



## Diversity of B Chromosomes in the Genus *Astyanax*

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### Review Article

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### Abstract

The *Astyanax* genus is composed of approximately 154 species, abundant in most of the Brazilian watersheds. In this sense, studies have revealed the presence of *B chromosomes* in populations of at least 12 species, which have different number, shape and size. *B chromosomes* are defined as additional elements, with particular evolution, transmission and maintenance, in addition to not following the Mendelian transmission laws. Thus, species of the *Astyanax* genus are presented as excellent models of evolutionary studies, maintenance and behavior of *B chromosomes* in fish; however, this information in this genre is often disconnected. In addition, several B-specific probes were isolated from B chromosomes of fish over the last decade, which together with genomic sequencing, have allowed for very detailed analyzes and results on its molecular structure. However for the *Astyanax* group there is no bibliographic survey relating the species or the populations carrying *B chromosomes*, nor the described morphologies, heterochromatic pattern, mitotic behavior, gene mapping, presence of functional genes, among others. Thus, this review compiles information aimed at the knowledge of supernumeraries in neotropical fish, more specifically those that comprise the genus *Astyanax*.

**Keywords:** B Chromosomes; Mitotic Instability; Morphotypes; Neotropical Fish; Supernumerary Chromosomes

**Abbreviations:** FISH: Fluorescent in Situ Hybridization; BM: Macro B Metacentric; Ba: Acrocentric B; BST: Macro Subtelocentric B; Phase S: Synthesis Phase; NGS: New Generation Sequences; SatDNA: Satellite DNA families.

### General Aspects of the *Astyanax* Genus

The Characiformes consist of 18 families, 270 genera and approximately 1674 species [1], with the family Characidae considered one of the most complex and numerous, with more than 1100 valid species [2]. The *Astyanax* genus is reported as the most diversified among the Neotropical Characidae [3], totaling 177 species [4]. Species that make up this genus are abundant along most of the headwaters of small rivers,

streams and Brazilian watersheds [5-7], distributing from the border Mexico-United States to the south Argentina [8], exposing diversities regarding the diploid number, since  $2n = 36$  chromosomes in *A. schubarti* [9] to  $2n = 50$  chromosomes in *A. bockmanni*.

In addition, one can notice a variation in the karyotype pattern, supernumerary chromosomes, natural triploidy, heterochromatin polymorphisms, NORs, ribosomal genes, among others [7,10-15]. These variations can be related to altitude, sexual differentiation or even environmental pollution [16]. Thus, with the advent of molecular cytogenetics in the mid-1980s, more detailed information about unrelated information among *Astyanax* species began

to emerge, mainly related to fluorescent in situ hybridization (FISH). Thus, the association between probes from different segments of DNA allowed for more robust studies related to the composition, evolution and structure of genetic material in fish [14,17,18]. It was precisely with this informative impulse that the term “species complexes” could be proposed in at least five major groups in the *Astyanax* genus: *A. scabripinnis* [19], *A. altiparanae* [20], *A. fasciatus* [21], *A. bimaculatus* [22] and *A. hastatus* [7].

Species complexes are closely related groups, difficult to differentiate by morphological characters, that is, the existence of species complexes is characterized when two or more species are classified under the name of only one [23]. This term was first mentioned in the species *A. scabripinnis* by Filho MO, et al. [19], based on cytogenetic and morphometric analyzes between populations of *A. scabripinnis* separately, where the morphological characteristics of the specimens were similar; however the presence of several cytotypes was observed for the different populations studied. The association of these cytogenetic characteristics with the geographic isolation of the studied populations led the authors to propose the existence of a “species complex”, today popularly known as “scabripinnis complex”.

In addition, studies with several species of the *Astyanax* genus have shown particular population dynamisms, which can lead to the formation of genetically highly variable populations from each other [7]. Moreover furthermore, cases of natural triploidy have been reported in populations comprised by the “scabripinnis complex” [24] and “fasciatus complex” [25].

Therefore, considering the intense karyotype variability in representatives of the *Astyanax* genus [17], wide geographical distribution [26], high phenotypic plasticity, great capacity to adapt to the most diverse environments [27], rapid ecological speciation and morphological differentiation [28], cytogenetic and cytogenetic-molecular studies still represent excellent tools in the search for better knowledge of the representatives of this group of fish.

## B Chromosomes

*B chromosomes* (or supernumeraries) are defined by Douglas RN, et al. [29] as additional elements to the standard chromosome set. They do not follow Mendelian inheritance laws, and have been widely described in several species of fungi, plants and animals, however, there are still doubts regarding their evolutionary role since their discovery

more than a century ago. They can be found only in some individuals of certain populations [30], and may vary in number and morphology between individuals [31-33].

Initially, *B chromosomes* were considered expendable non-functional elements and do not carry essential genes, thus not conferring any advantage on the organisms that maintain them [34]. Thus, they are thought of as parasitic or selfish elements, persisting in populations [35], doing use of the cellular machinery necessary for its maintenance, transmission and fixation [36]. When present in small numbers, they generally have little or no impact on carriers, however, on larger scales they can cause considerable phenotypic differences, and may even reduce fertility [29,37]. The largest number of *B chromosomes* reported in a single individual was observed in a kind of corn. In this case, 34 *B chromosomes* were reported and even surpassing the number of autosomes of the species [37].

Several hypotheses are raised regarding the origin of *B chromosomes* in their most diverse shapes and sizes among species. In this sense [38] propose two general hypotheses well accepted academically: a) *B chromosomes* would be products of structural rearrangements, evolutionary fruits of a common ancestor; b) supernumerary originated from chromosomal non-disjunction followed by genetic inactivation. There is another hypothesis proposed by Foresti PF, et al. [16] which suggests that these chromosomes could originate from construction processes and breakdown of genomic segments in normal cellular physiological processes, called again? In a nutshell, *B chromosomes* have aroused more and more interest in the most varied areas of knowledge aiming at information that allows understanding a little more of the mechanisms involved in their particular genetics.

