



## Cytogenetic characterization of F1, F2 and backcross hybrids of the Neotropical catfish species *Pseudoplatystoma corruscans* and *Pseudoplatystoma reticulatum* (Pimelodidae, Siluriformes)

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### Abstract

The cytogenetic characteristics of *Pseudoplatystoma corruscans* and *Pseudoplatystoma reticulatum* and their F1, F2 and backcross hybrids were assessed by using chromosome banding techniques. The diploid number of 56 chromosomes was constant in all species and lineages, with a karyotypic formula containing 20 metacentric, 12 submetacentric, 12 subtelocentric and 12 acrocentric chromosomes. Nucleolar organizer regions (NORs) were identified in two subtelocentric chromosomes in the parents and hybrids, with partial nucleolar dominance in F1 and F2 specimens. Heterochromatic blocks were detected in the terminal and centromeric regions of some chromosomes in all individuals. For parental and hybrid lineages, 18S ribosomal clusters corresponding to NORs and 5S ribosomal genes were identified in distinct pairs of chromosomes. The striking conservation in the chromosomal macrostructure of the parental species may account for the fertility of their F1 hybrids. Similarly, the lack of marked alterations in the chromosomal structure of the F1 hybrids could account for the maintenance of these features in post-F1 lineages.

**Key words:** backcrossing, cytogenetics, interspecific hybrids, post-F1 hybrids, *Pseudoplatystoma*.

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### Introduction

The genetic identification of hybrids is an important aspect of fish culture that helps to avoid problems associated with artificial hybridization (Toledo-Filho *et al.*, 1998; Porto-Foresti and Foresti, 2004). Among the currently available genetic methods, cytogenetics has contributed extensively to ichthyological studies and is an important tool for chromosomal, evolutionary and phylogenetic analyses and species identification (Foresti, 2008; Oliveira *et al.*, 2009). In hybridization studies, this technique is effective in assessing the levels of ploidy and in identifying gynogenetic and androgenetic lineages, the morphological and chromosomal structure of parental and hybrid species and lineages, structural alterations in the chromosomes of hybrids, and cytogenetic markers that allow discrimination

between pure species and interspecific hybrid lineages (Toledo-Filho *et al.*, 1994; Almeida-Toledo *et al.*, 1995; Porto-Foresti *et al.*, 2008).

Fishes of the genus *Pseudoplatystoma*, such as *P. corruscans* (pintado) and *P. reticulatum* (cachara), have been used in large scale interspecific hybridization programs in Brazil (Godinho, 2007; Campos, 2010). These species belong to the family Pimelodidae, an important group of Neotropical Siluriformes (Lundberg and Littmann, 2003; Ferraris Jr, 2007) with an ample Neotropical distribution, and occur in the Amazon River basin (*P. reticulatum*), San Francisco River basin (*P. corruscans*) and Prata River basin (*P. corruscans* and *P. reticulatum*) (Buitrago-Suarez and Burr, 2007).

Both of the reciprocal interspecific hybrids of these species, denominated “cachapinta” (produced by crossing cachara females with pintado males) and “pintachara” (produced by crossing pintado females and cachara males) are viable, commercially advantageous, and have good market acceptance (Godinho, 2007; Porto-Foresti *et al.*, 2008). These hybrids are fertile and are able to cross and produce

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post-F1 hybrids (F2 hybrids and backcrosses) in lineage cultivation, mainly involving the hybrid “cachapinta”.

Despite the fact that these hybrids are economically important and have been extensively raised in fish-farming environments, there are no chromosomal data for these lineages. The aim of this study was therefore to use chromosomal banding techniques to cytogenetically characterize F1 interspecific hybrids, F2 hybrids and individuals resulting from backcrosses involving *P. corruscans* and *P. reticulatum*, in order to assess potential differences that could be used to characterize the parental species and identify hybridization products.

