

## Occurrence of a B chromosome in *Cyphocharax modesta* (Pisces, Curimatidae)

Cesar Martins<sup>1\*</sup>, Lúcia Giuliano-Caetano<sup>2</sup> and Ana Lúcia Dias<sup>2</sup>

<sup>1</sup>Departamento de Genética e Evolução, Universidade Federal de São Carlos, Caixa Postal 676, CEP 13565-905 São Carlos, SP, Brazil, and <sup>2</sup>Departamento de Biologia Geral, Universidade Estadual de Londrina, Caixa Postal 6001, CEP 86051-970 Londrina, PR, Brazil

**Key words:** supernumerary chromosome, B chromosome, *Cyphocharax modesta*, Curimatidae

### Abstract

Specimens of *Cyphocharax modesta* (13 *in toto*) from the Três Bocas stream, municipality of Londrina, PR, Brazil, were analysed cytogenetically. A karyotypic structure consisting of 54 meta-submetacentric chromosomes was observed in all specimens. Three of the individuals studied, in addition to the basic chromosome structure presented a fully heterochromatic small supernumerary chromosome which did not show Ag-NOR-, mithramycin- or DAPI-positive bands. On the basis of these results, the origin, evolution and adaptive role of this chromosome type is discussed.

### Introduction

Many reports have shown that B chromosomes occur in fish from the neotropical region. According to Moreira-Filho (1994), at least 21 species belonging to eight families among the Ostariophysi have these additional chromosomes. Supernumerary chromosomes ranging in size from large chromosomes to microchromosomes have been described presenting widely varying forms and occurring as meta-, submeta- and acrocentrics. Supernumerary chromosomes vary enormously in number within the same population, as recorded for *Callichthys callichthys* (Callichthyidae, Pisces; Erdtmann *et al.*, 1990) and *Prochilodus lineatus* (Prochilodontidae; Pauls and Bertollo, 1983; Cavallaro, 1992).

The family Curimatidae comprises approximately 120 species belonging to eight genera (Vari, 1988) and 21 of them, representing six genera, have been studied cytogenetically. A conservative and symmetrical karyotype structure with 54 chromosomes has been identified for most of them. A supernumerary chromosome has been recorded in a female and in a male of *Cyphocharax modesta* from the municipality of Águas de São Pedro, SP, Brazil (Venere and Galetti, 1985) and from the Infernão pond (municipality of Luiz Antônio, SP, Brazil) (Venere, 1991), respectively. Another curimatid, *Steindachmerina insculpta*, collected from the Paparapanema river, Itatinga, SP, Brazil, also possessed a supernumerary chromosome (Oliveira and Foresti, 1992).

There are many accounts of the occurrence of supernumerary chromosomes in the most diverse fish groups, but these data have contributed little to ascertaining the origin and adaptive significance of this chromosome for the organism. A variety of techniques provides promising tools for understanding the structure, function and consequent adaptive role of this chromosome polymorphism in fish.

