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Nucleolar organizer regions, 18S and 5S rDNA clusters in the chromosomes of *Piabina argentea* (Characiformes: Characidae)

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Abstract: The *Piabina* genus was allocated in Stervadiinae subfamily after staying for years as an uncertain group in Characidae. Few cytogenetic studies are described in the literature for *Piabina argentea*, thus new analyzes in other populations are important to understanding of the chromosomal dynamics of them. The aim of this paper was to analyze the chromosomes of *P. argentea* from Corumbataí River basin focusing mainly the chromosomal locations of 18S rDNA and 5S rDNA clusters. The diploid chromosome number was $2n = 52$ for *P. argentea*, which were classified as 6 metacentrics (m), 8 submetacentrics (sm), 24 subtelocentrics (st) and 14 acrocentrics (a), and FN (fundamental number) value 90 in both sexes. Silver nitrate impregnation located the nucleolus organizer regions (Ag-NORs) on the short (p) arm of pair 16, however, an intra-individual variation of Ag-NORs sites was observed in two individuals. Fluorescence *in situ* hybridization (FISH) revealed six 18S rDNA sites (pairs 8, 10 and 16) and four 5S rDNA sites in terminal positions of the short arm of pairs 5 and 22 (one submetacentric and one acrocentric) in *P. argentea*. The results indicate that, despite the conserved diploid number, this group of fish presents a wide variability of the 18S and 5S rDNA sites.

Key words: repetitive DNA; FISH; chromosome evolution; Neotropical fish

Introduction

Many modifications have been made regarding the phylogenetic relationships of the *Piabina* genus, which have already belonged to the group *incertae sedis* in Characidae by Lima et al. (2003), as well as many other genera that remain uncertain to date. Studies based on analyzes of molecular characters have indicated that *Piabina* belong to the subfamily Stervadiinae (Oliveira et al. 2011; Thomaz et al. 2015).

The *Piabina* genus includes three recognized species, *Piabina argentea* Reinhardt, 1867, *Piabina anhembi* da Silva & Kaefer, 2003 and *Piabina thomasi* (Fowler, 1940) (Eschmeyer et al. 2017). Cytogenetic data are available for only *P. argentea* and *P. anhembi*, which possessed an invariably diploid chromosome number of $2n = 52$ and different karyotypes for individuals from different hydrographic basins (Portela et al. 1988; Peres et al. 2008; Fernandes et al. 2010; Pazian et al. 2012). Simple Ag-NORs were observed for *P. anhembi* (Pazian et al. 2012) and simple and multiple Ag-NORs were observed for *P. argentea* from different sites (Portela et al. 1988; Moreira et al. 2007; Peres et al. 2008; Fernandes et al. 2010; Pazian et al. 2012).

Cytogenetic studies on the distribution of ribosomal RNA (rRNA) genes were examined in *Piabina*. Physical mapping of 18S and 5S rDNAs in genome of *P. argentea* demonstrated variation of site numbers among different populations, ranging from 2 to 6 signals in karyotypes after using the fluorescence *in situ* hybridization (FISH) technique with 18S rDNA-probe and 4 to 6 signals with the 5S rDNA-probe (Peres et al. 2008; Pazian et al. 2012). This intraspecific variation of 18S rDNA sites also have been described for other species of the Stevardiinae subfamily, such as *Bryconamericus* spp. (Capistano et al. 2008; Santos et al. 2012; Piscor et al. 2013).

Considering the variation in the number of NORs and karyotype structure found for the different populations of *P. argentea* described in the literature, this study aims to investigate the karyotype in a population of *P. argentea*, utilizing different chromosome banding techniques, thereby contributing more information to improve the understanding of the karyotype evolution in the *P. argentea*.

Material and methods

Eleven individuals of *P. argentea* (five males and six females) were collected in the Passa-Cinco River tributary (22°23′25.4″ S; 47°50′47.8″ W) belonging to Corumbataí River basin from São Paulo state (SP), Brazil.