## Material and Methods

21 specimens of *P. corruscans* and 15 of *P. reticulatum* were used as the parental lineages. We also studied 15 specimens of the hybrid “pintachara” and 16 of the hybrid “cachapinta” as representing the F1 interspecific hybrid lineage, in addition to five F2 specimens from the “cachapinta” intercross and 10 backcross individuals from backcrosses involving a “cachapinta” female and a pintado male; the latter two groups belonging to a post-F1 hybrid lineage.

All of the specimens were obtained from the hatchery stocks of the Centro Nacional de Pesquisa e Conservação de Peixes Continentais do Instituto Chico Mendes da Conservação de Biodiversidade (CEPTA/ICMBio, Pirassununga, SP, Brazil), which is also where the hybrids were artificially produced. The low number of F2 hybrids analyzed reflected the high mortality during the first weeks of stockpiling.

The samples of *P. corruscans* and *P. reticulatum* were collected throughout the Paraguay River basin; additional samples of *P. corruscans* were also collected from the Mogi-Guaçu River (Upper Paraná River basin). The fish were identified and stocked in the collection of the Laboratório de Genética de Peixes, UNESP, Bauru, SP, Brazil.

Mitotic chromosomes were obtained by initially injecting the fish with a biological fermentation solution (Lozano *et al.*, 1988; Oliveira *et al.*, 1988) to stimulate mitosis, after which a kidney cell suspension was used to provide chromosomal preparations, essentially as described by Foresti *et al.* (1981). Constitutive heterochromatin was detected by the C-banding technique described by Sumner (1972) and nucleolar organizer regions (NORs) were identified by silver nitrate staining (Ag-NOR), according to Howell and Black (1980).

The 18S and 5S ribosomal genes were identified simultaneously by fluorescent *in situ* hybridization (FISH) using 18S and 5S rDNA (ribosomal DNA) probes. The probes were obtained by polymerase chain reaction (PCR) amplification using the primers NS1 (5'-GTAGTCATATGCTTGTCTC-3') and NS8 (5'-TCCGCAGGTTACCTACGGA-3') for the 18S gene

(White *et al.*, 1990) and primers A (5'-TACGCCCGATC TC GTCCGATC-3') and B (5'-CAGGCTGGTATGGCC GTAAGC-3') for the 5S gene (Pendás *et al.*, 1994). The probes were labelled with biotin-dATP (18S) and digoxigenin-dUTP (5S) by nick translation, according to the manufacturer's instructions (Bionick labelling system, Gibco BRL). After labeling, the hybridizations were done using the technique described by Pinkel *et al.* (1986). The slides were counterstained with 4' 6-diamidino-2-phenylindole (DAPI) and the metaphases then observed and photographed with an Olympus BX50 epi-fluorescence photomicroscope. The chromosomal morphology was defined based on the arm ratio, as described by Levan *et al.* (1964), and the chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a), and organized in decreasing order of size in the karyotype.

## Results

All of the samples of *P. corruscans* and *P. reticulatum* analyzed, *i.e.*, the parental lineages (Figure 1), F1 hybrids (“cachapinta” and “pintachara”) (Figure 2), and F2 hybrids and backcrosses (Figure 3), had a diploid number of 56 chromosomes arranged in a karyotype containing 20m+12sm+12st+12a, with no evidence of sex-related chromosomal heteromorphism. C-banding revealed heterochromatic markings in the pericentromeric and terminal portions of some chromosomes in both of the parental and all of the hybrid lineages (Figures 1B,D, 2B,D and 3B,D, respectively).

In the parental species, NORs were observed on the short arms of subtelocentric pair 18 (Figure 1A,C) which, in addition to the size polymorphism observed in some metaphases, showed positive C-banding (Figure 1B,D). In the F1, F2 and backcross hybrids, NORs were located on the short arm of two subtelocentric chromosomes of the same size that were polymorphic in some cells (Figures 2A,C and 3A,C) and totally heterochromatic (Figures 2B,D and 3B,D). In addition, silver nitrate staining revealed variations in the distribution of NORs in F1 and F2 hybrids, in which cells with only one active NOR and others with two active NORs were identified (Figure 4 and Table 1).

