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Cytogenetic characterization through chromosomic banding of *Pinirampus pirinampu* (Pisces, Pimelodidae) from the Tibagi river basin PR/Brazil.

ANA CLAUDIA SWARCA, LUCIA GIULIANO CAETANO and ANA LUCIA DIAS *

Departamento de Biologia Geral, Centre de Ciencias Biologicas, Universidade Estadual de Londrina, Londrina, Parana, Brazil.

Abstract — Specimens of *Pinirampus pirinampu* were analyzed cytogenetically. Fifteen individuals were obtained from the Tibagi river near Sertaneja, PR, Brazil. A karyotypic structure consisting of 50 chromosomes distributed as 26M+12SM+2ST+10A was observed. The nucleolar organizing regions (NORs) were identified on the short arm of a pair of subtelocentric chromosomes. A variation in size of the NOR regions was observed among the paired chromosomes. Chromomycin (CMA_3) staining established not only the nucleolar chromosome pair, but also fluorescent marking in the telomeric and centromeric regions of other chromosomes which seem to correspond to the distribution patterns of the constitutive heterochromatin. Restriction enzyme Alu I was employed and the reaction pattern obtained also corresponded to the heterochromatin constitutive distribution.

Key words: cytogenetics, C-bands, NOR, *Pinirampus*, Pimelodidae.

INTRODUCTION

Order Siluriformes is a group of fish that consists of 34 families with about 412 genera and 2405 species, of which 1300 inhabit the neotropical region and the remaining are distributed throughout the tropical regions of Af rica and Asia (NELSON 1994). Among these families, the Pimelodidae is the most diversified neotropical Siluriforme, possessing about 300 species distributed among 50 to 60 genera (MEES 1974). However, despite this diversity, cytogenetic data pertaining to this group of fish are scarce, and investigation are mainly at the level of chromosomic banding. The treatment of the chromosomic preparations by specific fluorochromes such as DAPI, chromomycin A_3 and mitramycin, is commonly used to identify chromosomic regions rich in A-T or G-C, depending on its specificity (SCHWEIZER 1980). When restriction enzymes are used on chromosomic preparations, they can produce highly specific patterns of bands for each type of enzyme. The analysis of these patterns facilitates chromosomic classification, the differentiation of the heterochromatin and the study of chromosomic polymorphisms in fish.

The objective of this work is to characterize the cytogenetics of *Pinirampus pirinampu* localized to the basin of the river Tibagi/PR, with intention to expand the karyotypic data in the Pimelodidae family.

* Corresponding author: fax +55-43-3714207.

MATERIALS AND METHODS

Fifteen specimens (7 females and 8 males) of *Pinirampus pirinampu* collected from the Tibagi river (Sertaneja, PR, Brazil) were used in the present cytogenetic investigation.

Mitotic chromosome preparations were obtained according to BERTOLLO *et al.* (1978). NOR silver staining was performed using the method of HOW-ELL and BLACK (1980). C-banding analyses were per formed using the method described by SUMNER (1972), and CMA, staining as described by SCHMID (1980). The standard reactions by restriction endo-nuclease Alu I were performed using the method de scribed by MEZZANOTTE *et al.* (1983) with some modifications as proposed by MAISTRO (1996). Chromosome morphology was determined on the basis of arm ratio (AR) as proposed by LEVAN *et al.* (1964) and chromosomes were classified as metacen-tric (M), submetacentric (SM), subtelocentric (ST) and acrocentric (A). NF (chromosome arm number)