## Supernumerary Chromosomes in *Astyanax*: Origin, Maintenance and Gene Organization

In fish, *B chromosomes* are reported in 113 species [39], and reported for the first time in the *Astyanax* genus by Salvador LB, et al. [40] in which the presence of an additional chromosome present in the species *A. scabripinnis* is reported. In the *Astyanax* group, research indicates the occurrence of these additional elements in at least 11 other species: *A. scabripinnis paranae* [41], *A. fasciatus* [10], *A. schubarti* [10], *A. sp.B* [42], *A. mexicanus* [43], *A. altiparanae* [11], *A. eigenmanniorum* [44], *A. bockmanni* [17], *A. goyacensis* [45], *A. paranae* [46] and *A. jordani* [47], arranged and distributed in various shapes, sizes and populations, as shown in Table 1.

Species	N/Size B	Municipality/State	Reference
<i>Astyanax Altiparanae</i>	1 B <sub>a</sub>	Bauru, SP	Hashimoto DT, et al. [11]
<i>A. Altiparanae</i>	1B <sub>a</sub>	Bauru, SP	Daniel SN, et al. [48]
<i>Astyanax Bockmanni</i>	1B <sub>M</sub>	Botucatu, SP	Silva DMZA, et al.[15]
<i>A. Bockmanni</i>	1 B <sub>a</sub>	Bauru, SP	Hashimoto, et al.[17]
<i>A. Bockmanni</i>	1-2 B <sub>a</sub>	Paulistânia, SP	Daniel SN, et al. [48]
<i>A. Bockmanni</i>	1 B <sub>M</sub> / 1 B <sub>m</sub> / 2 B <sub>a</sub>	Bauru, SP	Daniel SN, et al. [12]
<i>Astyanax Goyacensis</i>	1-9 B <sub>m</sub> / B <sub>a</sub>	Monte do Carmo, TO	Santos LPD, et al.[44]
<i>Astyanax Eigenmanniorum</i>	1-2 B <sub>M</sub>	Uberlândia. MG	Mariano AR, et al.[43]
<i>Astyanax fasciatus</i>	1 B <sub>M</sub> / 1 B <sub>SM</sub> / 1B <sub>ST</sub>	Botucatu, SP	Silva DM, et al.[47]
<i>A. Fasciatus</i>	1 B <sub>M</sub>	Botucatu, SP	Neto F
<i>A. Fasciatus</i>	1 B <sub>M</sub>	Mogi Guaçú, SP	Silva DM, et al.[47]
<i>A. Fasciatus</i>	1-2 B <sub>A</sub>	Bauru, SP	Daniel SN, et al. [48]
<i>A. Fasciatus</i>	1B <sub>A</sub>	Bauru, SP	Hashimoto DT, et al. [17]
<i>A. Fasciatus</i>	1 B <sub>M</sub>	Três Marias, MG	Filho MO, et al. [10]
<i>A. Fasciatus</i>	1-2 B <sub>ST</sub>	Bauru, SP	Daniel SN, et al. [48]
<i>Astyanax Paranae</i>	1 B <sub>M</sub> / 1 B <sub>SM</sub>	Botucatu, SP	Silva DM, et al. [47]
<i>A. Paranae</i>	1 B <sub>M</sub>	Maringa, PR	Abelini E, et al. [46]
<i>Astyanax Scabripinnis</i>	1 B <sub>M</sub>	Maringa, PR	Abelini E, et al. [46]
<i>A. Scabripinnis</i>	1 B <sub>SM</sub>	Itirapina, SP	Souza
<i>A. Scabripinnis</i>	1 B <sub>M</sub>	Pindamonhangaba, SP	Castro JP, et al. [49]
<i>A. Scabripinnis</i>	1 B <sub>M</sub> / 1 B <sub>SM</sub> / 1 B <sub>ST</sub> / 1 B <sub>A</sub>	Maringa, PR	Fernandes CA, et al.[20]
<i>A. Scabripinnis</i>	1-2 B <sub>M</sub>	Campos do Jordão,SP	Filho MO, et. al.[39]
<i>A. Scabripinnis</i>	2 B <sub>M</sub> / 1 B <sub>SM</sub>	Campos do Jordão,SP	Neo DM, et al. [50]
<i>A. Scabripinnis</i>	1 B <sub>M</sub>	Campos do Jordão,SP	Mestriner CA, et al. [51]
<i>A. Scabripinnis</i>	1 B <sub>M</sub> / 1-2 B <sub>SM</sub>	Campos do Jordão,SP	Ferro DAM, et al. [52]
<i>A. Scabripinnis</i>	1 B <sub>m</sub>	Paranavaí, PR	Santos M
<i>A. Scabripinnis</i>	1 B <sub>M</sub>	Pindamonhangaba, SP	Castro JP, et al. [49]
<i>A. Scabripinnis</i>	1 B <sub>M</sub>	Maringa, PR	Castro JP, et al. [49]
<i>A. Scabripinnis</i>	1 B <sub>M</sub>	Pindamonhangaba, SP	Barbosa P, et al. [53]
<i>A. Scabripinnis</i>	1-2 B <sub>M</sub>	Botucatu, SP	Machado
<i>A. Scabripinnis</i>	1 B <sub>M</sub>	Botucatu, SP	Maistro EL, et al. [54]
<i>A. Scabripinnis</i>	1-2 B <sub>a</sub>	Cerqueira César, SP	Mizoguchi
<i>A. Scabripinnis</i>	1 B <sub>M</sub>	Floresta, PR	Mizoguchi
<i>Astyanax Scabripinnis Paranae</i>	1 B <sub>M</sub>	Botucatu, SP	Foresti PF, et al. [16]
<i>A. Scabripinnis Paranae</i>	1 B <sub>M</sub>	Botucatu, SP	Maistro EL, et al.[41]
<i>Astyanax Schubarti</i>	1 B <sub>M</sub>	Misiones, Ar	Mestriner CA, et al. [51]
<i>Astyanax sp. B</i>	1 B <sub>M</sub> / 1 B <sub>SM</sub> / 1 B <sub>a</sub>	Capitao Leonidas Marques, PR	Fazoli LC, et al.[41]
<i>Astyanax Jordani</i>	1B <sub>m</sub>	Aquariofilia	Silva DM, et al.[47]
<i>Astyanax Mexicanus</i>	1 B <sub>a</sub>	Aquariofilia	Toledo LF, et al.[42]

**Table 1:** Relation of all populations and variants of *B* chromosomes reported in the *Astyanax* genus.

Among the several supernumeraries reported for *A. scabripinnis*, the BM variant (macro B metacentric) is the one that has the highest occurrence in natural populations. This variant is also observed in several other species and populations of the group and probably corresponds to a morphotype of B chromosome ancestral in the genus, according to Filho MO, et al. [55]. In addition, studies suggest that this BM is an isochromosome, with functional and structural homology between its two arms, a hypothesis initially raised by Vicente VE, et al. [56] and corroborated later by Vicari MR, et al. [57]. The other variants would probably derive from structural rearrangements of the BM chromosome [20], where most of them are totally heterochromatic [49-52].

