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Short Communication

Localization of ribosomal genes in three *Pimelodus* species (Siluriformes, Pimelodidae) of the São Francisco River: 5S genes as species markers and conservation of the 18S rDNA sites

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Abstract

Pimelodidae is one of the most representative of Neotropical catfish families. However, these fish are still poorly studied in terms of cytogenetics, especially regarding the application of more accurate techniques such as the chromosomal localization of ribosomal genes. In the present work, fluorescent *in situ* hybridization with 5S and 18S rDNA probes was employed for rDNA site mapping in *Pimelodus* sp., *P. fur* and *P. maculatus* from the São Francisco River in the Três Marias municipality – MG. The results from the application of the 18S probe confirmed the previous data obtained by silver nitrate staining, identifying a simple nucleolar organizing region system for these species. However, the labeling results from the 5S rDNA probe demonstrated a difference in the number and localization of these sites between the analyzed species. The obtained data allowed inferences on the possible processes involved in the karyotypic evolution of this genus.

Key words: Pimelodus, paracentric inversion, 5S rDNA, 18S rDNA, species marker.

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In higher eukaryotes, rDNA is organized into two distinct families: the large, 45S (18S, 28S and 5,8S) and the small, 5S (Pendas *et al.*, 1994), both formed by multiple tandemly organized copies. In contrast to the 45S rDNA which may also be localized by other techniques such as silver nitrate and GC-specific fluorochrome staining, 5S rDNA sites may only be visualized through *in situ* hybridization (Ferro *et al.*, 2001).

Little is known of the chromosome localization of the ribosomal genes in Neotropical fish, especially in relation to 5S rDNA (Martins and Galetti Jr., 1999). Even so, the few available data have shown that these sites may constitute an important cytotaxonomical marker in this group.

Pimelodidae, one of the largest Neotropical catfish families (Pinna, 1998). It is marked by a pronounced karyotypic variability with a predominance of 2n = 56 chromosomes (Swarça *et al.*, 2000). Nevertheless, only few studies report the application of more accurate cytogenetic techniques, such as *in situ* hybridization, in this group (Swarça *et al.*, 2001a, b; Souza *et al.*, 2004).

The localization of the 5S and 18S rDNA genes in three sympatric and syntopic *Pimelodus* species found in the São Francisco River is reported in the present work in an attempt to identify possible markers regarding localization and number of rDNA clusters, as well to confirm data concerning the nucleolar organizing regions (Ag-NORs) previously described by Garcia and Moreira-Filho (2005).

Specimens from three species belonging to the genus *Pimelodus* were analyzed: *Pimelodus* sp. (15 females, 10 males), *P. fur* (10 females, 3 males) and *P. maculatus* (10 females, 6 males), from the São Francisco River in the Três Marias municipality – MG, Brazil. The preparation of mitotic chromosomes was obtained from kidney cells through the direct preparation technique adapted by Bertollo *et al.* (1978) for fish studies. Chromosome morphology was determined according to the arm ratio proposed by Levan *et al.* (1964). The fundamental number (FN) was calculated considering metacentric (m), submetacentric (sm) and subtelocentric (st) chromosomes as bearers of two arms, and acrocentric (a) chromosomes as bearers of a single arm. Fluorescent *in situ* hybridization (FISH) followed the protocol described by Pinkel *et al.* (1986) using 18S (Hatanaka and Galetti Jr., 2004) and 5S rDNA probes (Martins and Galetti Jr., 1999).

Through the karyotypic analyses it was possible to verify that the three studied species differed in their diploid number and chromosome constitution with *P. fur* presenting 2n = 54 and a karyotypic formula of 32m + 8sm + 6st +

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8a, with an FN = 100 (Figure 1a). In *P. maculatus*, the 2n = 56 was distributed into 32m + 12sm + 12st with an FN = 112 (Figure 1b). *Pimelodus* sp. also presented 2n = 56 with the karyotype being composed of 32m + 12sm + 6st + 6a with a FN = 106 (Figure 1c).

The application of the 18S rDNA probe resulted in a pair of fluorescent signals located in the terminal portion of the long arm of chromosome pair 20 in the three species analyzed. In *P. fur* a size heteromorphism involving hybridization signals between the homologues could be observed in these chromosomes (Figure 2 - boxes).

