A NEW SYMPATRIC REGION FOR DISTINCT KARYOTYPIC FORMS OF *Hoplias malabaricus* (PISCES, ERYTHRINIDAE)

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(With 2 figures)

ABSTRACT

Specimens of *Hoplias malabaricus* from Lagoa Carioca, an isolated lake of the Rio Doce State Park (state of Minas Gerais, Brazil), were cytogenetically studied. The diploid number was found to be constant, *i.e.*, 2n = 42 chromosomes, although two karyotypic forms were found: karyotype A, characterized by 22M + 20SM chromosomes, observed only in a male specimen, and karyotype B, characterized by 24M + 16SM + 2ST and 24M + 17SM + 1ST chromosomes in female and male specimens, respectively. This sex difference found in karyotype B is related to an XX/XY sex chromosome system. Another female specimen of *H. malabaricus*, also carrying karyotype A, had previously been found in the same lake. The available data indicate that two sympatric cytotypes of *H. malabaricus* exist in the Lagoa Carioca, with cytotype A occurring at a lower frequency and differing from cytotype B by undifferentiated sex chromosomes.

Keywords: *Hoplias malabaricus*, fish, karyotypes, sympatric forms.

RESUMO

Uma nova região de simpatria para formas cariotípicas distintas de *Hoplias malabaricus* (Pisces, Erythrinidae)

Foram analisados, cromossomicamente, espécimes de *Hoplias malabaricus* provenientes da Lagoa Carioca, localizada no Parque Estadual do Rio Doce (Minas Gerais, Brasil). O número diplóide de cromossomos foi constante, 2n = 42, mas duas formas cariotípicas distintas foram encontradas: cariótipo A, caracterizado por 22M + 20SM, presente em apenas um dos exemplares machos e cariótipo B, caracterizado por 24M + 16SM + 2ST e 24M + 17SM + 1ST em fêmeas e machos, respectivamente, diferença esta devida a um sistema de cromossomos sexuais do tipo XX/XY. Contudo, um outro exemplar fêmea, apresentando também o cariótipo A, foi previamente detectado nessa mesma lagoa, o que permite caracterizar o cariótipo A como portador de 2n = 42 cromossomos, mas sem um sistema diferenciado de cromossomos sexuais. Os dados disponíveis evidenciam que dois citótipos distintos (A e B) são encontrados em simpatria e sintopia na Lagoa Carioca, tendo o citótipo A uma freqüência reduzida.

Palavras-chave: *Hoplias malabaricus*, peixes, cariótipos, formas simpátricas.

INTRODUCTION

The Erythrinidae family is a small freshwater Neotropical group comprising only three genera, *Hoplias, Hoplerythrinus* and *Erythrinus.* The genus *Hoplias* is the most widely distributed throughout several Brazilian hydrographic basins. Of this genus, the species *Hoplias malabaricus* has been the most extensively analyzed. The available data clearly demonstrate a non-conservative chromosomal evolution in *H. malabaricus*, with evidence of a species complex for this nominal species (Bertollo *et al*., 1986; Dergam & Bertollo, 1990; Scavone *et al*., 1995; Lopes & Fenocchio, 1994; Lopes *et al*., 1998; Bertollo *et al*., 2000).

H. malabaricus populations in the Juquiá river (São Paulo State, Brazil) and in two lakes of the Rio Doce State Park (Lagoa Dom Helvécio and Lagoa Carioca, MG, Brazil) were previously analyzed by Bertollo *et al.* (1979). All the specimens presented 2n = 42 chromosomes, and a probable XX/XY sex chromosome system, which was reanalyzed and confirmed for the fish from the Rio Doce State Park (Born & Bertollo, 2000a). In a complementary study involving specimens from Lagoa Carioca and Lagoa dos Patos, the latter also located in the Rio Doce State Park, Ferreira *et al*. (1989) found the same karyotypic structure previously described by Bertollo *et al*. (1979). However, of the 22 specimens from Lagoa Carioca, a single female individual presented a divergent karyotype, including a specific meta-submetacentric chromosome pair instead of a subtelocentric one.

This article reports on a similar finding in a male specimen from the same lake, analyzing its implications in terms of chromosome diversity in that fish group.

MATERIAL AND METHODS

Five *Hoplias malabaricus* specimens (4 males and 1 female) from Lagoa Carioca were used for chromosomal analyses. This lake belongs to a vast system of isolated lakes located in the Rio Doce State Park (MG, Brazil), which have been explored in several biological studies.

