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B chromosomes in Amazonian cichlid species

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Abstract. B chromosomes are reported in three different Amazonian cichlid species. One to three supernumerary microchromosomes were detected in the peacock bass *Cichla monoculus* (4 out of 28 specimens) and *Cichla* sp. (4 out of 13 specimens), and pike cichlids *Crenicichla reticulata* (2 out of 5 specimens), with no similar standard chromosomal morphology. C-banding revealed that B chromosomes are totally heterochromatic. We suggest two scenarios for the origin of these B chromosomes either by chromosomal breakdowns due to mutagenic action of methyl mercury present in the aquatic environment or by interspecific origin due to hybridization events.

Many animal and plant species possess B chromosomes, also known as supernumerary or accessory chromosomes, in addition to the standard complement. Since the first published report on B chromosomes in a neotropical freshwater fish species, *Prochilodus lineatus* (= *P. scrofa*), in the early 1980s (Pauls and Bertollo, 1983), several other occurrences have been reported in different representative groups of Characiformes (i.e., Anostomidae, Characidae, Characidiidae, Curimatidae, and Prochilodontidae), Siluriformes (Callichthyidae, Loricariidae, Pimelodidae, and Trichomycteridae), Perciformes (Cichlidae), Beloniformes (Belonidae), and Synbranchiformes (Synbranchidae), the number of fish species carrying B chromosomes barely reaching 5% of all neotropical freshwater fish already karyotyped (Salvador and Moreira Filho, 1992; Claudio Oliveira's Neotropical fish chromosomal database, unpublished). Among these fishes, the genus *Astyanax* is by far one of the best studied models, mainly concerning the species *A. scabripinnis* (Néo et al., 2000, among others).

In general, fish extra chromosomes vary from micro- to macro-chromosomes, and can be recognized as punctiform elements in the karyotype or as standard metacentric (M), submetacentric (SM), or subtelocentric (ST) chromosomes. They still vary in number and, in some cases, seem to be restricted to one gender (Salvador and Moreira-Filho, 1992).

The cichlids are Perciformes fish species that present the bimodal diploid number of 48 chromosomes in species from the New World, and 44 in those from the Old World (reviewed in Feldberg et al., 2003). This fish group has also provided some evidence for the presence of B chromosomes, and two distinct cases were reported previously in neotropical cichlids. The first one was described in male germ cells of *Gymnogeophagus balzanii* (Feldberg and Bertollo, 1984), and the second one as "chromatin corpuscles" in the somatic cells of the species: *Geophagus brasiliensis, Cichlasoma paranaensis*, and *Crenicichla niederleinii* (Martins et al., 1995).

As part of a long-term study developed with Amazonian fishes we have found scarce B chromosomal cases in cichlid species. Thus, besides reporting these findings, we intend to shed some light about the origin of the B chromosomes along the evolutionary history of this family.

Materials and methods

Peacock bass *(Cichla monoculus)* and pike cichlid *(Crenicichla reticulata)* were collected from two sampling sites: (1) Lake Balbina, an anthropogenic lake in the Uatumã River, formed about 20 years ago due to the construction of a hydroelectric power plant dam (59° 20′ W, 1° 00′ S); (2) in Lake Catalão, an ecotone formed by Solimões River "white water" and Negro River "black water" mixture (59° 54′ 29′ W, 3° 09′ 47′ S). *Cichla* sp. was only collected from Lake Balbina.

Chromosome preparations from kidney cells were obtained using the air-drying technique described by Bertollo et al. (1978), with modifications. The heterochromatin pattern was analysed according to C-banding (Sumner, 1972).

