

Cytogenetic characterization of B chromosomes in two populations of *Iheringichthys labrosus* (Pisces, Pimelodidae) from the Capivara Reservoir (Paraná, Brazil)

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Abstract — This study presents karyotype findings for *Iheringichthys labrosus* from two different municipalities near the Capivara Reservoir in Brazil. The 25 individuals studied showed a chromosome number of $2n=56$, with a chromosome formula of 26 metacentrics (M) + 12 submetacentrics (SM) + 6 subtelocentrics (ST) + 12 acrocentrics (A). Eighteen of these specimens studied showed the presence of a B microchromosome, with inter and intraindividual variation. By use of the C-banding technique, this B microchromosome was found to be completely heterochromatic and the other chromosomes of the complement showed the presence of heterochromatin preferentially at the telomeres. The fluorochrome chromomycin A₃ (CMA₃) produced fluorescent staining in some chromosomes of the normal complement, but not in the B chromosome. Meiotic analysis demonstrated in metaphase I the presence of 28 bivalents and one small univalent probably corresponding to the B microchromosome.

Key words: B-chromosome, heterochromatin, *Iheringichthys*, meiosis, Pimelodidae

INTRODUCTION

B chromosomes, also referred to as supernumerary or accessory chromosomes, are non-essential extra chromosomes, present in some individuals of some populations of some species, which probably originated from chromosome complement (or complement A) but had an evolutionary mechanism of their own (BEUKEBOOM 1994).

In the family Pimelodidae, *Rhamdia* is the genus that has been studied most for populations possessing supernumerary or B chromosomes (FENOCCHIO and BERTOLLO 1990; VISSOTTO *et al.* 1999; FENOCCHIO *et al.* 2000). Nevertheless, this type of chromosome has also been observed in *Pimelodella kronei* (ALMEIDA-TOLEDO *et al.* 1992), *Bergiaria westermanni* (DIAS and FORESTI 1993), *Iheringichthys labrosus* (DIAS and FORESTI 1990; VISSOTTO *et al.* 1999; CARVALHO *et al.* (2004); I. C. MARTINS-SANTOS, personal communication) and *Pimelodus ortmanni* and *Pimelodus* sp. (BORIN and MARTINS-SANTOS 2004).

In *Iheringichthys labrosus*, populations studied to date have shown $2n=56$ chromosomes (DIAS and FORESTI 1990; VISSOTTO *et al.* 1999), whereby the occurrence of up to three B chromosomes has been demonstrated in this species (CARVALHO *et al.* 2004). Generally, these chromosomes are comprised of heterochromatin (CARVALHO *et al.* 2004); however, VISSOTTO *et al.* (1999) found euchromatic B chromosomes using C-banding staining.

The present study describes the occurrence of one B chromosome in two populations of *Iheringichthys labrosus*, reports on its meiotic behavior and discusses some of its characteristics, making comparisons where possible with other populations of this species previously examined.

MATERIALS AND METHODS

Twenty five specimens of *Iheringichthys labrosus* collected in two different municipalities near the Capivara Reservoir in Brazil were used in the present study. Were studied 15 individuals (9 females and 6 males) from Sertãoópolis (PR, Brazil) and 10 individuals (5 females, 4 males and one of indetermined sex) from Porecatu (PR, Brazil).

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Mitotic chromosome preparations were obtained according to BERTOLLO *et al.* (1978). C-banding analyses were performed using the method described by SUMNER (1972) and treatment with the fluorochrome chromomycin A₃ (CMA₃) as described by SCHMID (1980). Meiotic chromosomes preparations were obtained according to KLIGERMAN and BLOOM (1977). Chromosomes were classified according LEVAN *et al.* (1964) as metacentrics (M), submetacentrics (SM), subtelocentrics (ST) and acrocentrics (A).

RESULTS

All of the individuals of *Iheringichthys labrosus* studied at two different locations showed $2n=56$ chromosomes, with a karyotype of $26M+12SM+6ST+12A$ (Fig. 1), whereby no difference was observed between sexes.

Of the 25 individuals examined in the two populations, 18 proved to have a supernumerary or B microchromosome (Fig. 2a, Table 1). Out of a total of 524 metaphases studied in the two populations, 74 showed the presence of this microchromosome, that is, 14.0% of the metaphases (Table 1).

Heterochromatin was visualized mainly in the telomeric regions of the chromosomes of *Iheringichthys labrosus* from the Capivara Reservoir, and the B chromosomes appeared heterochromatic (Fig. 2b).

Treatment with the fluorochrome CMA₃ produced fluorescent staining in some chromosomes, in the two populations of *Iheringichthys labrosus*, whereas B chromosomes showed a staining similar to the other chromosomes of the complement, that is, with no fluorescence (Fig. 2c).

