

Chromosome divergences between two sympatric characid fishes of the genus *Bryconamericus*

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ABSTRACT

Cytogenetic studies were performed on two sympatric species of *Bryconamericus* which are awaiting more precise taxonomic identification. Although both *Bryconamericus* sp A and *Bryconamericus* sp B had a chromosome number equal to 52 chromosomes, their karyotypic structures varied widely. The two species presented multiple nucleolar organizer regions (NORs), at times varying in size. Centromeric heterochromatin was the most frequent type in the species studied and C banding showed no significant differences between karyotypes. The data permitted us to propose that non-Robertsonian chromosome rearrangements of the pericentric inversion type led to the differences between the two species of *Bryconamericus*.

INTRODUCTION

The cytogenetics of Characiformes fish has revealed the occurrence of groups presenting considerably divergent chromosome evolution, whereas others present a more conservative karyotype macrostructure (for a review, see Galetti Jr. *et al.*, 1994). Among fishes that present marked karyotype transformations during the process of diversification are the characids. Nominally, the family Characidae consists of 30 subfamilies (Britski *et al.*, 1986) but, because of its diversity of forms, it probably does not represent a natural group. This same artificiality is also reflected in the arrangement of some of its subfamilies, Tetragonopterinae in particular (Britski, personal communication).

One of the first studies on the phylogenetic relations in the subfamily Tetragonopterinae was conducted by Eigenmann (1917). Characterized as an ancient group abundant in South America and Africa, this subfamily has since been considered to be of polyphyletic origin, i.e., to originate from divergent ancestral lines.

Among the various genera currently assigned to the subfamily Tetragonopterinae, more information is available on the karyotype structure of *Astyanax*, which shows wide chromosome diversity (Morelli *et al.*, 1983) even among populations of the same morphological species (Moreira Filho and Bertollo, 1991), as well as supernumerary chromosomes (Salvador and Moreira Filho, 1992; Maistro *et al.*, 1992).

Little is known about the chromosomes of the genus *Bryconamericus*. Portela *et al.* (1988) described the karyotype of *B. stramineus* from a site located in the Upper Paraná basin. In addition to this scarcity of cytogenetic information, taxonomic confusions exist about the *Bryconamericus* group which still await

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clarification and which in some cases prevent more precise identification.

In the present study we describe the karyotype structure of two sympatric species of *Bryconamericus*.

MATERIAL AND METHODS

Karyotypic studies were carried out on two species of the genus *Bryconamericus*, temporarily called A and B. Specimens of *Bryconamericus* sp A (two females) and *Bryconamericus* sp B (five females and 10 males) were collected from the Piracicaba river, municipality of Piracicaba, where they lived in sympatry and syntopy.

The two *Bryconamericus* species are morphologically quite similar but differ mainly with respect to the shape of the teeth of the internal series. *Bryconamericus* sp A has thicker teeth, about twice the size of the teeth of *Bryconamericus* sp B. Furthermore, the teeth of sp B have two small cusps in addition to the three major ones, not found in sp A (Britski, personal communication).

Chromosome preparations were obtained by the air-drying technique described by Bertollo *et al.* (1978) and by the short-term culture technique (Fenocchio *et al.*, 1991). The nucleolar organizer regions (NORs) were identified by silver nitrate staining as described by Howell and Black (1980). The regions of constitutive heterochromatin were detected by the method of Sumner (1972).

The chromosomes were arranged in groups (meta-submetacentrics, submetacentrics and acrocentrics). The chromosome pairs were arranged in decreasing

order of size in each group and morphology was determined on the basis of the arm ratio, as proposed by Levan *et al.* (1964).

RESULTS

The specimens of *Bryconamericus* sp A presented a karyotype with a diploid number equal to 52 chromosomes consisting of 6M + 30SM + 6ST + 10A, resulting in a fundamental number (FN) equal to 94 (Figure 1). In contrast, *Bryconamericus* sp B presented a chromosome formula of 10M + 6SM + 18ST + 18A and a consequent FN = 86, although its diploid number was the same as for *Bryconamericus* sp A, i.e., $2n = 52$ (Figure 2). No chromosome differences were observed between males and females of *Bryconamericus* sp B. Males of *Bryconamericus* sp A were not available for this study.

