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# Different cytotypes in fishes of the genus *Hypostomus* Lcépède, 1803, (Siluriformes: Loricariidae) from Xingu river (Amazon region, Brazil)

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**Abstract.** We analyzed the karyotypes of three specimens of fishes of the genus *Hypostomus* Lacépède, 1803 (Loricaridae) from Xingu River (Amazon region). We used conventional staining techniques, including C-banding, Ag-NOR staining, $CMA_3$- and DAPI-banding. Each specimen had a different cytotype: *Hypostomus* sp. Xingu-1 had 2n=64 (32M/SM, 32ST/A); *Hypostomus* sp. Xingu-2 has 2n=66 (32M/SM, 34ST/A), and *Hypostomus* sp. Xingu-3 had 2n=65 (38M/SM, 26ST/A + 1 B). The three cytotypes showed similar C-, $CMA_3$- and DAPI-banding patterns. The nucleolus organizing regions were located in the short arm of chromosome pair 25 of *Hypostomus* sp. Xingu-1 and pair 29 of *Hypostomus* sp. Xingu-2, and in the long arm of pair 30 of *Hypostomus* sp. Xingu-3, probably because of a pericentric inversion. A fusion/fission rearrangement explains the difference in the diploid number and number of M/SM and ST/A chromosomes between the 2n= 64 and 2n=66 cytotypes. The B chromosome most probably explains the difference between the 2n= 64 and 2n=65 cytotypes. The cytotype with 2n=65 had a significantly larger number of M/SM chromosomes, probably because of pericentric inversions. These three cytotypes may represent different species.

**Key words:** Siluriformes, *Hypostomus*, cytogenetics, heterochromatin, variability, C-banding, Ag-NOR, $CMA_3$ and DAPI.

## Introduction

The Loricariidae is one of the most diversified families within the order Siluriformes. It includes more than 600 species that may be divided into five subfamilies: Delturinae, Neoplecostominae, Hypoptopomatinae, Loricariinae and Hypostominae. These subfamilies are endemic to the Neotropical region with distributions extending from Panama to Uruguay (Isbrücker, 1980; Reis et al., 2003, 2006; Armbruster, 2004).

Using morpho-osteological analysis, Schaefer (1987) suggested that the subfamily Hypostominae is the most derived among the

Loricariidae. The same conclusion was made by Artoni, Bertollo (1996) using cytogenetic information. The subfamily Hypostominae includes numerous species that are broadly distributed throughout almost all rivers and lakes in South America (Armbruster, 2004; Alves et al., 2006). According to Armbruster (2004), this subfamily can be divided into five tribes: Corymbophanini, Rhinelepini, Hypostomini, Pterygoplichthini and Ancistrini. The tribe Hypostomini contains a single genus, *Hypostomus* Lacépède, 1803, which comprises 116 species and is found in most South American waters (Isbrücker, 1980).

The subfamily Hypostominae includes the largest number of karyotyped species within the Loricariidae (Table 1). The species of the Hypostominae show significant variations in chromosome number and morphology. The diploid number (2n) ranges from 52 to 80 chromosomes (Artoni, Bertollo, 1996, 2001). An XX/XY sex chromosome system has been described for some species (Michele et al., 1977), whereas others have a ZZ/ZW system (Artoni, 1996). The nucleolus organizer region (NOR) can be found as a single (Artoni, Bertollo, 1996, 2001; Artoni et al., 1998; Alves et al., 2006) or multiple pairs system (Artoni, 1996; Artoni, Bertollo, 1996; Alves et al., 2006). The composition of constitutive heterochromatin (CH) may be species-specific in *Hypostomus* with some blocks rich in A-T base pairs and others rich in G-C base pairs (Artoni, Bertollo, 1999).

In the present work, we describe the karyotypes of three specimens of *Hypostomus* from the Xingu River in the Amazon region of Brazil.

## Material and methods

The specimens were collected in the Amazon region, from the Xingu River in the town of Altamira (Para, Brazil; 03°12'48.0''S/52°12'41.7''W). Two females and a male were analyzed.

