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First Description of supernumerary Chromosomes in *Ictalurus punctatus* Rafinesque 1818 Reveals Active Ribosomal Genes in the B Complement

Fernanda Dotti do PRADO, Sandro Natal DANIEL, Manolo PENITENTE, Diogo Teruo HASHIMOTO, Fausto FORESTI, and Fábio PORTO-FORESTI

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The North American channel catfish *Ictalurus punctatus* Rafinesque 1818 is cultivated in the United States, Asia and Brazilian fish farms, and also utilized as a model species in aquaculture and genetic studies. In this work, cytogenetic analysis of *I. punctatus* from Brazilian aquaculture revealed for the first time the presence of extra elements (supernumerary or B chromosomes) in this species. These elements were characterized as *dot-like* micro B chromosomes and were found in three individuals (varying from 0 to 1) and in one individual with higher incidence per cell (varying from 0 to 5; mean number of Bs per cell = 2.01). More specific cytogenetic techniques in this individual revealed 58 A chromosomes (standard complement) containing heterochromatic bands in the centromeric regions, a single Ag-NOR in a subtelocentric pair (also positive for 18S rDNA using the FISH technique) and multiple 5S rDNA clusters in three different subtelocentric chromosomes. Four B chromosomes were entirely Ag-NOR positive (also fully heterochromatic) and three presented 18S rDNA clusters by FISH. The occurrence of Ag-NOR and 18S ribosomal genes in both A and B chromosome complements may indicate an intraspecific origin for these extra chromosomes. Additionally, the terminal location of 18S ribosomal clusters in the Ag-NOR-bearing chromosomes and the presence of active NOR in the B chromosomes suggested that breakage events may be related to a possible recent origin of these extra elements. We suggest this data may be useful as cytogenetic information for future elucidation of the composition, origin and evolution of extra chromosomes in fishes.

Key words: Cytogenetics, channel catfish, constitutive heterochromatin, Ag-NOR, B chromosomes.

Fernanda Dotti do PRADO, Sandro Natal DANIEL, Manolo PENITENTE, Fábio PORTO-FORESTI, Departamento de Ciências Biológicas, Faculdade de Ciências, Universidade Estadual Paulista (Unesp), Campus de Bauru, SP, Brazil.
E-mail: ferprado_bio@hotmail.com
sandro@fc.unesp.br
manolopenitente@gmail.com
fpforesti@fc.unesp.br
Diogo Teruo HASHIMOTO, Centro de Aquicultura da Unesp, Universidade Estadual Paulista (Unesp), Campus de Jaboticabal, SP, Brazil.
E-mail: diogohashimoto@caunsep.unesp.br
Fausto FORESTI, Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista (Unesp), Campus de Botucatu, SP, Brazil.
E-mail: fforesti@ibb.unesp.br

B chromosomes are extra elements found in the genome of several species groups (LECLAIR *et al.* 1996; LEVIN *et al.* 2005; VUJOSEVIC & BLAGOJEVIc 2004; CAMACHO 2005). In fishes, these elements have been widely studied, especially for Neotropical species (OLIVEIRA *et al.* 2009; HASHIMOTO *et al*. 2012; DANIEL *et al*. 2015). In this context, supernumerary chromosomes have aroused great interest from the scientific community, especially because of their apparently dispensable nature, and also because they still persist in populations without being eliminated by natural selection (CARVALHO *et al.* 2008).

Despite B chromosomes being present in a wide taxonomic coverage, until recently, knowledge concerning their origin, composition, regulation and accumulation mechanisms was very limited. Current technological advances involving sequencing and genomic analysis of B chromosomes (NOVAK *et al.* 2010; MAYER *et al.* 2011; SILVA *et al.*

2016; UTSUNOMIA *et al.* 2016) are helping to elucidate the genetic characteristics and molecular structure of these genomic elements, allowing to formulate hypotheses about their origin. Two main possibilities to explain the presence of B chromosomes in the organisms include intra- or interspecific origins. In the first case, B chromosomes originate from elements that compose the standard A complement, but later came to develop an independent evolutionary pathway (CAMACHO *et al.* 2000; TERUEL *et al.* 2009). In the second case, B chromosomes could emerge from a hybridization event, as a result of crosses between closely related species (CAMACHO *et al.* 2000; PERFECTTI & WERREN 2001).

The channel catfish (*Ictalurus punctatus* Rafinesque 1818) is native to the North American continent and dominates the United States aquaculture industry, representing over 50% of the total fisheries production (DUNHAM *et al.* 1998; REZK *et al.* 2003). In Brazil, the channel catfish was introduced in the 1970´s, initially in the northeast region, afterwards its cultivation expanded to the south in the 1980´s (PIEDRAS 1990), rapidly reaching various segments in fish farming, and becoming a fish of high economic representation in the country (GOMES & SCHLINDWEIN 2000). In the present study, we presented and discussed cytogenetic data for *I. punctatus* individuals collected in a Brazilian fish farm and described the first record of B chromosomes in this species. It is expected that these data may contribute to future studies involving B chromosomes in fishes and help to elucidate aspects of their evolution, composition and origin.