In meiotic cells of the *Iheringichthys labrosus* specimen labeled number 2430, 28 bivalents and one small chromosome could be seen in met-

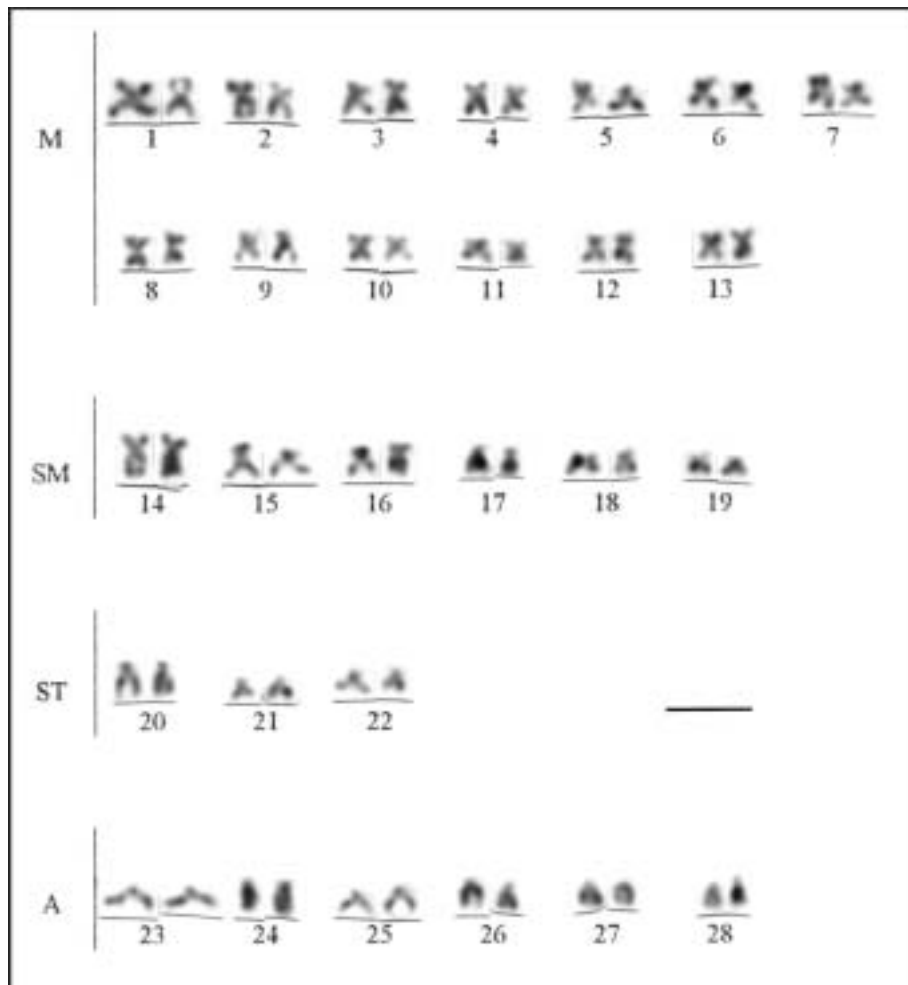


Fig. 1 — Giemsa-stained karyotype of *Iheringichthys labrosus*, showing no B chromosome.

Table 1 — B chromosome frequencies in the somatic cells of *Iheringichthys labrosus* from Porecatu and Sertanópolis (PR, Brazil)

Individual	Locality	Sex	B chromosome number		Total No. of cells
			0	1	
2424	Porecatu	Female	22	-	22
2425	Porecatu	Female	18	3	21
2426	Porecatu	Male	21	1	22
2427	Porecatu	Female	9	20	29
2428	Porecatu	Male	9	2	10
2430	Porecatu	Male	15	6	21
2431	Porecatu	Female	24	1	25
2432	Porecatu	Female	19	-	19
2433	Porecatu	Male	14	1	15
2434	Porecatu	Unknow	20	3	23
2339	Sertanópolis	Male	16	2	18
2340	Sertanópolis	Male	20	1	21
2397	Sertanópolis	Female	28	2	30
2458	Sertanópolis	Female	21	2	23
2459	Sertanópolis	Female	22	3	25
2460	Sertanópolis	Male	5	15	20
2461	Sertanópolis	Male	21	-	21
2463	Sertanópolis	Female	15	5	20
2464	Sertanópolis	Female	26	-	26
2465	Sertanópolis	Female	9	1	10
2466	Sertanópolis	Female	16	4	20
2467	Sertanópolis	Female	19	-	19
2468	Sertanópolis	Male	21	-	21
2470	Sertanópolis	Male	20	-	20
2471	Sertanópolis	Female	20	2	22
Total			450	74	524
(%)			85,9	14,0	100

aphase I (Fig. 3a). Also were observed cells in metaphase I with only the 28 bivalent chromosomes (Fig. 3b) and spermatogonial metaphases with 56 chromosomes (Fig. 3c).

