Cytogenetic Studies Comparing Three Characidae Fish Species from the Iguatemi River Basin, Brazil

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Summary Characidae is one of the predominant fish groups in the Neotropical region with approximately 30 highly diversified subfamilies, probably not representing a monophyletic group. Three Characidae species from the Iguatemi River Basin (Mundo Novo, MS, Brazil) were analyzed: *Astyanax bockmanni* with $2n=50$ chromosomes (6m+20sm+8st+16a), *Bryconamericus stramineus* with $2n=52$ chromosomes (6m+10sm+16st+20a) and *Piabina argentea* with $2n=52$ chromosomes (6m+24sm+12st+10a), all the species showing no differences between male and female karyotypes. The Ag-NORs were located in only a single chromosome pair in the three species. The chromosomal data obtained may be useful for the taxonomy and phylogenetic studies in these fish groups.

Key words *Piabina argentea*, *Bryconamericus stramineus*, *Astyanax bockmanni*, Animal cytogenetics.

The Characidae family has a large group of fishes in South America, comprising approximately 30 subfamilies. Tetragonopterinae was considered for a long time as the most species-rich and diversified Characidae subfamily. However, due to the lack of evidence that would support a monophyletic origin for this group (Weitzman and Malabarba 1998), it should be considered as only encompassing the genus *Tetragonopterus* and the species *T. argenteus* and *T. chalceus* (Reis 2003). The remaining genera were listed as “*Incertae Sedis*” in Characidae, including *Astyanax*, *Bryconamericus* and *Piabina* (Lima *et al.* 2003). *Piabina argentea* is small fish, with an elongated body, reaching up to 74 mm; *Bryconamericus stramineus* is small, with an elongated body, reaching up to 84 mm; *Astyanax bockmanni* is a small fish, with a high body, reaching up to 74.2 mm (Graça and Pavanelli 2007). *Astyanax bockmanni* is a widespread species in streams from the Upper Paraná River system in central, southeastern, and southern Brazil. It has been formerly identified as *A. eigenmanniorum*, which was described in southwards Brazil (Vari and Castro 2007).

Karyotype studies may become an important source of information for taxonomy and for the understanding of the phylogenetic relationships between species. Three Characidae species were cytogenetically analyzed in this work: *Astyanax bockmanni*, *Bryconamericus stramineus* and *Piabina argentea*. Some different methods were used in the search for chromosomal markers that could be useful for the taxonomy and phylogenetic studies in these fish groups.

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Fig. 1. Karyotype of *Astyanax bockmanni* (A), *Bryconamericus stramineus* (B) and *Piabina argentea* (C) after Giemsa staining.

Fig. 2. Metaphases of *Astyanax bockmanni* (A), *Bryconamericus stramineus* (B) and *Piabina argentea* (C). The arrows indicate the sites of Ag-NOR staining.

Materials and methods

Astyanax bockmanni (10 males and 1 female) and *Piabina argentea* (1 male and 3 females) were collected from Dourado stream (23°51' 04.9" S e 54° 25' 13.9" W) and the *Bryconamericus stramineus* (5 males, 18 females and 5 indeterminate sex) were collected from Guaçu stream (23° 54' 19.6" S e 54° 21' 43.4" W), both tributaries of the Iguatemi River (Mundo Novo, MS, Brazil). The fish were identified and deposited in the State University of Mato Grosso do Sul, Unit Mundo Novo. The mitotic chromosomes were obtained from kidney cells through the direct preparation technique, according to Bertollo *et al.* (1978). The nucleolar organizing regions (Ag-NORs) were detected following the methodology of Howell and Black (1980).

The chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a) according to their arm ratio (Levan *et al.* 1964). For the determination of the fundamental number (FN), or number of chromosome arms, the m, sm and st chromosomes were considered as bearing 2 arms and the acrocentric chromosomes only one arm.

Results

Astyanax bockmanni presented a modal diploid number of $2n=50$ chromosomes in males and females distributed in $6m+20sm+8st+16a$, with a FN of 84 (Fig. 1A). The Ag-NORs were located in a telomeric position on the short arm of the subtelocentric pair no. 17 (Fig. 2A), coinciding with

Table 1. Cytogenetic data in *A. bockmanni*, *B. stramineus* and *P. argentea* from different localities of Brazil

