

NEW CASES OF SUPERNUMERARY CHROMOSOMES IN CHARACIFORM FISHES

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ABSTRACT

Supernumerary chromosomes were described for five species of Neotropical characiform fishes. These extra chromosomes were small, acrocentric and fully heterochromatic in *Leporinus friderici* from two different localities as well as in *Leporinus* sp., but metacentric and fully heterochromatic in *Cyphocharax modesta* and *Prochilodus nigricans*. In *Characidium* cf. *zebra*, this element was small, acrocentric and euchromatic. GC-rich DNA blocks were observed in the supernumerary chromosome of *Leporinus* sp. using chromomycin A₃. The widespread occurrence of these extra chromosomal elements suggests their independent origins.

INTRODUCTION

Among vertebrates there are several descriptions of extra or supernumerary chromosomes, mainly in amphibians, reptiles and mammalians (Jones and Rees, 1982, for review). Their occurrence can be only related to sporadic events, and additional chromosomes might be an individual trait among the remaining individuals of a population. In fishes, an additional chromosome, for instance, was detected in only one individual of *Parodon* (Parodontidae) and in a specimen of *Oligosarcus* (Characidae), among several individuals (Falcão *et al.*, 1984). In several cases, however, they are a common feature within the population or species, becoming generation to generation fixed in that population, and often called B chromosomes. In characiform fishes, true B chromosomes were first described in *Prochilodus lineatus* (= *P. scrofa*), in which up to five small heterochromatic chromosomes were observed cell to cell variable but present in most studied individuals (Pauls and Bertollo, 1983).

Other cases of extra chromosomes in fish are now well known (Venere and Galetti, 1985; Hochberg and Erdtmann, 1988; Oliveira *et al.*, 1988; Foresti *et al.*, 1989; Fenocchio and Bertollo, 1990; Oliveira and Foresti, 1993; Pastori *et al.*, 1997). In *Astyanax scabripinnis* different populations have often shown one or two B chromosomes, or even up to five Bs, while several others showed none of these chromosomes (Salvador and Moreira-Filho, 1992; Maistro *et al.*, 1992; Stange and Almeida-Toledo, 1993). Recently, a female-biased sex ratio phenomenon was described in association with the presence of B chromosome

in this species. Moreover, in some of the B-bearing populations of *A. scabripinnis*, the B chromosomes appear to be constrained to the highest altitude along the extent of the stream where these fishes inhabit (Vicente *et al.*, 1996).

In the present, extra chromosomes are described in six species of Neotropical characiformes belonging to the families Anostomidae, Curimatidae, Prochilodontidae and Crenuchidae (Characidiinae) and an independent origin hypothesis is discussed.

MATERIAL AND METHODS

Five characiform species comprising four different families were cytogenetically studied.

Anostomidae: Sixteen specimens of *Leporinus friderici* collected in the Candeias River, near Porto Velho, RO, and two individuals from the Araguaia River, near Barra do Garças, MT. Three specimens of *Leporinus* sp. (new species, 4-3 dentary formula), from Dois de Agosto Stream, a tributary of Rio das Mortes, near Araguaiana, MT, Amazon basin.

Prochilodontidae: Three individuals of *Prochilodus nigricans* from the Araguaia River, near Barra do Garças, MT, Amazon basin.

Curimatidae: Seventeen individuals of *Cyphocharax modesta* collected in the Mogi-Guaçu River, near Luis Antonio, SP, Upper Paraná basin.

Crenuchidae (Characidiinae): Twenty-eight specimens of *Characidium* cf. *zebra* from Passa-Cinco River, in the municipality of Ipeúna, SP, also in the Upper Paraná basin.

Mitotic chromosomes were prepared from anterior kidney cells as described by Bertollo *et al.* (1978). C-banding was done according to Sumner (1972), and chromomycin A₃ (CMA₃) staining was as described by Schmid (1980).

RESULTS

The karyotypes of all five species studied here were previously described (Galetti *et al.*, 1981; Venere and

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Galetti, 1989; Pauls and Bertollo, 1990; Miyazawa and Galetti, 1994). In Anostomidae, the two *Leporinus* species studied had $2n = 54$ biarmed chromosomes in the normal karyotypes. However, among 16 specimens of *L. friderici* from Candeias River, one of them (unidentified sex) had an additional small acrocentric. Similarly, in the other locality sampled (Araguaia River), among two individuals of this species, one individual (unidentified sex) had an additional chromosome (small acrocentric). In *Leporinus* sp. from Dois de Agosto Stream one male among three individuals studied also showed one extra and small acrocentric (Figure 1a, b and c). Differently of *L. friderici* in which the extra chromosomes whenever detected were present in 100% of the analyzed cells, in *Leporinus* sp. it was observed in about 80% of the cells.

