



Population analysis of a chromosome polymorphism in *Astyanax* (Teleostei, Characiformes) species endemic to the Iguaçu River

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Abstract

Chromosomal analyses were performed in the fish *Astyanax* sp.D collected from three different points: two streams from the right bank and one from the left bank of the Upper Iguaçu River, Paraná State, Brazil. The individuals from all localities possess $2n = 50$ chromosomes and a $FN = 84$ ($4m+24sm+6st+16a$). The C-banding pattern was similar in all populations. However, within each population, an interindividual variation concerning the number and localization of heterochromatic bands was observed. Some of these variations were quantified in each population, and the results indicate that the samples were not different when studying the variable frequencies. Considering that *Astyanax* sp.D is typical in the headwaters of the Iguaçu River, these results were not expected. The data indicate that gene flow is occurring and that the Iguaçu River is not an ecological barrier among the *Astyanax* sp. D populations.

Key words: population cytogenetics, chromosome variations, *Astyanax* fish.

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Taxonomic studies in *Astyanax* of the Iguaçu River, southern Brazil, show that at least eleven different taxonomic entities may be differentiated in this locality: *Astyanax* sp. A (currently cited as *A. gimnogenis*) to *Astyanax* sp.F (Sampaio, 1988), *A. altiparanae* (Garutti and Britski, 2000), *Astyanax ita* (Almiróm *et al.*, 2002), *Astyanax leonidas* (Azpelicueta *et al.*, 2002), *Astyanax totae* (Haluch and Abilhoa, 2005) and *Astyanax varzeae* (Abilhoa and Duboc, 2007). Although lacking a valid scientific name, *Astyanax* sp. species of the Iguaçu River are considered natural taxonomic entities (Severi and Cordeiro, 1994; Agostinho and Gomes, 1997; Kantek *et al.*, 2003; Vitule and Abilhoa, 2003; Ingenito *et al.*, 2004).

The main habitats of *Astyanax* sp.D are the headwaters of the Upper Iguaçu River (Sampaio, 1988). Due to the isolation of many populations in streams, caused by the water volume in the inferior region of the basin, the detection of differences among populations caused by distinct selective pressures and also stochastic variations is expected.

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According to Buckup (1999), the stream species tend to present larger speciation rates, considering that their more restricted populations may genetically diverge with a faster rate than those typical of large rivers.

Cytotaxonomy is a useful tool for the comparison of populations of the same species. In *Astyanax*, interpopulational karyotypic differences were detected in many species, including *A. scabripinnis* (Martins *et al.*, 1984; Moreira-Filho and Bertollo, 1991; Mizoguchi and Martins-Santos, 1998; Maistro *et al.*, 1998), *A. fasciatus* (Morelli *et al.*, 1983; Centofante *et al.*, 2003), and *A. altiparanae* (Jim and Toledo, 1975; Morelli *et al.*, 1983; Fernandes and Martins-Santos, 2004). Sampaio (1988) and Kantek *et al.* (2007) consider *Astyanax* sp.D and *A. scabripinnis* as very similar species. Thus, bearing in mind the intense interpopulational karyotypic variability reported in the literature for *A. scabripinnis*, it is expected that variations could also occur in the same proportion in *A. sp.D*.

In this context, this work has the objective of verifying a possible populational structure in the samples of *Astyanax* sp.D from the Iguaçu River through the comparison of karyotypic data.

One hundred and eighteen *Astyanax* sp.D specimens were collected in the Upper Iguaçú River basin and were cytogenetically analyzed: 41 (4 males and 37 females; MHNCI - Museu de História Natural Capão da Imbuia - 9095) from the Bicudo River (Point 1 - UTM 22J0635133/7171020), 44 (12 males and 32 females; MHNCI 9153) from the Poço Claro River (Point 2 - UTM 22J0635327/7179957), and 33 (12 males and 21 females; MHNCI 9153) from a nameless stream located to the left bank of the Iguaçú River basin (Point 3 - UTM 22J0633551/7166935). The Bicudo and Poço Claro Rivers are on the right bank of the Iguaçú River. Mitotic metaphases were obtained according to the indirect method described by Fenocchio *et al.* (1991). C-banding was performed according to Sumner (1972). Chromosomes were classified according to Levan *et al.* (1964).