Because members of *Hypostomus* are very difficult to identify at the species level, we were unable to determine the species of our samples. Therefore, we called them

**Table 1.** Cytogenetic data of fishes of the genus *Hypostomus* in Brazil. 2n - diploid number, M - metacentric, SM - submetacentric, ST - subtelocentric, A - acrocentric, p - short arm, q - long arm, (*) - species with more than one karyotype, N - number of NOR bearing chromosomes.

| Species | Locality | 2n | Classification | | | Sex System | NOR | | References |
|---|---|---|---|---|---|---|---|---|---|
| | | | M | SM | ST/A | | Nº | Position | |
| *Hypostomus* sp. A | Rincão stream, Sao Paulo | **70** | 18 | 14 | 38 | – | 4 | q/A; p/M | Artoni, Bertollo, 1996 |
| *Hypostomus* sp. B | Mogi-Guaçu river, Sao Paulo | **72** | 12 | 18 | 42 | – | 2 | q/ST-A | Artoni, Bertollo, 1996 |
| *Hypostomus* sp. B (*) | Mogi-Guaçu river, Sao Paulo | **72** | 13 | 18 | 41 | – | 2 | – | Artoni, Bertollo, 1999 |
| *Hypostomus* sp. C | Mogi-Guaçu river, Sao Paulo | **72** | 10 | 18 | 44 | – | 3 | q/A | Artoni, Bertollo, 1996 |
| *Hypostomus* sp. $D_1$ | Mogi-Guaçu river, Sao Paulo | **72** | 10 | 26 | 36 | – | 2 | q/ST | Artoni, Bertollo, 1996 |
| *Hypostomus* sp. $D_2$ | Mogi-Guaçu river, Sao Paulo | **72** | 14 | 20 | 38 | – | 2 | q/A | Artoni, Bertollo, 1996 |
| *Hypostomus* sp. E | Mogi-Guaçu river, Sao Paulo | ***80*** | 8 | 16 | 56 | – | 4 | p/SM; p/ST | Artoni, Bertollo, 1996 |

**Table 1.** (Continuation).

| Species | Locality | 2n | Classification | | | Sex System | NOR | | References |
|---|---|---|---|---|---|---|---|---|---|
| | | | M | SM | ST/A | | Nº | Position | |
| *Hypostomus* sp. F | São Francisco river, Minas Gerais | **76** | 10 | 16 | 50 | – | 4 | q/SM; p/ST | Artoni, 1996 |
| *Hypostomus* sp. F (*) | São Francisco river, Minas Gerais | **75** | 10 | 17 | 48 | – | – | – | Artoni. Bertollo, 1999 |
| *Hypostomus* sp. G | Fundo brook, Araguaia river, Mato Grosso | **64** | 14 | 24 | 26 | ZZ/ZW | 2 | p/SM | Artoni et al., 1998 |
| *Hypostomus albopunctatus* | Mogi-Guaçu river, Sao Paulo | **74** | 10 | 20 | 44 | – | 4 | q/A; p/M | Artoni, Bertollo, 1996 |
| *Hypostomus ancistroides* | – | **68** | 10 | 28 | 30 | – | – | – | Michele et al., 1977 |
| *Hypostomus ancistroides* | Mogi-Guaçu river, Sao Paulo; Monjolinho brook, Sao Paulo | **68** | 16 | 18 | 34 | – | 6 | p/M; p/SM; p/A | Artoni, Bertollo, 1996 |
| *Hypostomus ancistroides* | Araquá river, Sao Paulo | **68** | 18 | 10 | 40 | – | 6 | p/M; p/SM; p/SM | Alves et al., 2006 |
| *Hypostomus affinis* | Jacuí brook, Sao Paulo | **66** | 14 | 14 | 38 | – | – | – | Kavalco et al., 2005 |
| *Hypostomus* prope *auroguttatus* | Mogi-Guaçu river, Sao Paulo | **76** | 8 | 30 | 38 | – | 2 | q/A | Artoni. Bertollo, 1996 |
| *Hypostomus emarginatus* | Araguaia river, Mato Grosso | **52** | 16 | 30 | 6 | – | 2 | q/M | Artoni, Bertollo, 2001 |
| *Hypostomus goyazensis* | Vermelho river, Goias | **72** | 10 | 16 | 46 | – | 2 | p/ST | Alves et al., 2006 |
| *Hypostomus macrops* | – | **68** | 10 | 14 | 44 | XX/XY | – | – | Michele et al., 1977 |
| *Hypostomus nigromaculatus* | Mogi-Guaçu river, Sao Paulo | **76** | 8 | 20 | 48 | – | 2 | p/ST; q/A | Rubert et al., 2008 |
| *Hypostomus paulinus* | – | **74** | 10 | 20 | 44 | – | – | – | Michele et al., 1977 |
| *Hypostomus plecostomus* | – | **54** | 36 | | 18 | – | – | – | Muramoto et al., 1968 |
| *Hypostomus regani* | Mogi-Guaçu river, Sao Paulo | **72** | 10 | 20 | 42 | – | 4 | q/A; p/ST | Artoni, Bertollo (1996) |
| *Hypostomus regani* | Araquá river, Sao Paulo | **72** | 12 | 18 | 42 | – | 4 | q/A; q/A | Alves et al., 2006 |
| *Hypostomus strigaticeps* | – | **74** | 8 | 4 | 62 | – | – | – | Michele et al., 1977 |
| *Hypostomus* sp. 1 | Quinta brook, Sao Paulo | **72** | – | – | – | – | – | – | Fenerich, 1998 |
| *Hypostomus* sp. 2 | Alambari brook, Sao Paulo | **68** | – | – | – | – | – | – | Fenerich, 1998 |
| *Hypostomus* sp. 3 | Paranapanema river, Sao Paulo | **66** | – | – | – | – | – | – | Fenerich, 1998 |
| *Hypostomus* sp. 4 | Hortelã brook, Sao Paulo | **76** | – | – | – | – | – | – | Fenerich, 1998 |
| *Hypostomus* sp. Xingu-1 | Xingu river, Para | **64** | 16 | 16 | 32 | – | 2 | p/A | Present study |
| *Hypostomus* sp. Xingu-2 | Xingu river, Para | **66** | 18 | 14 | 34 | – | 2 | p/A | Present study |
| *Hypostomus* sp. Xingu-3 | Xingu river, Para | **65** | 15 | 23 | 26+1B | – | 2 | q/A | Present study |