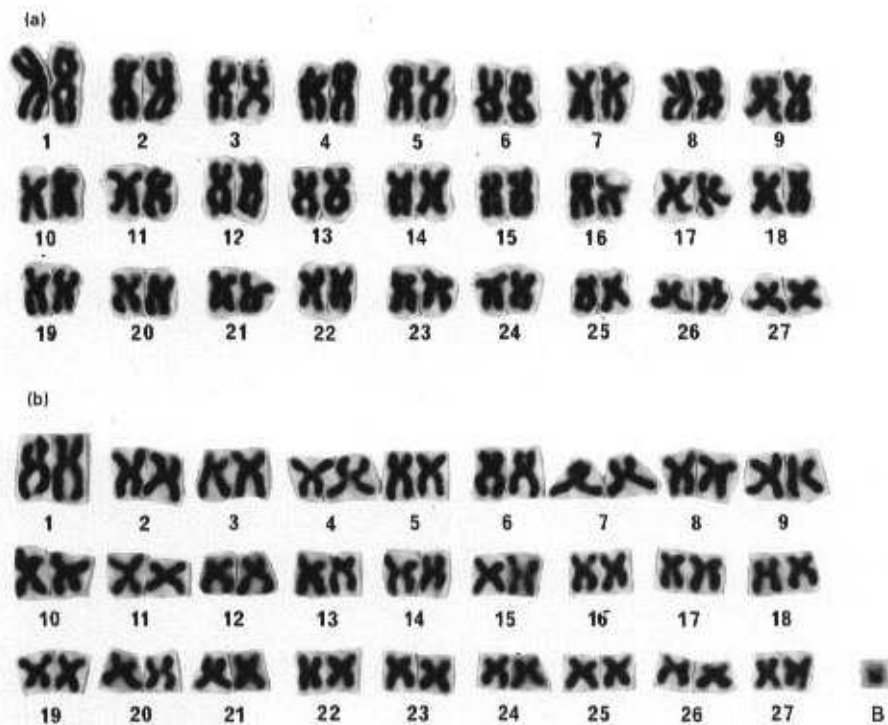
### Materials and methods

Specimens of *Cyphocharax modesta* (9 females and 4 males) were studied from the Três Bocas stream, municipality of Londrina, PR, Brazil. Metaphase chromosomes were obtained by the method of cell suspension in Hanks's salt solution (Foresti *et al.*, 1993). The nucleolar organizer regions (NORs) were observed using a colloidal silver nitrate solution (Ag-NOR bands) as described by Howell and Black (1980). Constitutive heterochromatin was analysed by the C-banding method of Sumner (1972), with some modifications. Moreover, we obtained banding using the GC-specific fluorochrome mithramycin (MM) in combination with the counterstain distamycin and with the AT-specific 4'-6-diamidino-2-phenylindole (DAPI) fluorochrome (Schweizer, 1980).

### Results

Chromosome analysis of *Cyphocharax modesta* from the Três Bocas stream showed a karyotypic structure composed of 54 chromosomes of the meta-submetacentric type (Figure 1a), in agreement with data reported for most Curimatidae. Of the thirteen specimens analysed, three females had a small supernumerary metacentric chromosome (Figure 1b), which was present in 100% of the cells analysed.

The chromosome banding data refer to individuals bearing the supernumerary chromosome. The NORs were present in the telomeric region of the second pair in the complement and did not show any relationship with the supernumerary chromosome (Figure 2a). Constitutive heterochromatin (C<sup>+</sup> band) was visualized in the pericentromeric regions of the most chromosomes and along the entire extension of the supernumerary chromosome (Figure 2b). Application of mithramycin produced results coinciding with those obtained with silver, with fluorescent bands only corresponding to the Ag-NOR<sup>+</sup> bands, while the remaining chromosomes in the complement, including the supernumerary chromosome, were barely fluorescent (Figure 2c). DAPI revealed negative bands coinciding with the NORs and homogeneous fluorescence in the remainder of the complement, including the supernumerary chromosome (Figure 2d).

