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Karyotypic diversity and distribution in *Hoplias malabaricus* (Pisces, Erythrinidae): Cytotypes with 2n = 40 chromosomes

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ABSTRACT

Data about karyotype diversification and its distribution among populations of *Hoplias malabaricus* are presented. Two cytotypes were denoted as types A and B. Although both had 2n = 40 meta-submetacentric chromosomes, these two cytotypes presented some characteristics that allow to differentiate them as distinct entities, the most evident being the relative size of the first chromosome pair. In cytotype A this chromosome was larger than the following ones in the karyotype, a fact not observed in cytotype B. This difference is not due to additional heterochromatic regions, as shown by the C-banded chromosomes. The geographic distribution of cytotype B appears to be larger than that of cytotype A, the former being more dispersed among Brazilian hydrographic basins, reaching Northeast Argentina. However, cytotype A was also found in other countries, as is the case of the Paramaribo population, in Surinam. This study supports the hypothesis that *H. malabaricus* is a species complex.

INTRODUCTION

Erythrinidae fishes include three recognized genera: *Erythrinus*, *Hoplerythrinus* and *Hoplias*. All of them are carnivores, preferentially living in lentic environments. Some species have a wide geographic distribution (Britski *et al.*, 1986). This applies to *Hoplias malabaricus*, commonly known as "traíra", which has been the species most intensively studied from a cytogenetic viewpoint. Analyzed populations from different hydrographic basins show karyotypic diversity in terms of numbers and types of chromosomes in their diploid complements (Bertollo *et al.*, 1979, 1983; Ferreira *et al.*, 1989; Dergam and Bertollo, 1990; Scavone *et al.*, 1994; Lopes and Fenocchio, 1994; Jorge, 1995). Five cytotypes have been identified: cytotype 2n = 42 (males and females), cytotype 2n = 40 (females) and 2n = 39 (males), cytotype 2n = 40 (females) and 2n = 41 (males), and cytotype 2n = 40 (males and females). Two distinct types were recognized in this last group, by their chromosomal features and were named as cytotypes A and B in the present study. This diversity has led to the hypothesis that different species may be recognized under the common name *H. malabaricus* (Bertollo *et al.*, 1986; Dergam and Bertollo, 1990), as observed in several other taxa of Brazilian freshwater fish in taxonomic reviews (Malabarba, 1989).

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MATERIAL AND METHODS

Thirteen populations of *H. malabaricus* were sampled, 12 from different Brazilian hydrographic basins and one from Surinam (Figure 1, Table I).

Table I - Number of *Hoplias malabaricus* specimens and number of metaphases analyzed per collection site.

Collection sites (Figure 1)	Locality (Brazilian State)	No. of specimens studied by sex	Number of metaphases analyzed
1	Manaus (Amazonas)	4 females	96
		5 males	118
2	Tucuruí (Pará)	4 females	33
		3 males	46
3	Porto Velho (Rondônia)	2 females	60
		2 males	43
4	Aripuanã (Mato Grosso)	1 female	22
5	Cuiabá (Mato Grosso)	4 females	195
		5 males	233
6	Goiás Velho (Goiás)	2 females	57
7	Corumbá (Mato Grosso do Sul)	1 female	80
		1 male	98
8	Miranda (Mato Grosso do Sul)	3 females	100
		5 males	284
9	Paramaribo - Surinam	1 female	13
		5 males	170
10	Tucuruí (Pará)	2 males	35
11	São Luiz (Maranhão)	1 female	33
		2 males	100
12	Natal (Rio Grande do Norte)	1 female	36
		2 males	42
13	Recife (Pernambuco)	2 females	59
		2 males	76

The animals were treated with 0.05% colchicine for 50-60 min (1 ml/100 g body weight) and mitotic chromosomes were obtained from kidney cells by the standard, air-drying technique (Bertollo *et al.*, 1978). In some cases, the specimens were first stimulated with a yeast suspension (Lee and Elder, 1980) for induction of mitosis.

Nucleolar organizer regions (NORs) and constitutive heterochromatin (C-bands) were visualized according to Howell and Black (1980) and Sumner (1972), respectively. The method of Kligerman and Bloom (1977), with small modifications, was used for the study of meiotic chromosomes from male gonads.