*B chromosomes* restricted to a specific sex are also reported in populations of *A. scabripinnis*, both macrochromosomes B restricted to females only [40] regarding B microchromosomes restricted to males [58], or even associated with distortion in the sex ratio in some populations [16,56]. In addition, studied through the use of microsatellites, a population of *A. scabripinnis* from the region of Serra da Mantiqueira (Brazil), reporting the recent occurrence of a bottleneck event, resulting in the presence of excess heterozygotes. According to the authors, this excess was driven by individuals with *B chromosomes*.

There are rarely records of two simultaneous supernumerary chromosomes in their genomes, and for some species, only one population has such elements [41]. In this sense, from cytogenetic characterization in *A. goyacensis*, Santos LPD, et al. [44] observed between 1 and 9 microchromosomes B (acrocentric and metacentric), which presented intra and interindividual polymorphisms, as well as a completely heterochromatic pattern in their majority. In addition, the authors isolated and produced a B-specific probe from one of the nine B microchromosomes, observing associations of this probe in eight of the nine B-microscopes, in addition to consistent markings on some autosome chromosomes, suggesting, therefore, that the *B chromosomes* of this species may have originated from fragments of standard chromosomes, with two acrocentric pairs being the most parsimonious candidates. This is the first and only mention of *B chromosomes* in this species, and still the largest record in number of *B chromosomes* among the entire group.

Multiple supernumerary chromosome records are also applied to *A. sp. B*: between 1 and 3 *B chromosomes*, of BM, Bst and Ba, *A. eigenmanniorum* morphologies: between one and two BM [43]; *A. altiparanae*: a small acrocentric B (Ba) [11]; *A. mexicanus*: between one and two Ba [42], *A. jordani*: between one and two Bm [47] and *A. schubart*: a metacentric B macro (BM) [10]. Excepting to *A. jordani* and *A. sp. B*, which do not have heterochromatic grade records, the other *B*

*chromosomes* demonstrate a totally heterochromatic pattern. Specifically in *A. mexicanus*, one of the Ba showed subtle microsatellite marking (CA)<sub>15</sub> and (GATA)<sub>8</sub>, in addition to stronger markings of (GA)<sub>15</sub>. None of these species showed associations of functional genes to *B chromosomes*, and little is known about the origin of these extra elements in these cases, since studies aimed at this purpose are still scarce and need to be carried out.

For *A. fasciatus* the first record of *B chromosomes* is credited to the Filho MO, et al. [10]. A metacentric B macro (BM) totally heterochromatic. Subsequently, other occurrences were reported: macro subtelocentric B (BST) [17] and subtelocentric mean B (Bst) [15]. In all B morphotypes of this species, there is the presence of microsatellite sequences (AC)<sub>15</sub>, however, the As51 satDNA is observed only in the BM. H1 sequences are restricted to Bst. This is the species that has the largest variety of morphologies described, as well as a greater number of supernumeraries carrying functional genes (18S and H1). As there are a large number of populations that present intraspecific and inter-specific cytotypes (from 2n = 45 to 2n = 50 chromosomes) [59], associations linking hybridization processes to the origin of supernumeraries in this species are being raised [60].

In relation to *A. scabripinnis paranae* Foresti PF, et al. [16] analyzed the frequency of the *B chromosomes* associated with population density in a stream used along three locations. In the first point, the authors inferred a population composed of approximately 212 individuals, of which 35 were analyzed, and of these, 23 presented BM. In the second, the population was estimated to be about 650 individuals, 20 were karyotypes, and only two had a BM. In the third point, the population was estimated at 107 individuals, 10 were analyzed, and only one had BM, conferring no relationship between the frequency of the BM and the population density; however the presence of adaptive effects provided by the *B chromosomes* to the living specimens of the first sampled point is not fully discarded. According to Maistro EL, et al. [41], most of the supernumeraries reported for this species are entirely heterochromatic, also showing a late replication pattern, evidenced by the incorporation of BrdU, a derivative of Uridine analogous to Thymidine, which gives it the ability to be incorporated into DNA during the synthesis phase (phase S).

Regarding its origin in *Astyanax*, Maistro EL, et al. [54] suggest that the *A. scabripinnis* BM chromosome would have arisen due to the non-disjunction of the first pair of metacentric chromosomes, forming a trisomic element, which would later have followed its own evolutionary path [51] analyzing meiotic cells of *A. scabripinnis* found that the *B chromosome* does not follow a pattern of Mendelian inheritance, forming univalent self-matched arms. In



addition, they mapped a type of satellite DNA on the *B chromosome* of *A. scabripinnis* obtained from the restriction enzyme KpnI, called As-51. The symmetrical position of this satDNA in both arms of the *B chromosome*, added to its self-pairing in meiosis, allowed the authors to propose that this B chromosome would be an isochromosome, formed, even, from the incorrect division and non-disjunction of the chromosome pair 24, rich in sequences As-51. Then Neo DM, et al. [50] observed similar patterns, corroborating the hypotheses suggested by Mestriner CA, et al. [51]. A few years later Vicari MR, et al. [57] strengthened the hypothesis of intraspecific origin of the BM of *A. scabripinnis* from the formation of an isochromosome based on results obtained by chromosomal painting with a B-specific probe and FISH with satDNA probes As-51 and Cot-1. More recently, Barbosa P, et al. [53] there were accumulations of microsatellites CA, CAC e GA in the telomeric and interstitial region of the B chromosome of this species.

