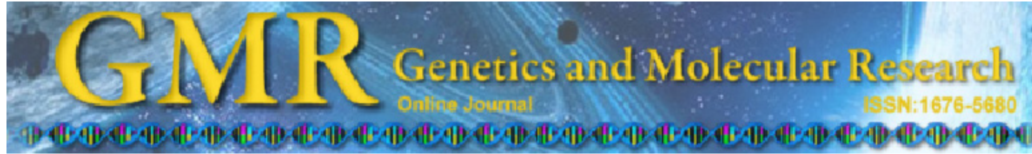

# Population divergence and peculiar karyoevolutionary trends in the loricariid fish *Hypostomus* aff. *unae* from northeastern Brazil

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**ABSTRACT.** Loricariidae (Siluriformes, Hypostominae) is one of the most diverse catfish families. In spite of the wide distribution of loricariids in South America, cytogenetic reports are available for only a few species, mostly from southern and southeastern Brazil. We made the first chromosomal analysis of *Hypostomus* aff. *unae* from the Contas River basin in northeastern Brazil. Four populations isolated by short distances but from distinct landscapes were studied based on conventional staining, C-banding, argyrophilic nucleolar organizer regions (Ag-NOR), $CMA_3$/DAPI fluorochrome staining, and fluorescent *in situ* hybridization with 18S rDNA probes. Although sharing the same diploid number (2n = 76) and NOR locations, each population presented exclusive karyotype formulae and specific patterns of heterochromatic and AT-rich regions. The derived karyotypes of *H*. aff. *unae* (2n >54; high number of acrocentrics bearing AT-rich interstitial heterochromatin) indicated a divergent karyoevolution, mostly driven by centric fissions, pericentric inversions and particular

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heterochromatin dispersion models. This finding of distinct evolutionary units in *H.* aff. *unae* will be useful for understanding the natural history of loricariids from relatively unexplored coastal basins in South America.

**Key words:** Cytogenetics; Biodiversity; Heterochromatin; Fluorochromes; Loricariidae

## INTRODUCTION

Loricariidae is the largest catfish family and the fifth most species-rich group of the 515 recognized fish families (Nelson, 2006) comprising about 710 species and 96 genera (Ferraris Jr., 2007). As expected, loricariids are regarded as a dominant and taxonomically complex freshwater fish group in the Neotropical region (Nelson, 2006). The genus *Hypostomus* stands out as one of the most abundant and puzzling ones in this family, with approximately 130 species and more species being described every year (Zawadzki et al., 2008).

On the other hand, cytogenetic studies in Neotropical fish have shown a unique genomic plasticity, higher than that of any other vertebrate group (Nirchio and Oliveira, 2006). In fact, chromosomal analyses have been successful in determining the real number of species among morphologically similar (cryptic) forms of several tropical fish groups and evolutionarily significant units (Oliveira et al., 2009a). Unfortunately, little emphasis has been placed on chromosomal variability in conservation genetics, for instance, although cytogenetic analyses cannot be replaced by DNA studies and chromosomal differences often affect fertility (Allendorf and Luikart, 2007).

A remarkable karyotypic variation has been identified within Loricariidae, with diploid numbers ranging from 2n = 34 in *Ancistrus* sp (Oliveira et al., 2009b) to 2n = 96 in *Upsilodus* sp (Kavalco et al., 2005) and quite distinct karyotype formulae. Such diversity suggests that centric fissions and pericentric inversions have played a major role in the karyoevolutionary divergence of this family (Artoni and Bertollo, 2001). Nevertheless, cytogenetic studies in Loricariidae are still scarce and the available data account for only about 17% of the described species.

Moreover, the cytogenetic reports in fish populations from northeastern basins in South America are incipient when compared to other regions, in spite of their threatened and endemic fauna (Jacobina et al., 2009). Thus, in the present study, we provide the first cytogenetic analysis in populations of a loricariid species (*Hypostomus* aff. *unae* Steindachner, 1878) from coastal rivers in northeastern Brazil and infer about the trends of chromosomal evolution within the genus and the family as well.

