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Comparative cytogenetics between three Characidae fish species from the São Francisco River basin

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Abstract — 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 São Francisco River basin (Três Marias, MG, Brazil) were analyzed: *Hasemania nana* with 2n=50 chromosomes (8m+42sm), *Orthospinus franciscencis* with 2n=50 chromosomes (22m+20sm+2st+6a) and *Piabina argentea* with 2n=52 chromosomes (8m+14sm+16st+14a), all the species showing no differences between male and female karyotypes. The heterochromatin was preferentially distributed in the centromeric region of the chromosomes and 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 those fish groups.

Key words: animal cytogenetics, Characidae, neotropical fish.

INTRODUCTION

In the beginning of the last century, when many Characidae subfamilies were described, the taxonomic studies emphasized the global similarities between the species, independently of the origin of each character. Thus, non-apomorphic characteristics were used to define many groups. Tetragonopterinae, for example, was considered for 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 *Piabina* and *Hasemania* (Lima *et al*. 2003). *Piabina* is a genus composed of small fish, with an elongated body, reaching up to 70 mm; *Hasemania* is also a genus composed of small fish that generally do not exceed 27 mm (Britski *et al.* 1988).

On the other hand, the subfamily Stethaprioninae, comprising the genera *Brachychalcinus*, *Orthospinus*, *Poptela* and *Stethaprion*, is considered a monophyletic group. Its monophyletism is based on the presence of a well-developed predorsal spine and modified hooks in the anal fin (Reis 1989).

Karyotype studies may become an important source of information for taxonomy and for the understanding of the phylogenetic relationships between the species. Three Characidae species were cytogenetically analyzed in this work: *Hasemania nana, Orthospinus franciscencis* and *Piabina argentea*, the first two being endemic to the São Francisco River basin. 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.

MATERIAL AND METHODS

Piabina argentea (15 males and 16 females), *Hasemania nana* (8 males and 9 females) and *Orthospinus franciscencis* (10 males and 17 females) were collected in a small lake at the margins of the São Francisco River (Três Marias, MG, Brazil). The fish were identified and deposited in the Museu de Zoologia da Universidade de São Paulo (Zoology Museum of the São Paulo University) under the registrations MZUSP 86912; MZUSP

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86917 and MZUSP 86913, respectively. The mitotic chromosomes were obtained from kidney cells through the direct preparation technique, according to BERTOLLO *et al*. (1978). The analysis of the constitutive heterochromatin (C-banding) and the nucleolar organizing regions (Ag-NORs) followed the methodologies of SUMNER (1972) and HOWELL and BLACK (1980), respectively. The same metaphase was sequentially analyzed in conventional Giemsa staining, C-banding and silver nitrate staining.

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 two arms and the acrocentric chromosomes only one arm.

RESULTS

Piabina argentea presented a modal diploid number of 2n=52 chromosomes in males and females (8m+14sm+16st+14a; FN=90), with no morphological chromosome difference between the sexes (Figure 1a). The constitutive heterochromatin was detected in few chromosomes in the pericentromeric region (Figure 1b). The Ag-NORs were located in a telomeric position on the short arm of the acrocentric pair no. 20, coinciding with a heterochromatic region (Figure 1, box).

Hasemania nana presented a modal diploid number of 2n=50 chromosomes in males and females (8m+42sm, FN=100), with no morphological chromosome difference between the sexes (Figure 2a). The constitutive heterochromatin was detected in few chromosomes in the pericentromeric region (Figure 2b). The Ag-NORs were located in a terminal position on the short arm of the submetacentric pair no. 7, adjacent to a heterochromatic region (Figure 2, box).

Orthospinus franciscensis presented a modal diploid number of 2n=50 chromosomes in males and females (22m+20sm+2st+6a, FN=94), with no morphological chromosome difference between the sexes (Figure 3a). The constitutive heterochromatin was detected in few chromosomes in the centromeric and in the distal regions of the short arms of the submetacentric pair no. 19 and the subtelocentric pair no. 22 (Figure 3b). The Ag-NORs were also located in a distal position on the short arm of the subtelocentric pair no. 22, coinciding with a heterochromatic region (Figure 3, box).

