

## NEW OCCURRENCE OF A MACRO B-CHROMOSOME IN *Astyanax scabripinnis paranae* (PISCES, CHARACIFORMES, CHARACIDAE)

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### ABSTRACT

Cytogenetic studies performed on 17 specimens (11 females and six males) of *Astyanax scabripinnis paranae* from the Cascatinha stream showed that this population has  $2n=50$  chromosomes ( $8M+22SM+10ST+10A$ ), two chromosome pairs with NORs and conspicuous C-band positive blocks in the terminal position of the long arm of five chromosome pairs. Three females presented  $2n=51$  chromosomes and the extra chromosome was a large metacentric similar in size and morphology to the first chromosome pair in the karyotype. This accessory chromosome was entirely heterochromatic in C-banded metaphases, which permitted its classification as a supernumerary chromosome. Some aspects related to the morphology of such macro B-chromosomes are discussed.

### INTRODUCTION

Supernumerary chromosomes are accessory genomic elements which occur in different families, species and populations of plants and animals at characteristically variable frequencies (Jones and Rees, 1982; Jones, 1991). Among fishes, the occurrence of B-chromosomes has been well documented for about 24 species of different Neotropical families (Salvador and Moreira-Filho, 1992). Karyotypic studies conducted on *Astyanax scabripinnis* have demonstrated the occurrence of extensive chromosome variability in this species, involving chromosome number and karyotypic formulae (Morelli *et al.*, 1983; Maistro, 1991; Moreira-Filho and Bertollo, 1991; Salvador and Moreira-Filho, 1992; Maistro *et al.*, 1992). In addition, many local populations are morphologically different, suggesting that a large number of species are still undescribed (Moreira-Filho and Bertollo, 1991; Maistro, 1991). The occurrence of macro B-chromosomes has been reported thus far in two of sixteen local populations of *A. scabripinnis* studied

(Maistro, 1991; Salvador and Moreira-Filho, 1992; Maistro *et al.*, 1992).

### MATERIAL AND METHODS

Seventeen specimens (11 females and six males) of *A. scabripinnis paranae* from the headwaters of the Cascatinha river, a small tributary of the Tietê river (Botucatu, State of São Paulo, Brasil) in the upper Paraná river basin, were analyzed (Table I). The specimens were identified by Dr. Valdener Garutti and MSc. Francisco Langeani Filho (UNESP, São José do Rio Preto) and were deposited in the fish collection of the Laboratory of Fish Biology, UNESP, Botucatu.

Chromosome preparations were obtained according to Oliveira *et al.* (1988). Chromosome morphology was determined on the basis of arm ratios as proposed by Levan *et al.* (1964) and the chromosomes were classified as metacentrics (M), submetacentrics (SM), subtelocentrics (ST) and acrocentrics (A). C-banding was performed by the method of Sumner (1972) and silver staining of nucleolus organizer regions (NORs) by the method of Howell and Black (1980).

### RESULTS AND DISCUSSION

*A. scabripinnis paranae* presented a diploid number of  $2n=50$  chromosomes ( $8M+22SM+10ST+10A$ ),

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Table I - Chromosome number in cells of males and females of *Astyanax scabripinnis paranae* from the Cascatinha river population.

Fish no. and sex		Chromosome numbers				
		47	48	49	50	51
		Number of cells				
862	♂	3	4	7	21	1
864	♂	2	1	2	14	-
865	♀	1	-	1	16	2
866	♀	1	2	3	35	1
867	♀	-	2	5	15	-
868	♀	3	1	3	20	-
872	♂	2	1	3	17	-
873	♀	2	2	6	29	1
874	♀	1	3	4	32	-
878	♂	1	4	2	27	1
879	♀	1	4	4	19	2
914	♀	-	1	2	43	-
915	♂	1	1	2	34	-
919	♀	-	3	1	9	82
920	♂	-	1	4	27	-
1842	♀	-	-	1	2	37
1843	♀	-	3	2	5	25

which is the same diploid number described for several populations of this species (Morelli *et al.*, 1983; Maistro, 1991; Moreira-Filho and Bertollo, 1991; Maistro *et al.*, 1992; Salvador and Moreira-Filho, 1992). However, the karyotypic formulae of the different local populations analyzed are quite different, a fact probably related to biological peculiarities of this species which is composed of many local populations restricted to headwaters of tributaries of small rivers. This characteristic probably permitted karyological modifications of different magnitudes to be fixed independently in the populations, resulting in the present cytogenetic diversity (Maistro, 1991; Moreira-Filho and Bertollo, 1991).

