

Evolutionary dynamics of the B chromosomes in the fish species *Prochilodus lineatus* Valenciennes, 1837 of the Paraná River Basin

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Abstract

The fish species *Prochilodus lineatus* has an interesting B chromosome system, with three morphological types as acrocentric, metacentric, and submetacentric. However, most cytogenetic studies on this species are restricted to the natural population of the Mogi Guaçu River. Given this, the present work aimed to study the structure karyotypic profile as well as the occurrence of supernumeraries in *P. lineatus* in several localities in the Paraná River basin, where this species is abundant. The results obtained showed a predominantly conserved karyotypic macrostructure and the presence of B chromosomes in all the seven localities studied, with the exception of the Apa River. Additionally, new variants of morphological characteristics were found in the population of the Batalha River (Reginópolis). These results allow us to infer that there is a large occurrence of B chromosomes in this species, with important differences in B chromosome frequency between the populations, especially in acrocentric and submetacentric B variants. Considering the possible origin and evolution of B chromosomes in *P. lineatus*, our results allow us to describe the dispersion of metacentric B variants, in contrast with the elimination observed in acrocentric and submetacentric variants.

Keywords

Cytogenetics, migratory fish, Neotropical fishes, supernumerary chromosomes

Introduction

The ichthyofauna of the Neotropical region is one of the richest in the world, with approximately 9100 species (Reis et al. 2016). One of the main orders of Neotropical fishes is Characiformes, which includes the family Prochilodontidae, comprising 21 species and divided into three genera: *Ichthyoelephas* Posada, 1909 with two species; *Prochilodus* Agassiz, 1829 with 13 species; and *Semaprochilodus* Fowler, 1941 with six species (Fricke et al. 2024; Froese and Pauly 2024). *Prochilodus* is the most abundant among these genera and is composed of detritivore fish of moderate size. In this context, *Prochilodus lineatus* Valenciennes, 1837 is characterized by its migratory behavior, with the shoals traveling hundreds of kilometers in the breeding season (Melo et al. 2017). *P. lineatus* populations usually exhibit low genetic variation (Garcez et al. 2011; Rueda et al. 2013), as also observed in other species of the Prochilodontidae (Machado et al. 2017; Landínez-García et al. 2020). In a similar way, cytogenetic studies have been demonstrating a diploid chromosome number ($2n = 54$) and monotonous karyotype structure with only metacentric and submetacentric pairs in *Prochilodus* (Pauls and Bertollo 1983, 1990; Voltolin et al. 2013), with the remarkable presence of B chromosomes in species such as *Prochilodus argenteus* Spix et Agassiz, 1829 (Penitente et al. 2015), *Prochilodus costatus* Valenciennes, 1850 (Melo et al. 2017), *Prochilodus brevis* Steindachner, 1875 (Pauls and Bertollo 1990), *Prochilodus mariae* Eigenmann, 1922 (Oliveira et al. 2003), and *P. lineatus* (Pauls and Bertollo 1983; Penitente et al. 2016; Stornioli et al. 2021). B chromosomes are selfish supernumerary elements that typically do not follow Mendelian inheritance laws (Camacho et al. 2000) and are found in the genomes of various plants, fungal species, and animal species (Camacho et al. 2000; Douglas and Birchler 2017). These elements are usually heterochromatic, but next-generation sequencing is demonstrating that their structure comprises fragments of A chromosomes (Ruban et al. 2017; Oliveira et al. 2024). In addition, recent transcriptomic analyses are registering the expression of these elements, including protein-encoding (Ruban et al. 2017; Oliveira et al. 2024). The origin of B chromosomes could be intraspecific, with these elements arising from A chromosomes of their host species (Mestriner et al. 2000; Jesus et al. 2003; Artoni et al. 2006), or interspecific, because of hybridization events (Schartl et al. 1995; Tosta et al. 2014), and repetitive elements can provide evidence of the origin mechanisms of these elements. In *P. lineatus*, it is assumed that the B chromosomes have an intraspecific origin due to the sharing of a satellite DNA (PliSat05-178) only between the B chromosomes and the telomeric region of the pair 4 (Stornioli et al. 2021).

