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Nuclear DNA content determination in Characiformes fish (Teleostei, Ostariophysi) from the Neotropical region

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Abstract

In the present study, nuclear DNA content was analyzed in 53 species of Characiformes fish from the Neotropical region. Diploid number ranged from 2n = 48 in *Astyanax fasciatus*, *Gymnocorymbus ternetzi* and *Hyphessobrycon griemi* to 2n = 102 in *Potamorhina squamoralevis*, with a modal number of 54 chromosomes. Nuclear DNA content ranged from 1.70 ± 0.04 pg of DNA per diploid nucleus in *Acestrorhynchus pantaneiro* to 3.94 ± 0.09 pg in *Tetragonopterus chalceus*. A general analysis showed a mean value of 2.9 pg of DNA per diploid nucleus. Very similar DNA content values were observed in the species of the family Cynodontidae which showed a variation of 3% between the two genera studied. Small variations were observed between populations of *Gymnocorymbus ternetzi*, Astyanax fasciatus and *Moenkhausia sanctaefilomenae* (Characidae, Tetragonopterinae). The subfamilies Tetragonopterinae and Acestrorhynchinae (Characidae) presented the widest range, about 96%. Even in those families in which diploid number and karyotypic formulae were conserved such as the families Anostomidae, Curimatidae, and Prochilodontidae, episodes leading to losses or gains of genetic material became fixed in their evolutionary history.

Key words: nuclear DNA, DNA content, chromosome, fish, evolution.

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Introduction

Studies of nuclear DNA content in living organisms in general and in fish in particular have been conducted using Feulgen-stained blood smears and microdensitometry analysis (Hinegardner and Rosen, 1972; Gold and Price, 1985; Majumdar and McAndrew, 1986; Gold and Amemiya, 1987; Oliveira *et al.*, 1992, 1993a and 1993b; Carvalho *et al.*, 1998), or blood cell samples in suspension, stained with base-specific fluorochromes and analyzed by flow cytometry (Thorgaard *et al.*, 1982; Johnson *et al.*, 1987; Tiersch and Chandler, 1989; Tiersch *et al.*, 1989a, 1989b and 1990).

Among vertebrates, DNA content has been observed to range from 0.78 to 280.00 pg per diploid nucleus (Olmo *et al.*, 1989). Among fishes, DNA content ranges from 0.78 pg per diploid nucleus in *Tetraodon fluviatis* (Hinegardner and Rosen, 1972) to 248.00 pg in *Lepidosiren paradoxa* (Ohno and Atkin, 1966). The data obtained by Hinegardner and Rosen (1972) on 275 teleostei showed that there is a clear modal value of about 2.0 pg of DNA per diploid nucleus. However, differences greater than two times may be found among specimens of the same family or even of the same genus, as is the case of cyprinids of the genus *Barbus* (Ohno *et al.*, 1967; Wolf *et al.*, 1969) and species of the genus *Corydoras* (Hinegardner and Rosen 1972; Oliveira *et al.*, 1992). Although some authors have suggested that this variation may be related to the number of genes in the organisms or to the complexity of their development (Kauffman, 1971 cited by Cavalier-Smith, 1978), many agree that there is no significant correlation between the amount of nuclear DNA and organic or genetic complexity (Cavalier-Smith, 1978). Thus, the analysis of nuclear DNA content poses a problem for evolutionary genetics regarding the interpretation of this quantitative variation in genome size or in DNA amount (Gold and Price, 1985).

The huge diversity of nuclear DNA content observed among fish led Ohno (1974) to suggest that comparative studies of the karyotype of different fish groups would

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make no sense if they were not followed by information regarding variation in genome size. Of about 900 species of Characiformes that live in the rivers and lakes of the Neotropical region, only 41 had their nuclear DNA content determined (Carvalho *et al.*, 1998). The main objective of the present study was to obtain nuclear DNA content data among representatives of the families and subfamilies of Characiformes that occur in the Neotropical region and provide new information for a better understanding of the process of chromosome evolution in this group.

Material and Methods

In the present study, 53 fish species were collected in Brazil from the Miranda river (Corumbá, Mato Grosso do Sul), Acre river and São Francisco stream (Rio Branco, Acre), São Francisco river (Três Marias, Minas Gerais) and Itimirim river (Iguape, São Paulo). Taxonomic status, collection site, number and sex of the specimens analyzed are presented in Table I. After processing, all specimens were fixed and kept at the fish collection of the Laboratório de Biologia de Peixes, Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil.