Three females with  $2n=51$  chromosomes were detected in the populations of Cascatinha stream and the extra element was characterized as a large metacentric chromosome, similar in size to the first pair in the karyotype (Table I and Figure 1).

The constitutive heterochromatin detected by the C-banding technique was frequently distributed as large and conspicuous blocks in the terminal position on the long arm of four acrocentric pairs. Additionally, the majority of chromosomes in the karyotype presented small pericentromeric regions weakly stained by this technique. Pairs 1 and 24 seem to be heteromorphic (Figure 2) but this difference found among the homologue was not observed in other individuals. The C-band patterns found among

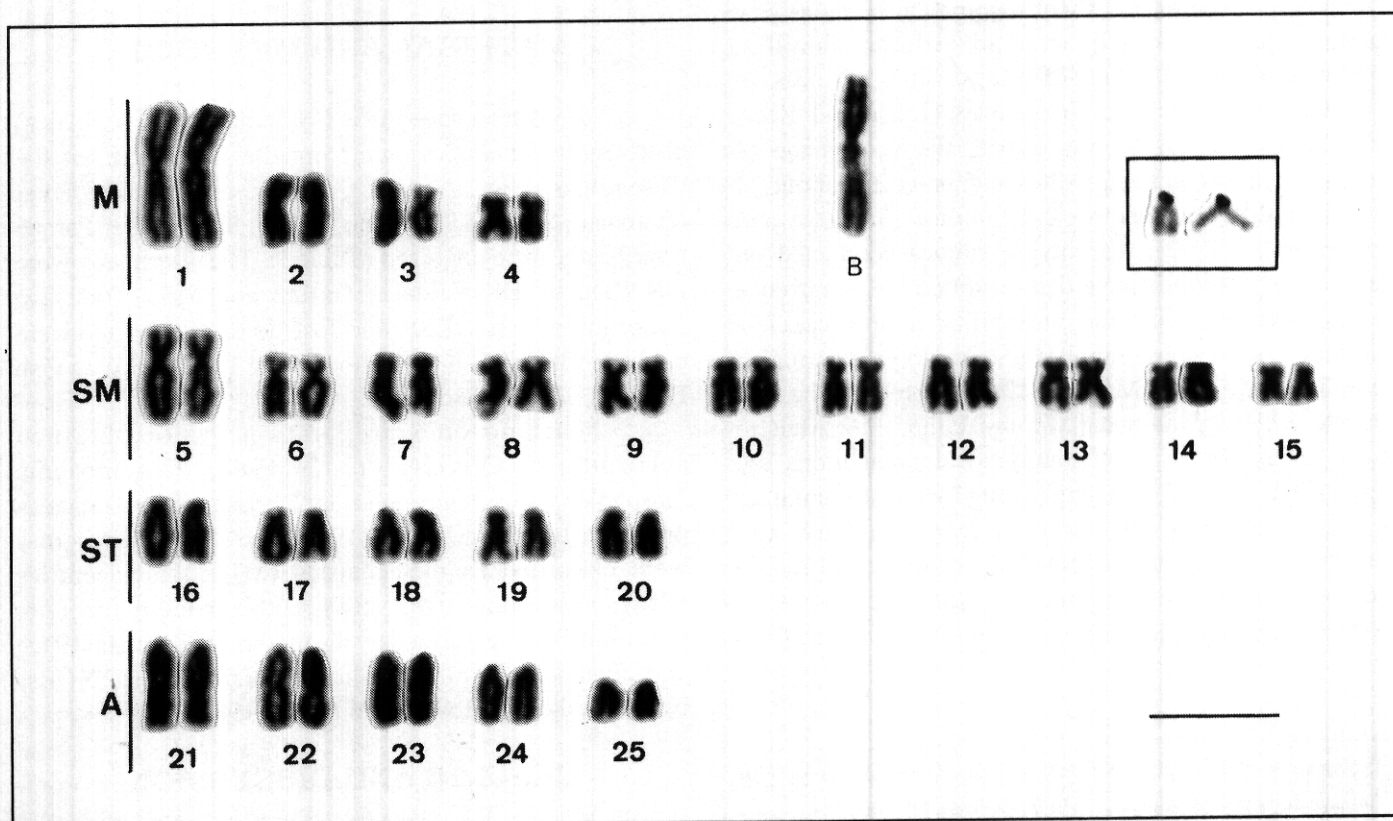


Figure 1 - Karyotype of *Astyanax scabripinnis paranae* with  $2n=51$  chromosomes, 50 of them being the normal elements of the karyotype and one a B-chromosome. In the inset, NOR bearing chromosome pair. Bar = 10  $\mu$ m.

