



## A comparative cytogenetic study of five piranha species (*Serrasalmus*, Serrasalminae) from the Amazon basin

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### Abstract

Cytogenetic studies were conducted on five piranha species belonging to the genus *Serrasalmus*, subfamily Serrasalminae (*Serrasalmus altispinis*, *S. compressus*, *S. elongatus*, *S. manuelli*, and *S. spilopleura*). All the species were collected in the Amazon basin: confluence of Negro and Solimões Rivers (Catalão Lake), Solimões River (Marchantaria Island – Camaleão Lake), Uatumã River (Hydroelectric Power Station of Balbina), and Pitinga River (Hydroelectric Power Station of Pitinga). All the five species possess  $2n = 60$  chromosomes with 5–12 subtelomeric and acrocentric chromosomes bearing nucleolar organizer regions. A proximal C-band positive heterochromatin block was evident on the long arms of a medium-sized metacentric chromosome pair in all the analyzed species, thus making it a cytogenetic marker for the genus. It is hypothesized that  $2n = 60$  chromosomes represents a derived feature in terms of the chromosomal evolution of piranhas because the basal lineages possess  $2n = 62$ . Both Robertsonian centric fusion and non-Robertsonian rearrangements such as pericentric inversions seem implicated in the chromosomal evolution of this group.

### Introduction

The fish commonly known as piranhas include five genera (*Catoprion*, *Pygopristis*, *Pygocentrus*, *Pristobrycon*, and *Serrasalmus*). Taxonomy and phylogeny of piranhas and their relatives have been studied elsewhere (Gosline, 1951; Nelson, 1961; Géry, 1976; Machado-Allison, 1983, 1985; Ortí et al., 1996), but are debatable, especially at subfamily or family level. However, it is well accepted that this group is monophyletic.

The genus *Serrasalmus*, according to Goulding (1980), is distributed throughout tropical portions of South America, east of the Andean Cordillera, being abundant in the basins of the Orinoco, Solimões–Amazonas, Paraná/Paraguai and São Francisco Rivers and inhabiting a wide variety of water bodies. They are characterized by the highly specialized predator nature and the feeding habits vary according to their ontogenetic development ranging from zooplankton, insects,

to fleshy tissues of fish and other animals (Goulding, 1980; Machado-Allison & Garcia, 1986; Nico & Taphorn, 1988; Nico, 1990; Machado-Allison & Fink, 1996).

Taxonomy of several species of the genus *Serrasalmus* is little known and quite confusing due to the fact that most analyses were carried out on small samples, usually consisting of young specimens. In addition, the color patterns of this genus usually change during the ontogenetic process and the reproductive period. Thus, the real number of valid *Serrasalmus* species is still unknown (Machado-Allison & Fink, 1996).

Cytogenetic information is available on several nominal species of *Serrasalmus* (Muramoto, Ohno & Atkins, 1968; Galetti, Silva & Cerminaro, 1985; Cestari & Galetti, 1992a, b; Martins-Santos, Julio Jr. & Santos, 1994; Nakayama, Porto & Feldberg, 2000; Nakayama et al., 2001; Centofante, Porto & Feldberg, 2002). Such data have proved to be of great cyto-