The diploid chromosome number (and sometimes also the haploid one) was determined for each population. The metaphases of the best quality were photographed and the karyotypes were arranged according to decreasing size.

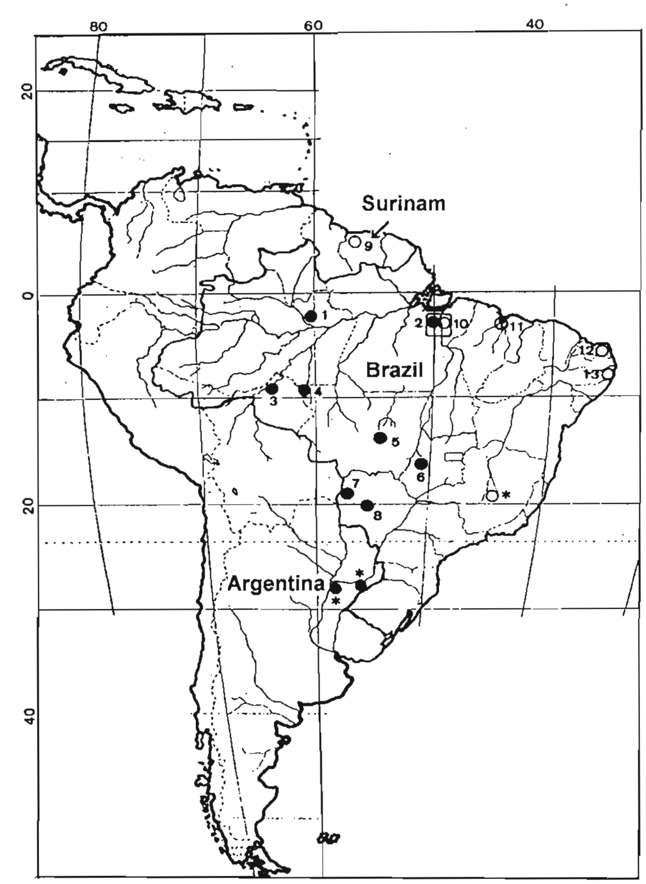

Figure 1 - Distribution of *Hoplias malabaricus* cytotypes A (open circle) and B (dark circles). The square represents a sympatric region of these two forms. The asterisks indicate the three populations previously studied, one belonging to cytotype A (Minas Gerais State, Brazil) and two belonging to cytotype B (Northeast Argentina). The numbered localities are indicated in Table I.

RESULTS AND DISCUSSION

Among the thirteen populations under study, five showed cytotype A and eight showed cytotype B (Figure 1).

Cytotype A is characterized by 2n = 40 meta-submetacentric chromosomes both in males and females, with no apparent sex-related heteromorphism, as previously described by Dergam and Bertollo (1990) for the population from the Três Marias region (São Francisco river, Minas Gerais State, Brazil). In the