Despite the similar content of repetitive sequences (Stornioli et al. 2021), *P. lineatus* presents three different variants of B chromosomes: acrocentric (small-sized), metacentric (medium-sized), and submetacentric (large-sized) (Artoni et al. 2006; Penitente et al. 2013). A large variation in the number of B chromosomes in an individual is documented, with individuals containing 0 to 9 B chromosomes (Voltolin et al. 2011). In addition, repetitive sequences are shared by *P. lineatus*, *P. costatus*, and *P. argenteus* B chromosomes, indicating a common origin of B chromosomes in *Prochilodus* (Melo et al. 2017).

Despite the widespread distribution of *P. lineatus* in South American watersheds, the majority of knowledge on the structure, inheritance, and evolution of this species' B chromosomes is limited to the Mogi-Guaçu River and Sapucaí River (Voltolin et al. 2011; Penitente et al. 2013, 2015, 2016; Stornioli et al. 2021), with few information available from other locations. The current study aimed to address this knowledge gap in other locations while also updating the B chromosome studies in *P. lineatus* with new data and statistical analyses on its quantity, presence, and morphology in seven sites within the Paraná River basin.

Materials and methods

We collected 148 individuals of *P. lineatus* in seven localities of the Paraná River basin (Table 1, Fig. 1). All samples were obtained with authorization from the relevant organizations (MMA/IBAMA/ICMBio/SISBIO-18884-1). Cytogenetic preparations were obtained through lymphocyte culture technique, as established by Fenocchio and Bertollo (1988). Nucleolus organization regions (NORs) are shown following the protocols established by Howell and Black (1980), and heterochromatic regions were visualised using the C-banding, as established by Sumner (1972). All images were captured using the software CellSens Standard 1.14 (Olympus) with a digital camera (Olympus Qcolor5) on a fluorescence microscope (BX50, Olympus).

Frequency comparisons of each B chromosome variant among populations were made using one-way ANOVA with the BioEstat 5.3 software (Ayres et al. 2007). Only values with P less than 0.05 were considered statistically significant ($P < 0.05$).

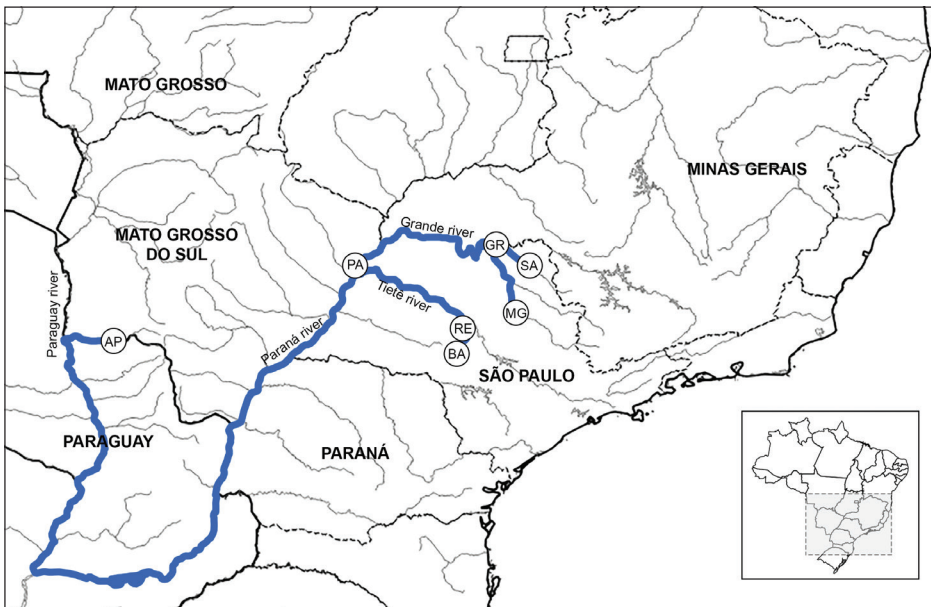


Figure 1. Collection sites of *P. lineatus* at Paraná River Basin.