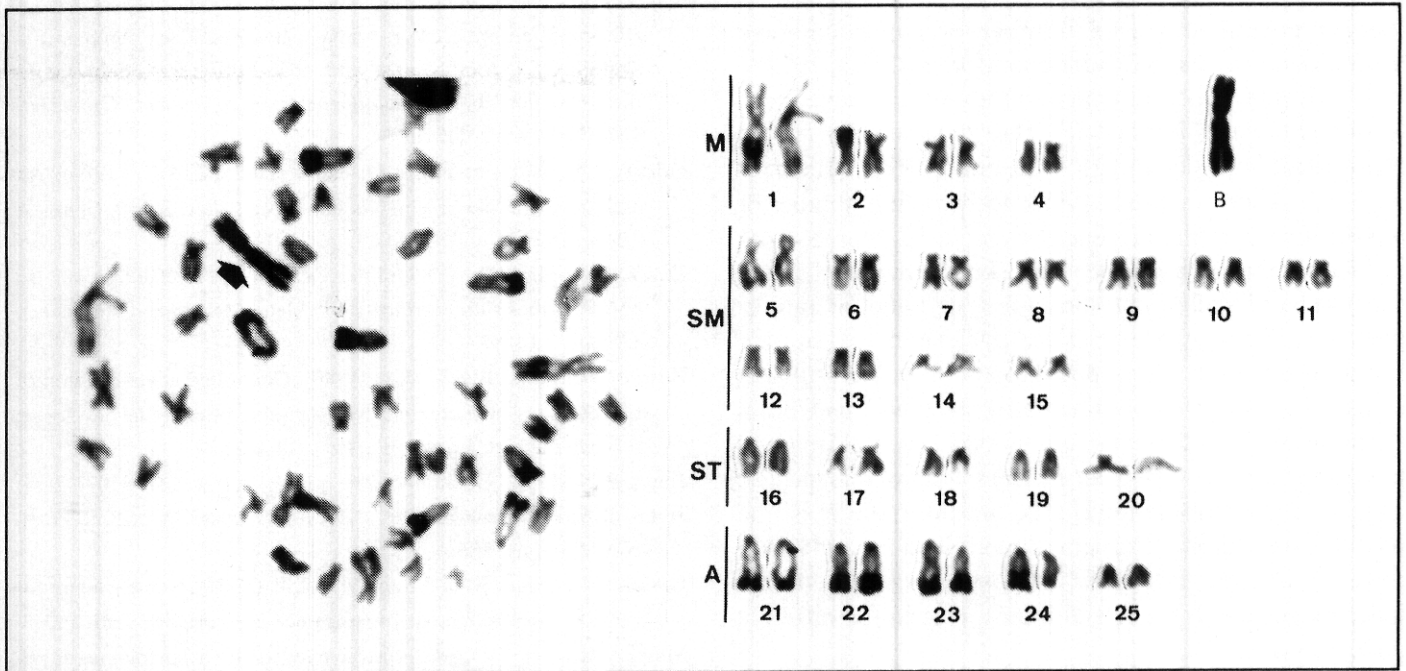


Figure 2 - Somatic metaphase and karyotype of *Astyanax scabripinnis paranae* showing C-banding. The arrow indicates the B-chromosome, which shows a conspicuous heterochromatic pattern.

different populations of *A. scabripinnis* are usually species specific, supporting the idea that the local populations are genetically isolated (Maistro, 1991; Moreira-Filho and Bertollo, 1991). In the specimens with  $2n=51$ , the extra metacentric chromosome was entirely heterochromatic (Figure 2). Previous observations of this type of chromosome were described in two populations of *A. scabripinnis*, where the extra chromosome was a large metacentric and entirely or partially heterochromatic (Salvador and Moreira-Filho, 1992; Maistro *et al.*, 1992), and showed a late replication pattern evidenced by BrdU incorporation (Maistro *et al.*, 1992).