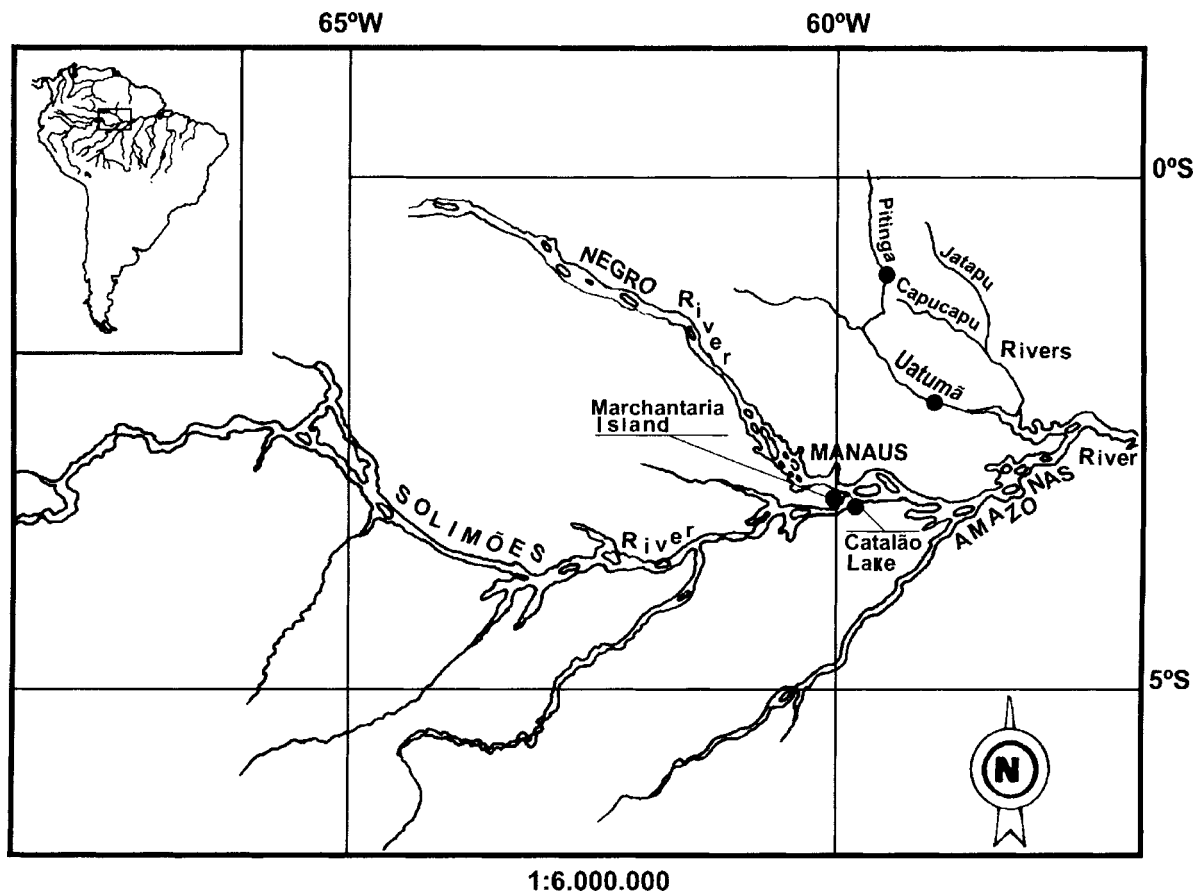


Figure 1. Map of central Amazon showing the location of sampling sites (black circles) of *Serrasalmus* species studied.

taxonomic value for the identification of species since each karyotypic formulae seems to be unique despite the same diploid number, that is,  $2n = 60$  chromosomes, except for *S. cf. rhombeus* (Nakayama et al., 2001) and *S. hollandi* (Muramoto, Ohno & Atkins, 1968).

The aim of this paper is to report the chromosome data for five Amazonian piranha species, including four cytogenetically new ones, from the genus *Serrasalmus* collected in central Amazon basin, and to discuss the chromosomal evolution in this genus.

### Material and methods

Karyotype analyses were performed on five species of the genus *Serrasalmus*: *S. altispinis* (two males, eight females), *S. compressus* (four males, three females), *S. elongatus* (seven males, four females), *S. manuelli* (five males, four females), and *S. spilopleura* (four

males, 11 females). The specimens were collected from the Amazon basin at the confluence of Negro and Solimões Rivers (Catalão Lake), Solimões (Marchantaria Island – Camaleão Lake), Uatumã River (Hydroelectric Power Station of Balbina), and Pitinga River (Hydroelectric Power Station of Pitinga) (Figure 1).

Chromosome preparations were obtained from a kidney cell suspension using the standard air-drying technique of Bertollo, Takahashi and Moreira Filho (1978). The nucleolar organizer regions (NORs) were identified by silver nitrate staining after Howell and Black (1980), and C-banding to locate heterochromatin was performed using barium hydroxide after Sumner (1972).

Chromosome analyses were performed on metaphase plates and the diploid number for males and females was determined. Chromosomes were measured and arranged in decreasing order of size into metacentrics (M), submetacentrics (SM), subtelocentrics (ST), and acrocentrics (A) according to Levan, Fredga and Sandberg (1964). The fundamental number (FN)

or arm number was determined by considering M, SM, and ST with two arms and A with only one.

## Results

Table 1 summarizes the karyotypic data obtained in the present study and those available in the literature. The species analyzed here displayed  $2n = 60$  chromosomes, with the FN ranging from 108 to 112. Overall the karyotypes are uniform (Figure 2(A)–(E)), but differ in the chromosome formulae (Table 1). No sex heteromorphism was detected in any piranha species analyzed.

The five species were characterized by multiple NOR-bearing chromosomes where the Ag-NORs

were located on terminal or proximal regions of the short arms of 5–12 ST and A chromosomes. Number, size, and staining intensity of the NORs were variable intraindividually and interspecifically (Figure 3(A)–(E)).

All the five species were characterized by C-band positive heterochromatin blocks in the pericentric region on all chromosomes, some more conspicuous than others. Also were observed chromosomes with different heterochromatic blocks, including those in the whole short arms, in the telomeric, and in the proximal regions. One pair of metacentric chromosomes had a proximal heterochromatic block on the long arms in all the species examined. In addition, all NORs were C-band positive (Figure 3(A)–(E)).