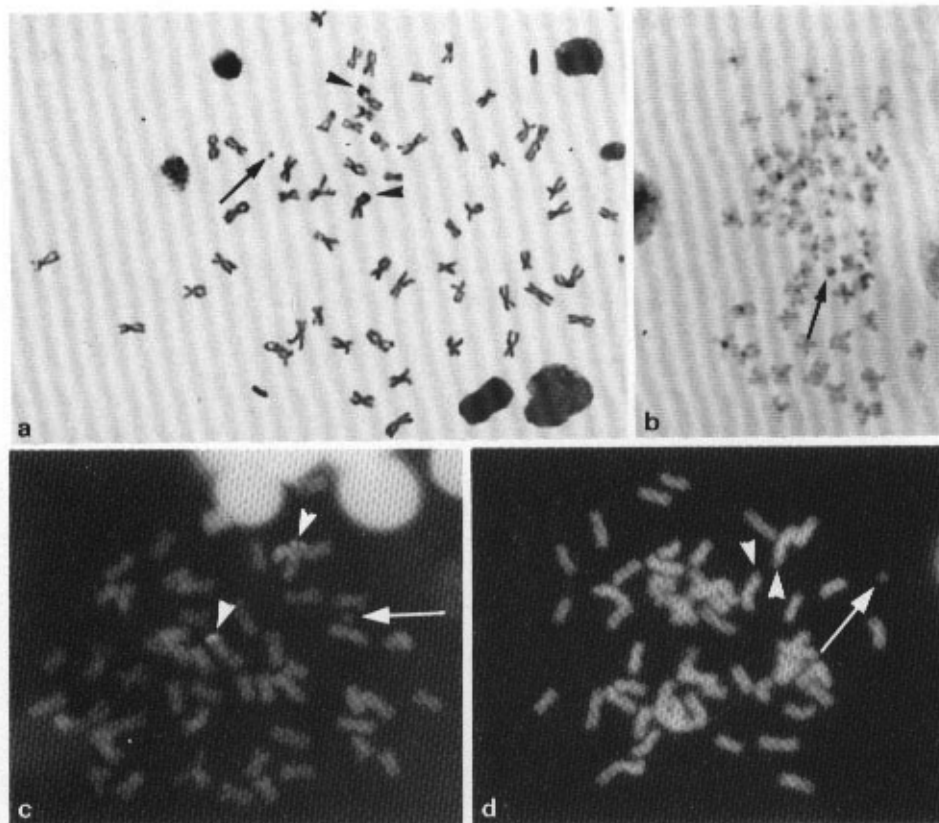


**Figure 1** Karyotypes of *Cyphocharax modesta* stained with Giemsa solution: (a)  $2n = 54$ ; (b)  $2n = 54 + 1 (B)$ .  $\times 3,300$ .

## Discussion

The present results, together with a previously described supernumerary chromosome in *Cyphocharax modesta*, and with reports by Jones (1975), Jones and Rees (1982) and Camacho (1993), enabled us to characterize this supernumerary chromosome as a B chromosome.

In contrast to the chromosomal conservatism previously described for Curimatidae, the occurrence of a B chromosome in *C. modesta* reveals new aspects of the chromosome evolution in this fish group. Despite the small number of individuals sampled, the total frequency of specimens bearing a B chromosome in the *C. modesta* population examined together with the constant occurrence of this chromosome, is significant, suggesting a high stability of the B chromosome in the karyotype complement of the species. These data, together with information available in published literature about the occurrence of supernumerary chromosomes in other populations of *C. modesta*



**Figure 2** Metaphases of a *Cyphocharax modesta* specimen bearing a supernumerary chromosome showing: (a) nucleolar organizer regions (Ag-NOR band); (b) constitutive heterochromatin (C<sup>+</sup> band); (c) and (d) mithramycin and DAPI banding, respectively. The arrow indicates a supernumerary chromosome and the arrowhead indicates Ag-NOR<sup>+</sup> (a), MM<sup>+</sup> (c) and DAPI<sup>-</sup> (d) bands.  $\times 3,000$ .

(Venere and Galetti, 1985; Venere, 1991), suggest that this chromosome represents a general trait of the species and not simply a specific occurrence in certain populations. Indeed, it may be attributed to the high mobility of these fish (Galetti, 1994).

The first report of B chromosomes associated with a given sex in fish was published by Feldberg and Bertollo (1984) and concerned *Gymnogeophagus balzanii* (Cichlidae) in which it was limited to the germ cells of males. Studying a population of *Astyanax scabripinnis paranae* (Characidae) from the Araquá river (a tributary of the Tietê river, Brazil), Maistro *et al.* (1992) recorded a B macrochromosome limited to females which, according to the authors, possibly had an effect

in terms of control of, or interference with, the adaptive process of these fish. Stange and Almeida-Toledo (1993), in a study of *Astyanax scabripinnis* from the Jucu river (municipality of Victor Hugo, ES, Brazil), observed a B chromosome limited to males, suggesting the elimination of B chromosomes from the somatic cells of the females of this population.

In *C. modesta*, the small number of individuals sampled is a limiting factor. However, if the occurrence of B chromosomes was restricted only to the females of the populations of *C. modesta* from the Três Bocas river and from Aguas de São Pedro, this chromosome may have some deleterious effect on males, leading to their inviability, or, as proposed by Green (1990), sex-determining genes may be present in this chromosome. However, the question of sex-restricted B chromosomes needs more in-depth analysis and further information is necessary.

Ag-NOR-banding did not reveal ribosomal cistrons associated with the B chromosome (Figure 2a). No data are available in published literature about the presence of ribosomal cistrons in the B chromosomes of fish. On the other hand, Green (1990) reported a large number of animal and plant organisms showing NORs in supernumerary chromosomes. This indicates that these chromosomes may have a more active role rather than being a non-functional structure, as usually proposed. Despite the large number of studies showing a heterochromatin pattern for B chromosomes, as also observed in the present study (Figure 2b), the possibility of the existence of functional genes associated or intercalated with the heterochromatin blocks cannot be ruled out (Bougourd, 1993).

