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Short Communication

First report of a B chromosome in a natural population of *Astyanax altiparanae* (Characiformes, Characidae)

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Abstract

Several species of the genus *Astyanax* have already been genetically studied, and B-chromosomes have been considered to be an interesting feature in some species of this group. In the present paper we report, for the first time, the occurrence of a B microchromosome in a natural population of *A. altiparanae*. This additional genomic element was identified as an acrocentric chromosome, similar in size to the smallest chromosomal pairs of the standard karyotype. Analysis of the constitutive heterochromatin pattern by C-banding evidenced heterochromatic blocks located on centromeric, pericentromeric, and interstitial regions of some chromosomes, and also positive marks in a subtelocentric chromosomal pair that presented the short arms entirely heterochromatic. The application of this methodology also revealed a heterochromatic pattern in the extra chromosome, a typical feature of supernumerary chromosomes.

Key words: B chromosomes, fish cytogenetics, *Astyanax altiparanae*.

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Several species of the genus *Astyanax* have already been genetically studied, mainly under a cytogenetical focus. The chromosomal number ranged from 2n = 36 in *A. schubarti* to 2n = 50 in *A. scabripinnis paranae* (Oliveira *et al.*, 1988), which demonstrates the high karyotype diversity related to chromosomal morphology and structure in this fish group. Cytogenetic studies have been performed in distinct populations of *A. altiparanae*, evidencing karyotypes composed of 50 chromosomes (Daniel-Silva and Almeida-Toledo, 2001; Fernandes and Martins-Santos, 2004; Daniel-Silva and Almeida-Toledo, 2005). Although different *A. altiparanae* populations present a conservative chromosomal number, differences in the number and location of the nucleolar organizer regions (NORs) show remarkable population variability (Pacheco *et al.*, 2001; Fernandes and Martins-Santos, 2006).

B-chromosomes represent an intriguing class of chromosomes, defined as dispensable supernumerary chromosomes that does not recombine with the A chromosomes and follow their own evolutionary pathway (Camacho *et al.*, 2000). B-chromosomes are found in all major taxonomic groups of both plants and animals (Beukeboom, 1994; Camacho, 2004). The occurrence of supernumerary chromosomes in Neotropical fishes seems to be an interesting feature in freshwater species, previously reported in 41 of 921 analyzed species (Oliveira *et al.*, 2000). The genus *Astyanax* is characterized by the presence of B microchromosomes (Mizoguchi and Martins-Santos, 1997) or B macrochromosomes (Porto-Foresti *et al.*, 1997; Moreira-Filho *et al.*, 2001).

The cytogenetic analyses were carried out in chromosomal preparations obtained from 29 specimens (8 females, 10 males, and 11 individuals of undetermined sex) of *A. altiparanae* from a population of the Campo Novo River (Tietê River Basin, Bauru, SP, Brazil). The fish specimens were identified and stored in the fish collection of the Laboratório de Genética de Peixes, UNESP, Bauru, SP, Brazil. Chromosomal preparations were obtained from gill and kidney tissues according to Foresti *et al.* (1993). C-banding was performed according to Sumner (1972). Chromosomal morphology was determined taking into account the arm ratio (AR) as suggested by Levan *et al.* (1964), and the chromosomes were classified as metacentric (M), submetacentric (SM), subtelocentric (ST), and acrocentric (A).

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Our results showed a diploid number equal to 2n = 50 chromosomes and a karyotypic formula composed of 12M+18SM+12ST+8A (fundamental number = 92) for both sexes (Figure 1) of *Astyanax altiparanae* from Campo Novo River. However, two specimens presented a diploid number of 2n = 51 chromosomes, bearing an extra chromosome, characterized as a small acrocentric chromosome, similar in size to the smallest chromosome pairs of the standard karyotype (Figure 1 - insert).

The diploid number of 2n = 50 chromosomes corroborates previous data reported for different *A. altiparanae* populations (Daniel-Silva and Almeida-Toledo, 2001; Fernandes and Martins-Santos, 2004; Daniel-Silva and Almeida-Toledo, 2005). This species has been regarded as presenting a high karyotypic stability within the genus *Astyanax*, especially when compared with *A. scabripinnis* and *A. fasciatus* that present variation in the diploid number and more pronounced differences in the macro and micro-structure of their chromosomes (Oliveira *et al.*, 1988; Moreira-Filho and Bertollo, 1991; Pazza *et al.*, 2006).

Despite the maintenance of the diploid number, the karyotypic formula of the present population is composed of 12 metacentric, 18 submetacentric, 12 subtelocentric, and 8 acrocentric chromosomes, with a fundamental number of 92, thus demonstrating some variation when compared to other populations (Daniel-Silva and Almeida-Toledo, 2001; Pacheco *et al.*, 2001; Fernandes and Martins-Santos, 2004; Daniel-Silva and Almeida-Toledo, 2005). Such differences can be explained by the occurrence of small non-Robertsonian chromosomal rearrangements, mainly pericentric inversions, although different levels of chromosomal condensation and/or morphological misclassification of the chromosomes cannot be excluded.

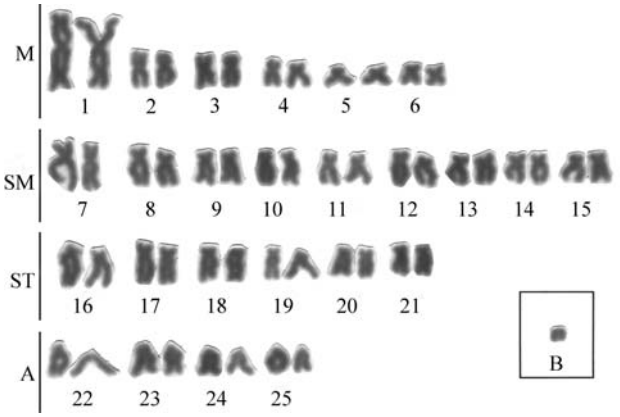

Figure 1 - Giemsa-stained karyotype of *Astyanax altiparanae* from the Campo Novo River. In evidence, one supernumerary chromosome.