Material and Methods

Twelve individuals of *Ictalurus punctatus* were cytogenetically analyzed. Fishes were collected in a fish farm in São Paulo State (Brazil) under authorization of the MMA/IBAMA/ICMBio/SISBIO (Protocol number 18884-1), and registration at IBAMA (Protocol number no. 2567470). Sampling procedures, animal maintenance and analysis were carried out according to international rules on animal testing followed by the São Paulo State University (CEEAA/IBB / UNESP).

The sampled animals were morphologically identified and vouchers of specimens were stored in the fish collection of the Laboratório de Genética de Peixes – UNESP (Bauru, São Paulo State, Brazil). Animals were anesthetized and dissected, and chromosomal preparations were obtained from kidney tissues using the technique described by FORESTI *et al*. (1981). Silver staining of the nucleolus organizer regions (Ag-NOR) followed the technique of HOWELL & BLACK (1980), and C-banding was performed according to SUMNER (1972).

Fluorescent *in situ* hybridization (FISH) followed the protocol described by PINKEL *et al*. (1986) using 77% of formamide stringency. The primer 5S A (5'-TCAACCAACCACAAAGACATTGGCAC-3') and 5S B (5'-TAGACTTCTGGGTGGCCAAAGGAATCA-3') (PENDÁS *et al.* 1994) were used for 5S rDNA (ribosomal DNA) amplification; and for the 18S rDNA segment the primers used were 18S A (5'-TACGCCCGATCTCGTCCGATC-3') and 18S B (5'-CAGGCTGGTATGGCCGTAAGC-3') (HATANAKA & GALETTI Jr 2004). Probes for 18S rDNA were labeled with biotin 14-dATP and with digoxigenin 11-dUTP (Roche Applied Science) for 5S rDNA by means of PCR (polymerase chain reaction). The hybridization signals were detected using Avidin-Fluorescein (FITC) and anti-digoxigenin-rhodamine (Roche Applied Science), respectively. Metaphases were counterstained with DAPI (4,6-diamidino-2-phenylindole dihydrochloride) for visualization of chromosomes.

Images were captured using the software QCapture Pro 5.1.1.14 and a digital camera Olympus Qcolor5 coupled to a fluorescence microscope (BX50, Olympus). Karyotype images were edited according to brightness and contrast levels of metaphases, as well as the relocation of the chromosomes to karyotype composition with the Adobe Photoshop CS5 program. Chromosomal morphology was determined on the basis of arm ratio, as proposed by LEVAN *et al*. (1964) and the chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a).

Results

Giemsa staining revealed the diploid number (2n) of 58 chromosomes as the standard A complement, classified in 8m+20sm+24st+6a and presenting a fundamental number (number of chromosome arms) of 110 (Fig. 1). Extra chromosomes occurred in four individuals. Among these fish, three presented intra-individual variation from zero to one B chromosome. For one individual (named 6378), supernumerary elements varied from zero to five in the cells (Fig. 2, Table 1). The mean frequency of B per cell was 2.01 (Table 1) and the occurrence of 3 B and 5 B were rare, only visualized for three and two metaphases, respectively. B chromosomes presented small size and were considered micro B chromosomes when compared to the chromosomes of the normal complement of the species. Their morphology was dot-like with an uncertain centromere position (Figs 2, 3 and 4).

Chromosome banding techniques as Ag-NOR, C-banding and FISH were applied in individual