NOR analysis in *Bryconamericus* sp A showed the presence of two to three silver-stained chromosomes, with a modal number equal to 3 (Table I and Figure 3). Terminal NORs were observed on the short arm of a pair of medium-sized submetelocentric chromosomes (pair 21), as well as on the short arm of a larger submetacentric (Figure 3). Nucleolus counts showed a variation from 1 to 4 nucleoli, with a modal number equal to 1 (Table II). NOR-bearing chromosomes ranged from 1 to 3 among males and females of *Bryconamericus* sp B, with a modal number equal to 2 (Table I and Figure 4). NORs were frequently observed in a terminal position on the short arm of a medium-sized submetelocentric pair corresponding to pair 13 (Figure 4B), which also showed clearly visible secondary constrictions in the

same region in Giemsa-stained preparations (Figure 2). Occasionally, only one of these chromosomes was silver stained in some of these cells (Figure 4A), whereas in others a third chromosome was visible, possibly a submetelocentric, bearing Ag-NOR sites on the short arm (Figure 4C). Analysis of the nucleoli showed a range of 1 to 4, with a modal number equal to 1 (Table II).

C banding revealed large heterochromatin blocks associated with the

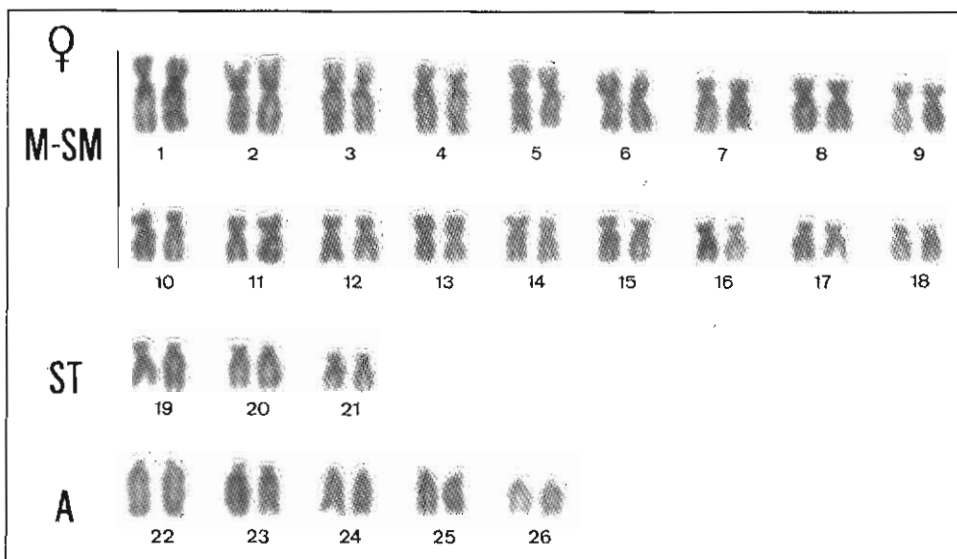


Figure 1 - Karyotype of *Bryconamericus* sp A females.