In a natural population of *A. bockmanni* Daniel SN, et al. [12] observed three morphotypes of *B chromosomes*: *A macrochromosome* BM and two microchromosomes: Ba and Bm, These variants showed significant intraindividual numerical variation, with records of two B microchromosomes in the same cell. In this population, cells carrying a macrochromosome B and a microchromosome B were not observed simultaneously, leading the authors to propose that the *B chromosomes* of this specie could have originated by isochromosome. High rates of mitotic instability were also recorded among the specimens analyzed. The authors also report that the observed microchromosomes B reveal a partial pattern of constitutive heterochromatin, and macrochromosome B outlines a totally heterochromatic pattern. Previously, Hashimoto DT, et al. [17] also reported the occurrence of Bm for this species, however, they did not verify mitotic instability or the presence of B macrochromosomes among the analyzed individuals. Additionally, the B microchromosomes of this species indicate a high incidence of transposable elements, more specifically those of the Rex family (Rex1, Rex3 and Rex6). Microsatellites (AC) 15 are also reported on the Bm and BM chromosomes of this species [15].

To investigate the origin of the *B chromosomes* of *A. paranae*, [15] used B-specific DNA probes obtained by chromosomal microdissection of only one arm of the BM chromosome. The application of FISH indicated signs of hybridization throughout the BM chromosome, that is, complete homology between the two arms of this supernumerary, indicating that the B chromosome of *A. paranae* has characteristics of an isochromosome. The authors also observed signs of histone H1 and 18S rDNA symmetrically in both arms of the BM chromosome, in addition to synthetic markings in pairs two and 23, which

could behave as possible chromosomes predecessors of the *B chromosome*, further supporting the hypothesis this element in *A. paranae* is an isochromosome. Based on sat DNA analysis Silva DMZA, et al. [61] corroborated this hypothesis. In addition, with some exceptions, this chromosome class is frequently observed in females, and rarely in males, as previously reported by Maistro EL, et al. [41], Foresti PF, et al. [16], Filho MO, et al. [10], Abelini E, et al. [46], suggesting that supernumeraries could play an important role in the sexual determination of this species. However, further studies are necessary in order to corroborate or refute this hypothesis.

	<i>Astyanax bockmanni</i> - B <sub>M</sub> /B <sub>m</sub> (macro/micro)
	<i>A. schubarti</i> - B <sub>M</sub> (macro)
	<i>A. fasciatus</i> - B <sub>M</sub> (macro)
	<i>A. paranae</i> - B <sub>M</sub> (macro)
	<i>A. scabripinnis</i> - B <sub>M</sub> /B <sub>m</sub> (macro/ middle/micro)
	<i>A. eigenmanniorum</i> - B <sub>M</sub> (macro)
	<i>A. jordani</i> - B <sub>m</sub> (micro)
	<i>A. sp. B</i> - B <sub>M</sub> (macro)
	<i>A. fasciatus</i> - B <sub>sm</sub> (middle)
	<i>A. paranae</i> - B <sub>SM</sub> (macro)
	<i>A. scabripinnis</i> - B <sub>SM</sub> /B <sub>sm</sub> (macro/ middle)
	<i>A. sp. B</i> - B <sub>SM</sub> (macro)
	<i>A. fasciatus</i> - B <sub>st</sub> (middle)
	<i>A. scabripinnis</i> - B <sub>ST</sub> (macro)
	<i>A. bockmanni</i> - B <sub>a</sub> (micro)
	<i>A. altiparanae</i> - B <sub>a</sub> (micro)
	<i>A. goyacensis</i> - B <sub>A</sub> /B <sub>a</sub> (macro/ middle/micro)
	<i>A. fasciatus</i> - B <sub>a</sub> (middle)
	<i>A. scabripinnis</i> - B <sub>A</sub> /B <sub>a</sub> (macro/micro)
	<i>A. mexicanus</i> - B <sub>a</sub> (micro)
	<i>A. sp. B</i> - B <sub>a</sub> (acro)

**Table 2:** Morphologies and size of *B chromosomes* reported among species of the genus *Astyanax*.

There is still no summary formula that expresses the origin of all morphotypes and sizes of *B chromosomes* in the 12 *Astyanax* species carrying these elements, however, Filo MO, et al. [10] defends the hypothesis of a common origin of the supernumeraries of type BM reported in *A. fasciatus*, *A. schubart* and *A. scabripinnis*. Similarly, Silva DMZA, et al. [15]

based on cyto-molecular analyzes, also proposed a common origin of the supernumeraries BM of *A. bockmanni*, *A. fasciatus* and *A. paranae*, however, more robust analyzes involving new generation sequencing and gene expression tests are necessary to verify the content of these *B chromosomes* at the sequence level, and thus corroborate or refute a hypothesis of common origin of BM among these species. The relationship between the shapes and size of the *B chromosomes* described in the *Astyanax* genus is shown in Table 2.

### Perspectives on the Knowledge of Supernumerary Chromosomes: Emphasis on *Astyanax*

Since its discovery, more than a century ago, studies involving supernumeraries have been the stage of research among several scholars worldwide. Such studies were conducted by classical cytogenetics throughout much of the 20th century, where, from the 1990s, molecular cytogenetics was added, which reached its peak throughout the 2000s through the insertion of chromosomal painting, a technique that was and is still recognized as an important ally in the search for information about sharing sequences between chromosomes of the same species or between related species [15,57,62-64]. This practice has since been improved, and isolation of specific chromosomal regions has become possible [65].

Currently, studies of this nature are more focused on the construction of band-specific libraries and physical mapping of chromosomes of interest in the form of chromosomal painting or cross-hybridization, when the genetic material of a species is used in the form of a probe to map sites or chromosomes of interest in another species, a process also called Zoo-FISH. In this sense, supernumerary chromosomes have gained studies [15,62,63,66]. Although these methodologies have provided relevant findings over the years, conclusive limits are found at the molecular level. Thus, genomic analysis and bioinformatics tools come as a complement in order to leverage massive investigations regarding the structures and functionalities related to *B chromosomes* [67].

In order to generate more and more knowledge about this chromosome class, massive new generation sequences (NGS) have provided a series of markers in organisms considered non-models [32,68]. Therefore, the combination of NGS and bioinformatics allows new ways to identify specific sequences present in *B chromosomes*, revealing a wealth of molecular data relevant to studies about its origin, maintenance and evolution review in Ruban A, et al. [64]. Conversely, both classic cytogenetic and cyto-molecular or molecular methodologies, obviously have their advantages and disadvantages, where the use of these markers together

can provide interesting information and answers according to the biological problem raised [69].