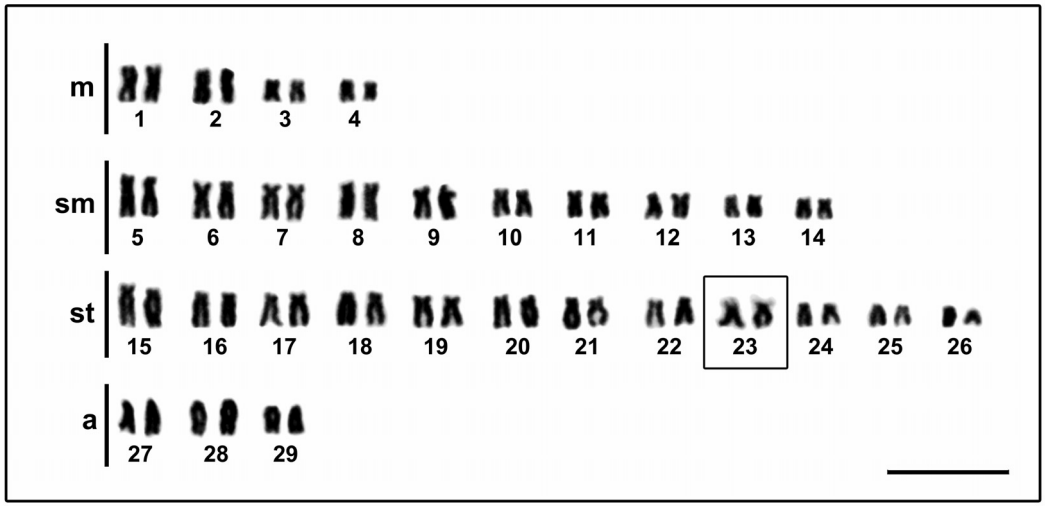

Fig. 1. *I. punctatus* karyotype after Giemsa staining. The box indicates the karyotype localization of the NOR-bearing chromosomes. Bar = 10 μm.

Table 1

B chromosome distribution in cells of one individual of *I. punctatus*

Specimen identification	Number of chromosomes per cell						Total of cells counted
	0	1	2	3	4	5	
3168 Mean = 2.01	12	10	13	3	12	2	51

6378 (Fig. 3) that showed the majority of B chromosomes in order to investigate the cytogenetic characteristics of these elements. However, in these analyses, no cell presenting 3 or 5B was found, probably due to the low frequency of this number of Bs per cell (Table 1). Thus, the maximum number of Bs per cell in posterior analyses was 4 (Figs 3 and 4).

Silver staining revealed active Ag-NORs on the terminal region of the short arms of two subtelocentric chromosomes (Fig. 3a) corresponding to the pair number 23 (Fig. 1). Constitutive heterochromatin was distributed through centromeric regions of the majority of A chromosomes (Fig. 3b). Four B chromosomes were totally Ag-NOR and C-band positive (Fig. 3).

The FISH technique using ribosomal probes revealed 18S rDNA genes located in the same subtelocentric pair bearing Ag-NORs and multiple 5S rDNA clusters in the terminal position of three distinct subtelocentric chromosomes (Fig. 4). 18S clusters were also present in three B chromosomes while one B remained without 18S signals (Fig. 4d'). Some differences in FISH intensity were also observed, with two B chromosomes fully and intensively marked (Figs 4b' and 4c') and one B poorly marked (Fig. 4d').

Discussion

In the present study, individuals presented the same diploid number (2n=58) previously reported for *I. punctatus*, which was considered conserved among related species (LEGRANDE 1981; LEGRANDE *et al*. 1984). The pattern of heterochromatic bands in the centromeric regions of A chromosomes and a unique NOR bearing pair (revealed by Silver staining and FISH technique) observed in this work was also similar to previous data for *I. punctatus* (ZHANG & TERRENCE 1998). In fishes, 5S rDNA clusters are most commonly observed in interstitial areas of the chromosomes and are considered more conserved when compared with 18S clusters (MARTINS & WASKO 2004). However, in this work, 5S signals were located in three chromosomes and one 5S signal is apparently missing. This result may be explained by the possibility of chromosomal rearrangements and breakages from the terminal position of this gene in the chromosomes. However, technical problems may not be discarded, as the presence of weak fluorescent signals from one of the homologous-bearing 5S clusters not detected using the FISH technique.