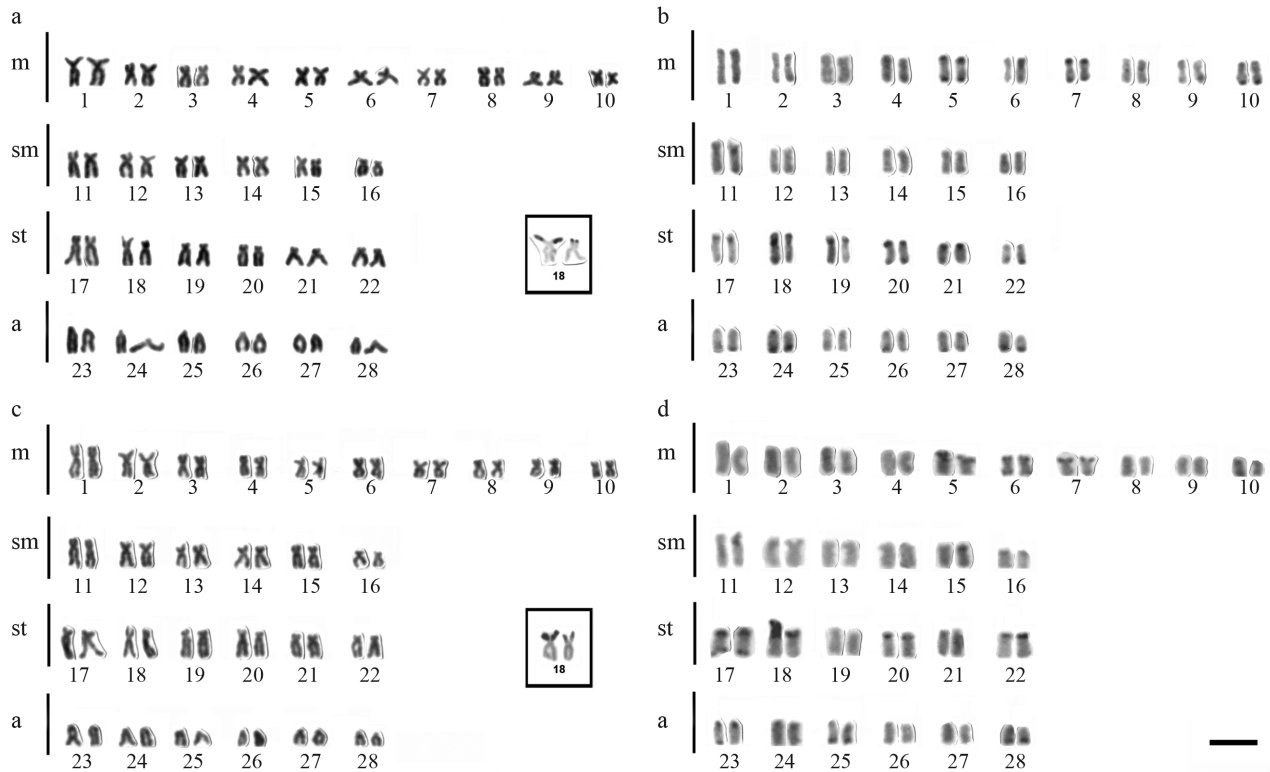
FISH confirmed the location of the 18S ribosomal genes in two subtelocentric chromosomes corresponding to the NOR-bearing chromosomes (Figure 5) in all of the samples studied. The 5S ribosomal genes were detected in the pericentromeric area of the short arm of a subtelocentric pair in *P. corruscans* and *P. reticulatum*. In F1 and post-F1 hybrids these genes occurred in the same area of two subtelocentric chromosomes of similar size and morphology (Figure 5).