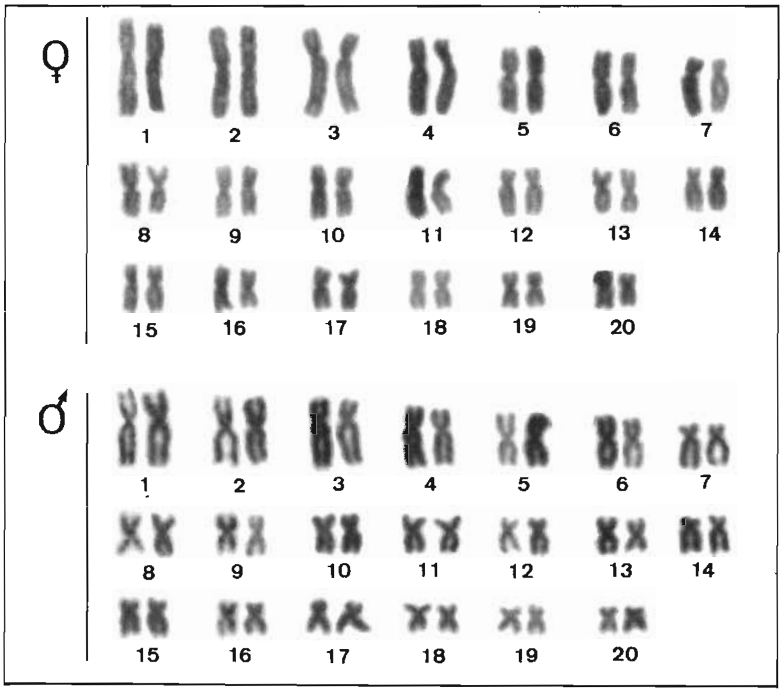

Figure 2 - Karyotypes of female and male *Hoplias malabaricus* with cytotype B, from Corumbá region, Mato Grosso do Sul State.

present study, an identical karyotype was observed for five additional populations (Figure 1).

Cytotype B also exhibited 2n = 40, the presence of meta-submetacentric chromosomes and absence of karyotypic differentiation between males and females, at least at the level of the analyses performed (Figure 2), as also observed by Lopes and Fenocchio (1994) and Jorge (1995) for two populations from Argentina. The data obtained from meiotic cells confirmed these results, with the identification of 40 chromosomes in spermatogonial cells, 20 bivalents in metaphases I and 20 chromosomes in metaphases II. No apparent atypical pairing was seen among the bivalents, supporting the presence of chromosomal homomorphism between sexes (Figure 3C-F).

Constitutive heterochromatin was not very abundant in either cytotype and was associated with the centromeric regions

