First description of B chromosomes in the Hyphessobrycon (Characiformes, Characidae) genus: a hypothesis for the extra element of Hyphessobrycon eques Steindachner, 1882

Diovani Piscor¹, Patricia Pasquali Parise-Maltempi¹

¹ Instituto de Biociências, Departamento de Biologia, Laboratório de Citogenética, Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Av. 24A, 1515, CEP: 13506-900, Rio Claro, SP, Brazil

Corresponding author: Patricia Pasquali Parise-Maltempi (parise@rc.unesp.br)

Academic editor: I. Kuznetcova | Received 30 April 2015 | Accepted 18 June 2014 | Published 3 July 2015

Abstract

The Hyphessobrycon are allocated in the incertae sedis group of the Characidae family, one of the genera with more species of the group. The chromosomes of some species of Hyphessobrycon are known, and the diploid number most common for genus is 2n = 50 chromosomes. The aims of this study were to examine the karyotype macrostructure in the Hyphessobrycon eques Steindachner, 1882, and show a new origin hypothesis for B chromosomes. The diploid number observed for H. eques was 2n = 52 chromosomes, and a karyotype formulae of 12m + 18sm + 8st + 14a, with FN (fundamental number) = 90 for both sexes. Only two females showed one B chromosome. The heterochromatin was observed mainly on centromeric regions, and in the long arm of the B chromosome. In this paper, the relationship of the B chromosome of H. eques with an occasional chromosome rearrangement was discussed.

Keywords

Karyotype, supernumerary chromosomes, C-banding, heteromorphism, chromosome evolution
Introduction

The *Hyphessobrycon* are allocated in the *incertae sedis* group of the Characidade family (Lima et al. 2003) with more than 130 species (e.g., Lima and Moreira 2003, Carvalho and Bertaco 2006). Among these, a species known as “Mato Grosso” has been considered *Hyphessobrycon callistus* (Boulenger, 1900) for a long time, however with the revision of Weitzman and Palmer (1997), it started to be classified as *Hyphessobrycon eques*.

The chromosomal data of the *Hyphessobrycon* genus are restricted primarily to the knowledge of the diploid number. Literature data showed that the diploid number vary between 2n = 42 and 52 chromosomes, being 2n = 50 chromosomes the most frequently observed number for the genus, i.e. *Hyphessobrycon scholzei* Ahl, 1937 (Arefjev 1990), *Hyphessobrycon reticulatus* Ellis, 1911 (Wlasiuk and Garcia 1996, Carvalho et al. 2002a), *Hyphessobrycon bifasciatus* Ellis, 1911 (Miyazawa 1997), *Hyphessobrycon aff. santae* Eigenmann, 1907 (Miyazawa 1997) and *Hyphessobrycon anisitsi* Eigenmann, 1907 (Centofante et al. 2003). According to Carvalho et al. (2002a) many species of the genus have a known chromosome set, though for many species only the haploid number has been described.

The B chromosomes have been described in many neotropical fish groups (see, for example, Maistro et al. 1992, Oliveira et al. 1997, Maistro et al. 2000, Torres-Mariano and Morelli 2008, Ferreira-Neto et al. 2012, Hashimoto et al. 2012, Silva et al. 2014). The occurrence of this type of chromosome among individuals of a population can be sporadic or commonly found for many individuals, and high frequency can be shown between them. It is also possible to find variations regarding to morphology, size, number and pattern of heterochromatin in the B chromosomes (Maistro et al. 1992, Venere et al. 1999, Cavallaro et al. 2000, Fernandes and Martins-Santos 2005, Artoni et al. 2006, Hashimoto et al. 2012, Barbosa et al. 2015).

Whereas the diversity of events described in an attempt to explain the origin and function of B chromosomes, the present study aims to demonstrate the probable origin of B chromosome in *Hyphessobrycon eques* through the study of heterochromatin, and describe for the first time the presence of an extra element in the *Hyphessobrycon* genus.

