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## KARYOTYPIC DIVERSIFICATION IN *Hoplias malabaricus* (OSTEICHTHYES, ERYTHRINIDAE) OF THE SÃO FRANCISCO AND ALTO PARANÁ BASINS, BRAZIL

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### ABSTRACT

Cytogenetic studies were made on two fish populations from the Monjolinho reservoir (Alto Paraná basin) and from the São Francisco river, belonging to the *Hoplias malabaricus* species group, widely distributed throughout Latin America. They possess different diploid numbers: 40 for both sexes in the São Francisco form and 39/40 for males and females, respectively, in the Monjolinho population. The former showed no differentiation of sex chromosomes, while the latter confirmed the  $X_1X_1X_2X_2/X_1X_2Y$  mechanism reported by Bertollo *et al.* (Cytologia 48: 1-12, 1983) for other populations of that basin. In both populations, NOR sites were multiple, telomeric and small, and C-banding was predominantly centromeric. The implications of the karyotypic diversity are discussed.

### INTRODUCTION

The superorder Ostariophysi, a morphologically well defined taxon, is basically restricted to freshwater, is distributed worldwide and includes the dominant fishes on all continents except Australia and Antarctica (Moyle and Cech, 1988). These characteristics make the group a good example of geographical diversification, still poorly understood.

Within the Ostariophysi, the family Erythrinidae belongs to the order Characiformes. The relationship of that family with the others is still ignored, though Fink and Fink (1981) suggest a possible relationship with the Ctenoluciidae and the

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African Hepsetidae. The Erythrinidae comprise three genera: *Hoplias*, *Hoplerythrinus* and *Erythrinus*, the former with some nominal species, *Hoplias malabaricus* and *H. lacerdae* being the most frequent.

For *Hoplias malabaricus* (Bertollo *et al.*, 1979; Ferreira *et al.*, 1989) reported forms with  $2n = 42$  from the Doce river and Juquiá river basins,  $2n = 39/40$  for males and females from the Alto Paraná basin and  $2n = 40/41$  for females and males from the Aripuanã river, the latter two had multiple sex chromosome systems,  $X_1X_1X_2X_2/X_1X_2Y$  and  $XX/XY_1Y_2$  types, respectively (Bertollo *et al.*, 1983).

Preliminary reports about the existence of a form with  $2n = 40$  chromosomes for both sexes in the São Francisco river stimulated us to compare it with specimens from the Alto Paraná basin.

#### MATERIALS AND METHODS

Fish were sampled from the Monjolinho reservoir (São Paulo State, Alto Paraná basin) and São Francisco river (Minas Gerais State, São Francisco basin).

Thirty-eight specimens (19 males and 19 females) from the São Francisco river and thirty specimens (13 males and 17 females) from the Monjolinho reservoir were studied. Each animal was treated with 0.025% Colchicine (1 ml/100 g body weight) and sacrificed one hour later. The anterior portion of the kidney was obtained, minced with forceps in 0.075M KCl and the cells suspended with a syringe. The cellular suspension was placed in a incubator for 20 minutes at 36°C, and then centrifuged. The supernatant was discarded and the cells fixed in methanol:acetic acid (3v:1v). After two centrifugations and fixations, drops of the cell suspension were placed on clean slides previously warmed at 28-30°C, air-dried and Giemsa stained.

C-banding patterns were obtained according to Sumner (1972). For detection of nucleolar organizer regions (NORs) the method of Howell and Black (1980) was followed.

#### RESULTS

The diploid number of the São Francisco population was determined by the modal frequency class of chromosome counts of 737 metaphase plates. Males and females showed a diploid number of 40 chromosomes ( $28 M + 12 SM$ ), with an arm number (NF) of 80 (Figure 1). Sex chromosomes differentiation was not detected.