The animals were previously treated with a yeast suspension for 24 to 48 hours to induce a higher number of mitotic cells (Lee & Elder, 1980). Using a conventional air-drying method, chromosomal preparations were obtained from cells of the anterior kidney after *in vivo* treatment with colchicine (Bertollo *et al*., 1978). The constitutive heterochromatin (C-bands) and the nucleolus organizing regions (NORs) were analyzed following the basic procedures of Sumner (1972) and Howell & Black (1980), respectively. Fluorochrome Mithramycin and counterstaining Distamycin A (DA/MM) were used to detect GC-rich regions in the chromosomes (Schmid, 1980).

The chromosomes were divided into three groups, namely, metacentric (M), submetacentric (SM) and subtelocentric (ST), according to the arm ratios (Levan *et al*., 1964), and arranged in decreasing order of size in each chromosome group.

RESULTS

All the specimens presented the diploid number 2n = 42 chromosomes. However, two distinct karyotypic forms, A and B, were detected. Karyotype A was found only in one male specimen and is characterized by 22M + 20SM chromosomes (Fig. 1a). Karyotype B was found in the remaining specimens and is characterized by 24M + 16SM + 2ST and 24M + 17SM + 1ST chromosomes in female and male specimens, respectively, this difference being related to an XX/XY sex chromosome system (Fig. 1b).

The constitutive heterochromatin, Ag-NORs (NORs detected by silver staining) and GC-rich chromosomal segments presented a similar pattern for both karyotypes. The constitutive heterochromatin showed a preferential location in the centromeric and telomeric regions of the chromosomes (Fig. 2a). Multiple Ag-NOR sites were found, in addition to the occurrence of bitelomeric NORs, *i.e.*, NORs located in both telomeric regions of the same chromosome (Fig. 2b). DA/MM staining highlighted the GC-rich segments, which are also located in the centromeric and telomeric regions of some chromosomes (Fig. 2c).

DISCUSSION

Karyotype B is the chromosomal form usually found in *H. malabaricus* from the Rio Doce State Park (Bertollo *et al*., 1979; Ferreira *et al*., 1989; Born & Bertollo, 2000a). With regard to karyotype A, besides the male specimen analyzed here, only one female specimen carrying this same karyotype

