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Karyotype description of two species of *Hypostomus* (Siluriformes, Loricariidae) of the Planalto da Bodoquena, Brazil

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ABSTRACT. *Hypostomus* sp 3-Córrego Salobrinha NUP 4247 and *Hypostomus* sp 2-Rio Perdido NUP 4249, collected in the Planalto da Bodoquena, Paraguay River basin, Brazil, were characterized cytogenetically. *Hypostomus* sp 3-Córrego Salobrinha showed two modal numbers. This polymorphism consists of the presence of two extrachromosomes. It was not possible to define the diploid number in four specimens, where cell lineages had 2n = 83 and 2n = 84 chromosomes in one individual, and 2n = 82, 2n = 83 and 2n = 84 chromosomes in the others. These results reveal the existence of a genetic mosaic due to the occurrence of one or two extrachromosomes in this species. *Hypostomus* sp 2-Rio Perdido NUP 4249 showed a 2n = 84, FN = 106 with size heteromorphism in one pair of chromosomes stained with $AgNO_3$. In both species, C banding showed a pattern of heterochromatin distribution with a few small bands in the centromeric and pericentromeric regions coinciding with chromomycin A_3 staining. Until now, the major diploid number for the genus *Hypostomus* was 2n = 80, but the species studied here had chromosomes that in-

creased this number and the variation for this genus. Our results are also the first cytogenetic data on *Hypostomus* from the Paraguay River basin.

Key words: Hypostominae; Cytogenetics; Chromosomal polymorphism; Heterochromatin; B chromosome.

INTRODUCTION

Although Reis et al. (2003) consider the Loricariidae as the largest family of catfishes in the world, little is known about the karyotypic organization in this group (Artoni and Bertollo, 2001). Meanwhile, some studies provide some cytogenetics information for Loricariinae (Scavone and Júlio Jr., 1994; Giuliano-Caetano, 1998; Artoni and Bertollo, 2001), Hypoptopomatinae (Andreata et al., 1992, 1993, 1994), Hypostominae (Artoni et al., 1998, 1999; Artoni and Bertollo, 1996, 1999, 2001; Alves et al., 2006), Ancistrini (Lara, 1998; Artoni and Bertollo, 2001) and Neoplecostominae (Alves, 2000; Kavalco et al., 2005).

In spite of the small amount of information compared with the number of the species already described for Loricariidae, the available data demonstrate that this is a group of great interest for cytogenetic studies, due not only to the variation in chromosome number, 2n = 36 in *Rineloricaria latirostris* (Giuliano-Caetano, 1998) to 2n = 96 in *Upsilodus* sp (Kavalco et al., 2005), but also to the occurrence of many chromosomal rearrangements, suggesting a divergent karyotypic evolution (Artoni and Bertollo, 2001).

Hypostomus is considered to be one of the most diversified groups of Neotropical fishes, and is one of the most studied genera from a cytogenetic point of view, showing a variation in chromosome number from 2n = 54 in *H. plecostomus* (Muramoto et al., 1968, in Artoni and Bertollo, 2001) to 2n = 80 in *Hypostomus* sp E (Artoni and Bertollo, 1999).

The Planalto da Bodoquena is situated in the cities of Bonito and Bodoquena and in part of the cities of Jardim and Porto Murtinho, in Mato Grosso do Sul, Brazil. The main rivers are the Formoso, da Prata, Perdido, and Salobra (Boggiani, 1999), belonging to the Paraguay River basin. Although Willink et al. (2000) report the uniqueness of the ichthyofauna, with many endemic species, there are no cytogenetic data recorded for fishes of this region. Thus, the present cytogenetic results on the two species of *Hypostomus* from Planalto da Bodoquena are the first data from its ichthyofauna and the first on *Hypostomus* from the Paraguay River basin.

