Occurrence of Multiple Euchromatic B Microchromosomes in *Moenkhausia sanctaefilomenae* (Pisces, Characidae) from the Upper Paraná River Basin, Brazil

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Received July 7, 2017; accepted August 25, 2017

**Summary** *Moenkhausia sanctaefilomenae* is a small freshwater characid fish that shows a conserved karyotype with respect to the standard genome, but also carries B microchromosomes. In the present study, one population of *M. sanctaefilomenae* from the Upper Paraná River basin was cytogenetically characterized, with emphasis on the analysis of B chromosomes. The *M. sanctaefilomenae* individuals showed a karyotype consisting of 2n=50 chromosomes with eight metacentrics, 36 submetacentrics, and six subtelocentrics, and a FN value of 100 in both sexes. In addition to the basic karyotype, all male and female individuals presented a variation from zero to eight B microchromosomes in mitotic metaphases. A secondary constriction was evident in the terminal region of the short arm of the subtelocentric pair 24, which corresponds to the Ag-NORs location. Analysis of the constitutive heterochromatin patterns by C-banding showed heterochromatic blocks in the centromeric and pericentromeric regions in most of the chromosomes. However, the B chromosomes were faintly stained with C-banding, similar to euchromatin in the A chromosomes. Aspects regarding the differences in the number and C-banding pattern of B chromosomes are discussed.

**Key words** Supernumerary chromosome, Karyotype, C-band, Ag-NOR, Karyotype evolution.

Eukaryote genomes are composed of the normal (A) set of chromosomes, and extra or supernumerary (B) chromosomes. B chromosomes do not always occur in pairs, their segregation does not conform to a Mendelian system, which may facilitate transmission rates higher than 0.5 among these chromosomes, resulting in transmission advantages (Camacho et al. 2000).

B chromosomes are dispensable genomic elements that are present in approximately 15% of eukaryotes. The origin of B chromosomes has been investigated in various organisms. Basically, they may arise from the A chromosomes of the current host species (intraspecific origin) or be derived interspecifically through hybridization (Camacho et al. 2000). In characid fish, an intraspecific origin of B chromosomes has been demonstrated, for instance, in *Astyanax paranae* (Silva et al. 2014) and *Moenkhausia sanctaefilomenae* (Scudeler et al. 2015, Utsunomia et al. 2016).

*M. sanctaefilomenae* is a small freshwater characid fish that shows a conserved karyotype with respect to the standard genome (2n=50 biarmed chromosomes) but also carries B microchromosomes in almost all populations hitherto analyzed (Foresti et al. 1989, Portela-Castro et al. 2000, Dantas et al. 2007, Hashimoto et al. 2012, Scudeler et al. 2015, Utsunomia et al. 2016), except for those from the Novo River (Paranapanema River Basin) in which B chromosomes are lacking (Utsunomia et al. 2016). This species possesses from zero to eight B chromosomes, with numbers varying intra- and inter-individually and either euchromatic or partially or fully heterochromatic B chromosomes (Foresti et al. 1989, Dantas et al. 2007, Hashimoto et al. 2012, Scudeler et al. 2015, Utsunomia et al. 2016), whereas one population collected in the Paraná River shows only the euchromatic variant, which is restricted to males (Portela-Castro et al. 2000).

Considering the notoriety of *M. sanctaefilomenae* in studies focused on the investigation of the origin and structure of B chromosomes, the present work, reports the occurrence of multiple euchromatic B microchromosomes in *M. sanctaefilomenae* males and females of the Paraná River Basin and addresses aspects of the B chromosome evolution, contributing to the recent discussions about this theme.

**Materials and methods**

We analysed six individuals (three males and three females) of *M. sanctaefilomenae* collected from Guaçu stream, upper Paraná River basin (Mundo Novo-MS; 23°54′19.6″S and 54°21′43.4″W).

The fishes were identified and deposited in the State University of Mato Grosso do Sul, Mundo Novo, Bra-

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DOI: 10.1508/cytologia.82.547
The experimentation followed the ethical conduct protocols, with fishes being anesthetized by an overdose of clove oil prior to evisceration (Griffiths 2000). Metaphasic chromosomes were obtained from anterior kidney cells using the air-drying technique (Bertollo et al. 1978). Analysis of the C-positive heterochromatin (C-bands) followed the basic procedures of Sumner (1972), with some minor adaptations. NORs were detected by means of silver nitrate staining (Ag-NORs), according to Howell and Black (1980).

