Vol.50, n. 1 : pp.67-74, January 2007
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

Cytotaxonomy, Heterochromatic Polymorphism and Natural Triploidy of a Species of *Astyanax* (Pisces, Characidae) Endemic to the Iguaçu River Basin

Daniel Luis Zanella Kantek[1]*, Rafael Bueno Noleto[1], Alberto Sérgio Fenocchio[2] and Marta Margarete Cestari[1]

[1]Laboratório de Citogenética Animal; Universidade Federal do Paraná; pastelson@yahoo.com; 81531-990; Curitiba - PR - Brasil.[2]Laboratório de Citogenética; Universidad Nacional de Misiones; 3300 Posadas - Misiones - Argentina

ABSTRACT

Cytogenetic analysis with Astyanax *sp. D revealed a karyotype of 2n=50 with 2M+26SM+6ST+16A, besides a triploid specimen showing 2n=75 chromosomes (3M+39SM+9ST+24A). C-banding strongly stained the terminal regions of several SM-ST-A chromossomes. Two pairs of acrocentric chromosomes presented interstitial heterochromatin, this state being polymorphic and occuring due to possible paracentric inversions. The results obtained with the* Alu*I restriction enzyme and A_3 chromomycin were similar to the C-banding. Relationships were proposed between* Astyanax *sp. D and* A. scabripinnis*, as well as considerations for a possible origin of the triploid specimen (2n=3x=75). When comparing the present results with cytogenetic features of other endemic* Astyanax *species in the Iguaçu river (*A. *sp. B and C), a clear differentiation was observed between them, indicating cytogenetics as an important cytotaxonomic tool.*

Key words: Cytogenetics; triploidy; paracentric inversion

INTRODUCTION

The genus *Astyanax* has received special attention due to its biological and cytogenetic characteristics, with cases where different populations of the same species demonstrate cytogenetic and morphological differences. This genus shows a broad karyotypic variability, being observed from 2n=36 in *A. schubarti* (Morelli et al.,1983; Daniel-Silva and Almeida-Toledo, 2001) to 2n=50 in other species of the genus (Souza and Moreira-Filho, 1995). Variations in the diploid number have been detected within single *Astyanax* species, as is the case of *A. fasciatus* (Morelli et al., 1983; Justi, 1993) and *A. scabripinnis* (Moreira-Filho and Bertollo, 1991; Souza and Moreira-Filho, 1995; Néo et al., 2000; Alves and Martins-Santos, 2002).

According to Sampaio (1988), the Iguaçu river has at least six *Astyanax* species. These species received the denominations of *Astyanax* sp.A, B, C, D, E, F. Althugh they lack a valid scientific name, these species are considered as real taxonomic entities in the literature concerning the Iguaçu river (Severi and Cordeiro 1994; Agostinho and Gomes 1997; Kantek et al., 2003; Ingenito et al., 2004). Garutti and Britski (2000) described the ocurrence of *Astyanax altiparanae* in the river. The objective of this study was to cytogenetically characterize *Astyanax* sp.D from the first plateau

* Autor for corresponce

of the Iguaçu river (Paraná, Brasil), comparing with other taxa and reporting one more case of natural triploidy.

MATERIAL AND METHODS

Seven *Astyanax* sp. D specimens (5 males and 2 females) from the first plateau of the Iguaçu river, in the township of Piraquara (PR), were studied. The mitotic metaphases were obtained by the indirect method described by Fenocchio et al. (1991). C-banding was performed as described by Sumner (1972), while chromomycin A_3 (CMA_3) and the restriction endonuclease *Alu*I assays were done according to Schmid (1980) and Mezzanote et al. (1983), respectively.