MATERIAL AND METHODS

Both species studied are new to science, and are deposited at the ichthyological collection of the Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (NUPELIA) at the Universidade Estadual de Maringá, under the catalog number NUP 4247 for *Hypostomus* sp 3-Córrego Salobrinha and NUP 4249 for *Hypostomus* sp 2-Rio Perdido. Thirteen specimens of *Hypostomus* sp 3-Córrego Salobrinha NUP 4247 were analyzed, including three males, four females and six young individuals, which were collected in the Salobra River located at 20º 41' 34''S and 56º 44' 25''W, and in the Salobrinha Stream at 20º 41' 07''S and 56º 46' 44''W. For analysis of *Hypostomus* sp 2-Rio Perdido NUP 4249, there were five specimens including four males and one female, collected in the Perdido River at 21º 17' 09''S and 56º 41' 46''W. The rivers and stream cited above are located in the Planalto da Bodoquena, State of Mato Grosso do Sul (Figure 1).

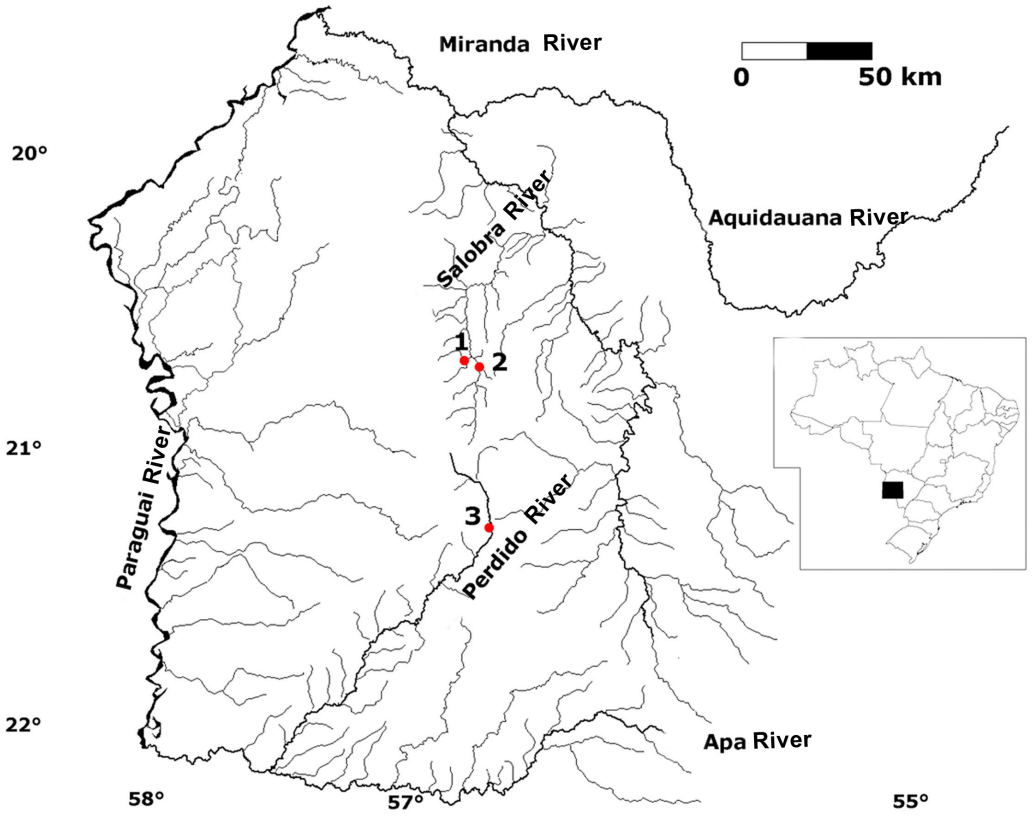

Figure 1. Localization of the area of study on the map of Brazil (inset) and localization of the collection points: Salobrinha Stream (1), Salobra River (2) and Perdido River (3).