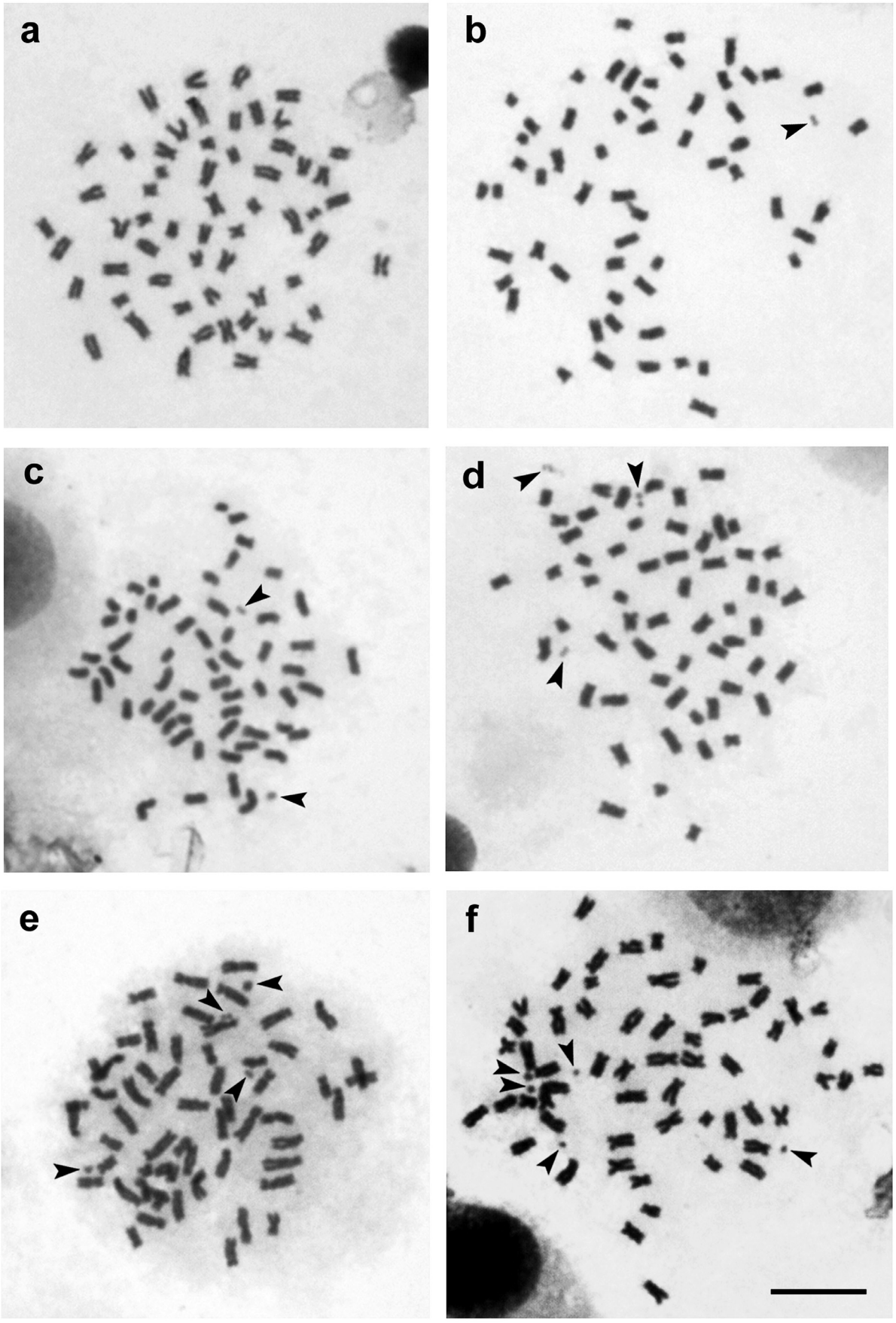

Fig. 2. Metaphases of *I. punctatus* presenting zero (a), one (b), two (c), three (d), four (e) and five (f) B chromosomes. Arrowheads indicate B chromosomes. Bar =10 µm.

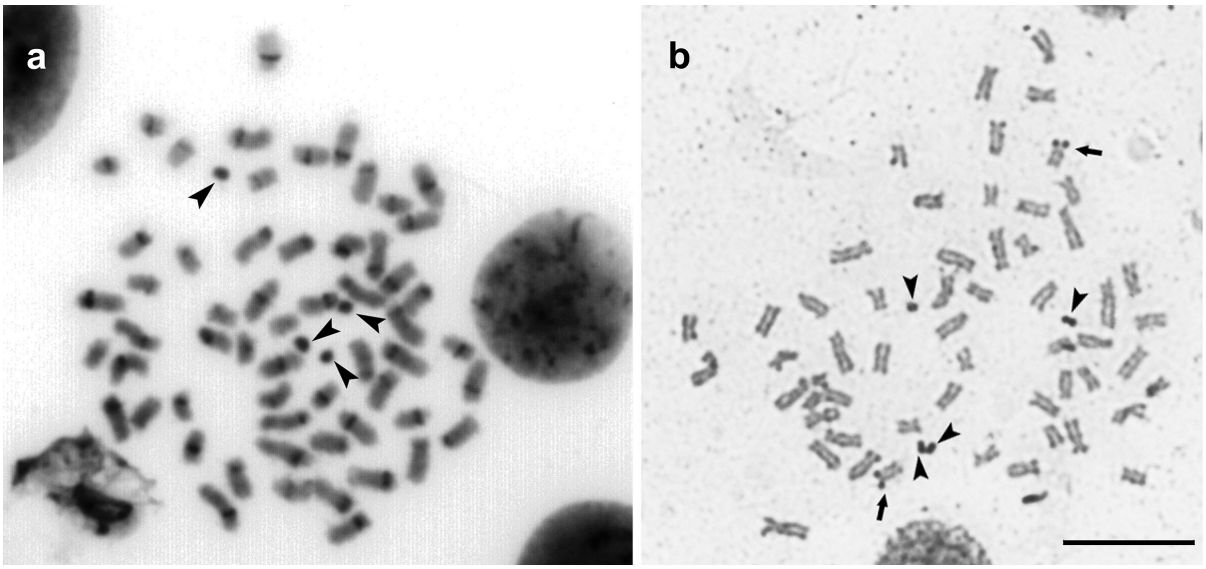

Fig. 3. Metaphases of *I. punctatus* presenting 4 B chromosomes after banding treatment. (a) C-band; (b) Ag-NOR. Arrows indicate Ag-NOR bearing chromosomes and arrowheads indicate B chromosomes. Bar =10 μm.