## Discussion

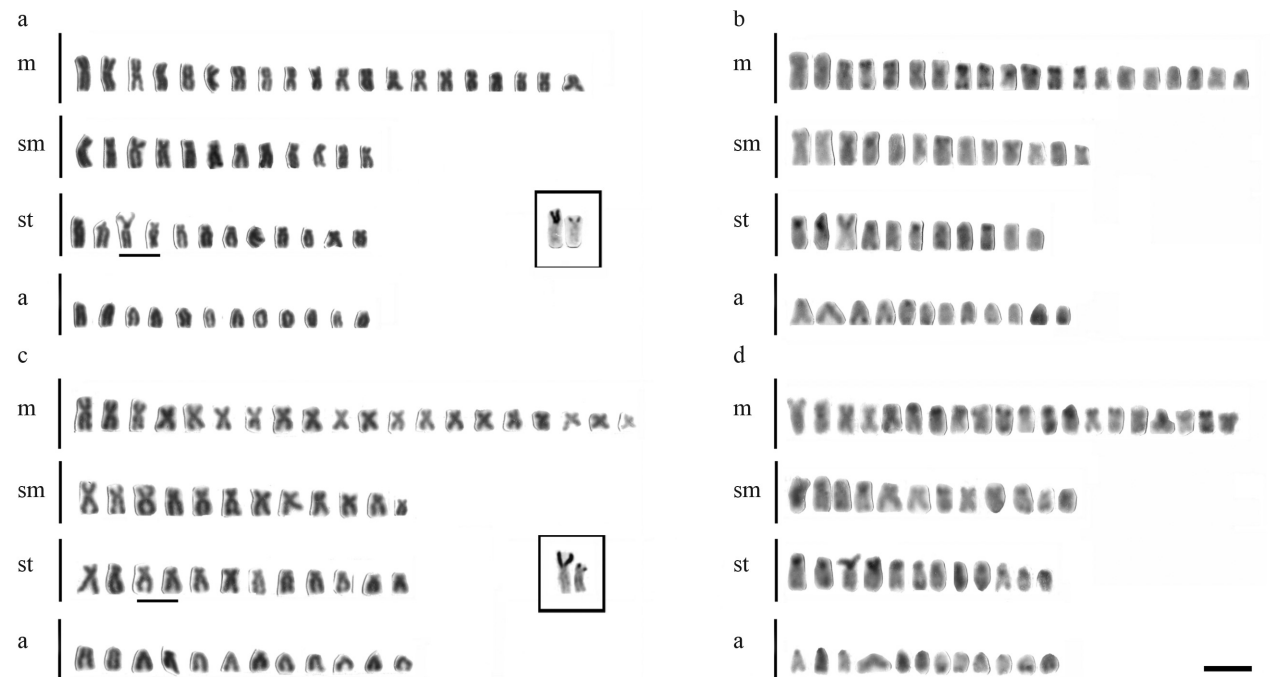
The genus *Pseudoplatystoma* is characterized by a highly stable number of chromosomes, with all of the spe-

cies studied so far having 56 chromosomes (Klinkhardt *et al.*, 1995; Arai, 2011). The results described here confirmed this characteristic for *P. corruscans* and *P. reticulatum*, both of which had 56 chromosomes arranged