For acquisition of mitotic metaphasic chromosomes, the conventional technique (Bertollo et al., 1978) or the alternative technique (Moreira Filho and Bertollo, 1990) was used. The chromosomes were classified into three groups: metacentrics (m), submetacentrics (sm), and subtelocentrics-acrocentrics (st-a), according to Levan et al. (1964), with modifications. C banding was used for determination of distribution pattern of heterochromatin (Sumner, 1972). Chromomycin A_3 / DAPI staining was according to Christian et al. (1998), with some modification.

RESULTS

A specimen of *Hypostomus* sp 3-Córrego Salobrinha NUP 4247 showed two distinct modal numbers, 2n = 82 and 2n = 84 (Figure 2) with variation in chromosome formula and fundamental number. One male and one female showed a 2n = 82, with a formula of 6m + 12sm + 64st-a, and FN = 100 (Figure 2A), while one male, one female and five juveniles showed a 2n = 84, with karyotype 6m + 12sm + 66st-a and FN = 102 (Figure 2B). In four

specimens (one male, two females and one juvenile), it was not possible to define the diploid number, where two females and one juvenile had 2n = 82 (Figure 2A), 2n = 84 (Figure 2B) and 2n = 83, 6m + 12sm + 65st-a, and FN = 101 (Figure 2C), characterizing a case of genetic mosaicism due to extrachromosomes.

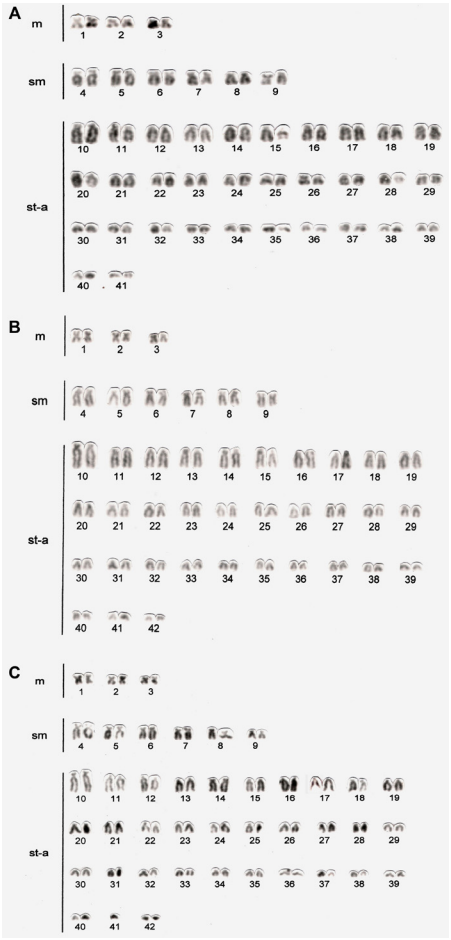

Figure 2. Cytotypes with 2n = 82 chromosomes (A), 2n = 84 chromosomes (B) and 2n = 83 chromosomes (C) *Hypostomus* sp 3-Córrego Salobrinha NUP 4247. m = metacentric; sm = submetacentric; st-a = subtelocentric-acrocentric.

The polymorphism encountered in *Hypostomus* sp 3-Córrego Salobrinha NUP 4247 may be due to the occurrence of one or two extrachromosomes, where the basic cytotype of the species is 2n = 82 chromosomes. There was no evidence of heterochromatic chromosomes, which suggests that these extrachromosomes would be euchromatic.

All specimens of *Hypostomus* sp 2-Rio Perdido NUP 4249 showed a modal diploid number of 2n = 84, with karyotype formula of 6m + 16sm + 62st-a, and FN = 106 (Figure 3A).

In four specimens, the secondary constriction in one of the homologs of pair 35 appeared very conspicuous after Giemsa staining (Figure 3A and B.1), $AgNO_3$ impregnation (Figure 3B.2) and CMA_3 staining, which also indicated size heteromorphism in the same pair (Figure 3B.3).