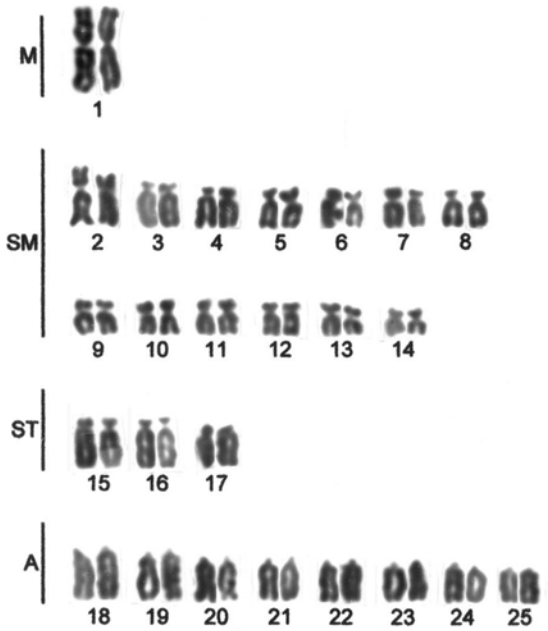

Figure 1 - Giemsa-stained karyotype of *Astyanax* sp. D.

RESULTS

The studied fish had a modal diploid number of 50 chromosomes, comprising 2 metacentric (M), 26 submetacentric (SM), 6 subtelocentric (ST) and 16 acrocentric (A) chromosomes, adding up to the fundamental number (FN) of 84 (Fig. 1). Heteromorphic sex chromosomes were not detected. The constitutive heterochromatin was detected (Fig. 2A, 3A) on weak little centromeric C^+ marks in some chromosomes and on large interstitial and terminal blocks located on the long arms of all ST chromosomes, on several A chromosomes and some SM chromosomes. Pairs 18 and 19 showed interindividual C-banding variations in the following situations: (1) both homologues with C^+ interstitial blocks; (2) one of the chromosomes with the C^+ terminal block and its homologue with an interstitial mark; (3) both homologues with C^+ terminal blocks (Fig. 2B). It was possible to observe a size heteromorphism of the heterochromatic blocks between homologues (Fig. 2A-pair 21), as well as heterochromatic marks that were located at a short distance above the telomere (Fig. 2A-pair 5). The number of C-bands was also variable. Figure 2A shows only seven A chromosomes bearing such regions, while on Figure 3A it is possible to visualize ten A chromosomes with C-bands. The *Alu*I^+ pattern was identical to the C-banding distribution (Fig. 3A,B). A triploid specimen was also identified, with 2n=3x=75 chromosomes: 3M+39SM+9ST+24A (Fig. 4A). Its karyotype showed the same features observed for the diploid specimens. C-banding, *Alu*I and CMA_3 data showed that triads 18 and 19 were formed by two identical chromosomes plus a third distinct chromosome (Fig. 4B, 5A,B).