In this sense, Ruano RFJ, et al. [70] described the locust satellitoma *Locusta migratoria*, that certainly opened up new perspectives on the distribution of this class of DNA in animals. Subsequently, through high-performance analyzes, Ruano RFJ, et al. [70] showed an ancestry of a morphological type of *B chromosome* in the grasshopper *Eumigus monticola*, proving that *B chromosomes* of intraspecific origin can harbor several *B-specific* satDNA sequences. In another manuscript, Vozdova M, et al. [71] discovered an extensive heterogeneity of satellite sequences between *Canidae*, indicating close evolutionary relationships between red and arctic foxes. In fish, the greater focus on satDNAs has been used to better understand the origin and behavior of *B chromosomes*. In this sense, one of the first isolated satDNAs is the so-called As-51, isolated from the *B chromosome* of *A. scabripinnis*, which was symmetrical in both arms of this supernumerary [51]. Combining Sanger and NGS sequencing with PCR and FISH, Utsunomia R, et al. [32] analyzed the variation and abundance of MsaSat01-177 sequences in ten species of *Characidae*, reporting extensive variation of this marker among them, in addition to a wide knowledge of this satDNA family in the analyzed species. Therefore, studying these sequences means advancing the knowledge of genomic organization and the dynamics with which these sequences have evolved in the most varied organisms.

For the species that make up the *Astyanax* group, the joint use of these methodologies has generated interesting knowledge of the mechanisms related to evolutionary processes, origin, rearrangements and differentiation of *B chromosomes*. Like this, Silva DMZA, et al. [61] isolated and validated 45 satellite DNA families (satDNA) obtained from the *Astyanax paranae* genome by Illumina sequencing. Simultaneous analysis of the chromosomal location of 35 (out of 45) satDNAs in *A. paranae*, *A. fasciatus* and *A. bockmanni* revealed that most of these satellites are shared among the three species, even with highly similar patterns of chromosomal distribution. The isochromosomal nature of the *B chromosomes* in these species has been corroborated by the symmetrical location of many satDNAs in both arms of the supernumeraries. In addition, the authors also verified a lower symmetry observed on the *A. chromosome* of *A. fasciatus*, suggesting that the metacentric *B chromosome* of this species is older than the other *B chromosomes* of the same morphology reported in *A. paranae* and *A. bockmanni*, reinforcing the hypothesis that these *B chromosomes* descended from an ancestor common to the three species. Also according to the authors, these results raise new questions about the different hypotheses previously suggested about the origin of the *B chromosomes* in *Astyanax*: the *B chromosomes* found in different species of

this genus may indeed have been derived from an ancestral *B chromosome*, but they may have evolved at different rates between these species, that is, more quickly in *A. fasciatus* than in the others.

Therefore, in this animal group, the hypothesis those *B chromosomes* may originate from hybridization processes has been recurrent, as already confirmed in bees Tosta VC, et al, [72] and even suggested for *Astyanax* [15]. Hybridization is conceptualized as the union of distinct genetic heritage, resulting in offspring with intermediate characteristics of both parents [73]. In fish, evidence of the appearance of supernumeraries by this mechanism was observed in *Poecilia formosa*, a hybrid involving *Poecilia mexicana* and *Poecilia latipinna* species [74].

In this sense, aiming to analyze the hypothesis that hybridization processes occur between close species and genera Goes CAG, et al. [4] analyzed cytogenetically hybrid strains between *A. altiparanae* (2n=50) x *A. fasciatus* (2n=46), resulting in individuals with 2n = 48 chromosomes; *A. altiparanae* (2n=50) x *A. schubarti* (2n=36), resulting in individuals with 2n = 43 chromosomes; *A. altiparanae* (2n=50) x *A. mexicanus* (2n=50), with individuals carrying 2n = 50 chromosomes, and *A. altiparanae* (2n=50) x *Hypphessobrycon anisitsi* (2n=50), resulting in offspring with 2n = 50 and 51 chromosomes, associating their cytogenetic patterns with species complexes. In addition, in recent paper, hybrid products involving species of *Astyanax* and species of close genera developed by Piva LH, et al. [75] proved to be sexually viable.

Therefore, hybrid products from *Astyanax* species with different diploid numbers would be a completely viable process, presenting gonads capable of producing gametes, and in this way, they could cause the accumulation of additional chromosomes, which could be transmitted to the later generation, and in a hypothetical situation, settle in the population in the form of *B chromosomes*. Added to that, recently Gavazzoni M, et al. [76] reported the presence of natural hybrids using cytogenetic and molecular techniques.

In short, techniques until recently applied and focused only on other areas of study (such as niche modeling, massive sequencing of *B chromosomes*, qPCR markers and targeted crosses) today have gained more space in building knowledge about additional chromosomes, mainly in species of the genus *Astyanax* due to the wide geographical distribution, presence and polymorphisms of shape and size of *B chromosomes* in different species and different populations. Certainly, the applicability of these modern sequencing methods associated with bioinformatics will launch a new perspective on *B chromosomes*, significantly increasing our knowledge of their genomic and evolutionary

dynamics, providing subsidies for more complete inferences about this chromosomal class in *Astyanax* and other species of neotropical fish, since several factors involving this chromosomal class still remain a mystery, among them, its physiological importance to the host, what has already been evidenced in works developed by Castro JP, et al. [77], Castro JP, et al. [78], to ascertain the presence of *B chromosomes* associated with the expression of certain genes, mainly regarding the influence of the *B chromosomes* on sexual differentiation in *Astyanax* species [79,80].