## MATERIAL AND METHODS

Forty-six specimens of *H.* aff. *unae* from the Contas River basin, Bahia, northeastern Brazil, were analyzed: 3 males, 2 females and 5 juveniles from the main channel of the Contas River (13°51'51"S/40°04'54"W), 6 males, 1 female and 3 immature specimens from the Preto do Costa River (13°45'84"S/39°56'47"W), 9 males and 6 juveniles from the Oricó River (14°08'03"S/39°21'30"W), and 4 males, 4 females and 3 juveniles from the Preto do Criciúma River (13°55'45"S/39°57'57"W) (Figure 1). These populations were correspondingly named A, B, C, and D. Collection sites B, C, and D are about 17, 80 and 6 km apart from the collection site in the Contas River (site A), respectively. Voucher specimens were identified by Dr.

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Claudio Zawadski (UEM) and deposited in the ichthyological collection at the Universidade Estadual de Maringá (UEM - NUPELIA), PR, Brazil (NUP 9811, 9814).

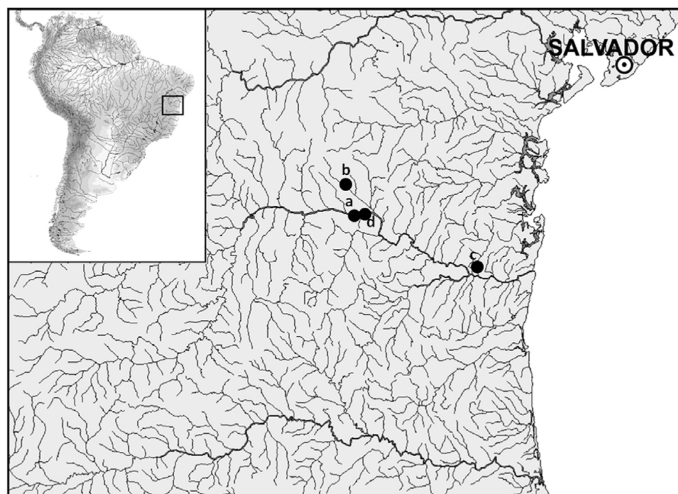


**Figure 1.** Map of the Contas River basin in Bahia State, Brazil, northeastern South America, indicating the collection sites of *Hypostomus* aff. *unae*. Contas River (**a**), Preto do Costa River (**b**), Oricó River (**c**), and Preto do Criciúma Stream (**d**).

Mitotic chromosomes were obtained from kidney cells according to Bertollo et al. (1978) after mitotic stimulation using a commercial bacterial and fungal antigen (Munolan®) as proposed by Molina (2001). The chromosomes were classified as: metacentric (m), submetacentric (sm), subtelocentric (st), and acrocentric (a), as elsewhere described in fish cytogenetics (Artoni and Bertollo, 2001; Alves et al., 2006). The fundamental arm number (FN) was calculated taking into account that m/sm chromosomes are bi-armed and st/a chromosomes are uni-armed.

The nucleolar organizer regions (NORs) were detected by silver nitrate staining (Howell and Black, 1980) and fluorescent *in situ* hybridization (FISH) (Pinkel et al., 1986) with slight modifications. The 18S rDNA probe was obtained from genomic DNA of *Prochilodus argenteus* (Prochilodontidae) by PCR (Hatanaka and Galetti, 2004) using NS1 (5'-GTAGTCATATGCTTGTCTC-3') and NS8 (5'-TCCGCAGGTTCACCTACGGA-3') primers (White et al., 1990). The probe was indirectly labeled via nick translation, using biotinylated adenine (14dATP-biotin; Invitrogen) and then detected by avidin-FITC (conjugated fluorescein isothiocyanate-avidin; Sigma).

The heterochromatin distribution was analyzed by C-banding (Sumner, 1972). GC- and AT-rich chromosomal regions were visualized by fluorochrome staining, using chromomycin $A_3$ ($CMA_3$) and DAPI, respectively (Schweizer, 1980).