In the Monjolinho population, our results confirmed the findings of Bertollo *et al.* (1983) for other populations from that basin. Males showed a diploid number of 39 ( $27 M + 12 SM$ ), NF = 78, and females a  $2n = 40$  ( $28 M + 12 SM$ ), NF = 80, with an  $X_1X_1X_2X_2/X_1X_2Y$  sex chromosome mechanism (Figure 2). For both

populations, NOR sites were multiple, small and telomeric (Figures 3 and 4), as characterized by Bertollo (1988) for other populations of *H. malabaricus*. Their total number was variable among metaphases.

C-banding was mainly pericentromeric (Figures 5 and 6) though in some cases we observed some telomeric bands, perhaps related to the presence of NORs. In the males of the Monjolinho reservoir, the probable Y chromosome bears a strong interstitial C-positive band on its long arm (Figure 7).

#### DISCUSSION

In the genus *Hoplias* karyotypic diversity has been observed since the studies of Bertollo (1978). As more karyotypical data of *H. malabaricus* have become available, we are able to recognize two major groups of forms: one with a basic diploid number of 40 and another with 42 (Bertollo *et al.*, 1986), referred to hereafter as "G40" and "G42", respectively, the ones studied here being part of the former group. While the "G40" group shows tendencies to develop multiple sex chromosome systems, the "G42" displays a less obvious system of the XX/XY type, which still awaits confirmation (Bertollo *et al.*, 1979; Ferreira *et al.*, 1989). The cases of sex chromosome differentiation in the "G40" group involves non-reciprocal translocations, in contrast to the mechanism observed in some other fish species, as in the genus *Leporinus*, in which a heterochromatinization process is involved (Galetti Jr. and Foresti, 1986).

It seems likely that the karyotype of the São Francisco population ( $2n = 40$  for both sexes) represents a more primitive condition compared to that of the Monjolinho population, which has a multiple sex chromosome system. The presence of a C-positive interstitial band on the long arm of the probable Y chromosome of these fishes may be involved in the differentiation process of this chromosome. The other chromosomes, in both populations, have the constitutive heterochromatin located in a preferential pericentromeric region. Studies in other Characiformes (Falcão, 1988; Feldberg and Porto, 1988; Mestriner and Galetti Jr., 1988; Nakayama *et al.*, 1988) also indicate a clear preponderance of pericentromeric C-banding.

Besides the sex chromosomes, the karyotypes of *H. malabaricus* studied here differ significantly in the relative size of the first chromosome pair, which is larger in the São Francisco form. Preliminary studies indicate the presence of this latter configuration in *H. malabaricus* from other localities, such as São Luiz (MA), Recife (PE), Natal (RN) and Suriname (Bertollo, unpublished data).

In relation to NORs, the two populations analyzed possess multiple, telomeric sites which are variable in number. While this latter feature may be related to gene activity, it seems reasonable to consider the presence of multiple NORs as a derived character state (Hsu *et al.*, 1975; Gold and Amemiya, 1986, 1988). Interestingly, within

Figure 2 - Karyotypes of a female and a male of *Hoplias malabaricus* from the Monjolinho reservoir. Apparently, chromosomes of the 6th and the 19th pairs were involved in the formation of the Y chromosome in the male's karyotype.