Table 1. Summary of the chromosome findings of the species of the genus *Serrasalmus* studied so far ( $2n$  = diploid number; FN = fundamental number; M = metacentric, SM = submetacentric; ST = subtelocentric; A = acrocentric)

Species	Locality	$2n$	M	SM	ST	A	NF	Reference
<i>S. altispinis</i>	Uatumã and Pitinga Rivers	60	20	28	2	10	110	Present paper
<i>S. brandti</i>	Três Marias Dam	60	18	24	8	10	110	Cestari and Galetti (1992b)
<i>S. compressus</i>	Catalão and Camaleão Lakes	60	18	30	2	10	110	Present paper
<i>S. elongatus</i>	Catalão and Camaleão Lakes	60	22	22	4	12	108	Present paper
<i>S. hollandi</i>	Unknown	64	30	–	16	18	110	Muramoto, Ohno and Atkins (1968)
<i>S. humeralis</i>	Corumbá	60	16	26	6	12	108	Cestari, unpublished data
<i>S. manuelli</i>	Catalão and Camaleão Lakes	60	22	24	6	8	112	Present paper
<i>S. marginatus</i>	Baia River and Corumbá	60	16	26	6	12	108	Martins-Santos, Júlio Jr. and Santos (1994), Cestari and Galetti (1992b)
<i>S. spilopleura</i> 'A'	Catalão and Camaleão Lakes	60	24	20	4	12	108	Nakayama, Porto and Feldberg (2000), Centofante, Porto and Feldberg (in press), present paper
<i>S. spilopleura</i> 'B'	Catalão and Camaleão Lakes	60	24	26	–	10	110	Nakayama, Porto and Feldberg (2000)
<i>S. spilopleura</i> 'C'	Catalão Lake	60	23	21	4	12	108	Centofante, Porto and Feldberg (2002)
<i>S. spilopleura</i> 'D'	Manacapuru River	60	24	20	4	12	108	Centofante, Porto and Feldberg (2002)
<i>S. spilopleura</i> 'a'	Mogi-Guaçu River; Grande River; Baia River	60	20	26	4	10	110	Galetti, Silva and Cerminaro (1985), Cestari and Galetti (1992a), Martins-Santos, Júlio Jr. and Santos (1994)
<i>S. spilopleura</i> 'b'	Aguapei Stream; Poconé; Corumbá	60	18	26	4	12	108	Cestari and Galetti (1992a)
<i>S. spilopleura</i> 'c'	Aguapei Stream	60	19	26	4	11	109	Cestari and Galetti (1992a)
<i>S. cf. rhombeus</i>	Catalão and Camaleão Lakes	58	30	16	2	10	106	Nakayama et al. (2001)
<i>S. rhombeus</i>	Catalão Lake; Uatumã River	60	20	24	6	10	110	Nakayama et al. (2001)

Capital and lower case alphabets in *S. spilopleura* represents the Amazon and Paraná/Paraguay cytotypes, respectively.



Figure 2. Conventional Giemsa-stained karyotypes of: (A) *S. altispinis*, (B) *S. compressus*, (C) *S. elongatus*, (D) *S. manuelli* and (E) *S. spilopleura*. Scale bar represents 5 µm.

## Discussion

The relationships among piranhas have been the subject of evolutionary studies using morphological (Machado-Allison, 1985), karyological (Porto et al., 1989; 1991), parasitological (Van Every & Kritsky, 1992), and molecular (Ortí et al., 1996) characters. Both morphological and molecular data agrees that the 'piranha' clade is rooted by the genus *Mettnis* (not piranha). However, a consensus has not been obtained with respect to the internal relationships. Partially different from morphological data, the molecular data indicate that the species *Catoprion mento* and *Pygopristis denticulatus* plus *Pristobrycon striolatus* form a well supported clade, sister to a paraphyletic