Here we reported the first occurrence of B chromosomes in *I. punctatus*, indicating mitotic instability, and variable number of B chromosome number per metaphase. These supernumeraries were presented as dot-like elements. Supernumerary chromosomes showing a well defined morphology are commonly observed for catfish species (PANDEY & LAKRA 1997; MORAES *et al.* 2009). However, as found in the present work, micro chromosome Bs have already been reported for other fish such as the widely studied species *Prochilodus lineatus* Valenciennes 1837 (ARTONI *et al.* 2006; PENITENTE *et al.* 2016).

The B chromosomes in *I. punctatus* were considered heterochromatic, which is the most common pattern observed for fishes (VENERE *et al.* 1999). Concerning ribosomal genes, a notable feature found herein was the presence of positive NOR signals, detected by Silver staining and the 18S rDNA FISH method in the B complement, indicating that some areas of these elements remain functional in ribosome activity. Ag-staining provides a simple method to detect ribosomal gene transcription (HOWELL & BLACK 1980). B chromosomes in several species carry rDNA genes (CAMACHO 2005), including fish species (BARONI *et al*. 2009; POLETTO *et al*. 2010; HASHIMOTO *et al*. 2012), and in some cases, rDNA has been detected by Ag-staining evidencing the presence of active genes, as demonstrated in the present study.

In relation to the origin of these B chromosomes, as 18S ribosomal genes were detected, we suggest a possible intraspecific source from the A complement. This hypothesis has been proposed for other fish species, e.g. VICARI *et al*. (2011) studied the distribution of As51, 18S and 5S rDNA markers on the chromosomes of *Astyanax scabripinnis* Jenyns 1842 and suggested an intraspecific origin for the B chromosomes. Chromosomal breaks may contribute to the emergence of B chromosomes (HOUBEN *et al.* 2014; BANAEI-MOGHADDAM *et al.* 2015). The location of 18S ribosomal genes in the terminal portion of chromosomes in *I. punctatus* as observed in this study may favor modifier events, confirming the possibility of an intra-specific origin of B chromosomes derived from chromosomal rearrangements involving chromosome breakage of heterochromatic segments and repetitive sequences of 18S rDNA.

SILVA *et al*. (2016) verified that only two of seven B-types analyzed in *Astyanax* species carried 18S rDNA. In the present work, despite the evident correspondence between B chromosomes carrying active NORs and 18S rDNA clusters, one B remained without 18S signals. Considering the small size, similar morphology and the variable quantity of Bs per cell observed in this study, it is possible that this B was not detected by the Ag-NOR technique, i.e., it may be a different B variant in this species that does not carry ribosomal genes. The dispensability of B chromosomes explains why their DNA sequences evolve at a high rate, which makes possible the presence of a variant with active 18S regions and another kind of B with a different origin or posterior genomic modifications.

These results have shown that the B chromosomes may be important for the genome organization of *I. punctatus*. Our data will be useful for further analyses to determine whether the B chromosome context can influence the NOR activity in order to provide an extra amount of rRNA for ribosome assembly, and consequently if individuals