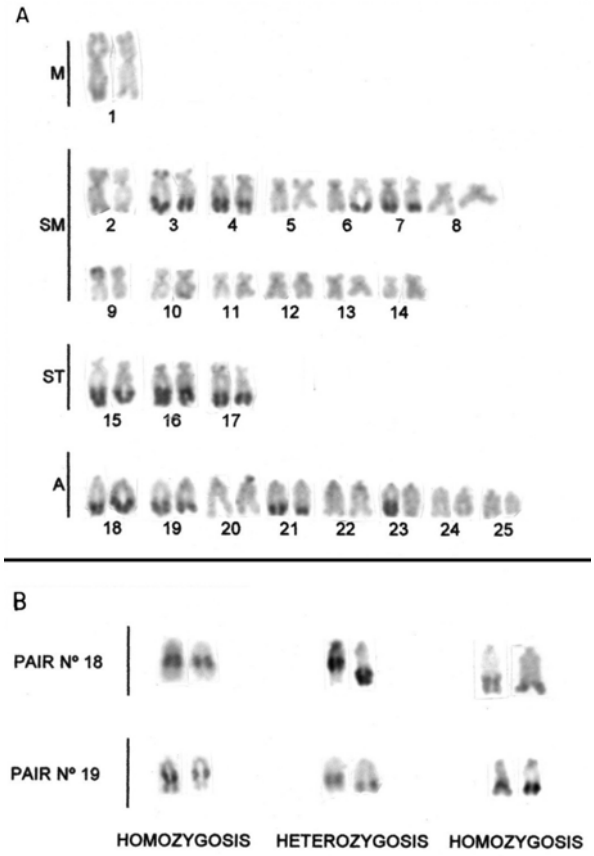

Figure 2 - A) C-banded karyotype of *Astyanax* sp. D; B) Chromosome pairs 18 and 19 showing heterochromatic blocks in heterozygosis and homozygosis.

DISCUSSION

The diploid number of 2n=50 for *Astyanax* sp. D did not differ from those of most species of this genus, including *A. scabripinnis* (Moreira-Filho and Bertollo 1991; Maistro et al., 1998; Souza et al., 1996), *Astyanax* sp.C (Kantek et al., 2003), *Astyanax* sp.B (Fazoli et al., 2003) and *A. altiparanae* (Cenci and Margarido, 2003; Kantek et al., 2004). The C-banding pattern could be considered equilocal on the chromosomes. A possible explanation for this pattern would be based on the model proposed by Schweizer and Loidl (1987), which disserted on the orientation of the chromosomes during the meiotic interphase (Rabl orientation), allowing the transference of heterochromatin between equidistant positions of non-homologue chromosomes. The similarity between the C^+ blocks, also demonstrated by the *Alu*I and CMA_3 techniques, supported this hypothesis.

When comparing patterns of *Astyanax.* sp.D, some *A. scabripinnis* populations (Mantovani et al., 2000; Maistro et al., 2001) and *A. janeiroensis* (Carvalho et al., 2002) showed a similar C-banding pattern. These species presented large terminal heterochromatic blocks located on the long arm of the ST and A chromosomes. The pattern obtained with *Alu*I was also similar for *Astyanax* sp. D and *A. scabripinnis* from the Cascatinha river (Maistro et al., 2001). However, other populations differed in C-band distribution (Maistro et al., 1998; Moreira-Filho and Bertolo, 1991), the same occurring with *Astyanax* sp. B. (Fazoli et al., 2003), *Astyanax.* sp.C (Kantek et al., 2003) and *A. altiparanae* (Cenci and Margarido, 2003; Kantek et al., 2004) from the Iguaçu river, which had less heterochromatin than *Astyanax.* sp. D.