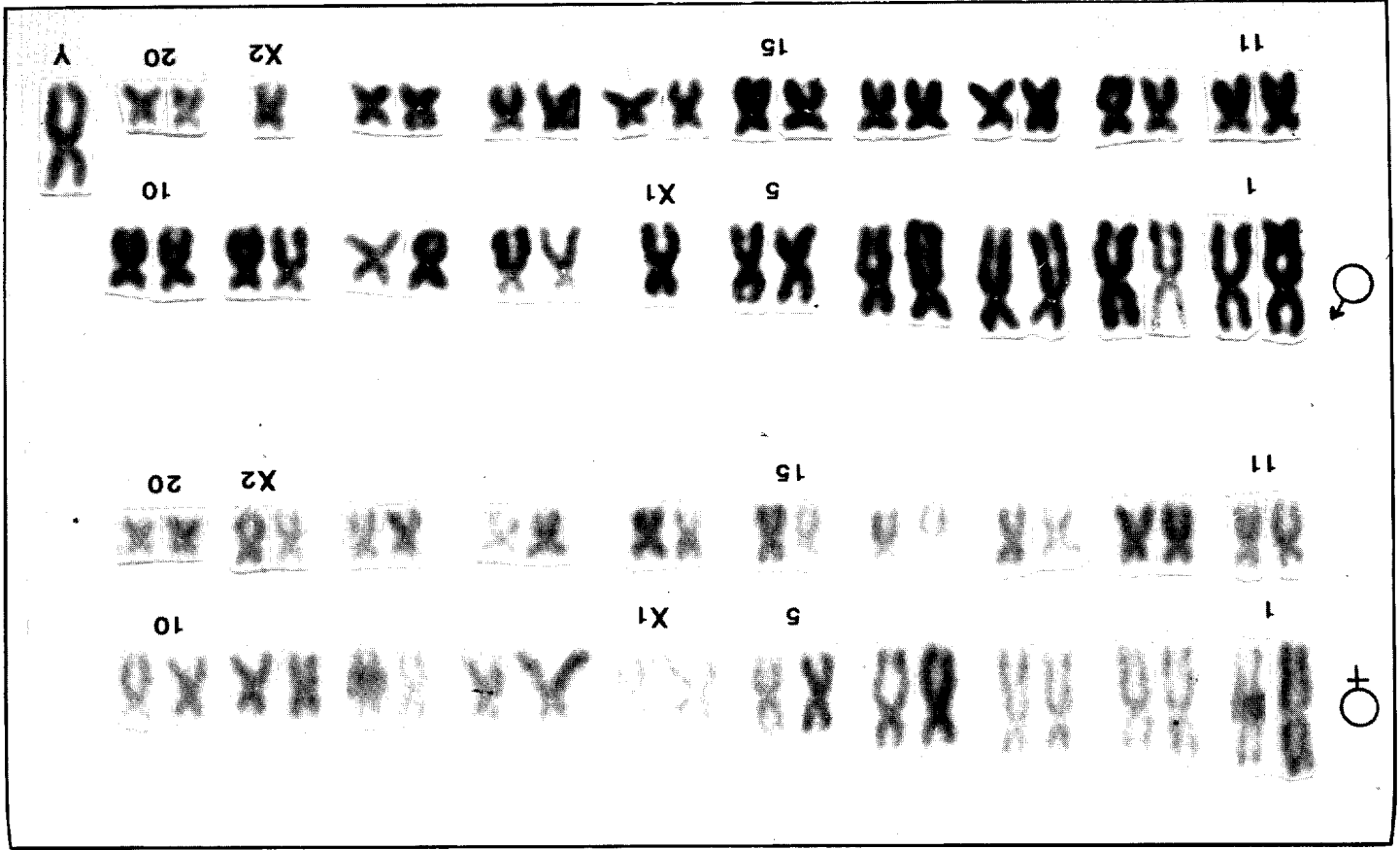


Figure 1 - Karyotypes of a female and a male of *Hoplias malabaricus* from the São Francisco river. No sex chromosome differentiation was detected.



Figure 4 - Two karyotypes of *Hoplias malabaricus* (males) from the Monjolinho reservoir, treated with silver salts, also showing some variability in the NORs

