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Cytogenetic Analysis of Three Sympatric *Gymnotus* Species (Teleostei: Gymnotidae) from the Fundo Stream, MG, Brazil

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Summary Three sympatric species of *Gymnotus* from the Fundo stream, a small tributary of the Sapucaí river, Minas Gerais State, Brazil, were studied in relation to their karyology. *Gymnotus sylvius* presented $2n=40$ chromosomes (36 m/sm+4 st/a), *Gymnotus* sp. presented $2n=50$ (26 m/sm+24 st/a) and *Gymnotus paraguensis* had $2n=54$ (50 m/sm+4 st/a). C-banding demonstrated positively stained heterochromatic blocks in the centromeric position of few chromosomes on *G. sylvius* and in the centromeric region of all chromosomes on *G. paraguensis* samples. The nucleolus organizer region (NOR) was located on the short arm of one st chromosome pair in *G. sylvius* and *Gymnotus* sp., and in the interstitial position on the short arm of the pair number one and below the centromere on a third chromosome on *G. paraguensis*. The cytogenetic data obtained indicate that *Gymnotus* sp. represent a new *Gymnotus* specie with a karyotypic constitution never observed on others species from this genus. Some aspects related to the chromosome diversification of these *Gymnotus* are discussed.

Key words *Gymnotus paraguensis*, *Gymnotus sylvius*, *Gymnotus* sp., NOR-banding, C-banding.

Gymnotus is the most geographically widespread of all Gymnotiformes, extending from the Rio Salado in the Pampas of Argentina (36°S) to the Rio San Nicolás of southeastern Chiapas, Mexico (18°N), and is present in the continental waters of all South and Middle American countries except Chile and Belize (Albert 2001). Is the only genus of Gymnotidae and encompasses about 32 valid species (Albert and Crampton 2003, Albert *et al.* 2005) although many undescribed species probably still exist (Nelson 1994). *Gymnotus* is most diverse in the Amazon Basin where 18 species are known to reside including three undescribed forms (Albert *et al.* 2005). Fishes of this group present nocturnal habits, are extremely territorial and aggressive (Lundberg *et al.* 1987).

Gymnotus species have been studied from the cytogenetic viewpoint. *G. carapo* present $2n=54$ chromosomes, *G. sylvius* $2n=40$, *G. pantherinus* and *G. inaequilabiatus* sharing $2n=52$ chromosomes, *Gymnotus pantanal* (cited as *Gymnotus* sp.) present $2n=40$ for females and $2n=39$ for males being the unique *Gymnotus* specie with multiple sex chromosome system, and high intraspecific karyological conservation have been observed when different *Gymnotus* populations are compared (Foresti *et al.* 1984, Fernandes-Matioli *et al.* 1998, Fernandes *et al.* 2005, Sánchez *et al.* 2004, Silva and Margarido 2005). An extensive chromosome variability was initially described for *G. carapo*, including chromosome numbers varying from $2n=52$, $2n=48$, $2n=46$ to $2n=42$, that were described for different populations on this species sampled from Amazon basin, the Paraná basin and from the East basin (Almeida-Toledo 1978, Foresti *et al.* 1984, Foresti 1987). However, Albert and Crampton (2003) after the re-examination of these *G. carapo* populations made a re-description of them and described some new species. Natural triploidy was observed on *G. carapo*

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samples from Mogi Guaçu river (Upper Paraná basin) (Fernandes-Matioli *et al.* 1998) and a wide variability of the NOR-bearing chromosomes was observed on six *G. carapo* populations from the Upper Paraná river system in Brazil (Fernandes-Matioli *et al.* 1997), now redescribed as a new species, *G. paraguensis* (Albert and Crampton 2003). In order to understand better the *Gymnotus* evolution, some species also were analyzed considering its electric organ discharge (Fernandes-Matioli 1996), nuclear $(GGAC)_n$ microsatellite (Fernandes-Matioli *et al.* 2000) and the partial sequence of the subunit 2 of the NADH-dehydrogenase mitochondrial gene (Fernandes-Matioli and Almeida-Toledo 2001).

The purpose of this paper is to describe the diploid number, chromosome formula, constitutive heterochromatin pattern, and NOR location of three sympatric *Gymnotus* species collected in a small tributary of Sapucaí river, Minas Gerais, Brazil.

Materials and methods

A cytogenetic survey was performed on eleven specimens of *Gymnotus sylvius* (5 females, 2 males and 4 with undetermined sex), 16 specimens of *G. paraguensis* (7 females, 2 males and 7 with undetermined sex) and 2 specimens of *Gymnotus* sp. (2 males) collected in the Fundo stream, Alfenas town, State of Minas Gerais, Brazil. Voucher specimens are deposited in the fish collection of the Laboratory of Genetics, UNIFENAS, Alfenas, Minas Gerais, Brazil.

