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Chromosomal polymorphism in *Serrasalmus spilopleura* Kner, 1858 (Characidae, Serrasalminae) from Central Amazon Basin

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Abstract - Cytogenetic studies were performed in *Serrasalmus spilopleura*, a piranha species, from three sampling sites in the Central Amazon basin: 1. Catalão; 2. Manacapuru, 3. Itacoatiara. All specimens displayed $2n$=60 chromosomes and no sexual chromosomal heteromorphism was found. However, by considering the karyotypic formula, three cytotypes were identifyed: cytotype A consisted of 24M+20SM+4ST+12A, and was the most common being found in all three sampling sites; cytotype C consisted of 23M+21SM+4ST+12A, was found only in Catalão and differ from cytotype A by the presence of a heteroziguous inversion in the first chromosome pair and a distal heterocromatinization in the long arm of this same pair, probably caused by the rearrangement or due to a chromosomal adjustment; cytotype D consisted of 24M+20SM+4ST+12A, was found only in Manacapuru and differ from cytoytpe A by a heteroziguous size polymorphism on the second chromosome pair, probably due to a translocation. C-banding revealed the same constitutive heterochromatin pattern for the three cytotypes except for the chromosome pair that underwent pericentric inversion (cytotype C) which possessed different heterochromatic blocks. Analysis in the number of active nucleolar organizing regions (NORs) revealed no differentiation among cytotypes and NOR-bearing chromosomes varied from 10–12 acrocentric chromosomes.

Key words: Amazon Basin, chromosomal polymorphism, cytotypes, fish, piranha, Serrasalminae.

INTRODUCTION

Subfamily Serrasalminae comprises two groups of fishes (lineages A and B), popularly known as pacus and piranhas (MACHADO-ALLISON 1983). They are very specialized fishes and are characterized by a compressed and, usually, deep body with a ventral sawn keel. Collouring pattern can vary from solid red to yellow and from dark to pale grey. They are a highly diverse group, with a wide distribution throughout South America, and despite their fame as voracious carnivore, most species are considered to be omnivorous (GÉRY 1972; GOULDING 1980; MACHADO-ALLISON and FINK 1996).

Cytogenetic studies performed on this subfamily, lineage B (piranhas), have shown a diploid number ranging from $2n$=58 (*Serrasalmus* sp., NAKAYAMA *et al.* in press) to $2n$=64 (*Serrasalmus hollandi*, MURAMOTO *et al.* 1968). Nevertheless, the most frequent diploid number is equal to 60 chromosomes (GALETTI *et al.* 1985; CESTARI and GALETTI 1992a, b; MARTINS-SANTOS *et al.* 1994; NAKAYAMA 1997), in most derived genera, and $2n$=62 in most basal ones. Furthermore, chromosomal analyses have revealed the occurrence of intraspecific differences due to numerical and

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structural variability (NAKAYAMA *et al.* 2000, in press).

Among piranhas *Serrasalmus* might be considered the most well studied genus, concerning chromosomal analysis. Multiple nucleolar organizing regions (NORs) system, located on subtelo-acrocentric chromosomes, in which number, size and intensity of markings vary intraindividualy, seem to be a rule (GALETTI *et al.* 1985; CESTARI and GALETTI 1992a, b; MARTINS-SANTOS *et al.* 1994; CESTARI 1996; NAKAYAMA 1997).

In *Serrasalmus spilopleura*, a species widespread throughout the Amazon basin as well as in Paraná-Paraguay and Uruguay basins, five cytotypes have been described so far. Three of them were found in Paraná-Paraguay basin (CESTARI and GALETTI 1992a; CESTARI 1996) and two in the Catalão Lake in the Amazon basin (NAKAYAMA *et al.* 2000).

Since variability due to chromosomal rearrangements can determine changes in the diploid number of a species (by chromosomal fission and fusion) or changes only in the chromosome structure (by inversions, deletions, duplications and/or translocations), to understand these alterations and its association to the speciation process, is one of the major challenges in karyoevolutionary studies (WHITE 1977; JOHN 1980; GUERRA 1988). By recognizing that *S. spilopleura* is chromosomaly polymorphic and that might exist a species "complex" (NAKAYAMA *et al.* 2000), the present study aimed to increase the sampling sites in the Central Amazon basin and to document the cytogenetic variation found in nominal forms of *S. spilopleura*.