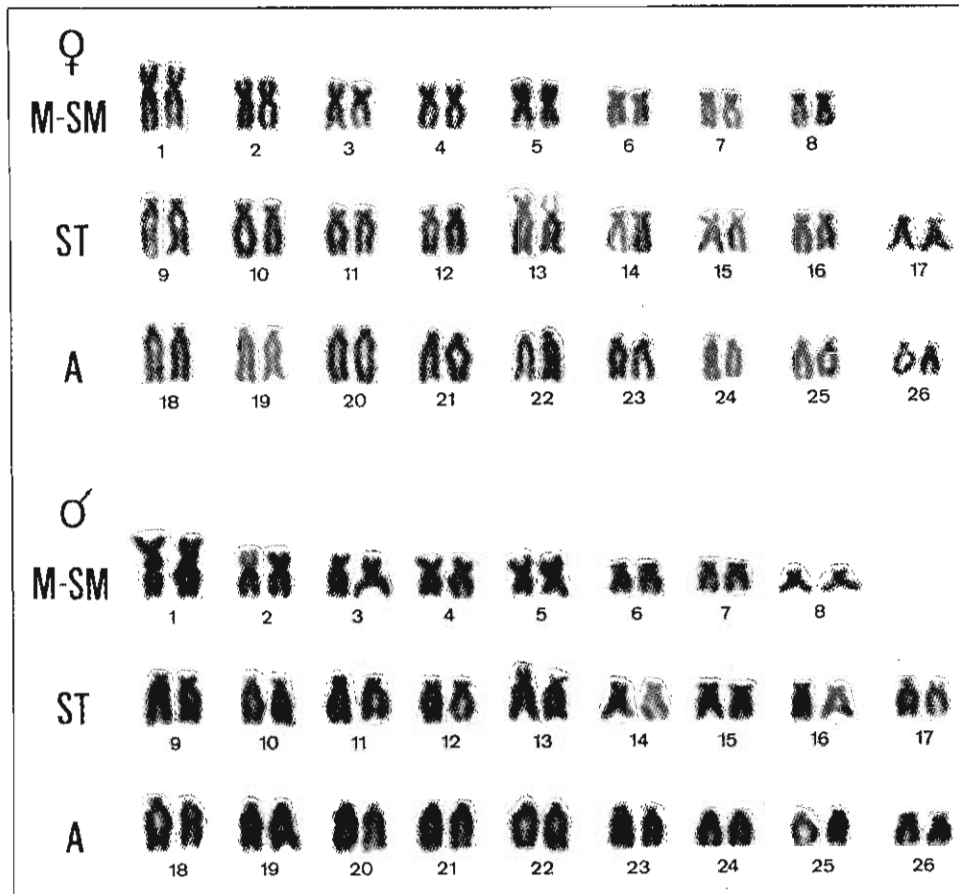


Figure 2 - Karyotypes of *Bryconamericus* sp B females and males.

Table I - Frequency of NOR-bearing chromosomes in the species studied.

Species	Number of NOR-bearing chromosomes			Total number of cells
	1	2	3	
<i>Bryconamericus</i> sp A	-	35	66	101
<i>Bryconamericus</i> sp B	51	288	18	357

Table II - Frequency of nucleoli observed in the species studied.

Species	Number of nucleoli				Total number of cells
	1	2	3	4	
<i>Bryconamericus</i> sp A	49	46	30	16	141
<i>Bryconamericus</i> sp B	364	290	58	8	720

NORs, especially those detected on chromosome pair 21 of *Bryconamericus* sp A and on chromosome pair 13 of *Bryconamericus* sp B (Figure 5). C-band positive blocks were also observed in the centromeres of all

chromosomes, as well as in some telomeres, especially those of the short arms, in both species.

Analysis of some Giemsa-stained preparations revealed the presence of interstitial secondary constrictions in several chromosomes of *Bryconamericus* sp B, as illustrated in chromosome 1 of the male karyotype (Figure 2), which appear to coincide with some small interstitial heterochromatin blocks, also visible in the karyotypes of both species (Figure 5).

DISCUSSION

The diploid number for the two species studied is 52 chromosomes, with no karyotypic differences between males and females, at least in *Bryconamericus* sp B, for which both sexes were available for analysis. Previous studies on *Bryconamericus stramineus* have also revealed $2n = 52$, with the occurrence of 13 meta-submetacentric chromosome pairs and 13 subtelocentric pairs (Portela *et al.*, 1988). However, in order to standardize the results obtained by Portela *et al.* with those obtained here, we reorganized the karyotypes presented by the above authors using the same criteria