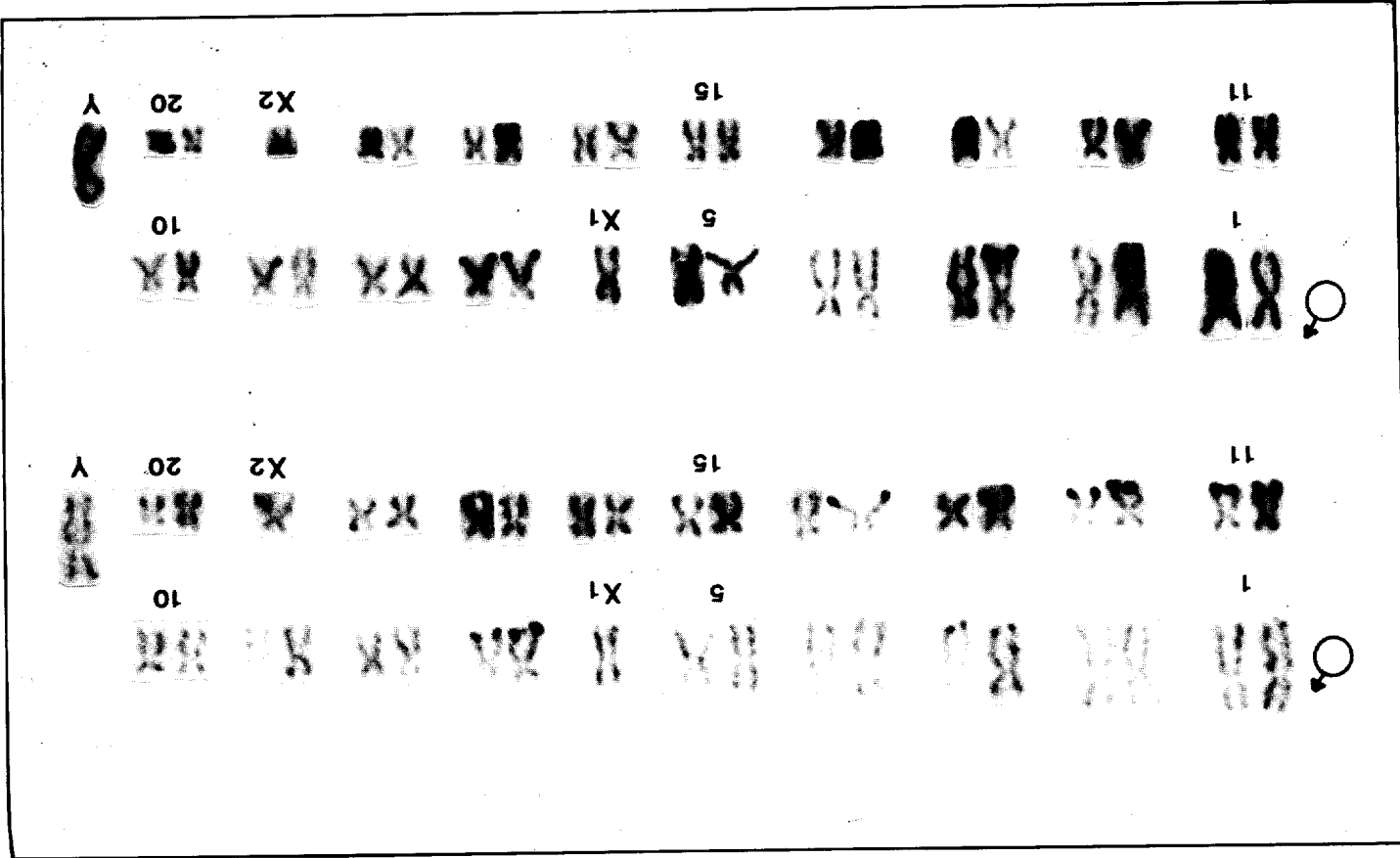


Figure 3 - Two karyotypes of *Hoplias malabaricus* (females) from the São Francisco river, treated with silver salts, showing variability in the number of NOR