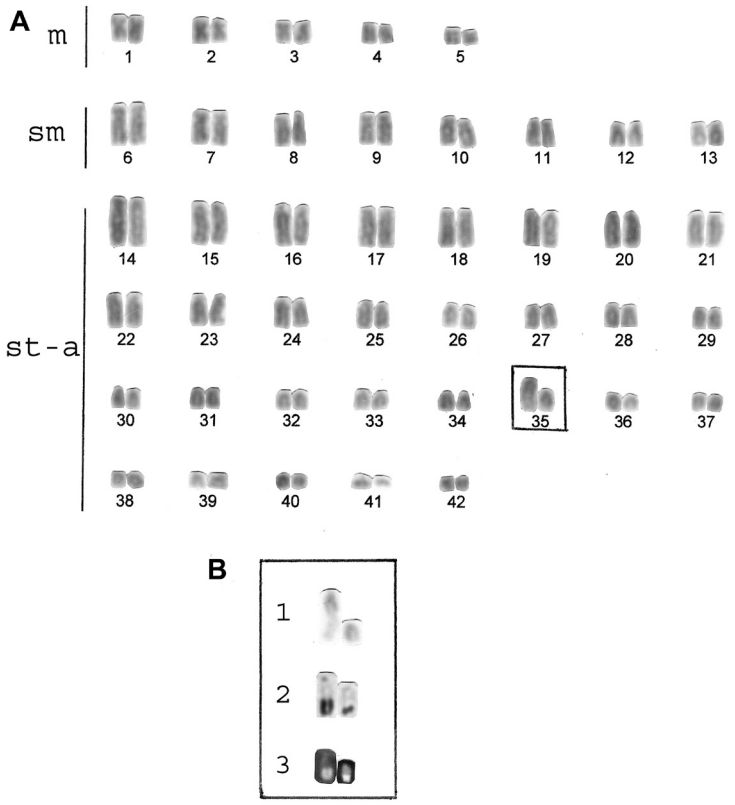

Figure 3. Karyotype of *Hypostomus* sp 2-Rio Perdido NUP 4249 with 2n = 84 chromosomes (A); secondary constriction observed in pair 35 (B): with Giemsa staining (B.1), impregnation with $AgNO_3$ (B.2) and staining with fluorochrome CMA_3 (B.3). m = metacentric; sm = submetacentric; st-a = subtelocentric-acrocentric.

In the two species, banding showed evidence of a heterochromatin distribution pattern characterized by the presence of a few small bands in the pericentromeric region, and by the very evident block in the terminal position of the long arms of small acrocentric chromosomes (Figure 4A.a and B.a). Staining with CMA_3 showed in *Hypostomus* sp 3-Córrego Salobrinha NUP 4247 at least two bands on the small acrocentric chromosomes, one of them being more evident than the other (Figure 4B.b). In *Hypostomus* sp 2-Rio Perdido NUP 4249, staining was very strong in the terminal region of the long arm of two small acrocentric chromosomes (Figure 4A.b). DAPI staining (Figure 4A.c and B.c) did not reveal any fluorescent bands in any of the two species studied.

