



B chromosomes in a population of *Astyanax eigenmanniorum* (Characiformes, Characidae) from the Araguari River Basin (Uberlândia, MG, Brazil)

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Abstract

A cytogenetic study was conducted on an *Astyanax eigenmanniorum* population from the Caetano Stream (18° 44' 56" S/ 048° 18' 39" W) - in Uberlândia, MG, Brazil - showing a modal diploid number of 48 chromosomes in the standard male and female karyotypes. However, in several specimens it was also possible to observe metaphases with one or two B chromosomes, increasing the diploid number to 49 or 50 chromosomes, respectively. The supernumerary chromosomes were totally heterochromatic and highlighted after C-banding. The silver-stained nucleolus organizing regions (Ag-NORs) were located in at least five chromosomes of the standard karyotype, thus characterizing a multiple NOR system in the species. This is the first occurrence of an *A. eigenmanniorum* population with $2n = 48$ chromosomes, bearing supernumerary chromosomes.

Key words: Characidae, *Astyanax eigenmanniorum*, supernumerary chromosome, heterochromatin, nucleolus organizing regions (NORs).

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Preliminary cytogenetic studies in Brazilian *Astyanax* species were initiated in the late 1970s by Foresti *et al.* (1977). Further studies confirmed that the chromosomal number in this fish group ranges from $2n = 36$ in *A. schubarti* (Morelli *et al.*, 1983) to $2n = 50$ in *A. altiparanae* (Pacheco *et al.*, 2001; Fernandes and Martins-Santos, 2004) and *A. eigenmanniorum* (Stripecke *et al.*, 1985; Fauaz *et al.*, 1994; Pfister and Moreira-Filho, 1997). *A. scabripinnis* (Morelli *et al.*, 1983; Araujo and Morelli, 2006), *A. fasciatus* (Morelli *et al.*, 1983; Torres-Mariano and Morelli, 2006), and *A. altiparanae* correspond to the species with the highest number of cytogenetic studies.

Karyotypic variability among different *Astyanax* fish populations is very common and, in some cases, the presence of different numbers of chromosomes has been found among individuals of the same population, such as in *A. fasciatus* (Pazza *et al.*, 2006) and *A. scabripinnis* (Kavalco and Moreira-Filho, 2003; Fernandes and Martins-Santos, 2005).

Supernumerary or B chromosomes are additional dispensable chromosomes that are present in some individuals from some populations in some species, which have probably arisen from the A (standard) chromosomes but follow their own evolutionary pathway (Camacho *et al.*, 2000).

These chromosomes may constitute an alternative form of organization of the genetic material and can be considered true parasites, remaining in populations exclusively by means of their distinctive transmission mechanisms (Rejón *et al.*, 1987).

B chromosomes can be found in haploid or diploid organisms, and can be seen totally or partially heterochromatic (Jones, 1991), after the C-banding technique (Sumner, 1972). However, although very common, these characteristics are not present in all B chromosomes (Beukeboom, 1994). Among fishes, the most studied species is *A. scabripinnis*, which presents macro- and micro-supernumerary chromosomes with a frequency that varies between males and females and according to the altitude at which populations occur (Salvador and Moreira-Filho, 1992; Vicente *et al.*, 1996; Néó *et al.*, 2000; Mestriner *et al.*, 2000; Ferro *et al.*, 2003; Moreira-Filho *et al.*, 2004; Araujo and Morelli, 2006).

This paper gives the description of a large metacentric supernumerary chromosome in *A. eigenmanniorum* from the Caetano Stream (18° 44' 56" S/ 048° 18' 39" W, Uberlândia, MG, Brazil). Thirteen specimens (five males, seven females, and one unsexed) were cytogenetically analyzed. The animals were identified by Dr. Valdener Garutti (UNESP, São José do Rio Preto, SP), and further registered and deposited in the fish collection of the Laboratory of Animal Cytogenetics, at the Federal University of Uberlândia.

Mitotic chromosomes were obtained according to Bertollo *et al.* (1978). Nucleolus organizer regions (Ag-NORs) were identified following the procedure described by Howell and Black (1980). Constitutive heterochromatin was detected using the C-banding technique (Sumner, 1972). The chromosome morphology was determined on the basis of the arm ratio (AR), as proposed by Levan *et al.* (1964), and the chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st), and acrocentric (a).

A. eigenmanniorum presented a standard karyotype with a diploid number of $2n = 48$ chromosomes, both in males and females (Table 1) and a karyotype composed of 7 pairs of metacentric (m), 9 pairs of submetacentric (sm), 5 pairs of subtelocentric (st), and 3 pairs of acrocentric (a) chromosomes (Figure 1a). However, in several specimens it was also possible to observe metaphases with one or two B chromosomes, increasing the diploid number up to 49 chromosomes or 50 chromosomes (Table 1). In three females the proportion between 48 and 49 chromosomes was very similar (Table 1). The metaphases with 49 and 50 chromosomes showed the presence of one or two large

metacentric supernumerary chromosomes, respectively (Figure 1b, c), corresponding in size to the second chromosome pair of the A complement, which proved to be totally heterochromatic when subjected to C-banding (Figure 1e). The other chromosomes showed heterochromatin located at the telomeric and/or centromeric regions, as well as in the whole short arm (Figure 1e). Although two male specimens (n. 584 and 623) showed $2n = 49$ chromosomes, a low metaphase index was found with this chromosome number (Table 1), and they did not present completely heterochromatic chromosomes after C-banding. Thus, the extra chromosome found in these cases appears only to be a lost chromosome from another standard metaphase.

A. eigenmanniorum is characterized by a multiple NOR system. Indeed, Ag-NORs were seen in different chromosome pairs of the standard karyotype. In the first pair of submetacentric chromosome it is also evident a size polymorphism between the homologous NORs, but with a low frequency (Figure 1d).