Mitotic chromosome preparations were obtained from kidney and gill cells using the air-drying technique described by Foresti *et al.* (1993). Individual relative DNA content was determined according to the technique described by Gold and Price (1985) with some minor modifications. Blood was collected by caudal puncture and

Table I - Diploid nuclear DNA content values, in picograms (pg), observed in the Characiformes species analyzed.

Species	Collection sites	Fishes analyzed M/F/?	2n	Nuclear DNA content (pg)
ANOSTOMIDAE				
Leporinus elongatus	São Francisco river	1/3/0	54	2.94 ± 0.11
Leporinus piau	São Francisco river	4/0/0	54	2.89 ± 0.07
Leporinus reinhardti	São Francisco river	3/3/0	54	3.05 ± 0.06
Leporinus sp.	São Francisco river	1/1/0	-	3.02 ± 0.05
Schizodon borellii	Miranda river	3/3/0	-	2.97 ± 0.24
Schizodon sp.	Acre river	1/0/0	-	3.68 ± 0.06
CHARACIDAE				
ACESTRORHYNCHINAE				
Acestrorhynchus pantaneiro	Miranda river	1/2/0	-	1.70 ± 0.04
Acestrorhynchus sp.	Acre river	1/0/0	-	3.10 ± 0.06
Oligosarcus hepsetus	Itimirim river	1/0/0	50	3.33 ± 0.06
APHYOCHARACINAE				
Aphyocharax anisitsi	Miranda river	1/4/0	-	2.66 ± 0.08
Aphyocharax dentatus	Miranda river	1/3/0	-	2.45 ± 0.19
BRYCONINAE				
Brycon microlepis	Miranda river	1/2/0	50	2.40 ± 0.04
CHARACINAE				
Charax leticiae	Miranda river	0/1/0	-	2.88 ± 0.06
Roeboides bonariensis	Miranda river	0/1/0	52	2.18 ± 0.09
Roeboides prognathus	Miranda river	1/0/0	-	3.07 ± 0.04
GLANDULOCAUDINAE				
Mimagoniates microlepis	Itimirim river	8/2/0	52	3.06 ± 0.14
Pseudocorynopoma heterandria	Itimirim river	2/0/0	-	2.52 ± 0.09
IGUANODECTINAE				
Piabucus melanostomus	Miranda river	2/3/0	-	2.39 ± 0.04
SERRASALMINAE				
Serrasalmus brandti	São Francisco river	4/1/0	60	3.28 ± 0.06
Mylossoma paraguayensis	Miranda river	1/1/0	54	2.91 ± 0.09

Table I. (cont)

Serrasalmus spilopleura	Miranda river	0/1/0	-	3.15 ± 0.09
Serrasalmus sp.	Acre river	1/0/0	-	3.58 ± 0.06
STETAPRIONINAE				
Brachychalcinus copei	Acre river	1/1/0	-	3.47 ± 0.03
Poptella paraguayensis	Miranda river	4/0/0	-	3.47 ± 0.16
Species	Collection sites	Fishes analyzed M/F/?	2n	Nuclear DNA content (pg)
TETRAGONOPTERINAE				
Astyanax abramis	Miranda river	2/2/0	50	3.23 ± 0.09
Astyanax asuncionensis	Miranda river	1/4/0	50	2.38 ± 0.08
Astyanax bimaculatus lacustris	São Francisco river	2/0/0	50	2.94 ± 0.12
Astyanax fasciatus	São Francisco river	1/2/0	48	2.75 ± 0.10
Astyanax cf. *ribeirae*	Itimirim river	6/1/0	-	3.28 ± 0.11
Creatocanus affinis	São Francisco river	1/1/0	-	2.20 ± 0.04
Gymnocorymbus ternetzi	Miranda river	2/3/0	48	3.33 ± 0.16
Hyphessobrycon griemi	Itimirim river	1/6/0	48	2.56 ± 0.27
Hyphessobrycon reticulatus	Itimirim river	0/1/1	50	2.29 ± 0.09
Markiana nigripinnis	Miranda river	1/0/0	52	2.16 ± 0.03
Moenkhausia dichroura	Miranda river	4/1/0	-	2.01 ± 0.16
Moenkhausia sanctaefilomenae	Miranda river	4/0/0	-	2.39 ± 0.09
Tetragonopterus argenteus	Miranda river	2/3/0	-	2.99 ± 0.21
Tetragonopterus chalceus	São Francisco river	2/3/0	52	3.94 ± 0.09
TRIPORTHEINAE				
Triportheus paranaensis	Miranda river	3/1/0	-	2.67 ± 0.10
Triportheus pictus	Acre river	0/1/0	-	3.46 ± 0.06
CURIMATIDAE				
Curimata elegans	São Francisco river	6/1/0	54	3.45 ± 0.34
Curimata vittata	Acre river	2/0/0	-	2.98 ± 0.30
Curimatella dorsalis	Miranda river	2/1/0	54	2.83 ± 0.26
Cyphocharax gilberti	Itimirim river	1/1/0	54	3.31±0.12
Potamorhina squamoralevis	Miranda river	4/1/0	102	3.80 ± 0.23
Steindachnerina guentheri	Acre river	6/6/0	54	3.24 ± 0.14
CYNODONTIDAE				
Hydrolycus scomberoides	Acre river	0/1/3	-	2.09 ± 0.28
Rhaphyodon vulpinus	Acre river	0/2/0	-	2.02 ± 0.06
GASTEROPELECIDAE				
Thoracocharax cf. *stellatus*	Acre river	0/1/0	-	2.57 ± 0.10
Thoracocharax cf. *stellatus*	São Francisco stream	2/3/0	52	2.18 ± 0.09
PARODONTIDAE				
Apareiodon affinis	Miranda river	1/1/0	-	2.04 ± 0.12
PROCHILODONTIDAE				
Prochilodus affinis	São Francisco river	1/3/0	54	3.12 ± 0.09
Prochilodus marggravii	São Francisco river	3/1/0	54	3.08 ± 0.07
Semaprochilodus binotatus	Acre river	1/1/0	-	3.72 ± 0.05