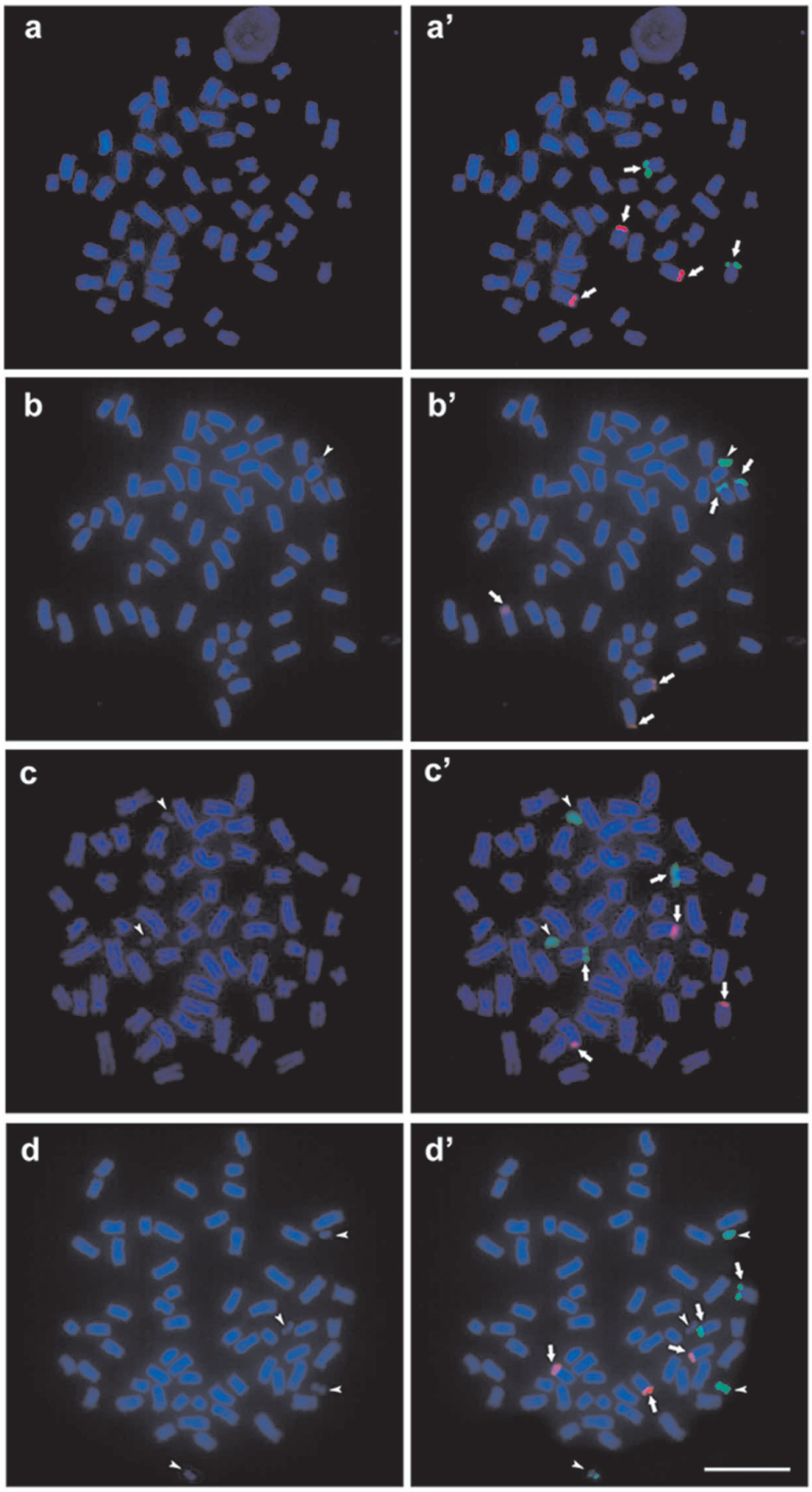

Fig. 4. Metaphases of *I. punctatus* after DAPI staining presenting 0 (a), 1 (b), 2 (c) and 4 (d) B chromosomes; and the same metaphases after the double FISH (a', b', c', d') with rRNA 18S (green) and 5S (red) probes. Arrowheads indicate B chromosomes and arrows indicate chromosomes of the A complex carrying ribosomal genes. Bar =10 μm.

bearing B chromosomes with nucleolar activity could have better performance in aquaculture. Our results revealed at least three B chromosomes containing active NORs corresponding to 18S sequences, suggesting an intraspecific and recent origin of these elements in this individual, probably resulting from breaks of terminal NORs from the A complement. These results contribute to the study of the channel catfish and also add information to the future elucidation of evolution and origin of supernumerary chromosomes in fishes.

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References

ARTONI R.F., SHIBATTA O.A., GROSS M.C., SCHNEIDER C.H., ALMEIDA M.C. 2006. *Astyanax* aff . *fasciatus* Cuvier, 1819 (Teleostei, Characidae): evidences of a species complex in the upper rio Tibagi basin (Paraná, Brazil). Neotrop. Ichthyol. **4**: 197-202.

BANAEI-MOGHADDAM A.M., MARTIS M.M., MACAS J., GUNDLACH H., HIMMELBACH A., ALTSCHMIED L., MAYER K.F.X., HOUBEN A. 2015. Genes on B chromosomes: old questions revisited with new tools. BBA, Gene Regulatory Mechanisms **1849**: 64-70.

BARONI S., LOPES C.E., ALMEIDA-TOLEDO L.F. 2009. Cytogenetic characterization of *Metynnis maculatus* (Teleostei; Characiformes): the description in Serrasalminae of a small B chromosome bearing inactive NOR-like sequences. Caryologia. **2**: 95-101.

