

## HETEROCHROMATIN PATTERNS AND KARYOTYPE RELATIONSHIPS WITHIN AND BETWEEN THE GENERA *Brycon* AND *Salminus* (PISCES, CHARACIDAE)

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### ABSTRACT

Chromosomes of two *Brycon* species (*B. lundii* and *B. microlepis*) and *Salminus hilarii* were analyzed. Based on constitutive heterochromatin distribution patterns, karyotypic relationships within and between Bryconinae and Salmininae were examined. A monophyletic origin for the genus *Brycon*, comprising at least two chromosome synapomorphies (presence of two large submetacentric bearing paracentromeric and telomeric heterochromatin), is suggested. Moreover, Bryconinae and Salmininae may represent a monophyletic unit among Characidae, as they share several chromosome features.

### INTRODUCTION

Characidae, the largest family among the Characiformes, includes nearly 55% of the fishes belonging to this freshwater fish order (Fink and Fink, 1981), and may not assemble a monophyletic group of species (Lucena, 1993). The genus *Brycon* has about 40 species (Howes, 1982), and together with *Triporthus* and *Chalceus*, had been included in the tribe Bryconini (Weitzman, 1960). Currently, *Brycon* appears alone in the subfamily Bryconinae (Gery, 1977), and has been considered more closely related to *Triporthus* and *Salminus* than other South American characids (Uj, 1990; Portugal, 1990).

Previous chromosome studies on *Brycon* species from various hydrographic basins revealed significant karyotype stability, characterized by the same chromosome number ( $2n = 50$ ) and great similarity in chromosome structure among species. Nucleolar organizer region (NOR) sites also appear to be conserved within this genus, and a common chromosome pair has been detected carrying these sites in all species thus far studied (Almeida-Toledo *et al.*, 1996; Margarido and Galetti Jr., 1996). In contrast, changes in constitutive heterochromatin seem to have an important role in the chromosome evolution of these fishes, and two major patterns of heterochromatin distribution have been reported for this genus (Margarido and Galetti Jr., 1996). First, a group consisting of *B. lundii*, *B. breviceauda* and *B. insignis* was characterized by predominantly paracentromeric C bands, mainly in the submetacentric chromosomes. Another group, comprising *Brycon* sp., *B. orbignyanus*, *B. microlepis* and *B. cephalus*, was characterized by telomeric bands in some metacentric chromosomes.

In the present study, representative species of both

heterochromatin major patterns were reanalyzed through C banding and base-specific fluorochrome mithramycin and DAPI staining (new data). Chromosomes of *Salminus hilarii* were also analyzed and a phylogenetic relationship between Bryconinae and Salmininae is discussed.

### MATERIAL AND METHODS

Mitotic chromosomes obtained from anterior kidney cells (Bertollo *et al.*, 1978; Fenocchio *et al.*, 1991) of *Brycon lundii* (5 females and 5 males) from the São Francisco River (Três Marias, MG) and *Brycon microlepis* (7 females and 3 males) from the Cuiabá River (Cuiabá, MT), as well as Bryconinae and *Salminus hilarii* (Salmininae, 5 females and 3 males) from the Mogi-Guaçu River (Pirassununga, SP), were analyzed using C banding (Sumner, 1972) and fluorescent staining with mithramycin A (MM) and 4'-6-diamidin-2-phenylindole (DAPI) and counterstained with distamycin A (DA) (Schweizer, 1976; Schmid, 1980).

