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Short Communication

Identification and description of distinct B chromosomes in *Cyphocharax modestus* (Characiformes, Curimatidae)

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Abstract

Cytogenetic analyses were performed in *Cyphocharax modestus*, collected at Paranapanema River and Tietê River (São Paulo State, Brazil). A karyotype with 2n = 54 chromosomes was observed in the animals from both Brazilian freshwater river systems. One to four B chromosomes were also detected in individuals from the Paranapanema River, which represents the probable first report of more than a single supernumerary element in a species of the Curimatidae group. C-banding revealed centromeric and telomeric heterochromatin blocks in several chromosomes of the normal karyotype complement of *C. modestus*. Moreover, while some B chromosomes were characterized by the complete absence of C-bands, others were totally heterochromatic. Although there was a prevalence of B chromosomes in males of *C. modestus*, at least one supernumerary element was found in males and/or females of several other populations of the species, which suggests that the presence of these chromosomes seems to represent a general trait of *C. modestus*. A possible origin of the described B chromosomes may be related to the occurrence of a chromosome non-disjunction followed by the loss of euchromatic segments, an event that should have occurred in chromosomes that present conspicuous centromeric heterochromatic blocks and even in chromosomes that lack C-bands in this region, resulting in small supernumerary elements.

Key words: B chromosomes, C-banding, Curimatidae, *Cyphocharax modestus*, supernumerary chromosomes.

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In addition to the standard chromosome complement, B chromosomes, also known as supernumerary or accessory elements, can be found in several animal and plant species (Jones, 1975). As fish represent one of the most numerous and diverse vertebrate groups, comprising species that can be found in almost all aquatic environments (Nelson, 1994), it is expected that different species could present B chromosomes. The occurrence of extra chromosomes has been reported in individuals and populations of different fish groups as Characiformes, Siluriformes, Perciformes, Beloniformes, and Synbranchiformes, representing approximately 5% of all Neotropical freshwater fish already cytogenetically studied (C. Oliveira, personal communication).

The Neotropical members of the order Characiformes present a wide chromosome diversity characterized by the occurrence of stable karyotype groups as well as divergent ones (Bertollo *et al.*, 1986). The Curimatidae family can be included in the first group (Galetti *et al.*, 1994) since most studied species present a diploid number of 54 chromosomes and the karyotype composed by metacentric and submetacentric elements (Scheel, 1973; Galetti *et al.*, 1981, Galetti *et al.*, 1991; Pauls and Bertollo, 1990; Feldberg *et al.*, 1992; Navarrete and Julio, 1997). Despite its macro-structural karyotype stability, the occurrence of B chromosomes has been described in some curimatids, such as *Cyphocharax modestus* (Venere and Galetti, 1985; Martins *et al.*, 1996), *Steindachnerina insculpta* (Oliveira and Foresti, 1993), and *Cyphocharax spilotus* (Fenocchio *et al.*, 2003).

The aim of the present study was to verify the occurrence of supernumerary chromosomes in *Cyphocharax modestus* from the Paranapanema and Tietê Rivers in order to compare the results with those obtained for other populations of the species.

Samples of *Cyphocharax modestus* Fernándes-Yépes 1948 were collected at Paranapanema River (Jurumirim Hydroelectric Reservoir, border municipality of Paranapanema and Angatuba, São Paulo State, Brazil), represent-

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ing twenty males and nine females, and at Tietê River (municipality of Botucatu, São Paulo State, Brazil), representing three males and nine females.Mitotic chromosomes were obtained from kidney cells, as described by Foresti *et al.* (1993). Detection of constitutive heterochromatin (C-banding) was performed as described by Sumner (1972), with some minor modifications. The analyzed animals were deposited at the museum of the Laboratório de Biologia e Genética de Peixes, Instituto de Biociências, UNESP, Botucatu, SP, Brazil.

All analyzed individuals of *C. modestus* from Paranapanema and Tietê Rivers had a karyotype with a diploid number of 2n = 54, consisting exclusively of metacentric and submetacentric chromosomes (De Rosa *et al.*, *in press*). Although no differences were observed in the normal karyotype complement of *C. modestus* from the two collecting sites, one to four small supernumerary chromosomes could be evidenced only in individuals of this species collected at the Paranapanema River (Figure 1 and Tables 1 and 2). The identification of a high number of individuals with only one supernumerary chromosome may be related to the reduced number of analyzed animals. However, we could not discard the possibility that the presence of more than a single extra element in the organism of this fish species can be related to a deleterious effect.

The occurrence of a supernumerary chromosome was previously detected in different Curimatidae species, such as *C. modestus* from the Tietê River (Venere and Galetti, 1985), Piracicaba River (Venere *et al.*, 1999), and Tibagi River (Martins *et al.*, 1996), *Steindachnerina insculpta* from the Paranapanema River (Oliveira and Foresti, 1993), and *Cyphocharax spilotus* from the Paraná-Plata basin (Fenocchio *et al.*, 2003; Brassesco *et al.* 2004). The presence of more than one B chromosome in Curimatidae, as ob-

Table 1 - B chromosome distribution in *Cyphocharax modestus* from the Paranapanema River.