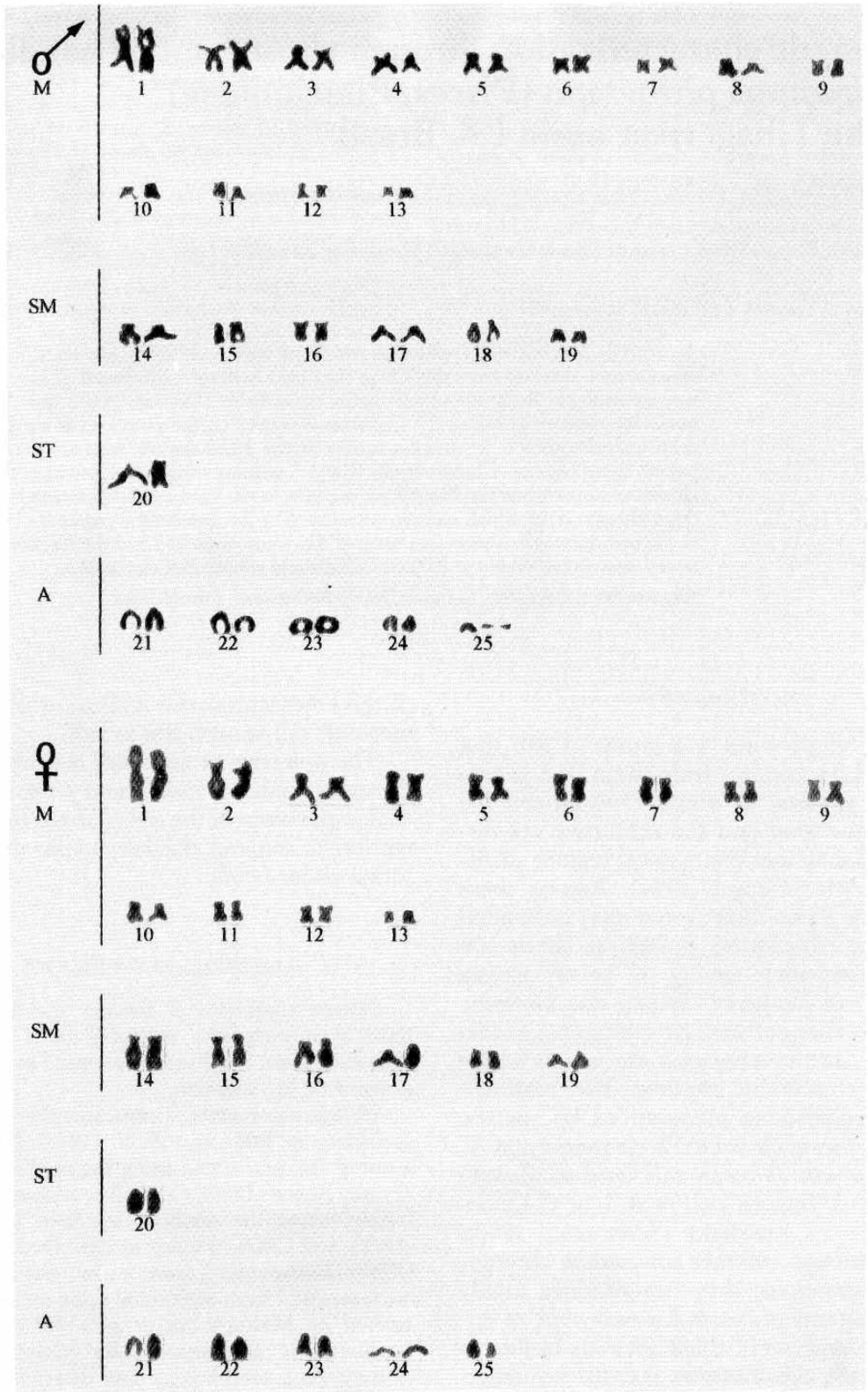

Fig. 1. — Karyotypes of male and female of *Pmirampus pinnampu* stained with Giemsa solution.

was determined considering M/SM/ST chromosomes to possess two arms and acrocentric chromosomes to consist of one arm.

RESULTS AND DISCUSSION

The number of diploid pairs for the ana lyzed individuals of *P. pirinampu* was 50 chromosomes, distributed as 26 metacentric (M), 12 submetacentric (SM), 2 subtelocentric (ST) and 10 acrocentric (A) with a fundamental number (NF) equal to 90, the same for males as in females (Fig. 1). VASCONCELOS (1994) also ana lyzed the same species from the Parana river with 2^=50. However, it was distributed as 22M+12SM+4ST+12A. The difference in the karyotypic formulae between the individuals of the two regions was most likely due to different degrees of condensation of the chromosomes, leading the different karyotypical measure ments. It must be pointed out that the *Pinirampus* genus is monospecific in that *P. pirinampu* is the only known species belonging to the genus. The Pimelodidae family is characterized by an equal diploid number of 56 and 58 chromo somes, a diploid number of 2n=50 was only ob served in *Callophysus macropterus* (GIL 1993).

The order Siluriformes possess simple NORs, where most frequently, only one pair of chromosomes carries ribosomal genes. Then main variation being in its localization on the specific chromosomes (FENOCCHIO and BER-TOLLO 1990; BIAS and FORESTI 1993; VISSOTTO *et al.* 1997; ABUCARMA 1998). In *Pinimmpus pirinampu* the NOR is located in the short arm of 1 pair of subtelocentric chromosomes. Through the impregnation of silver nitrate, a