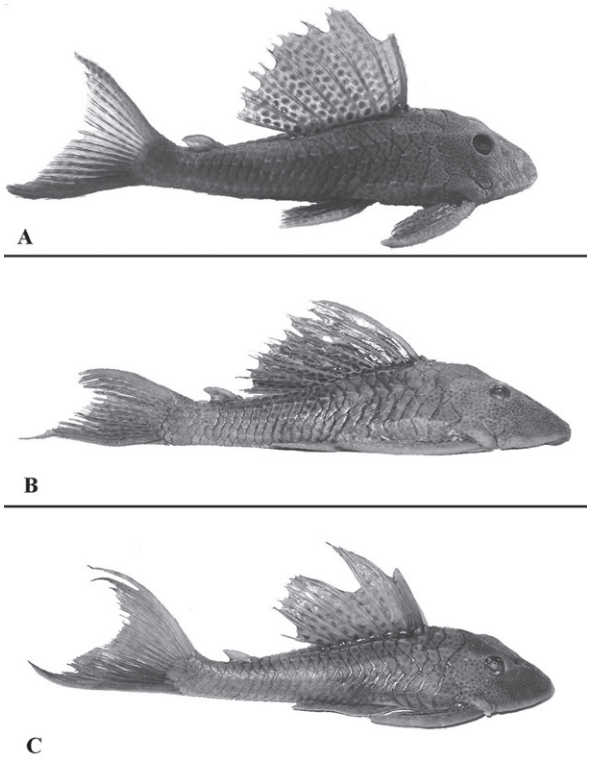


**Fig. 1**, **a-c. a** - *Hypostomus* sp. Xingu-1. **b** - *Hypostomus* sp. Xingu-2. **c** - *Hypostomus* sp. Xingu-3.

*Hymostomus* sp. Xingu-1, *Hymostomus sp.* Xingu-2 and *Hymostomus sp.* Xingu-3.

Chromosome preparation was performed as described in Bertollo et al. (1978). The chromosomes were classified as described in Guerra (1986). We used conventional staining techniques, including C-banding (Sumner, 1972), Ag-NOR staining (Howell, Black, 1980), and the fluorochromes chromomycin $A_3$ ($CMA_3$) (Schweizer, 1980) and 4'6'-diamidino-2-phenylindole (DAPI) (Pieczarka et al., 2006).

The fish specimens studied were vouchered at the Museu Paraense Emilio Goeldi (MPEG) under the following numbers: *Hymostomus* sp. Xingu-1, MPEG 13431; *Hymostomus* sp. Xingu-2, MPEG 13430; and *Hymostomus* sp. Xingu-3, MPEG 13429.