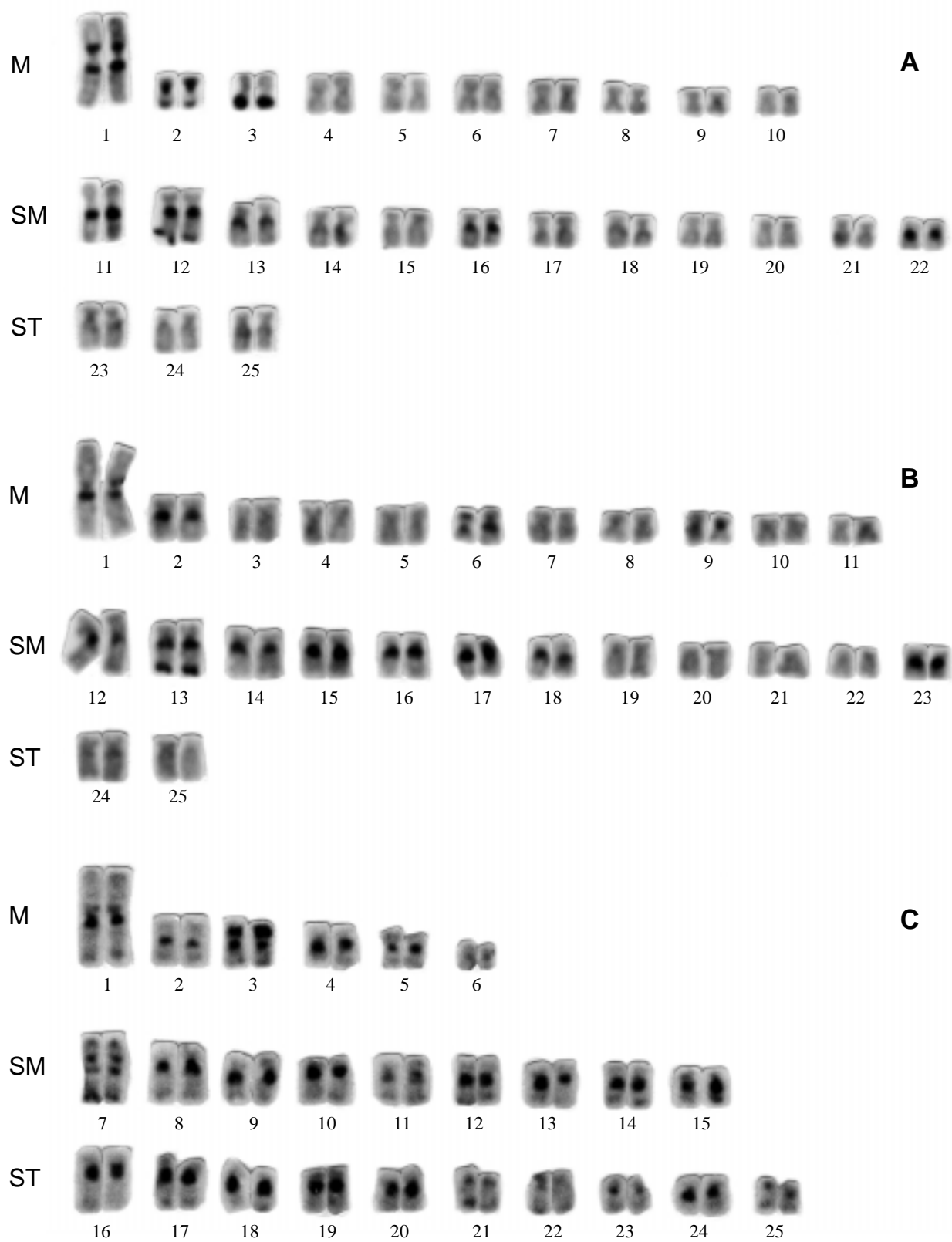
### RESULTS

*Brycon* species studied here presented less heterochromatin than *S. hilarii*. *B. microlepis* showed pale C bands in centromeric regions of most chromosomes. Dark heterochromatic blocks appeared to be equilocally distributed in the paracentromeric region of both arms of the first metacentric (M) pair, apparently in the entire short arm and telomeres of the long arm of the second M pair, and in the telomeres of the long arm of the third M pair. Darkly stained heterochromatic blocks were also detected on the long arm, near the centromere of the two largest submetacentric (SM) (11 and 12) and three other SM pairs (13, 16, 22) (Figure 1A).

Equilocal dark C bands could also be observed in the paracentromeric region of both arms of the first M pair in *B. lundii*. Paracentromeric dark C bands were also observed in at least two M pairs (2, 6) and eight SM pairs

