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Analysis of the nucleolus organizer regions in 5 species of the genus *Pimelodus* (Siluriformes, Pimelodidae), using $AgNO_3$, CMA_3 and FISH with the 18S rDNA probe

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Abstract — A cytogenetic study was conducted on five species of the genus *Pimelodus* collected from various regions in Brazil: *Pimelodus heraldoi*, *Pimelodus* sp, *Pimelodus argenteus*, *P. maculatus* and *P. mysteriosus.* All the species had 2n=56 chromosomes. AgNOR was found in one pair of subtelocentric (ST) chromosomes with staining at the terminal position in all the five species. However, *Pimelodus* sp, *P. heraldoi* and *P. maculatus* showed NOR staining on long arm and *P. argenteus* and *P. mysteriosus* showed NOR staining on the short arm. AgNOR size heteromorphism was observed in *Pimelodus* sp, *P. maculatus* and *P. argenteus*. The CMA_3 fluorochrome staining pattern in all the species corresponded with AgNOR sites, also showing heteromorphism, including *P. mysteriosus*. FISH revealed the presence of one pair of chromosomes with ribosomal cistrons and confirmed the presence of the structural heteromorphism of these sites, except in *P. heraldoi.*

Key words: AgNOR, CMA_3, 18S rDNA, *Pimelodus*.

INTRODUCTION

Nucleolus organizer regions (NORs) constitute the regions of chromosomes involved in the transcription of ribosomal RNA. These regions can be identified by impregnation with silver nitrate, which reacts with the acidic proteins associated with rDNA (Pendás et al., 1993a) and by GC-specific fluorochromes such as chromomycin A_3 and mithramycin, since NORs are associated with DNA families rich in GC bases (Salvadori, et al., 1995) in almost all groups, except mammals (Gold *et al*., 1990).

Fluorescence *in situ* hybridization (FISH) is a combination of cytogenetic and molecular techniques to obtain information about specific regions of chromosomes. This technique utilizes rDNA probes to reveal with precision the chromosomes involved in the organization of the nucleolus, as well as the exact localization of ribosomal genes (Almeida-Toledo et al., 1988).

Variations in the number, position and size of NORs have been found in different groups of fishes and, according to Klinkhardt (1998), this could furnish valuable information about intra- and interspecies differences, which could help in defining the taxonomic position of a species in terms of its karyotypic evolution.

In neotropical fishes, some groups have their ribosomal cistrons localized in a single pair of chromosomes (Parodontidae, Anostomidae, Curimatidae, Pimelodidae), while others have NORs in various chromosomes of the complement (Characidae, Loricariidae, Erythrinidae, Callichthyidae).

In the family Pimelodidae, the presence of at least one NOR, generally at the terminal position of chromosomes, is the most commonly observed circumstance, with possible variations occurring in the type of chromosomes in which the NOR is localized and in the presence or absence of size heteromorphism of these regions, between homologous chromosomes such as in: *Pseudoplatystoma fasciatum, P. tigrinum* and *Sorubim lima* (Fenocchio and Bertollo, 1992), *Bergiaria westermanni* (Dias and Foresti, 1993), *Pimelodella avanhandavae*, *Microglanis cottoides* and *Cetopsorhamdia iheringi* (Vissotto et al., 1999), *Pinirampus pirinampu* (Swarca et al., 1999), and *Rhamdia voulezi* (Abucarma and Martins-Santos, 2001), among others.

The aim of the present study was to analyze and compare the nucleolus organizer regions (NORs) in five species of fish of the genus *Pimelodus*, family Pimelodidae, utilizing three different staining tech-

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niques: $AgNO_3$, CMA_3 and FISH with an 18S rDNA probe.