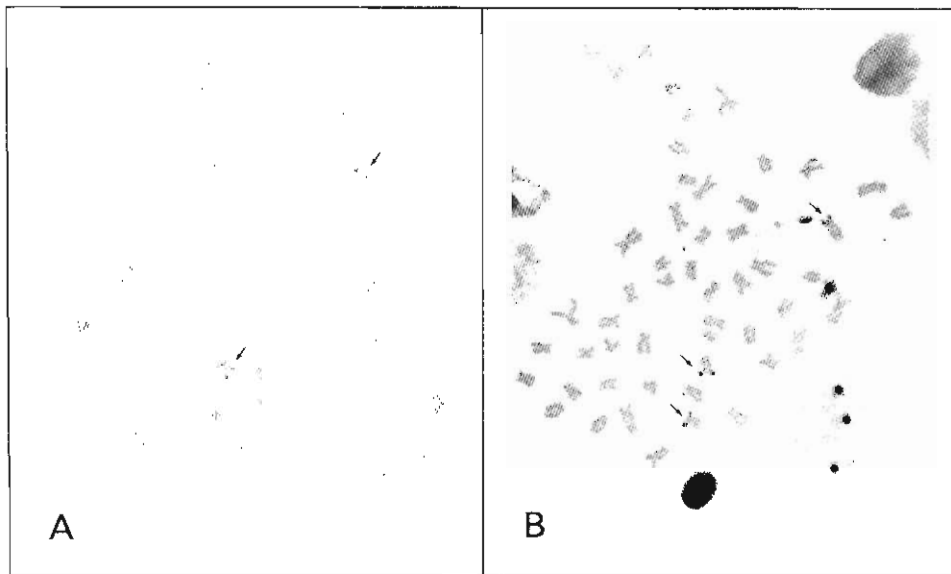


Figure 3 - NOR patterns observed in *Bryconamericus* sp A females. A, Metaphase showing a nucleolar chromosome pair. B, Metaphase showing three NOR-bearing chromosomes.

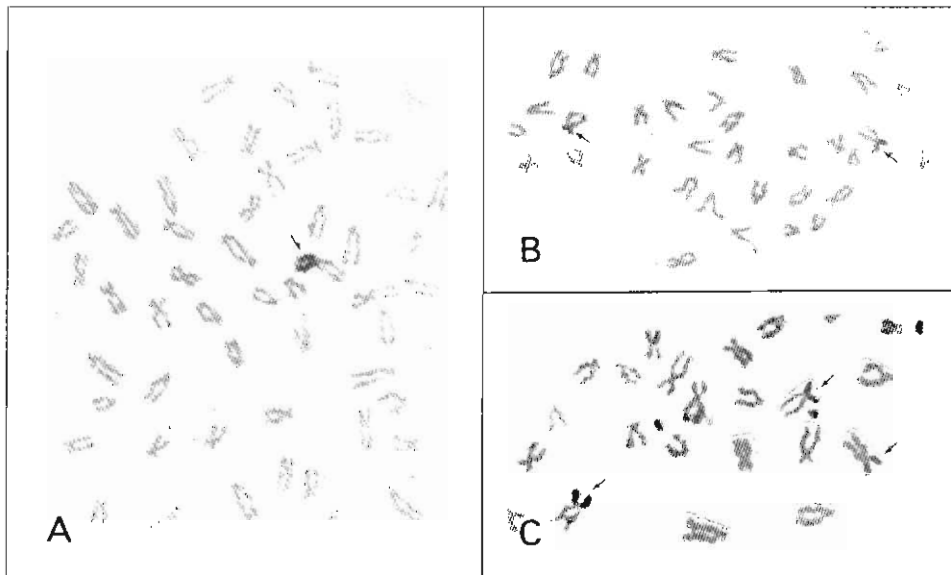


Figure 4 - NOR patterns observed in *Bryconamericus* sp B females and males. A, Metaphase showing a NOR-bearing chromosome. B, Partial metaphase showing a pair of nucleolar chromosomes. C, Partial metaphase showing three NOR-bearing chromosomes.

as for the species described here. On this basis, *B. stramineus* seems to have six metacentric (M) chromosomes, 10 submetacentrics (SM), 16 subtelo-centrics (ST) and 20 acrocentrics (A), for a total FN = 84.