Many supernumerary chromosomes bear active gene cistrons (Green, 1988, 1990), as observed in plumose supernumeraries of frogs which present lateral loops, an indication of transcriptional activity (Green, 1990). The fluorescent bands coinciding with the NORs obtained with the use of mithramycin (Figure 2c) support the observations made on amphibians by Schmid *et al.* (1987) who found that these regions were rich in GC sequences. On the other hand, the absence of fluorescent labelling in chromosome B both with the use of mithramycin (GC-specific; Figure 2c) and of DAPI (AT-specific; Figure 2d) demonstrates the homogeneous nature of this chromosome in which AT- or GC-rich regions are not organized in clusters.

One of the possible origins proposed for the chromosome B detected in *C. modesta* may be related to non-disjunction of a given chromosome, as proposed for the B chromosome of *Astyanax scabripinnis* (Pisces, Characidae) by Salvador and Moreira-Filho (1992). In the case of *C. modesta*, this non-disjunction may be followed by a loss of chromatin material resulting in a small B chromosome with pericentromeric segments. In support of this idea is the presence



of heterochromatin in chromosome B and also in the pericentromeric region of most chromosomes in the standard complement. McQuade *et al.* (1994), using fluorescence *in situ* hybridization, observed that the B chromosome of *Petauroides volans* (Marsupialia) had DNA sequences unique to this chromosome and sequences exhibiting homology with centromeric regions of other chromosomes in the complement.

The fact that B chromosomes are distributed among different taxa of the neotropical fish fauna suggests that they were probably of polyphyletic origin. With respect to Curimatidae (*Steindachnerina* and *Cyphocharax*), two hypotheses may be considered about the origin of this chromosome. The first suggests that this trait arose in the ancestors of the family Curimatidae (plesiomorphy) and was eliminated from present species which do not bear a B chromosome. Another hypothesis is that of a recent and independent origin of this chromosome (apomorphy) in the species which bear it.

B chromosomes have long been regarded as simply an accessory, or a dispensable complement without much value for the organism. However, many gene sequences have been identified in the supernumeraries of different organisms, mainly among plants, where they have a profound influence on development (Green, 1990). According to Rejón *et al.* (1987), B chromosomes represent an alternative pathway for the evolution of genetic material and have acquired mechanisms which permit them to escape from Mendelian control and from natural selection, leading to their survival and propagation. This leads B chromosomes to evolve autonomously regardless, up to a point, of their phenotypic effects, and giving rise to an accumulation of mutations which would not be permitted by natural selection in A chromosomes. On this basis, B chromosomes represent a depository of genetic variability which may be incorporated into the A complement, thus participating in the evolution of the genome as a whole (*op. cit.*).

### Acknowledgements

The authors wish to thank Dr L. A. C. Bertollo for helpful suggestions, and the Cytogenetics Laboratory of the Federal University of São Carlos for providing facilities for this work which was supported by CNPq (Brazil).

### References

- BOUGOURD S. M. 1993. Phenotypic effects. The First B-chromosome Conference. Madrid, Spain. pp 58-62.
- CAMACHO J. P. M. 1993. Polymorphisms and geographic distribution. The First B-chromosome Conference. Madrid, Spain. pp 2-3.
- CAVALLARO Z. I. 1992. Estudo comparativo sobre os cromossomos B de *Prochilodus scrofa* Steindachner, 1881 (Pisces, Prochilodontidae) de diferentes localidades. Master's Thesis. Universidade Federal de São Carlos, São Carlos, SP.