Number of B chromosomes	males	females
0	12	8
1	4	1
2	2	-
3	1	-
4	1	-

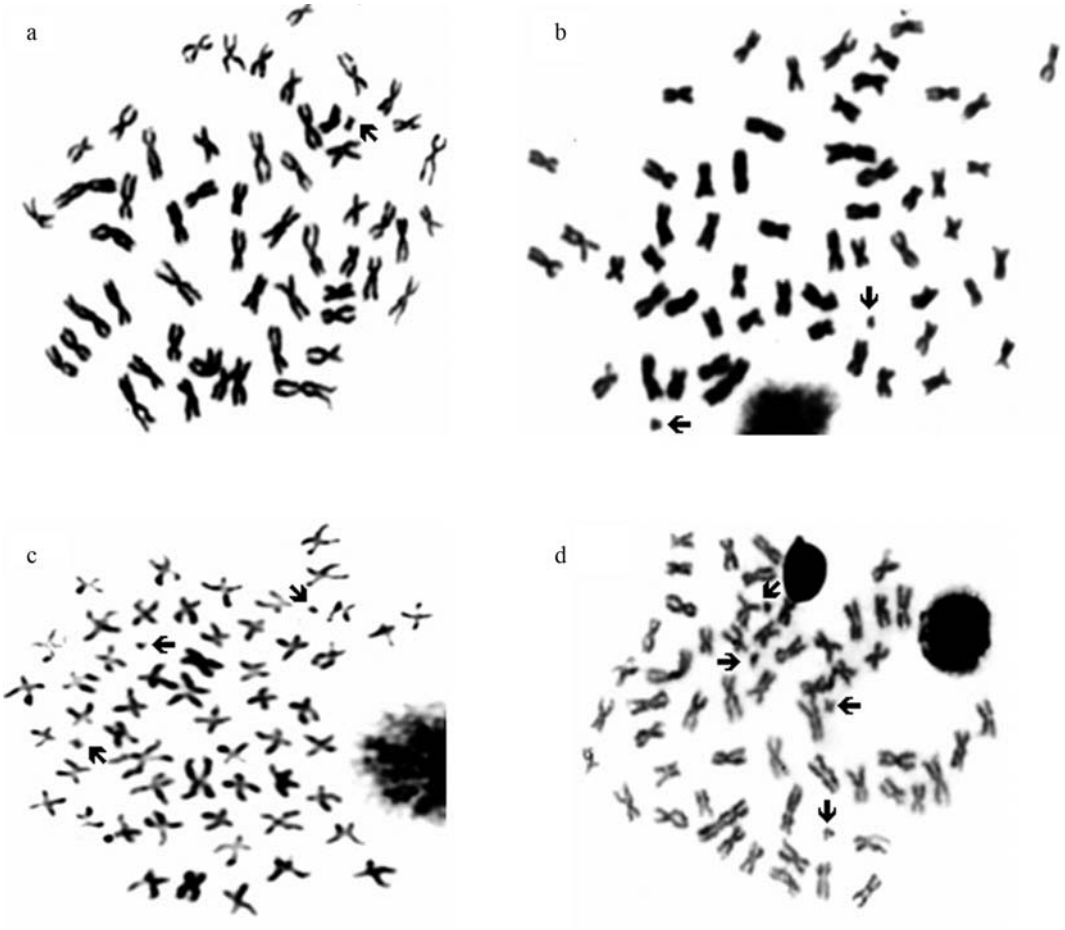

Figure 1 - Giemsa-stained metaphases of *Cyphocharax modestus* from the Paranapanema River evidencing (a) one supernumerary chromosome, (b) two supernumerary chromosomes, (c) three supernumerary chromosomes, and (d) four supernumerary chromosomes.

Table 2 - Distribution of B chromosomes in specimens of *Cyphocharax modestus* from the Paranapanema River.

Specimen identification	Sex	2n + Bs	0	1	2	3	4	B cell frequency (%)	Total number of cells
12211	male	54 + 1	2	17	-	-	-	0.89	19
12215	male	54 + 1	3	23	-	-	-	0.88	26
12232	male	54 + 1	4	23	-	-	-	0.85	27
12235	male	54 + 4	-	1	-	-	31	1	32
12412	male	54 + 2	-	2	16	-	-	1	18
13604	male	54 + 1	2	13	-	-	-	0.86	15
13627	female	54 + 1	-	24	-	-	-	1	24
13797	male	54 + 2	-	6	36	-	-	1	42
13643	male	54 + 3	2	-	-	15	-	0.88	17

served in the present study in *C. modestus* from the Paranapanema River system, appears to constitute an uncommon feature for this fish group.