## Results

*Hymostomus* sp. Xingu-1, a female (Fig. 1, a), shows 2n=64 and a karyotype formula (KF) with 32M/SM+32ST/A (Fig. 2, a). *Hymostomus* sp. Xingu-2, a male (Fig. 1, b), shows 2n=66 and KF with 32M/SM+34ST/A (Fig. 3, a). *Hymostomus* sp. Xingu-3, a female (Fig. 1, c), shows 2n=65, KF with 38M/SM+26ST/A + 1 B microchromosome (Fig. 4, a).

*Hymostomus* sp. Xingu-1 has blocks of CH in the distal region of both arms of metacentric pair 14, in the proximal region of the long arm and the distal region of the short arm of submetacentric pair 6, and at the distal region of the long arm of pair 25 (Fig. 2, B). *Hymostomus* sp. Xingu-2 has evident heterochromatic blocks found in the distal regions of both arms of metacentric pair 13 and in the distal region of the long arm of pair 29. There is a size heteromorphism of the CH block in the proximal region of the long arm of pair 4 (Fig. 3, B). In *Hymostomus* sp. Xingu-3 chromosome pair 9 is heteromorphic: one of the homologues is metacentric and the other is submetacentric, due to a heteromorphism of the heterochromatin in the proximal region of the long arm: there is an interstitial heterochromatic block in only one of the homologues (Fig. 4, b). Chromosome pair 5 has small blocks of heterochromatin in the distal regions of both arms (Fig. 4, b).

In *Hymostomus* sp. Xingu-1, the NOR is of the simple type, and it is located on the distal short arm of pair 25 (Fig. 5, a). Heteromorphism in the NOR size was found, and the NOR is C-band negative. In *Hymostomus* sp. Xingu-2, the NOR is present in the short arm of pair 29 (Fig. 5, b). In *Hymostomus* sp. Xingu-3 the NOR is found

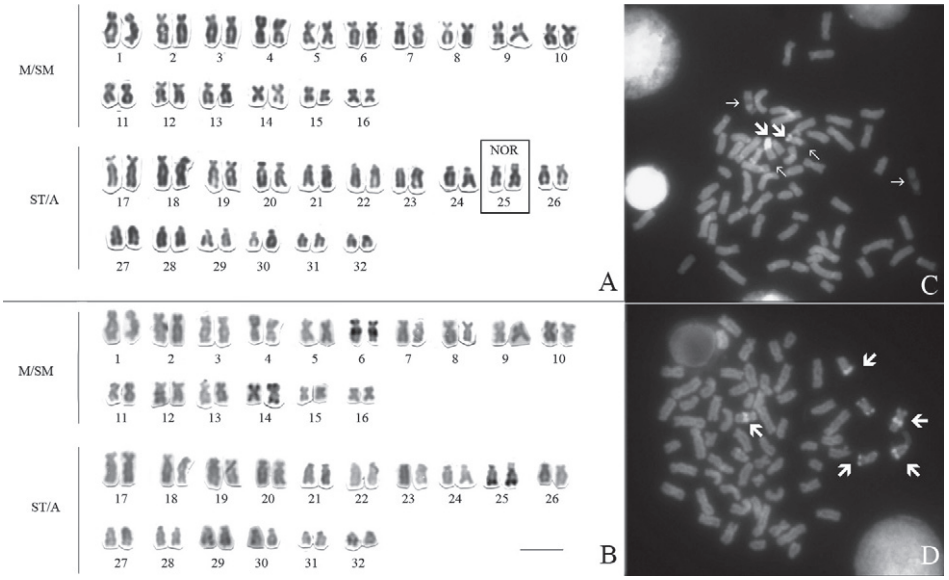


**Fig. 2, a-d.** Karyotype of *Hypostomus* sp. Xingu-1. **a** - Giemsa staining, showing the NOR-bearing pair. **b** - C-banded karyotype. **c** - metaphase spread stained with $CMA_3$ (larger arrows show the NOR region, smaller arrows show the $CMA_3$-quenched regions). **d** - DAPI-banding (arrows show the positive C-banded regions that are A-T rich). Bar = 10 μm.