CAMACHO J.P.M. 2005. *B chromosomes*. (In: The Evolution of the Genome. Gregory T.R. ed. San Diego, California, USA): 223-286.

CAMACHO J.P.M., SHARBEL T.F., BEUKEBOOM L.W. 2000. B-chromosome evolution. Philos. Trans. Roy. Soc. B (London) **355**: 163-178.

CARVALHO R.A., MARTINS-SANTOS I.C., DIAS A.L. 2008. B chromosomes: an update about their occurrence in freshwater Neotropical fishes (Teleostei*).* J. Fish Biol. **72**: 1907-1932.

DANIEL S.N., PENITENTE M., HASHIMOTO D.T., SILVA D.M.Z.A., FORESTI F., PORTO-FORESTI F. 2015. New insights into karyotypic relationships among populations of *Astyanax bockmanni* (Teleostei, Characiformes) of different watersheds. Zebrafish **12**: 181-188.

DUNHAM R.A., LIU Z., ARGUE B.J. 1998. The effect of the absence or presence of *channel catfish* males on induced ovulation of channel catfish females for artificial fertilization with blue catfish sperm. Prog. Fish Cult. **60**: 297-300.

FORESTI F., ALMEIDA TOLEDO L.D., TOLEDO F.S.A. 1981. Polymorphic nature of nucleolus organizer regions in fishes. Cytog. Gen. Res. **31**: 137-144.

GOMES S.Z., SCHLINDWEIN A.P. 2000. Efeitos de períodos de cultivo e densidades de estocagem sobre o desempenho do Catfish (*Ictalurus punctatus* sp) nas condições climáticas do litoral de Santa Catarina. Rev. Bras. Zootechn. **29**: 1266-1272.

HASHIMOTO D.T., VOLTOLIN T.A., PAES A.D.N.V.A., FORESTI F., BORTOLOZZI J., PORTO-FORESTI F. 2012. Cytogenetic analysis of B chromosomes in one population of the fish *Moenkhausia sanctaefilomenae* (Steindachner, 1907) (Teleostei, Characiformes). Comp. Cytogen. **6**: 141.

HATANAKA T., GALETTI Jr. P.M. 2004. Mapping of the 18S and 5S ribosomal RNA genes in the fish *Prochilodus argenteus* Agassiz, 1829 (Characiformes, Prochilodontidae). Genetica **122**: 239-244.

HOUBEN A., BANAEI MOGHADDAM A.M.,·KLEMME S., TIMMIS J.N. 2014. Evolution and biology of supernumerary B chromosomes. Cell. Mol. Life Sci. **71**: 467-478.

HOWELL W.T., BLACK D.A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia **36**: 1014-1015.

LECLAIR S., ANSAN-MELAYAH D., ROUXEL T., BALESDENT M. 1996. Meiotic behaviour of the minichromosome in the phytopathogenic ascomycete *Leptosphaeria maculans*. Current Genet. **30**: 541-548.

LEGRANDE W.H. 1981. Chromosomal evolution in North American catfishes (Siluriformes: Ictaluridae) with particular emphasis on the madtoms, *Noturu*s. Copeia **1981**: 33-52.

LEGRANDE W.H., DUNHAM R.A., SMITHERMAN R.O. 1984. Karyology of three species of catfishes (Ictaluridae: *Ictalurus*) and four hybrid combinations. Copeia **1984**: 873-878.

LEVAN A., FREDGA K., SANDBREG A.A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201-220.

LEVIN D.A., PALESTIS B.G., JONES R.N., TRIVERS R. 2005. Phyletic hot spots for B chromosomes in angiosperms. Evolution **59**: 962-969.

MARTINS C., WASKO A.P. 2004. Organization and evolution of 5S ribosomal DNA in the fish genome. Focus on Genome Res. 335-363.

MAYER K.F., MARTIS M., HEDLEY P.E., SIMKOVA H., LIU H., MORRIS J.A., STEUERNAGEL B., TAUDIEN S., ROESSNER S., GUNDLACH H., KUBALAKOVA M., SUCHANKOVA P., MURAT F., FELDER M., NUSSBAUMER T., GRANER A., SALSE J., ENDO T., SAKAI H., TANAKA T., ITOH T., SATO K., PLATZER M., MATSUMOTO T., SCHOLZ U., DOLEZEL J., WAUGH R., STEIN N. 2011. Unlocking the barley genome by chromosomal and comparative genomics. The Plant Cell **23**: 1249-1263.

MORAES V.P.O., CARNEIRO J.S., DIAS A.L. 2009. B Chromosomes in Four Different Populations of *Rhamdia quelen* (Siluriformes, Heptapteridae): A Comparative Study of Frequency and Distribution. Folia Biol. (Kraków) **57**: 165-169.

