Cytogenetic Characterization and NOR Polymorphism in *Astyanax* sp. C (Tetragonopterinae, Characidae) from the First Plateau of the Iguaçu River (Paraná State, Brazil)

Daniel Luis Zanella Kantek¹, Alberto Sergio Fenocchio^{1,2} and Marta Margarete Cestari^{1,*}

¹ Universidade Federal do Paraná, Departamento de Genética, Laboratório de Citogenética Animal, 81531–990 Curitiba, PR, Brazil

² Universidad Nacional de Misiones, Departamento de Genética, Laboratório de Citogenética, 3300-Posadas, Misiones, Argentina

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Summary The characids belong to the greater freshwater fish family of the world with around 700 recognized species. Within this group the genus *Astyanax* includes several species with unclear phylogenetic relationships, showing a wide karyotypic diversity. In the highly endemic Iguaçu river basin these problems are also observed being distinguished 6 unnamed *Astyanax* species (A to F). In the present work were cytogenetically studied 2 populations of *A. sp. C* from the first plateau of the Iguaçu river (Paraná State, Brazil). Fishes of both populations are similar in chromosome number, having $2n=50$, however were evidenced clear differences in C-banding and nucleolar organizing regions (NORs) patterns. With the use of the restriction enzyme Alu I were obtained the same results as C-bands. These results indicate the complexity of *Astyanax* and the importance of further taxonomic revisions joining morphological, cytogenetic and molecular approachings.

Key words Fish cytogenetics, Neotropical region, Characiformes.

Fishes of the family Characidae (Pisces, Characiformes) show a great diversity in shape, size and ecological features, being the largest group of fresh water from South America, performing around of 170 genera and 885 species (Nelson 1994).

Previous cytogenetic studies have demonstrated that some subfamilies of Characidae show almost conservative karyotypes, as is the case of Salmininae (Marco 1986) and Bryconinae (Vascon *et al.* 1984), and others present variable or “plastic” karyotypes generally associated with several taxonomic problems, as occurs in Serrasalminae (Arefjev 1990a, Cestari and Galetti Jr. 1992), Cheirodontinae (Arefjev 1990b, Wasko and Galetti Jr. 1994) and Tetragonopterinae (Portela *et al.* 1988, Arefjev 1990b).

The genus *Astyanax* (Tetragonopterinae) have shown a wide karyotypic variability, either in chromosome number and structure, being reported from $2n=36$ in *A. schubarti* (Morelli *et al.* 1983) to $2n=50$ in other species (Kirby *et al.* 1977, Souza and Moreira-Filho 1995). Variations in chromosome number also has being identified within some species of *Astyanax*, i.e. *A. fasciatus* (Morelli *et al.* 1983, Justi 1993, Heras 1998) and *A. scabripinnis* (Moreira-Filho 1989, Moreira-Filho and Bertollo 1991).

The Iguaçu river constitute an interesting area for biological studies because is divided in several “plateaus” separated by waterfalls that represent barriers, constituting a practically isolated system with a restricted and almost endemic fish fauna. Taxonomic studies in *Astyanax* from Iguaçu

* Corresponding author, e-mail: margaces@bio.ufpr.br

river demonstrated that at least 6 different specific entities could be identified (Sampaio 1988), but only one have been named: *A. gymnogenys* (= *A. sp. A*), the others have named provisionally as *Astyanax sp. B, C, D, E* and *F*.

The objective of the present study was to characterize cytogenetically *Astyanax sp. C* from the first plateau of the Iguaçu river (Paraná State, Brazil) by means of conventional Giemsa staining and C, AgNOR and RE bandings and compare the results with other available data.

Materials and methods

38 individuals of *Astyanax sp. C* from the first plateau of the Iguaçu river (Paraná State, Brazil) were studied at 2 localities, São José dos Pinhais (13 males, 18 females, 1 specimen of indetermined sex) and Piraquara (4 males, 2 females).

Chromosome preparations were obtained by direct methods (Bertollo *et al.* 1978) and by short term cultures of kidney cells (Fenocchio *et al.* 1991). NORs and C bandings were visualized respectively by Howell and Black (1980) and Sumner (1972) techniques. Restriction enzyme (RE) banding were performed according Mezzanotte *et al.* (1983) with some modifications (Maistro 1996).