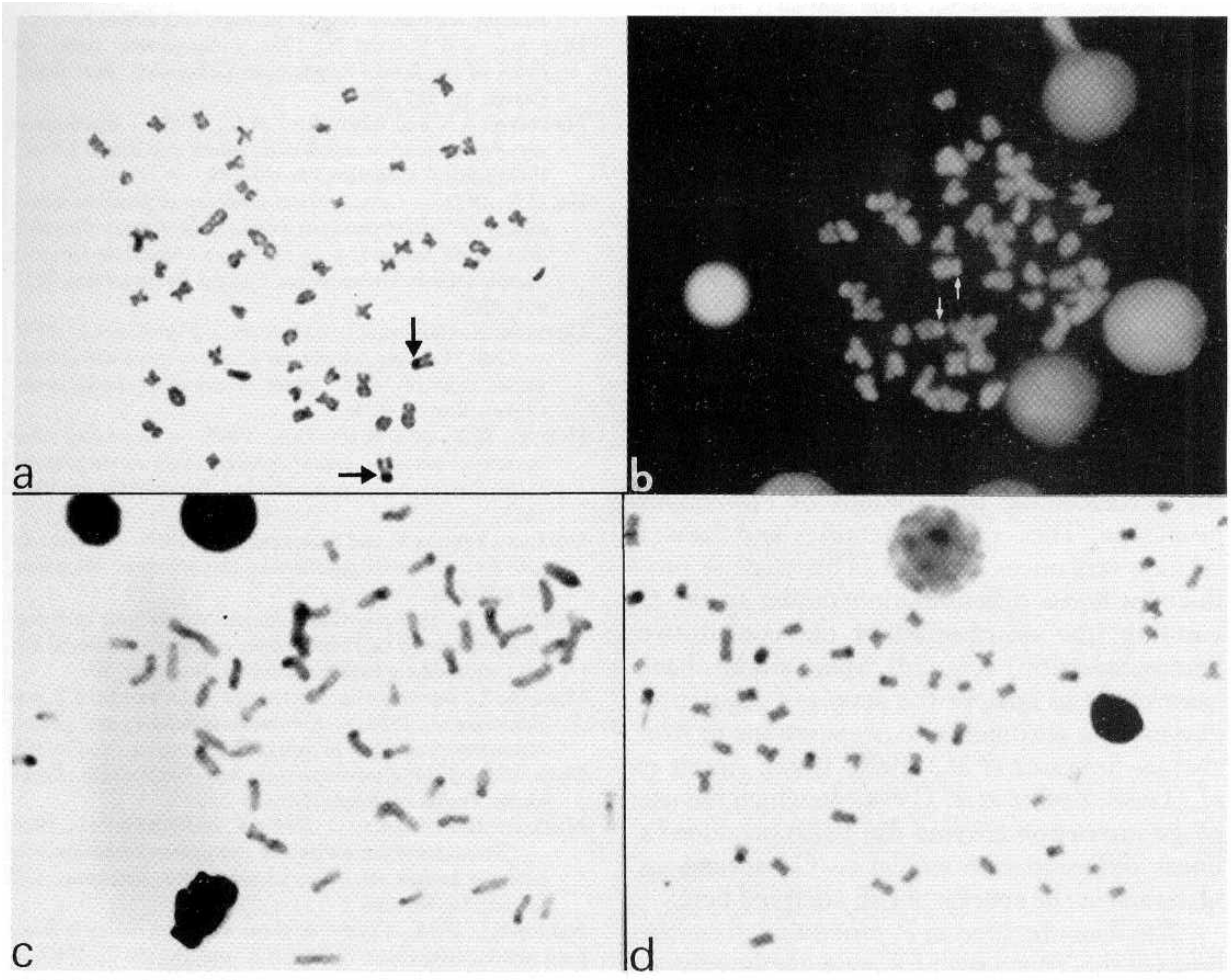

Fig. 2. — Metaphase plates showing: (a) Ag-NORbanding; (b) CMA $_3$-banding; (c) constitutive heterochromatin (C banding); (d) C banding pattern after digestion with Alu I. The arrows indicate the NOR-bearing pair.

heteromorphism in the size of this region was observed between homologous chromosomes (Fig. 2a). That variation may have occurred due to amplification of ribosomal cistrons in that region. VASCONCELOS (1994) did not obvserve the heteromorphism in size of the NOR in the specimens of P. *pirinampu* from the Parana river.

The treatment with chromomycin (CMA_3), in addition to indicating the nucleolar chromosomic pair, also revealed innumerable centromeric and telomeric markings on almost all of the chromosomes (Fig. 2b). The same pattern was observed during the analysis of distribution of the constitutive heterochromatin through the technique of C banding. It showed strong centromeric and telomeric markings in almost all chromosomes of P. *pirinampu* (Fig. 2c). This suggests that the heterochromatic regions in this species are CMA_{3^+}. This pattern was also observed in other species, as in *D. rerio*, where GORNUNG *et a I.* (1997) had used fluorochrome CMA_3 to demonstrate the C banding pattern in its chromosomes. According to MEDRANO *et al.* (1988), segments rich in G-C are responsible for the strong compartmentalization of the genome in the higher vertebrates. In the great majority of the fish, there exists small amounts, or the complete absence of these segments. It is probable, that by producing a pattern of clear-cut banding, suggesting sequences rich in G-C, *Pinirampus pirinampu* possesses a more compartmentalized genome.

The restriction enzyme Alu I was applied to the chromosomic preparations of *Pinirampus pirinampu.* This enzyme identifies and cleaves specific sequences of DNA. This enzyme produced a linear differentiation similar to the C banding (the distribution of the constitutive heterochromatin) (Fig. 2d). Some studies have been made to analyze the action of restriction enzymes on chromosomic preparations of fish, such as SANCHEZ *et al,* (1990, 1991), ABUIN *et al.* (1994), BOUZA *et al.* (1994), in which the use of the restriction enzyme Alu I also produced a linear differentiation similar to C banding, as observed in the species of fish analyzed here.

The data obtained in *P. pirinampu,* from the basin of the Tibagi river PR, extends the cytogenetic data base in Pimelodidae, mainly through the use of fluorochromes and restriction enzymes; thus contributing to the karyotypic structure and evolution of this group of fish.

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