Paraná river Basin, Brazil. **Genetics and Molecular Biology**, v.29, n.3, p.464-468, 2006.
- FERREIRA, I. A.; BERTOLLO, L. A. C.; MARTINS, C. Comparative chromosome mapping of 5S rDNA and 5S HindIII repetitive sequences in Erythrinidae fishes (Characiformes) with emphasis on the *Hoplias malabaricus* 'species complex'. **Cytogenetic and Genome Research**, v.118, p.78-83, 2007.
- FERREIRA NETO, M.; VICARI, M. R.; CAMARGO, E. F.; ARTONI, R. F.; MOREIRA-FILHO, O. Comparative cytogenetics among populations of *Astyanax altiparanae* (Characiformes, Characidae, *Incertae sedis*). **Genetics and Molecular Biology**, v. 32, p. 792-796, 2009.
- FERRO, D. M.; MOREIRA-FILHO, O.; BERTOLLO, L. A. C. Nucleolar Organizing regions, 18S and 5S rDNA in *Astyanax scabripinnis* (Pisces, Characidae): populations distribution and functional diversity. **Genetica**, v.110, p.55-62, 2001.
- FONTELES, S. B. A.; LOPES, C. E.; AKAMA, A.; FERNANDES, F. M. C.; PORTO-FORESTI, F.; SENHORINI, J. A.; DANIEL-SILVA, M. F. Z.; FORESTI, F.; ALMEIDA-TOLEDO, L. F. Cytogenetic characterization of the strongly electric Amazonian eel, *Electrophorus electricus* (Teleostei, Gymnotiformes), from the Brazilian rivers Amazon and Araguaia. **Genetics and Molecular Biology**, v.31, p.1-9, 2008.
- FORESTI, F.; ALMEIDA-TOLEDO, L. F.; TOLEDO-FILHO, A. S. Chromosome studies in *Gymnotus carapo* and *Gymnotus sp.* (Pisces, Gymnotidae). **Caryologia**, v.37, p.141-146, 1984.
- GALETTI Jr., P. M.; FORESTI, F.; BERTOLLO, L. A. C.; MOREIRA-FILHO, O. Characterization of eight species of Anostomidae (Cypriniformes) fish on the basis of the nucleolar organizing region. **Caryologia**, v.37, p.401-406, 1984.
- HATANAKA, T.; GALLETTI Jr., P. M. Mapping of the 18S and 5S ribosomal RNA genes in the fish *Prochilodus argenteus* Agassiz, 1829 (Characiformes, Prochilodontidae). **Genetica**, v.122, p.239-244, 2004.
- HOWEL, W. M.; BLACK, D. A. Controlled silver sataining of organizer regions with a protective colloidal developer: a 1-step method. **Experientia**, v.36, p.1014-1015, 1980.
- KANTEK, D. L. Z.; NOLETO, R. B.; FENOCCHIO, A. S.; CESTARI, M. M. Cytotaxonomy, heterochromatic polymorphism and natural triploidy of a species of *Astyanax* (Pisces, Characidae) endemic to the Iguaçú River Basin. **Brazilian Archives of Biology and Technology**, v.50, p.67-74, 2007.
- _____.; VICARI, M. R.; PERES, W. A. M.; CESTARI, M. M.; ARTONI, R. F.; BERTOLLO, L. A. C.; MOREIRA-FILHO, O. Chromosomal location and distribution of As51 satellite DNA in five species of the genus *Astyanax* (Teleostei, Characidae.). **Journal of Fish Biology**, v. 75, p. 408-421, 2009.
- KULLANDER, S. O.; FERREIRA, E. J. G. A new *Satanoperca* species (Teleostei, Cichlidae) from the Amazon River Basin in Brazil. **Cybium**, v.12, n.4, p.343-355, 1988.