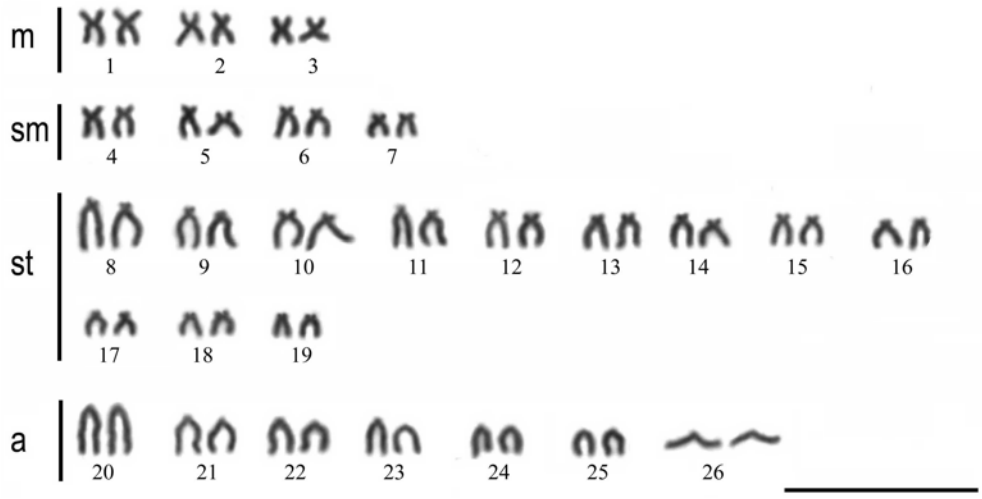

Fig. 1. Karyotype of *Piabina argentea* mounted after staining by Giemsa. Scale 10 μm.

Chromosomes were obtained from cells of the kidney, according to the methodology proposed by Foresti et al. (1981). The NORs were detected using the silver nitrate impregnation technique described by Howell & Black (1980). Chromosomes were classified according to the most common classification system used for fish chromosomes in Brazil (Piscor et al. 2013). This classification comprises the calculation of the ratio between long (q) and short (p) arms, e.g., the metacentric (m) chromosomes had the ratio between 1−1.7, submetacentric (sm) ratio between 1.71−3, subtelocentric (st) ratio between 3.01−7, and acrocentric (a) the ratio >7. The fundamental number (FN) was calculated according to the chromosomal arm numbers (the chromosomes m, sm and st were considered to contain two arms – p and q arms – and the a with one arm – only q arm).

Genomic DNA was extracted from fin samples of *P. argentea* according to Sambrook & Russell (2001). The 5S rDNA probe was prepared using polymerase chain reaction (PCR) with primers described by Pendás et al. (1994) and Martins & Galetti (1999). The 18S rDNA probe was labeled by PCR with digoxigenin-11-dUTP (Roche, Mannheim, Germany), and 5S rDNA probe was labeled by PCR with biotin-14-dATP (Invitrogen, San Diego, CA, USA). Probes labeled with digoxigenin-11-dUTP were detected using anti-digoxigenin-rhodamine (Roche) and the probes labeled with biotin-14-dATP were detected using avidin-FITC (Sigma, Poole, UK). Single and two-color FISH experiments were performed using mitotic metaphasic chromosomes according to Pinkel et al. (1986) and with modifications as described by Piscor et al. (2013). Chromosomes were counterstained with Vectashield Mounting Medium (Vector, Burlingame, CA, USA) containing DAPI. Chromosomes and fluorescent signals were visualized with an Olympus BX51 microscope coupled to a digital camera (Olympus model D71). Images were captured using DP Controller software.

Results

All the 11 individuals of *P. argentea* had $2n = 52$ chromosomes, with the karyotype composed of 6 m, 8 sm, 24 st and 14 a, and FN value 90 in both sexes (Fig. 1). Two individuals exhibited intra-individual variation of Ag-NORs site numbers, i.e., showed metaphasic cells with two, three and four Ag-NORs sites (Fig. 2A). The other nine individuals showed Ag-NORs regions located at the terminal position on the p arm of pair 16 (Fig. 2A3).