For population analyses, chromosome variants (inversions) were detected by C-banding. In order to obtain the mean number of inversions per individual in each population, only the chromosomes belonging to pairs 18 and 19 with large interstitial heterochromatic blocks (IB) were considered. A contingency table was constructed to verify whether individual frequencies with zero, one, two, or three IB differ among the *Astyanax* sp.D samples. The Kolmogorov-Smirnov normality test was used to evaluate the distribution data. A Kruskal-Wallis non-parametric test was performed to compare the mean number of inversions per

individual in each population. InStat 3.05 program was used in the analyses.

The three analyzed populations possess a diploid number of 50 chromosomes, made up of 2 metacentric pairs (m), 12 submetacentric pairs (sm), 3 subtelocentric pairs (st), and 8 acrocentric pairs (a), with a FN = 84 (Figure 1a, 1b and 1c). No sex chromosome dimorphism was detected. The telomeric regions of many sm, st, and a chromosomes were more strongly evidenced by C-banding (Figure 1d). However, interindividual variations occur within each population. By comparing the acrocentric chromosomes of the individuals, it was possible to reveal the variation in the number and localization of heterochromatic blocks (Figure 2a, 2b and 2c). Some chromosomes bearing the IB possess another heterochromatic band located below the IB (for an example, see Figure 2a, individual B, pair 18).

The frequency of specimens with 0, 1, 2, or 3 IBs in each sample is shown in Table 1. The chi-square independence test indicated that at least one of the samples differs from the others in this parameter ($p = 0.0476$). The p -values referring to comparisons between sample pairs are summarized in Table 2.

The IB average values are: Point 1 = 1.585 (SE - Standard error of mean = 0,139; SD - Standard deviation = 0,894), Point 2 = 1.523 (SE = 0,147; SD = 0,976), and Point 3 = 1.242 (SE = 0,151; SD = 0,867). The Kolmogorov-Smirnov test indicated that the data were not normally distributed ($p = 0.0032$ - Point1; $p = 0.0022$ - Point 2;

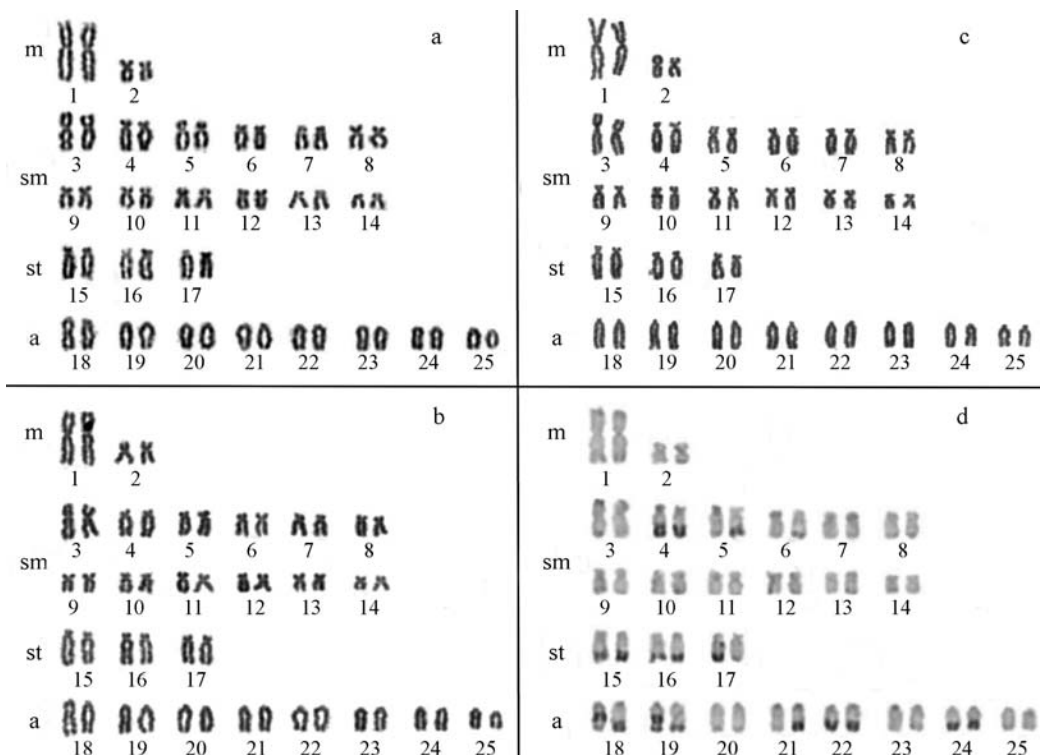


Figure 1 - Karyotypes of *Astyanax* sp.D. Giemsa staining of specimens collected in the Bicudo (a) and Poço Claro (b) Rivers and in the nameless stream from the left bank of the Iguaçú River basin (c). General C-banding pattern (d).