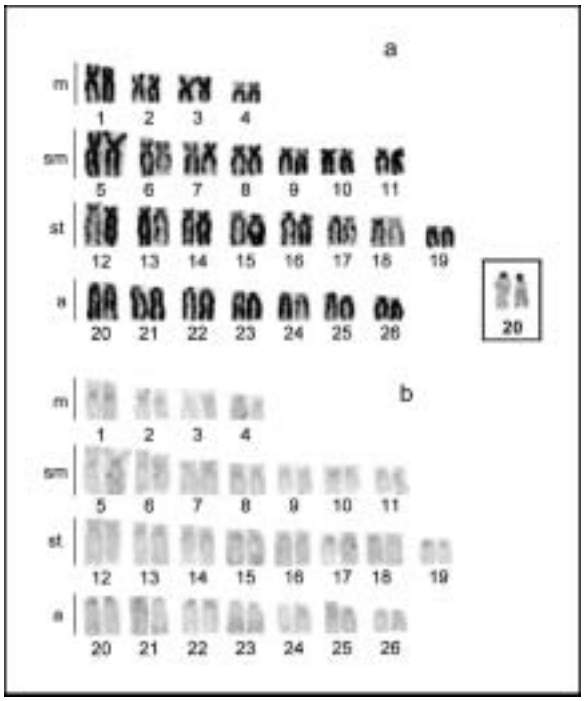

Fig. 1 — Sequential karyotype of *Piabina argentea:* (a) Conventional Giemsa staining, (b) C-banding, (box) Ag-NORs.

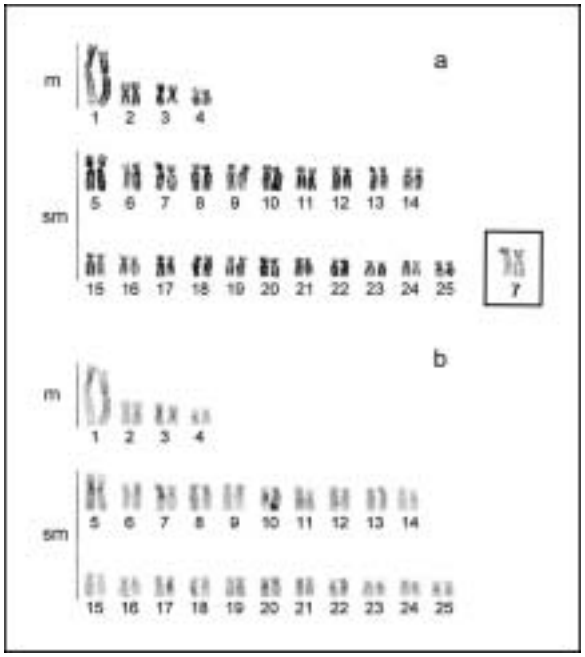

Fig. 2 — Sequential karyotype of *Hasemania nana:* (a) Conventional Giemsa staining, (b) C-banding, (box) Ag-NORs.

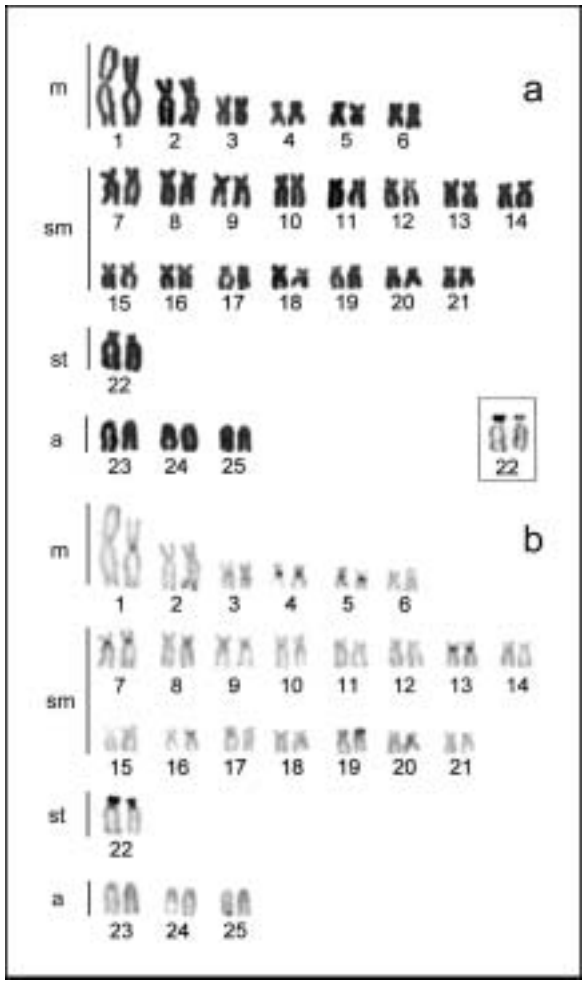

Fig. 3 — Sequential karyotype of *Orthospinus franciscensis:* (a) Conventional Giemsa staining, (b) C-banding, (box) Ag-NORs.