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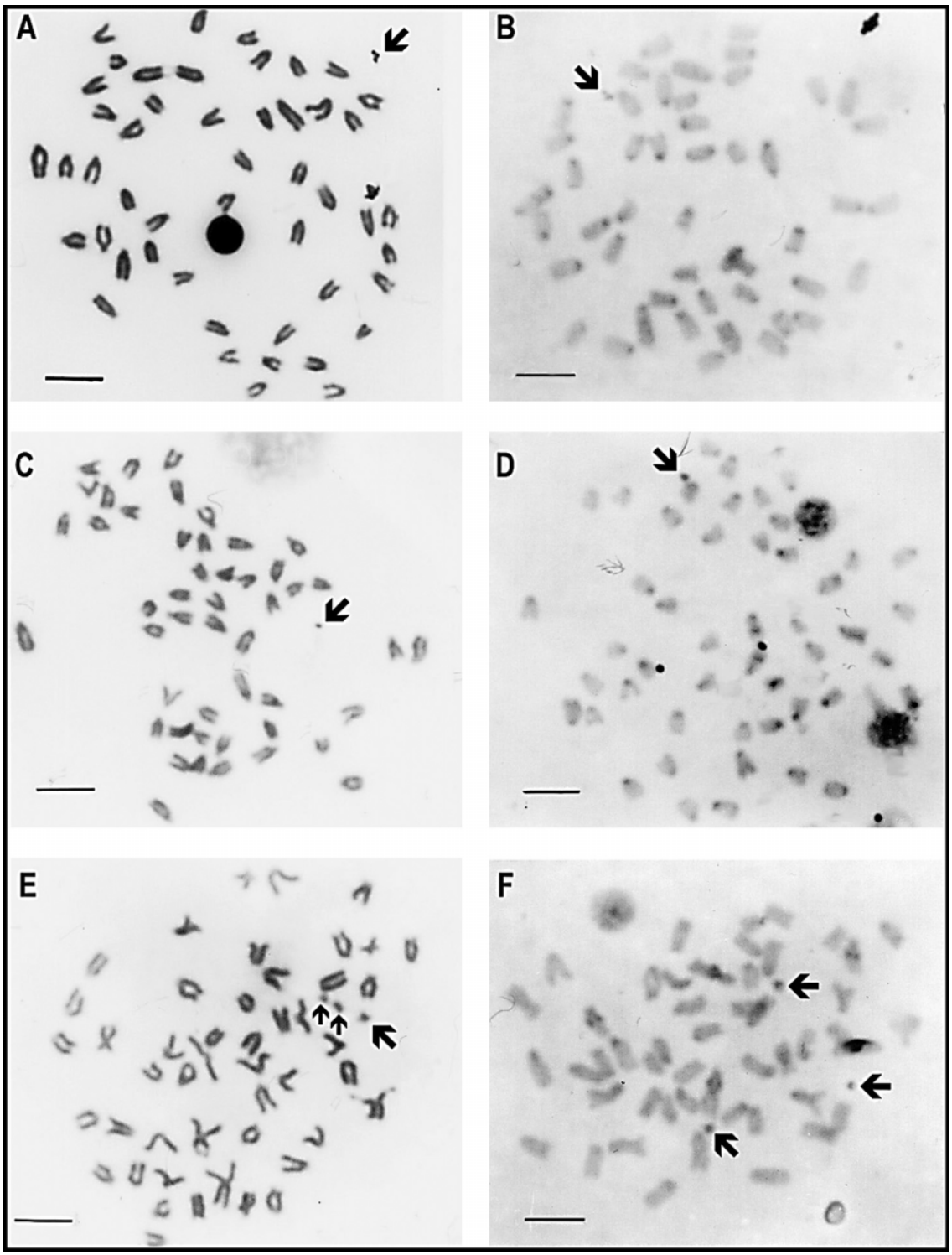

Fig. 1. Mitotic metaphases of cichlids showing B microchromosomes. (**A**) and (**B**): Giemsa staining and C-banding of *Cichla monoculus*, respectively; (**C**) and (**D**): Giemsa staining and C-banding of *Cichla* sp., respectively; (**E**) and (**F**): Giemsa staining and C-banding of *Crenicichla reticulata*, respectively. Arrows point to B chromosomes. Scale bar represents 5 μm.