## References

1. Nelson JS (2006) *Fishes of the world*, John Wiley e Sons, New York, pp: 601.
2. Eschmeyer WN, Fong JD (2014) *Species by family/subfamily*.
3. Lucena CAS, Castro JB, Bertaco VA (2013) Three new species of *Astyanax* from drainages of southern Brazil (Characiformes: Characidae). *Neotropical Ichthyology* 11(3): 537-552.
4. Goes CAG, Daniel SN, Piva LH, Yasui GS, Artoni RF, et al. (2020) Cytogenetic markers as a tool for characterization of hybrids of *Astyanax* Baird & Girard, 1854 and *Hypphessobrycon* Eigenmann, 1907. *Comp Cytogenet* 14(2): 231-242.
5. Garutti V, Britski HA (2000) Descrição de uma espécie nova de *Astyanax* (Teleostei: Characidae) da bacia do alto rio Paraná e considerações sobre as demais espécies do gênero na bacia. *Comunicações do Museu de Ciências e Tecnologia da PUCRS série Zoologia* 13: 65-88.
6. Lima FCT, Malabarba LR, Buckup PA, Silva JFP, Vari RP, et al. (2003) *Genera incertae sedis in Characidae*. In: Check list of the freshwater fishes of South and Central America. Edipucrs, Porto Alegre, Rio Grande do Sul, Brasil.
7. Kavalco KF, Pazza R, Almeida-Toledo LF (2009) *Astyanax bockmanni* Vari and Castro, 2007: An ambiguous karyotype in the *Astyanax* genus. *Genetica* 136(1): 135-139.
8. Bertaco VA, Lucena, CA (2010) Redescription of *Astyanax obscurus* (Hensel, 1870) and *A. laticeps* (Cope, 1894) (Teleostei: Characidae): two valid freshwater species originally described from rivers of Southern Brazil. *Neotropical Ichthyology* 8(1): 7-20.
9. Morelli S, Bertollo LAC, Foresti F, Moreira FO, Toledo AFS (1983) Cytogenetic considerations on the genus *Astyanax* (Pisces, Characidae). I. Karyotypic variability. *Caryologia* 36(3): 235-244.

10. Filho MO, Fenocchio AS, Pastori MC, Bertollo LAC (2001) Occurrence of a metacentric macrochromosome B in different species of the genus *Astyanax* (Pisces, Characidae, Tetragonopterinae). *Cytologia* 66(1): 59-64.
11. Hashimoto DT, Gonçalves VR, Bortolozzi J, Foresti F, Foresti FP (2008) First report of a B chromosome in a natural population of *Astyanax altiparanae* (Characiformes, Characidae). *Genetic and Molecular Biology* 31: 275-278.
12. Daniel SN, Hashimoto DT, Alves JCP, Foresti F, Foresti FP (2012) Cytogenetic characterization of distinct B chromosomes in a population of the fish *Astyanax bockmanni* (Teleostei, Characiformes). *Caryologia* 65(3): 229-233.
13. Daniel SN, Penitente M, Hashimoto DT, Silva DMA, Foresti F, et al. (2015) New insights into karyotypic relationships among populations of *Astyanax bockmanni* (Teleostei, Characiformes) of different watersheds. *Zebrafish* 12(2): 181-188.
14. Silva DMZA, Pansonato-Alves JC, Utsunomia R, Daniel SN, Hashimoto DT, et al. (2013) Chromosomal organization of repetitive DNA sequences in *Astyanax bockmanni* (Teleostei, Characiformes): dispersive location, association and co-localization in the genome. *Genetica* 141(7-9): 329-336.
15. Silva DMZA, Daniel SN, Camacho JPM, Utsunomia R, Ruano FJR, et al. (2016) Origin of B chromosomes in the genus *Astyanax* (Characiformes, Characidae) and the limits of chromosome painting. *Molecular Genetics and Genomics* 291(3): 1407-1418.
16. Foresti FP, Oliveira C, Maistro EL, Foresti F (1997) Estimated frequency of B-chromosomes and population density of *Astyanax scabripinnis* paranae in a small stream. *Revista Brasileira de Genética* 20(3): 377-380.
17. Hashimoto DT, Smith MF, Rens W, Foresti F, Porto-Foresti F (2011) Chromosome mapping of H1 histone and 5S rRNA gene clusters in three species of *Astyanax* (Teleostei, Characiformes). *Cytogenetic and Genome Research* 134(1): 64-71.
18. Daniel SN, Penitente M, Silva DMZA, Hashimoto DT, Ferreira DC, et al. (2015b) Organization and chromosomal distribution of histone genes and transposable Rex elements in the genome of *Astyanax bockmanni* (Teleostei, Characiformes). *Cytogenetic and Genome Research* 146(4): 311-318.
19. Filho MO, Bertollo LAC (1991) *Astyanax scabripinnis* (Pisces Characidae): a species complex. *Revista Brasileira de Genética* 14(2): 331-357.
20. 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 Parana, Brazil. *Genetica* 124(2-3): 301-306.
21. Artoni RF, Shibatta OA, Gross MC, Schneider CH, Almeida MC (2006) *Astyanax* aff. *fasciatus* Cuvier, 1819 (Teleostei, Characidae): evidences of a species complex in the upper rio Tibagi basin (Paraná, Brazil). *Neotropical Ichthyology* 4(2): 197-202.
22. Garutti V, Langeani F (2009) Redescription of *Astyanax goyacensis* Eigenmann, 1908 (Ostariophysi: Characiformes: Characidae). *Neotropical Ichthyology* 7(3): 371-376.
23. Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, et al. (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* 22(3): 148-155.
24. Maistro EL, Dias AL, Foresti F, Oliveira C, Moreira-Filho O (1994) Natural triploidy in *Astyanax scabripinnis* (Pisces, Characidae) and simultaneous occurrence of macro B-chromosomes. *Caryologia* 47(3-4): 233-239.
25. Peres WAM, Buckup PA, Kantek DLZ, Bertollo LAC, Moreira-Filho O (2009) Chromosomal evidence of downstream dispersal of *Astyanax fasciatus* (Characiformes, Characidae) associated with river shed interconnection. *Genetica* 137(3): 305-311.
26. Zanata AM, Camelier P (2009) *Astyanax vermillion* and *Astyanax burgerai*: new characid fishes (Ostariophysi: Characiformes) from Northeastern Bahia, Brazil. *Neotropical Ichthyology* 7(2): 175-184.
27. Dowling P (2002) The sociology of mathematics education: Mathematical myths/pedagogic texts. Routledge.
28. Reeves RG, Bermingham E (2006) Colonization, population expansion, and lineage turnover: phylogeography of Mesoamerican characid fish. *Biological Journal of the Linnean Society* 88(2): 235-255.
29. Douglas RN, Birchler JA (2017) B Chromosomes. In *Chromosome structure and Aberrations*. Springer India, pp: 13-39.
30. Moghaddam AMB, Martis MM, Macas J, Gundlach H, Himmelbach A, et al. (2015) Genes on B chromosomes: old questions revisited with new tools. *Biochim, Biophys Acta Biomembranes* 1849(1): 64-70.