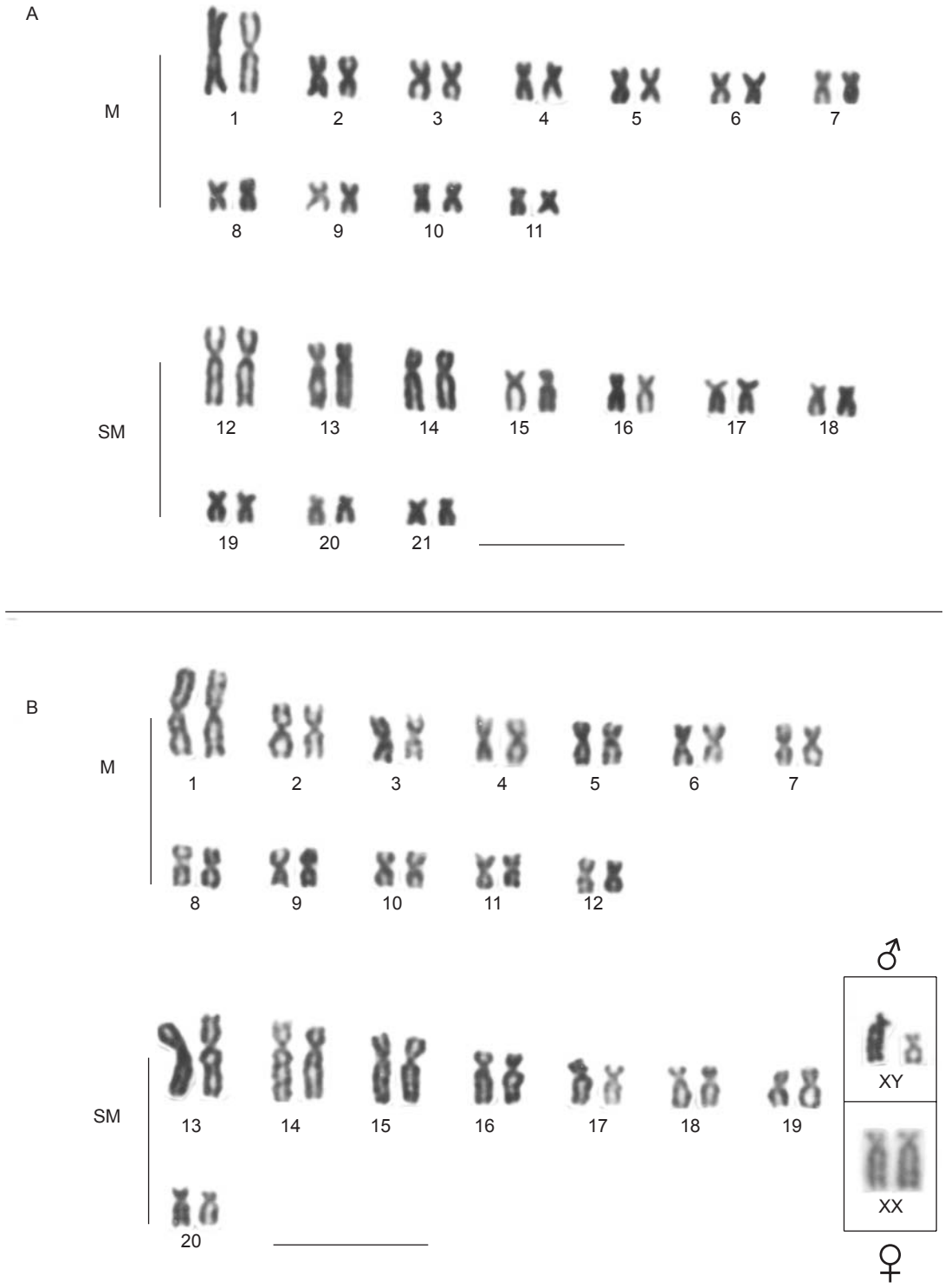

Fig. 1 — *Hoplias malabaricus* karyotypic forms found in the Lagoa Carioca, both with 2n = 42 chromosomes. a) Karyotype without a sex chromosome system; and b) Karyotype with an XX/XY sex chromosome system. The X (subtelocentric) and Y (submetacentric) chromosomes are shown in detail. Bar = 5µm.