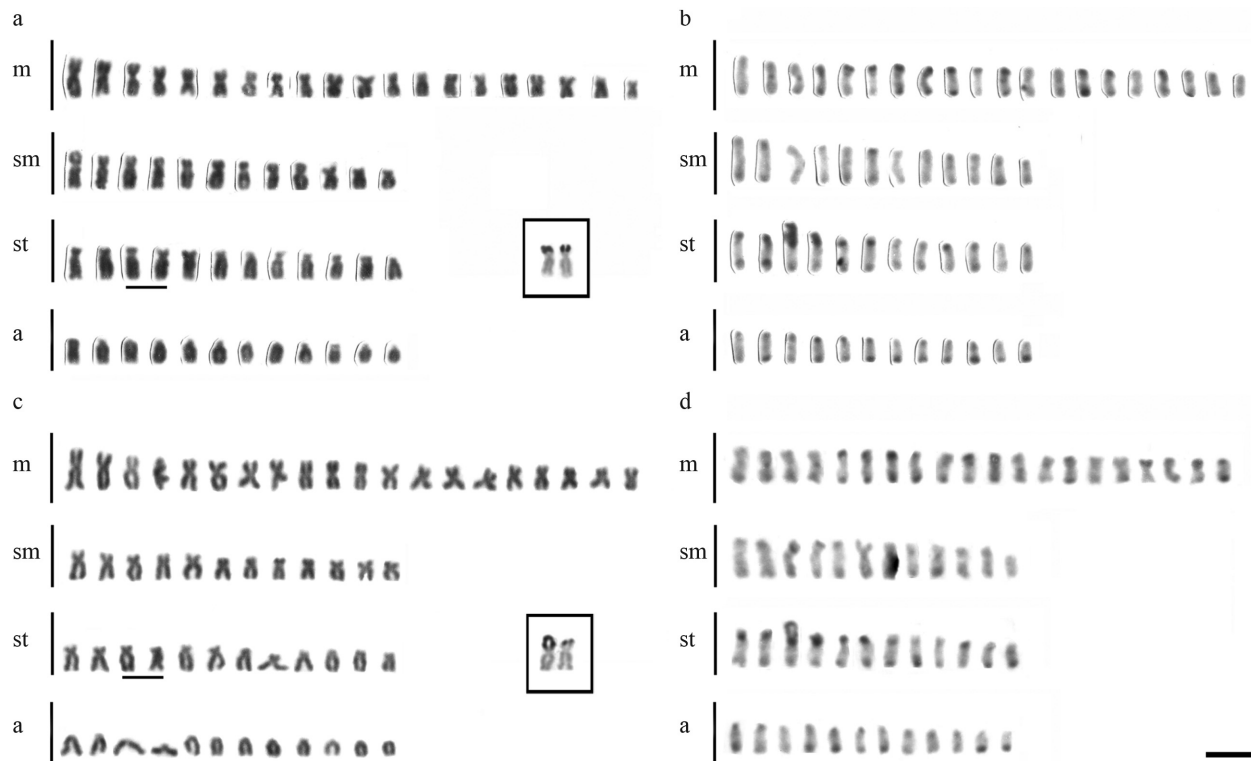
in a karyotype composed of 20m+12sm+12st+12a, in agreement with previous reports for these two species (Porto-Foresti *et al.*, 2000; Foresti *et al.*, 2007). However, different karyotypic formulae have been described for *P.*



**Figure 1** - Karyotypes of the parental species *P. corruscans* (A,B) and *P. reticulatum* (C,D) after Giemsa staining (A,C) and C-banding (B,D). The chromosomes bearing NORs are shown in detail (boxes). m, metacentric; sm, submetacentric; st, subtelocentric; a, acrocentric chromosomes. Scale bar = 10  $\mu$ m.



**Figure 2** - Karyotypes of the F1 hybrids "pintachara" (A,B) and "cachapinta" (C,D) after Giemsa staining (A,C) and C-banding (B,D). The chromosomes bearing NORs are shown in detail (boxes). Bars indicate the karyotype localization of the NOR-bearing chromosomes. Scale bar = 10  $\mu$ m.



**Figure 3** - Karyotypes of the F2 (A,B) and backcross (C,D) hybrids after Giemsa staining (A,C) and C-banding (B,D). The chromosomes bearing NORs are shown in detail (boxes). Bars indicate the karyotype localization of the NOR-bearing chromosomes. Scale bar = 10  $\mu$ m.

*corruscans* (Martins-Santos *et al.*, 1996; Souza *et al.*, 1997; Swarça *et al.*, 2005) and *P. reticulatum* (Neto *et al.*, 2011), indicating the existence of chromosomal differentiation in these species. Since the differences are subtle and involve few chromosomal pairs, and considering the small size and similar morphology of the chromosomes involved, these discrepancies may be related to difficulties in accurately identifying the chromosomal morphology and in assembling the karyotypes. A similar observation applies to the

**Table 1** - Distribution of NOR-bearing chromosomes in the parental species, F1 and post-F1 hybrid lineages based on silver nitrate staining (Ag-NOR).