Material and methods

The *H. eques* (seven males and four females) specimens were obtained from Ribeirão Claro river (22°21′36″S, 47°30′42″W) in the state of São Paulo (SP), Brazil. The individuals were anesthetized with benzocaine (5%) and then used for cytogenetic analysis. The individuals were fixed in formaldehyde 10% and then in ethanol 70%, and placed in the ichthyological collection from Departamento de Biologia do Instituto de Biociências da UNESP, campus de Rio Claro. The chromosomes were obtained as described by Foresti et al. (1981). Chromosome morphologies were determined according to the ratio of the arms (the most frequently used classification system for
First description of B chromosomes in the Hyphessobrycon (Characiformes, Characidae)...

Fish chromosomes. Briefly, the length of the long arm (q) was divided by the length of the short arm (p) as cited by Piscor et al. (2013). Therefore, the chromosomes with two arms and an arm ratio (AR) of 1–1.7 were classified as metacentric (m), those with two arms and AR of 1.71–3 were classified as submetacentric (sm), and those with two arms and AR of 3.01–7 were classified as subtelocentric (st). Chromosomes with a single arm (AR >7) were considered to be acrocentric (a). Heterochromatin was observed using the C-band technique proposed by Sumner (1972).

Results

The *H. eques* specimens had 2n = 52 chromosomes, and the karyotype contained 12 metacentric, 18 submetacentric, 8 subtelocentric, and 14 acrocentric chromosomes (12m + 18sm + 8st + 14a), yielding a FN of 90 for both sexes (Figure 1a, Table 1). A region of secondary constriction was evident on the short arm of one homolog of pair 19 (Figure 1a, b). One subtelocentric B chromosome was observed in all cells of two females (Figures 1b, 2a). Heterochromatic regions were observed mainly on centromeric regions, and a large block was observed in the short arm on one homolog of pair 19 (individuals with and without B chromosome) (Figure 2b). The B chromosome showed the long arm fully heterochromatic (Figure 2b).

A summary diagram indicating a possible origin mechanism of the B chromosome in *H. eques* by heterochromatin blocks is shown in Figure 3.

Table 1. Cytogenetic data and presence of B chromosomes in the *Hyphessobrycon* genus.

| Species               | 2n  | Karyotype formulae | Presence of Bs | References             |
|-----------------------|-----|--------------------|----------------|------------------------|
| *H. minor*            | 52  | 14m+20sm+16st      | –              | Arefjev (1989)         |
| *H. scholzei*         | 50  | 8m+20sm+8st+14a    | –              | Arefjev (1990)         |
| *H. flammeus*         | 52  | 18m/sm+32st+2a     | –              | Arefjev (1990)         |
| *H. herbertaxelrodi*  | 52  | 10m/sm+42st/a      | –              | Arefjev (1990)         |
| *H. reticulatus*      | 50  | 20m+14sm+16st/a    | –              | Wlasiuk and Garcia (1996) |
| *H. bifasciatus*      | 50  | 16m+10sm+12st+12a  | –              | Miyazawa (1997)        |
| *H. aff. santae*      | 50  | 12m+10sm+10st+18a  | –              | Miyazawa (1997)        |
| *H. reticulatus*      | 50  | 14m+20sm+16st      | –              | Carvalho et al. (2002a) |
| *H. reticulatus*      | 50  | –                  | –              | Carvalho et al. (2002b) |
| *H. griemi*           | 48  | –                  | –              | Carvalho et al. (2002b) |
| *H. anisitsi*         | 50  | 6m+16sm+12st+16a   | –              | Centofante et al. (2003) |
| *H. anisitsi*         | 50  | 18m+10sm+6st+16a   | –              | Mendes et al. (2011)   |
| *H. luetkenii*        | 50  | 6m+8sm+36a         | –              | Mendes et al. (2011)   |
| *H. eques*            | 52  | 14m+16sm+4st+18a   | 0–1♀/0♂        | Martinez et al. (2012) |
| *H. eques*            | 52  | 12m+18sm+8st+14a   | 0–1♀/0♂        | Present study           |
Figure 1. Giemsa stained chromosomes of *H. eques*. **A** Karyotype without B chromosome **B** Karyotype with B chromosome. Inset show the B chromosome. Bar = 10 µm.

**Discussion**

The heterochromatin was observed mainly in the centromeric regions on chromosomes of *H. eques* in this present paper. On the other hand, Carvalho et al. (2002a) detected small heterochromatin blocks in the pericentromeric regions in all chromosomes of *H. reticulatus* from Juquiá river (state of São Paulo, Brazil). Centofante et al. (2003) studied two populations of *H. anisitsi* from adjacent hydrographic basins (upper Paraná river basin and Paraíba do Sul river basin) and also observed heterochromatic blocks mainly on pericentromeric regions of most chromosomes.