MATERIALS AND METHODS

Seven specimens of *Pimelodus heraldoi* (six females and one male) were collected from the Tibagi River, in the region of Limoeiro/PR, and sixteen specimens of *Pimelodus* sp (ten females and six males) from the Iguaçu River, in Salto Segredo/PR, these two rivers belonging to the hydographic basin of the upper Paraná. Eighteen specimens of *Pimelodus argenteus* (two females and sixteen males), seven specimens of *P. maculatus* (three females and four males) and thirty-nine specimens of *P. mysteriosus* (seventeen females and twenty-two males) were collected from the Paraguai River, in the region of Corumbá/MS, belonging to the hydrographic basin of the Paraguai River

Mitotic chromosome preparations were obtained from lymphocyte culture in *Pimelodus* sp according to FENOCCHIO and BERTOLLO (1988) and from kidney cells in the others species by the method direct according BERTOLLO et al. (1978). NOR silver staining was perfomed using the method of HOWELL and BLACK (1980) and chromomycin A_3 (CMA_3) staining as decribed by SCHMID (1980). The 18S rDNA segment containing 1700pb of the fish *Oreochromis niloticus* was used for fluorescence *in situ* hybridization (FISH) and labeled with biotin-14-dATP by nick translation (Gibco cat N° 18247-015), according to the manufacturer's instructions. The hybridization technique, post-hybridization washes and visualization were carried out as reported by Swarça et al. (2001a).

RESULTS AND DISCUSSION

All of the species of *Pimelodus* examined in the present study have 2n=56 chromosomes and different karyotype formulas, as previously determined by SOUZA (2003). Silver nitrate ($AgNO_3$) impregnation revealed a pair of subtelocentric (ST) chromosomes with staining at the terminal position in all five species of *Pimelodus*. *Pimelodus* sp., *P. heraldoi* and *P. maculatus* (Fig. 1A, B, D) displayed the NOR on the long arm, while in *P. mysteriosus* and *P. argenteus* (Fig. 1C, E) the NOR was seen on the short arm.

In the genus *Pimelodus*, what is commonly observed is that NOR is localized at the terminal position of one pair of ST chromosomes on the long arm, such as in *P. absconditus* and *P. maculatus* from the Paraná River basin, in the Porto Rico region (BORIN and MARTINS-SANTOS, 2002), *P. maculatus* from the Tibagi River (SWARCA et al., 2001b), *P. maculatus* from the Paranapanema River (VISSOTTO *et al.,* 1999), among others. However, *P. ornatus* from the Paraná River basin, in the Porto Rico region (BORIN and MARTINS-SANTOS, 2002) and *P. blochii* from the Araguaia River, in Mato Grosso (FARIA et al., 2000), have the NOR on the short arm as observed in *P. mysteriosus* and *P. argenteus*, from the Paraguai River basin, which were studied here.

The data cited above demonstrate the variability of the chromosomal localization of the NOR in the genus *Pimelodus*, a fact that could be due to the rearrangements in this region, such as translocations, which occurred and became stable in this group of fish. BORIN and MARTINS-SANTOS (2002) suggested that the localization of the NOR on the short arm in *P. ornatus* which they studied and in *P. blochii* investigated by FARIA *et al.* (2000), could be caused by a translocation of the rDNA genes of the long arm to the short arm during the process of speciation.

In *Pimelodus* sp (Fig. 1A) from the Paraná basin, *P. maculatus* and *P. argenteus* (Fig. 1D, E) from the Paraguai basin, there was evidence of size heteromorphism of the AgNOR, where in *P. argenteus* this region was strongly stained in one of the homologous chromosomes, probably a secondary constriction observed with the conventional Giemsa technique by SOUZA (2003). The occurrence of size heteromorphism of this region is not very frequent in the genus *Pimelodus*. To date, it has been reported in *Pimelodus maculatus* from Tibagi River (SWARCA et al., 2001b), from the Rio Paranapanema and from the Jurumirim reservoir (VISSOTTO et al., 1999). *P. maculatus* from the Mogi-Guaçu River and São Francisco River do not display size heteromorphism for NOR, demonstrating that there may be differences among populations of the same species.

In some species of the family Pimelodidae, $AgNO_3$ impregnation also showed size heteromorphism of the NOR between the homologous chromosomes, as observed in *Zungaro zungaro* from the Paraná River (SWARCA et al., 2001c), *Pinirampus pirinampu* from the Tibagi River (SWARCA et al., 1999), *Pimelodella* sp from the Mogi-Guaçu River (DIAS and FORESTI, 1993), among others.