Lineage	n	Cells with two Ag-NORs (%)	Cells with one Ag-NOR (%)
Parental species			
<i>P. corruscans</i>	21	300 (100.00)	-
<i>P. reticulatum</i>	15	225 (100.00)	—
F1 hybrids			
“pintachara”	15	86 (41.75)	120 (58.25)
“cachapinta”	16	108 (57.75)	79 (42.25)
Post-F1 hybrids			
F2	5	36 (20.45)	140 (79.55)
Backcross	10	325 (100.00)	-

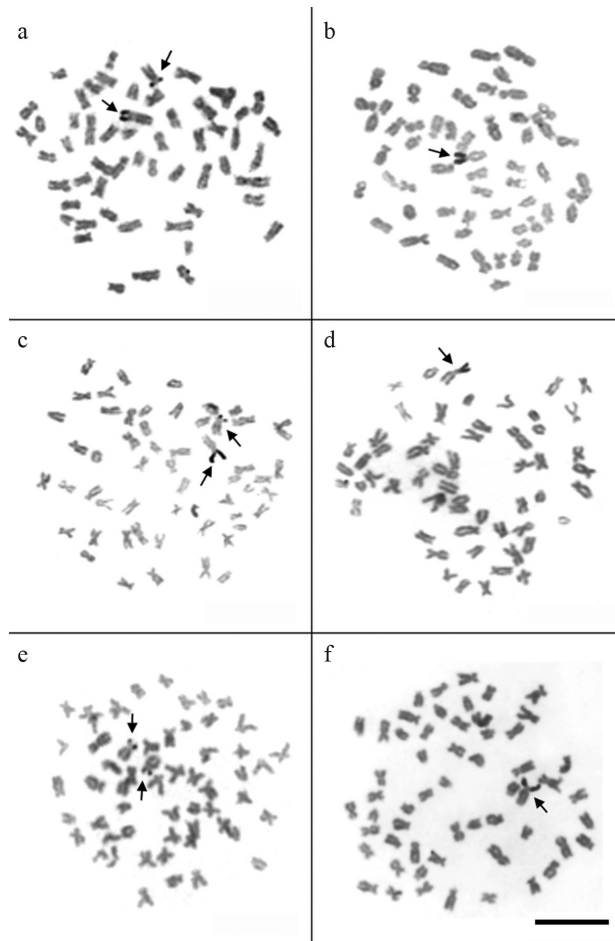
n - number of individuals analyzed.

F1 hybrids analyzed here, both of which maintained the diploid number and chromosomal morphology of their parental lineages.

Closely related species can have the same chromosome number and karyotype that are maintained in the hybrids, as shown for *Piaractus mesopotamicus*, *Colossoma macropomum* and their interspecific hybrid “tambacu” (Almeida-Toledo *et al.*, 1988), as well as species of the genus *Cichla* and their F1 hybrids (Brinn *et al.*, 2004) and hybrids of *Oreochromis mossambicus* and *Oreochromis urolepis hornorum* (Zhu *et al.*, 2010).

Hybridization among different fish species can generate not only simple diploid hybrids, with an equal contribution of the parental genomes, but also gynogenetic (containing only the maternal chromosomal group) or androgenetic (containing only the paternal chromosomal group) offspring, as well as haploid, triploid or tetraploid individuals (Toledo-Filho *et al.*, 1994). In the present study, despite the impossibility of identifying the haploid groups of each parental species within the chromosomal group of the interspecific hybrids, these groups can be classified as simple diploid hybrids since molecular studies have shown that these fish inherited specific gene fragments from both of the parental species (Prado *et al.*, 2011).