The 5S rDNA probes were located on four chromosome sites in terminal regions on the p arm of the pairs 4 and 22 (Fig. 2B). The 18S rDNA clusters were found in terminal position in the p arm of the pairs 8 and 16, confirmed by Ag-NORs results, and one additional pair was also identified in the p arm of the pair 10 (Fig. 2C). The results are summarized in ideogram (Fig. 2D).

Discussion

The diploid number ($2n = 52$) of *P. argentea* described here is coincident to the diploid number of other studied populations of this species (Portela et al. 1988; Peres et al. 2008; Fernandes et al. 2010; Pazian et al. 2012). However, the karyotype structure of *P. argentea* (6m + 8sm + 24st + 14a) differs from the other populations and, so far, appears to be a unique feature of this species. In fact, despite the maintenance of the diploid number, rearrangements modifying the chromosomal morphology, such as pericentric inversions, have played a major role in the karyotypic evolution of *P. argentea*.

An intra-population variation of NOR-bearing chromosomes was exhibited in *P. argentea* in this study, ranging of two to four chromosomes revealed by Ag-impregnation. We believe that this activity difference could be explained by the need for cells to produce ribosomes, which are essential units for the protein production process. Thus, the individuals that presented more than one pair of Ag-NOR would require a greater production of ribosomes, activating other rDNA regions. Multiple Ag-NORs also were observed in the karyotypes of *P. argentea* from Tietę and Mogi-Guaçu rivers (Portela et al. 1988; Pazian et al. 2012), as well as simple Ag-NORs in the karyotypes of *P. argentea* from São Francisco (Peres et al. 2008), Paranapanema and Tietę rivers (Pazian et al. 2012).

Physical mapping of 18S rDNA clusters in genome of *P. argentea* demonstrated six chromosome pairs, i.e.,