MATERIAL AND METHODS

A total of sixty-two (62) specimens of the nominal species *Serrasalmus spilopleura* were collected in three different areas in the Central Amazon basin (Fig. 1): (1) at the confluence of Solimões and Negro Rivers (Catalão Lake), 31 specimens (13 males, 16 females and two undetermined sex ones); (2) in the lower Manacapuru River, a tributary of Solimões River, 15 specimens (three males and 12 females); (3) 16 specimens (10 males and six females) in the Amazon River, near to Itacoatiara township.

Mitotic stimulation was done with biological yeast (LOZANO *et al.* 1988). Mitotic chromosomes, nucle-

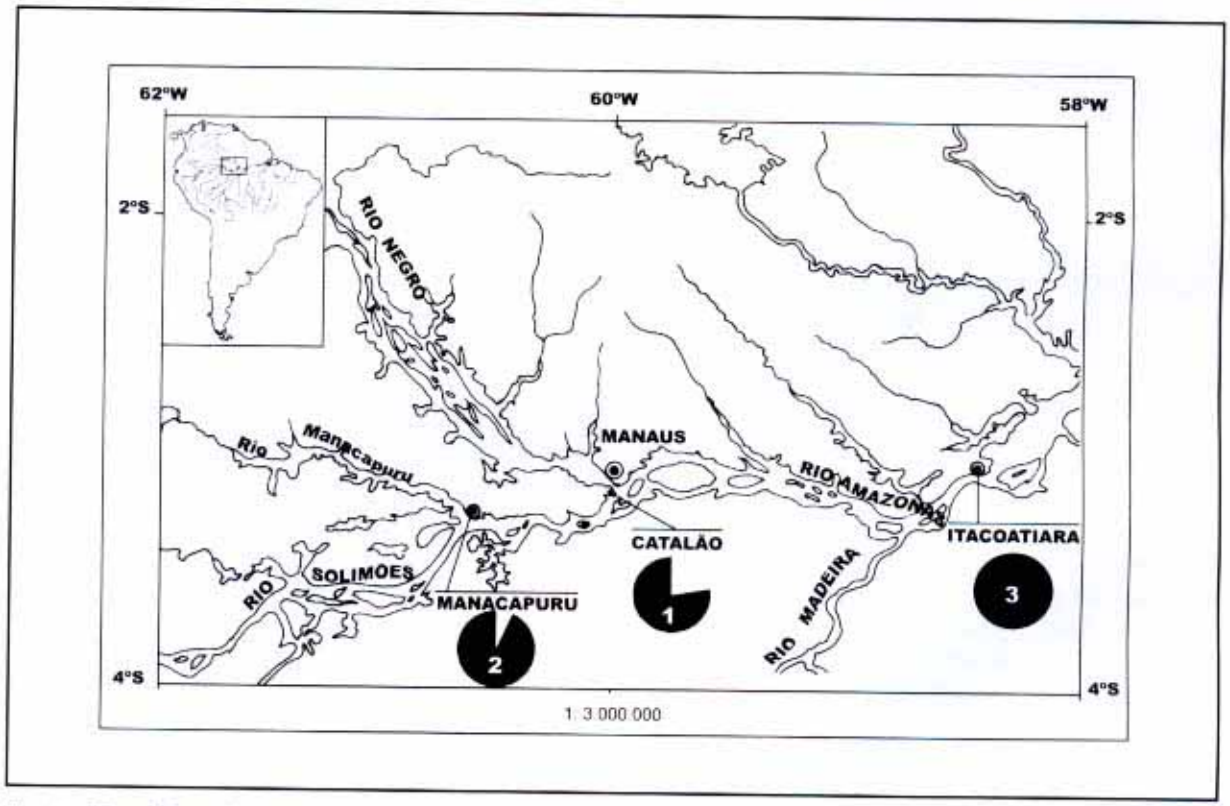

Fig. 1 – Map of Central Amazon showing the location of sampling sites and geographical distribution of Amazonian *S. spilopleura* cytotypes: 1) Catalão Lake (77% cytotype A and 23% cytotype C); 2) Manacapuru River mouth (93% cytotype A and 7% cytotype D); and 3) Amazonas River, Itacoatiara township (100% cytotype A).