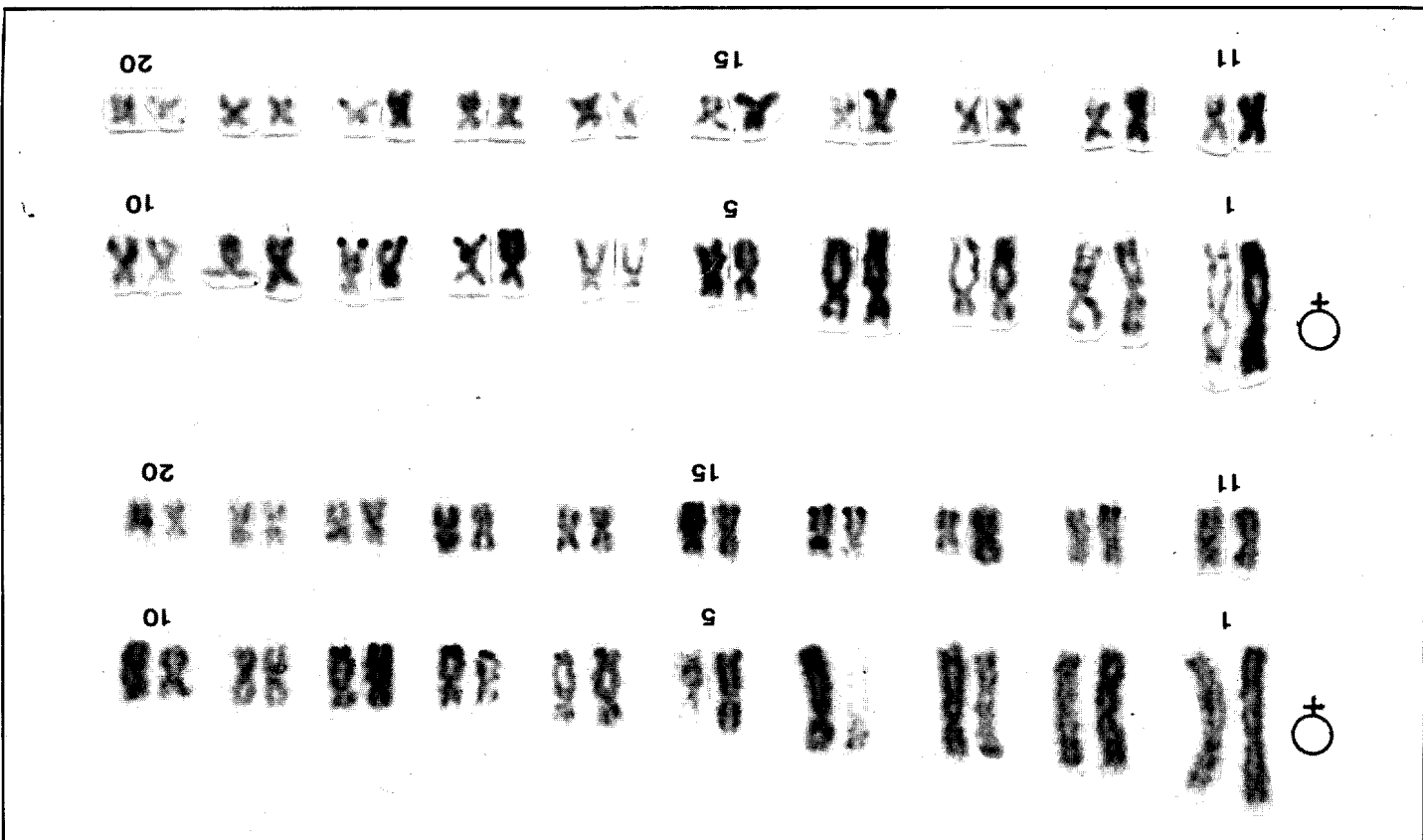


Figure 6 - C-banding pattern in a female and male *Hoplias malabaricus* from the Monjolinho reservoir. Most of the bands are pericentromeric and few are

