

Occurrence of B chromosomes in *Cyphocharax modestus* (Fernández-Yépez, 1948) and *Steindachnerina insculpta* (Fernández-Yépez, 1948) (Characiformes, Curimatidae) from the Tibagi River basin (Paraná State, Brazil)

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(With 3 figures)

Abstract

We cytogenetically analyzed 18 individuals of *Cyphocharax modestus*, and 41 individuals of *Steindachnerina insculpta* from the Tibagi River basin (Paraná State, Brazil). All the specimens had $2n = 54$ chromosomes, all meta-submetacentric (M-SM). The presence of 1 or 2 B chromosomes was seen in some individuals of *C. modestus* of the two sampled populations, showing inter- and intra individual variation. In *S. insculpta*, B chromosomes were also observed but only in one population, also showing inter- and intra individual variation. C-banding was used to show that the identified B chromosomes were totally heterochromatic in the two examined species.

Keywords: supernumerary chromosomes, karyotype, C-banding, fish populations.

Ocorrência de cromossomos B em *Cyphocharax modestus* e *Steindachnerina insculpta* (Characiformes, Curimatidae) da bacia do Rio Tibagi (Paraná, Brasil)

Resumo

Foram analisados, citogeneticamente, 18 indivíduos de *Cyphocharax modestus* e 41 indivíduos de *Steindachnerina insculpta* da bacia do rio Tibagi (Paraná, Brasil). Todos os espécimes apresentaram $2n = 54$ cromossomos, todos do tipo meta-submetacêntrico (M-SM). A presença de 1 ou 2 cromossomos B foi detectada em alguns indivíduos de *C. modestus* de duas populações amostradas, evidenciando uma variação inter e intraindividual. Em *S. insculpta*, também foram observados cromossomos B, embora em somente uma população, mostrando também uma variação inter e intraindividual. Bandamento C foi usado para mostrar que os cromossomos B identificados apresentaram-se totalmente heterocromáticos nas duas espécies examinadas.

Palavras-chave: cromossomos supranumerários, cariótipo, bandamento C, populações de peixes.

1. Introduction

B chromosomes, also referred to as supernumerary chromosomes, are extra elements to the standard complement and have been described in more than 1,300 species of plants and almost 500 species of animals (Camacho et al., 2000). In the family Curimatidae, the first work to record the presence of a supernumerary chromosome was published by Venere and Galetti (1985) in *Cyphocharax modestus* (cited as *Curimata modesta*). The presence of this chromosome was intra- as well as inter individually variable, and it appeared to be totally heterochromatic.

Since then, other populations of *Cyphocharax modestus* have shown to present B chromosomes (Venere, 1991; Martins et al., 1996).

Steindachnerina insculpta was also found to have 1 or 2 B chromosomes in a population studied by Oliveira and Foresti (1993), whereby these chromosomes were totally euchromatic, differing from the populations of *Cyphocharax modestus* cited above. To date, this has been the only report of B chromosomes in *S. insculpta*.

The aim of the present study was to determine the occurrence of supernumerary or B chromosomes in *Cyphocharax modestus* and *Steindachnerina insculpta* from the Tibagi River basin (Paraná State, Brazil).

2. Material and Methods

We analyzed twelve specimens of *Cyphocharax modestus* (9 males and 3 females), and fourteen specimens of *Steindachnerina insculpta* (7 males and 7 females) collected from Três Bocas Stream; six specimens of *C. modestus* (2 males, 3 females and 1 individual of unknown sex), and seven specimens (4 males, 2 females and 1 individual of unknown sex) of *S. insculpta* collected from Taquari Stream; eight specimens (5 males and 3 females), and two females of *S. insculpta* from the Tibagi and Água da Floresta Rivers, respectively. All the water bodies belong to the Tibagi River basin (Paraná State, Brazil). Mitotic chromosome preparations were obtained according to Bertollo et al. (1978). C-banding was performed using the method described by Sumner (1972).