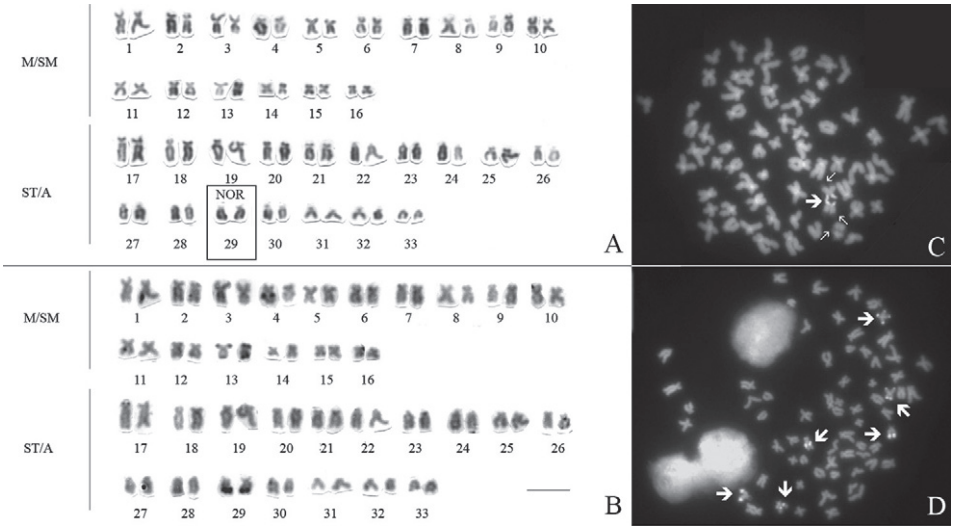


**Fig. 3**, **a-d.** Karyotype of *Hypostomus* sp. Xingu-2. **a** - Giemsa staining, showing the NOR-bearing pair. **b** - C-banded karyotype. **c** - metaphase spread stained with $CMA_3$ (larger arrows show the NOR region, smaller arrows show the $CMA_3$-quenched regions). **d** - DAPI-banding (arrows show the positive C-banded regions that are A-T rich). Bar = 10 μm.

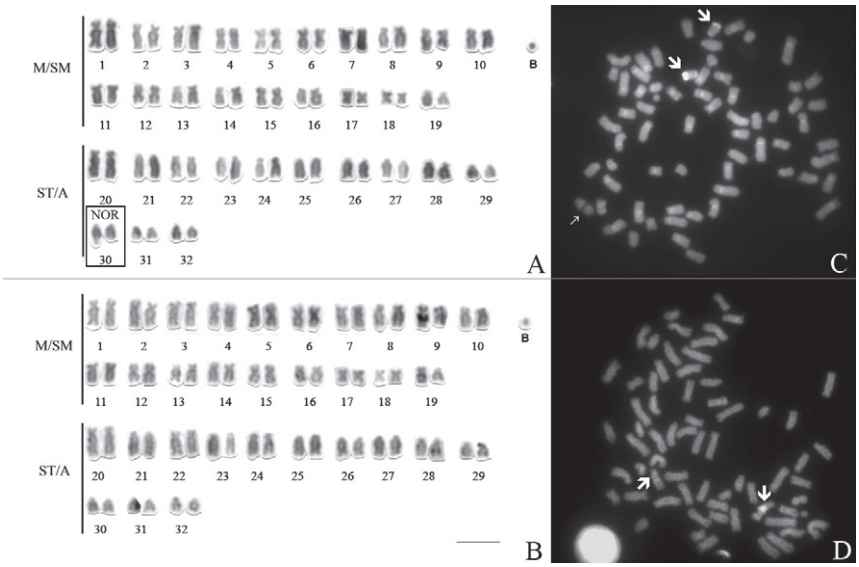


**Fig. 4**, **a-d.** Karyotype of *Hypostomus* sp. Xingu-3. **a** - Giemsa staining, showing the NOR-bearing pair (B represents the minichromosome). **b** - C-banded karyotype. **c** - metaphase spread stained with $CMA_3$ (larger arrows show the NOR region, smaller arrows show the $CMA_3$-quenched regions). **d** - DAPI-banding (arrows show the positive C-banded regions that are A-T rich). Bar = 10 μm.