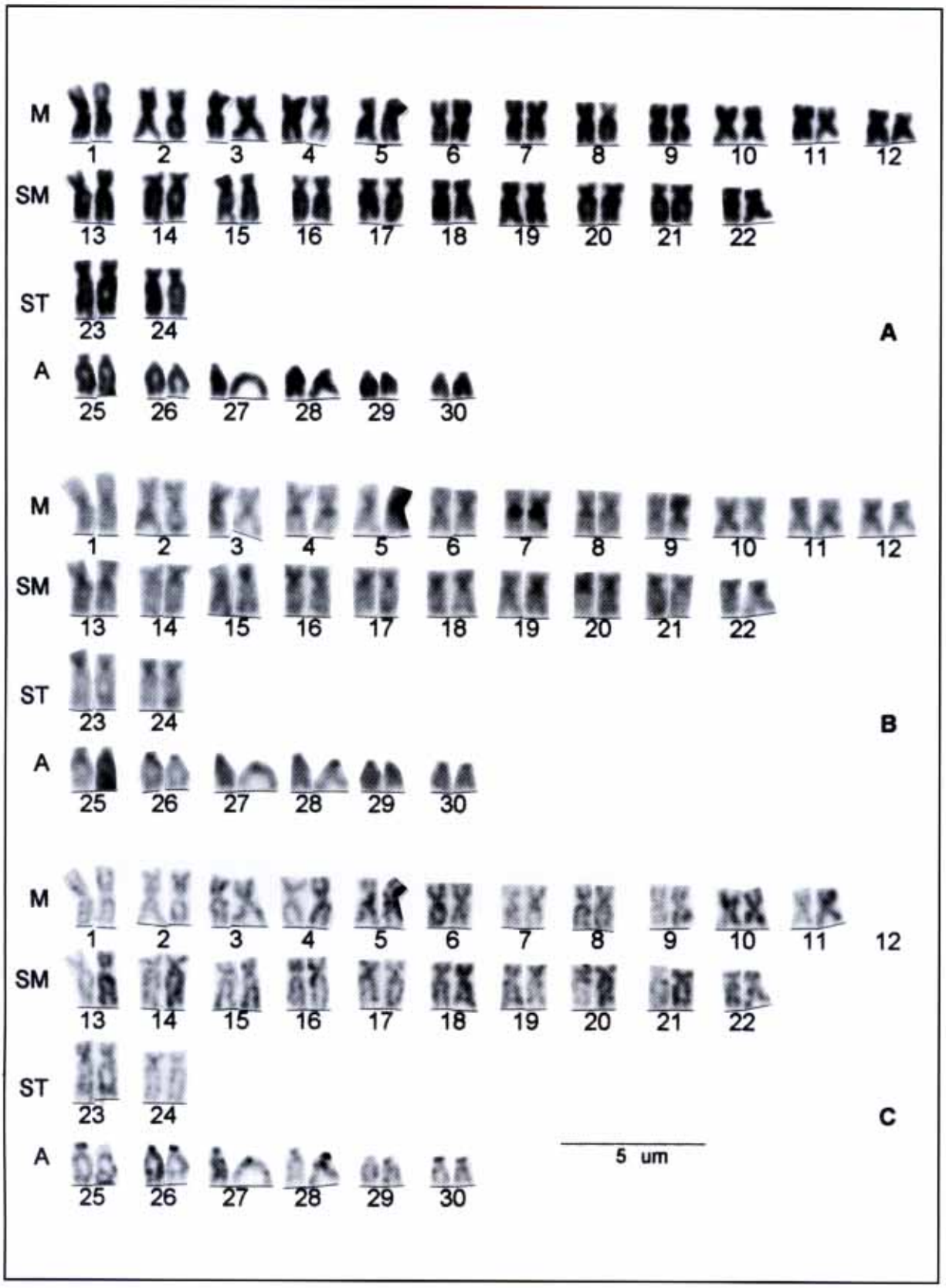

Fig. 2 – Sequential karyotype of *S. spilopleura* (cytotype A) from Catalão Lake. (A) Conventional staining, (B) C-banding, (C) $AgNO_3$ staining (NORs).