The karyotype of *B. stramineus* is quite similar to that of *Bryconamericus* sp B, practically presenting the same chromosome formula, especially with respect to the number of subtelo-centric and acrocentric chromosomes. An interesting inversion is noted in the number of meta- and submetacentric chromosomes

between these species. Whereas *Bryconamericus* sp B presents 10 M and six SM, *B. stramineus* inversely shows six M and 10 SM. Although some artifacts of the methodology for chromosome measurement may have occurred, it seems quite likely that chromosome rearrangements of the pericentric inversion type were responsible for these differences. Also, the size relations among some chromosomes in the complement are clearly seen to be modified. Chromosome 1 of *Bryconamericus* sp B is considerably larger than the second chromosome in the complement. In contrast, in *B. stramineus* this difference is practically nonexistent, and the morphology of chromosome 1 differs from the comparable chromosome of *Bryconamericus* sp B. More marked differences in karyotype morphology are observed in *Bryconamericus* sp A when compared with either of the species discussed above.

In *Bryconamericus* sp A, the number of meta- and submetacentric chromosomes is much larger than that observed in the two above species. This causes a much more

heterogeneous distribution of chromosome number in the different morphology classes (M, SM, ST and A). It is quite probable that this distribution observed in *Bryconamericus* sp A is a derived condition compared to that observed in *Bryconamericus* sp B and in *B. stramineus*. Other Tetragonopterinae genera also showing $2n = 52$ (*Tetragonopterus* and *Piabina*, for example) also have a chromosome formula of more homogeneous distribution (Portela et al., 1988), supporting the hypothesis that the karyotype of an ancestral *Bryconamericus* is closer to that observed in