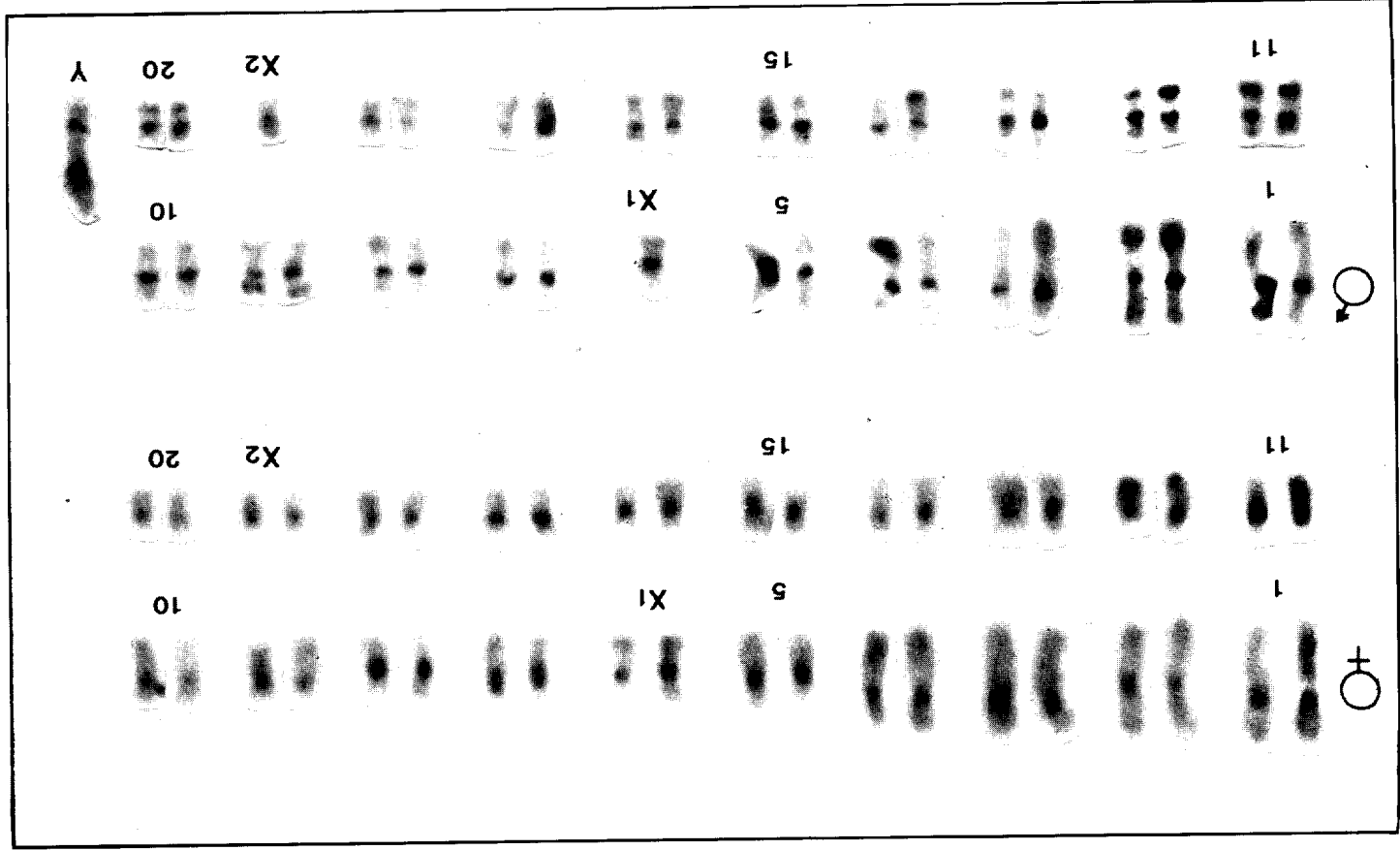