FISH with the 5S rDNA probe demonstrated that these ribosomal sites vary in position and number (4 to 6) in the *Pimelodus* species analyzed (Figures 1 and 2). The occurrence of a pair of fluorescent signals interstitially located in the long arm of the first chromosome was observed for the three species; however in *P. fur* these sites were relatively smaller, hindering their observation. Another pair of signals was also observed in a pericentromeric position of the long arm of chromosome pair 17 in *P. fur* and *Pimelodus* sp., while in *P. maculatus* these markings were located in a terminal position of the same chromosome pair. Besides these sites, the existence of a third pair of markings was observed in *Pimelodus* sp. and *P. maculatus*.. It was located in a pericentromeric position of the long arm of the 21st chromosome pair.

Previous cytogenetic studies performed by Garcia and Moreira-Filho (2005) involving the species here analyzed suggested the existence of a simple NOR system, based on data obtained from silver nitrate and chromomycin A_3 staining,. The results of the present work obtained by FISH using a 18S rDNA probe confirm this hypothesis, as well as the occurrence of a size heteromorphism involving the NOR region in *P. fur*. This is possibly related to the different number of copies of these genes in the homologues resulting from an unequal crossover or some other kind of chromosomal rearrangement

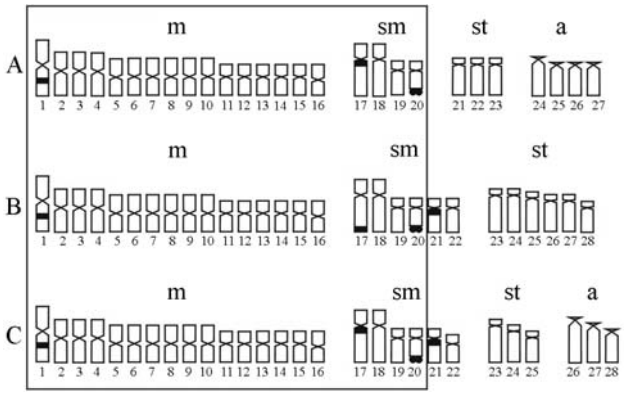

Figure 1 - Idiogram representative of the chromosome set in A) *P. fur*, B) *P. maculatus* and C) *Pimelodus* sp.. The dark circles correspond to nucleolar organizing regions and the shaded regions to the 5S rDNA gene localizations. Highlighted is the part of the chromosome set macroscopically similar in the three analyzed species.

