

# Karyotypic studies on two *Hoplias malabaricus* populations (Characiformes, Erythrinidae) of the $2n=42$ group, from the first plateau of the Iguaçu river basin (Paraná State, Brazil)

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**Abstract** - *Hoplias malabaricus* represent a clear example of a species complex, including several karyotypic forms with chromosome numbers ranging from  $2n=39/40$  to  $2n=42$ , including cases of simple and multiple sex chromosome systems. In this work were cytogenetically analyzed two populations of this "species". The samples were obtained at the first plateau of the Iguaçu river (Paraná State, Brazil). The results show that both populations have the same chromosome number ( $2n=42$ ) but one of them appears to be characterized by a sex chromosome system with male heterogamety (XX/XY). A such system was also previously described in *H. malabaricus* from another Brazilian region. Thus, the presence of this differentiated sex chromosome system in this river basin suggests that it was originated before the geographic isolation of the studied populations. The present data represent an additional support for the proposition that *Hoplias malabaricus* constitutes a species complex.

**Key words:** geographic distribution, *Hoplias malabaricus* fish, karyotypic diversity.

## INTRODUCTION

Characiformes represents the dominant fish group in Neotropical Region, with a large geographic distribution from Central America to Patagonia, including a very wide diversity of forms, either in morphology, physiology and adaptation traits. However, several taxonomic aspects and phyletic relations between and within families of this order remain obscure. According to NELSON (1994), this fish group comprises about 10 families, with several subfamilies. Its also presents interesting cytogenetical features, such as variability in chromosome number and formula, sex determining systems, structural and/or numeric and NOR polymorphisms, B-chromosomes, among others. Some families, as Prochilodontidae,

are characterized by an apparent "conservatism" in their karyotypic macrostructure, while other groups, as Tetragonopterinae, show great karyotypic variability accompanying their morphological diversity.

Until the beginning of the cytogenetic studies, the Erythrinidae family was seen without major taxonomic problems, with only three genera (*Hoplias*, *Erythrinus*, and *Hoplerythrinus*) and few species largely distributed in lentic body waters. However, from the first reports of BERTOLLO *et al.* (1978, 1979) it was evident that *Hoplias malabaricus* does not represent a "single" taxonomic entity, but rather a "species complex" comprising forms without sex chromosome heteromorphism, forms with an XX/XY sex chromosome system and a third group with multiple sex chromosome systems (with multiple X or multiple Y chromosomes) (BORN and BERTOLLO 2000; BERTOLLO *et al.* 2000). Several cases of sympatric

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and even syntopic distinct karyotypes were already found (SCAVONE *et al.* 1994; LOPES and FENOCCHIO 1994; LOPEZ *et al.* 1998; BERTOLLO *et al.* 2000).

In the present work two populations of *H. malabaricus* from the first plateau of the Iguaçú river basin (Paraná State, Brazil) were cytogenetically studied, and new informations on the distribution of the chromosomal diversity in this fish group were obtained.

## MATERIALS AND METHODS

Specimens from two populations of *Hoplias malabaricus* from the first plateau of the high Iguaçú river were cytogenetically analyzed. The 1<sup>st</sup> sample was composed of nine individuals (5 females and 4 males) from Canguiri farm (Piraquara). The 2<sup>nd</sup> one was composed by 12 individuals (6 females and 6 males) from Chácara da Paz (São José dos Pinhais). Both localities are not far from Curitiba city (Paraná State, Brazil).

Chromosomal preparations were obtained by the direct air drying technique (BERTOLLO *et al.* 1978) and short term kidney cells culture (FENOCCHIO *et al.* 1991). The chromosomes were stained with Giemsa (5%, 10 minutes) and C- and NOR-banded according to SUMNER (1972) and HOWELL and BLACK (1980), respectively. The karyotypes were arranged as BORN and BERTOLLO *et al.* (2001).

## RESULTS

### *Canguiri Farm, Piraquara*

This population has a diploid number of 42 banded chromosomes, comprising 10 metacentric (M) pairs and 11 submetacentric (SM) pairs. No heteromorphism between males and females was observed (Fig. 1 a, b). The nucleolar organizing regions (NORs) were polymorphic in number and located at telomeric sites of 2 to 6 chromosomes. In some cases, NORs were found on both telomeres of a metacentric chromosome (Fig. 1 d). C-banding showed pericentromeric heterochromatin in most of the chromosomes, as well as some interstitial heterochromatic segments (Fig. 1 c).