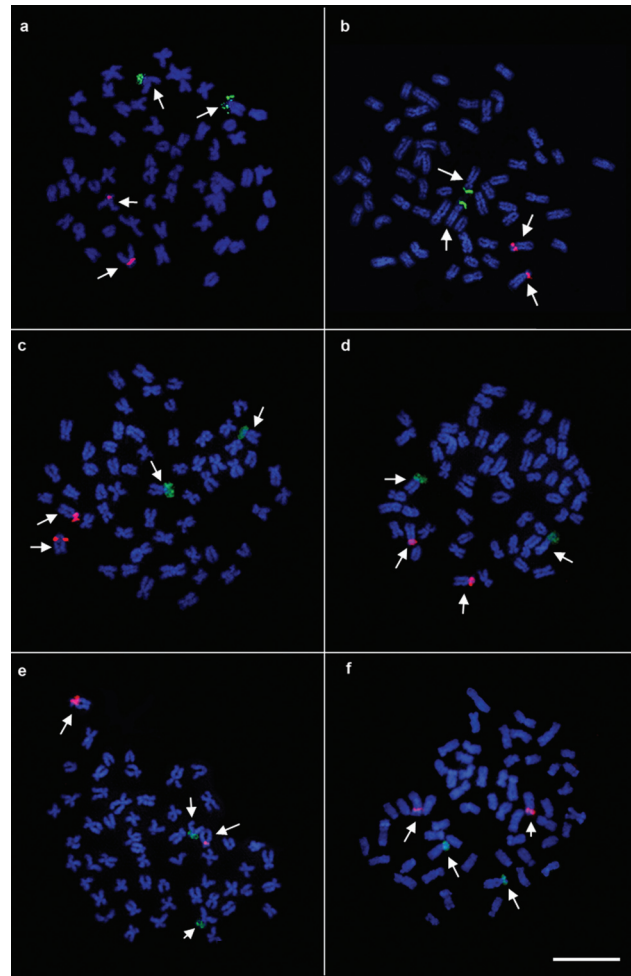
Fertile hybrids have been described for several fish species (reviewed in Bartley *et al.*, 2001), with the production of post-F1 lineages being more common than in other animals. This reflects the fact that in fish even gametes that



**Figure 4** - Metaphases of the F1 hybrids “pintachara” (A,B) and “cachapinta” (C,D) and F2 hybrids (E,F) after silver nitrate staining. Panels (A), (C) and (E) show cells with two Ag-NORs and panels (B), (D) and (F) show cells with only one Ag-NOR. Arrows indicate the NOR-bearing chromosomes. Scale bar = 10  $\mu$ m.

have unbalanced chromosomal complements maintain part of their genetic and functional capabilities and can generate viable descendants (Toledo-Filho *et al.*, 1994). However, there have been few cytogenetic studies of post-F1 lineages in fish. One of the few such studies for Neotropical species involved the backcrossing of the hybrid “tambacu” with the parental pacu (Almeida-Toledo *et al.*, 1996), which resulted in a lineage with a cytogenetic pattern identical to that of the parental pacu. The cytogenetic data described here for post-F1 lineages also showed the occurrence of a diploid number and karyotypic constitution identical to the parental species and F1 hybrids.

The C-banding in *P. corruscans* and *P. reticulatum* revealed a band pattern characteristic of the family Pimelodidae, with a small amount of constitutive heterochromatin distributed in the terminal and pericentromeric areas of some chromosomes (Fenocchio and Bertollo, 1992; Garcia and Moreira-Filho, 2005). The existence of small differences in the areas of heterochromatin between the parental lineages of cachara and pintado cannot be ex-



**Figure 5** - Metaphases of the parental species *P. corruscans* (A) and *P. reticulatum* (B), F1 hybrids “pintachara” (C) and “cachapinta” (D), F2 hybrids (E) and backcross hybrids (F) after FISH with 18S and 5S rDNA probes. Arrows indicate the chromosomes bearing 18S (green) and 5S (red) rDNA. Scale bar = 10  $\mu$ m.

cluded, although peculiar characteristics of the chromatin of these fish (Fenocchio and Bertollo, 1992) could hinder the detection of discriminatory banding patterns. The finding that the F1 and post-F1 hybrid lineages studied here had the same heterochromatic banding pattern agreed with similar results described by Brinn *et al.* (2004) for interspecific hybrids of *Cichla monoculus* and *C. temensis*.

The NORs in *P. corruscans* and *P. reticulatum* were restricted to terminal areas of the chromosomes, as frequently observed in Pimelodidae, and this may represent the ancestral condition for this character in Siluriformes (Oliveira and Gosztonyi, 2000). Differences in the size of the Ag-NOR band among homologous chromosomes are common in Neotropical fish (Foresti *et al.*, 1981) and may be related to variation in NOR activity, to the number of repetitions of rDNA genes, or to other decisive rearrangements involved in determining polymorphism.