- ERDTMANN B., Calcagnoto D., Rabolini L. and Malabarba L. R. 1990. Variabilidade cromossômica em *Callichthys callichthys* (Callichthyidae, Pisces). *Abstr. Ciên. Cult.* 452.
- FELDBERG E. and Bertollo L. A. C. 1984. Discordance in chromosome number among somatic and gonadal tissue cells of *Gymnogeophagus balzanii* (Pisces: Cichlidae). *Rev. Bras. Genet.* 7 639-45.
- FORESTI F., Oliveira C. and Almeida-Toledo L. F. 1993. A method for chromosome preparations from large fish specimens using *in vitro* short-term treatment with colchicine. *Experientia* 49 810-3.
- GALETTI P. M. Jr. 1994. Tendências da evolução cromossômica dos nossos peixes. Uma síntese. V Simpósio de Citogenética Evolutiva e Aplicada de Peixes Neotropicais. UNESP, Botucatu, SP, Brasil. pp 31-2.
- GREEN D. M. 1988. Cytogenetics of the endemic New Zealand frog, *Leiopelma hochstetteri*; extraordinary supernumerary chromosome variation and a unique sex-chromosome system. *Chromosoma* 97 55-70.
- GREEN D. M. 1990. Muller's ratchet and the evolution of supernumerary chromosomes. *Genome* 33 818-24.
- HOWELL W. M. and Black D. A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* 36 1014-5.
- JONES R. N. 1975. B-chromosomes systems in flowering plants and animal species. *Int. Rev. Cytol.* 40 1-100.
- JONES R. N. and Rees H. 1982. *B chromosome*. Academic Press, London, Great Britain.
- MAISTRO E. L., Foresti F., Oliveira C. and Almeida-Toledo L. F. 1992. Occurrence of macro B chromosome in *Astyanax scabripinnis paranae* (Pisces, Characiformes, Characidae). *Genetica* 87 101-6.
- McQUADE L. R., Hill R. J. and Francis D. 1994. B-chromosome systems in the greater glider, *Petauroides volans* (Marsupialia: Pseudocheiridae). *Cytogenet. Cell Genet.* 66 155-62.
- MOREIRA-FILHO O. 1994. Cromossomos supranumerários em peixes. V Simpósio de Citogenética Evolutiva e Aplicada de Peixes Neotropicais. UNESP, Botucatu, SP, Brasil. pp 62-3.
- OLIVEIRA C. and Foresti F. 1992. Occurrence of supernumerary microchromosomes in *Steindachnerina insculpta* (Pisces, Characiformes, Curimatidae). *Cytobios* 42 183-6.
- PAULS E. and Bertollo L. A. C. 1983. Evidence for a system of supernumerary chromosomes in *Prochilodus scrofa* Steindachner, 1881 (Pisces, Prochilodontidae). *Caryologia* 36 307-14.
- REJÓN M. R., Rejón C. R. and Oliveira J. L. 1987. Evolución de los cromosomas B. *Invest. Ciéncia* 133 92-101.
- SALVADOR L. B. and Moreira-Filho O. 1992. B chromosome in *Astyanax scabripinnis* (Pisces, Characidae). *Heredity* 69 50-6.
- SCHMID M., Vitelli L. and Batistoni R. 1987. Chromosome banding in Amphibia. XI. Constitutive heterochromatin, nucleolus organizers, 18S + 28S and 5S ribosomal RNA genes in Ascaphidae, Pipidae, Discoglossidae and Pelobatidae. *Chromosoma* 95 271-84.
- SCHWEIZER D. 1980. Simultaneous fluorescent staining of R bands and specific heterochromatic regions (DA-DAPI bands) in human chromosomes. *Cytogenet. Cell Genet.* 27 190-3.
- STANGE E. R. and Almeida-Toledo L. F. 1993. Supernumerary B chromosomes restricted to males in *Astyanax scabripinnis* (Pisces, Characidae). *Rev. Bras. Genet.* 16 601-15.
- SUMNER A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. *Exp Cell Res.* 75 304-6.
- VARI R. P. 1988. The Curimatidae, a lowland Neotropical fish family (Pisces, Characiformes): distribution, endemism and phylogenetic biogeography. In Neotropical Distribution Patterns. pp 343-7. Edited by W. R. Heyer and P. E. Vanzolini. Academia Brasileira de Ciências, Rio de Janeiro, Brasil.
- VENERE P. C. 1991. Citogenética comparativa de peixes da família Curimatidae (Characiformes). Master's thesis, Universidade Federal de São Carlos, São Carlos, SP.
- VENERE P. C. and Galetti P. M. Jr. 1985. Natural triploidy and chromosome B in the fish *Curimata modesta* (Curimatidae, Characiformes). *Rev. Bras. Genet.* 8 681-7.

Accepted 29 June 1996