- LACERDA, M. C. V.; MAISTRO, E. L. Cytogenetic Analysis of Three Sympatric *Gymnotus* Species (Teleostei: Gymnotidae) from the Fundo Stream, MG, Brazil. **Cytologia**, v.72, p.89-93, 2007.
- LARISSA, P. B.; GIULIANO-CAETANO, L.; DIAS, A. N. Karyotype similarities among two populations of *Geophagus brasiliensis* (Perciformes, Cichlidae) from the Tibagi river basin/PR/Brazil. **Caryologia**, v.6, n.1/2, p.135-138, 2008.
- LEVAN, A.; FREDGA, K.; SANDBERG, A. A. Nomenclature for centromeric positions on chromosomes. **Hereditas**, v.52, p.202-220, 1964.
- MANTOVANI, M.; ABEL, L. D. S.; MESTRINER, C. A.; MOREIRA-FILHO, O. Accentuated polymorphism of heterochromatin and nucleolar organizer regions in *Astyanax scabripinnis* (Pisces, Characidae): tools for understanding karyotypic evolution. **Genetica**, v.109, p.161-168, 2000.
- _____.; ABEL, L. D. S.; MESTRINER, C. A.; MOREIRA-FILHO, O. Evidence of the differentiated structural arrangement of constitutive heterochromatin between two populations of *Astyanax scabripinnis* (Pisces, Characidae). **Genetics and Molecular Biology**, v.27, p.536-542, 2004.
- _____.; ABEL, L. D. S.; MOREIRA-FILHO, O. Conserved 5S and variable 45S rDNA chromosomal localization revealed by FISH in *Astyanax scabripinnis* (Pisces, Characidae). **Genetica**, v.123, p.211-216, 2005.
- MARGARIDO, V. P.; BELLAFRONT E.; MOREIRA-FILHO, O. Cytogenetic analysis of three sympatric *Gymnotus* (Gymnotiformes, Gymnotidae) species verifies invasive species in the Upper Paraná River basin, Brazil. **Journal of Fish Biology**, v.70, p.155-164, 2007.
- MARTINS, C.; PORTELLA-CASTRO, A. L. B.; JULIO Jr., H. F. Chromosome analysis of 5 species of the Cichlidae family (Pisces, Perciformes) from the Paraná river. **Cytologia**, v.42, p.535-537, 1995.
- MARTINS, C.; GALETTI Jr., P. M. Chromosomal localization of 5S rDNA genes in *Leporinus* Fish (Anostomidae, Characiformes). **Chromosome Research**, v.7, p.363-367, 1999.
- MILHOMEM, S. S. R.; PIECZARKA, J. C.; CRAMPTON, W. R.; SOUZA, A. C. P.; CARVALHO, JR.; J. R.; NAGAMACHI, C. Y. Differences in karyotype between two sympatric species

- of *Gymnotus* (Gymnotiformes:Gymnotidae) from the eastern amazon of Brazil. **Zootaxa**, v.1397, p. 55-62, 2007.
- OLIVEIRA, C.; ALMEIDA-TOLEDO, L. F.; FORESTI, F.; BRITSKI, H. A.; TOLEDO-FILHO, S. A. Chromosome formulae of neotropical freshwater fishes. **Brazilian Journal of Genetics**, v.11, p.577-624, 1988.
- PACHECO, R. B.; GIULIANO-CAETANO, L.; DIAS, A. L. Cytotypes and multiple NORs in an *Astyanax altiparanae* population (Pisces, Tetragonopterinae). **Chromosome Science**, v.5, p.109-114, 2001.
- PERAZZO, G. X.; NOLETO, R. B.; VICARI, M. R.; MACHADO, P. C.; GAVA, A.; CESTARI, M. M. Chromosomal studies in *Crenicichla lepidota* and *Australoheros facetus* (Cichlidae, Perciformes) from extreme Southern Brazil. **Review in Fish Biology and Fisheries**, (in press). Doi: 10.1007/s11160-010-9170-x
- PERES, W. A. M.; BERTOLLO, L. A. C.; MOREIRA-FILHO, O. Physical mapping of the 18S and 5S ribosomal genes in nine Characidae species (Teleostei, Characiformes). **Genetics and Molecular Biology**, v.31, p.222-226, 2008.