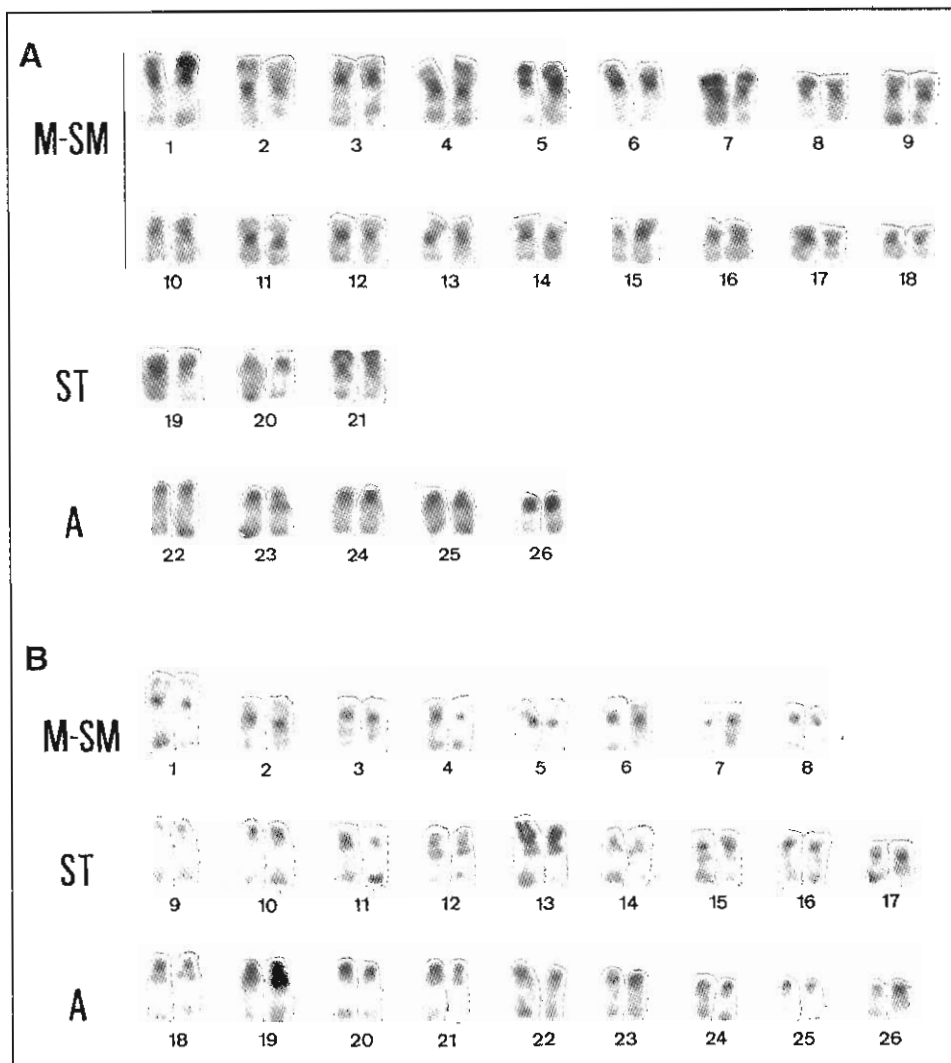


Figura 5 - C-banding pattern distribution. A, *Bryconamericus* sp A. B, *Bryconamericus* sp B.

Bryconamericus sp B and *B. stramineus* than to that observed in *Bryconamericus* sp A.

The two *Bryconamericus* species studied here present variations in the number of NOR-bearing chromosomes, thus being characterized as fishes presenting multiple NORs (Galetti Jr. *et al.*, 1985; Almeida Toledo and Foresti, 1985). Variations related to NOR number have been previously identified in other fishes of the family Tetragonopterinae by several investigators (Morelli, 1981; Portela *et al.*, 1988, among others). In addition to variations in the number of NOR-bearing chromosomes, *Bryconamericus* sp A and *Bryconamericus* sp B also present inter- and intraindividual variations with respect to the size of these NORs. When present in a single chromosome the NORs appear to be triplicate and when present in two chromosomes they appear to be duplicate compared to the NORs present in three chromosomes.

Heteromorphisms of this segment are also clearly visible in the C-banded karyotypes, especially in

chromosome pair 21 of *Bryconamericus* sp A and chromosome pair 13 of *Bryconamericus* sp B, suggesting that a good part of the polymorphism observed in these fishes may be due to structural changes along these cistrons and may also involve the heterochromatin segment.

In both species heterochromatin preferentially appears in the centromeres and telomeres of the chromosomes, in addition to being present in small interstitial blocks, especially on the short arm of some subtelocentric and acrocentric chromosomes. Thus, there are no great differences in the amount of heterochromatin between the karyotypes of the species studied.

On this basis, contrary to other fish groups that maintain a conserved karyotype macrostructure but that are quite divergent in terms of NOR and heterochromatin distribution (Pauls and Bertollo, 1991; Galetti Jr. *et al.*, 1991, for example), in the genus *Bryconamericus*, whereas karyotype morphology is considerably diverse, the same does not appear to occur

with the pattern of NOR and constitutive heterochromatin distribution, or with the diploid number ($2n = 52$), which remain constant.

ACKNOWLEDGMENTS

The authors are indebted to CNPq for financial support and to Heraldo A. Britski for the identification of the species studied.

Publication supported by FAPESP.

RESUMO

Foram realizados estudos citogenéticos em duas espécies simpátricas de *Bryconamericus*, as quais aguardam identificação taxonômica mais segura. Apesar de *Bryconamericus* sp A e *Bryconamericus* sp B mostrarem um número diplóide igual a 52 cromossomos, existem grandes diferenças em suas estruturas cariotípicas. As duas espécies em questão apresentam regiões organizadoras de nucléolos (NORs) múltiplas, algumas vezes com variações no tamanho. A heterocromatina centromérica foi a mais freqüente nas espécies estudadas e o bandeamento C mostrou que não há diferenças significativas entre seus cariótipos. Os dados obtidos no presente trabalho permitiram-nos propor que provavelmente rearranjos cromossômicos não-Robertsonianos, do tipo inversões pericêntricas, levaram às diferenças entre as duas espécies de *Bryconamericus*.

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(Received July 14, 1995)