According to GOLD *et al.* (1990), NOR size heteromorphism may arise from differences in expression between the homologous chromosomes or may even show a true genetic polymorphism. Further studies are needed to be confirm this using other methods because, according to PENDÁS et al. (1993b), silver nitrate is not recommended for the localization of rRNA genes, although it is quite appropriate for the study of NOR expression.

In all the species examined in the present study, CMA_3 staining corresponded with AgNOR sites, evidencing that these regions are rich in GC base pairs (Fig. 2). With the exception of *P. heraldoi* (Fig. 2B), the other four species of *Pimelodus* displayed a

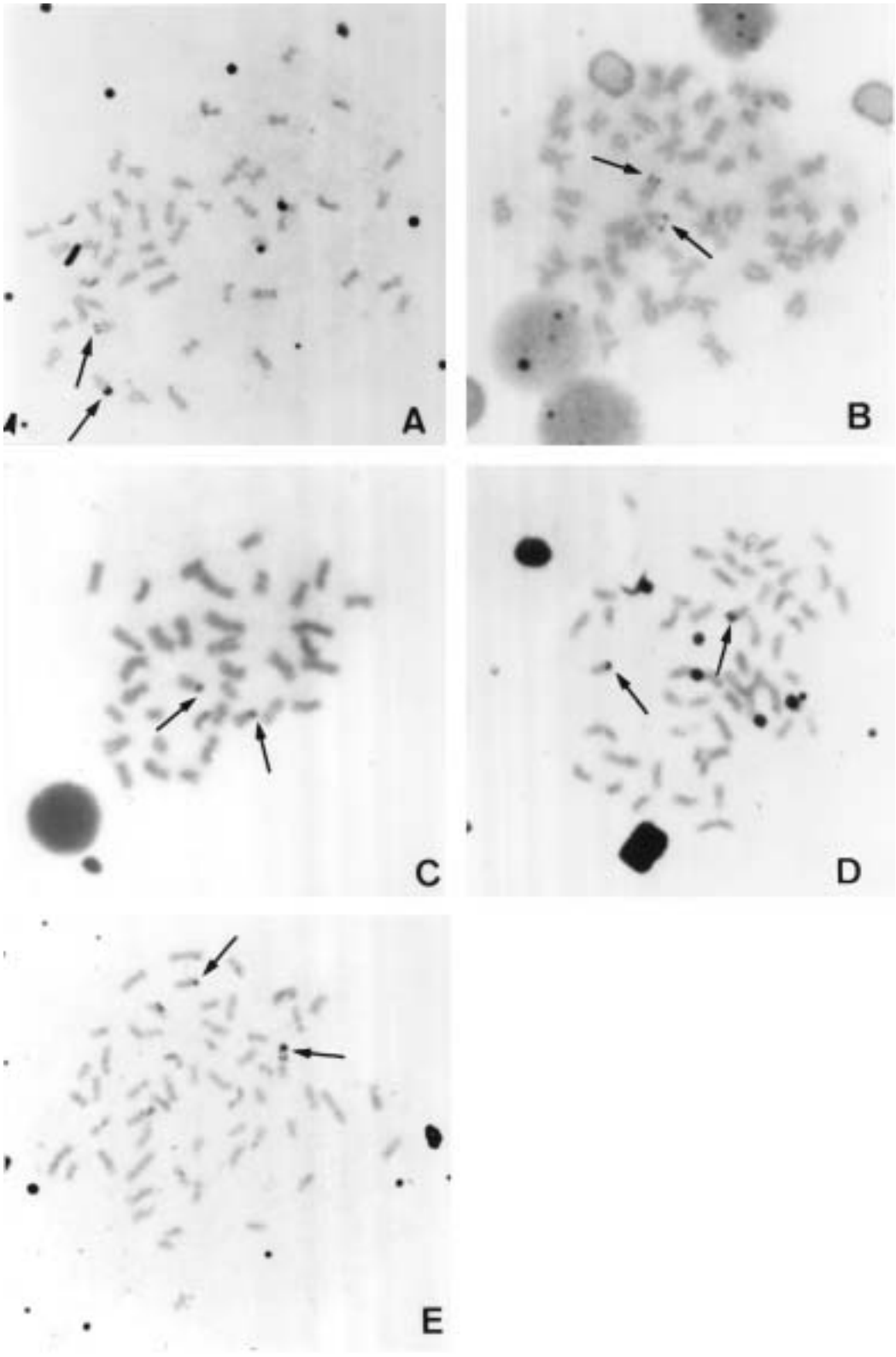

Fig. 1 — Somatic metaphases treated with silver nitrate ($AgNO_3$), in *Pimelodus* sp (A), *P. heraldoi* (B), *P. mysteriosus* (C), *P. maculatus* (D) and *P. argenteus* (E). The arrows indicate NOR-bearing chromosomes.