Species	River/stream, state	2n	FN	Formulae	Reference
<i>A. bockmanni</i>	Paranapanema basin (SP)	50	84	10m+12sm+12st+16a	Kavalco <i>et al.</i> (2009)
<i>A. bockmanni</i>	Dourado stream (MS)	50	84	6m+20sm+8st+16a	Present study
<i>B. stramineus</i>	Mogi-Guaçu River (SP)	52	—	26m/sm+26st/a	Portela <i>et al.</i> (1988)
<i>B. stramineus</i>	Guaçu stream (MS)	52	84	6m+10sm+16st+20a	Present study
<i>P. argentea</i>	Mogi-Guaçu River (SP)	52	—	26m/sm+26st/a	Portela <i>et al.</i> (1988)
<i>P. argentea</i>	São Francisco River (MG)	52	90	8m+14sm+16st+14a	Moreira <i>et al.</i> (2007)
<i>P. argentea</i>	Dourado stream (MS)	52	94	6m+24sm+12st+10a	Present study

m, metacentric; sm, submetacentric; st, subtelocentric; a, acrocentric; FN, fundamental number

a secondary constriction visualized in Fig. 1A.

Bryconamericus stramineus presented a modal diploid number of $2n=52$ chromosomes in males and females (6m+10sm+16st+20a; FN=84), with no morphological chromosome difference between the sexes (Fig. 1B). The Ag-NORs were located in a telomeric position on the short arm of one subtelocentric pair (Fig. 2B).

Piabina argentea presented a modal diploid number of $2n=52$ chromosomes in males and females (6m+24sm+12st+10a; FN=94), with no morphological chromosome difference between the sexes (Fig. 1C). The Ag-NORs were located in a telomeric position on the short arm of one subtelocentric pair (Fig. 2C).

Discussion

The diploid numbers in Characidae vary from $2n=28$ to $2n=64$, with $2n=50$ more often, suggesting it is a plesiomorphic condition for this family (Oliveira *et al.* 1988, Portela *et al.* 1988). The diploid number found in *B. stramineus* and *P. argentea* ($2n=52$) and that found in *A. bockmanni* ($2n=50$) are in agreement with those reported in the previous studies for these species (Portela *et al.* 1988, Moreira *et al.* 2007, Kavalco *et al.* 2009). However, their karyotypic structures were divergent (Table 1). As for *B. stramineus*, 6m+10sm+16st+20a chromosomes were identified in our studies, while 26m/sm+26st/a chromosomes were reported previously (Portela *et al.* 1988). As for *P. argentea*, 6m+24sm+12st+10a chromosomes were identified in our studies, while 26m/sm+26st/a chromosomes (Portela *et al.* 1988) and 8m+14sm+16st+14a chromosomes (Moreira *et al.* 2007) were previously found. Two different samples of *B. stramineus* examined in 2 different studies and 3 different samples of *P. argentea* examined in 3 different studies were considered to belong to the respective single species. This suggests that chromosome rearrangements, like pericentric inversions, have played a major role in their karyotypic differentiation in each species.

Although the karyotype of *A. bockmanni* also showed some divergence from those described by Kavalco *et al.* (2009), the differences are more subtle for this species and may be attributed to the difficulty in characterizing with precision the morphology of some chromosomes because they are very condensed, which complicates the organization of the karyotype, since the samples have the same number of acrocentric chromosomes and fundamental number (Table 1). Daniel *et al.* (2009) observed the presence of B chromosomes in *A. bockmanni* which was not detected in the present study.

Some characins may present both single and multiple chromosomes bearing NORs, such as *A. bockmanni*, *B. stramineus* and *P. argentea*, while others are characterized by presenting only a single pair of NORs, such as [Bryconinae (Almeida-Toledo *et al.* 1996, Margarido and Galetti Jr. 1999)], or multiple NORs, such as [Serrasalminae (Galetti Jr *et al.* 1985)]. Thus, the number and

localization of Ag-NORs have exhibited a broad variability in this group. The 3 species here analyzed presented only a pair of Ag-NOR sites. Moreira *et al.* (2007) also found a single Ag-NOR pair in *P. argentea*, corroborating the present study. On the other hand, a *P. argentea* population described by Portela *et al.* (1988) and a population of *A. bockmanni* analyzed by Kavalco *et al.* (2009), presented up to 4 chromosomes with Ag-NORs. In the present study, the chromosomal location of Ag-NORs in *B. stramineus* population was investigated for the first time.

According to Hsu *et al.* (1975), species with a single pair of NORs could be considered ancestors of related species whose NORs are distributed among several chromosomes. Therefore, regarding this trait, the *A. bockmanni* and *P. argentea* populations studied up to now could be considered more recent compared to the *P. argentea* population of the São Francisco River and *A. bockmanni* and *P. argentea* populations of the Dourado stream. Nevertheless, as Ag-NORs indicate only the functionally active rDNA sites in the preceding interphase nuclei (Miller *et al.* 1976), a complementary analysis using fluorescent *in situ* hybridization with a probe for 18S rDNA is necessary for a conclusive NOR study.

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