Among 17 specimens sampled of *Cyphocharax modesta* (Curimatidae), one male showed a small biarmed extra chromosome besides the chromosomes of the normal complement ($2n = 54$) (Figure 1d), and in prochilodontids, one male had two extra chromosomes in 85% of the analyzed cells, among three individuals of *Prochilodus nigricans* (Figure 1e). Finally, in *Characidium* cf. *zebra*, all of the specimens showed $2n = 50$ biarmed chromosomes in the normal karyotype, except for one in which an additional small acrocentric was detected in about 25% of the cells (Figure 1f).

C-banding revealed that these supernumerary chromosomes were fully heterochromatic (Figure 2a, b, c and d), except in *Characidium* cf. *zebra* (Figure 2e). A small bright CMA_3 band was detected in an interstitial position on the long arm of the extra chromosome of *Leporinus* sp. (Figure 3).

DISCUSSION

A main question about the extra chromosomes described here in different characiform fishes might be if they have or not a common origin. Undoubtedly, they came from different events, even though the mechanisms of origin of most of these extra chromosomes might be the same. Among Characiformes they appear to spring up several times, since they are morphologically and probably functionally diverse. Their frequencies also seem different among all species studied. In *Leporinus*, *Cyphocharax* and *Characidium*, for instance, the apparent rare occurrence of extra chromosomes might indicate recent events with incipient fixation in the populations. On the other hand, the morphologically similar small heterochromatic acrocentrics detected in three *Leporinus* species suggest an earlier and unique origin of this chromosome in this genus. In this case, their probably rare occurrence in the populations might indicate a selective disadvantage. In curimatids, previous studies in different populations of *Cyphocharax modesta* had also shown an extra chromosome, morphologically similar to that here reported (Venere and Galetti, 1985; Martins *et al.*, 1996). This might sug-

gest that this chromosome is ancient in this species, widely spread in the distribution area, despite to it occurs in a low frequency within those populations. In the genus *Prochilodus* two other species also share the presence of extra chromosomes. Up to five B chromosomes were previously reported in *Prochilodus lineatus* (Pauls and Bertollo, 1983). Small B chromosomes were also detected in *P. cearensis* (Pauls and Bertollo, 1990) and here in *P. nigricans*. The presence of these supernumerary chromosomes was not detected in other population of *P. nigricans* from the Amazon basin (Pauls and Bertollo, 1990). So, these chromosomes may represent a population characteristic in the Araguaia River or the frequency of this element in the Amazon population is very low. It seems that these small and extra chromosomes assemble a specific clade separated from the remaining species from the genus *Prochilodus*.

Different mechanisms have been proposed to explain the origin and maintenance of extra chromosomes in several organisms (Jones and Rees, 1982, for review). The heterochromatinization, however, appears to be a common process occurring during the differentiation of these chromosomes, since the extra chromosomes have often been shown fully heterochromatic. The extra chromosome here detected in *Characidium* cf. *zebra*, however, showed no heterochromatic segments, suggesting that the heterochromatin should have no role on the origin and development of this chromosome in this species. An absence of heterochromatin was also reported in supernumerary microchromosome of a curimatid fish, *Steindachnerina insculpta* (Oliveira and Foresti, 1993). Thus, it is strongly indicative that these extra chromosomes occurring in different characiform groups have arisen several times among these fishes, although in a minor taxon (*Leporinus*, for instance), they can be shared by several species.

The occurrence of a small bright CMA_3 band in extra chromosome of *Leporinus* sp. is a very interesting feature. In fishes, as well as in amphibians, the chromomycin A_3 has been useful to detect NOR independently of its activity (Schmid and Guttenbach, 1988). Since Ag staining was unable to detect any Ag-NORs in this extra chromosome, one might suppose that if the CMA^+ band is actually related to NOR sites, they should be inactive in this extra chromosome. Otherwise, these interstitial CMA^+ band observed in the extra chromosome of *Leporinus* sp. might be related to a particular heterochromatin stainability, instead any presence of NOR sites.

Interestingly, most of the cases of extra chromosomes thus far reported in fishes is related to characiform species, even though other major groups such as Siluriformes may show some species with these chromosomes (Fenocchio and Bertollo, 1990; Dias and Foresti, 1993, among others). Moreover, it could be accounted for that all those fishes showing accessory chromosomes exclusively inhabit the Neotropical region. Similar report was made for various sex chromosome systems which are much