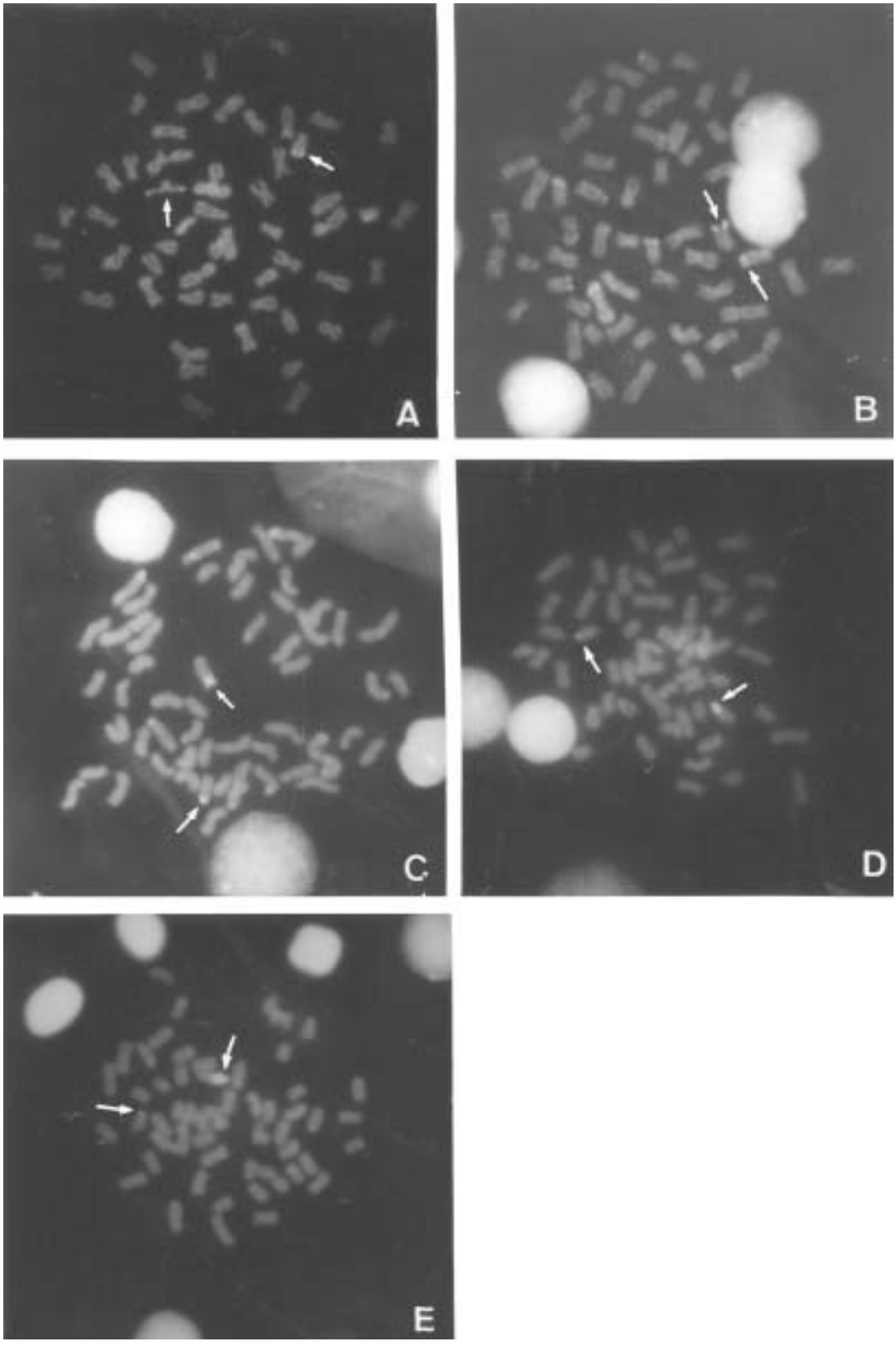

Fig. 2 — Somatic metaphases treated with chromomycin A_3 (CMA_3), in *Pimelodus* sp (A), *P. heraldoi* (B) *P. maculatus* (C) *P. mysteriosus* (D) and *P. argenteus* (E). The arrows indicate chromosomes with fluorescent regions corresponding to NORs.