3. Results and Discussion

All the analyzed specimens of *Cyphocharax modestus* and *Steindachnerina insculpta* had a diploid number of 54 meta-submetacentric (M-SM) chromosomes, for both sexes (Figure 1a and 1b, respectively). This finding confirms the karyotypic stability in the family Curimatidae, since this diploid number has been found in the majority of species so far studied (Navarrete and Júlio, 1997; Brassesco et al., 2004; among others).

In addition to the normal basic complement, there was a variation of 1 to 2 B microchromosomes in the somatic cells of *Cyphocharax modestus* (Figure 2a, b) from

two populations (Três Bocas and Taquari Streams), and in the somatic cells of *Steindachnerina insculpta* from Três Bocas Stream (Figure 2c, d). This occurrence has also been reported in other populations of *C. modestus* (Venere and Galetti, 1985; Venere, 1991; Martins et al., 1996), of *S. insculpta* (Oliveira and Foresti, 1993), and in one specimen of *Cyphocharax spilotos* (Brassesco, 2000).

In the population of *Cyphocharax modestus* from Três Bocas Stream, it was possible to detect the presence of one B microchromosome in 6 individuals of the 12 examined, in 13.33% of the metaphases in both males and females (Table 1). In the population from Taquari Stream, one B was found in 5 of the 6 examined individuals, in 80.92% of the metaphases (Table 2), demonstrating a high frequency of this chromosome in this population and thus differing from the population from Três Bocas Stream. This difference in frequency of B chromosomes can be an indication of a population differentiation of *C. modestus* from the Tibagi River basin.

Both populations had a low incidence of two B chromosomes. In *C. modestus* from Três Bocas Stream, 5.34% of the metaphases had two Bs, and only 1.27% of the metaphases of *C. modestus* from Taquari Stream evidenced two extra chromosomes. Inter- and intra individual variation of B chromosomes in the two populations of *C. modestus* demonstrates the mitotic instability of this supernumerary chromosome, probably due to the non-Mendelian behavior of the B chromosomes during cell division.

Martins et al. (1996) also studied *C. modestus* from Três Bocas Stream and found B chromosomes only in females. This finding led the authors to propose that this

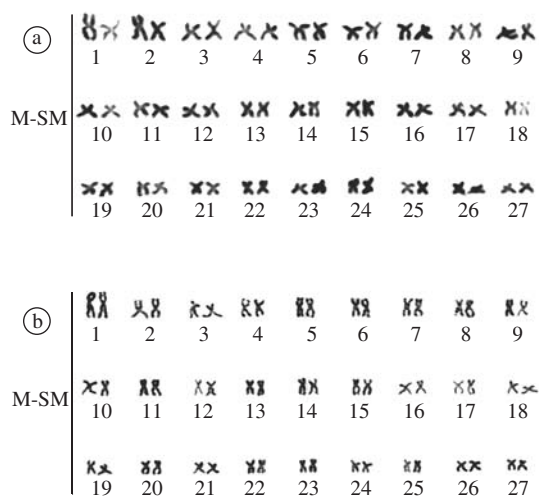


Figure 1. Giemsa-stained metaphases of a) *Cyphocharax modestus*, and b) *Steindachnerina insculpta*.

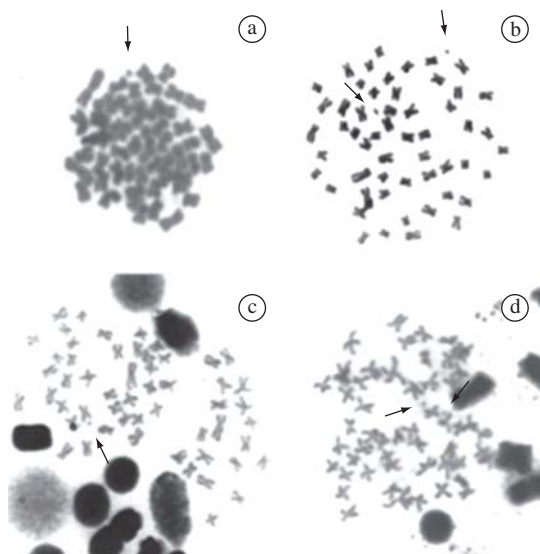


Figure 2. Giemsa-stained metaphases of a, b) *Cyphocharax modestus*, and *Steindachnerina insculpta* c, d) showing B chromosomes (arrows).

chromosome could be deleterious to males of this species. However, according to the data of the present study, it is evident that these chromosomes are not harmful to males in the examined population.