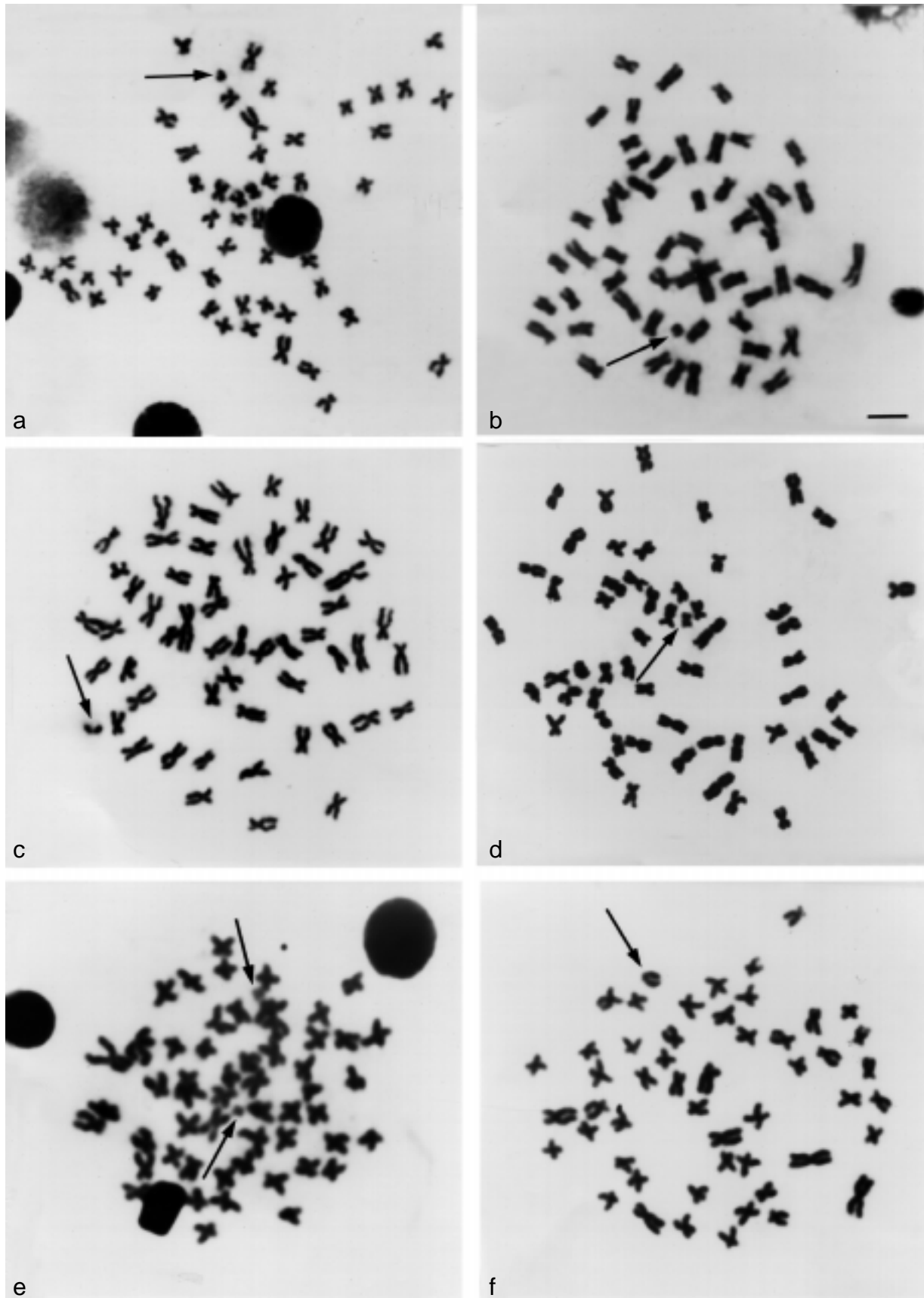


Figure 1 - Giemsa-stained metaphases of (a) *Leporinus friderici* from the Candeias River, (b) *Leporinus friderici* from the Araguaia River, (c) *Leporinus* sp., (d) *Cyphocharax modesta*, (e) *Prochilodus nigricans* and (f) *Characidium* cf. *zebra*. The arrows indicate the supernumerary chromosomes. Bar = 5 μ m.