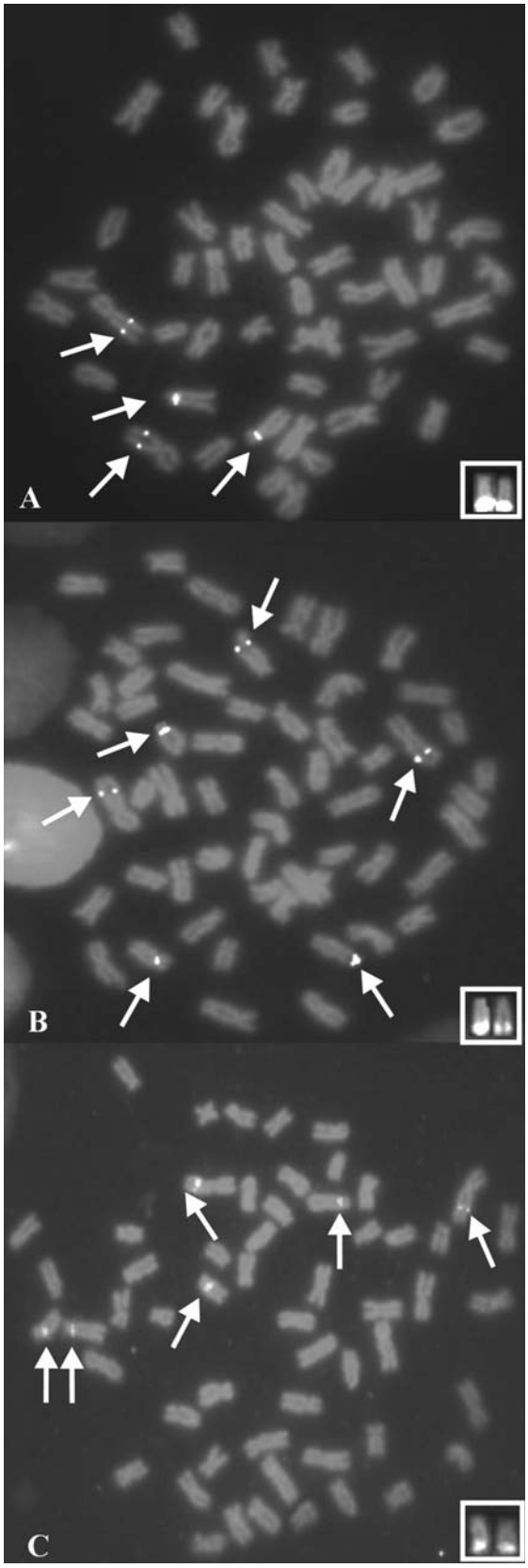

Figure 2 - Metaphases in A) *P. fur*, B) *P. maculatus* and C) *Pimelodus* sp., submitted to FISH with a 5S rDNA probe. The arrows indicate the fluorescent signals. In detail, the nucleolar organizing chromosomes identified through FISH with an 18S rDNA probe.

(Foresti *et al.*, 1981). The occurrence of simple NORs and size heteromorphisms involving this region have already been confirmed by FISH with an 18S probe in other *Pimelodus* species (Souza *et al*, 2004). These data reinforce the idea that the occurrence of a terminal simple NOR system is a conserved characteristic for the genus.

Pimelodus sp. and *P. maculatus* are karyotypically similar in relation to diploid number, NOR number and position and number of 5S rDNA sites. Despite this, a few differences were observed, such as the localization of the 5S rDNA gene in the 17th chromosome pair. This localization difference indicates that a probable paracentric inversion, or even a transposition event, occurred in *P. maculatus*, resulting in the transportation of these genes from a pericentromeric position to a terminal position.

In *P. fur*, a reduction in the diploid number and in the number of 5S rDNA sites (only two pairs) and a differential intensity of the markings in the first chromosome pair were observed. The absence of markings in pair 21 could derive from deletions or possible processes that resulted in the reduction in chromosome number. The intensity difference of the fluorescent signal involving these regions after FISH could be explained by the existence of a smaller number of copies of the 5S rDNA genes in this species.

In the present work, the predominance of 5S rDNA clusters in an interstitial or pericentromeric position was observed. According to Martins and Galetti Jr. (1999), the localization of these sites in an interstitial position would be related to a higher degree of protection of these sequences, avoiding transposition and crossing events that are more frequent in more terminal positions.

Among the Siluriformes, there are few available data on the localization of 5S genes. Previous studies involve members of the families Loricariidae (Kavalco *et al.*, 2004) and Heptapteridae (Garcia *et al.*, 2003), and demonstrate the predominance of only a single chromosome pair bearing the 5S ribosomal sites located in an interstitial position. In *Hypostomus* and *Upsilodus* a greater number of sites were identified, eight and four, respectively (Kavalco *et al.*, 2004). The few cytogenetic studies performed in the genus *Pimelodus* do not allow the discussion of whether the presence of up to six 5S rDNA sites is a plesiomorphic or apomorphic characteristic and the localization difference demonstrate that the identification of these sites for these *Pimelodus* species from the São Francisco River may be considered a species marker.

The data here gathered indicate a high degree of conservation involving the NOR region in terms of number and position in the three analyzed *Pimelodus* species. Another characteristic that indicates a strong evolutionary relationship between these species is the conservation of the karyotypic macrostructure of the 20 first chromosome pairs and the differences that appeared regarding numerical and structural terms in the remaining karyotype (Figure 1). These could easily be explained by the occurrence of non-Robertsonian rearrangements where the inversions stand out and explain, for example, the difference in the localization of the 5S rDNA genes in the 17th chromosome pair of *P. maculatus*, indicating that these rearrangements could have had a major importance in the karyotypic evolution of the genus.

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