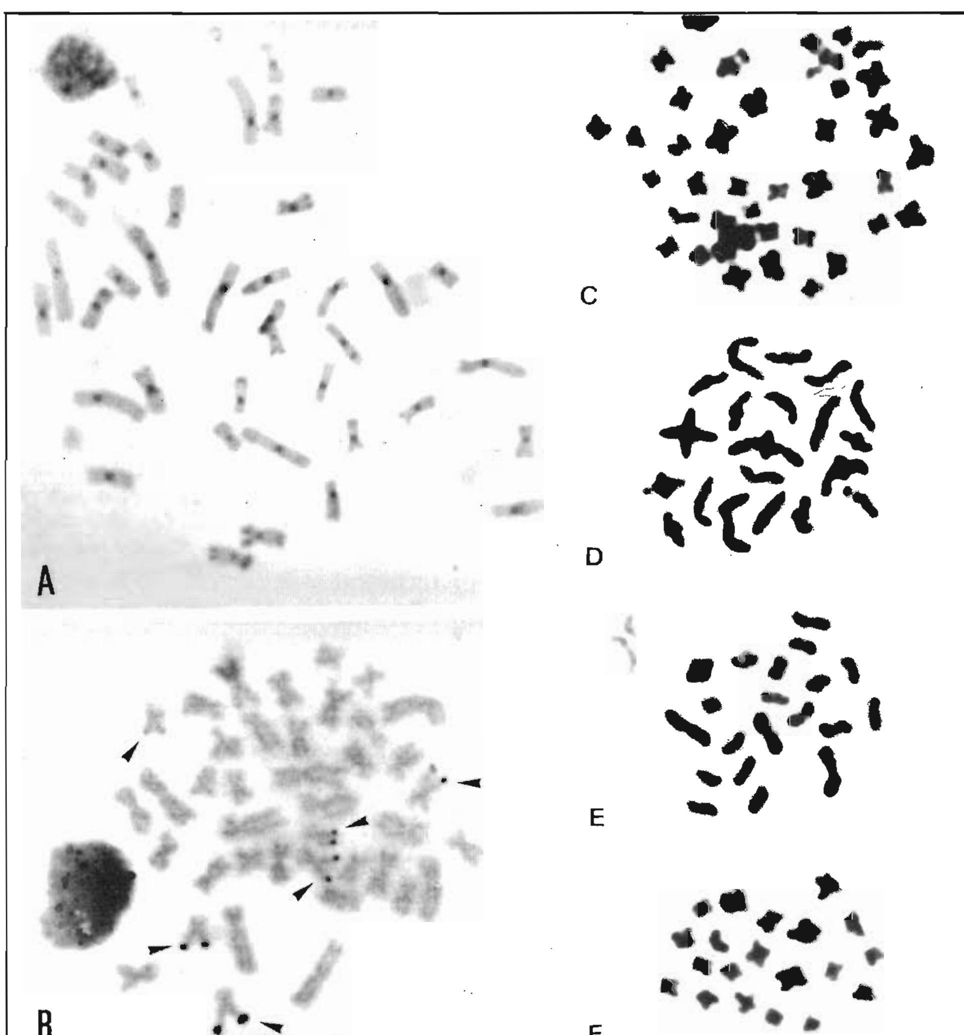

Figure 3 - Constitutive heterochromatin (A), NORs (B) and meiotic stages (C-F) representative of *Hoplias malabaricus* cytotype B (A,B: Corumbá region, Mato Grosso do Sul State; C-F: Manaus region, Amazonas State). The two dark chromosome arms in A and B are due to technical artefacts.

of all chromosomes, as well as with some telomeric regions (Dergam and Bertollo, 1990; Figure 3A, present paper). Interstitial heterochromatin segments were seen sporadically in some chromosomes. Similarly, the NORs were multiple in both cytotypes, located on different chromosomes of medium to small size, in a terminal position on the long arm. Figure 3B illustrates six of these regions, apparently located on three homologue pairs. However, the NORs can vary in number both intra- and interindividually and in some cases are bi-telomeric, i.e., located on both telomeres of the same chromosome, as discussed in detail by Bertollo (1996). Among fishes, the NORs can occur in a single chromosome pair or in several, according to the groups considered (Moreira-Filho *et al.*, 1984; Almeida Toledo and Foresti, 1985; Porto *et al.*, 1992; Galetti Jr. *et al.*, 1995). Thus, *H. malabaricus* belongs to the group with multiple NORs, a condition that also prevails in other previously studied erythrinids (Bertollo, 1996).

However, despite the similarities mentioned above, cytotypes A and B have distinctive characteristics that permit their unequivocal differentiation. The most outstanding among them is definitely the relative size of the first chromosome pair when compared to the size of the subsequent ones in the karyotype. Whereas in cytotype A the first pair is approximately 1/4 larger than the second, in cytotype B these pairs are nearly the same size. The distribution of constitutive heterochromatin in these "marker" chromosomes is similar in both cytotypes, which excludes the possibility that the larger size in cytotype A is due to heterochromatin accumulation in this chromosome (Figure 4).

Minor differences between the two cytotypes could also be observed, such as location of the centromeres in some chromosome pairs. Similar differences also seem to occur among some populations of the same cytotype, in which certain chromosomes are meta- or submetacentrics. Whether these differences actually exist or are only the products of technical limitations still remains to be clarified. However, the basic karyotypic structures of cytotypes A and B can be perfectly observed in all populations under study.

The distribution of cytotype B appears to be larger than that of cytotype A, the former being somewhat more dispersed among the Brazilian hydrographic basins according to the available data (Figure 1). Considering the results of Lopes and Fenocchio (1994) and Jorge (1995), cytotype B extends to northeast Argentina, reaching the regions of Posadas and Corrientes, respectively. Cytotype A is also found in the Paramaribo population, in Surinam. However, the area of distribution of each of these two cytotypes is