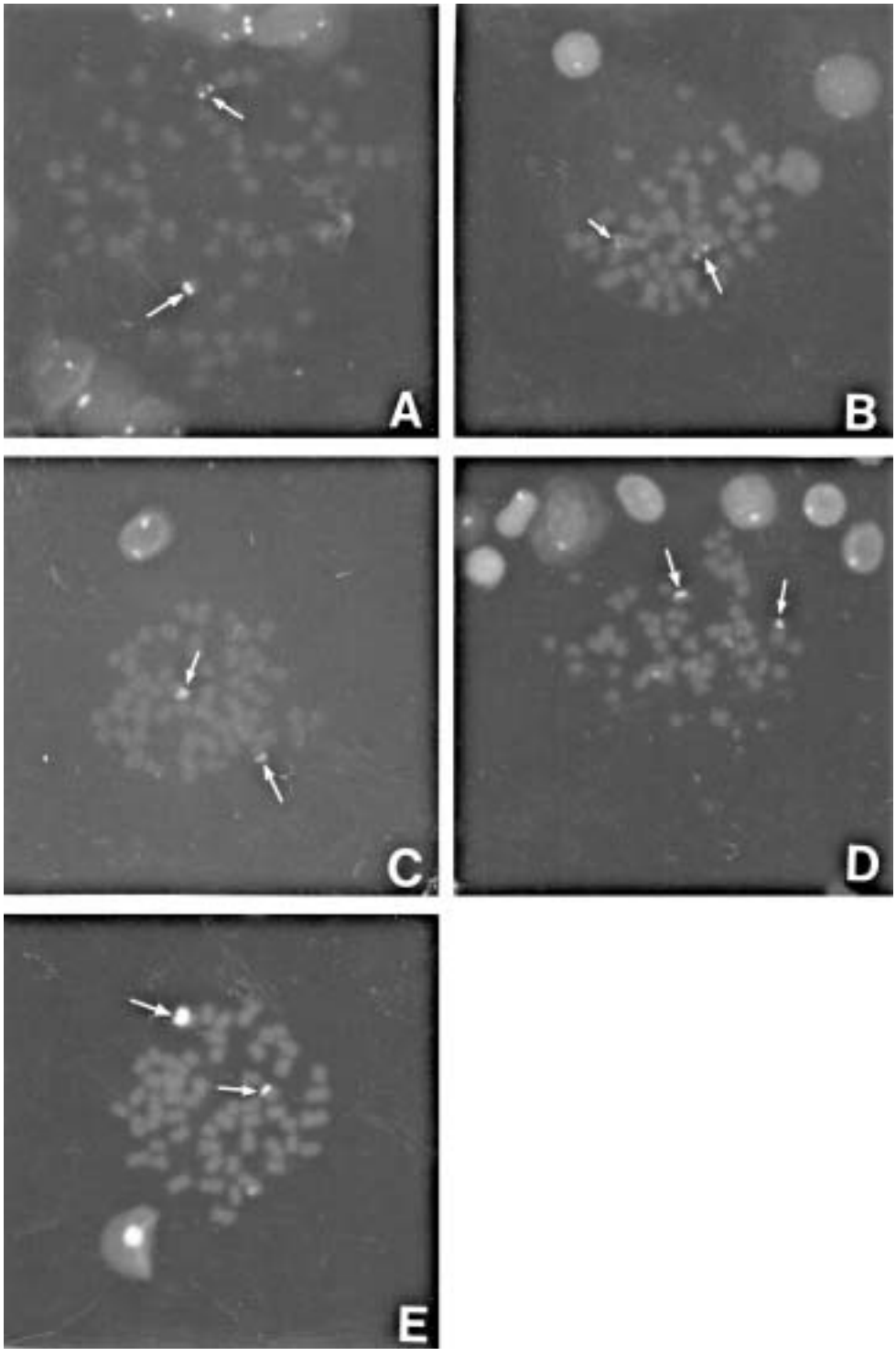

Fig. 3 — Somatic metaphases submitted to *in situ* hybridization using 18S rDNA probe in *Pimelodus* sp (A), *P. heraldoi* (B), *P. maculatus* (C), *P. mysteriosus* (D) and *P. argenteus* (E). The arrows indicate the localization of ribosomal genes in the chromosomes.

size heteromorphism between the homologous chromosomes of the NOR pair, in which only in *P. mysteriosus* this heteromorphism was not demonstrable with silver nitrate.

This correspondence between NORs and CMA_3 staining has already been observed in various species of *Pimelodus* such as *P. maculatus* (BORIN and MARTINS-SANTOS, 2002; SWARCA *et al.*, 2001b), *P. abscon-*

ditus and *P. ornatus* (BORIN and MARTINS-SANTOS, 2002), among others.

In addition to showing the pair of chromosomes corresponding to NOR in *Pimelodus* sp and *P. heraldoi* from the Paraná basin, other fluorescent stained areas at telomeric positions were observed in various chromosomes of the complement of these species, as well as in *P. maculatus* from the Paraguai basin which showed a pair of chromosomes with interstitial staining (Fig. 2C). In *P. heraldoi* and *Pimelodus* sp from the Paraná basin, there was an interesting finding that among the other fluorescent stained locations that did not stain for NOR, a pair of chromosomes were observed with staining at both telomeres, whereby fluorescence was stronger in one of the homologous chromosomes.

The technique of *in situ* hybridization with an 18S rDNA probe showed that there was only one pair of chromosomes with ribosomal cistrons in the 5 species of *Pimelodus* studied here (Fig. 3). This indicates that in *Pimelodus* sp, *P. heraldoi* and *P. maculatus*, the positive CMA_3 staining observed in addition to NOR, could be related to heterochromatin rich in GC bases.