Many supernumerary chromosomes bear active gene cistrons, as found in some amphibian species (*e.g.* Green, 1990). However, previous data using Ag-staining and 18S rDNA-FISH showed that the Nucleolar Organizer Regions (NORs) were localized in a single chromosome pair of the normal karyotype complement of the species and no ribosomal cistrons could be associated with the B elements of *C. modestus* from the Paranapanema River (De Rosa *et al.*, *in press*), confirming other previous data on individuals of *C. modestus* captured in other freshwater systems which also presented B chromosomes (Venere and Galetti, 1985; Venere, 1991; Martins *et al.*, 1996). FISH experiments also revealed that 5S rDNA cistrons are not present in the supernumerary chromosomes of the species (De Rosa *et al.*, 2006).

Heterochromatic B chromosomes represent a common feature in several fish species, as observed in *Prochilodus scrofa* (Pauls and Bertollo, 1983), *Pimelodella kronei* (Almeida-Toledo and Foresti, 1985), *Rhamdia* sp. (Hochberg *et al.*, 1985), *Corydoras aeneus* (Oliveira *et al.*, 1988), *Leporinus friderici*, *Leporinus* sp. and *Prochilodus nigricans* (Venere *et al.*, 1999) among others. However, some cases of euchromatic supernumerary chromosomes, after C-banding treatments, have also been described in fish, such as *Moenkhausia sanctaefilomenae* (Foresti *et al.*, 1989), and *Characidium* cf. *zebra* (Venere *et al.*, 1999).

C-banding revealed centromeric and telomeric heterochromatin blocks in several chromosomes of the normal complement of *C. modestus* from the Paranapanema River (De Rosa *et al.*, *in press*). Moreover, while some B chromosomes of this species were characterized by the complete absence of C-bands, other supernumerary elements were totally heterochromatic (Figure 2). The supernumerary chromosomes found in individuals with just one of these elements were always entire heterochromatic. However, heterochromatic and euchromatic supernumerary chromosomes could be identified in animals bearing two or more of these chromosomes. Whereas Venere and Galetti (1985) found a single B chromosome in *C. modesta* in a sample from the Tietê River system that was fully heterochromatic, a euchromatic supernumerary chromosome was described in *Steindachnerina insculpta* (Oliveira and Foresti, 1993). Although the occurrence of both totally heterochromatic or euchromatic B chromosomes in the same individual has been described in other fish species, such as *Moenkhausia sanctaefilomenae* (Foresti *et al.*, 1989), the occurrence in *C. modestus* from the Paranapanema River seems to be the first report for Curimatidae.

It has been proposed that the presence of B chromosomes could be related to ecological factors. In fish species, autosomal breaks due to water pollutants could lead to the occurrence of B elements, as suggested for *Charax leticiae* (Miyazawa and Mondin-Freitas, 2004) and *Sphoeroides greeley* (Alves *et al.*, 2002). The present identification of B chromosomes in several individuals of *C. modestus* from the Paranapenema River and their absence in samples from the Tietê River also could suggest a correlation between B chromosomes and different environmental conditions in these two hydrographic regions. However, as Venere and Galetti (1985) had already found a B chromosome in some individuals of *C. modestus* from the Tietê River system, and considering that a restricted number of individuals from this region were analyzed, we could not discard that the population differences may be due to small sampling size.

Although we have analyzed a larger number of males than females, it seems that the frequency of the B chromosomes is higher in males than in females (Table 1). This contrasts with findings by Martins *et al.* (1996) who identified a B chromosome only in females of *C. modestus*. Supernumerary chromosomes associated with a given sex have been described in some fish species such as *Astyanax scabripinnis* (Stange and Almeida-Toledo, 1993), and *Astyanax scabripinnis paranae* (Maistro *et al.*, 1992). The presence of a B chromosome in different percentages between sexes could be related to deleterious effects in one of the sexes, leading to its partial or total non-viability or, could be related to the occurrence of sex-determining genes in these chromosomes (Green, 1990).

Figure 2 - C-banding metaphases of *Cyphocharax modestus* from the Paranapanema River, evidencing (a) a heterochromatic supernumerary chromosome, (b) euchromatic and heterochromatic supernumerary chromosomes.

Cytogenetic data compilation on *C. modestus* revealed that small B chromosomes were identified in females and/or males and in samples from several populations of the species - Tietê River (Venere and Galetti, 1985), Piracicaba River (Venere and Galetti, 1989), Tibagi River (Martins *et al.*, 1996), and Paranapanema River (present data). Theses facts suggest that at least one B chromosome could represent a general trait of *C. modestus* and its widespread distribution is probably due to the high mobility of this species.

Moreover, another Curimatidae species, such as *Steindachnerina insculpta* and *Cyphocharax spilotus*, also had a B chromosome (Oliveira and Foresti, 1993; Fenocchio *et al.*, 2003). Therefore, supernumerary elements could have arisen in an ancestor of this fish family and were eliminated from the present species that do not bear B chromosomes. Alternatively, they could have had a recent and independent origin in the family. A possible origin of the two kinds of B chromosomes now described may be related to the occurrence of a chromosome non-disjunction, followed by the loss of euchromatic segments. If so, this event should have occurred in chromosomes presenting conspicuous centromeric heterochromatic blocks and even in chromosomes with the lack of C-bands in this region, resulting in small heterochromatic and euchromatic B chromosomes, respectively.

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