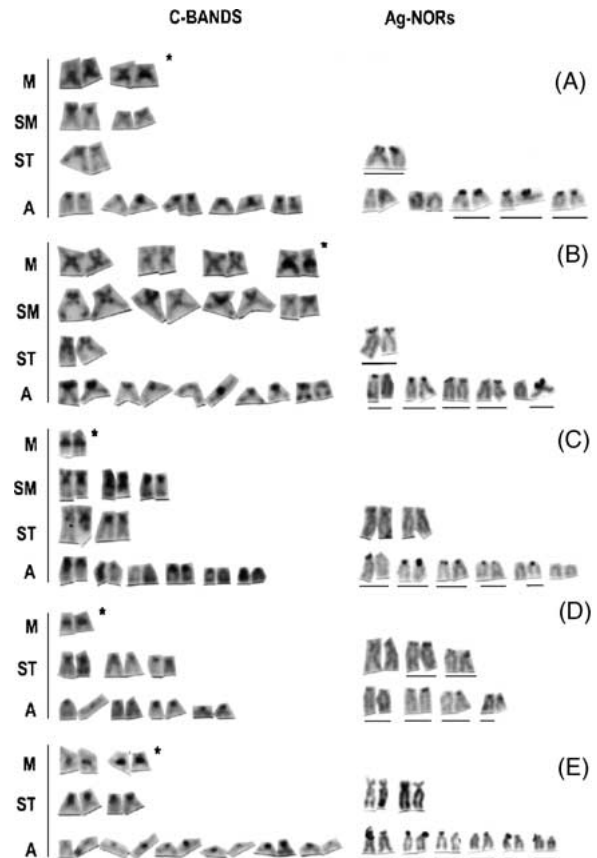


Figure 3. Partial karyotypes with C-bands (left) and Ag-NORs (right) of: (A) *S. altispinis*, (B) *S. compressus*, (C) *S. elongatus*, (D) *S. manuelli* and (E) *S. spilopleura*. All the subtel- and acrocentric chromosomes with or without proximal, terminal or other interstitial C-blocks besides centromeric C-bands are aligned for comparison with those chromosomes bearing Ag-NORs. Also, meta- and submetacentric chromosomes with similar characteristic C-bands are arranged. Asterisk indicate the pair with a proximal C-band positive heterochromatic block considered common for all the examined *Serrasalmus* species. The underline indicates the NOR-bearing chromosomes.

group that includes species of the genera *Pristobrycon*, *Serrasalmus*, and *Pygocentrus*.

Chromosomally, *Serrasalmus* shows a diploid number ranging from  $2n = 58$  to 64 chromosomes, with  $2n = 60$  being the most common among the species, but with usually distinct chromosome formulae (Table 1). Cestari and Galetti (1992a) suggested that the basic diploid number for the genus *Serrasalmus* could be  $2n = 60$  chromosomes, and that other chromosome numbers might represent a derived state. Apparently, karyotypes with  $2n = 58$  (Nakayama et al., 2001) and  $2n = 64$  (Muramoto, Ohno & Atkins, 1968) must represent autapomorphies since each one was

detected in only one species, suggesting that the ancestral diploid number for this genus is  $2n = 60$  chromosomes. This putative ancestral diploid number for *Serrasalmus* is also shared with species of the genera *Pygocentrus* and *Pristobrycon* (Nakayama, unpublished master's thesis), but not with *P. striolatus* and the basal monotypic genera *Catoprion* and *Pygopristis* (Nakayama, Porto & Feldberg, unpublished data), all of which possessed  $2n = 62$ . Thus, considering the present and previous chromosome data of piranhas, it is possible to suggest the evolution of  $2n = 60$  from  $2n = 62$  chromosomes by both Robertsonian centric fusion and non-Robertsonian rearrangements (such as pericentric inversions) in this group.

With respect to the NORs, all the species analyzed in this study presented several ribosomal sites that vary in number and size. The present data evidence that piranhas are characterized by a multiple NOR system located exclusively in ST and A chromosomes, and corroborate the previous reports that only the Serrasalminae possesses exclusively multiple NORs, compared to other characids (see checklists of Oliveira et al., 1988; Porto et al., 1992). The phenetic NOR characters seem to be a good tool for differentiating clades within the Serrasalminae as reported elsewhere (Porto et al., 1989, 1991), but apparently is not applicable in piranhas species, particularly in those belonging to the genus *Serrasalmus*, because of no discriminating NOR patterns among the species studied here.

Centric and pericentric C-bands appear to be usual in the examined piranhas, being more evident in some chromosomes than in others. Telomeric C-bands were observed in some chromosome pairs, but they were usually fainter than the pericentric bands. Most intriguing finding is the common occurrence of a medium-sized metacentric pair with a proximal heterochromatin block on the long arms in the five species examined herein (Figure 3). This observation is in agreement with the previous findings in other species of the genus *Serrasalmus* (Cestari & Galetti, 1992a, b; Martins-Santos, Júlio Jr. & Santos, 1994; Nakayama et al., 2001; Centofante, Porto & Feldberg, 2002; present study). However, this characteristic was not well observed in other piranha genera where C-banding is available (Souza & Nakayama, 2000), suggesting that the proximal C-block may be a chromosome marker, probably a synapomorphy of the genus *Serrasalmus*. Unfortunately we have not yet been able to determine the homeology of this chromosome pair in all the examined species. The observed

C-band positive NORs seem to be a common feature among *Serrasalmus* (present study) and other Neotropical fish groups (Galetti, 1998).

Thus, chromosome evolution in the genus *Serrasalmus* is somewhat divergent from other piranha genera in terms of the karyotypic formulae as well as C-band positive heterochromatin; whereas the NORs seem to be more conservative than C-bands.

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