

Cytogenetics and Biogeography: Considerations about the Natural Origin of *Hoplias malabaricus* (Characiformes, Erythrinidae) on the Iguaçú River

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ABSTRACT

Hoplias malabaricus (trahira) is a widespread fish species over the Neotropical region with diversified inter-population karyotypes (cytotypes), which may correspond to a species complex. Despite the wide distribution in the South American basins, some authors have questioned its natural origin in the Iguaçú river, an important Brazilian river basin which is characterized by several endemic fish species. We have analyzed the karyotype of *H. malabaricus* from different collection sites of this river, by conventional and banding methods. Our results, in addition to our previous data concerning geographic distribution of the cytotypes, contribute to better understand the origin of *H. malabaricus* on the Iguaçú river, reinforcing the proposition that it is a natural fish species in this river basin.

Key words: Chromosome evolution, karyotypes distribution, endemism

INTRODUCTION

The fish fauna of the Iguaçú river is well known for its high degree of endemism and for the absence of several fish families common in other Brazilian basins (Júlio Jr. et al., 1997). The Iguaçú falls have acted as an efficient geographical barrier between the ichthyofauna from the first and the second plateaus along most of the river length (Maack, 1968). Garavello et al. (1997) described 36 endemic species, 11 introduced species and 3 exotic species at Iguaçú river. The occurrence of *Hoplias malabaricus* in this basin, popularly

known as traíra, is quite controversial being referred as an introduced species (Garavello et al. op. cit.). Dergam et al. (1998) stated that the introduction of *H. malabaricus* in the Iguaçú river could have been done from the Tibagi headwaters, based on the similar genetic patterns of the populations, as well as in the proximity of both rivers.

Cytogenetical data clearly indicate that *H. malabaricus* is a complex species, probably related to its wide distribution over Neotropical region, requiring a careful taxonomic revision (Bertollo et al., 1983; Dergam and Bertollo, 1990; Scavone et

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al., 1994; Bertollo et al., 2000). Bertollo et al. (2000) differentiated seven distinct cytotypes in this fish group, based on their chromosome numbers and morphology and the absence or occurrence of distinct sex chromosome systems. The present paper aimed to contribute for a better understanding of the biogeography of *H. malabaricus* and its probable natural origin on the Iguaçu river basin.

MATERIAL AND METHODS

Forty-six specimens of *Hoplias malabaricus* were sampled in the main stream and in marginal lakes belonging to the Iguaçu river. Twelve specimens were collected from a population in the western region of the Paraná State (Brazil), in the township of Nova Prata do Iguaçu. Thirty-four specimens were captured from a population in the eastern region of the same state, in the township of Palmeira (Fig. 1).