The chromosomal analyses were carried out on an epifluorescence microscope Olympus BX51 and the micrographs were obtained using the Image Pro-Plus 6.1 software (Media Cybernetics).

## RESULTS

Regardless of the collection site, the modal diploid number observed in *H.* aff. *unae* was 2n = 76. However, interpopulation differences with exclusive karyotype formulae

were detected for each locality, as follows: 12m+16sm+48st/a (FN = 104) for population A, 12m+20sm+44st/a (FN = 108) for population B, 10m+14sm+52st/a (FN = 100) for population C, and 10m+20sm+46st/a (FN = 106) for population D (Figure 2).

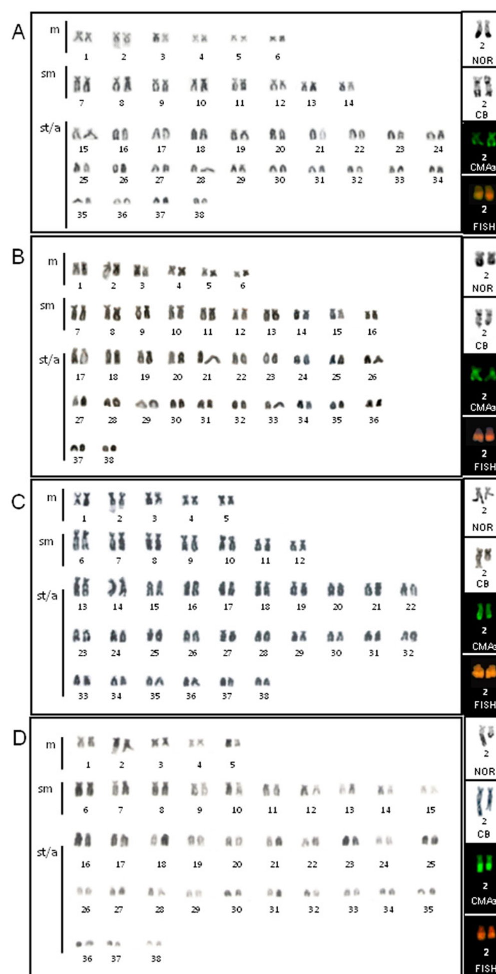


**Figure 2.** Karyotypes of *Hypostomus* aff. *unae* populations. (**A**) Contas, (**B**) Preto do Costa, (**C**) Oricó, and (**D**) Preto do Criciúma Rivers. Close-up of the NOR-bearing pairs after silver nitrate staining, C-banding, $CMA_3$ staining and 18S rDNA FISH. Notice the absence of NOR-associated heterochromatin in C.

Heteromorphic secondary constrictions located at the terminal position on the 2nd metacentric pair and equivalent to NORs were visualized in all specimens (Figure 2, close-up). The active rDNA sites detected by silver nitrate were confirmed by FISH using 18S rDNA probes, characterizing a single-NOR system. Similarly to secondary constrictions and Ag-NORs, a size heteromorphism of ribosomal cistrons between homologues was also observed by FISH (Figure 2, close-up).

Besides centromeric heterochromatin, all populations presented conspicuous interstitial and/or terminal C-bands in several acrocentric chromosomes (Figure 3). However, the dis-

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tribution of heterochromatic regions was specific for each collection site, as described below.