At least 30 metaphases were analyzed for each individual and those with better chromosome morphology were used in the karyotype analysis. The chromosomes were classified as metacentric (m), submetacentric (sm), and subtelocentric (st) according to their arm ratio (Levan et al. 1964). For the determination of the fundamental number (FN), or number of chromosome arms, the m, sm, and st chromosomes were considered as bearing two arms.

**Results**

The *M. sanctaefilomenae* individuals presented 2n=50 chromosomes with a karyotypic constitution of eight metacentrics, 36 submetacentrics, and six subtelocentrics, and a FN value of 100 in both sexes. In addition
to the basic karyotype, all male and female individuals presented a variation from zero to eight B microchromosomes in the somatic cells without homology with the other chromosomes (Fig. 1). These elements are smaller than any chromosome of the normal A complement.

A secondary constriction was evident in the terminal region of the short arm of the subtelocentric pair 24, which corresponds to the Ag-NORs location (Fig. 1a, in box). Analysis of the constitutive heterochromatin patterns by C-banding showed heterochromatic blocks in the centromeric and pericentromeric regions in most of the chromosomes (Fig. 1b). However, similar to euchromatin in the A chromosomes, the B chromosomes were faintly stained with C-banding (Fig. 2).

**Discussion**

The diploid chromosome number \((2n=50)\) and karyotypes composed mainly of metacentric and submetacentric chromosomes, which were described in the present study, seem to be a conserved characteristic of *M. sanctaefilomenae* populations (Foresti et al. 1989, Portela-Castro et al. 2000, Portela-Castro and Júlio Jr. 2002, Dantas et al. 2007, Hashimoto et al. 2012, Scudeler et al. 2015, Utsunomia et al. 2016). However, variations among different populations emerge when the number and C-banding pattern B chromosomes are analyzed.

The *M. sanctaefilomenae* population from Guaçu stream (Paraná River basin) presented with zero to eight B microchromosomes, with numbers varying intra- and inter-individually for males and females. For a population belonging to the same hydrographic basin, only males presented with zero to two B microchromosomes (Portela-Castro et al. 2000). On the other hand, the specimens analyzed by Foresti et al. (1989) and Hashimoto et al. (2012) belonging to the Tietê River basin, presented one to eight B microchromosomes in both sexes. These polymorphisms regarding the occurrence of B microchromosomes likely indicate a process of genetic divergence in distinct *M. sanctaefilomenae* populations, as reported for species of *Astyanax* (Moreira-Filho and Bertollo 1991, Fernandes and Martins Santos 2005, Vicari et al. 2008, Hashimoto et al. 2011) which are restricted to small tributaries and streams.

*M. sanctaefilomenae* under study presented similar distribution pattern of heterochromatin to other populations, with interstitial and centromeric conspicuous blocks in the majority of the chromosomes. However, similar to euchromatin in A chromosomes, all B microchromosomes were faintly stained with C-banding. This result partially corroborates with Foresti et al. (1989), Hashimoto et al. (2012) and Utsunomia et al. (2016) that observed euchromatic B microchromosomes as found in our study and also partially or totally heterochromatic B microchromosomes. This is an indication that these B chromosomes can have a different DNA composition, mainly with respect to repetitive sequences.

These observations highlight that the simple Ag-NORs phenotypes observed in our study is a contrasting result from other populations that presented multiple Ag-NORs phenotypes (Foresti et al. 1989, Portela-Castro et al. 2000, Hashimoto et al. 2012). The B microchromosomes detected here did not present Ag impregnation, unlike the population of *M. sanctaefilomenae* from Tietê River basin, in which B chromosomes were entirely marked with silver nitrate (Hashimoto et al. 2012). On the other hand, FISH experiments using 18S rDNA probes revealed the presence of non-active ribosomal genes in some B chromosomes of a population from the Tietê River basin (Scudeler et al. 2015). In addition, it was recently demonstrated that the two B types, euchromatic (designated B1) and heterochromatic (designated B2), contain the same tandem repeat DNA sequences (18S ribosomal DNA, H3 histone genes, MS3 and MS7 satellite DNA) in a population of *M. sanctaefilomenae* from the Tietê River basin (Utsunomia et al. 2016).

We emphasize the need for FISH studies with repeatable DNA sequences in the populations of *M. sanctaefilomenae* of the Guaçu stream, in order to confirm the DNA composition of these euchromatic B microchromosomes, which apparently present a different composition from those individuals previously analyzed from the Tiete River Basin.

**Acknowledgements**

The authors thank the Brazilian agency Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT) for financial support. Besides, we are grateful to Ministério do Meio Ambiente/Instituto Chico Mendes de Conservação da Biodiversidade (MMA/ICMBio—License number 54199-1) for authorization to collect the biological material.

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