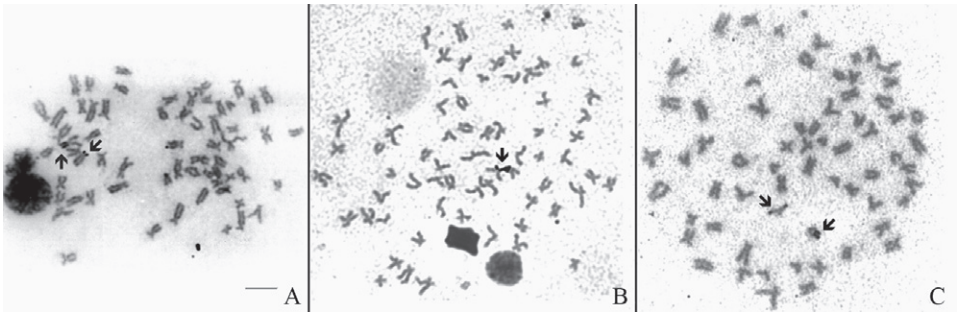


**Fig. 5**, **a-c.** Metaphases submitted to Ag-NOR technique. The arrows show the NOR-bearing chromosomes. **a** - *Hypostomus* sp. Xingu-1. **b** - *Hypostomus* sp. Xingu-2. **c** - *Hypostomus* sp. Xingu-3. Bar = 10 μm.

at the distal region of the long arm of pair 30 and shows size heteromorphism (Fig. 5, c).

The heterochromatic blocks of pairs 6, 14 and 25 produce bright DAPI fluorescence (Fig. 2, d) in *Hymostomus* sp. Xingu-1, whereas the heterochromatic blocks of pairs 14 and 25 have quenched $CMA_3$ fluorescence. As they also are DAPI bright, these regions are likely to be rich in A-T base pairs. In *Hymostomus* sp. Xingu-2 the heterochromatic blocks of pairs 4, 13 and 29 are DAPI-bright (Fig. 3, d). $CMA_3$ staining is bright at the centromeres and at the NOR (Fig. 3, c), while the CH blocks of pairs 4 and 29 are $CMA_3$-quenched (Fig. 3, c). *Hymostomus* sp. Xingu-3 has DAPI staining bright in the heterochromatin blocks of pairs

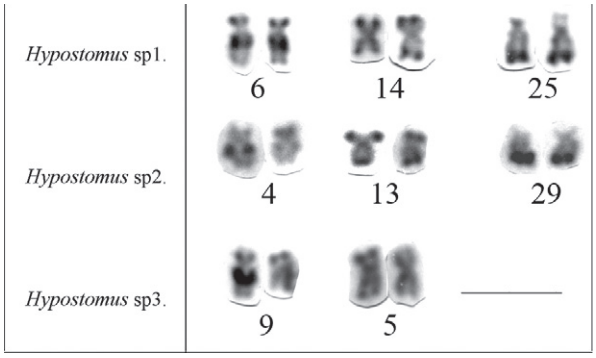


**Fig. 6.** Probable homologies among the specimens studied herein, as suggested by C-banding. Bar = 10 μm.

5 and 9 (Fig. 4, d). $CMA_3$ is bright in most of the centromeres and in the NOR (Fig. 4, c), whereas the above mentioned heterochromatic block in pair 9 is $CMA_3$-quenched (Fig. 4, c). The additional small chromosome (a "B" or supernumerary chromosome) is neither heterochromatic nor bright with DAPI or $CMA_3$ staining.

## Discussion

A comparative analysis of the three karyotypes suggests that a fusion/fission-type rearrangement could explain the differences in the 2n and in the number of M/SM and ST/A chromosomes between the cytotypes with 2n=64 and 2n=66. The presence of a B chromosome explains the difference in the 2n of the cytotypes with 2n=65 and 2n=64. In addition, the cytotype with 2n=65 has more M/SM chromosomes than the other cytotypes, probably because of pericentric inversions.

When comparing our results with prior descriptions in the literature, we have found that *Hymostomus* sp. Xingu-1 (2n=64; 32M/SM+32ST/A) has the same diploid number as *Hymostomus* sp. G (2n=64; 14M+24SM+26ST/A, Artoni et al., 1998), while *Hymostomus* sp. Xingu-2 (2n=66; 32M/SM+34ST/) has the same diploid number as *H. affinis* Steindachner, 1877 (2n=66; 14M+14SM+38ST/A, Kavalco et al., 2005). However, the karyotypic formulae differ in both cases, probably because of pericentric inversions, which can change chromosome morphology without changing the diploid number.