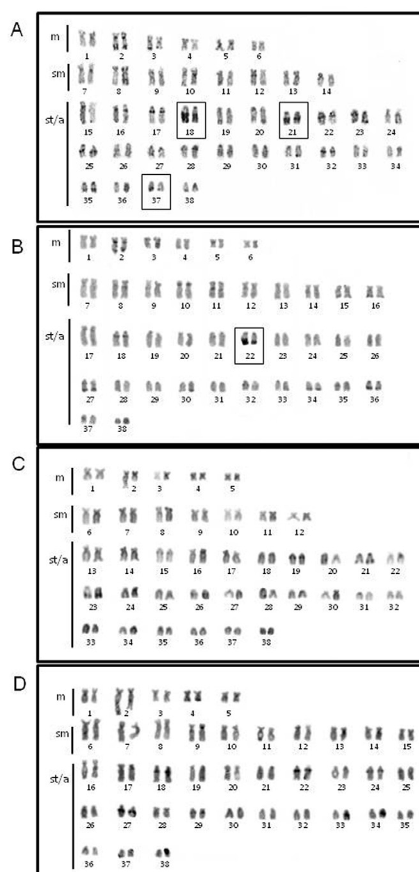

**Figure 3.** Karyotypes of *Hypostomus* aff. *unae* after C-banding in populations **A**, **B**, **C**, and **D**. The chromosomal pairs bearing heteromorphic heterochromatin are highlighted in A and B.

Population A differs from the others by presenting evident C-bands and a large number of chromosomes bearing heteromorphic blocks (pairs 18, 21 and 37) (Figure 3A). Population B was characterized by a single noticeable pair with heteromorphic heterochromatin (pair 22) (Figure 3B). On the other hand, heterochromatin differences between homologues were absent in populations C and D (Figure 3C and D, respectively). All specimens, excepting those from population C, showed NOR-associated heterochromatin.

Base-specific fluorochrome staining revealed a single chromosomal pair bearing $CMA_3^+$/$DAPI^-$ signals coincident to NORs, indicating that this region is GC-rich (Figure 4). Furthermore, several AT-rich sites ($DAPI^+$) were detected in the four populations, mainly distributed over interstitial and terminal regions of acrocentric chromosomes (Figure 5).

Based on the cytogenetic methodologies applied in the present study, there is no evidence of heteromorphic sex chromosomes in *H.* aff. *unae*.

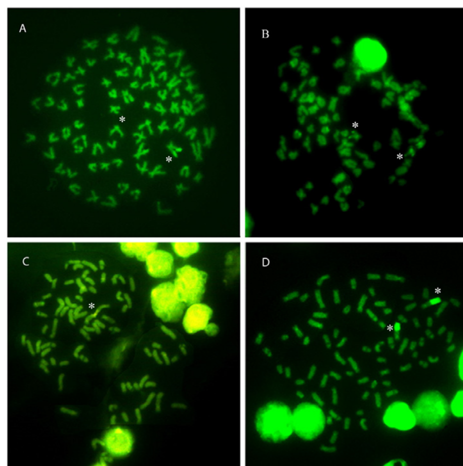


**Figure 4.** Metaphases of *Hypostomus* aff. *unae* from populations **A**, **B**, **C**, and **D** after $CMA_3$ staining. The asterisks indicate the NOR-bearing pair.

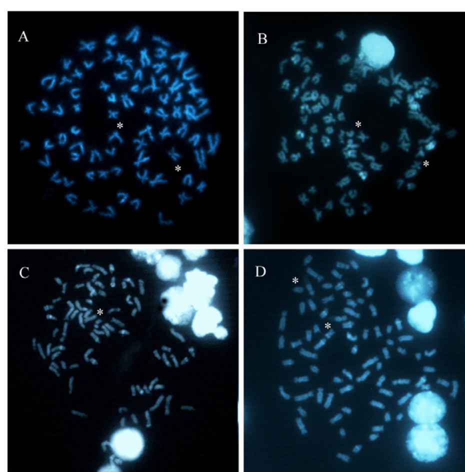


**Figure 5.** Metaphases of *Hypostomus* aff. *unae* from populations **A**, **B**, **C**, and **D** after DAPI staining, showing several chromosomes bearing positively stained AT-rich regions. The asterisks indicate the NOR-bearing pair.

## DISCUSSION

So far, cytogenetic reports are available for 31 of the 130 recognized *Hypostomus* species, including the present study (Table 1). Based on these data, 2n = 76 and 2n = 72 are the most frequent condition in this genus, representing 25.8% of all analyzed species each, followed by 2n = 74 (12.9%) and 2n = 68 (9.6%).