Chromosome preparation: Animals were injected with 0.025% colchicine (1 ml/100 g body weight) 50 min before sacrifice. Anterior kidney was extracted and minced in a 0.075 M KCl solution, placed in a incubator at 37°C for 23 min and then centrifuged. The supernatant was discarded and the cell pellet was fixed twice in a methanol : acetic acid (3 : 1) solution, ressuspended in fresh fixative and dropped on heated slides (Foresti *et al.*, 1993). Chromosome preparations were stained with a 5% Giemsa staining solution. Chromosome morphology was determined on the basis of arm ratios as proposed by Levan *et al.* (1964) and the chromosomes were classified as metacentric/submetacentric (m/sm) and subtelocentric/acrocentric (st/a), and were paired by decreasing size order. C-banding was performed by the method of Sumner (1972), and silver-staining of the nucleolus organizer regions was performed by the technique of Howell and Black (1980).

Results

Gymnotus sylvius analyzed in this study presented $2n=40$ chromosomes, being 36 m/sm+4 st/a; *G. paraguensis* presented $2n=54$, being 50 m/sm+4 st/a; and *Gymnotus* sp. presented $2n=50$ (26 m/sm+24 st/a) (Figs. 1a, 2a and 3a, respectively). In *Gymnotus* sp., the first chromosome pair is clearly bigger than the second pair (Fig. 3a). A secondary constriction was evidenced on the terminal position on the short arm of the 19th st chromosome pair in *G. sylvius* and in the same position on the first chromosome pair of the karyotype in *G. paraguensis* (Figs. 1a and 2a, respectively).

Ag-NORs was located in the terminal region on the short arm of the 19th st pair of *G. sylvius* with an evident size heteromorphism (coinciding with the region of secondary constriction) (Fig. 1, in the inset), on the same position on a st chromosome pair of *Gymnotus* sp. with size heteromorphism (Fig. 3b), and on the short arm of the first chromosome pair also with size heteromorphism (coinciding with the region of secondary constriction) of *G. paraguensis* being observed additionally one sm chromosome with Ag-NOR below the centromere in about 5% of the cells of the 2 examples (Fig. 2, in the inset).

With regard to C-band pattern, heterochromatic blocks were detected in the centromeric or pericentromeric regions of few chromosomes, and in the terminal position on the short arm of the pairs 10 and 19 on *G. sylvius* (Fig. 1b). In *G. paraguensis* C-band blocks were located in the cen-

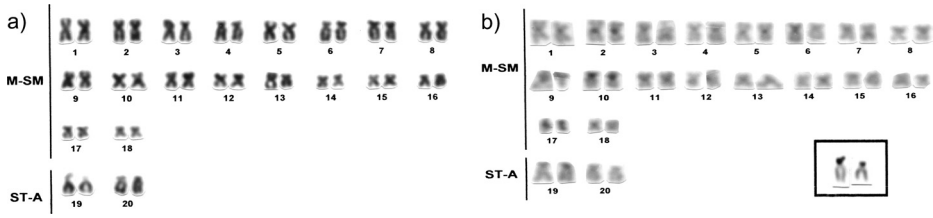

Fig. 1. Karyotypes of *Gymnotus sylvius* from the Fundo stream after conventional Giemsa staining (a) and C-banding (b). In the inset, NOR-bearing chromosomes.

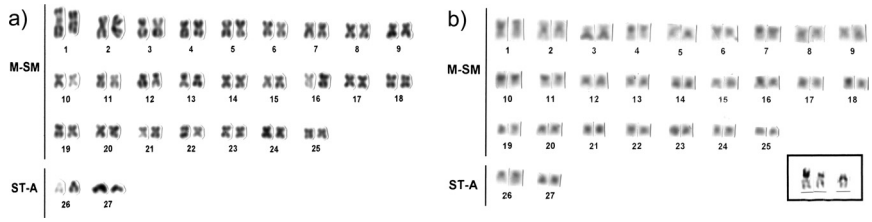

Fig. 2. Karyotypes of *Gymnotus paraguensis* from the Fundo stream after conventional Giemsa staining (a) and C-banding (b). In the inset, NOR-bearing chromosomes.

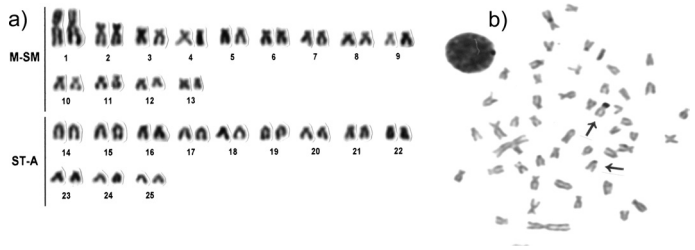

Fig. 3. Karyotype of *Gymnotus* sp. from the Fundo stream after conventional Giemsa staining (a), and (b) somatic metaphase showing NOR-bearing chromosomes (arrows).

tromeric position of all chromosomes, in the short arm of the pairs 1 and 10, and in the long arm of the pair 3 (Fig. 2b). It was not possible to obtain C-banding pattern from *Gymnotus* sp. samples.