The presence of a dot type B microchromosome was also observed in 9 of the 14 individuals of *Steindachnerina insculpta* from Três Bocas Stream, in 12.74% of the metaphases (Table 3). Two B chromosomes were seen in one of the individuals in just 0.47% of the metaphases. This is the second occurrence of B chromosomes in *S. insculpta*. The presence of one or two B chromosomes has also been demonstrated in another population of this species from Paranapanema River, studied by Oliveira and Foresti (1993).

One interesting fact is that four populations of *S. insculpta* from the Tibagi River basin were examined

in the present study and only one had B chromosomes. Venere (1991) and Oliveira and Foresti (1993) studied this same species from the Mogi-Guaçu River and Paranapanema River (São Paulo State, Brazil), respectively, and as noted above, B chromosomes were only found in the latter region. This strongly indicates that this type of chromosome is a local characteristic and that its absence in one population of *S. insculpta* does not exclude its occurrence in other populations.

The B chromosome, when present, appeared totally heterochromatic in the two studied species (Figure 3a, b, respectively), therefore indicating the absence of functional genes in these chromosomes. In all the other investigated populations of *Cyphocharax modestus*, the B chromosome has been shown to be heterochromatic (Venere and Galetti, 1985; Martins et al., 1996), while it has been reported by Oliveira and Foresti (1993) to be euchromatic in *Steindachnerina insculpta*.

There are two hypotheses that could explain the origin of B chromosomes in *C. modestus* and *S. insculpta*, as suggested by Martins et al. (1996). The first suggests that these chromosomes arose in some ancestor of the family, and were eliminated in species where they are not found today. The second suggests that B chromosomes have a more recent and independent origin in the species that bear it. The latter is supported by the fact that the B chromosomes found in all the populations of *Cyphocharax modestus*, including those in the present study, and in the population of *Steindachnerina insculpta* from Três Bocas Stream are heterochromatic, while those of *S. insculpta* from

Table 1. B chromosome frequencies in the somatic cells of *Cyphocharax modestus* from Três Bocas Stream (Paraná State, Brazil).

| Individual | Sex | B chromosome number | | | Total no. of cells |
|------------|--------|---------------------|-------|------|--------------------|
| | | 0 | 1 | 2 | |
| 1928 | Male | 9 | 1 | 1 | 11 |
| 1933 | Female | 2 | - | - | 2 |
| 2082 | Male | 4 | - | - | 4 |
| 2083 | Male | 4 | - | - | 4 |
| 2085 | Male | 7 | 1 | 1 | 9 |
| 2086 | Male | 1 | 1 | 1 | 3 |
| 2367 | Male | 4 | - | - | 4 |
| 2368 | Female | 4 | - | - | 4 |
| 2531 | Male | 7 | 1 | - | 8 |
| 2539 | Male | 8 | 3 | 1 | 12 |
| 2579 | Male | 6 | - | - | 6 |
| 2583 | Female | 5 | 3 | - | 8 |
| Total | | 61 | 10 | 4 | 75 |
| % | | 81.33 | 13.33 | 5.34 | |

Table 2. B chromosome frequencies in the somatic cells of *Cyphocharax modestus* from Taquari Stream (Paraná State, Brazil)

| Individual | Sex | B chromosome number | | | Total no. of cells |
|------------|---------|---------------------|-------|------|--------------------|
| | | 0 | 1 | 2 | |
| 1233 | Male | - | 23 | 1 | 24 |
| 1234 | Female | 3 | 15 | - | 18 |
| 1235 | Female | 4 | 20 | 1 | 25 |
| 1237 | Female | 7 | 19 | - | 26 |
| 1238 | Unknown | - | 29 | - | 29 |
| 1443 | Male | 9 | - | - | 9 |
| Total | | 23 | 106 | 2 | 131 |
| % | | 17.55 | 80.92 | 1.53 | |