Nevertheless, comparative analyses with related basal groups show 2n = 54 to be the plesiomorphic diploid number in Loricariidae (Artoni and Bertollo, 2001). Although shared by several subfamilies, this condition has been reported in a single *Hypostomus* species and a

**Table 1.** Cytogenetic data in *Hypostomus* species.

| Species | Locality | 2n | Chromosomal formula | B | Sex system | Ref. |
|---|---|---|---|---|---|---|
| *Hypostomus* | | | | | | |
| *H. affinis* | Jacuí Stream (SP) | 66 | 14m+14sm+12st+26a | - | - | 10 |
| *H. ancistroides* | Tributaries of Tibagi River (PR) | 68 | 16m+18sm+34st/a | - | - | 3 |
| *H. ancistroides* | - | 68 | 10m+26sm+32st/a | - | - | 13 |
| *H. ancistroides* | Mogi-Guaçu River (SP) | 68 | 16m+18sm+34st/a | - | - | 3 |
| *H. ancistroides* | Araquá River (SP) | 68 | 18m+10sm+12st+28a | - | - | 1 |
| *H. ancistroides* | - | 68 | 10m+27sm+31st/a (M)<br>10m+28sm+30st/a (F) | - | XX/XY | 11 |
| *H. albopunctatus* | Mogi-Guaçu River (SP) | 74 | 10m+20sm+44st/a | - | - | 3 |
| *H. albopunctatus* | Piracicaba River (SP) | 74 | 10m+20sm+44st/a | - | - | 7 |
| *H.* aff. *auroguttatus* | Mogi-Guaçu River (SP) | 76 | 8m+30sm+38st/a | - | - | 3 |
| *H. emarginatus* | Araguaia River (MT) | 52 | 16m+30sm+6st/a | - | - | 6 |
| *H. goyazensis* | Vermelho River (GO) | 72 | 10m+16sm+10st+36a | - | - | 1 |
| *H. macrops* | - | 68 | 10m+14sm+44st/a | - | - | 11 |
| *H. paulinus* | - | 74 | 10m+20sm+44st/a | - | - | 11 |
| *H. paulinus* | Três Bocas Stream | 76 | 6m+16sm+54st/a | - | - | 13 |
| *H. plecostomus* | - | 54 | 24m/sm+12st+18a | - | - | 12 |
| *H. regani* | Mogi-Guaçu River (SP) | 72 | 10m+20sm+42st/a | - | - | 3 |
| *H. regani* | Araquá River (SP) | 72 | 12m+18sm+26st+16a | - | - | 1 |
| *H. regani* | Jacutinga Stream (PR) | 72 | 10m+18sm+44st/a | - | - | 13 |
| *H. regani* | Piumhi - São Francisco River (MG) | 72 | 8m+16sm+20st+28a | - | - | 15 |
| *H. strigaticeps* | Três Boca Stream, Jacutinga and Taquari River (PR) | 72 | 10m+16sm+46st/a | - | - | 13 |
| *H. strigaticeps* | Mogi-Guaçu River (SP) | 74 | 8m+4sm+62st/a | - | - | 11 |
| *Hypostomus* sp 3- *Ribeirão Salobrinha NUP 4247* | Salobrinha Stream (MS) | 82<br>83<br>84 | 6m+12sm+64st/a<br>6m+12sm+65st/a<br>6m+12sm+66st/a | 1-2 | - | 8 |
| *Hypostomus* sp *2-Rio Perdido NUP 4249* | Perdido River (MS) | 84 | 6m+16sm+62st/a | - | - | 8 |