olar organizing regions (NORs) and constitutive heterochromatin (C-band) were obtained through the methods of BERTOLLO *et al.* (1978), HOWELL and BLACK (1980), and SUMNER (1972), respectively. An effort was done to analyse the same metaphases after a sequential Giemsa, C- and Ag-NOR banding, by using the following procedures: 1) take photographs of Giemsa conventional stained metaphases; 2) wash the slide in xylol for two minutes; 3) incubate the slide in Carnoy's fixative for 15 minutes; 4) incubate the slide in 0.2N HCl for 15 minutes; 5) wash it in distilled water and let it air dry; 6) incubate the slide in 2XSSC solution at 60° C for 30 minutes; 7) wash it in distilled water and let it air dry; 8) incubate the slide in a fresh and filtered 5% $Ba(OH)_2 8H_2O$, at 46°C for two minutes; 9) pass it quickly through 0.2N HCl, wash it in distilled water and leave it to dry; 10) incubate it in 2XSSC solution at 60° C for 30 minutes; 11) wash it in distilled water and leave it to air dry; 12) stain the slide with 5% Giemsa solution for eight minutes; 13) take a new photograph of the selected metaphases, now showing a C-band pattern; 14) repeat steps 2 to 5; 15) add a drop of 2% gelatin solution (added to formic acid on a ratio of 1ml per 100ml of water) on the slide; 16) add two drops of 50% silver nitrate solution to this drop. Mix it carefully and cover it with a coverslip; 17) incubate it in a humid chamber in a 60°C waterbath, for about three minutes; 18) wash it in distilled water, take off the coverslip and let it dry; 19) the same metaphases should be photographed again, and now they should be stained by the silver nitrate, revealing the NOR sites. Chromosomes were identified by the arm ratio criteria proposed by LEVAN *et al.* (1964), where the metacentric, submetacentric and subtelocentric were considered to be biarmed chromosomes, and acrocentric one-armed elements.

RESULTS

All specimens displayed $2n$= 60 chromosomes and FN= 108. However, we detected structural polymorphisms without sexual chromosomal heteromorphism leading us to propose two new cytotypes for this species. Cytotype A (*sensu* NAKAYAMA *et al.* 2000) is composed of 24M+20SM+4ST+12A and its frequency in all three populations was 77%, 93% and 100% in Catalão, Manacapuru and Itacoatiara, respectively (Fig. 2A); cytotype C is composed of 23M+21SM+4ST+12A and was present in seven individuals (23%) only at Catalão lake population (Fig. 3A); and cytotype D is composed of 24M+20SM+4ST+12A, where the second pair was formed by different sized chromosomes, and it was found only in one individual (7%) from Manacapuru River mouth (Fig. 4A). During collection trips from July to December, 1998, in these three areas, cytotype B *sensu* NAKAYAMA *et al.* (2000), was not found.

The heterochromatin pattern was very similar among the different cytotypes. In cytotype A, the constitutive heterochromatin was detected at the pericentromeric region of all chromosomes, in the telomeric region on short and long arms of some chromosomes, and on one pair of metacentric homologue chromosomes, probably the seventh pair, where a conspicuous proximal block on long arms could be seen. All NOR sites were C-band positive (Fig. 2B). For cytotype C, the heterochromatin pattern was the same as described for cytotype A, except for the first and seventh pairs, where in the latter they did not seem to be composed of homologues while the inverted first pair chromosome showed a distal block on the long arm (Fig. 3B). The bearing proximal heterochromatic blocks chromosomal pair also seemed to be formed by non homologue elements for cytotype D, (Fig. 4B).

Nucleolar organizing regions (NORs), in all specimens, were located on the short arms of 10 to 12 acrocentric chromosomes (Figg. 2C, 3C and 4C).

DISCUSSION

Chromosomal studies on fish have been providing more and more information everyday, about karyotypical variability at inter and intraspecific levels, which can be of great interest to phylogenetic systematics and taxonomy.

In the present study, three cytotypes were found and the cytogenetical analyses revealed two typical cases of chromosomal rearrangements occurring on *Serrasalmus spilopleura*. One case is related to pericentric inversion in one of the homologue chromosomes from the first metacentric pair (cytotype C, Fig. 3) and the other is related to a non balanced translocation, involving one of the homologue chromosomes from the second metacentric pair (cytotype D, Fig. 4).