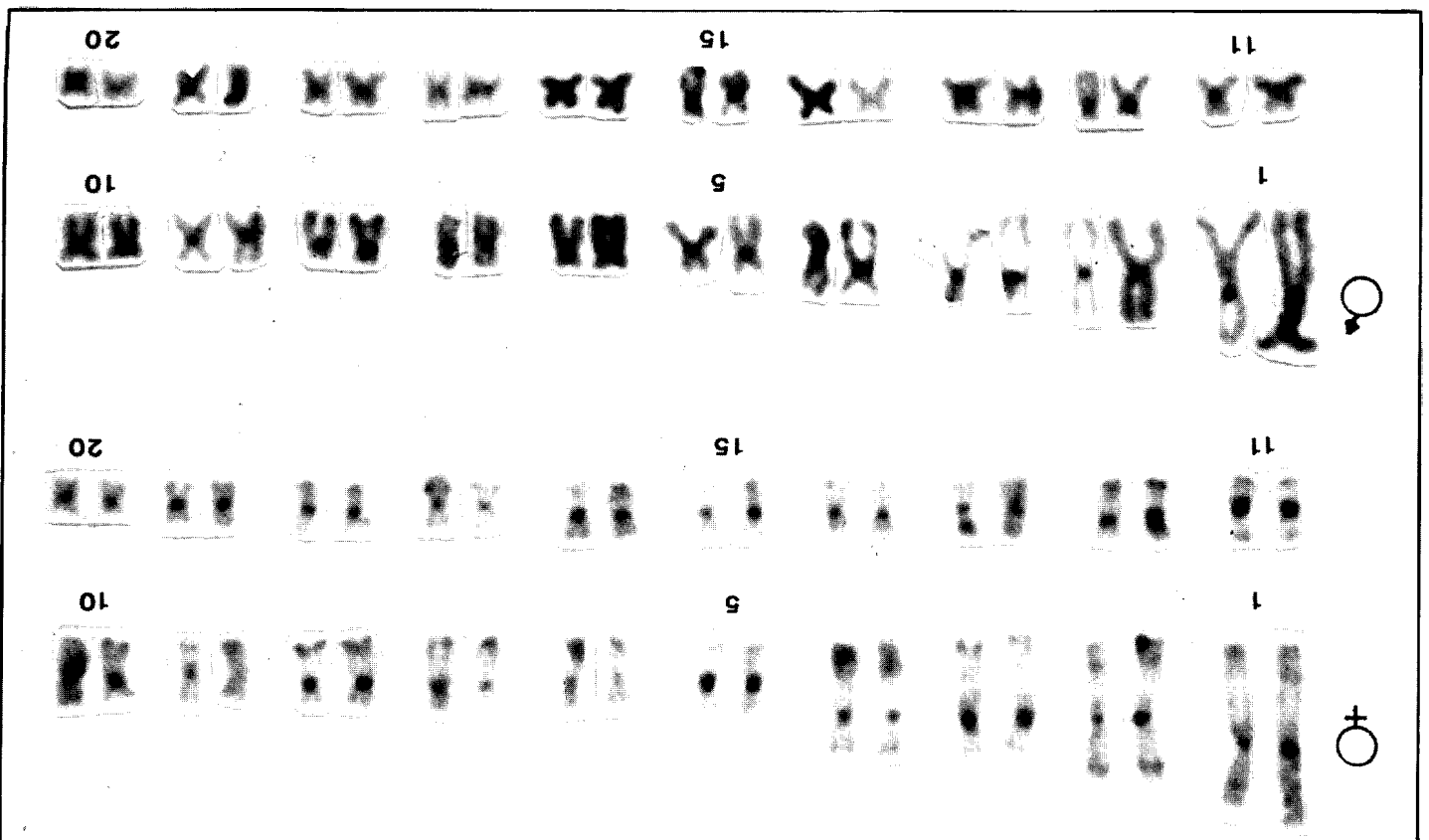