Table 1. Number and geographic origin of *P. lineatus* specimens collected at each point of the Paraná River Basin.

| Population | Code | N | Geographic coordinates |
|-------------|------|----|--------------------------|
| Mogi Guaçu | MG | 32 | -21.9267528, -47.3669111 |
| Sapucaí | SA | 23 | -20.5724083, -47.7827416 |
| Grande | GR | 20 | -20.1258167, -48.5738056 |
| Paraná | PA | 30 | -20.7767028, -51.6404721 |
| Reginópolis | RE | 15 | -21.8441917, -49.2194721 |
| Bauru | BA | 8 | -22.3820556, -49.1153610 |
| Apa | AP | 20 | -22.2497111, -56.9268693 |

Results

All the individuals collected in this work had $2n = 54$, with only meta- or submetacentric chromosomes and a fundamental number of 108 (Fig. 2). The C-banding demonstrates the presence of heterochromatin only in the centromeric portion of all chromosomes, without differences between populations (Fig. 2). In addition, all B chromosomes observed in the specimens were completely heterochromatic (Fig. 2). The Ag-NOR technique also demonstrated conservation in the karyotype of *P. lineatus* in all populations analyzed, with all individuals demonstrating just one block in the long arm of one pair of submetacentric chromosomes (Fig. 2). None of the B chromosomes had Ag-NOR signals.

The populations of *P. lineatus* analyzed here have demonstrated heterogeneity related to the presence of B chromosomes (Table 2). The Apa River population (APA) was the only one without the presence of any B chromosome in all the individuals analyzed (Table 2). The three morphologies of B chromosomes ever described were found in the locations Mogi Guaçu (MG), Sapucaí (SA), and Grande (GR), representing the Rio Grande basin. The Paraná River (PA) demonstrated the prevalence of metacentric B chromosomes, with only one individual having an acrocentric B chromosome. In two points of the Rio Batalha (RE and BA), representing the Tietê River basin, only metacentric B chromosomes were found. However, in RE, one individual presented a metacentric macrochromosome B, representing a new variation of this element in *P. lineatus* (Fig. 3), also completely heterochromatic.

Table 2. Statistical analyses of the types of B found in *P. lineatus* in the seven sampled points. (M – metacentric, SM – submetacentric, A - acrocentric). SD = Standard deviation.

| B variant | | MG | SA | GR | PA | RE | BA | AP |
|-----------|------|--------|--------|--------|--------|--------|--------|--------|
| M | Mean | 1.8750 | 2.0435 | 2.3000 | 1.2000 | 2.0667 | 3.0000 | 0.0000 |
| | SD | 0.1665 | 0.1831 | 0.2524 | 0.1390 | 0.2063 | 0.6547 | 0.0000 |
| S | Mean | 0.5000 | 0.2609 | 0.4500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| | SD | 0.1004 | 0.1128 | 0.1535 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| A | Mean | 0.0625 | 0.3478 | 0.6000 | 0.0333 | 0.0000 | 0.0000 | 0.0000 |
| | SD | 0.0435 | 0.1194 | 0.1522 | 0.0333 | 0.0000 | 0.0000 | 0.0000 |
| Total | Mean | 2.4375 | 2.6364 | 3.3500 | 2.4300 | 2.0667 | 3.0000 | 0.0000 |
| | SD | 1.2684 | 1.0272 | 1.5652 | 0.7279 | 0.7988 | 1.8516 | 0.0000 |