Chromossomal rearrangements involving pericentric inversions have been reported in some fish species. In *Ilyodon furcidens* from Coahuayana River basin in Southern Mexico, TURNER *et al.* (1985) detected a diploid number of $2n$=48 chromosomes

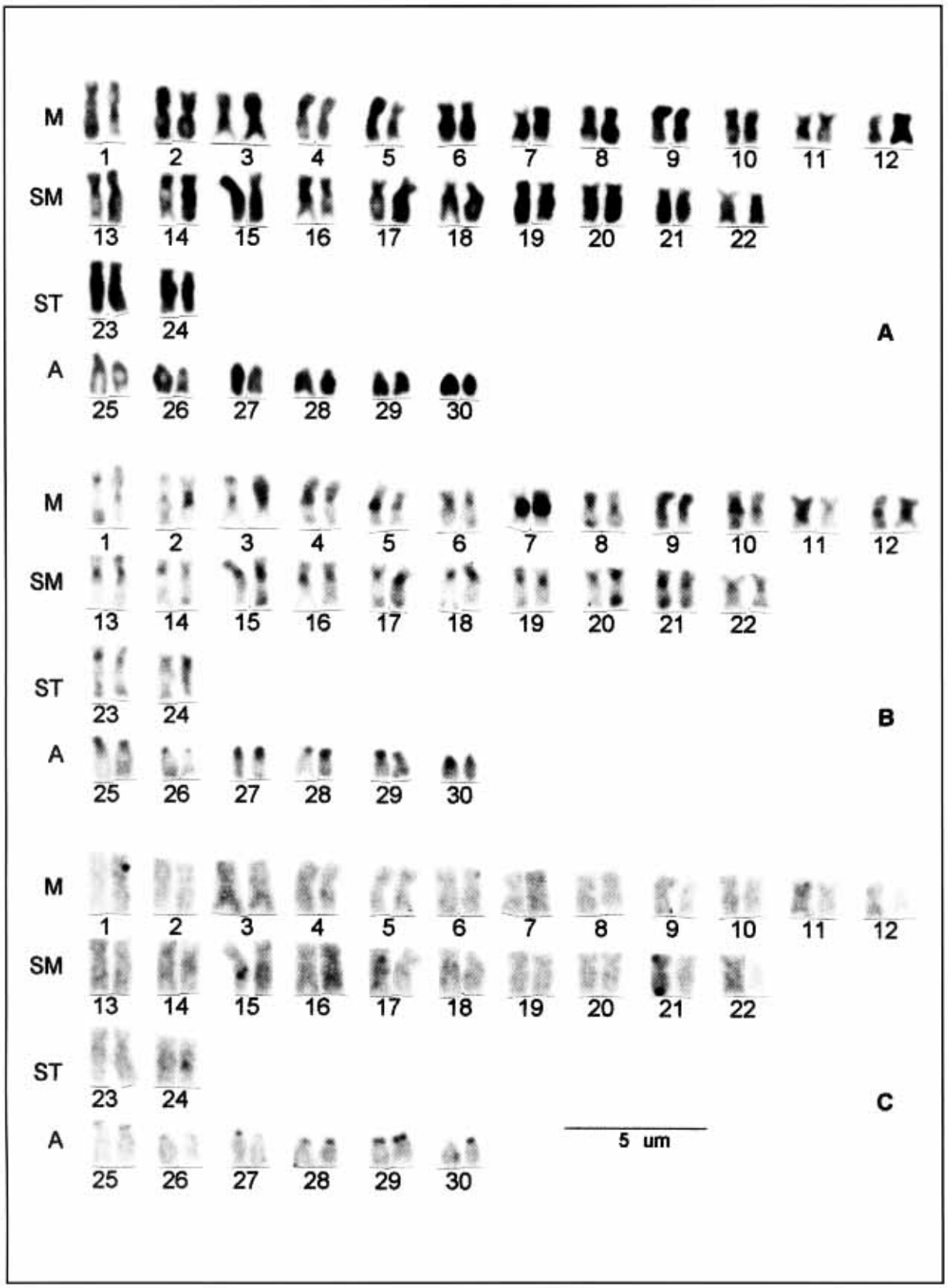

Fig. 3 – Sequential karyotype of *S. spilopleura* (cytotype C) from Catalão Lake. (A) Conventional staining, (B) C-banding, (C) $AgNO_3$ staining (NORs).