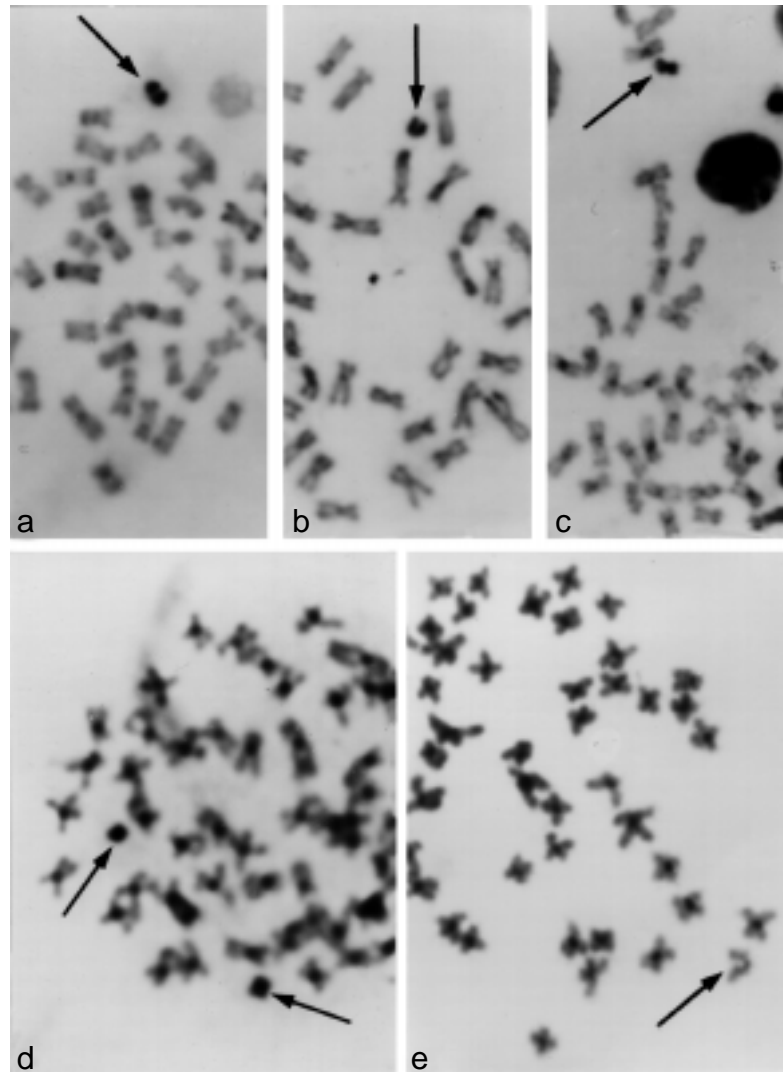


Figure 2 - C-banded partial metaphases of (a) *Leporinus friderici* from the Araguaia River, (b) *Leporinus* sp., (c) *Cyphocharax modesta*, (d) *Prochilodus nigricans* and (e) *Characidium cf. zebra*. The arrows indicate the supernumerary chromosomes.

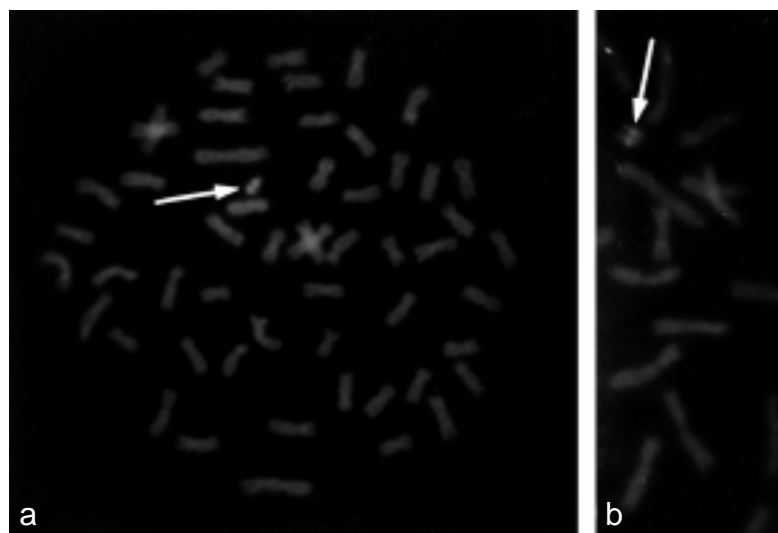


Figure 3 - Completely (a) and partially (b) CMA₃-stained metaphases of *Leporinus* sp. The arrows indicate the supernumerary chromosome with a bright band on the long arm.

more frequent in this geographic area (Moreira-Filho *et al.*, 1993), and independently of why it occurs, the chromosome diversity appears to be facilitated in this area. The findings of the present study and of the previous ones, therefore, are strongly indicative that the nature has found in Characiformes a pool of species on which different ways of origin and development of supernumerary chromosomes might have led to the general picture now observed.

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RESUMO

São descritos os cromossomos supranumerários observados em cinco espécies de peixes pertencentes a quatro famílias distintas de caracíformes neotropicais. Esses cromossomos mostraram-se pequenos, totalmente heterocromáticos e acrocêntricos em *Leporinus friderici* e *Leporinus* sp. e metacêntricos e totalmente heterocromáticos em *Cyphocharax modesta* e *Prochilodus nigricans*. Em *Characidium* cf. *zebra* um pequeno extra acrocêntrico é visto totalmente eucromático. Um pequeno segmento rico em pares de bases GC pôde ser observado no cromossomo extra de *Leporinus* sp. após a coloração com cromomicina A₃. Alguns aspectos relacionados à origem desses cromossomos extras entre os caracíformes são discutidos.

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