One chromosome pair, a small-sized ST, presented Ag-stained NORs in the terminal position on the short arm (Table II and Figure 1). In a few cells another chromosome pair, a large ST with the NOR in the interstitial position on the long arm was identified (Table II). The presence of multiple NORs is common among Characid fishes (Almeida-Toledo and Foresti, 1985) and has already been reported in *A. sacabripinnis* (Moreira-Filho, 1989; Maistro, 1991). The presence of a large B chromosome in three specimens apparently did not interfere with NOR regulation since the frequency of marked NOR in the fishes with  $2n=51$  was not different from that found in fish with  $2n=50$  (Table II), as previously demonstrated by Maistro *et al.* (1992).

The occurrence of an extra chromosome in three specimens of *A. s. paranae* from the Cascatinha river and the fact that this chromosome was entirely heterochromatic strongly suggests that it is a supernumerary chromosome. Among Neotropical fishes, three classes of species far can

Table II - NOR frequency in chromosomes of *Astyanax scabripinnis paranae* of the Cascatinha river population.

Fish no. and sex	Number of NORs			
	1	2	3	4
	Number of cells			
862 ♂	5	9	4	-
865 ♀	3	19	3	-
866 ♀	-	18	8	1
874 ♀	6	11	4	-
878 ♂	9	33	3	2
*919 ♀	1	23	4	2
920 ♂	3	24	5	1
*1842 ♀	7	25	-	-
*1843 ♀	5	17	1	-
%	15.23	69.92	12.50	2.35

\*Fish specimens with  $2n=51$  chromosomes.

be characterized with respect to B-chromosomes: the first comprises species with one or a few large extra chromosomes, usually as large as the largest chromosome pair in the karyotype; the second group comprises species with some small B-chromosomes, usually having the size of the smallest pair in the karyotype; in the third class,

species present a varied number of supernumerary microchromosomes (Salvador and Moreira-Filho, 1992).

Large supernumerary chromosomes have been reported in several fish species (Hafez et al., 1981; Falcão et al., 1984; Salvador and Moreira-Filho, 1992; Maistro et al., 1992; Andreato et al., 1993). the growing number of reports involving the presence of macro B-chromosomes in Neotropical fish species seems to indicate that the occurrence of such a chromosome type is not as rare as previously supposed. The presence of macro B-chromosomes in three populations of *A. scabripinnis* collected from different streams in the Tietê river basin could mean that a large supernumerary chromosome was present in the karyotype of an ancestral form of this species. This B-chromosome could be maintained in some populations with small structural modifications and lost in several others. Another hypothesis suggests an independent origin for this chromosomes in the different populations.

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## RESUMO

Estudos citogenéticos foram realizados em 17 exemplares (11 fêmeas e 6 machos) de *Astyanax scabripinnis paranae* coletados no Córrego Cascatinha (Botucatu, SP). As análises citogenéticas mostraram que esta população apresenta um número diplóide  $2n=50$  cromossomos (8M+22SM+10ST+10A), dois pares de cromossomos portadores de NORs e conspicuos blocos de heterocromatina constitutiva localizados em posição terminal do braço longo de quatro pares de cromossomos. Três fêmeas desta população apresentaram um número diplóide de  $2n=51$  cromossomos, onde o cromossomo extra corresponde a um grande metacêntrico, de tamanho e morfologia similares ao do primeiro par do cariótipo. Este cromossomo acessório mostrou-se totalmente heterocromático pela técnica de banda-C, o que permitiu sua classificação como um cromossomo supernumerário. Alguns aspectos relacionados a morfologia destes macrocromossomos supernumerários são discutidos.

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