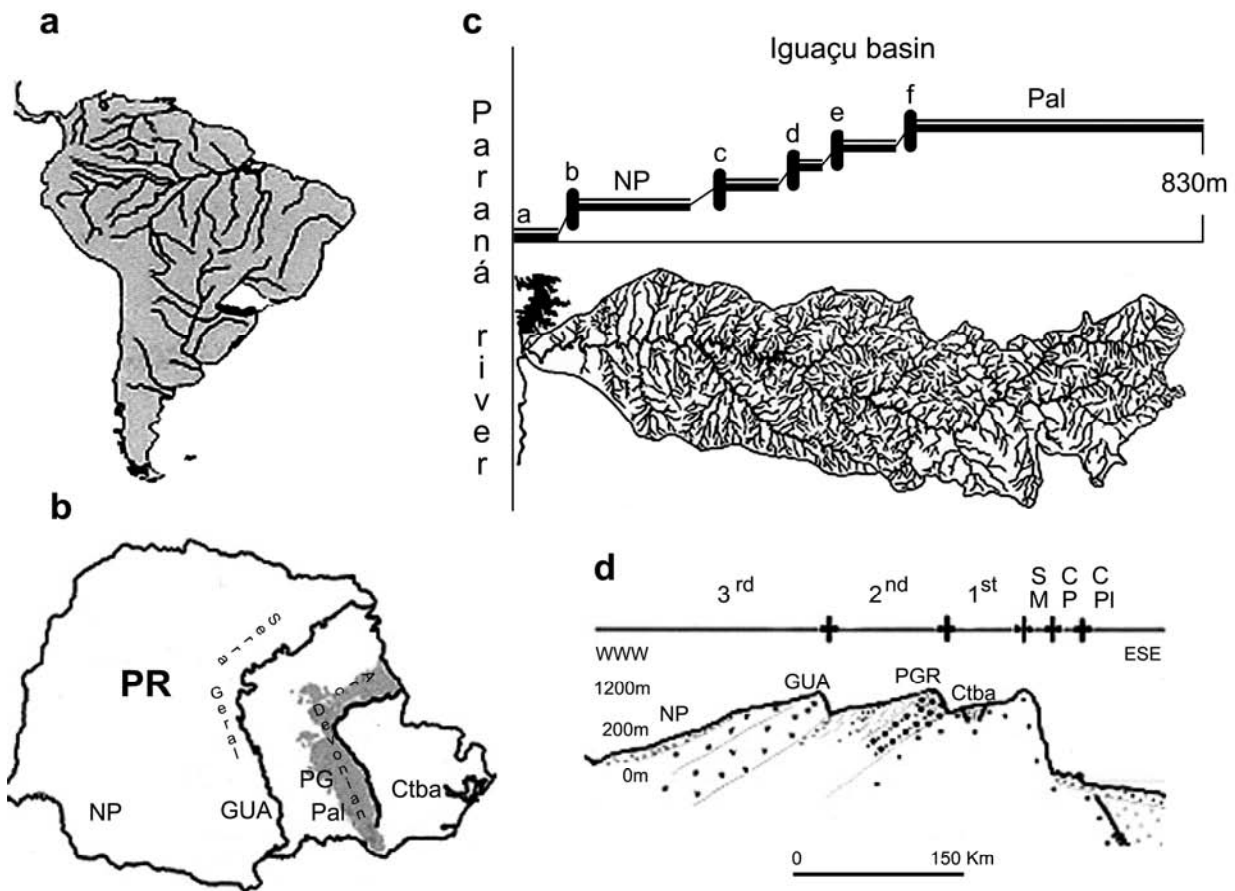


Figure 1 - (a) South American hydrographic map showing the Brazilian Paraná state location. (b) Paraná (PR) state map showing the geographical divisors between the 1st and 2nd plateaus (Devonian Arc) and the 2nd and 3rd plateaus (Serra Geral). Pal=Palmeira, NP=Nova Prata do Iguaçu cities. (c) Hydrographic Iguaçu basin profile showing the sampling sites in Palmeira (Pal) and Nova Prata do Iguaçu (NP) cities, in the eastern and western regions of the Paraná state, respectively. The points a-f are natural barriers (falls) that impede the dispersal of the fishes in the basin. (d) Geological profile of the Paraná state showing the divisors between the 3rd, 2nd and 1st plateaus (PI). SM=Serra do Mar; CP=coastal plain; CPI=continental platform. GUA=Guarapuava, PGR=Ponta Grossa and Ctba=Curitiba cities. (b e d, modificado de MS Melo baseado em Maack, 1948)

Metaphases were obtained according to Bertollo et al. (1978), using a cell suspension from the anterior kidney, after *in vivo* treatment with colchicine (1ml/100g body weight). The detection of the nucleolar organizing regions (NORs) and the constitutive heterochromatin followed the methods described by Howell and Black (1980) and Sumner (1972), respectively. Chromomycin A₃ staining was performed according to Schmid (1980). Chromosomes were arranged by size and shape, according to Levan et al. (1964) and the cytotypes were classified as stated by Bertollo et al. (2000).

RESULTS

The two populations analyzed, from now named eastern and western populations, showed the same karyotypic macrostructure, with a diploid number of $2n=42$, with 24 metacentric (M) plus 18 submetacentric (SM) chromosomes. The karyotypes were identical between the sexes, without any evidence of heteromorphic sex chromosomes (Figs. 2a, c).