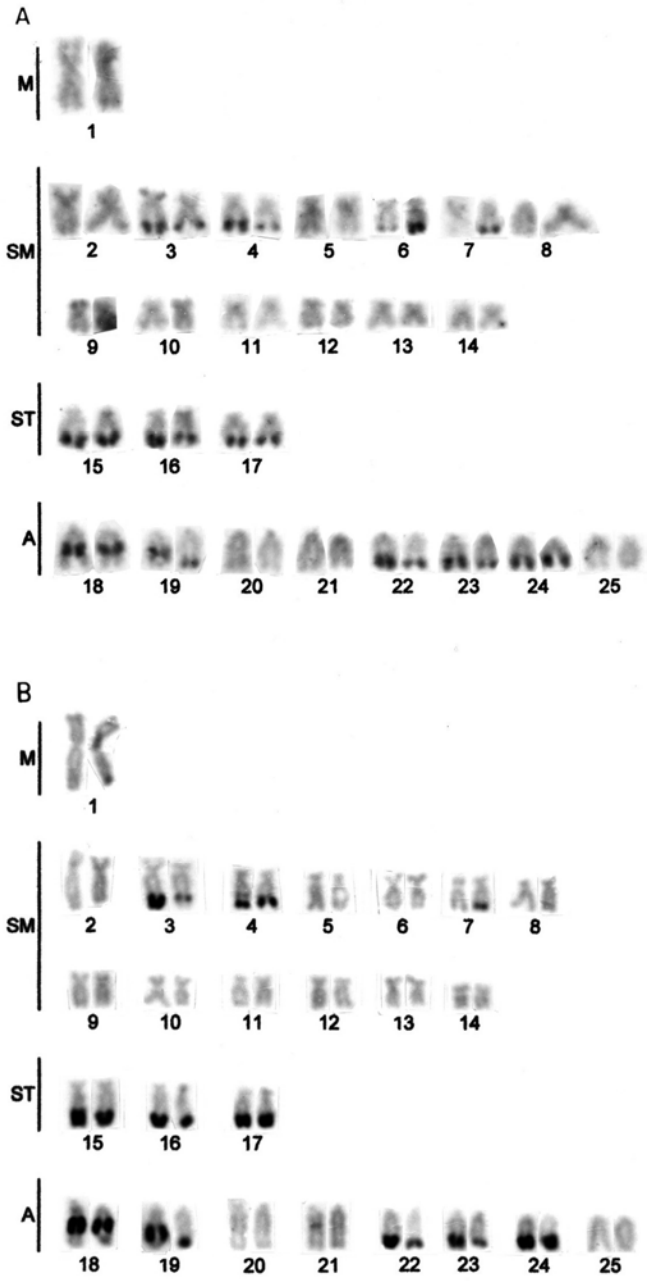

Figure 3 - Differential staining on only one specimen of *Astyanax* sp. D. A) C-banded karyotype; B) Restriction enzyme *Alu*I-treated karyotype.

The C-band polymorphism on the 18th and 19th chromosome pairs of *Astyanax* sp.D seemed to be related to paracentric inversions. Such polymorphism was not detected in other *Astyanax* species thus far. *A. janeiroensis* (Carvalho et al., 2002) also showed interstitial C^+ blocks, but without a polymorphic condition.

CMA_3 staining showed that C^+ regions of *Astyanax* sp. D were GC-rich, as occured in *A. scabripinnis* (Souza et al., 1996; Daniel-Silva, 1996). The differences in the amount of heterochromatin between homologues were also registered for *A. scabripinnis* (Mantovani et al., 2000; Maistro et al., 2000).