| *Hypostomus* sp 1 | Paranapanema River (SP) | 64 | - | - | - | 9 |
| *Hypostomus* sp 1a | Patos Stream (MG) | 76 | 6m+8sm+16st+46a | - | - | 15 |
| *Hypostomus* sp 1b | Araras Stream (MG) | 76 | 6m+8sm+16st+46a | - | - | 15 |
| *Hypostomus* sp 2 | Araras Stream (MG) | 74 | 10m+6sm+16st+42a | - | - | 15 |
| *Hypostomus* sp 2 | Jacutinga Stream (SP) | 68 | - | - | - | 9 |
| *Hypostomus* sp 2 | Alambari Stream (SP) | 68 | - | - | - | 9 |
| *Hypostomus* sp 3 | Quinta Stream (SP) | 72 | - | - | - | 9 |
| *Hypostomus* sp 3 | Edgardia Stream (SP) | 72 | - | - | - | 9 |
| *Hypostomus* sp 3 | Paranapanema River (SP) | 72 | - | - | - | 9 |
| *Hypostomus* sp 4 | Hortelã Stream (SP) | 76 | - | - | - | 9 |
| *Hypostomus* sp 4 | Paranapanema River (SP) | 76 | - | - | - | 9 |
| *Hypostomus* sp *A* | Rincão River (SP) | 70 | 18m+14sm+38st/a | - | - | 3 |
| *Hypostomus* sp *B* | Mogi-Guaçu River (SP) | 72 | 12m+18sm+42st/a | - | - | 3 |
| *Hypostomus* sp *B* | Mogi-Guaçu River (SP) | 72 | 13m+18sm+41st/a | - | - | 5 |
| *Hypostomus* sp *C* | Mogi-Guaçu River (SP) | 72 | 10m+18sm+44st/a | - | - | 3 |
| *Hypnstomus* sp *D1* | Mogi-Guaçu River (SP) | 72 | 10m+26sm+36st/a | - | - | 3 |
| *Hypostomus* sp *D2* | Mogi-Guaçu River (SP) | 72 | 14m+20sm+38st/a | - | - | 3 |
| *Hypostomus* sp *E* | Mogi-Guaçu River (SP) | 80 | 8m+16sm+56st/a | - | - | 3 |
| *Hypostomus* sp *F* | São Francisco River (MG) | 76 | 10m+16sm+50st/a | - | - | 2 |
| *Hypostomus* sp *F* | São Francisco River (MG) | 75 | 10m+17sm+48st/a | - | - | 5 |
| *Hypostomus* sp *G* | Araguaia River (MT) | 64 | 14m+24sm+26st/a (M)<br>15m+24sm+25st/a (F) | - | ZZ/ZW | 4 |
| *H. nigromaculatus* | Três Bocas and Apertados Stream (PR) | 76 | 6m+20sm+50st/a | - | - | 14 |
| *H. nigromaculatus* | Mogi-Guaçu River (SP) | 76 | 8m+20sm+48st/a | - | - | 14 |
| *Hypostomus* sp *Xingu-1* | Xingu River (PR) | 64 | 16m+16sm+32st/a | - | - | 16 |
| *Hypostomus* sp *Xingu-2* | Xingu River (PR) | 66 | 18m+14sm+34st/a | - | - | 16 |
| *Hypostomus* sp *Xingu-3* | Xingu River (PR) | 64<br>65 | 15m+23sm+26st/a<br>15m+23sm+27st/a | 1 | - | 16 |
| *H.* aff. *unae* | Contas River (BA) | 76 | 12m+16sm+48st/a | - | - | 17 |
| *H.* aff. *unae* | Preto do Costa River (BA) | 76 | 12m+20sm+44st/a | - | - | 17 |
| *H.* aff. *unae* | Oricó River (BA) | 76 | 10m+14sm+52st/a | - | - | 17 |
| *H.* aff. *unae* | Preto do Criciuma River (BA) | 76 | 10m+20sm+46st/a | - | - | 17 |

