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Cytogenetic Analysis of A-, B-chromosomes and ZZ/ZW Sex Chromosomes of *Characidium gomesi* (Teleostei, Characiformes, Crenuchidae)

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Summary Different cytogenetic techniques were used to analyze the chromosomes of *Characidium gomesi* with the main objective of comparing the base composition of ZZ/ZW sex-chromosomes, B-chromosomes and the heterochromatin of A-chromosomes. The results of digestion of chromosomes with *Alu*I restriction endonuclease (RE), silver and CMA_3 staining, C-banding and fluorescence *in situ* hybridization (FISH) with the 18S rDNA probe suggested the existence of compositional differences between the heterochromatin of ZZ/ZW sex-chromosomes, A- and B-chromosomes, and indicated the presence of different numbers and morphology of B-chromosomes in the samples of this population.

Key words Fish cytogenetics, *Characidium gomesi*, B-chromosomes, ZZ/ZW sex chromosome system, Heterochromatin differentiation.

The genus *Characidium* belongs to the Crenuchidae family and comprises small sized fishes which rarely exceed a standard length of 100 mm (Buckup 1991). This group includes about 58 nominal species but cytogenetic data are available for only few species of the genus *Jobertina* and *Characidium* (Miyazawa and Galetti-Jr. 1994, Maistro *et al.* 1998, Centofante *et al.* 2001). Karyotypic data for the genus *Characidium* have shown a relatively stable pattern, with all species having a diploid number of $2n=50$ and biarmed chromosomes (meta-submetacentrics). Sex chromosome systems, presence of B chromosomes as well as natural triploidy were verified in some of the populations and species analyzed (Miyazawa and Galetti-Jr. 1994, Maistro *et al.* 1998, Centofante *et al.* 2001).

Several reports have shown the occurrence of B chromosomes among Neotropical fish species (Salvador and Moreira-Filho 1992, Vicente *et al.* 1996). These chromosomes vary in size from microchromosomes, such as those in *Prochilodus lineatus* (Maistro *et al.* 2000), to medium-sized chromosomes, as in *Rhamdia hilarii* (Fenocchio and Bertollo 1990, Maistro *et al.* 2002), and even macrochromosomes, as in some *A. scabripinnis* populations (Salvador and Moreira-Filho 1992, Maistro *et al.* 2001). In spite of several new reports about B-chromosomes, little information is available about their structure in fish (Vicente *et al.* 1996, Maistro *et al.* 2000, 2001, 2002).

In the present study several cytogenetic techniques were employed to analyze the chromosomes of *Characidium gomesi* in order to compare the general structures and base compositions of A- and B-sets of chromosomes of this species.

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Materials and methods

Thirty-one specimens (21 females and 10 males) of *Characidium gomesi* from the Pardo River, Botucatu, State of São Paulo, Brazil, were analyzed. The fish were identified and have been deposited in the fish collection of the Laboratory of Genetics and Fish Biology, UNESP, Botucatu, São Paulo, Brazil.

Mitotic chromosomes were obtained from a cell suspension of anterior kidney and gill tissues, as described by Foresti *et al.* (1993). The arm ratio limits suggested by Levan *et al.* (1964) were employed for the classification of chromosome types. 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). Chromomycin A_3 staining was performed by the method of Schweizer (1980). Restriction endonuclease (RE) *Alu*I suspended in the appropriate buffer was applied at the concentration of 0.3 U/μl to air-dried chromosome preparations, and slides were incubated in a moist chamber at 37°C for 4 h, washed in distilled water and stained with 5% Giemsa for 5–10 min. As a control, cells on other slides were treated with *Alu*I buffer without enzyme. The 18S rDNA probe was produced by the polymerase chain reaction (PCR) using genomic DNA from *Prochilodus affinis* and the procedure for fluorescent *in situ* hybridization (FISH) was carried out as described by Wasko and Galetti-Jr. (2000).

Results and discussion

The specimens of *Characidium gomesi* analyzed in the present study had $2n=50$ chromosomes (32M+18SM) confirming the results obtained by Maistro *et al.* (1998) in which the species was identified as *Characidium* cf. *fasciatum*. A characteristic of this species is the presence of biarmed chromosomes, usually meta-submetacentrics (Miyazawa and Galetti-Jr. 1994, Maistro *et al.* 1998, Centofante *et al.* 2001). The only exception was observed for *Characidium pterostictum* which showed a medium-sized subtelocentric pair in its complement (Miyazawa and Galetti-Jr. 1994). Some fishes presented 1 to 4 ST/A B-chromosomes (Figs. 1, 2). The interindividual numerical chromosome variation observed in *C. gomesi* from the Pardo river is probably due the occurrence of meiotic instability of the supernumerary chromosomes, as was proposed for *Prochilodus lineatus* (Pauls and Bertollo 1990, Maistro *et al.* 2000) and *Rhamdia hilarii* (Maistro *et al.* 2002) populations.