Discussion

G. sylvius analyzed in the present study showed the chromosome number, morphology and C-banding pattern very similar to that described for *G. sylvius* from different collected sites from Upper Paraná system (Fernandes-Matioli *et al.* 1998). However, the Ag-NOR signals observed on other populations studied by Fernandes-Matioli *et al.* (1998) were always located on interstitial position on the short arm of one sm pair, while in the population from the Fundo stream, the Ag-NOR signals were detected on the terminal position. Since heterochromatic regions are known to contain highly repetitive DNA and to present heteromorphisms (Sumner 1990), our observation reinforces the suggestion of Fernandes-Matioli *et al.* (1998) that heterochromatic regions bordering NOR tends to favor chromosome rearrangements in these particular chromosome areas.

On the same way, karyological data obtained on *G. paraguensis* from Fundo stream were similar to that observed on *Gymnotus carapo* (re-described as *G. paraguensis*, Albert and Crampton 2003) from others Southeastern Brazilian basins (Fernandes-Matioli *et al.* 1998) and indicate to

correspond to the same specie. However, some individuals from Fundo stream showed a third NOR-bearing chromosome, not observed on this other populations studied. The karyological data obtained of this two *Gymnotus* species from Fundo stream confirm the high intraspecific karyotypic conservation of some *Gymnotus* species from different localities on Southeastern Brazilian basins observed by Fernandes-Matioli *et al.* (1998). Chromosome analysis of *Gymnotus* species/populations using FISH with rDNA probes could clarify whether heteromorphism of the NOR number represents a new feature to the *G. paraguensis* from the Fundo stream or corresponds only to technical artifacts related to NOR banding.

On the other hand, *Gymnotus* sp. from Fundo stream presented $2n=50$ chromosomes, being 26 m/sm and 24 st/a. This chromosome number and morphology were never described for others *Gymnotus* species/populations cytogenetically studied and clearly show that this population represents a new *Gymnotus* specie. This result reinforces the idea proposed by Nelson (1994) that many undescribed *Gymnotus* species probably still exist. The karyological data available in literature shows that *G. pantherinus* from Upper Paraná system and from East basins, with $2n=52$ chromosomes (46 m/sm+6 st/a) and NOR sites also on one st chromosome pair (Fernandes-Matioli *et al.* 1998), is a *Gymnotus* species that present karyotypic constitution more similar to this new specie from Fundo stream. If this correlation is correct, and considering the proposition that $2n=52$ could be considered a basal feature for *Gymnotus* group (Fernandes-Matioli and Almeida-Toledo 2001), we may assume the hypothesis that a centric fusion involving 2 st/a chromosome pairs and pericentric inversions on some m/sm chromosomes could be responsible for the karyotypic diversification observed between *Gymnotus* sp. and *G. pantherinus*. The centric fusion proposed also could explain the big size of the first chromosome pair of *Gymnotus* sp. from Fundo stream which is not observed on others *Gymnotus* species till now cytogenetically studied.

Several *Gymnotus* species represent cryptical species with hard identification by conventional taxonomic parameters (Nelson 1994). The three sympatric *Gymnotus* species studied in the present work could be discriminated by the Ag-NOR-bearing chromosomes showing that NOR sites are specie/specific for then. This point of view evidenced that NOR-banding is a good cytotaxonomic tool for this fish group as well as observed on *Leporinus* species, when different species presenting $2n=54$ m/sm chromosomes can be differentiated by NOR sites location (Galetti-Jr. *et al.* 1984, Pereira *et al.* 2002).

The cytogenetic data obtained in the present study shows the existence of three species of *Gymnotus* living in sympatry, one of them still undescribed. This is not a new situation on fishes. Maistro *et al.* (2000) analyzing specimens of *Astyanax scabripinnis* from the Tamanduá stream, State of São Paulo, Brazil, observed specimens with $2n=50$ and $2n=48$ chromosomes, also with differences in the chromosome morphology. Paintner-Marques *et al.* (2002) observed the presence of 2 cytotypes on specimens of *Bryconamericus* aff. *exodon* from Três Bocas stream, Paraná State, Brazil, where the examples not differentiated taxonomically presented clear differences in the chromosome morphology.

The data presented here and others in the literature clearly show the importance of association of cytogenetic, molecular and others parameters with the morphological analysis for a better understanding of phylogenetic and chromosome evolution relationship, specially on groups that present taxonomic complexity like *Gymnotus*.

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