1 = Alves et al. (2006); 2 = Artoni (1996); 3 = Artoni and Bertollo (1996); 4 = Artoni et al. (1998); 5 = Artoni and Bertollo (1999); 6 = Artoni and Bertollo (2001); 7 = Camilo (2004); 8 = Cereali et al. (2008); 9 = Fenerich and Oliveira (2004); 10 = Kavalco et al. (2005); 11 = Michelle et al. (1977); 12 = Muramoto et al. (1968); 13 = Rubert (2007); 14 = Rubert et al. (2008); 15 = Mendes-Neto (2008); 16 = Milhomem et al. (2010); 17 = This study. m = metacentric; sm = submetacentric; st = subtelocentric; a = acrocentric.

few other Hypostominae (Artoni and Bertollo, 2001; Alves et al., 2005). Instead, the subfamily Hypostominae is characterized by a remarkable diversity of karyotypic forms, with 2n ranging from 34 (Oliveira et al., 2009b) to 84 (Cereali et al., 2008).

In general, loricariids exhibit a strong correlation between high numbers of meta/submetacentric chromosomes and low diploid number (i.e., the higher the 2n, the higher the frequency of subtelo/acrocentric chromosomes). For instance, a karyotype formula of 32m/sm+2st is reported for *Ancistrus* sp Purus (2n = 34) (Oliveira et al., 2009b) while *Upsilodus* sp (2n = 96) presents 16m+8sm+72a (Kavalco et al., 2005). This pattern indicates that centric fissions have played a major role in the karyotypic evolution of this family (Artoni and Bertollo, 2001) and can be inferred to explain the derived karyotype observed in *H.* aff. *unae* (2n = 76 and the presence of several st/a pairs).

However, other chromosomal rearrangements can also be identified in the genus *Hypostomus.* As observed in the present study, the maintenance of 2n = 76 with distinctive karyotype formulae in the four analyzed populations indicates that pericentric inversions are involved as well. Similarly, variation in karyotype formulae has been identified among several populations of *Hypostomus* (Table 1). Nonetheless, a misidentification of close chromosomal types because of their reduced size and/or differential chromatin condensation might interfere with the precision of karyotypic analyses. To minimize putative technical artifacts, we divided the chromosomal pairs of each population into bi-armed and one-armed groups (m/sm and st/a, respectively). Nevertheless, the karyotypic divergence among the studied populations remained evident (Table 2).

**Table 2.** Karyotype formulae of *Hypostomus* aff. *unae* populations divided into m/sm and st/a chromosomal types.

| Population | Karyotype formula |
|---|---|
| A | 28m/sm+48st/a |
| B | 32m/sm+44st/a |
| C | 24m/sm+52st/a |
| D | 30m/sm+46st/a |

m = metacentric; sm = submetacentric; st = subtelocentric; a = acrocentric.

Terminal NORs on long arms of a single chromosomal pair, a basal feature within loricariids (Kavalco et al., 2005), were observed in *H*. aff. *unae*. Moreover, an NOR size heteromorphism was usually observed between homologs in all samples, although more evident in population D. Polymorphic conditions related to differences in NOR size between chromosomes are frequent in fish species bearing single NORs (Artoni and Bertollo, 2001; Affonso and Galetti Jr., 2005).

FISH using 18S rDNA probes confirmed both the location of major ribosomal genes and the heteromorphic NOR size between homologues. Thus, the size differences in rDNA clusters are indeed structural and not just transcriptional as assumed after silver nitrate staining (Figure 2, close-up) and are likely to result from duplications/deletions or unequal crossovers (Affonso and Galetti Jr., 2005).

A co-localization of NORs and heterochromatin is commonly reported for *Hypostomus* (Rubert et al., 2008). However, this correspondence is not a ubiquitous condition inasmuch as it is absent in some loricariids such as *Upsilodus* sp, *Neoplecostomus microps* Steindachner, 1877 (Kavalco et al., 2005) and population C of *H.* aff. *unae* analyzed in the

present study. However, the latter also presented GC-rich NORs as shown by $CMA_3^+$ signals, following the general pattern among Hypostominae (Rubert et al., 2008).

Heterochromatic blocks distributed at interstitial regions are considered to be an ancestral condition in Loricariidae and frequently observed in this family and in the closely related Callichthyidae fish (Frehner et al., 2004). In the subfamily Hypostominae, conspicuous heterochromatic segments at interstitial regions of acrocentric chromosomes are regarded as the common pattern for species with high diploid numbers (Artoni and Bertollo, 2001). Nevertheless, the populations A, B, and C of *H*. aff *unae*, although presenting 2n = 76, do not fit this hypothetical pattern since they bear large terminal heterochromatin blocks of acrocentric chromosomes. This evidence suggests that heterochromatin distribution within *Hypostomus* is more complex than previously thought.