DISCUSSION

Despite that the individuals of the two populations were from different locations, separated by about 67 km, there were no significant differences in karyotype for the two populations. The two populations showed $2n=56$ chromosomes with a distribution of $26M+12SM+6ST+12A$, demonstrating that these individuals were in fact part of a single population or from distinct populations that could show a gene flow between them.

CARVALHO *et al.* (2004), VISSOTTO *et al.* (1999) and DIAS and FORESTI (1990) found the same diploid number ($2n=56$) for others populations of *I. labrosus*, but with variations in the karyotypic formula that could be attributed to differences in chromosome condensation leading to confusion in classification of chromosome types.

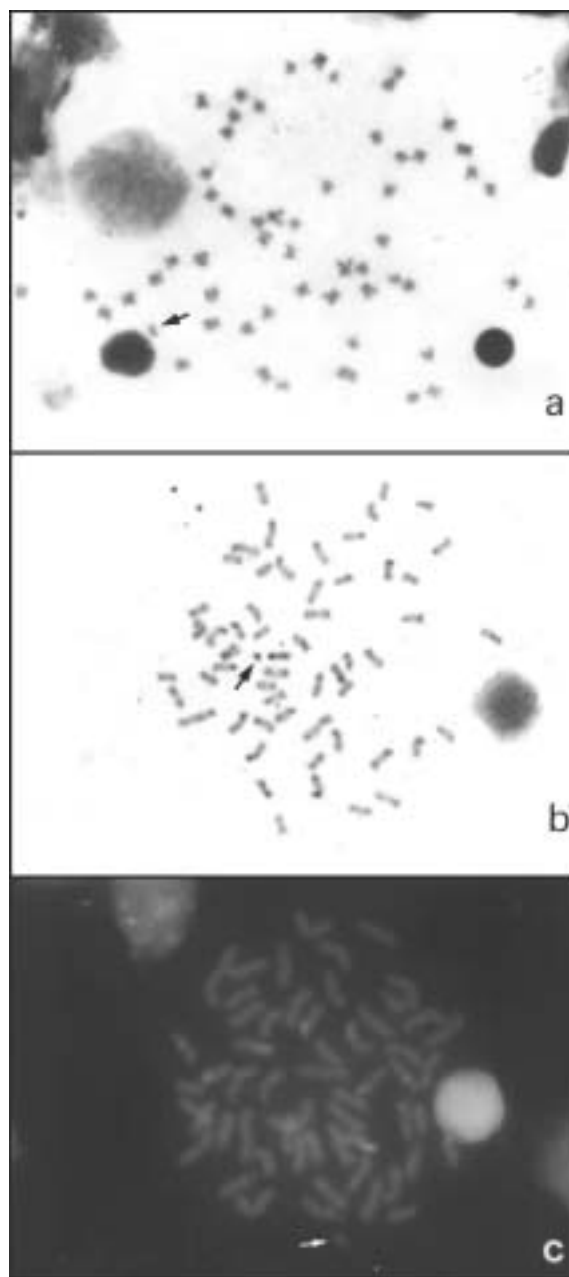


Fig. 2 — Differential by stained metaphases of *Iheringichthys labrosus*. Giemsa-stained metaphase (a), C-banded metaphase (b) and CMA₃ stained metaphase (c). The arrows show the B chromosome.

In both populations of *I. labrosus* examined in the present study, an inter and intraindividual variation in the number of B microchromosomes was evident. The two populations showed 14.0% of metaphases with B chromosomes. This demonstrates a very low frequency of B chromosomes in the two populations, although in an individual from Porecatu, labeled number 2427, had B chromosomes in 69.0% of metaphases examined and