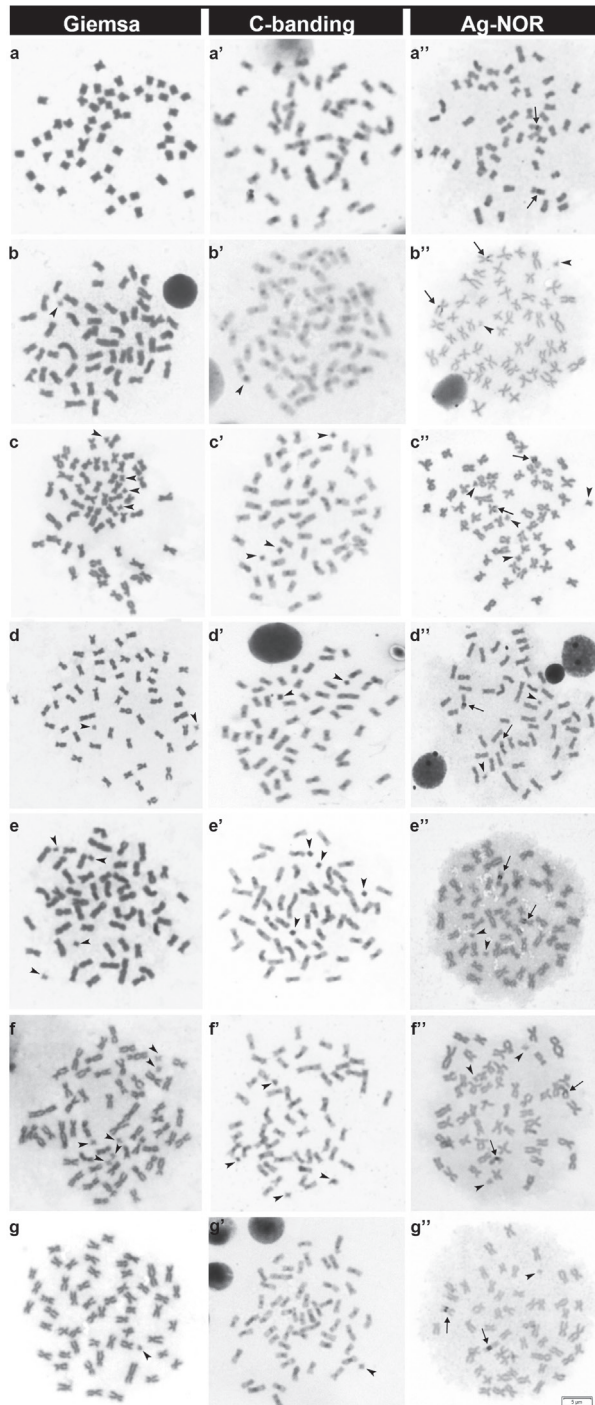


Figure 2. Metaphases of all analyzed populations of *P. lineatus* stained totally (Giemsa) or differentially (C bands, Ag-NORs). The locations are Apa (**a**), Mogi Guaçu (**b**), Sapucaí (**c**), Batalha – Reginópolis (**d**), Grande (**e**), Batalha – Bauru (**f**), and Paraná (**g**). Arrowheads indicate B chromosomes, and arrows indicate NOR regions.

The statistical analyses using one-way ANOVA on the frequencies of different types of B chromosomes demonstrated differentiation among localities, with $F = 18.1307$, $P < 0.0001$ for metacentric B chromosome, with degrees of freedom ($df = 6$), $F = 6.5932$, $P < 0.0001$ for submetacentric B chromosome ($df = 6$), and $F = 8.1826$, $P < 0.0001$ for acrocentric B chromosome ($df = 6$) (Fig. 4).

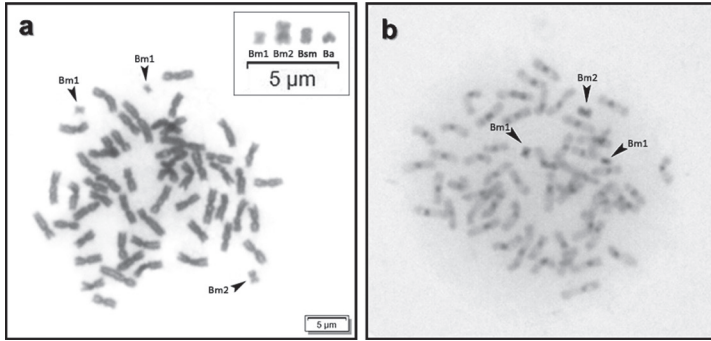


Figure 3. Metaphases with B chromosome variants of the species *Prochilodus lineatus* (Bm1 = metacentric, Ba = acrocentric, Bsm = submetacentric) and the discovery of a new metacentric macrochromosome (Bm2), present in the RE population **a** giemsa staining **b** c banding showing this variant completely heterochromatic.