The studied specimens also presented an equilocal distribution of interstitial heterochromatin, more evident in population D. These C bands, located equidistantly throughout acrocentric chromosomes, are in accordance with the model of heterochromatin distribution proposed by Schweizer and Loidl (1987) and seem to be a common feature of Loricariidae species (Frehner et al., 2004).

Moreover, the positional differences of C-bands among the studied populations from terminal to interstitial location might be explained by paracentric inversions or transpositions. Afterwards, these segments could have been amplified and/or accumulated and evolved independently in each population.

$DAPI^+$ (AT-rich) signals are rarely found in fish but detected in a few *Hypostomus* species (Artoni and Bertollo, 1999) including populations A, C and D of *H.* aff. *unae* (Figure 5A, C and D). The equivalent location of AT-rich blocks on several chromosomal pairs points towards a common origin of this segment that was distributed to equilocal sites by heterochromatin dispersion within a specific chromosomal group (Schweizer and Loidl, 1987). Similar hypotheses have been proposed to explain the preferential pattern of heterochromatin distribution in some fish species with derived karyotypes (Mantovani et al., 2000; Affonso and Galetti Jr., 2005).

AT-richness is usually associated with a pronounced DNA curvature and subsequent chromosomal stability, as reported in some fish species like *Salmo trutta* Linnaeus, 1758 (Caputo et al., 2009). On the contrary, several AT-rich segments were coupled with chromosomal variation in the genome of *H*. aff. *unae* suggesting that such correlation remains to be confirmed in Neotropical fish species. In specimens from population B, equally $CMA_3^+$ and $DAPI^+$ marks were characterized, suggesting that GC and AT-rich regions are interspersed, although visualized as a single overlapped signal (Figures 4B and 5B).

Dispersal constraints might favor the fixation of chromosomal rearrangements by genetic drift or differential selective pressures (if some adaptive value in the chromosomal structure is present) leading to interpopulation divergence and high endemism (Oliveira et al., 2009a). Because of their non-migratory benthic behavior, very little gene flow between populations of Hypostominae would be expected (Zawadzki et al., 2005). In fact, relatively low levels of genetic variability and significant population structure assessed by allozyme and DNA markers have been reported for different species of *Hypostomus* that could be related to the sedentary habit of these fish (Zawadzki et al., 2005).

The Contas River basin is divided into three physiographic regions (Upper, Middle and Lower), encompassing distinct landscapes: semiarid biome, transition zones and Atlantic forest (Pamponet et al., 2008). All sampling sites of *H*. aff. *unae* belong to the Middle Contas

sub-basin but they can be readily distinguished by environmental/physical features: the main river channel (site A) is a highly damaged area, affected upstream by the Pedras dam and industrial and domestic sewage from the city of Jequié (Pamponet et al., 2008); site B is a narrow river located on a transition zone between semiarid and tropical climates; site C is a comparatively large, fast flowing river located in a typical Atlantic forest region, and site D is a headwater stream in Atlantic forest, with several small waterfalls and separated from the Contas River (6 km apart) by a reservoir.

Thus, the cytogenetic differences among the studied populations might be associated with both the low vagility of *Hypostomus* and the environmental particularities of each site but not the geographical distance *per se*. Similar hypotheses were inferred to explain genetic differences in other fish species along the Middle Contas sub-basin (Pamponet et al., 2008).

Considering that *Hypostomus* is a taxonomically controversial group, the present results may actually be related to the occurrence of a cryptic species along the Contas river basin, as commonly observed in other *Hypostomus* (Milhomem et al., 2010).

Finally, in spite of their importance to our understanding of karyotypic evolution, studies focusing on heterochromatin are scarce in Siluriformes, as well as in many Neotropical fish species from isolated Brazilian coastal basins. The present study represents an effort to change this scenario, providing the first cytogenetic report in Loricariidae from a northeastern South America basin with identification of distinctive evolutionary units of *Hypostomus* aff. *unae*.

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