An interesting feature observed by C-band technique in the *H. eques* was a heteromorphic block of heterochromatin always presents on short arm (pair 19) in all specimens (with and without B chromosomes), which another population of *H. eques* studied by Martinez et al. (2012) not showed. Nevertheless, we believe that the B
First description of B chromosomes in the Hyphessobrycon (Characiformes, Characidae)...

329

chromosome (observed in two *H. eques* females) may be related with chromosomal rearrangements (see a possible mechanism in the Figure 3).

This study reported for the first time the presence of B chromosomes in the Hyphessobrycon genus. According to Leach et al. (2004) analyses of the molecular structure have shown that B chromosomes are subject to gene silencing, repetitive DNA accumulation and heterochromatinization. Thus, most of the heterochromatic of B chromosomes are due to the presence of chromatin characterized by a high degree of condensation during the cell cycle, and this natural condensation results from the high

---

**Figure 2.** Mitotic metaphase chromosomes. **A** Giemsa stained **B** C-banding. The arrow indicates the B chromosome and the arrowhead indicates the secondary constriction. Inset show the pair 19 C-banded of an individual without B chromosome. Bar = 10 µm.

**Figure 3.** Scheme showing a possible origin of the B chromosome in *H. eques*. **A** Pair 19 not fissioned **B** The short arm of one homologous underwent fission and inversion **C** One homologous of pair 19 without the secondary constriction and a B chromosome formed.
content of the repetitive DNA of many types, especially satellite and ribosomal DNAs (Camacho 2005).

Different postulations have been formulated to explain the independent evolution of B chromosomes in the genome of organisms that possess them. Camacho et al. (2000) claim that, subsequent to synaptic isolation of the B chromosome and regardless of their origin, processes of molecular evolution also can occur and determine a degenerate morphology for these genomic segments. Thus, the morphological and structural features would be more a reflex of molecular evolution processes than the way in which they originated. However, it appears that the supernumerary chromosomes do not present a model of common origin, i.e. they may have originated independently following different evolutionary paths.

One hypothesis proposed to explain the presence and function of the B chromosomes is the isochromosome (Vicente et al. 1996, Mestriner 2000, Silva et al. 2014). According to Sumner (2003), isochromosomes are chromosomes with two homologous arms, i.e. which are structurally and genetically equal and may be originated by different ways. The author explains that one of the hypotheses suggested for the emergence of this type of chromosome is the fusion between two identical acrocentric chromosomes, which most likely did not occur with the B chromosome in the *H. eques* studied in this paper.

Nevertheless, the presence of one B chromosome in females may be less likely due to the sex chromosome system in the *H. eques* (even if only one sex) than involved with possible chromosomal break. However, we cannot rule out the possibility that this occasional chromosome break, from now on, may have resulted in the maintenance of this element in the females and drive to differentiation of a sex chromosome system for *H. eques*.

**Acknowledgments**

The authors are grateful to Coordenadoria de Aperfeiçoamento de Ensino Superior (CAPES) for the financial support.

**References**

Arefjev VA (1989) Chromosome complements of four characid species (Characidae, Teleostei). Zoologicheskii Zhurnal 68: 82–91.
Arefjev VA (1990) Problems of karyotypic variability in the family Characidae (Pisces, Characiformes) with the description of somatic karyotypes for six species of tetras. Caryologia 43: 305–319. doi: 10.1080/00087114.1990.10797009
Artoni RF, Vicari MR, Endler AL, Cavallaro ZI, Jesus CM, Almeida MC, Moreira-Filho O, Bertollo LAC (2006) Evolution of B chromosomes in the Prochilodontidae fish, *Prochilodus lineatus*. Genetica 127: 277–284. doi: 10.1007/s10709-005-4846-1
Barbosa P, oliveira LA, Pucci MB, Santos MH, Moreira-Filho O, Vicari MR, Nogaroto V, Almeida MC, Artoni RF (2015) Identification and chromosome mapping of repetitive elements in the Astyanax scabripinnis (Teleostei: Characidae) species complex. Genetica 143: 55–62. doi: 10.1007/s10709-014-9813-2