Results and discussion

The three species examined, *Cichla monoculus*, *Cichla* sp., and *Crenicichla reticulata* showed a diploid number equal to 48 chromosomes plus one to three B microchromosomes. In *C. monoculus*, four specimens out of 28 sampled carried B chromosomes (14.3 %). In *Cichla* sp., 4 out of 11 individuals analysed carried Bs (36.4 %). In *C. reticulata*, two out of the five animals sampled carried Bs. Unlike *Cichla*, where only specimens from Lake Balbina carried B chromosomes, *Crenicichla* showed B chromosomes in specimens from Lake Balbina and Lake Catalão (Fig. 1). C-banding revealed that B chromosomes in the three species are completely heterochromatic (Fig. 1).

B chromosomes have already been detected in different neotropical fish groups, with a predominance for the Characidae family (Portela-Castro et al., 2001), and they may be found just in one or more populations of a same species (Moreira Filho et al., 2001).

As far as we know, only two fish species bearing B chromosomes have been reported in the Amazon region, i.e., *Callichthys callichthys*, a Callichthyidae armoured catfish (Porto and Feldberg, 1993), and *Metynnis lippincottianus*, a Serrasalminae species (Souza et al., 1999). Thus, there seems to be a remarkable bias in the geographic distribution of B chromosomes in Brazil, since most B chromosome records have been reported in southern populations. However this bias seems to be more related to a sampling effect than to other probable cause.

In mammals, supporting the theory of centromeric drive, the B chromosomes are more frequent in animals with monoarmed chromosomes (Palestis et al., 2004). However, this is not the case for Cichlidae fishes, even though their karyotypes are mainly formed by acrocentric chromosomes. In fact, considering the available chromosomal data on more than 135 Cichlidae species (Feldberg et al., 2003), only seven B-carrying species (including the three described in the present paper) have been reported. This represents about 5.2% of the karyotyped cichlids, which is consistent with B frequency in fish in general.

B chromosomes can originate intraspecifically from the standard A complement or interspecifically as the result of interspecies mating (Camacho et al., 2000). B chromosomes could be either a by-product of chromosomal rearrangements or a by-product of injured chromosomes. A scenario where B chromosomes are originated by lagging chromosome fragments during the cell division can not be discarded and the mutagenic heavy metal mercury could be the causative agent. It is well known that mercury interferes with the mitotic spindle (Miura and Imura, 1987) and, particularly in *Cichla* species from Lake Balbina, a certain degree of mercury contamination has been found (Kehrig et al., 1998). Mercury has been released in the Amazon Basin during events of gold mining, deforestation, damning of rivers, and when associated with natural pedogeochemical and atmospherical transformation processes have severely affected the Amazonian biota (Artaxo et al., 2000). Moreover, chromosome damage has been reported in Amazonian people exposed to methyl mercury contamination (Amorim et al., 2000).

Regarding the hypothesis of B chromosome interspecific origin, we have some clues that *Cichla* species, but not *Crenicichla*, have experienced hybridization in the wild, as evidenced by mtDNA (Andrade et al., 2001), esterase enzymes (Teixeira and Oliveira, personal communication), and chromosomal data (Alves-Brin, Porto and Feldberg, manuscript in preparation). Thus, *Cichla* fits well to this model. Chromosomal data has demonstrated a probable hybridization between *C. monoculus* and *C. temensis* and the origin of *Cichla* sp. from this process. Thus, we can speculate that during chromosome introgression from one *Cichla* species into the other, several rearrangements might have occurred and that fragmentation of the alien chromosomes due to cell instability resulted in B chromosome generation. However, the mechanisms that influenced these small pieces of chromosome to be kept in the cells as extra elements or B chromosomes are still unclear. This premise is supported by case studies in the monocot *Coix* (Sapre and Deshpande, 1987), fruit fly (Braverman et al., 1992), fish (Schartl et al., 1995) and wasps (Perfectti and Werren, 2001) where the B chromosomes were probably introduced by interspecies mating.

The fact that B chromosomes are found in different neotropical fish groups does not necessarily mean they have a common origin or are product of a single event. The likely explanation for the presence of B chromosomes in the Amazonian cichlids might be due to changes in the aquatic environment that led to a heavy metal bioaccumulation, as well as to the failure of the reproductive barrier among some species. These changes could have triggered the origin of B chromosomes in both *Crenicichla* and *Cichla* species.

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