Results and discussion

The karyotype of *Astyanax sp. C* from the 2 populations are constituted by 2 pairs of metacentrics (M), 11 pairs of submetacentrics (SM), 4 pairs of subtelocentrics (ST) and 8 pairs of acrocentrics (A), summing $2n=50$ and arm number (NF)=84 (Fig. 1). As is characteristic in other Characidae and *Astyanax* species, 2 large metacentric chromosomes constitute the first pair. Karyotypes of males and females from São José dos Pinhais (similar that those of Piraquara) are shown in Fig. 2. Heteromorphisms related to sex were not detected.

Individuals of the 2 populations show a secondary constriction at NOR sites on the short arm of one of the chromosomes of the pair 16 (Figs. 2A, 3D, E).

In *Astyanax sp. C* from Piraquara only one NOR bearing chromosome pair were observed, the bands were detected on the short arm of the pair 16 (Fig. 4B). C bands were scarce and the most conspicuous ones are associated with the NORs.

Specimens from São José dos Pinhais had multiple NORs and frequently showed associations. For instance, an NOR association between 3 and 16 chromosomes, shown in the Fig. 3 (A, B). The number of NORs bearing chromosomes varied from 1 to 4 per metaphase plate (Fig. 4A). A total of 8 non-homologous chromosomes beared NORs (Fig. 4B–G). In the sample of São José dos Pinhais a SM chromosome showing a interstitial secondary constriction on the long arm were detected and it is also related to NORs (Figs. 3C, 4C).

Fig. 1. Frequencies of diploid numbers of the studied specimens from: A) São José dos Pinhais and B) Piraquara.

Fig. 2. Karyotypes of males (A) and females (B) of *Astyanax* sp. C from Sao José dos Pinhais.

Only chromosomes of the pair 16 always showed silver impregnation at their decondensed secondary constriction (Fig. 3E). The other silver positive bands had a high intra- and inter-individual variation, either in number and location. In some individuals a strong band was detected on the short arm of one of the homologues of the third chromosome pair (Figs. 3A, B, 4B).

The C-bands of *Astyanax* sp. C from Sao José dos Pinhais showed a wide polymorphic variation and were coincident with the NORs (Figs. 3D, E, 5A, B). Centromeric bands were pale and only apparent in decondensed chromosomes (Fig. 5B), also some telomeric bands on the acrocentric chromosomes (p arm) were detected.

When restriction enzymes are applied to several fish species the band pattern was generally like

Fig. 3. Metaphase plates of *A. sp. C* from São José dos Pinhais. A, B) sequential Giemsa-AgNO₃ staining showing a secondary constriction and chromosome association; C) Giemsa staining, the arrow-head indicates a secondary constriction related to NORs; D, E) sequential staining C-band and AgNO₃.

Fig. 4. *Astyanax sp. C*, AgNO₃ staining. A) Piraquara population, single NORs; B-G) São José dos Pinhais, multiple NORs; in the box, several NOR bearing chromosomes.

the C banding (Maistro *et al.* 1999, Swarça *et al.* 1999). The digestion with Alu I in the chromosome preparations from São José dos Pinhais showed a staining pattern almost similar to the C banding, with a strong Giemsa staining on the NORs secondary constrictions and heterochromatic regions (Fig. 5C).

In spite of the karyotypic diversity of the family Characidae, several species and populations of the genus *Astyanax* shared some basic cytogenetic traits *i.e.* the diploid number ($2n=50$) and a

Fig. 5. Metaphase plates of *Astyanax* sp. C from Sao José dos Pinhais. A, B) C-banding; C) Alu I digestion.

large first metacentric pair, as observed in *A. bimaculatus* (Scheel 1973, Paganelli and Galetti Jr. 1986), *A. taeniatus* (Rocon-Stange *et al.* 1986) and *A. eigenmanniorum* (Fauaz *et al.* 1994).

Similarly, *Astyanax* sp. C of the 2 populations studied here presents these features, but some differences could be pointed between them in relation to the number of NOR bearing chromosomes, only 1 pair in Piraquara and multiple NORs in Sao José dos Pinhais. Likewise, the C-bands were different among these populations, being polymorphic in Sao José dos Pinhais and monomorphic in the individuals from Piraquara, that showed a single C-band pattern.

The absence of conspicuous and larger heterochromatic blocks distinguishes *Astyanax* sp. C from other species of the genus and this trait would be considered a marker character.

These data represent the first contribution to the cytogenetic knowledge of the complex genus *Astyanax* in the Iguaçú river and further works joining diverse approaches (cytogenetic, molecular, morphologic) about the other related species (*A.* sp. A, B, D, E, F) would help to clarify their systematic relationships.

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