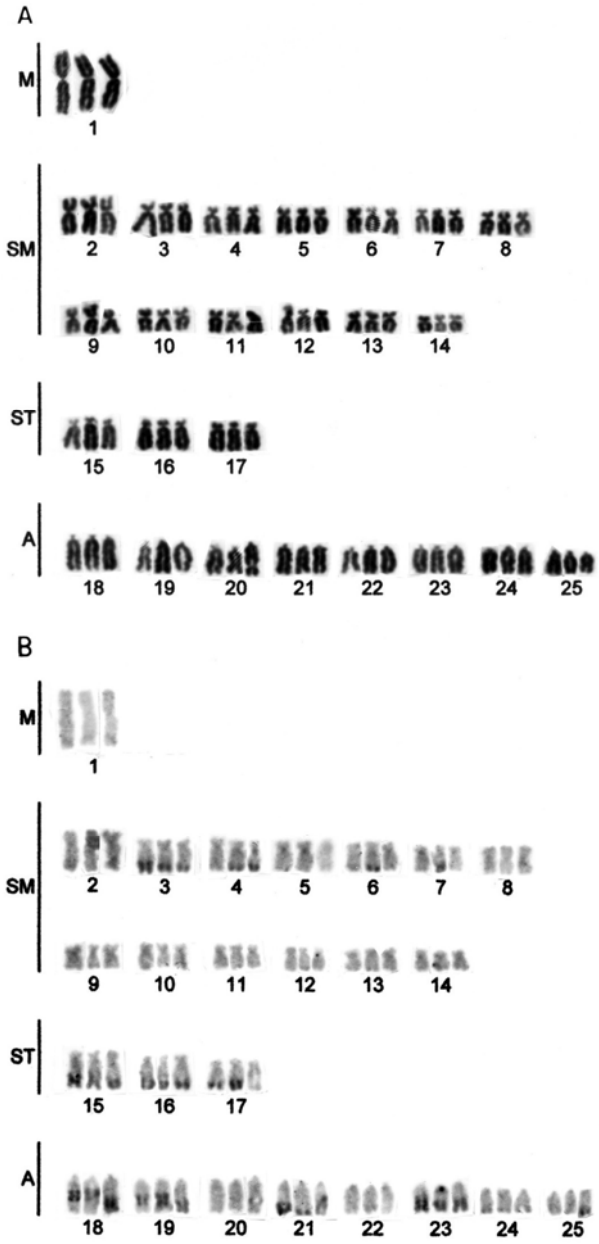

Figure 4 - A triploid *Astyanax* sp. D specimen. A) Giemsa-stained karyotype; B) C-banded karyotype

The identification key for *Astyanax* species elaborated by Eigenmann (1921) identified *Astyanax* sp. D as *A. scabripinnis paranae*. However, Sampaio (1988), through morphological measurements, considered these two fish forms as distinct species. The current diagnosis for *A. scabripinnis* (Eigenmann, 1921) was considered to be little specific as it joined many forms of *Astyanax* that inhabited headwaters, making it not informative for the taxonomy of this group. The high diversity recognized for this taxon would be a reflex of this flaw on its taxonomy. Many populations identified before as *A. scabripinnis* subspecies could now be considered new species. Future analysis would result in the recognition of other taxa (Bertaco and Malabarba, 2001).

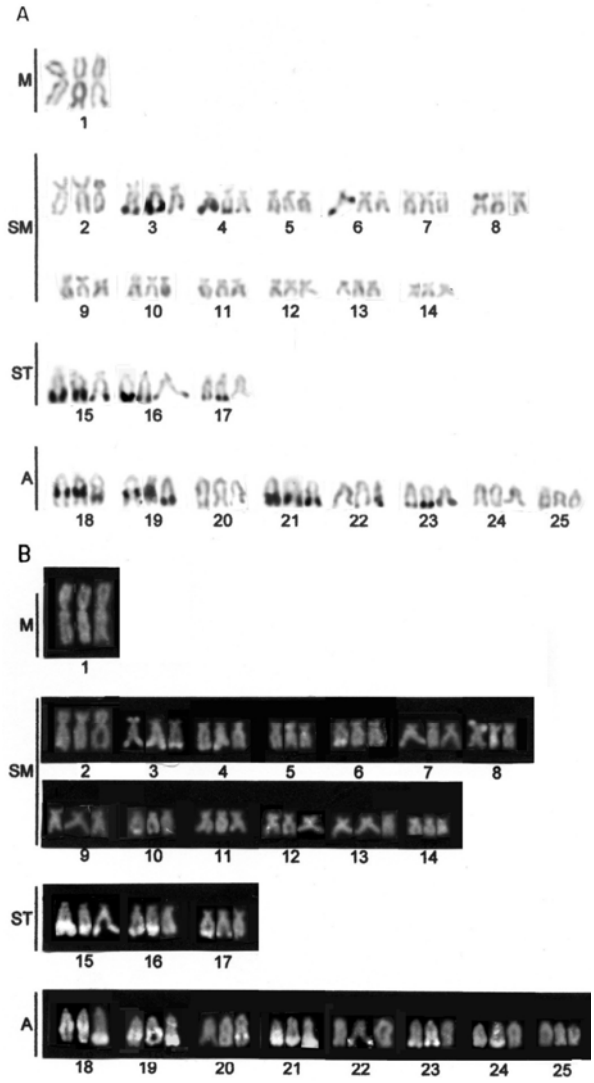

Figure 5 - *Astyanax* sp. D. triploid A) Restriction enzyme *Alu*I-treated karyotype; B) CMA_3-stained karyotype.

Astyanax, as well as most fishes, exhibited external fertilization, with the oocyte finishing the second mitotic division in the external medium (water), resulting in a non-disjunction of the sister chromatids (II meiosis) in adverse environment conditions (low temperatures, for example), producing one oocyte with 2n chromosomes (Fauaz et al., 1994).