Figure 5 - C-banding pattern in female and male of *Hoplias malabaricus* from the São Francisco river. Positive bands are mainly pericentromeric and few are located on the telomeres.



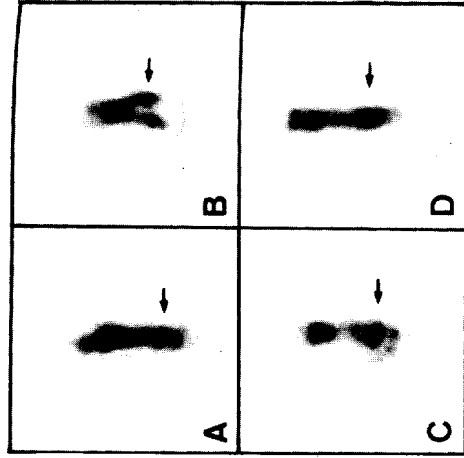


Figure 7 - The probable Y chromosome of male *Hoplias malabaricus* from the Monjolinho reservoir, showing a interstitial C-band (arrows). (A-B) and (C-D): chromosomes of two different specimens.

the family Erythrinidae few cases of a single pair of NORs have been detected (Bertollo, unpublished data).

Following Nelson and Platnick's (1981) definition of species ("... the smallest detected samples of self-perpetuating organisms that have unique sets of characters"), we can consider by means of unique karyotypical characters, the specimens of the two populations studied, to belong to distinct species of the *H. malabaricus* species group.

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#### RESUMO

Duas populações de peixes pertencentes à represa do Monjolinho (bacia do Alto Paraná) e ao rio São Francisco, integrantes do complexo de espécies *Hoplias malabaricus*, foram submetidas a estudos citogenéticos. Os números diplóides obtidos foram 40, para ambos os sexos na população do São Francisco e 39/40, para machos e fêmeas, respectivamente, na população da represa Monjolinho. Os espécimens do São Francisco não apresentaram evidências de cromossomos sexuais. Naquelas da represa do Monjolinho foi confirmado o mecanismo  $X_1X_1X_2X_2/X_1X_2Y$ , já descrito por Bertollo *et al.* (Cytologia 48: 1-12, 1983) para outra população também da bacia do Alto Paraná. Em ambas as populações as regiões organizadoras de nucléolos apresentaram-se múltiplas, teloméricas e de pequeno tamanho. O padrão de bandas C foi predominantemente pericentromérico. São discutidas as implicações da diversidade cariotípica apresentada.

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## NUCLEOLAR ORGANIZER REGIONS, G-AND C-BANDS IN SOME BRAZILIAN SPECIES OF DIDELPHIDAE

Maria José de Souza, Vilna Maia and José Ferreira dos Santos

### ABSTRACT

Karyotypes of four didelphid marsupial species (*Marmosa murina*, *M. cinerea*, *Caturomys philander* and *C. lanatus*) showed a similar complement of  $2n = 14$ ,  $NF = 20$ . The results demonstrate an extensive homology of the G-banding patterns but there are considerable differences in the constitutive heterochromatic patterns and nucleolar organizer regions (NORs), as shown by C-banding and silver staining techniques.

### INTRODUCTION

A few more than 20 didelphid marsupial species have been so far cytogenetically described, based mainly on the studies of standard nondifferentially stained karyotypes. These data show that three distinct types of chromosomal complements characterize marsupials from this family:  $2n = 14$  found in *Caturomys*, *Metachirus*, *Marmosa* and *Dromiciops* (Reig *et al.*, 1977; Rofe and Hayman, 1985);  $2n = 18$  found in *Monodelphis* (Reig *et al.*, 1977; Merry *et al.*, 1983; Langguth and Lima, 1987) and  $2n = 22$ , found in *Didelphis*, *Chironectes*, *Lutreolina* and *Philander* (Reig *et al.*, 1977; Yonenaga-Yassuda *et al.*, 1982).

Data concerning longitudinal differentiation of G bands, distribution of constitutive heterochromatin and nucleolar organizer regions in the karyotypes of