M - Male; F - Female; ? Not sexed.

smeared near the frosted end on each of three slides. Blood smears from chicken, common carp, and rainbow trout served as standard references for DNA quantification. Absorbance values of fish nuclei from each slide were standardized as a percentage of the mean absorbance value of the three controls. To express DNA amount in picograms (pg) the standardized data were multiplied by the known values for these species (2.5 pg, 3.4 pg, and 5.5 pg, respectively, according to Tiersch *et al.*, 1989b). Chicken blood was obtained from a Hampishare male, and rainbow trout and common carp blood was obtained from domesticated stocks. The slides were fixed for 20 min in 9:1 methanol-formaldehyde (37%), rinsed twice (10 min each) in distilled water, dehydrated in 70% ethanol (2 min) and 95% ethanol (2 min), and stored overnight under desiccated conditions at 4 °C. On the following day, individual batches of 20 (randomized) slides were hydrolyzed for 15 min in 1.0 N HCl at 60 °C, rinsed briefly in distilled water, and stained for 2 h in Schiff's reagent (Feulgen stain). Hydrolysis time was empirically determined as the point of maximum absorbance in a hydrolysis curve. Following staining, the slides were rinsed twice (10 min each) in SO_2 water and once (10 min) in distilled water, air dried in the dark and analyzed.

Microdensitometry analysis was performed under a Zeiss microscope using a 100x oil-immersion objective. Analyses were done using the OPTIMAS software, version 4.1. For each individual fish, 15 nuclei were measured from each of two slides (30 nuclei per individual). The third slide prepared from each specimen served as a backup in case of breakage. Only the nuclei that were roughly spherical, homogeneously Feulgen-stained and found in clear areas of the slide were selected for measurement.

Results and Discussion

The results obtained among the species of Characiformes analyzed are shown in Table I. Diploid number ranged from 2n = 48 to 2n = 102, with 2n = 54 being the modal number. Nuclear DNA content ranged from 1.70 ± 0.04 pg of DNA per diploid nucleus in *Acestrorhynchus pantaneiro* to 3.94 ± 0.09 pg in *Tetragonopterus chalceus.* The lowest DNA content observed in the present study for Characiformes was lower than the lowest values previously observed: 2.2 pg in *Chalceus macrolepidotus* (Hinegardner and Rosen, 1972), and 2.32 ± 0.09 pg in *Hoplias malabaricus* (Carvalho *et al.*, 1998).

An overall analysis of our data showed a mean value of 2.9 pg, very similar to that reported by Carvalho *et al.* (1998), 3.0 pg, and 50% higher than the value described by Hinegardner and Rosen (1972), 2.0 pg. No significant variation in DNA amount was observed among fish of different sexes.

According to Hinegardner and Rosen (1972), during genome evolution, the amount of DNA may remain constant, or increase by duplication, or reduced by deletion. The analysis of Table I suggests that all these events occurred among the different groups of Characiformes.