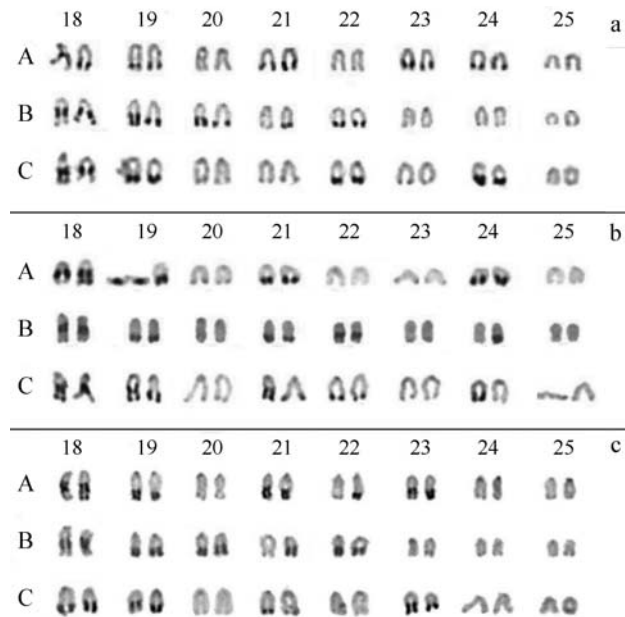


Figure 2 - Interindividual variation of heterochromatic blocks in *Astyanax* sp.D samples from the Bicudo (a) and Poço Claro (b) Rivers and from the nameless stream (c) from the left bank of the Iguaçu River basin. The letters at left represent some of the analyzed individuals. The numbers show the acrocentric chromosome pairs. Note that individual “B” of Poço Claro has an interstitial band on pair 22.

$p = 0.0128$ - Point 3). According to the Kruskal-Wallis test, the averages do not differ ($p = 0.229$).

The presence of the interstitial bands on pairs 18 and 19 was interpreted by Kantek *et al.* (2007) as a consequence of possible paracentric inversions in chromosomes bearing telomeric heterochromatic blocks. The occurrence of these alterations in three chromosome pairs (18, 19, and 22) may be the indication of a tendency for the occurrence of similar chromosomal rearrangements (karyotypic ortoselection) in *Astyanax* sp.D.

If *Astyanax* sp.D was restricted to the headwaters of the Iguaçu River (Sampaio, 1988), it would be expected for the populations to be isolated in such a way as to prevent, at least partially, the gene flow among them. The present work showed that there are no cytogenetic differences between the populations, and that all variations are intrapopulation. Furthermore, when the present results are compared to those of Kantek *et al.* (2007), who analyzed an *A.* sp.D sample from a distinct river located in the eastern region of Curitiba (PR), it is seen that the karyotypic pattern is faithfully repeated, including the presence of the interstitial heterochromatic bands and the intrapopulation variation. Thus, these results suggest the existence of gene flow among the populations.

Despite the result of the contingency test, which indicated that the distribution of the IBs is not equal among the three populations, the p -value was very close to 0.05. Thus, it is possible to assume that the three populations are similar. Moreover, when comparing only two populations

Table 1 - Frequency of specimens with 0, 1, 2, or 3 interstitial heterochromatic blocks in each sample.

Number of heterochromatic blocks	0	1	2	3
Bicudo River	3	19	11	8
Poço Claro River	9	9	20	6
Nameless stream	6	16	8	3

Table 2 - p -values of the contingency test between sample pairs. Point 1 - Bicudo River, Point 2 - Poço Claro River, Point 3 - nameless stream.

	Point 1	Point 2	Point 3
Point 1	-	0.0247	0.3653
Point 2	-	-	0.0635

through the contingency test, only the comparison between Point 1 and Point 2 suggests differences. The sample collected in the stream located on the left bank of the Iguaçu River (Point 3) did not differ from the other two samples, suggesting the occurrence of gene flow between the *Astyanax* sp.D populations. The Kruskal-Wallis test result indicates that the mean number of IBs per individual in each sample does not differ, reaffirming the hypothesis that the samples are elements of a single population. Another evidence in favor of the gene flow hypothesis is that, according to Abilhoa, V. (personal communication), some *A.* sp.D specimens are also sporadically collected in the waters of the Iguaçu River.

Morphological and/or molecular marker analyses should be used in future approaches in order to confirm the equality similarity between the analyzed populations, as proposed in the present work through chromosomal data.

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