but with metacentric chromosomes numerical variation, thus identifing four cytotypes. In *Hoplerythrinus unitaeniatus*, from Negro River, GIULIANO-CAETANO and BERTOLLO (1988) detected a $2n$=48 chromosome diploid number and identified four cytotypes related to having or not having acrocentric chromosomes. In *S. spilopleura*, from Paraná-Paraguay basin, CESTARI and GALETTI (1992a), detected a $2n$=60 chromosome diploid number identifying three cytotypes related to the metacentric and acrocentric chromosome presence, in allopatry. NAKAYAMA *et al.* (2000) also observed the occurrence of two cytotypes (cytotype A and B) with equal diploid numbers ($2n$=60), but in simpatry at Catalão Lake for this same species.

Most chromosomal inversions determine a genetic variation caused by gene segregation, and they are disadvantageous when in homozygosity. On the other hand, a small proportion leads to a selective advantage (LANDE 1984). Thus, inversions also play an important role in speciation process, by altering gene sequences, sometimes activating or disactivating them, allowing or not for new habitats and/or niches exploitation (JOHN 1980).

According to JOHN (1980), the occurrence of rearrangements is not a common event, happenning seldomly on homologue chromosomes. Thus, structural rearrangements are introduced in wild populations in heterozygous ones where the homozygous offspring is established by interbreeding.

Catalão Lake is a mixed waters ecotone, formed by waters from Solimões River (white waters) and Negro River (acid and dark waters). In this environment, other chromosomal rearrangement situations such as fusions and pericentric inversions were detected by NAKAYAMA *et al.* (in press) in *S. rhombeus* and by FELDBERG *et al.* (1999) in *Plagioscion* sp. According to GUERRA (1988), some chromosomal rearrangements are more frequent under certain environmental conditions, since higher levels of polymorphism occur for species inhabiting their most favorable environment. So, we can not exclude the hypothesis that aquatic environments, like the one from Catalão Lake, could favor the fixation of individuals bearing chromosomal rearrangements.

The observed rearrangement on cytotype D (one individual), from Manacapuru population, can be a result of an unequal crossing-over during meiosis or of a unbalanced translocation. A similar fact was also characterized in *Gobius fallax* where, in addition to Robertsonian translocations (one of the responsible events for speciation), THODE *et al.* (1988) also identified three individuals bearing an unbalanced translocation in a metacentric chromosome. According to JOHN (1980) translocation cases are rarer in wild populations.

Nucleolar organizing regions were multiple in all individuals, being present on 10 to 12 acrocentric chromosomes. These results are similar to those previously described for other piranha species (GALETTI *et al.* 1985; CESTARI and GALETTI, 1992a, b; MARTINS-SANTOS *et al.* 1994; CESTARI 1996; NAKAYAMA 1997), confirming the system of multiple NORs for this group.

The constitutive heterochromatin pattern was very similar on the three cytotypes at different collection sites. This implies that the observed rearrangements on *S. spilopleura* were not followed by heterochromatic differentiation. However, in cytotype C, the pericentric inversion bearing chromosome presented a distal heterochromatic block on the long arm, which must have arisen as a consequence of heterochromatinization, i.e., a possible tandem duplication for a chromosomal adjustment following inversion.

In general, all species of genus *Serrasalmus* present a chromosomal pair with heterochromatic blocks on long arms, which is considered to be a cytogenetic marker for genus *Serrasalmus* (NAKAYAMA 1997). However, in C and D cytotypes, this pair appears not to be composed by homologue chromosomes. This lack of homology might be related to rearrangements.

In fish, the amount of heterochromatin and its distribution along chromosomes, has been described as an evolutionary karyotypical mechanism for some groups, such as Anostomidae where the karyotype macrostructure is stable but significant differences related to constitutive heterochromatin are found between genus and species (GALETTI *et al.* 1991). In Curimatidae, FELDBERG *et al.* (1993), suggested that the heterochromatinization process could be playing an important role in the karyotypic evolution of the genus *Potamorhina*.

What is evident by this work along with other reports, is the important role of non Robertsonian chromosomal rearrangements in the chromosomal evolution of the subfamily Serrasalminae, and that chromosomal polymorphism cases detected in *Serrasalmus spilopleura* may indicate a speciation process when compared to samples from Amazon and Paraná-Paraguay basins.