### *Chácara da Paz, São José dos Pinhais*

All specimens of this sample have also  $2n=42$  banded chromosomes. However, while a median-sized submetacentric (ST) pair occurs in the female karyotype (12M + 8SM + 1ST), the male

specimens show only one of such chromosome type, together with a single small SM, characterizing a possible sex chromosome system of the XX/XY type (Fig 2 a, b). As in the former population, the NORs were polymorphic and the C-bands were seen in a preferential pericentromeric location (Fig. 2 c, d).

## DISCUSSION

According to several reports, *Hoplias malabaricus* appears to constitute a “species complex”, including at least seven groups with distinct karyotypic features (BERTOLLO *et al.* 2000). Some of these groups showing  $2n=40$  and  $2n=42$  chromosomes, and without heteromorphism between sexes, can be characterized on the basis of their chromosomal morphology and formulae. On the other hand, a form that also presents  $2n=42$  chromosomes, but with an XX/XY sex chromosome system, can also be found (BORN and BERTOLLO 2000). In addition, there are forms that show multiple sex chromosome systems, including multiple X (X1X1X2X2 / X1X2Y) or multiple Y (XX / XY1Y2) chromosomes (BERTOLLO *et al.* 1997a, b, 2000). Some situations in which distinct cytotypes occur in sympatry and/or in syntopy have been reported (BERTOLLO *et al.* 2000). They include both cytotypes without a sex chromosome heteromorphism, such as  $2n=40 + 2n=42$  (LOPEZ and FENOCCHIO 1994; LOPEZ *et al.* 1998) as well as cytotypes without a sex chromosome system together with cytotypes showing differentiated sex chromosomes, such as  $2n=40 + 2n=39/40$ : X1X1X2X2 / X1X2Y (SCAVONE *et al.* 1994) or  $2n=42 + 2n=42$ : XX/XY (BORN 2000).

*Hoplias malabaricus* from Canguiri farm has  $2n=42$  chromosomes without a sex heteromorphism. Its karyotype is quite similar to that described by BORN and BERTOLLO (2001), showing a chromosomal formula composed by 20 metacentric + 22 submetacentric chromosomes. The population from Chácara da Paz also shows  $2n=42$  chromosomes, but with a probable XX/XY sex heteromorphism. These data are in agreement with the report of BORN and BERTOLLO (2000), the females having a median sized submetacentric pair, corresponding to the X chromosome, and the males showing only one of these chromosomes besides another small submetacentric, the Y chromosome. It was not possible to identify if the X chromosome carries ribosomal