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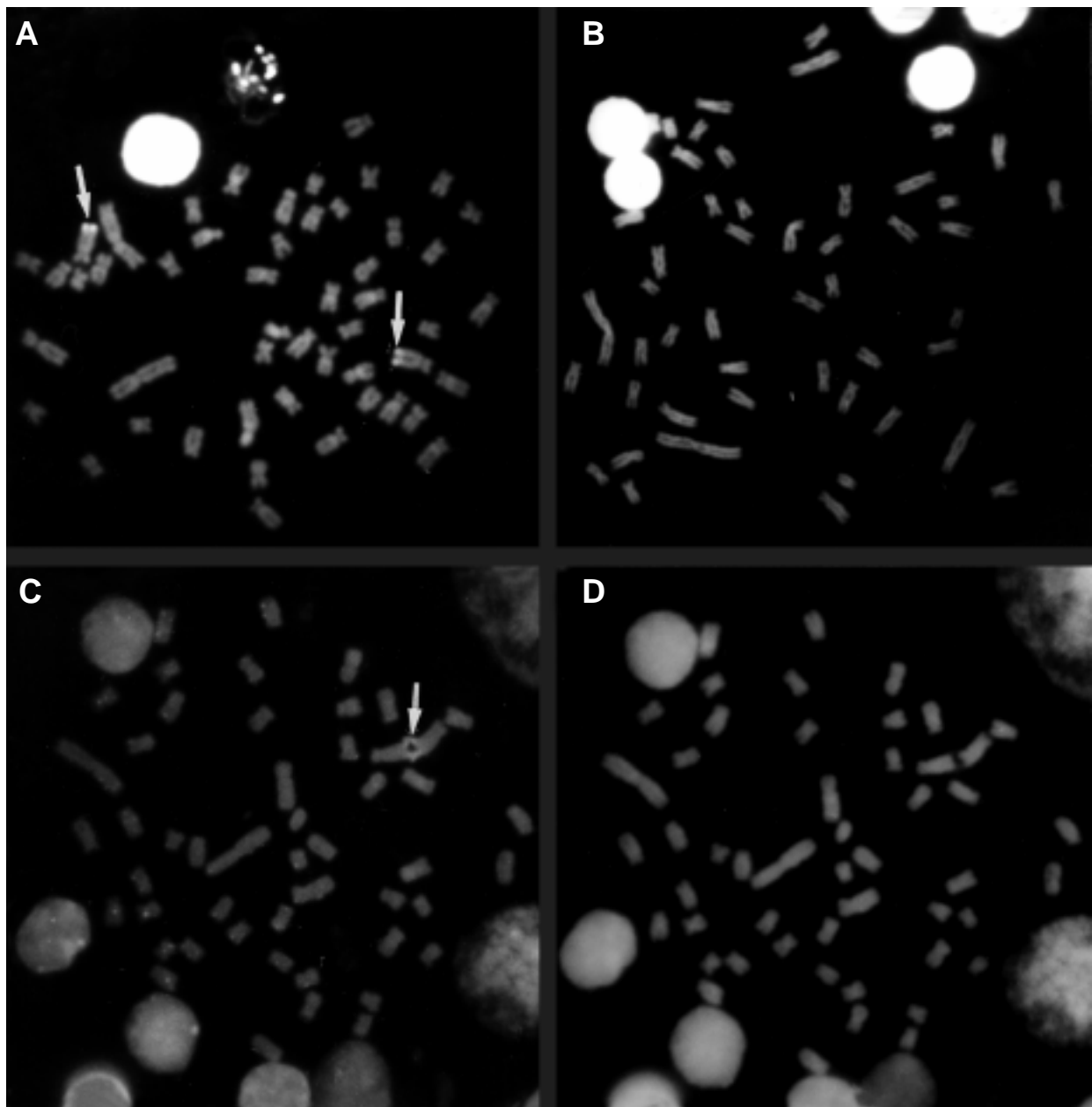
**Figure 1** - C banding karyotypes of (A) *Brycon microlepis*, (B) *Brycon lundii* and (C) *Salminus hilarii*.

(12, 13, 14, 15, 16, 17, 18, 23). In one of these (13), dark C bands were detected in the telomeres of its long arm (Figure 1B). Larger amounts of heterochromatin could be observed in *S. hilarii*, with paracentromeric dark C bands in most chromosomes of the complement. Like Bryconinae species, the largest M pair also showed equilocal paracentromeric heterochromatic blocks on both chromosome arms. A large SM pair showed a particular C banding pattern with a pale C band on the telomere of the short arm, a conspicuous centromeric heterochromatic block, a proximal dot-like C band on the long arm, and dark C bands on its telomeres (Figure 1C).

Mithramycin staining revealed two fluorescent MM<sup>+</sup> bands in the chromosome complement of *B. lundii* (Figure 2A), located in the telomeric region of the long arm of the second SM pair. DAPI staining showed no differential fluorescence among these chromosomes (Figure 2B). Similar results were obtained in *B. microlepis* (Figure 2C, D).