31. Oliveira C, Foresti F, Hilsdorf AWS (2009) Genetics of Neotropical fish: from chromosomes to populations. *Fish Physiol Biochem* 35(1): 81-100.
32. Utsunomia R, Silva DMZA, Ruiz-Ruano FJ, Jaime CA, Pansonato-Alves JC, et al. (2016) Uncovering the ancestry of B chromosomes in *Moenkhausia sanctaefilomenae* (Teleostei, Characidae). *PLoS One* 11(3): e0150573.
33. Serrano EA, Utsunomia R, Scudeller PS, Oliveira C, Foresti F (2017) Origin of B chromosomes in *Characidium alipioi* (Characiformes, Crenuchidae) and its relationship with supernumerary chromosomes in other *Characidium* species. *Comparative Cytogenetics* 11(1): 81-95.
34. Houben A, Banaei-Moghaddam AM, Klemme S, Timmis JN (2013) Evolution and biology of supernumerary B chromosomes. *Cellular and Molecular Life Sciences* 71(3): 467-478.
35. Camacho JPM, Sharbel TF, Beukboom LW (2000) B-chromosome evolution. *Philosophical Transactions of the Royal Society of London* 355: 163-178.
36. Cabrero J, Leon L, Estévez MR, Gomez R, Petitpierre E, et al. (2014) B1 was the ancestor B chromosome variant in the western mediterranean area in the grasshopper *Eyprepocnemis plorans*. *Cytogenetic and Genome Research* 142(1): 54-58.
37. Jones R, Rees H (1982) B Chromosomes. Academic Press, London.
38. Volobujev VT (1981) B-chromosomes system of the mammals. *Caryologia* 34(1): 1-23.
39. D'Ambrosio U, Alonso-Lifante MP, Barros K, Kovařík A, Mas de Xaxars G, et al. (2017) B-chrom: a database on B-chromosomes of plants, animals and fungi. *New Phytologist* 216(3): 635-642.
40. Salvador LB, Moreira-Filho O (1992) B chromosomes in *Astyanax scabripinnis* (Pisces, Characidae). *Heredity* 69(1): 50-56.
41. Maistro EL, Foresti F, Oliveira C, Almeida-Toledo LF (1992) Occurrence of macro B chromosome in *Astyanax scabripinnis paranae* (Pisces, Characiformes, Characidae). *Genetica* 87(2): 101-106.
42. Fazoli LC, Silva VDAB, Castro ALBP, Júlio HF (2003) Chromosome characterization of *Astyanax* sp B (Characidae, Tetragonopterinae), an endemic species of the Iguaçu river, Paraná, Brazil. *Cytologia* 68(4): 389-394.
43. Kavalco KF, Toledo A LF (2007) Molecular cytogenetics of blind mexican tetra and comments on the karyotypic characteristics of genus *Astyanax* (Teleostei, Characidae). *Zebrafish* 4(2):103-111.
44. Mariano ART, Morelli S (2008) B chromosomes in a population of *Astyanax eigenmanniorum* (Characiformes, Characidae) from the Araguari River Basin (Uberlândia, MG, Brazil). *Genetic and Molecular Biology* 31(1): 246-249.
45. Santos LPD, Castro JP, Francisco CMD, Vicari MR, Almeida MCD, et al. (2013) Cytogenetic analysis in the Neotropical fish *Astyanax goyacensis* Eigenmann, 1908 (Characidae, incertae sedis): karyotype description and occurrence of B microchromosomes. *Molecular Cytogenetics* 6(1): 1-10.
46. Abelini E, Santos ICM, Fernandes CA (2014) Cytogenetic analysis in three species from genus *Astyanax* (Pisces; Characiformes) with a new occurrence of B chromosome in *Astyanax paranae*. *Caryologia* 67(2): 160-171.
47. Silva DM, Utsunomia R, Alves JCP, Oliveira C, Foresti F (2015) Chromosomal mapping of repetitive DNA sequences in five species of *Astyanax* (Characiformes, Characidae) reveals independent location of U1 and U2 snRNA sites and association of U1 snRNA and 5S rDNA. *Cytogenetic and Genome Research* 146(2): 144-152.
48. Daniel SN (2018) Cromossomos B no gênero *Astyanax* (Characiformes, Characidae): estudos genéticos e modelagem de nicho.
49. Castro JP, Moura MO, Filho OM, Shibatta OA, Santos MH, et al. (2015) Diversity of the *Astyanax scabripinnis* species complex (Teleostei: Characidae) in the Atlantic Forest, Brazil: species limits and evolutionary inferences. *Reviews in Fish Biology and Fisheries* 25(1): 231-244.
50. Néo DM, Bertollo LAC, Filho OM (2000) Morphological differentiation and possible origin of B chromosomes in natural Brazilian population of *Astyanax scabripinnis* (Pisces, Characidae). *Genetica* 108: 211-215.
51. Mestriner CA, Galetti PM, Valentini SR, Ruiz IRG, Abel LDS, et al. (2000) Structural and functional evidence that a B chromosome in the characid fish *Astyanax scabripinnis* is an isochromosome. *Heredity* 85: 1-9.
52. Ferro DAM, Filho OM, Bertollo LAC (2003) B chromosome polymorphism in the fish, *Astyanax scabripinnis*. *Genetica* 119(2): 147-153.
53. Barbosa P, Leal E, Silva M, Almeida M, Filho OM, et al. (2017) Variability and evolutionary implications of repetitive DNA dynamics in genome of *Astyanax*