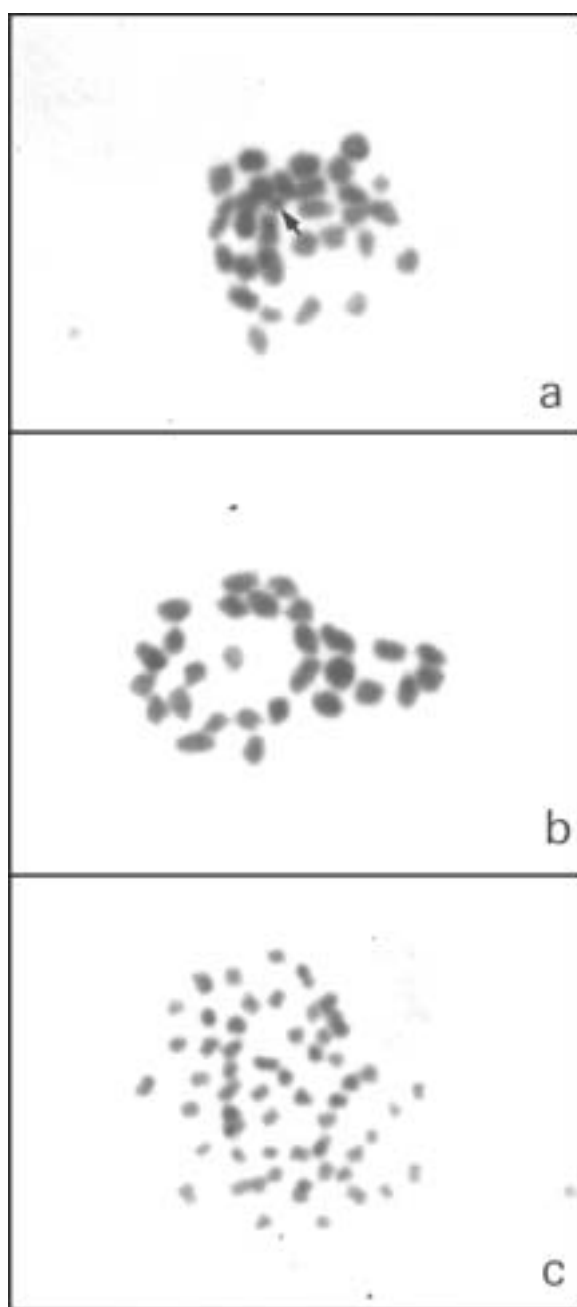


Fig. 3 — Meiotic cells of *Iheringichthys labrosus*: (a, b) metaphase I and (c) spermatogonial metaphase. The arrow shows the univalent B chromosome.

an individual from Sertanópolis, labeled number 2460, had B chromosomes in 75.0% of metaphases studied (Table 1). In other individuals, the frequency of B chromosomes surpassed 20%, evidence of the high mitotic instability of this chromosome in this population.

These inter- and intraindividual variations in B chromosomes in this population, demonstrating the mitotic instability of this chromosome, prob-

ably are due to its non-mendelian behavior during cell division, as proposed by JONES and REES (1982). VOLOBUJEV (1981) suggested that this irregular behavior of supernumerary chromosomes during mitosis and meiosis is not uncommon.

CARVALHO *et al.* (2004) reported on the occurrence of up to three B microchromosomes in a population of *Iheringichthys labrosus* from the Tibagi River, also indicating a low frequency, however with two individuals showing a higher frequency in relation to the others. VISSOTTO *et al.* (1999) observed two B microchromosomes in the population from the Jurumirim Reservoir (SP) and DIAS and FORESTI (1990) reported findings in all of the individuals studied of *I. labrosus* from the Mogi-Guaçu River cells with 0, 1 or 2 B microchromosomes.

The finding of heterochromatin distributed in the telomeric regions of the chromosomes of *I. labrosus*, is in agreement with the data available for the family Pimelodidae, while the B chromosome has been shown to be totally heterochromatic, as evidenced by CARVALHO *et al.* (2004) and DIAS and FORESTI (1990). Conversely, VISSOTTO *et al.* (1999) described the B chromosomes in the population from the Jurumirim Reservoir as euchromatic, with no C-banding staining.

With regard to the origin of supernumerary chromosomes in these populations of *Iheringichthys labrosus*, it is speculated that these chromosomes originated from a species ancestral to all these populations. The finding of a population with euchromatic B chromosomes (VISSOTTO *et al.* (1999), can be related to a process of euchromatinization, which through specific environmental conditions make it possible for this process to occur.

Treatment with the fluorochrome CMA₃, which preferentially stains GC-rich regions, did not show evidence of fluorescent regions in B chromosomes of *I. labrosus*. Thus, these chromosomes do not possess a large amount of GC, but rather balanced amounts of GC and AT base pairs.

The meiotic study of the individual of *I. labrosus* labeled number 2430 showed that the B chromosome was not homologous to any component of the chromosome complement, since it appeared to be univalent. The frequency of B chromosomes in mitotic metaphases of this individual was 28.6% (Table 1). Due to this low frequency, it was only possible to view two cells in metaphase I (MI) that showed this univalent chromosome. The other cells in MI that were examined showed only the 28 bivalent chromosomes, and the sper-

matogonial metaphases did not show the presence of B microchromosomes. The intraindividual variation observed in the meiotic cells of this individual showed that, besides a mitotic instability, B chromosomes show a meiotic instability in this species, albeit in the one individual studied.

The findings presented here demonstrate another occurrence of B microchromosomes in *Iheringichthys labrosus*. The inter- and intraindividual variation and the low frequency of B chromosomes observed in this population from the Capivara Reservoir, suggest that this chromosome could be undergoing processes of maintenance and accumulation in this population, just as in others studied, eventually becoming a characteristic of this group of fish.

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