## DISCUSSION

The karyotypic macrostructure observed in *Brycon* is quite similar to the chromosome complement of *S. hilarii*



**Figure 2** - Metaphases stained by mithramycin in (A) *Brycon lundii* and (C) *Brycon microlepis*, and by DAPI in (B) *Brycon lundii* and (D) *Brycon microlepis*. Mithramycin positive bands are indicated by arrows.

(Marco, 1986; present study) and other *Salminus* species (*S. maxillosus* and *S. brasiliensis*) studied through Giemsa and silver staining (Marco, 1986). This strongly suggests that this karyotype pattern may be ancestral for some Characidae. Although some Tetragonopterinae (e.g. *Astyanax*), Acestrorhynchinae and Cynopotaminae have  $2n = 50$ , they show a karyotype structure divergent from Bryconinae and Salmininae, bearing several uniarmed chromosomes. Most groups currently defined in the family Characidae show a different karyotype pattern than the one apparently shared by *Brycon* and *Salminus*. *Chalceus* (Chalcidiinae), for instance, shows  $2n = 52-54$  (Muramoto *et al.*, 1968; Ojima *et al.*, 1976). *Triportheus* (Triportheinae) also shows  $2n = 52$  and cytologically differentiated sex chromosomes (Falcão *et al.*, 1985; Bertollo and Cavallaro, 1992).

C banding shows significant differences within and between the genera *Brycon* and *Salminus*. Diverse heterochromatin patterns have already been reported within the genus *Brycon* (Margarido and Galetti Jr., 1996), and were confirmed in the present paper. Heterochromatin appears reduced to approximately 11 chromosome pairs in the karyotype of *B. lundii*, and no more than 8 pairs in *B. microlepis*. In contrast, large paracentromeric heterochromatins are abundant in the karyotype of *S. hilarii*. The two largest submetacentric pairs, which showed similar C banding between *B. lundii* and *B. microlepis*, have been reported as very conservative among *Brycon* species (Margarido and Galetti Jr., 1996). These chromosomes seem to be modified in the C-banded karyotype of *S. hilarii* and could characterize a synapomorphy in the genus *Brycon*. A large metacentric pair bearing a typical equilocal paracentromeric heterochromatin in both chromosome arms, as observed here in *Brycon* species and *S. hilarii*, has been reported in other characids (Scheel, 1973; Daniel-Silva, 1996) and may represent a synapomorphy for the family.

Silver staining detected a large submetacentric pair bearing the nucleolar organizer region in the telomeres of its long arm in both genera. In *Brycon* these chromosomes present C bands restricted to both paracentromeric and telomeric regions of the long arm, while in *S. hilarii* the first appears modified to a proximal dot-like C band, suggesting a divergence related to heterochromatin along the NOR-bearing chromosomes between both genera.

DAPI staining showed no differential fluorescence among the chromosome complement of *B. lundii* and *B. microlepis*, and mithramycin staining revealed only two bright MM<sup>+</sup> bands along their karyotypes. As, in general, reported for fishes (e.g. Mayr *et al.*, 1985), these MM<sup>+</sup> bands appear coincident to AgNOR sites and also to C banded heterochromatin of both *Brycon* species. *S. hilarii* similarly showed only two MM<sup>+</sup> bands/cell, corresponding to AgNORs (data not shown), and again indicates a common pattern of chromosome evolution among these fish groups.

In conclusion, chromosome data strongly suggest that Bryconinae and Salmininae may form a monophyletic unit among characids, and heterochromatin may have an important role in their karyotype diversification.

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## RESUMO

Foram analisados os cromossomos de duas espécies de peixes do gênero *Brycon* (*B. lundii* e *B. microlepis*) e de *Salminus hilarii*. Principalmente baseado no padrão de distribuição da heterocromatina constitutiva, foram discutidas as relações cariotípicas dentro e entre os grupos Bryconinae e Salmininae. É sugerida uma origem monofilética para o gênero *Brycon*, com pelo menos duas sinapomorfias cromossômicas (presença de dois grandes submetacêntricos apresentando blocos pericentromérico e telomérico de heterocromatina constitutiva). Ainda, Bryconinae e Salmininae, compartilhando vários caracteres cromossômicos, podem formar uma unidade monofilética entre os Characidae, na qual outros caracídeos não estão incluídos.

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