- scabripinnis* (Teleostei, Characidae). Comparative Cytogenetics 11(1): 143-162.
54. Maistro EL, Foresti F, Oliveira C (1999) R-and G-band patterns in *Astyanax scabripinnis* paranae (Pisces, Characiformes, Characidae). Genetics and Molecular Biology 22(2): 201-204.
  55. Moreira-Filho O, Galetti PM, Bertollo LAC (2004) B chromosomes in the fish *Astyanax scabripinnis* (Characidae, Tetragonopterinae): An overview in natural populations. Cytogenetic and Genome Research 106(2-4): 230-234.
  56. Vicente VE, Filho OM, Camacho JPM (1996) Sex-ratio distortion associated with the presence of a B chromosome in *Astyanax scabripinnis* (Teleostei, Characidae). Cytogenet Cell Genet 74(1-2): 70-75.
  57. Vicari MR, Pistune HFM, Castro JP, Almeida MC, Bertollo LAC, et al. (2011) New insights on the origin of B chromosomes in *Astyanax scabripinnis* obtained by chromosome painting and FISH. Genetica 139(8): 1073-1081.
  58. Stange EAR, Toledo LFA (1993) Supernumerary B chromosomes restricted to Revista. brasileira de genética 16(3-4): 601.
  59. Kavalco KF, Pazza R, Brandão, KO, Toledo LFA (2013) Biogeographic patterns in the chromosomal distribution of a satellite DNA in the banded tetra *Astyanax fasciatus* (Teleostei: Characiformes). Organisms Diversity and Evolution 13(1): 67-76.
  60. Pazza R, Kavalco KF, Bertollo LAC (2006) Chromosome polymorphism in *Astyanax fasciatus* (Teleostei, Characidae). 1. Karyotype analysis, Ag-NORs and mapping of the 18S and 5S ribosomal genes in sympatric karyotypes and their possible hybrid forms. Cytogenetic and Genome Research 112(3-4): 313-319.
  61. Silva DMZA, Utsunomia R, Ruiz Ruano FJ, Daniel SN, Porto Foresti F, et al. (2017) High-throughput analysis unveils a highly shared satellite DNA library among three species of fish genus *Astyanax*. Scientific Reports 7(1): 12726.
  62. Voltolin TA, Pansonato Alves JC, Senhorini JA, Foresti F, Camacho JPM, et al. (2013) Common descent of B chromosomes in two species of the fish genus *Prochilodus* (Characiformes, Prochilodontidae). Cytogenet. Genome Research 141(2-3): 206-211.
  63. Penitente M, Daniel SN, Scudeler PES, Foresti F, Foresti FP (2016) B chromosome variants in *Prochilodus lineatus* (Characiformes, Prochilodontidae) analyzed by microdissection and chromosome painting techniques. Caryologia 69(2): 181-186.
  64. Ruban A, Schmutzer T, Scholz U, Houben A (2017) How Next-Generation sequencing has aided our understanding of the sequence composition and origin of B chromosomes. Genes 8(11): 294.
  65. Senger DR, Connolly DT, Van De Water L, Feder J, Dvorak HF (1990) Purification and NH2-terminal amino acid sequence of guinea pig tumor-secreted vascular permeability factor. Cancer Res 50(6): 1774-1778.
  66. Diniz D, Filho OM, Bertollo LAC (2008) Molecular cytogenetics and characterization of a ZZ/ZW sex chromosome system in *Triporthus nematurus* (Characiformes, Characidae). Genetica 133(1): 85-91.
  67. Valente GT, Nakajima RT, Fantinatti BEA, Marques DF, Almeida RO, et al. (2017) B chromosomes: from cytogenetics to systems biology. Chromosoma 126(1): 73-81.
  68. Valente GT, Conte MA, Fantinatti BEA, Cabral de Mello DC, Carvalho RF, et al. (2014) Origin and evolution of B chromosomes in the cichlid fish *Astatotilapia latifasciata* based on integrated genomic analyses. Mol Biol Evol 31(8): 2061-2072.
  69. Yakovlev SS, Pustahija F, Vivic V, Robin O (2014) Molecular cytogenetics (FISH and fluorochrome banding): resolving species relationships and genome organization. Methods Mol Biol 1115: 309-323.
  70. Ruiz Ruano FJ, Cabrero J, López León MD, Camacho JPM (2016) Satellite DNA content illuminates the ancestry of a supernumerary (B) chromosome. Chromosoma 126(4): 487-500.
  71. Vozdova M, Kubickova S, Cernohorska H, Frohlich J, Rubes J (2017) Satellite DNA sequences in Canidae and their chromosome distribution in dog and red fox. Cytogenet Genome Res 150(2): 118-127.
  72. Tosta VC, Marthe JB, Tavares MG, Fernandes Salomao TM, Pompolo SG, et al. (2015). Possible introgression of B chromosomes between bee species (genus *Partamona*). Cytogenet Genome Res 144(3): 220-226.
  73. Mayr E (1973) The recent historiography of genetics. Journal of the History of Biology 6(1): 125-154.
  74. Schedina IM, Hartmann S, Groth D, Schlupp I, Tiedemann R (2014) Comparative analysis of the gonadal transcriptomes of the all-female species *Poecilia formosa* and its maternal ancestor *Poecilia mexicana*. BMC Res

Notes 7(1): 249.

75. Piva LH, Siqueira Silva DH, Goes CAG, Fujimoto T, Saito T, et al. (2018). Triploid or hybrid tetra: Which is the ideal sterile host for surrogate technology? *Theriogenology* 108: 239-244.
76. Gavazzoni M, Pavanelli CS, Graça WJ, Melo BF, Gubiani ÉA, et al. (2020) Detection of natural hybridization and delimitation of two closely related operational taxonomic units of the *Astyanax fasciatus* (Teleostei: Characidae) complex through integrative approaches. *Biological Journal of the Linnean Society* 129(3): 687-700.
77. Castro JP, Hattori RS, Yoshinaga TT, Silva DMZDA, Foresti F, et al. (2019) Differential expression of *dmrt1* in *Astyanax scabripinnis* (Teleostei, Characidae) Is Correlated with B chromosome occurrence. *Zebrafish* 16(2): 182-188.
78. Castro JP, Hattori RS, Yoshinaga TT, Silva DMZDA, Ruiz Ruano FJ, et al. (2019) Differential Expression of genes related to sexual determination can modify the reproductive cycle of *Astyanax scabripinnis* (Characiformes: Characidae) in B chromosome carrier individuals. *Genes* 10(11): 909.
79. Foresti F, Almeida Toledo LF, Toledo Filho SA (1981) Polymorphic nature of nucleous organizer regions in fishes. *Cytogenetics Cell Genet* 31: 137-144.
80. Vari RP, Castro RMC (2007) New species of *Astyanax* (Ostariophysi: Characiformes: Characidae) from the Upper Rio Paraná System, Brazil. *Copeia* 1: 150-162.