Analysis of the constitutive heterochromatin patterns by C-banding showed heterochromatic blocks on centromeric, pericentromeric, and interstitial regions of some chromosomes, besides consistent marks on a pair of subtelocentric chromosomes that presented the short arms entirely heterochromatic. Such general heterochromatin pattern has frequently been observed in distinct populations of *A. altiparanae* (Daniel-Silva and Almeida-Toledo, 2001; Fernandes and Martins-Santos, 2004), demonstrating that these chromosomal regions present a highly conservative distribution in this species. The supernumerary chromosome was totally heterochromatic (Figure 2), in accordance with a common feature reported for B chromosomes in the genus *Astyanax* (Salvador and Moreira-Filho, 1992; Mizoguchi and Martins-Santos, 1997; Néo *et al.*, 2000a, b; Moreira-Filho *et al.*, 2001).

B chromosomes have already been described in other three *Astyanax* species, such as *A. scabripinnis* (Salvador and Moreira-Filho, 1992; Vicente *et al.*, 1996; Ferro *et al.*,

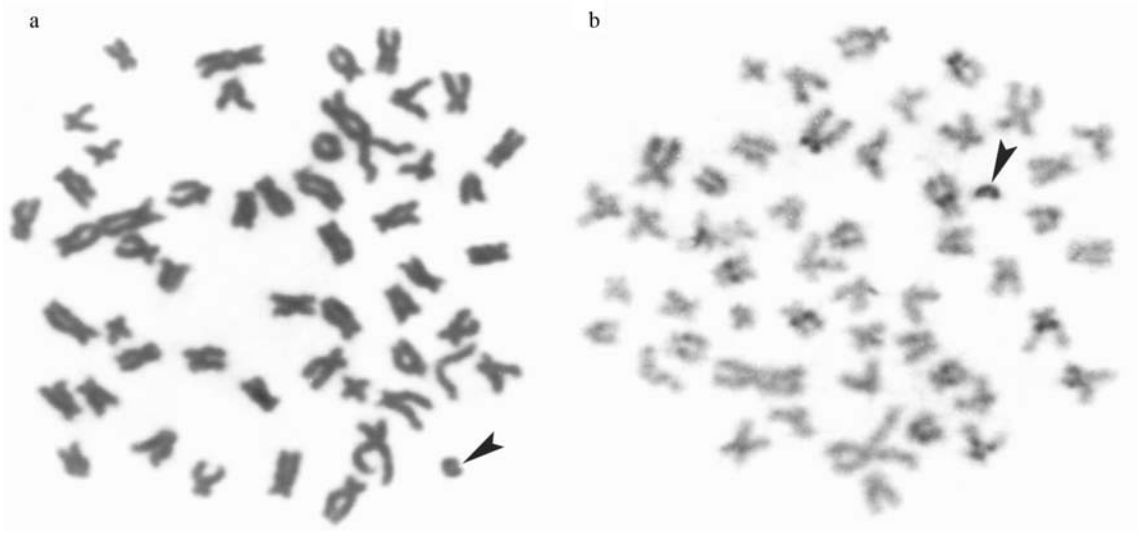

Figure 2 - Metaphases of *Astyanax altiparanae* from the Campo Novo River. Arrows indicate the supernumerary chromosome (conventional Giemsa staining) (a) and the heterochromatic supernumerary chromosome after C-banding (b).

2003), *A. fasciatus*, and *A. schubarti* (Moreira-Filho *et al.*, 2001). In *A. scabripinnis*, supernumerary elements are very frequent in distinct populations (for review, see Moreira-Filho *et al.*, 2004), with several studies related to the distribution of B chromosomes according to altitude (Néo *et al.*, 2000b), sex (Vicente *et al.*, 1996; Mizoguchi and Martins-Santos, 1997), morphology and size (Néo *et al.*, 2000a, b; Ferro *et al.*, 2003), and origin in natural populations (Salvador and Moreira-Filho, 1992; Vicente *et al.*, 1996; Mestriner *et al.*, 2000; Néo *et al.*, 2000a). Nevertheless, there are no reports about B chromosomes in *Astyanax altiparanae*, and this represents the first occurrence reported for this species.

Although *A. scabripinnis* reveals B chromosomes of different morphology and size, a metacentric B macrochromosome (B_M) has been found to be a common feature in most of the analyzed populations with supernumerary chromosomes (Moreira-Filho *et al.*, 2004). Additionally, a similar B_M chromosome has been found in other three species of the same genus (*A. eigenmanniorum*, *A. fasciatus*, and *A. schubarti*), suggesting that this B_M variant might have preceded the differentiation of these species (Moreira-Filho *et al.*, 2001). Thus, considering this hypothesis, the B microchromosome of *A. altiparanae* appears to have an independent origin of the B_M variant found in other *Astyanax* species and can represent a sporadic case in this species.

Even though the diploid number remains unchanged and the heterochromatin pattern appears similar amongst different populations of *A. altiparanae*, the presence of B chromosomes, as well as variation in both karyotypic formula and fundamental numbers, show that a plenty of work is still required in order to provide a better understanding of the chromosomal diversification of this fish group.

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