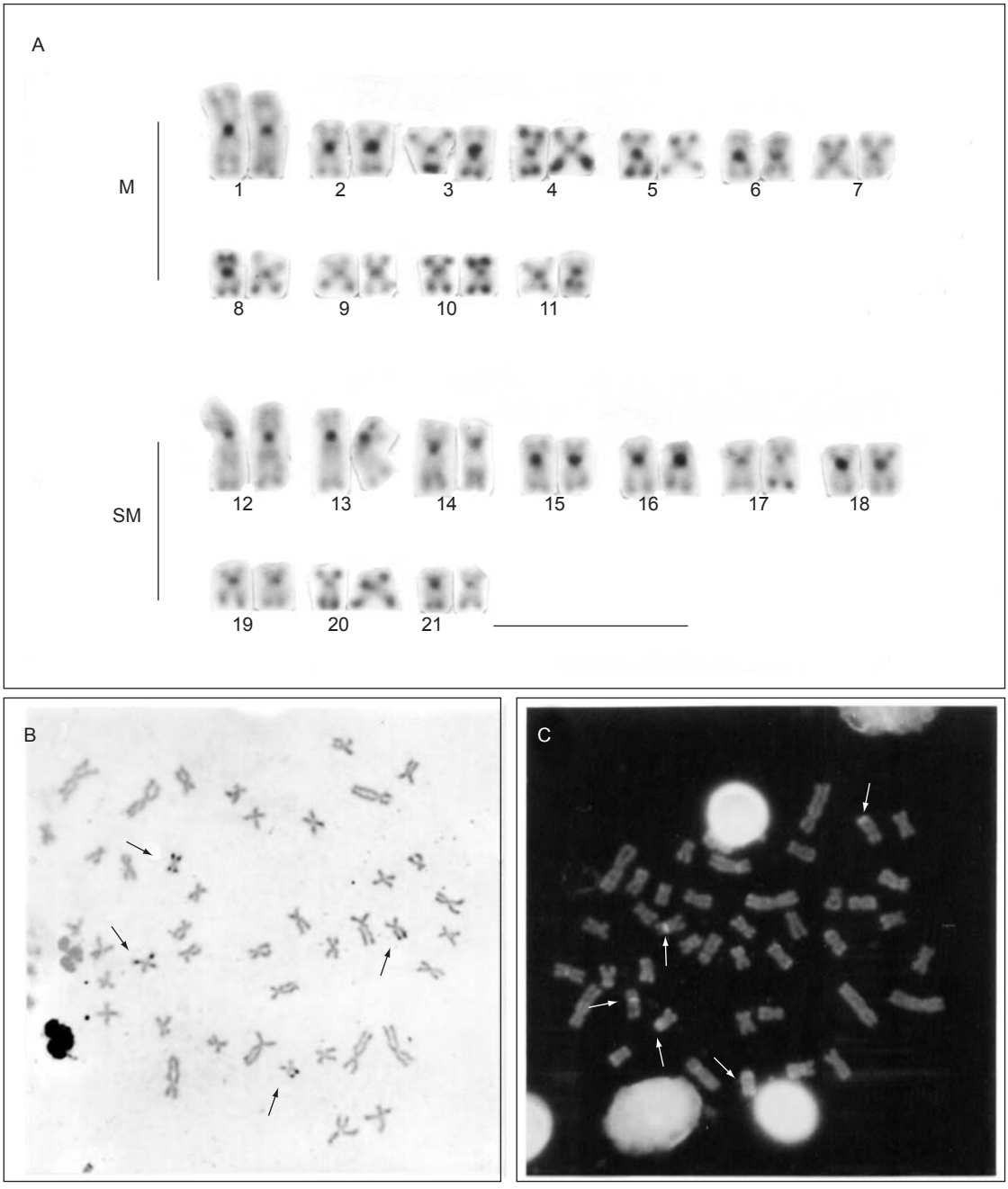

Fig. 2 — *Hoplias malabaricus* karyotypic form without sex chromosomes; a) C-banded karyotype; b) Metaphase showing 3 chromosomes bearing Ag-NORs (arrows), one with bitelomeric sites; and c) Mithramycin-stained metaphase showing fluorescent signals in the telomeric and centromeric regions of some chromosomes. Bar = 5μm.

had been previously reported for specimens from the Lagoa Carioca (Ferreira *et al.*, 1989). In this study, the probable occurrence of a pericentric inversion from the standard karyotype – 40M/SM + 2ST – originating the variant karyotypic form – 42M/SM (Ferreira *et al.*, 1989) was proposed.

However, in a subsequent comparative analysis among populations, seven cytotypes, *i.e.*, distinct karyotypic forms, were characterized for *H. malabaricus*, three of them bearing 2n = 42 chromosomes (Bertollo *et al.*, 2000). The first of these three displayed an exclusive acrocentric chromosome pair (cytotype E), which represents an uncommon feature for *H. malabaricus* and has been found in only one northern population of Brazil. The second one had an XX/XY sex chromosome system (cytotype B) which appears to be restricted to some populations of the Rio Doce State Park and the first plateau of the Iguaçu river (Born & Bertollo, 2000a; Lemos *et al*., 2002). The third one showed only M/SM chromosomes without differentiated sex chromosomes (cytotype A), and is widely distributed among several Brazilian hydrographic basins, presenting minor differentiations among populations (Born & Bertollo, 2000b; Vicari, 2003). In fact, the two karyotypic forms, A and B, from the Lagoa Carioca present chromosome features of cytotypes A and B, respectively. Thus, the available data allow for the characterization of two *H. malabaricus* sympatric cytotypes in the Lagoa Carioca. The occurrence of both male (present paper) and female (Ferreira *et al.*, 1989) specimens carrying cytotype A reinforces this proposition.

Both cytotypes A and B retain the general pattern for constitutive heterochromatin demonstrated by most *H. malabaricus* populations, with C-bands located mainly in the centromeric and telomeric regions of the chromosomes (Fig. 2a). In cytotype B, the subtelocentric X chromosome shows a conspicuous heterochromatic block along a great extent of its long arm (Born & Bertollo, 2000a).

H. malabaricus is a fish group possessing a multiple NOR system located in the telomeric region of distinct chromosome pairs. Bi-telomeric NORs, *i. e.*, NORs located in both telomeric regions of a chromosome, can also be found in some populations (Bertollo, 1996), as is the case of the specimens analyzed here (Fig. 2b). In cytotype A, the number of chromosomes with Ag-NORs ranged from 3 to 4, with a modal number equal to 4, while in cytotype B the modal number was 6 for females and 5 for males (Born & Bertollo 2000a). These differences between sex and cytotypes are related to the subtelocentric X chromosome, which is also a NOR bearing chromosome in cytotype B. GC-rich chromosome regions (Fig. 2c) also showed an identical pattern in both cytotypes, except for the conspicuously labeled segment on the long arm of the X chromosome of cytotype B, which coincides with the heterochromatic segment located here (Born & Bertollo, 2000a).

Some cases of sympatry and syntopy have been reported for different cytotypes of *H. malabaricus* (Scavone *et al*., 1995; Bertollo *et al.*, 1997; Lopes *et al*., 1998; Bertollo *et al*., 2000), without the detection of hybrid forms, thus reinforcing evidence that this fish group corresponds to a species complex (Bertollo *et al.*, 2000). In this context, the Lagoa Carioca emerges as a new sympatric region for two *H. malabaricus* cytotypes, one of them (cytotype A) displaying a lower frequency.

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