Electrophoretic analysis of hybrids from the crossing of two carp species demonstrated that triploid individuals possesed two maternal alleles and only paternal allele (Tsukomo and Rigolino, 1993), supporting the hypothesis that the natural triploids came through a diploid egg x haploid sperm fertilization.

The identification of two identical chromosomes and one differentiated on the 18^{th} and 19^{th} triads of the triploid specimen (Figs. 4, 5) corroborated this hypothesis, which had been suggested for other fish species (Morelli et al., 1983; Almeida-Toledo et al., 1985; Giuliano-Caetano and Bertollo, 1990; Maistro et al., 1994). However, the alternative hypothesis, haploid egg x diploid sperm fertilization, could not be ruled out in the origin of the triploidy.

These results supported the proximity of the *A.* sp. D and *A. scabripinnis* groups. However presently,

it would not be possible to differentiate between equal or distinct biological units. When confronting *Astyanax* sp. D with *Astyanax* sp. C (Kantek et al., 2003), *Astyanax* sp. B (Fazoli et al., 2003) and *Astyanax altiparanae* (Cenci and Margarido, 2003; Kantek et al., 2004) from the Iguaçu river, these species were clearly distinguishable by their cytogenetic features, showing the probable lack of gene flow between them as well as the importance of karyotypic data for the taxonomy of this fish group.

ACKNOWLEDGMENTS

We thank Vinicius Abilhoa from the Museu de Historia Natural Capão da Imbuia (Curitiba-PR) for the sample identification and Gil Caveira for translation of this paper. Financial support was provided by CNPq (National Council for Scientific and Technologic Development).

RESUMO

Análises citogenéticas em *Astyanax* sp. D evidenciaram 2n=50 cromossomos e um cariótipo com 2M+26SM+6ST+16A. O bandamento C destacou as regiões teloméricas de diversos cromossomos SM-ST-A. Dois pares de cromossomos acrocêntricos possuem heterocromatina intersticial, sendo este estado polimórfico decorrente de prováveis inversões paracêntricas. O resultados obtidos com a enzima de restrição *Alu*I e a Cromomicina A_3 foram semelhantes aos do bandamento C. São propostas relações de parentesco entre *Astyanax* sp. D e *Astyanax scabripinnis*, bem como considerações sobre a possível origem do exemplar triplóide (2n=3x=75). Ao comparar os resultados deste trabalho com outras espécies de *Astyanax* do Rio Iguaçu, estas espécies se tornam claramente distinguíveis, evidenciando a citogenética como uma importante ferramenta taxonômica.

REFERENCES

Agostinho, A.A. and Gomes, L. C. (1997), Reservatório de Segredo: bases ecológicas para o manejo. Eduem, Maringá, Brasil.

Almeida-Toledo, L.F.; Foresti, F. and Toledo-Filho, S.A. (1985), Spontaneous triploidy and NOR activity in *Eigenmannia* sp. (Pisces, Sternopygidae) from de Amazon basin. *Genetica*, **66**, 85-88.

Alves, A.L. and Martins-Santos, I.C. (2002), Cytogenetics studies in two populations of *Astyanax scabripinnis* with 2n=48 chromosomes (Teleostei, Characidae). *Cytologia*, **67**, 117-122.

Bertaco, V. A. and Malabarba, L.R. (2001), Description of two new especies of *Astyanax* (Teleostei, Characidae) from headwater streams of Southern Brazil, with comments on the "*A. scabripinnis* species complex". *Ichthyological Exploration of Freshwaters*, **12**, 221-234.

Carvalho, M.L.; Oliveira, C. and Foresti, F. (2002), Cytogenetic analysis of five species of the subfamily Tetragonopterinae (Teleostei, Characiformes, Characidae). *Caryologia*, **55**, 181-188.