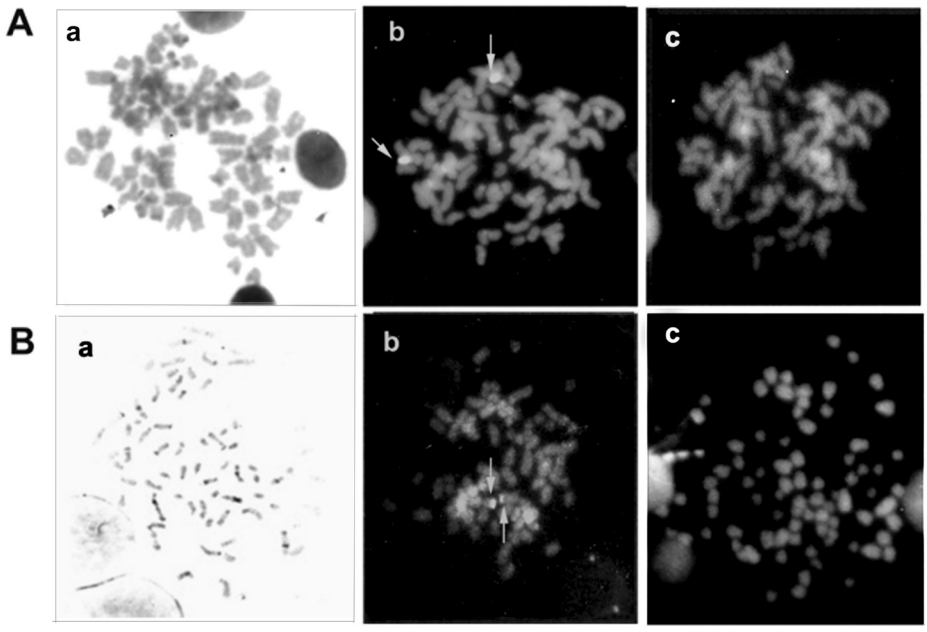

Figure 4. Metaphases showing pattern of distribution of heterochromatin in *Hypostomus* sp 2-Rio Perdido NUP 4249 (A) and *Hypostomus* sp 3-Córrego Salobrinha NUP 4247 (B) with C banding (a), with CMA_3 staining (b) and DAPI staining (c).

DISCUSSION

The largest diploid number described, until now, for genus *Hypostomus* was 2n = 80 chromosomes, in *Hypostomus* sp E (Artoni and Bertollo, 1996). However, *Hypostomus* sp 3-Córrego Salobrinha NUP 4247 and *Hypostomus* sp 2-Rio Perdido NUP 4249 showed 2n = 82 and 2n = 84 chromosomes, respectively, widening the range of variation for this genus.

In Hypostominae, the meta/submetacentric chromosomes are more frequent in species with lower diploid numbers, whereas the subtelo/acrocentric chromosomes prevail in species with higher diploid numbers (Artoni and Bertollo, 2001). This was evident in *Hypostomus* sp 3-Córrego Salobrinha NUP 4247 and *Hypostomus* sp 2-Rio Perdido NUP 4249 which were found to have many subtelo/acrocentric chromosomes, in contrast to *Hypostomus cochliodon* with 2n = 64 chromosomes which showed more meta/submetacentrics (Cereali, 2006).

In *Hypostomus* sp 3-Córrego Salobrinha NUP 4247, a chromosomal polymorphism with two cytotypes was observed, that is 2n = 82 and 2n = 84, due to the presence of extrachromosomes. These data are the first of such report for this genus. The difference between the two cytotypes is that there was one pair of small acrocentric chromosomes in 2n = 84, but in cytotype 2n = 83 the non-matched chromosome was one of the smallest acrocentrics of the complement. Because the chromosomes were not heterochromatic and due to the fact that they resembled the smaller sized acrocentrics, it was not possible to determine which were the extrachromosomes. Extrachromosomes non-heterochromatic as those found in *Hypostomus* sp 3-Córrego Salobrinha

NUP 4247 were also described in *Characidium* cf. *zebra* (Venere et al., 1999), *Steindachnerina insculpita* (Oliveira and Foresti, 1993) and *Rhamdia quelen* (Moraes et al., 2007).

Extrachromosomes, supernumeraries and B chromosomes have been reported most frequently in Characiformes (Venere et al., 1999; Neo et al., 2000; Maistro et al., 2004). However, few cases are described in Siluriformes (Fenocchio and Bertollo, 1990; Dias and Foresti, 1993). B chromosomes are described in a few species of Loricariidae: *Microlepdogaster leucofrenatus*, from 1 to 2 Bs, in two locations (Andreata et al., 1993, 1994); *Loricaria* sp and *L. prolixa*, from 1 to 5 Bs (Scavone and Júlio Jr., 1994).