The third cytotype, *Hymostomus* sp. Xingu-3 (2n=65; 38M/SM+26ST/A + 1 B) has a karyotype similar to that of *Hymostomus* sp. G, described above. However, *Hymostomus* sp. Xingu-3 differs from *Hymostomus* sp. G in the heteromorphism of pair 9 and the presence of a B chromosome. Notably, this is the first report of a B chromosome in *Hypostomus*. This comparison suggests that *Hymostomus* sp. Xingu-3 is more similar to *Hymostomus* sp. G from the Araguaia River (which is linked to the Amazon basin), than to the other two cytotypes obtained from the Xingu River.

All the three specimens have NORs of a simple type located respectively on the short arms of pairs 25 and 29, and the long arm of pair 30. The difference in the NOR position in *Hymostomus* sp. Xingu-3 can be explained by a pericentric inversion. In fishes, NORs are often found on a single pair of chromosomes, as seen in *Hypostomus* (Artoni, Bertollo 1996, 2001; Artoni et al., 1998, Alves et al., 2006), although there are many inter- and intraspecific deviations from this trend (Oliveira, 1987; Andreata, 1991; Fenocchio, 1993; Artoni, 1996; Souza, 2003). Artoni (1996) claimed that most of the Hypostominae species with a single pair of NORs have heteromorphism in NOR size. This is consistent with the pattern we found in *Hymostomus* sp. Xingu-1 and *Hymostomus* sp. Xingu-3 (Fig. 5).

The NORs showed bright $CMA_3$ banding, indicating that these sequences are G-C rich. This fluorochrome stains regions independently of the transcriptional activity in the previous interphase (Howell, 1977; Almeida-Toledo, 1998).

*Hypostomus* has relatively little constitutive heterochromatin (Artoni, 1996). The cytotypes studied by us have very similar patterns of heterochromatin distribution, with only few chromosome pairs showing small heterochromatic blocks. It is possible to compare the homologies among the pairs based on their heterochromatin patterns (Fig. 6). Our $CMA_3$ and DAPI staining results suggest that the three cytotypes have a heterogeneous composition of constitutive heterochromatin, with some blocks rich in A-T base pairs (DAPI bright) and other rich in G-C base pairs ($CMA_3$ bright). The same result was found in some species of *Hypostomus* by Artoni and Bertollo (1999). However, these results differ from that obtained by Kavalco et al. (2005), who demonstrated that *H. affinis* has its CH exclusively rich in GC pairs.

According to Camacho et al. (2000) the B chromosomes are typically heterochromatic. Andreata et al (1993) described two large heterochromatic B chromosomes in *Microlepidogaster leucofrenatus* (Miranda Ribeiro, 1908) (Siluriformes: Loricariidae). In our study the B-chromosome was neither heterochromatic nor especially rich in AT or GC-bp. More detailed molecular studies may help to understand its nature.

Artoni and Bertollo (2001) proposed that the karyotypes with 2n=54 should be an ancestral condition in *Hypostomus* karyotypes, since this diploid number is found in different genera and even families. As a consequence, centric fissions must be important rearrangements for the evolution of karyotypes of *Hypostomus*, in which the diploid numbers range from 2n=52 to 2n=80 (Table 1). Also, the number of ST/A chromosomes increases in karyotypes with higher diploid numbers, with a consequent reduction in the number of M/SM chromosomes. Kavalco et al. (2005) suggest that Robertsonian rearrangements, as well as pericentric inversions, were the main changes related with the karyotypic diversification of the Hypostominae. Our data are in agreement with this suggestion, since the differences among the three karyotypes studied can be a consequence of these rearrangements.

Despite the differences in diploid numbers and karyotypic formulae, the three cytotypes share similar patterns of heterochromatin distribution and the presence of a single NOR. These results suggest that: a) the three specimens could be members of a single species that maintains the observed chromosome differences as polymorphisms; b) the population could be undergoing a chromosome-based speciation event through which individuals are diverging from a common ancestor; or c) the specimens may represent members of different species that arose from a common ancestor. Additional studies will be required to distinguish among these possibilities, but at the moment the option "c" seems to be the most reasonable. The lack of cytogenetic information and the lack of knowledge of most of the species morphology (see Table 1, where many *Hypostomus* are "sp.") turns the *Hypostomus* genus one of the most interesting for any kind of study. New information is always helpful, like that described here.

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