Data from only three *A. eigenmanniorum* populations have been described to date (Stripecke *et al.*, 1985; Fauaz *et al.*, 1994; Pfister and Moreira-Filho, 1997), all with $2n = 50$

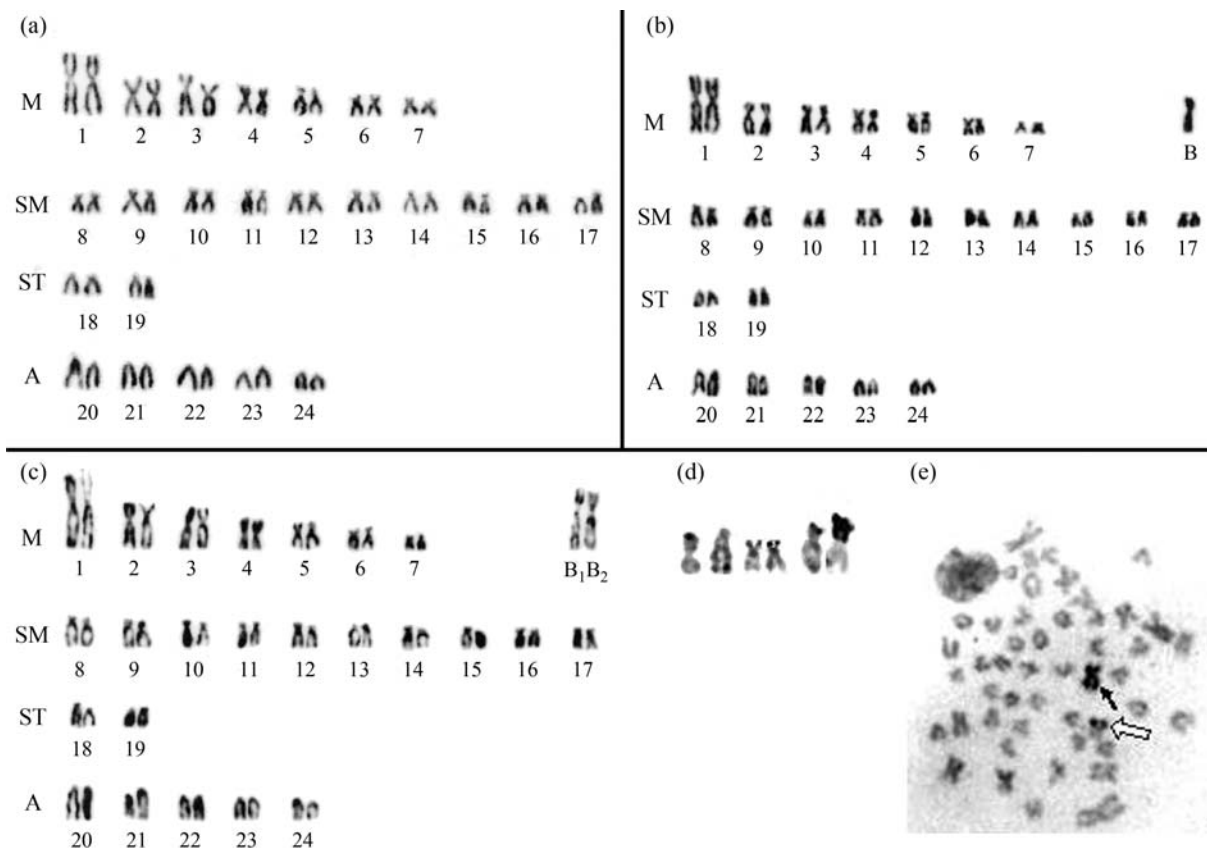


Figure 1 - Karyotypes of *A. eigenmanniorum* from the Caetano Stream, presenting 48 chromosomes (a), 49 chromosomes with 1 B chromosome (b), and 50 chromosomes with 2 B chromosomes (c). In (d) Ag-NOR bearing chromosomes - from left to right: 3rd metacentric chromosome, an acrocentric chromosome (which is rarely detected), 4th metacentric pair, and 1st submetacentric pair, highlighting the size polymorphism of the NOR site. In (e) C-banded metaphase: the small black arrow indicates the heterochromatic supernumerary chromosome; the thick white one indicates the metacentric NOR bearing chromosome.

Table 1 - Chromosome number frequency found in *Astyanax eigenmanniorum* from the Caetano Stream.

Specimen registration	Sex and diploid chromosome number					
	sex	46	47	48	49	50
577	M	8	11	18		
578	M	8	7	20		
584	M	6	2	29	1	
623	M	6	9	22	2	
581	F	7	4	18	6	
583	F	12	7	33	39	1
613	F	0	3	7	18	2
617	F	6	4	13	14	6
620	F	3	8	4	21	4
622	F	6	8	9	19	1
1314	F	4	4	17	12	1
Total number of cells		66	67	190	132	15

M: male. F: female.

chromosomes. Some triploid specimens were found in populations of the Grande River, MG (Fauaz *et al.*, 1994), and only one female from Atibaia River population showed a B chromosome whose size corresponds to that found in the present paper (Stripecke *et al.*, 1985). In the *A. eigenmanniorum* population from the Caetano Stream, the presence of at least one totally heterochromatic B chromosome was evidenced in several specimens. It is also interesting to note that these specimens presented inter- and intra-individual variation in chromosome number (Table 1), suggesting a recent origin for the B chromosomes in this population.

This paper also describes, for the first time, the presence of supernumerary chromosomes in an *A. eigenmanniorum* population with the diploid number equal to 48 chromosomes.

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