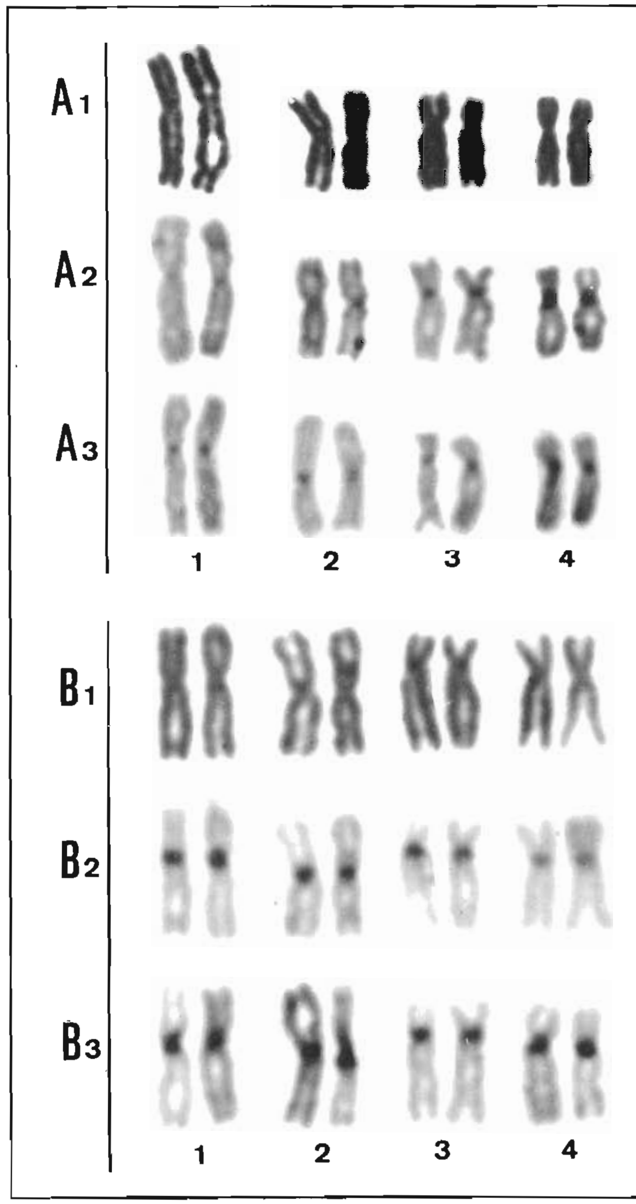

Figure 4 - Partial karyotypes of *Hoplias malabaricus* arranged from Giemsa-stained (A_1, B_1) and C-banded (A_2, A_3, B_2, B_3) chromosomes of the first four pairs demonstrating cytotypes A and B. (A_1, A_2: Natal region, Rio Grande do Norte State; A_3: Paramaribo region, Surinam; B_1, B_2, B_3: Manaus region, Amazonas State).

shared by other distinct cytotypes also found in *H. malabaricus* (Bertollo, L.A.C., unpublished results). In the Tocantins river (Tucuruí, Pará State), cytotypes A and B were found in sympatry (Figure 1), with no evidence of hybridization, reinforcing the hypothesis that the chromosomal differentiation observed in *H. malabaricus* reflects the presence of distinct species with species specific karyotypes.

Two major patterns of karyotype evolution can be observed among neotropical Characiformes: a more homogeneous one characterized by a greater stability, at least at the karyotypic macrostructure level, and a more heterogeneous one characterized by an apparent numerical and/or morphological chromosome diversity (Galetti Jr. *et al.*, 1994). The latter situation applies to *H. malabaricus*, together with some other nominal "species" such as *Astyanax scabripinnis* (Moreira-Filho and Bertollo, 1991), *Astyanax fasciatus* (Morelli *et al.*, 1983; Justi, 1993), *Corydoras nattereri* (Oliveira *et al.*, 1990), *Eigenmannia virescens* (Almeida Toledo *et al.*, 1993), among others, supporting the necessity for a re-examination of their present taxonomy.

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RESUMO

São apresentados dados adicionais sobre a diversidade cariotípica e sua distribuição geográfica em populações de *Hoplias malabaricus*, considerando dois citótipos distintos aqui denominados de A e B. Embora estes dois citótipos possuam 2n = 40 cromossomos meta-submetacêntricos, eles apresentam algumas particularidades que permitem distinguí-los claramente entre si, como é o caso do tamanho relativo do primeiro par de cromossomos no cariótipo. No citótipo A este cromossomo é bem maior que os pares imediatamente seguintes, fato este que não ocorre no citótipo B. Esta diferença não pode ser atribuída a regiões heterocromáticas adicionais, como mostrado pelo bandamento C. Os dados até então disponíveis mostram que a distribuição geográfica do citótipo B apresenta-se mais ampla, comparativamente àquela do citótipo A, sendo mais disperso entre diferentes bacias hidrográficas brasileiras, podendo também atingir o nordeste da Argentina. Contudo, o citótipo A também pode ser encontrado em países fronteiriços ao Brasil, como é o caso do Suriname. Os dados obtidos suportam a hipótese de que *H. malabaricus* pode representar um complexo de espécies.

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