The karyotype of *Characidium gomesi* showed a small amount of heterochromatin after C-banding, with a few chromosomes showing some more clearly visible bands (Fig. 1b). C-banding patterns were basically characterized by the presence of small heterochromatic blocks in the centromeric region of most chromosomes. B chromosomes were heterochromatic. This pattern is also a general feature observed in the chromosomes of other *Characidium* samples (Miyazawa and Galetti-Jr. 1994, Centofante *et al.* 2001). A conspicuous block was observed in the interstitial position near the centromere in the two chromosomes of pair 19 in males; in females, one of the chromosomes of pair 19 was totally heterochromatic (Fig. 1b). This heteromorphic chromosome pair observed in females indicate the occurrence of a ZZ/ZW system of sex chromosome, confirming the results initially obtained by Maistro *et al.* (1998). A similar kind of sex chromosome mechanism was detected in *Characidium gomesi* from the Paiol Grande stream, where the W chromosome is also completely heterochromatic but, differently from what was observed in the present study, the W is smaller than the Z chromosome (Centofante *et al.* 2001).

One NOR-bearing chromosome pair was detected in the most of the samples investigated by silver staining. NORs were located terminally on the long arm of pair 17 and showed positive C-banding. In one specimen, few cells showed a third chromosome with the Ag-NOR in the terminal position (Fig. 2a). Comparable Ag-NOR positions were described for a *C. gomesi* population from