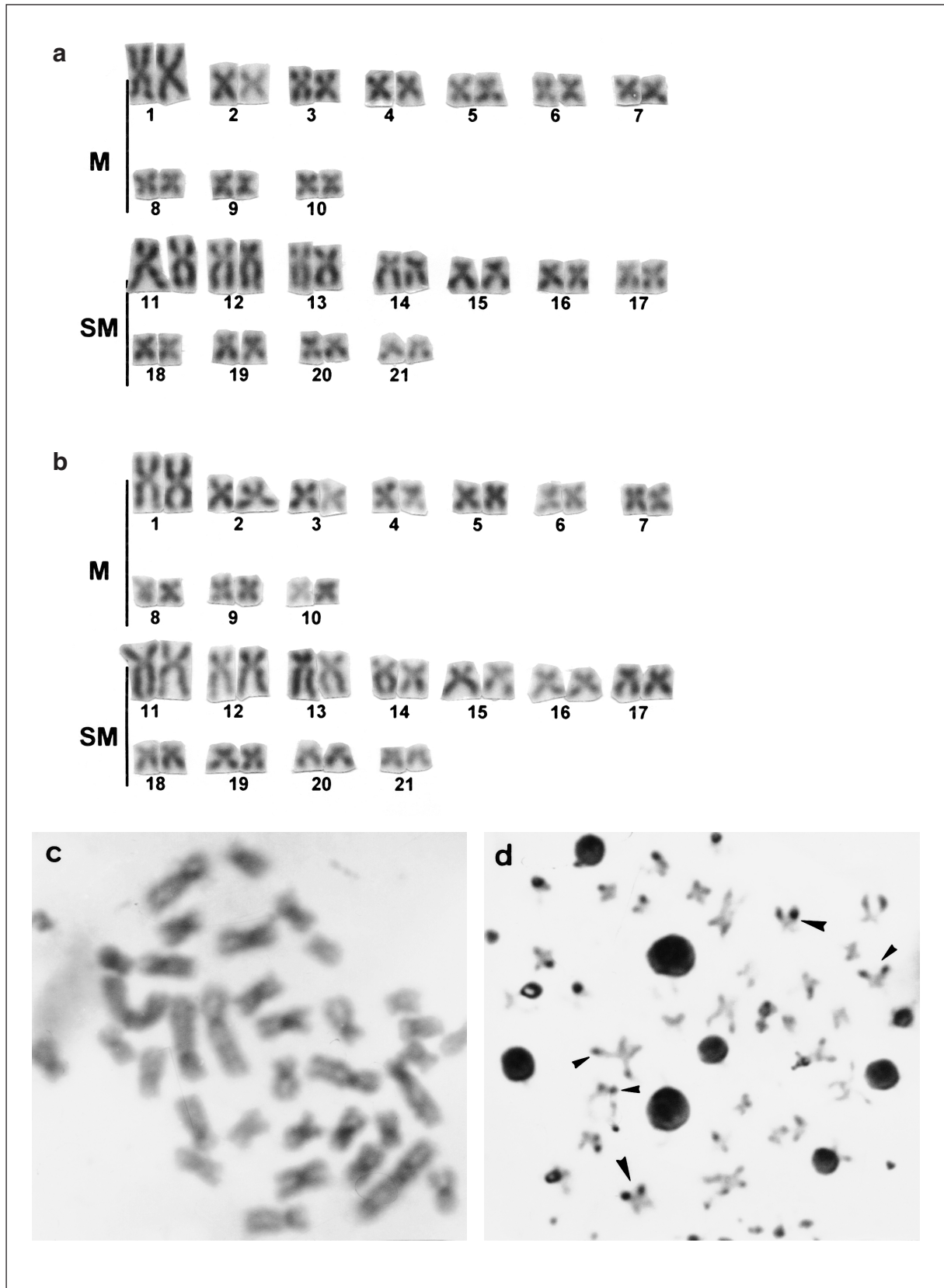


Fig. 1 – Chromosomes of *Hoplias malabaricus* from Fazenda Canguiri. a: female karyotype; b: male karyotype (Giemsa stain); c: C-banding; d: Ag-NORs.