Very similar DNA content values were observed in the species of the family Cynodontidae, which showed a variation of about 3% between the two genera studied (Table I). This small variation is likely to be related with the fact that both species have DNA contents similar to the minimum values observed in Characiformes (about 2.0 pg). In this case, further data on the DNA content of other species would provide a better view of the evolutionary tendencies of the group.

The presence of supernumerary chromosomes has usually produced a high coefficient of variation in DNA content values (Carvalho *et al*., 1998). In the present study, *Moenkhausia sanctaefilomenae* specimens from the Miranda river (Mato Grosso do Sul) presented 2.39 ± 0.09 pg (Table I) while studies conducted in specimens of the same species from the Capivara river (São Paulo) showed that this latter sample exhibited 2.75 ± 0.25 pg (Carvalho *et al*., 1998). This difference may be related with the presence of 1 to 8 supernumerary microchromosomes observed in the sample from the Capivara river (Foresti *et al*., 1989). However, future studies are necessary to confirm the karyotypes of specimens from the Miranda river.

Apareiodon affinis from the Miranda river (Paraguai river basin) displayed one of the smallest amount of DNA (2.04 ± 0.12 pg - Table I) among the Neotropical species. Carvalho *et al*. (1998) observed that the specimens of this species collected from the Paranapanema river (Upper Paraná river basin) showed 2.53 ± 0.17 pg. Moreira Filho *et al*. (1985) and Jesus *et al*. (1999) reported that in local populations from the state of São Paulo (Brazil), the fish exhibited 2n = 54-55 and a ZZ/ZW_1W_2 sex chromosome system. On the other hand, Jorge and Moreira Filho (2000) on local populations from the Lower Paraná river basin demonstrated that the fish had no sex chromosomes. In this species, the higher amount of DNA found in the sample from the Paranapanema river may be related to the presence of sex chromosomes in this sample.

The DNA content of the *Gymnocorymbus ternetzi* population studied by Hinegardner and Rosen (1972) and that studied herein differ in about 26%. Both populations of *G. ternetzi* exhibited the same diploid number (2n = 48). The *Astyanax fasciatus* population studied by Carvalho *et al.* (1998) displayed 2n = 46 chromosomes and 3.50 ± 0.18 pg of DNA per nucleus whereas, in the present study, a local population of *A. fasciatus* presented 2n = 48 and 2.75 ± 0.10 pg, a difference of 27%. In these two cases, the differences in DNA content do not appear to be related to chromosome number.

Among the groups analyzed, the subfamily Tetragonopterinae (Characidae) showed one of the greatest variations. *Tetragonopterus chalceus* showed 3.94 ± 0.09 pg whereas *Moenkhausia dichroura* exhibited 2.01 ± 0.16 pg, differing in 96%. Cytogenetic studies carried out in fish of this group have shown a considerable variation in diploid number and karyotypic structure. Weitzman and Fink (1983), based on morphological data, suggested that the subfamily Tetragonopterinae may represent an artificial group within the family Characidae. The existence of differences in DNA content and karyotype emphasizes the necessity of further studies in order to achieve a better definition of the fish of this subfamily.

In this work, the genus *Astyanax* (Tetragonopterinae, Characidae) showed the greatest variation in nuclear DNA content (79%) as previously observed by Carvalho *et al.* (1998). Among six species whose DNA content had been determined (Carvalho *et al*., 1998; present study) it was observed a range from 2.09 ± 0.15 pg in *A. altiparanae* (identified as *A. bimaculatus*) to 3.74 ± 0.13 pg in *A. scabripinnis*, and 2n = 50 chromosomes in both species. The diploid number of the species analyzed range from 2n = 46 to 2n = 50. In this case the variation in DNA content can not be directly related to chromosome number. Considering that the fish of this genus exhibit chromosomes of all types (metacentric, submetacentric, subtelocentric, and acrocentric) the amount of DNA can not be related with karyotypic constitution either.