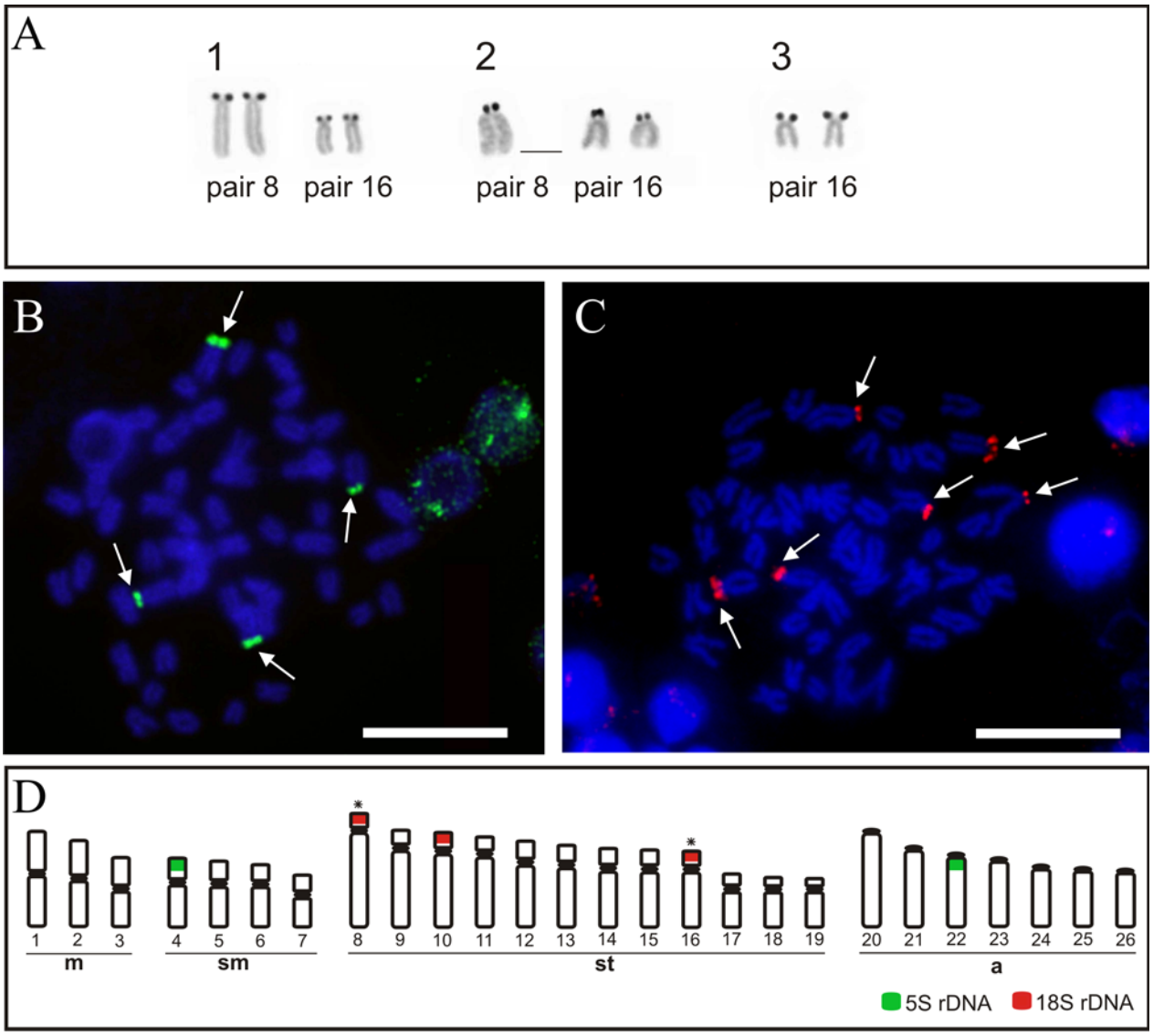

Fig. 2. Cytogenetic techniques in chromosomes of *Piabina argentea*. A: Variations of Ag-NORs sites (A1 – four chromosomes, A2 – three chromosomes and A3 – two chromosomes); B: The arrows indicate the 5S rDNA clusters; C: The arrows indicate the 18S rDNA clusters; D: Ideogram with the repetitive DNA clusters (the asterisks indicate the possible chromosomes bearing Ag-NORs sites). Scales 10 μm.

two sites more than the number visualized by Ag-NORs. Similar patterns were observed in karyotypes of *P. argentea* from São Francisco (Peres et al. 2008) and Tietę rivers (Pazian et al. 2012). On the other hand, in individuals of *P. argentea* from Itatinga two 18S rRNA gene sites were detected, i.e., confirmed the data obtained by silver nitrate impregnation. In individuals from Botucatu four 18S rRNA gene sites were observed, two sites more than the number visualized by Ag-NORs (Pazian et al. 2012). The differences in marking by these techniques may reflect the activity of the NORs during the previous interphase. Moreover, transposable elements have been proposed as one of the mechanisms responsible for the process of mobility of rDNA sequences to new sites, which could cause this inter-population variation (Raskina et al. 2004; 2008).

Physical mapping of 5S rDNA in genome of *P. argentea* showed these clusters located in four chromosomes. Multiple 5S rDNA sites were also observed in karyotypes of *P. argentea* from São Francisco (Peres et al. 2008), Paranapanema and Tietę rivers (Pazian et al. 2012), ranging from 4 to 6 signals in the karyotypes. Thus, this variation of the number 5S rDNA clusters among different *P. argentea* populations also could be related to transposable elements. In a recent study, Moraes et al. (2017) showed co-localization of *Rex3*-5S rDNA on the chromosomes of *Pyrrhulina australis* Eigenmann & Kennedy, 1903 and *Pyrrhulina* aff. *australis*, suggesting that such association may have played a significant role in the dispersion of the 5S rDNA sequences in the genome of *Pyrrhulina* species.

The data observed in the present work could contribute to the understanding of the variation of number and positions of the rDNA sequences associated to the nucleolus among the different populations of *P. argentea* and to understanding of the dynamics of these sequences within of Stevardiinae subfamily. An evalu-

ation of the cytogenetic data obtained in this paper, together with the results described in the literature, reinforces the wide variability of the 18S and 5S rDNA sites within of *P. argentea*.

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