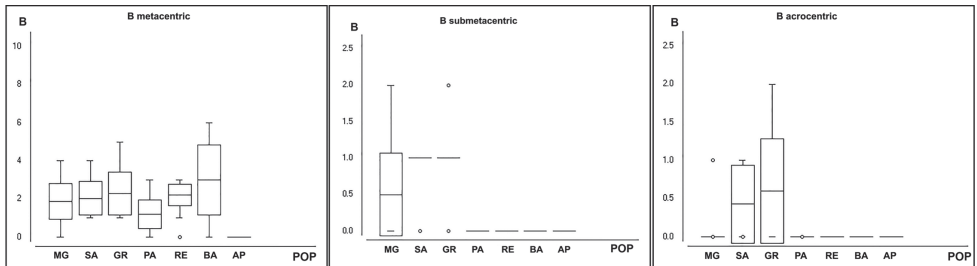


Figure 4. Frequency of metacentric, submetacentric, and acrocentric variants of B chromosomes in locations, respectively.

Discussion

Pioneering studies conducted by Pauls and Bertollo (1983, 1990) on the cytogenetics of fish of the genus *Prochilodus* Agassiz, 1829 suggested the existence of homogeneity of basic karyotypic characters. Several studies have shown that different species of *Prochilodus* analyzed cytogenetically presented a diploid number composed of metacentric and submetacentric chromosomes, with a fundamental number equal to 108 (Pauls and Bertollo 1983; 1990; Oliveira et al. 1997; Cavallaro et al. 2000; Jesus and Moreira-Filho 2003; Voltolin et al. 2009, 2013). Our data corroborates this affirmation, with all individuals analyzed in this work demonstrating the same pattern of NOR, heterochromatin regions, and fundamental number (Fig. 2).

Although diploid number and basic cytogenetic markers are conserved, B chromosomes follow a directly opposite path. We found a large variation related to the frequency of B chromosomes in the analyzed populations, especially in the acrocentric and submetacentric variations, which could mean a population structure in *P. lineatus*. However, several studies related to the population dynamics of *P. lineatus* demonstrated a panmictic population for this fish (Garcez et al. 2011; Rueda et al. 2013). Behavior studies indicate that *P. lineatus* can migrate more than 1000 km, but this distance is significantly smaller to reproductive goals, varying between 100 and 400 km (Godoy 1975). So, the B chromosomes can demonstrate different meiotic drives in each locality observed in this work. Despite the differences in species, B chromosome systems, and the environment, different B chromosome frequencies are also observed in a population of *Psalidodon paranae* Eigenmann 1914. The animals of the Cascatinha stream (Botucatu, São Paulo, Brazil -22°53'22.5"S, 48°29'22.4"W), collected in different stretch of the river, demonstrated an accentuated difference in B chromosome frequency, varying from 65.7% of B chromosome carriers in stretch 1, next to the source of the river, in contrast to 10% of B chromosome carriers in the other two stretches of the river along (Porto-Foresti et al. 1997).

Intensive B chromosome analysis in *Prochilodus* has focused on its structure, origin, and inheritance (Voltolin et al. 2011; Penitente et al. 2013, 2016; Stornioli et al. 2021). The FISH mapping of satellite DNAs points to a common origin of these chromosomes in the genus *Prochilodus*, before the speciation of at least three carrier species (*P. lineatus*, *P. costatus* and *P. argenteus*). In addition, all variants of the B chromosomes of *P. lineatus* have the same repetitive composition and are probably derived from the autosomal pair 4 (Stornioli et al. 2021). This fact reinforces the hypothesis that there is a single origin to the variations of B in *P. lineatus*, from an ancestral acrocentric and the formation of an isochromosome, resulting in the actual metacentric. The other variants would be derived from the metacentric (Penitente et al. 2016), as the new metacentric macrochromosome B described in the Reginópolis population (present work). So, the possibility of a new event of origin that could modify the frequencies of Bs in the analyzed populations seems to be very improbable.