DNA content variation was also very high (about 96%) in the subfamily Acestrorhynchinae (Characidae). In the members of this subfamily, the diploid number and karyotypic macrostructure remain unchanged (Falcão and Bertollo, 1985; Miyazawa, 1997). Regarding the genus *Acestrorhynchus,* Miyazawa (1997) suggested that pericentric inversions may be the major factor responsible for chromosomal diversification in the group. In this study, it was observed that nuclear DNA content ranged from 1.70 ± 0.04 in *Acestrorhynchus pantaneiro* to 3.10 ± 0.06 in *Acestrorhynchus* sp. This variation of 82% may suggest that, besides the pericentric inversions suggested by Miyazawa (1997), other events such as duplications and deletions may also have occurred and become fixed during the evolutionary history of the group. A higher number of species need to have their nuclear DNA content quantified to permit a better view of the possible changes that may be associated with DNA during the evolutionary process of the group.

The variation of DNA content obtained in representatives of the other families studied showed that, even in some families with evident conservation of diploid number and karyotypic formulae such as Anostomidae, Curimatidae, and Prochilodontidae, episodes leading to losses or gains of genetic material may have been fixed in the groups, explaining the variation observed in the nuclear DNA content.

In the family Anostomidae, the DNA content ranged from 2.57 ± 0.14 pg in *Leporinus friderici* (Carvalho *et al*., 1998) to 3.68 ± 0.06 pg in *Schizodon* sp. from the Acre river (Table I). This difference of about 1.11 pg cannot be related to the karyotypic macrostructure of the species in that all the species studied that belong to these two genus exhibit 2n = 54 chromosomes and metacentric and submetacentric chromosomes (Galetti Junior *et al*., 1991; Galetti Junior *et al*., 1995; Martins and Galetti Junior, 1998).

In three *Leporinus* species whose DNA content is known, *L. elongatus*, *L. reinhardti* (Table I) and *L. obtusidens* (Carvalho *et al*., 1998), systems of sex chromosomes of the type ZZ/ZW were found, with the W chromosome usually bigger than the Z chromosome and almost entirely heterochromatic (Galetti Junior *et al*., 1991; Galetti Junior *et al*., 1995). The analysis of the DNA content of these species showed that they have about 3.0 pg, which is an intermediary value among the others found in the species that show no sex chromosomes such as *L. friderici* (2.57 ± 0.14 pg) and *L. octofasciatus* (3.48 ± 0.15 pg) (Carvalho *et al*., 1998). These data suggest that the presence of large sex chromosomes is not the most important factor in the determination of the amount of DNA in these species.

Extensive studies conducted with species of the family Curimatidae (Venere and Galetti Junior, 1989; Feldberg *et al*., 1993; Navarrete and Júlio Junior, 1997) have shown that the diploid number range from 2n = 46 in *Curimatopsis myersi* (Navarrete and Júlio Junior, 1997) to 2n = 102 in *Potamorhina altamazonica* (Feldberg *et al*., 1993) and *P. squamoralevis* (Navarrete, 1996). The analysis of the DNA content of eight species of this family (Carvalho *et al*., 1998; Table I) showed that among the species with 2n = 54 chromosomes there is a range from 2.83 ± 0.26 in *Curimatella dorsalis* to 3.45 ± 0.34 pg in *Curimata elegans*, and the species *Potamorhina squamoralevis* with 2n = 102 has 3.80 ± 0.23 pg. Considering that the species with 2n = 46 to 2n = 56 exhibit almost exclusively metacentric and submetacentric chromosomes and that the species *P. altamazonica* has 49 acrocentric pairs, Feldberg *et al.* (1993) suggested that a large number of centric fission events have been fixed in the evolutionary process of this species, and that may also have occurred with *P. squamoralevis* (Navarrete, 1996). A general analysis of the amount of nuclear DNA in the family shows that the highest value found for the species with 2n = 46 to 2n = 56 was 3.31 ± 0.12 pg in *Cyphocharax gilberti* (Table I) whereas in *Potamorhina squamoralevis* (2n = 102) the amount observed was 3.80 ± 0.23 pg (Table I). Thus, besides an increase in chromosome number, an increase in DNA amount also occurred in the evolutionary process of this group.

The data available in the literature on 275 species of teleostei show that differences greater than two times may be found among the members of a genus, as observed in cyprinids of the genus *Barbus* (Ohno *et al.*, 1967; Wolf *et al.*, 1969) and the members of the genus *Corydoras* (Hinegardner and Rosen 1972; Oliveira *et al.*, 1992). The great variation in DNA content observed in the *Corydoras* species (Hinegardner and Rosen, 1972; Oliveira *et al.*, 1992) was not followed by remarkable anatomical changes

(Strauss, 1985). Thus, it seems that a considerable amount of DNA might be lost or gained without significant changes in the appearance of the individuals (Hinegardner and Rosen, 1972). These observations seems to be valid for the order Characiformes.

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