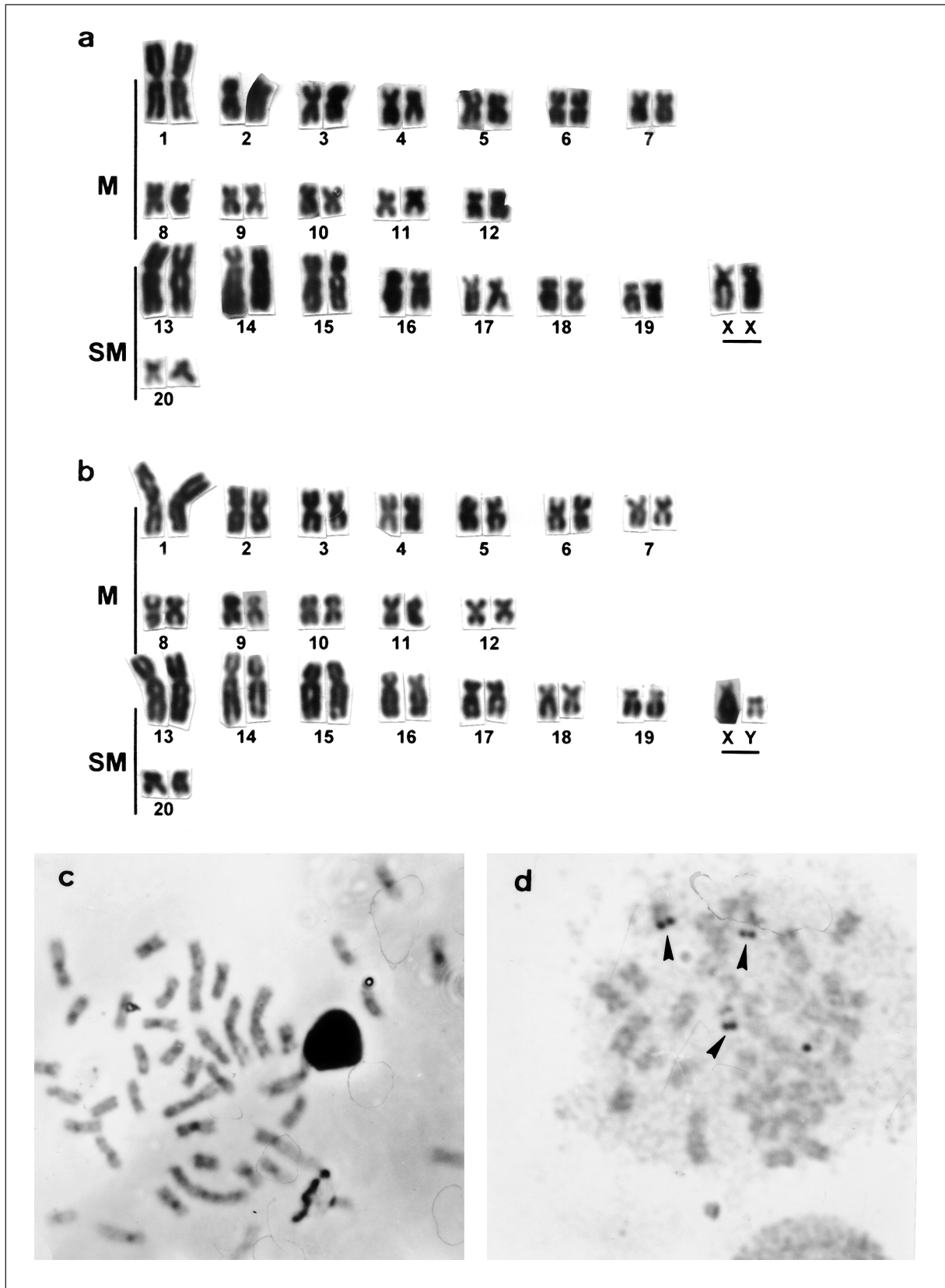


Fig. 2 – Chromosomes of *Hoplias malabaricus* from Chácara da Paz. a: female karyotype; b: male karyotype (Giemsa stain); c: C-banding; d: Ag-NORs. Sex chromosomes are shown in the details.



genes, as occur with the X chromosome of the *Hoplias malabaricus* population reported by BORN and BERTOLLO (op. cit.).

The present data clearly indicate that at the first plateau of the Iguaçú river, as observed in other Brazilian regions, distinct cytotypes are found for “*H. malabaricus* species”, both belonging to the group with  $2n=42$  chromosomes. The Iguaçú basin shows a high endemism rate for several fish groups and it was proposed that the occurrence of *Hoplias malabaricus* in this river could be due to human introduction (SAMPAIO 1988; DERGAM *et al.* 1998). However, some other possibilities can also be considered concerning its presence in this basin. Indeed, the *H. malabaricus* cytotype with  $2n=42$  chromosomes, without a sex heteromorphism, shows a large distribution through the southeast and south Brazil, which is present in several rivers from the Paraná state, reaching Uruguay and Argentina (BERTOLLO *et al.* 2000). Thus, the occurrence of this cytotype in the Iguaçú river does not discard its presence in this hydrographic system before the geographical isolation of the basin. On the other hand, the cytotype  $2n=42$ , with an XX/XY sex chromosome system, appears to have a more restricted distribution, being till now detected in some populations from the Brazilian East basins (BERTOLLO *et al.* 1979; BORN and BERTOLLO 2000, 2001) and in the Iguaçú river (present study). Thus, the presence of this cytotype in these basins could be better explained through dispersion, although this occurrence seems not to have been frequent in the Iguaçú river (SAMPAIO 1988). The hypothesis that cytotype  $2n=42$  (XX/XY) would be derived from the cytotype  $2n=42$  (homomorphic), through differentiations from isolated populations inside the Iguaçú basin, is not supported by the above evidences. It not seems also probable that two distinct karyotypic forms have been equally introduced in this river system. Thus, while the endemism of the Iguaçú basin is a valid occurrence for several fish species, it appears to be not applied for some other species, as the two *H. malabaricus* cytotypes, which are also represented in other Brazilian hydrographic basins.

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