NOVAK P., NEUMANN P., MACAS J. 2010. Graph-based clustering and characterization of repetitive sequences in next generation sequencing data. BMC Bioinformatics **11**: 378.

OLIVEIRA C., FORESTI F., HILSDORf A.W.S. 2009. Genetics of neotropical fish: from chromosomes to populations. Fish Physiol. Biochem. **35**: 81-100.

PANDEY N.L.W.S., LAKRA W.S. 1997. Evidence of female heterogamety, B-chromosome and natural tetraploidy in the Asian catfish, *Clarias batrachus*, used in aquaculture. Aquaculture **149**: 31-37.

PENDÁS A.M., MORÁN P., FREIJE J.P., GARCIA-VÁSQUEZ E. 1994. Chromosomal mapping and nucleotide sequence of two *tandem* repeats of Atlantic salmon 5S rDNA. Cytogenet. Cell Genet. **67**: 31-36.

PENITENTE M., DANIEL S.N., SENHORINI J.A., FORESTI F., PORTO-FORESTI F. 2016. Transmission Behavior of B Chromosomes in *Prochilodus lineatus* (Characiformes, Prochilodontidae). Cytogenet. Genome Res. **147**: 179-185.

PERFECTTI F., WERREN J.H. 2001. The Interspecific Origin of B chromosomes: Experimental Evidence. Evolution **55**: 1069-1073. 1-12.

PIEDRAS S.R.N. 1990. Manual prático para o cultivo do channel catfish. EDUCAT-UCPEL, Pelotas, RS, Brazil. Pp 72.

PINKEL D., STRAUME T., GRAY J.W. 1986. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. P. Nat. Acad. Sci. USA, 83, Pp 2934-2938.

POLETTO A.B., FERREIRA I.A., MARTINS C. 2010. The B chromosomes of the African cichlid fish *Haplochromis obliquidens harbour* 18S rRNA gene copies. BMC Genetics **11**: 1.

REZK M.A., SMITHERMAN R.O., WILLIAMS J.C., NICHOLS A., KUCUKTAS H., DUNHAM R.A. 2003. Response to three generations of selection for increased body weight in channel catfish, *Ictalurus punctatus*, grown in earthen ponds. Aquaculture **228**: 69-79.

SILVA D.M.Z.A., DANIEL S.N., CAMACHO J.P.M., UTSUNOMIA R., RUIZ-RUANO F.J., PENITENTE M., PANSONATO-ALVES J.C., HASHIMOTO D.T.,· OLIVEIRA C., PORTO-FORESTI F., FORESTI F. 2016. Origin of B chromosomes in the genus *Astyanax* (Characiformes, Characidae) and the limits of chromosome painting. Mol. Genet. Mol. Genet. Genomics **291**: 1407-1418.

SUMNER A.T. 1972. A simple technique for demonstrating centromExperimental Evidence. Evolution **55**: 1069-1073.

TERUEL M., CABRERO J., MONTIEL E.E., ACOSTA M.J., SÁNCHEZ A., CAMACHO J.P.M. 2009. Microdissection and chromosome painting of X and B chromosomes in *Locusta migratória*. Chromosome Res. **17**: 11-18.

UTSUNOMIA R., SILVA D.M.Z.A., RUIZ-RUANO F.J., ARAYA-JAIME C., PANSONATO-ALVES J.C., SCACCHETTI P.C., HASHIMOTO D.T., OLIVEIRA C., TRIFONOV V.A., PORTO-FORESTI F., CAMACHO J.P.M., FORESTI F. 2016. Uncovering the ancestry of B chromosomes in *Moenkhausia sanctaefilomenae* (Teleostei, Characidae). PloS One **11**: e0150573.

VENERE P.C., MIYAZAWA C.S., GALETTI Jr P.M. 1999. New cases of supernumerary chromosomes in characiform fishes. Genet. Mol. Biol. **22**: 345-349.

VICARI M.R., PISTUNE H.F.M., CASTRO J.P., ALMEIDA M.C., BERTOLLO L.A.C., MOREIRA-FILHO O., CAMACHO J.P.M., ARTONI R.F. 2011. New insights on the origin of B chromosomes in *Astyanax scabripinnis* obtained by chromosome painting and FISH. Genetica **139**: 1073-1081.

VUJOSEVIC M., BLAGOJEVIC J. 2004. B chromosomes in populations of mammals. Cyt. Gen. Res. **106**: 247-256.

ZHANG Q., TERRENCE R.T. 1998. Standardization of the channel catfish karyotype with localization of constitutive heterochromatin and restriction enzyme banding. T. Am. Fish Soc. **127**: 551-559.