DISCUSSION

The diploid number in Characidae may vary from 2n=28, for a *Hemigramus* species (Schell 1973) to 2n=64, for *Serrasalmus* (Muramoto *et al.* 1968). Nevertheless, 2n=50 predominates, suggesting it is a plesiomorphic condition for this family (Oliveira *et al.* 1988; Portela *et al.* 1988), besides evidencing the role of non-Robertsonian rearrangements, especially pericentric inversions, in the chromosomal evolution of this group.

The diploid numbers 2n=50 chromosomes found in *H. nana* and *O. franciscensis*, and 2n=52 chromosomes found in *P. argentea*, agree with previous studies in these species (Arefjev 1990, cited as *H. marginata*; Pfister *et al.* 1997; Portela *et al.* 1988). However, their karyotypic structures were divergent, especially in *H. nana*. In this species 12m+18sm+10st+10a chromosomes were previously found (Arefjev, 1990), while 8m+42sm chromosomes were identified in our studies. These very distinct karyotypes may indicate the occurrence of two different *Hasemania* species. Unfortunately, we have no information about the region of origin for the specimens analyzed by Arefjev (1990), in his referred work. Otherwise, if we admit that the two samples correspond to the same species, *H. nana* a great deal of chromosome rearrangements, like pericentric inversions, have played a major role in their karyotypic differentiation. Although the karyotypes of *O. franciscensis* and *P. argentea* also showed some divergence from those described by Pfister *et al.* (1997) and Portela *et al.* (1988), respectively, the differences are more subtle for these species and may be attributed to the difficulty in characterizing with precision the morphology of some chromosomes, whose arm ratios are very close to the class limits established by Levan *et al.* (1964).

In this work, we described for the first time the C-band distribution in *P. argentea* and *H. nana.* In the three analyzed species, positive C-bands were observed mainly in a pericentromeric position on the chromosomes. Terminal positive bands were also observed in *O. franciscensis*, corroborating previous studies (Pfister *et al.* 1997). C-banding has been widely used in cytogenetical studies of fish (Almeida-Toledo *et al.* 1996; Margarido and Galetti Jr. 1999; Mantovani *et at*. 2004), and changes in the heterochromatin distribution seem to have played an important role in their chromosome diversification. *Astyanax scabripinnis* populations, for example, may be characterized by their heterochromatic distribution pattern (Moreira-Filho and Bertollo 1991). Some of these populations present large C-banded blocks in the terminal regions of subtelocentric and acrocentric chromosomes (Moreira-Filho and Bertollo 1991; Souza *et al.* 1996; Mantovani *et al.* 2000; Souza *et al.* 2001; Mantovani *et al.* 2004), which are not present in other populations.

The Ag-NOR sites located in the subtelocentric pair no. 22 of *O. franciscensis* and in the acrocentric pair no. 20 of *P. argentea* were also heterochromatic. In *H. nana*, however, the Ag-NOR was not heterochromatic but presented an adjacent heterochromatic region on the submetacentric pair no. 7. Among fish, a correspondence between Ag-NORs and positive C-band sites is a relatively common feature (Galetti Jr. and Rash 1993; Souza *et al.* 1996), with the presence of heterochromatin in/or adjacent to the Ag-NOR possibly facilitating chromosomal rearrangements related to these sites (Moreira-Filho *et al.* 1984).

Some Characidae subfamilies 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). Otherwise, other Characidae groups may present both single and multiple chromosomes bearing NORs, such as *Astyanax*, where some populations can present up to fifteen Ag-NORs (Rocon-Stange and Almeida-Toledo 1993), one of the largest numbers ever described in Characidae. Thus, the number and localization of Ag-NORs has exhibited a broad variability in this group. The three species here analyzed presented only a pair of Ag-NOR sites. Pfister *et al.* (1997) also found a single Ag-NOR pair in *O. franciscensis*, corroborating the present study. On the other hand, a distinct *P. argentea* population analyzed by Portela *et al.* (1988), presented up to four chromosomes with Ag-NORs. Nevertheless, as Ag-NORs indicate only the functionally active rDNA sites in the preceding interphase nuclei (Miller 1976), a complementary analysis using fluorescent *in situ* hybridization with a 18S rDNA probe is indicate for a conclusive NOR study.

Reis (1989), considered Stethaprioninae as a group related to Tetragonopterinae and Serrasalminae. The cytogenetic data suggest a greater proximity of Stethaprioninae to species previously grouped in Tetragonopterinae. In these two groups we may find species with a diploid number of 2n=50 chromosomes, a single Ag-NOR pair and a much larger first metacentric chromosome pair when compared to other chromosomes of the complement. On the other hand, the Serrasalminae species possess a diploid number varying from 2n=54 to 2n=64, multiple Ag-NORs and the absence of the first marker metacentric chromosome.

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