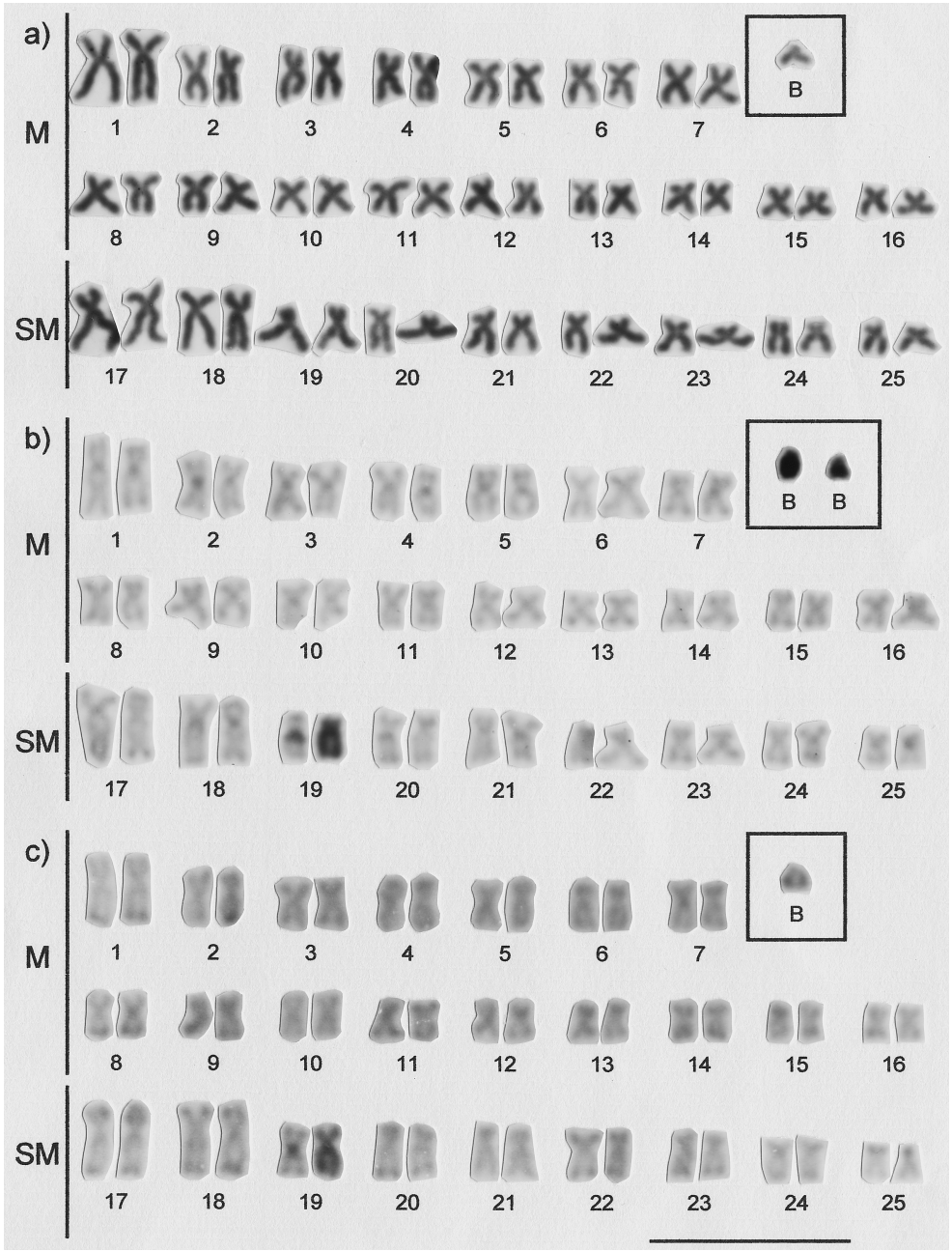

Fig. 1. Karyotypes of *Characidium gomesi* from the Pardo River. a) Giemsa staining (in the inset, the B-chromosome), b) C-banding (in the inset, two B-chromosomes), c) After *Alu*I restriction enzyme cleavage (in the inset, the B-chromosome); Bar=10 μm.

the Paiol Grande stream (Centofante *et al.* 2001). These authors also could distinguish the karyotypes of *C. gomesi* and *C.* cf. *gomesi* by the NOR position. Though each population/species of *Characidium* shows more frequently one NOR-bearing chromosome pair, some specimens from some populations have shown multiple Ag-NORs (Miyazawa and Galetti-Jr. 1994, Maistro *et al.* 1998). Miyazawa and Galetti-Jr. (1994) suggest that the multiplicity of NORs observed in some *Characidium gomesi* populations could be a particular chromosomal characteristic of differentia-