In *Pinirampus pirinampu* SWARCA et al. (1999), besides the NOR-bearing pair of chromosomes, also observed other chromosomes of the complement with fluorescent staining at the telomeres and centromeres, following a pattern corresponding to C-banding. SWARCA et al. (2001a), utilizing the 18S rDNA probe, observed only one pair of chromosomes bearing ribosomal genes in *Pinirampus pirinampu* cited above.

The data obtained after *in situ* hybridization (FISH) in the species examined, confirmed the presence of size heteromorphism between the homologous chromosomes bearing NORs, in *Pimelodus maculatus*, *P. argenteus* and *Pimelodus* sp. This had already been observed with silver nitrate and CMA_3, and in *P. mysteriosus,* whose heteromorphism was detected with just CMA_3, confirming that these regions are made up of a variable number of genes in these species.

The difference in the copy number of ribosomal cistrons between homologous chromosomes has been attributed to various mechanisms, including unequal crossing over, transposition, tandem amplification and other rearrangements involving homologous segments, yielding structural modifications in the NOR (CASTRO et al., 1998; GALETTI et al., 1995).

It should be pointed out that the FISH technique with the 18S rDNA probe, was recently utilized for the first time in the genus *Pimelodus* and quite recently in the family Pimelodidae such as in *Pinirampus pirinampu* and *Zungaro zungaro* (SWARCA et al., 2001a, c, respectively).

Differences in the size of NORs between homologous chromosomes was observed in all of the species of *Pimelodus* from the Paraguai River and in *Pimelodus* sp, species endemic to the Iguaçu River, suggesting that this heteromorphism could be a characteristic of these populations.

The genus *Pimelodus* is conservative in relation to the number of NORs, that is, simple NOR, and in chromosomal type (1 pair of ST). However, our findings and those in the literature indicate variability in the localization of this region in the chromosome, being on the short or long arm, and in absence or presence of size heteromorphism.

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