- PEREIRA M. A.; OLIVEIRA, C.; FORESTI, F.; MAISTRO, E. L. Cytogenetic and Nucleolar Content Analysis in Anostomidae Fishes from the Sapucaí River, Minas Gerais State, Brazil. **Cytologia**, v.67, p.289-296, 2002.
- PINKEL, D.; STRAUME, T.; GRAY, J. W. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. **Proc Natl Acad Sci, USA**, v.83, p.2934-2938, 1986.
- PIRES, L. B.; GIULIANO-CAETANO, L.; DIAS, A. L. Karyotype similarities among two populations of *Geophagus brasiliensis* (Perciformes, Cichlidae) from the Tibagi river basin/PR/Brazil. **Caryologia**, v.61, p.135-138, 2008.
- RONCATI, H. A.; PASTORI, M. C.; FENOCHIO, A. S. Cytogenetic studies and evolutive considerations on fishes of the family Cichlidae (Perciformes) from Parana River (Argentina). **Cytologia**, v.72, p.379-384, 2007.
- SILVA, E. B.; MARGARIDO, V. P. An X1X1X2X2/X1X2Y multiple sex chromosome system in a new species of the genus *Gymnotus* (Pisces, Gymnotiformes). **Environmental Biology of Fishes**, v.73, p.293-297, 2005.
- SILVA, M. **Evolução da diferenciação cromossômica entre os sexos no gênero *Gymnotus* (Gymnotiformes, Gymnotidae)**. Programa de Pós-Graduação em Biologia Evolutiva (dissertação de mestrado). Universidade Estadual de Ponta Grossa, Ponta Grossa, 2009.
- SUMNER, A. T. A simple technique for demonstrating centromeric heterocromatin. **Experimental Cell Research**, v.75, p.304-306, 1972.
- THOMPSON, K. W. Cytotaxonomy of 4 species of Neotropical Cichlidae. **Copeia**, v.1979, p.679-691, 1979.
- VICARI, M. R.; ARTONI, R. F.; BERTOLLO, L. A. C. Comparative cytogenetics of *Hoplias malabaricus* (Pisces, Erythrinidae): A population analysis in adjacent hydrographic basins. **Genetics and Molecular Biology**, v.28, p.103-110, 2005.
- _____; ARTONI, R. F.; MOREIRA-FILHO, O.; BERTOLLO, L. A. C. Basic and Molecular Cytogenetics in freshwater Cichlidae (Osteichthyes, Perciformes). Karyotypic conservatism and divergence. **Caryologia**, v.59, n.3, p.260-266, 2006.
- _____; NOLETO, R. B.; ARTONI, R. F.; MOREIRA-FILHO, O.; BERTOLLO, L. A. C. Comparative cytogenetics among species of the *Astyanax scabripinnis* complex. Evolutionary and biogeographical inferences. **Genetics and Molecular Biology**, v. 31, p.173 - 179, 2008a.
- _____; ARTONI, R. F.; MOREIRA-FILHO, O.; BERTOLLO, L. A. C. Co-localization of repetitive DNAs and silencing of major rDNA genes. A case report in the fish, *Astyanax janeiroensis*. **Cytogenetic and Genome Research**, v.122, p.67-72, 2008b.
- _____; NOGAROTO, V.; NOLETO, R. B.; CESTARI, M. M.; CIOFFI, M. B.; ALMEIDA, M. C.; MOREIRA-FILHO, O.; BERTOLLO, L. A. C.; ARTONI, R. F. Satellite DNA and chromosomes in Neotropical fishes: Methods, applications and perspectives. **Journal of Fish Biology**, v.76, p.1094-1116, 2010.