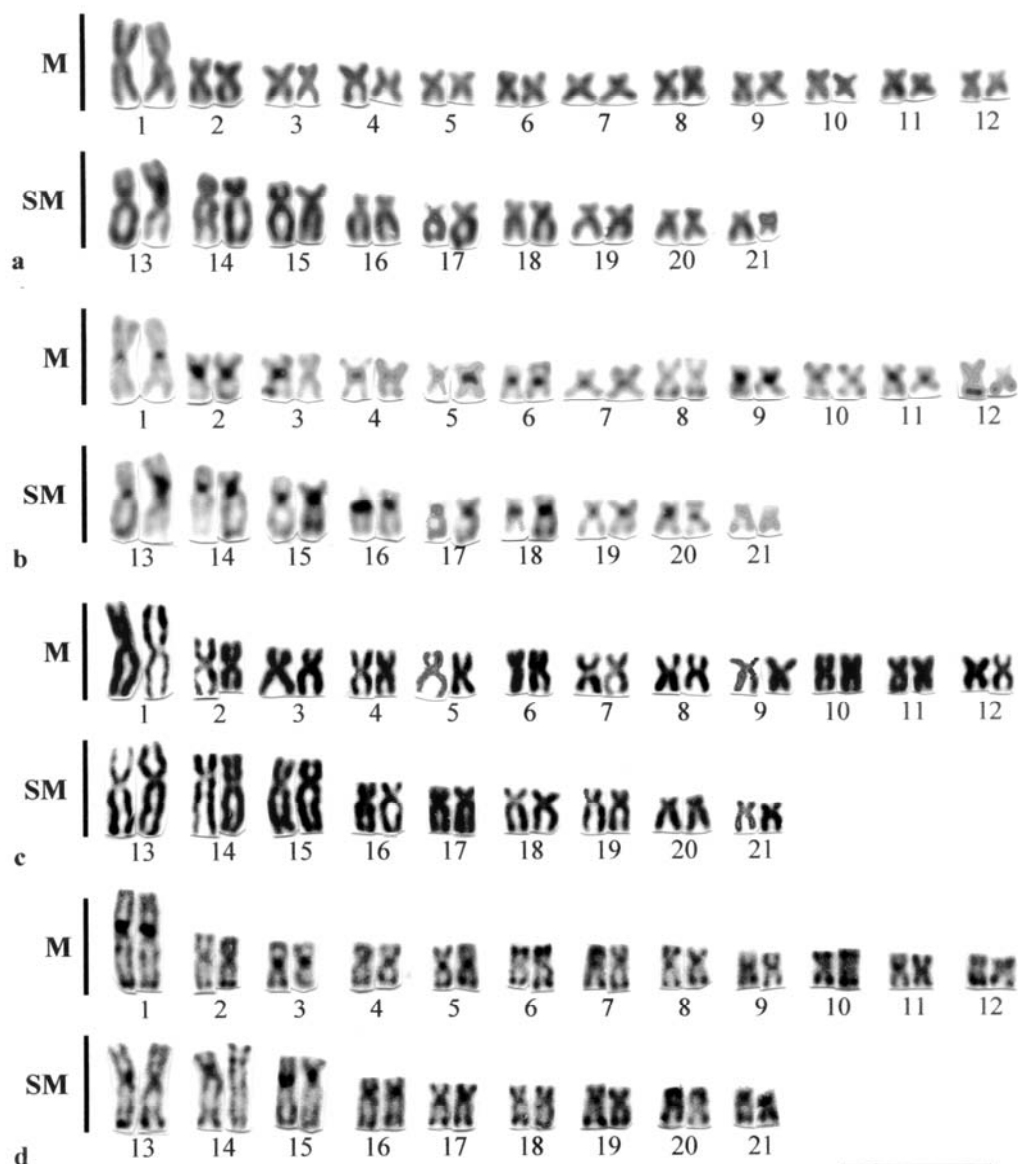


Figure 2 - Giemsa stained (a,c) and C-banded (b,d) karyotypes of *Hoplias malabaricus* from the eastern and western populations, respectively. Bar=5 μ m.

Ag-NORs (silver stained nucleolar organizing regions) were variable in number in both populations. Two to seven Ag-NORs were evidenced in the eastern population and three to eight in the western population, generally located on the telomeric region of the long arm of meta/submetacentric chromosomes. Specifically in the eastern population, it was found Ag-NOR in the telomeric region of the long arm of the smaller submetacentric chromosome pair and an interstitial Ag-NOR in the long arm of the only one homologue of the 16th pair (Fig. 3b). Bi-telomeric Ag-NORs (NORs located on the two telomeric

regions of a same chromosome) were also found in both populations (Fig. 3b).