B chromosomes are considered selfish parasitic elements (Camacho et al. 2000; Douglas and Birchler 2017), and their inheritance mechanisms are a big question in carrier species. In grasshoppers, an elimination process during spermiogenesis is documented in the form of nonfunctional microspermatids (Cabreró et al. 2017). In *P. paranae*, females with B chromosomes have an inheritance pattern lower than B carrier males, suggesting an elimination process of Bs by the elimination of polar bodies (Goes et al. 2021). In *P. lineatus*, the variants have different transmission rates, with the metacentric being the only variation with inheritance rates above the Mendelian expectations ($kb = 0.587$), in contrast to the acrocentric and submetacentric variants ($kb = 0.333$ and 0.385 , respectively), characterized by an elimination of B chromosomes process, following the B chromosome evolution cycle (Camacho et al. 2000). This data corroborates the data of this work, with the metacentric variation dispersed by almost all populations analyzed and the other two variants being very rare, restricted to some individuals of three populations. So, our hypothesis is that the acrocentric

and submetacentric variants have already been lost in PA, RE, BA, and AP populations, persisting only in populations of MG, SA, and GR. This process seems to be more accentuated in submetacentric variation, with expressive presence only in the MG population. In contrast, the metacentric variant persists in all populations, with the exception of AP, due to the accumulation inheritance pattern of the metacentric.

Only the AP population shows individuals without B chromosomes (Stornioli et al. 2021). This population was isolated by the Sete Quedas waterfall, which was destroyed by the construction of the Itaipu hydroelectric plant. So, the absence of Bs in these animals can be explained by two hypotheses: 1) Bs were eliminated in this population and can appear again due to contact with animals from other populations; 2) Bs never existed in the AP population and now can appear for the first time due to contact with other populations. We consider hypothesis two invalid due to the origin of Bs anterior of the diversification of *P. lineatus* and two other species (Melo et al. 2017), showing the very old origin of these elements.

Several studies have been dedicated to the B chromosomes of *P. lineatus*, generating insights about their origin (Stornioli et al. 2021), repetitive sequence content (Jesus et al. 2003; Melo et al. 2017; Stornioli et al. 2021), and inheritance (Penitente et al. 2015). The present work reveals the evolutionary dynamic along the Parana River basin, revealing the manutention of metacentric variants and potential disappearance of the acrocentric and submetacentric variants. The perspectives about the studies of B chromosomes of *P. lineatus* include the genome assembling at chromosome level, which can potentially demonstrate genomic differences between the three variants and bring new light about the evolution of supernumeraries in *P. lineatus*.

Conclusion

Our work presents an extensive panorama of B chromosome frequency in the *P. lineatus* population of the Alto Paraná Basins, corroborating theories about the evolution of these elements. We could verify the dispersion of metacentric variation, agreeing with the accumulation inheritance pattern, in contrast with the elimination process observed in acrocentric and submetacentric B chromosomes.

Author contributions

Manolo Penitente - conceptualization, methodology, formal analysis, writing of original draft; Caio Augusto Gomes Goes - validation, formal analysis, data Curation, writing of original draft; Rodrigo Zeni dos Santos - formal analysis, data curation, writing of original draft; Ricardo Utsunomia - validation, formal analysis, data curation, writing of original draft, writing of review; Fausto Foresti - validation, writing of review and editing, visualization; Fabio Porto-Foresti - conceptualization, validation, resources, writing of review and editing, visualization, supervision, project administration, funding acquisition.

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Supplementary material I

Number of B chromosome in *P. lineatus* of Mogi Guaçu population

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Data type: docx

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