Hypostomus sp 3-Córrego Salobrinha NUP 4247 showed intraindividual numerical variation due to the presence of extrachromosomes with very similar frequency, which could be considered a case of chromosomal mosaicism. Borin and Martins-Santos (2000), described intraindividual numerical polymorphism in *Thichomycterus davisi*, where the variation was attributed to a probable post-zygotic nondisjunction of a metacentric chromosome of medium/small size, followed by centric fission, producing a mosaic individual. Torres et al. (2002) use the expression "mosaicism" in the title of a study with fishes. The authors found an individual of *Thichomycterus paolence* suggesting the occurrence of aneuploidy due to post-zygotic nondisjunction during the first blastomere divisions.

According to Artoni and Bertollo (1999, 2001), there are two general patterns of heterochromatin distribution among the Loricariidae: 1 - few heterochromatin regions located in telomeric and/or centromeric regions, mostly associated with species with a smaller diploid number; 2 - many of these regions, besides heterochromatic segments in interstitial regions, mostly associated with species with a larger diploid number. The distribution of heterochromatin in *Hypostomus* sp 3-Córrego Salobrinha is consonant with the second group, while that in *Hypostomus* sp 2-Rio Perdido is close to the description of the first group.

Size heteromorphism among homologs of chromosomes stained with $AgNO_3$ has been reported in species of *Hypostomus* by Artoni and Bertollo (1996), and also observed in *Hypostomus* sp 2-Rio Perdido NUP 4249.

In Loricariidae, regions rich in GC are described in *Hypostomus* (Artoni et al., 1998; Artoni and Bertollo, 1999; Kavalco et al., 2004) and *Rineloricaria* (Giuliano-Caetano, 1998) among others. AT-rich regions are rarely described in fishes. Although some species of *Hypostomus* studied by Artoni and Bertollo (1999) show fluorescent bands with DAPI staining, *Hypostomus* sp 3-Córrego Salobrinha NUP 4247 and *Hypostomus* sp 2-Rio Perdido NUP 4249 did not show such staining with DAPI, which indicates that AT-rich regions were very small or did not exist in our study.

Chromosomal polymorphisms generally do not cause any effect on phenotype, where they are normally detected only cytogenetically. Various examples found in nature show both structural and numerical chromosomal polymorphism (Dias and Giuliano Caetano, 2002). Beçak et al. (1966) showed three karyotypes for *Lepomis cyanellus*, 2n = 48, 2n = 47 and 2n = 46. The standard karyotype for this species is 2n = 48, all acrocentric chromosomes. The karyotype 2n = 46 presented 44a + 2m, while 2n = 47 presented 46a and 1m. The autors concluded that this variation was due to a centric fusion between acrocentric chromosomes, with the formation of the metacentric chromosome. Intra-individual polymorphism was observed in the heterozygous specimens with the three karyotypes (2n = 48, 2n = 47, 2n = 46) and somatic segregation favoring the reconstitution of homozygous cells was proposed to explain this fact.

The development of polymorphic populations is important as much for the population as the individual. In heterogeneous environments, a uniform population generally

can exploit efficiently only some of the available ecological niches, while a polymorphic population is able to use more habitats when exploring the environment for those best suited (Ford, 1980). However, in the case of fishes, it is likely that periods of flood and drought, as well as climatic and physical chemistry alterations, have a greater influence on the occupation of niches than on the availability of habitats.

At the site where specimens of *Hypostomus* sp 2-Rio Perdido NUP 4249 were collected, the stretch of river is practically isolated by natural physical barriers. This is very unusual not only due to its physiognomy and the presence of only a few species (only nine) but also because among these, four were not found in any other place of the Planalto da Bodoquena and all were new to science, including the two species of *Hypostosmus* studied here. The Salobrinha Stream and Salobra River also have their own peculiarities, where *Hypostomus* sp 3-Córrego Salobrinha NUP 4247, in spite of being abundant there, is also a species not described previously.

These facts, combined with the characteristics of the region, make these populations of great interest. The continuity of cytogenetic studies of these and other species in the region is essential for a better comprehension of the evolutionary trends of the genus *Hypostomus* as a whole and especially in the Paraguay River basin.

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