Table 3. B chromosome frequencies in the somatic cells of *Steindachnerina insculpta* from Três Bocas Stream (Paraná State, Brazil)

| Individual | Sex | B chromosome number | | | Total no. of cells |
|------------|--------|---------------------|-------|------|--------------------|
| | | 0 | 1 | 2 | |
| 1929 | Female | 26 | - | - | 26 |
| 1930 | Female | 22 | 9 | - | 31 |
| 2084 | Male | 15 | - | - | 15 |
| 2323 | Female | 3 | 3 | - | 6 |
| 2408 | Male | 14 | - | - | 14 |
| 2411 | Female | 2 | - | - | 2 |
| 2412 | Female | 7 | - | - | 7 |
| 2560 | Female | 10 | 1 | 1 | 12 |
| 2572 | Male | 19 | 5 | - | 24 |
| 2573 | Male | 12 | 1 | - | 13 |
| 2574 | Male | 5 | 1 | - | 6 |
| 2575 | Female | 12 | 2 | - | 14 |
| 2576 | Male | 16 | 3 | - | 19 |
| 2578 | Male | 21 | 2 | - | 23 |
| Total | | 184 | 27 | 1 | 212 |
| % | | 86.79 | 12.74 | 0.47 | |

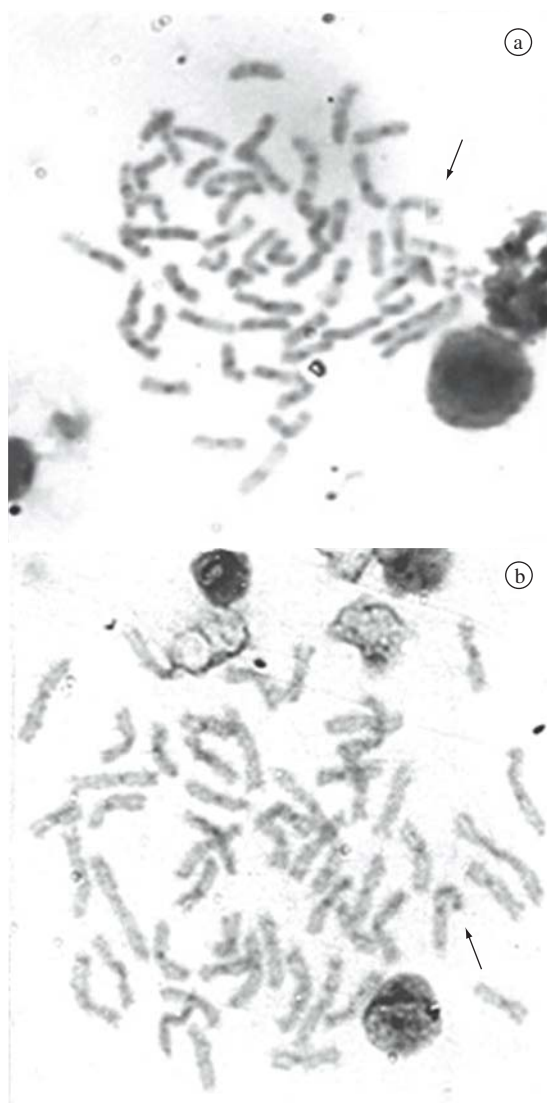


Figure 3. C-banded metaphases of a) *Cyphocharax modestus*, and b) *Steindachnerina insculpta* showing heterochromatic B chromosomes (arrows).

the Paranapanema River are euchromatic. In addition, it should be noted that of the 35 species of curimatids cytogenetically studied, only *C. modestus* and *S. insculpta* have B chromosomes, and therefore, the second hypothesis appears to be more in line with the presented data.

The findings presented here provide additional evidence for the occurrence of B chromosomes in different populations of two species of curimatids, demonstrating that the presence of this chromosome in some populations of *S. insculpta* and at distinct frequencies in *C. modestus* at certain locations may be indicative of population differentiation.

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