Cenci, M.A. and Margarido, V.P. (1999), Estudos citogenéticos comparativos de duas populações de *Astyanax bimaculatus* (PISCES, CHARACIDAE, TETRAGONOPTERINAE) coletados nas bacias dos rios Iguaçu e Piquiri. In: Anais do 45 Congresso Nacional de Genética, Gramado, Brasil.

Daniel-Silva, M.F.Z. (1996), Estudos citogenéticos comparativos em quatro espécies do gênero *Astyanax* (Pisces, Characidae). Masters Thesis. Universidade de São Paulo, SP, Brasil.

Daniel-Silva, M.F.Z. and Almeida-Toledo, L.F (2001), Chromosome R-banding pattern and conservation of a marker chromosome in four species, genus *Astyanax* (Characidade, Tetragonopterinae). *Caryologia*, **54**, 209-215.

Eigenmann, C.H. (1921), The American Characidae. *Memoirs of the Museum of Comparative Zoology*, **43**, 209-310.

Fauaz, G.; Vicente, V. and Moreira-Filho, O. (1994), Natural triploidy and B chromosomes in the neotropical fish genus *Astyanax* (CHARACIDAE). *Revista Brasileira de Genética*, **17**, 157-163.

Fazoli, L.C.; Silva, V.A.B.; Portela-Castro, A.L.B and Júlio Jr, H.F. (2003), Chromosome characterization of *Astyanax* sp B (Characidae, Tetragonopterinae) an endemic species of the Iguaçu River, Paraná, Brazil. *Cytologia*, **68**, 389-394.

Fenocchio, A.S.; Venere, P.C.; Cesar, A.C.G.; Dias, A.L. and Bertollo, L.A.C. (1991), Short term culture from solid tissues of fishes. *Caryologia*, **44**, 161-166.

Garutti, V. and Britski, H.A. (2000), Descrição de uma nova espécie de *Astyanax* (Telestei: Characidae) da bacia do alto rio Paraná e considerações gerais sobre as demais espécies do gênero na bacia. *Comum. Museu de Ciência e Tecnologia PUCRS Série Zoologia*, **13**, 65-88.

Giulino-Caetano, L. and Bertollo, L.A.C. (1990), Karyotypic variability in *Hoplerythrinus unitaeniatus* (Pisces, Characiformes). II. Ocurrence of natural triploidy. *Revista Brasileira de Genética*, **13**, 231-237.

Ingenito, L.F.S.; Duboc, L.F. and Abilhoa V. (2004), Contribuição ao conhecimento da ictiofauna da bacia do alto rio Iguaçu, Paraná, Brasil. *Arq. Ciênc. Vet. UNIPAR*, **7,** 23-26.

Justi, A.J. (1993), Caracterização cariotípica de populações de *Astyanax fasciatus* (Cuvier, 1819), Pisces, Characidae, em três bacias hidrográficas. Masters Thesis. Universidade Federal de São Carlos, São Paulo, Brasil.

Kantek, D.L.Z.; Fenocchio, A.S. and Cestari, M.M. (2003), Cytogenetic characterization and NOR polymorphism in *Astyanax* sp. C (Tetragonopterinae, Characidae) from First Plateau of Iguaçu River (Paraná State, Brazil). *Cytologia*, **68**, 19-24.

Kantek, D.L.Z.; Domingues, M.S.; Artoni, R.F.; Noleto, R.B.; Cipriano, R.R.; Cestari M.M. (2004), Caracterização citogenética de *Astyanax altiparanae* (PISCES, TETRAGONOPTERINAE) da lagoa da fazenda Canguiri, bacia do alto rio Iguaçu (PR). In: Anais do X Simpósio de Citogenética e Genética de Peixes, Natal, Brasil.

Maistro, E.L.; Dias, A.L.; Foresti, F.; Oliveira, C. and Moreira, O. (1994), Natural triploidy in *Astyanqx scabripinnis* (Pisces, Characidae) and simultaneous ocurrence of macro B-chromosomes. *Caryologia*, **47**, 233-239.