Constitutive heterochromatin was evidenced in the centromeric/pericentromeric regions of all chromosomes and, eventually, in a telomeric position (Figs. 2b, d). In the eastern population, the 16th chromosome pair showed a polymorphic GC-rich heterochromatic region in agreement with the NOR location (Fig. 3a). In the western population, GC-rich heterochromatic regions were detected in the centromeric region of the 6th, 13th and 15th chromosome pairs, without association with NORs (Fig. 3c).

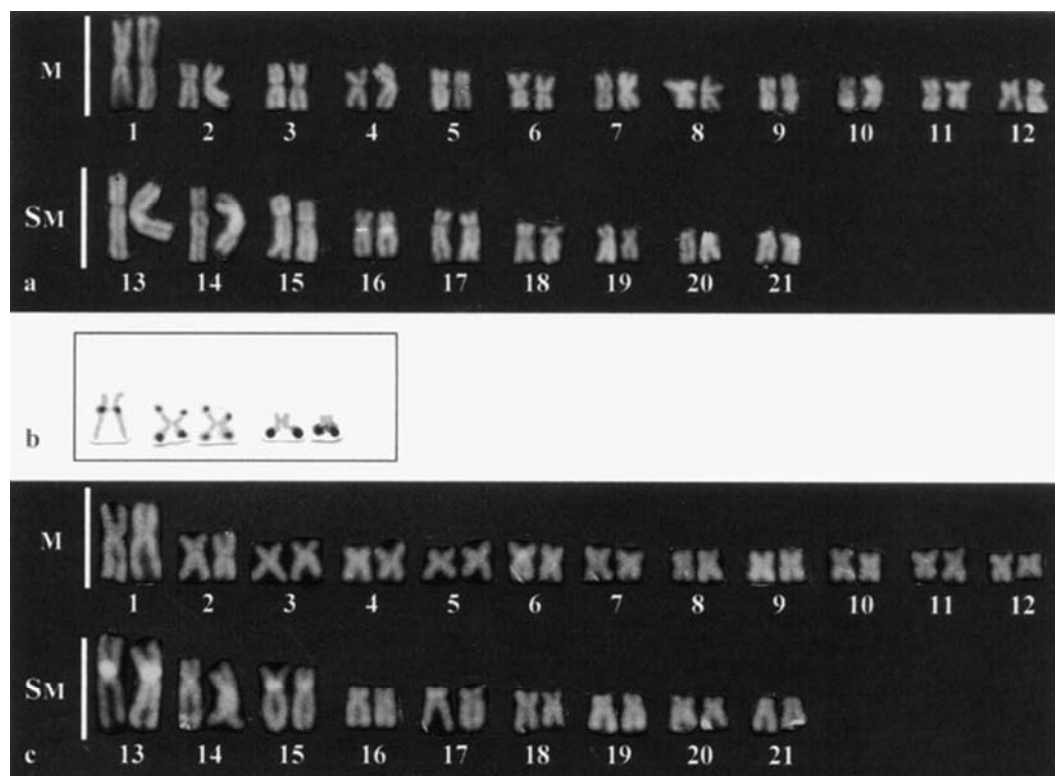


Figure 3 - Chromomycin A₃ stained karyotypes (a,c) of *Hoplias malabaricus* from the eastern and western populations, respectively, showing the GC-rich chromosome segments. (b) Representative chromosomes bearing Ag-NORs (from the eastern population) showing bitelomeric NORs in two metacentric ones. Bar=5 μ m.

DISCUSSION

Both eastern and western populations showed a karyotypic structure that corresponds to cytotype A in the Bertollo's classification (Bertollo et al., 2000). However, some chromosomal differentiation was found between the populations,

particularly in respect of the Ag-NORs and GC-rich regions locations. Indeed, the Ag-NORs located on the telomeric region of the smaller submetacentric chromosomes and in the interstitial region of the 16th chromosome (Fig. 3b) were only seen in the eastern population. In fact, interstitial NOR is an uncommon feature for *H. malabaricus*.