The analysis of NORs in the hybrid lineages revealed demarcations in chromosomes of equal size and morphol-

ogy that hindered identification of the origin of the parental chromosomes. The variation in the number of chromosomes with NORs observed in the F1 and F2 hybrid metaphases was possibly related to differences in the activity of this region since silver nitrate staining is restricted to transcriptionally active NORs (Howell, 1977; Jordan, 1987). Alternatively, such variations may reflect the effect of partial nucleolar dominance in which the NOR of one of the parentals is active while the other one is inactive. This phenomenon is commonly observed in interspecific hybrids and has been described in *Drosophila* (Durica and Krider, 1978), *Xenopus* (Reeder and Roan, 1984) and, more recently, in fish (Gold *et al.*, 1991; Hashimoto *et al.*, 2009).

The fact that the backcross hybrids assessed here showed no such variation in their NORs can be explained by the possible “rescue” of the nucleolus lost during backcrossing of the hybrid “cachapinta” with the parental pintado, resulting in active NORs in all of the cells analyzed. In plants of the genus *Crepis* it was possible “to recover” the repressed nucleolus of interspecific hybrids through appropriate backcrossing, indicating that the nucleolar formation of the chromosome was not altered or lost in the hybrid (Pikaard, 2000).

FISH revealed 18S rDNA clusters in the regions corresponding to NORs in both *P. corruscans* and *P. reticulatum*, thus confirming the existence of only one chromosome pair bearing this gene, as is usually the case in the Pimelodidae (Vasconcelos and Martins-Santos, 2000; Swarça *et al.*, 2008; Carvalho *et al.*, 2010). Regardless of the Ag-NOR variation, *in situ* hybridization in the F1 and F2 hybrids indicated that the 18S ribosomal genes were not lost or altered, as also reported for hybrids of *Leporinus* (Hashimoto *et al.*, 2009).

Although few chromosomal mapping studies have examined the 5S gene in the Pimelodidae, the evidence indicates the prevalence of only two chromosomes related to these ribosomal sites (Swarça *et al.*, 2009; Carvalho *et al.*, 2010). As shown here, the 5S ribosomal gene was located pericentromerically on one chromosomal pair in *P. reticulatum* and *P. corruscans*, as also observed by Swarça *et al.* (2005) and Neto *et al.* (2011); the hybrid lineages showed 5S demarcations at the same chromosomal positions as their parental lineages, thus conforming previous observations for this fish group. According to Martins and Galetti Jr (2001), the preferential pericentromeric location of the 5S rDNA sites seen here in the parental and hybrid metaphases may be related to the protection of these sequences from transposition and exchange events that are more frequent in terminal areas. This protection may be an advantageous characteristic in the genomic organization of these organisms.

The techniques used here revealed identical cytogenetic patterns in *P. corruscans* and *P. reticulatum* and their maintenance in subsequent F1 and post-F1 hybrid lineages, indicating that chromosomal conservation is an im-

portant phenomenon in species of Pimelodidae. A high degree of homology in the chromosomal constitution of the parental species frequently results in correct pairing and segregation during meiosis and the consequent production of viable gametes in interspecific hybrids (Nikoljukin, 1946). This phenomenon could explain the fertility of the F1 hybrids seen here.

Although there may be subtle karyotypic differences between *P. corruscans* and *P. reticulatum*, the techniques used here were not sufficiently sensitive to detect such differences among the chromosomal markers of the parental and hybrid lineages. This work represents the first cytogenetic study of F1 and post-F1 hybrids of the pimelodid species *P. corruscans* and *P. reticulatum* and the results obtained may be useful in future genetic and evolutionary analyses of the parental species and their hybrid lineages. The findings regarding the reproduction and fertility of the “cachapinta” hybrid represent one of the few studies that have examined the fertility of F1 hybrids and the viability and survival of post-F1 hybrids in Brazilian fish.

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