Camacho JPM (2005) B chromosomes. In: Gregory TR. The evolution of the genome. San Diego, California, USA, 223–286. doi: 10.1016/b978-012301463-4/50006-1

Camacho JPM, Sharbel TF, Beukeboom LW (2000) B chromosome evolution. Philosophical Transactions of the Royal Society of London 355: 163–178. doi: 10.1098/rstb.2000.0556

Carvalho ML, Oliveira C, Foresti F (2002a) Cytogenetics analysis of five species of the subfamily Tetragonopterinae (Teleostei, Characiformes, Characidae). Caryologia 55: 181–188. doi: 10.1080/00087114.2002.10589275

Carvalho ML, Oliveira C, Navarrete MC, Froehlich O, Foresti F (2002b) Nuclear DNA content determination in Characiformes fish (Teleostei, Ostariophysi) from the Neotropical region. Genetics and Molecular Biology 25: 49–55. doi: 10.1590/S1415-47572002000100010

Carvalho TP, Bertaco VA (2006) Two new species of Hyphessobrycon (Teleosteii: Characidae) from upper rio Tapajós basin on Chapada dos Parecis, central Brazil. Neotropical Ichthyology 4: 301–308. doi: 10.1590/S1679-62252006000300001

Cavallaro ZI, Bertollo LAC, Perfecttì F, Camacho JPM (2000) Frequency increase and mitotic stabilization of a B chromosome in fish Prochilodus lineatus. Chromosome Research. 8: 627–634. doi: 10.1023/A:1009242209375

Centofante L, Bertollo LAC, Miyazawa CS, Moreira-Filho O (2003) Chromosomal differentiation among allopatric populations of Hyphessobrycon anisitsi (Pisces, Tetragonopterinae). Cytologia 68: 283–288. doi: 10.1508/cytologia.68.283

Fernandes CA, Martins-Santos IC (2005) Sympatric occurrence of three cytotypes and four morphological types of B chromosomes of Astyanax scabripinnis (Pisces, Characiformes) in the river Ivaí basin, state of Paraná, Brazil. Genetica 124: 301–306. doi: 10.1007/s10709-005-4751-7

Ferreira-Neto M, Artoni RF, Vicari MR, Moreira-Filho O, Camacho JPM, Bak kali M, Oliveira C, Foresti F (2012) Three sympatric karyomorphs in the fish Astyanax fasciatus (Teleostei, Characidae) do not seem to hybridize in natural populations. Comparative Cytogenetics 6(1): 29–40. doi: 10.3897/CompCytogen.v6i1.2151

Foresti F, Almeida-Toledo lf, Toledo-Filho SA (1981) Polymorphic nature of nuclear organizer regions in fishes. Cytogenetics and Cell Genetics 31: 137–144. doi: 10.1159/000131639

Hashimoto DT, Voltolin TA, Paes ADNV, 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). Comparative Cytogenetics 6(2): 141–151. doi: 10.3897/CompCytogen.v6i2.1769

Leach CR, Houben A, Timmis JN (2004) The B chromosome in Brachycome. Cytogenetic and Genome Research 106: 199–209. doi: 10.1159/000079288

Lima FCT, Malabarba LR, Buckup PA, Silva JFP, Vari RP, Harold A, Benine R, Oyakawa OT, Pavanelli CS, Menezes NA et al. (2003) Genera Incertae Sedis in Characidae. In: Reis RE, Kullander SE, Ferraris Jr CJ (Eds) Check List of the Freshwater Fishes of South and Central America. 1st edition. Edipucrs, Porto Alegre, 106–169. doi: 10.1590/S1679-62252003000100003
Lima FCT, Moreira CR (2003) Three new species of *Hyphessobrycon* (Characiformes: Characidae) from the upper rio Araguaia basin in Brazil. Neotropical Ichthyology 1: 21–33.