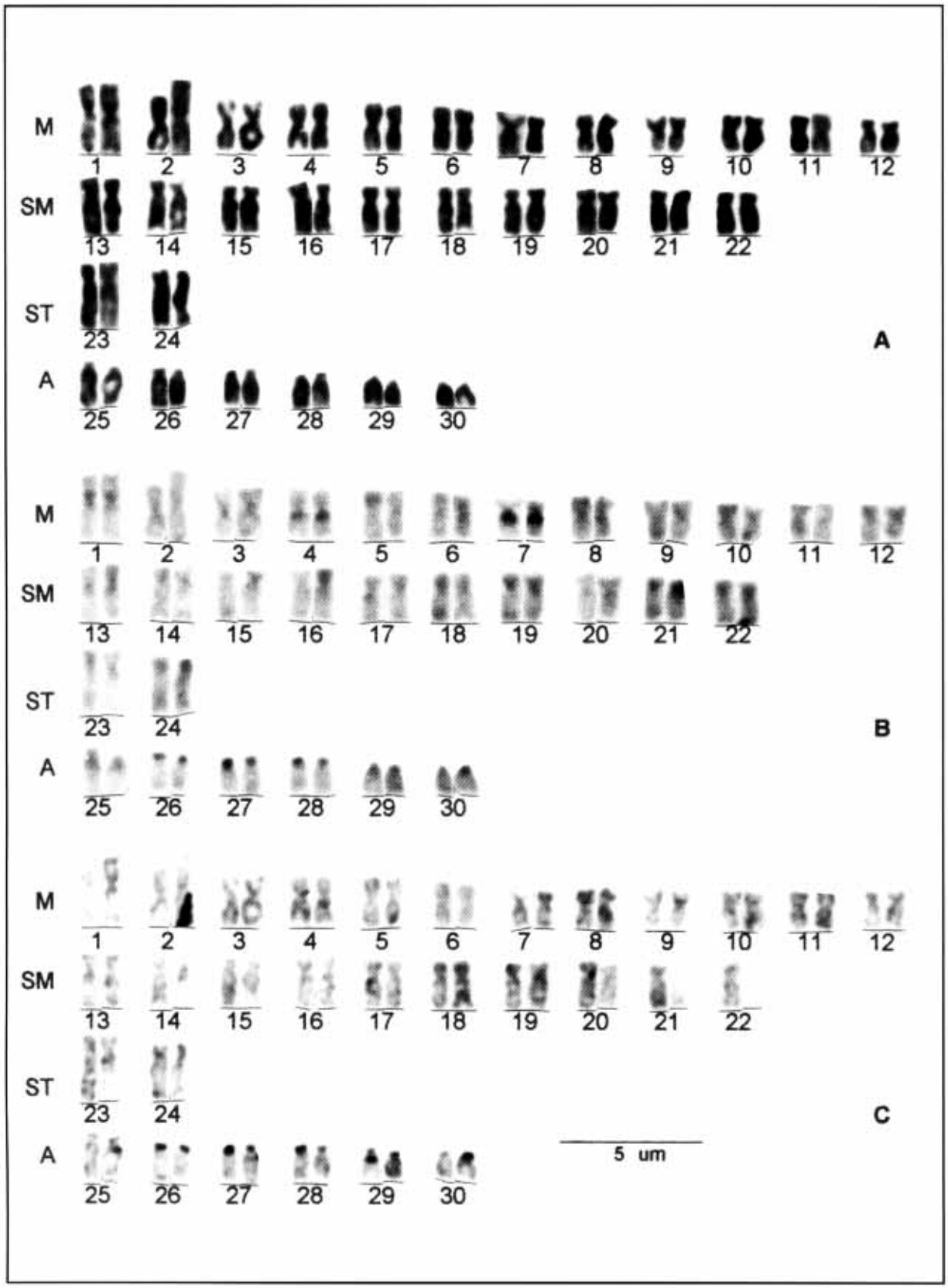

Fig. 4 – Sequential karyotype of *S. spilopleura* (cytotype D) from Manacapuru River mouth. (A) Conventional staining, (B) C-banding, (C) $AgNO_3$ staining (NORs).

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