Maistro, E.L.; Oliveira, C. and Foresti, F. (1998), Comparative cytogenetic and morphological analysis of *Astyanax scabripinnis paranae* (Pisces, Characidae, Tetragonopterinae). *Genetics and Molecular Biology*, **21**, 201-206.

Maistro, E.L.; Oliveira, C. and Foresti, F. (2000), Sympatric occurrence of two cytotypes of *Astyanax scabripinnis* (Characiformes, Characidae). *Genetics and Molecular Biology*, **23**, 356-369.

Maistro, E.L.; Oliveira, C. and Foresti, F. (2001), Cytogenetic characterization of a supernumerary chromosome segment and of B-chromosomes in *Astyanax scabripinnis* (Teleostei, Characidae). *Genetica*, **110**, 177-183.

Mantovani, M.; Abel, L.D.S.; Mestriner, C.A. and Moreira-Filho, O. (2000), Accentued polymorphism of heterochromatin and nucleolar organizer regions in *Astyanax scabripinnis* (Pisces, Characidae): tools for understanding karyotypic evolution. *Genetica*, **109**, 161-168.

Mezzanote, R.; Bianchi,U.; Vanni, R. and Ferruci, L. (1983), Chromatin organization and restriction endonuclease activity on human metaphase chromosomes. *Cytogenetics and Cell Genetics*, **36**, 562-566.

Moreira-Filho, O. and Bertollo, L.A.C. (1991), *Astyanax scabripinnis* (Pisces, Characidae): a species complex. *Revista Brasileira de Genética*, **14**, 331-357.

Morelli, S.; Bertollo, L.A.C.; Foresti, F.; Moreira-Filho, O. and Toledo-Filho, A.S. (1983), Cytogenetic considerations on the genus *Astyanax* (Pisces, Characidae). I. Kariotypic variability. *Caryologia*, **36**, 235-244.

Néo, D.M.; Moreira-Filho, O. and Camacho, J.P.M. (2000), Altitudinal variation for B chromosome frequency in the characid fish *Astyanax scabripinnis. Heredity*, **85**, 136-141.

Sampaio, F.A.A. (1988), Estudos taxonômicos preliminares dos Characiformes (Teleostei, Ostariophysi) da Bacia do Rio Iguaçu, com comentários sobre o endemismo desta fauna. Masters Thesis. Universidade Federal de São Carlos, São Paulo, Brasil.

Schmid, M. (1980), Chromosome banding in Amphibia. IV. Diferentiation of GC- and AT-rich chromosome region in Anura. *Chromosoma*, **77**, 83-103.

Schweizer, D. and Loidl, J. (1987), A model for heterochromatin dispersion and the evolution of C-band patterns. *Chromosomes Today*, **9**, 61-74.

Severi, W. and Cordeiro, A.A.M. (1994), Catálogo de Peixes da Bacia do Rio Iguaçu. IAP/GTZ, Curitiba, Paraná, Brasil.

Souza, I.L. and Moreira-Filho, O. (1995), Cytogenetic diversity in the *Astyanax scabripinnis* species complex (Pisces, Characidade). I. Allopatric distribution in a small stream. *Cytologia***, 60**, 1-11.

Souza, I.L.; Moreira-Filho, O. and Galetti Jr, P.M. (1996), Heterochromatin differentiation in the characid fish *Astyanax scabripinnis*. *Brazilian Journal of Genetics*, **19**, 405-410.

Sumner, A.T. (1972), A simple technique for demonstrating centromeric heterochromatin. *Experimental Cell Research*, **75**, 304-306.

Tsukamoto, R.Y. and Rigolino, M.G. (1993), Aplicação da biotecnologia à aqüicultura. In: Anais do X Encontro Brasileiro de Ictiologia, São Paulo, Brasil.

Received: March 11, 2005;
Revised: October 03, 2005;
Accepted: August 31, 2006.