Maistro EL, Foresti F, Oliveira C, Almeida-Toledo LF (1992) Occurrence of macro B chromosomes in *Astyanax scabripinnis paranae* (Pisces, Characiformes, Characidae). Genetica 87: 101–106. doi: 10.1007/BF00120999

Maistro EL, Oliveira C, Foresti F (2000) Sympatric occurrence of two cytotypes of *Astyanax scabripinnis* (Characiformes, Characidae, Tetragonopteridae). Genetics and Molecular Biology 2: 365–369. doi: 10.1590/S1415-47572000000200021

Martínez ERM, Alves AL, Silveira SM, Foresti F, Oliveira C (2012) Cytogenetic analysis in the *incertae sedis* species *Astyanax altiparanae* Garutti and Britzki, 2000 and *Hyphessobrycon equestris* Steindachner, 1882 (Characiformes, Characidae) from the upper Paraná river basin. Comparative Cytogenetics 6(1): 41–51. doi: 10.3897/CompCytogen.v6i1.1873

Mestriner CA, Galetti Jr PM, Valentini SR, Ruiz IRG, Abel LDS, Moreira-Filho O, Camacho JPM (2000) Structural and functional evidence that a B chromosome in the characid fish *Astyanax scabripinnis* is an isochromosome. Heredity 85: 1–9. doi: 10.1046/j.1365-2540.2000.00702.x

Miyazawa CS (1997) Citogenética de carácidos da bacia do rio Paraguai: Análises citotaxonômicas, evolutivas e considerações biogeográficas. PhD Thesis, Universidade Federal de São Carlos, São Carlos.

Mendes MM, Rosa R, Giuliano-Caetano L, Dias AL (2011) Karyotype diversity of four species of the *incertae sedis* group (Characidae) from different hydrographic basins: analysis of AgNORs, CMA3 and 18S rDNA. Genetics and Molecular Research 10(4): 3596–3608. doi: 10.4238/2011.November.22.5

Oliveira C, Saboya SMR, Foresti F, Senhorini JA, Bernardino G (1997) Increased B chromosome frequency and absence of drive in the fish *Prochilodus lineatus*. Heredity 79: 473–476. doi: 10.1038/hdy.1997.186

Piscor D, Ribacinko-Piscor DB, Fernandes CA, Parise-Maltempi PP (2013) Cytogenetic analysis in three *Bryconamericus* species (Characiformes, Characidae): first description of the 5S rDNA-bearing chromosome pairs in the genus. Molecular Cytogenetics 6: 13. doi: 10.1186/1755-8166-6-13

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromation. Experimental Cell Research 75: 304–306. doi: 10.1016/0014-4827(72)90558-7

Sumner AT (2003) Chromosome: Organization and Function. BlackWell Publishing, United Kingdom.

Silva DMZdA, Pansonato-Alves JC, Utsunomia R, Araya-Jaime C, Ruiz-Ruano FJ et al (2014) Delimiting the origin of a B chromosome by FISH mapping, chromosome painting and DNA sequence analysis in *Astyanax paranae* (Teleostei, Characiformes). PLoS ONE 9(4): e94896. doi: 10.1371/journal.pone.0094896

Torres-Mariano AR, Morelli S (2008) B chromosomes in a population of *Astyanax eigenmanniorum* (Characiformes, Characidae) from the Araguarí River Basin (Uberlândia, MG, Brazil). Genetics and Molecular Biology 31(1): 246–249. doi: 10.1590/S1415-47572008000200015
Venere PC, Miyazawa CS, Galetti Jr PM (1999) New cases of supernumerary chromosomes in characiform fishes. Genetic and Molecular Research 22: 345–349. doi: 10.1590/s1415-47571999000300010

Vicente VE, Moreira-Filho O, Camacho JPM (1996) Sex-ratio distortion associated with the presence of a B chromosome in Astyanax scabripinnis (Teleostei, Characidae). Cytogenetics and Cell Genetics 74: 70–75. doi: 10.1159/000134385

Weitzman SH, Palmer L (1997) A new species of Hyphessobrycon (Teleostei: Characidae) from the Neblina region of Venezuela and Brazil, with comments on the putative 'rosy tetra clade'. Ichthyological Exploration of Freshwaters 7(3): 209–242.

Wlasiuk G, Garcia G (1996) Análisis Preliminar del cariótipo y del proceso meiótico em Hyphessobrycon reticulatus (Characidae, Tetragonopterinae), procedente de Rocha, Uruguay. VI Simpósio de Citogenética Evolutiva e Aplicada de Peixes Neotropicais, Rio de Janeiro, Brazil, 56 pp.