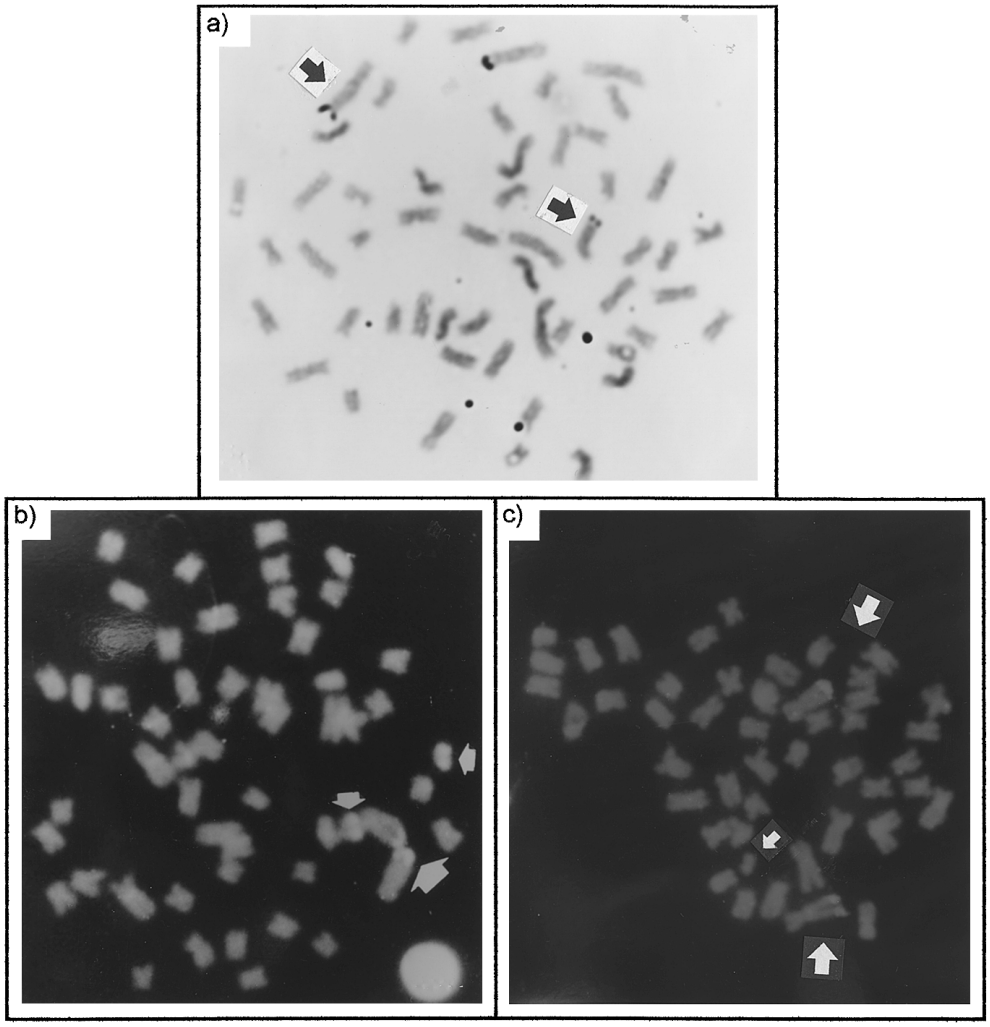

Fig. 2. Somatic metaphases of *Characidium gomesi* from the Pardo River. a) Ag-NOR bearing chromosomes (arrows), b) CMA_3 staining, c) FISH with the 18S rDNA probe (On b and c, small arrows shows B-chromosomes and large arrows shows NOR bearing chromosomes).

tion, or may be due to processes of regulation of genetic activity of ribosomal cistrons, since only active cistrons in the preceding interphase are stained by silver (Miller *et al.* 1978).

The Ag-NORs were positively stained by the CMA_3 fluorochrome (Fig. 2b), suggesting that the rDNA loci of *C.* cf. *gomesi* may contain spacer sequences or NOR-associated heterochromatin rich in GC base pairs, as found in other fish species (Amemiya and Gold 1986, Maistro *et al.* 2002, among others). CMA_3 staining also revealed that the heterochromatin located at the terminal position on the long arm of the micro B-chromosomes was rich in GC base pairs. Similar results were obtained for the *Astyanax scabripinnis* species from the Cascatinha stream (Maistro *et al.* 2001).

Since some animals and plants have NORs in the B-chromosomes (Green 1990, Beukeboom 1994) we applied FISH with an 18S rDNA probe to check if the strong staining of the extremities of the B-chromosomes of *C. gomesi* after CMA_3 staining could represent inactive NORs in this population. FISH applied to slides from four specimens revealed only one chromosome pair as described after Ag-NOR staining and did not show the presence of rDNA sites on B-chromosomes (Fig. 2c). Maistro *et al.* (1998) previously observed the presence of multiple NORs in some speci-

Table 1. Different types of heterochromatin in *Characidium gomesi*

Types	C-band	CMA_3	*Alu*I restriction enzyme	FISH 18S probe	Chromosome location
1	+ totally	+ only at the end of the long arm	+ only at the end of the long arm	−	B-chromosome
2	+ totally	−	+	−	W sex chromosome
3	+	+	+	+	NOR-bearing chromosomes
4	+	−	+ majority of the chromosomes	−	Telomeric bands
5	+	−	− except on Z chromosome	−	Centromeric bands

+, Presence of differential staining (band); −, negative band.

mens of *C. gomesi* (identified as *C.* cf. *fasciatum*). Since the FISH technique was applied only to few specimens and revealed only a single chromosome pair with rDNA sites, the divergence observed could indicate the existence of interindividual polymorphism of the NOR chromosome number. Further studies are necessary to clarify this question.

When fixed metaphase chromosomes were digested with *Alu*I restriction endonuclease, the telomeric regions on A-chromosomes, the heterochromatin attached to the centromere the Z and W chromosomes and the terminal region of the long arm of the ST/A B-chromosome were not digested, virtually resembling the C-banding pattern, except for a few differences (Fig. 1c). These findings suggest that the heterochromatin of the telomeres, Z, W and the extremity of the long arm of B-chromosomes are poor in *Alu*I sequences. The dark staining of some chromosome regions after restriction endonuclease treatment may be related to the absence of DNA sequences recognized by the enzyme in these regions or to inaccessibility of highly compacted heterochromatin for digestion, or to both factors (Mezzanotte and Ferrucci 1984). The results obtained with the different techniques applied in the present study suggest the existence of some compositional differences between the heterochromatin present in the A-, B- and sex chromosomes of *Characidium gomesi* (Table 1). Similar findings were reported for other fish species when these combined techniques were used (Maistro *et al.* 2000, 2001, 2002, Swarça *et al.* 2001, among others) and have contributed to a better characterization of the fish chromosomes.

The fact that only two close populations of *Characidium* showed B-chromosomes (Maistro *et al.* 1998) and that *Characidium gomesi* fish represent local populations restricted to small tributaries of some rivers, permit us to suggest an independent origin of these chromosomes in some populations. Moreover, considering that B-chromosomes probably arose from A-chromosomes (Green 1990, Beukeboom 1994), the existence of a different chromatin composition between them may indicate that the origin of B-chromosomes in *Characidium gomesi* is not a recent event, with enough time having elapsed for these chromosomes to accumulate some modifications on their structure during evolution.

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