On the other hand bitelomeric NORs, as those observed in both eastern and western populations, have been commonly found in *H. malabaricus* cytotypes (Bertollo, 1996; Born and Bertollo, 2001), representing a probable sinapomorphic condition for this fish group. Concerning the distinct numbers of Ag-NORs found in the eastern (2-7) and in the western (3-8) populations, although this variation may be related to a differential expression of some rDNA cistrons, the results may also represent real karyotypic differences, as verified by Born and Bertollo (2001) and Vicari et al. (2003) for distinct *H. malabaricus* populations belonging to cytotype A. Two classes of heterochromatin were observed in both populations. The first one possesses a GC-rich DNA, which is present only in few chromosomes. This heterochromatin was not found to be related to NORs in the western population, which might represent an exception feature amongst fishes (Souza et al., 1996; Artoni et al., 1999; Margarido and Galetti Jr., 2000). Indeed, GC-rich heterochromatin associated with NORs is a common character in fish species (Mayr et al., 1985; Schmid and Guttenbach, 1988; Phillips and Hartley, 1988; Sola et al., 1992). The location of this heterochromatin in distinct chromosome pairs, besides its relation with NOR only in the eastern population, are good evidences that a karyotypic diversification have already occurred at some degree between the populations. A second heterochromatin class, present on the centromeric region of most chromosomes, does not present any fluorescent signal after chromomycin A₃ staining.

Lemos et al. (2002) found sympatric populations of *H. malabaricus* belonging to cytotype A (2n=42 chromosomes without a sex chromosome system) and cytotype B (2n=42 chromosomes with a XX/XY sex chromosome system) in the Iguaçú river, the latter being also found in coastal Brazilian basins (Bertollo et al., 2000). The occurrence of the cytotype B in the Iguaçú basin could be explained by dispersion from the coastal basins, as a consequence of a preterit contact between the basins, a headwater capture in small streams or even an occasional communication during the wet seasons (Lemos et al., 2002). Whereas cytotype A show a wide distribution along the Iguaçú basin (Bertollo et al., 2000; Lemos et al., 2002; present study), cytotype B was found thus far only in a restricted area of the first plateau of that basin (Fig. 1d). Thus, it is likely

that the dispersal event of cytotype B have a recent origin, in addition to the low vagility of *H. malabaricus* and the possible geological barrier formed by the Ponta Grossa arc, that is, the Devonian scarp that delimits the first and the second plateaus of the Paraná State (Fig. 1b).

The Iguaçú river basin is not recent in origin. As other tributaries of the Paraná river, as well as the Paraná river itself, its origin dates from the basaltic settlement during the formation of Serra Geral, in the Cretaceous (Potter, 1997). Its fish fauna finds itself isolated from that of the Paraná river due to the formation of the Iguaçú falls, approximately 22 millions years ago (Oligo-Miocene period). This fact may have favored the speciation and the considerable degree of endemism of that basin (Severi and Cordeiro 1994). *H. malabaricus* has been considered a non-native species in the Iguaçú basin. However, cytogenetics data show that cytotype A finds itself broadly distributed in Brazilian southeast and southern regions (where the Iguaçú basin is inserted), reaching Uruguay and Argentina (Bertollo et al., 2000). A priori, it seems very unlikely that *H. malabaricus* was originally absent from the Iguaçú basin, although present in many other neighboring basins. Thus, the endemism of the Iguaçú river appears to be valid for many fish species, but does not seem to apply for others, as *H. malabaricus* (Lemos et al., 2002). Our hypothesis is that *H. malabaricus* cytotype A is a native form in the Iguaçú river, and that the karyotypic diversification of the populations here analyzed is a throughout time consequence of vicariant events.

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RESUMO

Hoplias malabaricus (traíra) é uma espécie de peixe com ampla distribuição pela região Neotropical e alta diversidade cariotípica interpopulacional (citótipos), que podem corresponder a um complexo de espécies. Apesar da sua ampla distribuição pelas bacias hidrográficas da América do Sul, alguns autores têm questionado sua origem natural no rio Iguazu, uma importante bacia hidrográfica brasileira que é caracterizada pela presença de várias espécies de peixes endêmicas. Assim, nós temos analisado o cariótipo de *H. malabaricus* de diferentes locais de coleta neste rio por métodos convencionais e bandamentos cromossômicos. Nossos resultados, em adição a dados prévios a respeito da distribuição geográfica dos citótipos contribuem para um melhor entendimento da origem de *H. malabaricus* no rio Iguazu, reforçando a proposta que esta é uma espécie de peixe natural desta bacia hidrográfica.

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