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Cytotaxonomic diagnosis of *Trichomycterus diabolus* (Teleostei: Trichomycteridae) with comments about its evolutionary relationships with co-generic species

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The karyotype and the Ag-NOR location of a sample of *Trichomycterus diabolus* collected in the córrego Hortelã (Botucatu, São Paulo, Brazil) are described. The species exhibited 2n=56 chromosomes (42 metacentrics, 12 submetacentrics and 2 subtelocentrics) and the nucleolus organizing region located near to the centromere on the long arm of the largest metacentric pair. The presence of 2n=56 chromosomes in *T. diabolus* is an interesting characteristic since until now all cis-Andean species karyotyped presented 2n=54 chromosomes while almost all trans-Andean species presented different diploid numbers. The possible origin of this unexpected karyotypic form is discussed.

São descritos o cariótipo e a localização das regiões organizadoras de nucléolo (Ag-NOR) de uma amostra de *Trichomycterus diabolus*, coletada no córrego Hortelã (Botucatu, São Paulo, Brasil). A espécie apresentou 2n=56 cromossomos (42 metacêntricos, 12 submetacêntricos e 2 subtelocêntricos) e as regiões organizadoras de nucléolo localizadas próximas ao centrômero, no braço longo do maior par metacêntrico. A ocorrência de 2n=56 cromossomos em *Trichomycterus diabolus* é uma característica interessante, uma vez que, até o momento, todas as espécies cis-Andinas cariotipadas apresentaram 2n=54 cromossomos, enquanto que quase todas as espécies trans-Andinas apresentaram números diplóides diferentes. É discutida a possível origem desta inesperada estrutura cariotípica.

Keywords: Trichomycterinae, karyotype, chromosome evolution, fish cytogenetic

Introduction

The family Trichomycteridae, with about 200 species described, is one of the most diverse Neotropical catfishes group (de Pinna & Wosiacki, 2003). The genus *Trichomycterus* has about 120 species, distributed in Central and South America from Costa Rica to north Argentina, in both sides of Andes (Wosiacki & Garavello, 2004). The genus is not monophyletic and has many taxonomic and systematic problems (Wosiacki, 2002; de Pinna & Wosiacki, 2003).

Until now cytogenetic studies were carried out with about 18 species of the family Trichomycteridae (reviewed by Sato *et al.*, 2004). The diploid numbers range from 2n=52 in *Hatcheria macraei* to 2n=64 in *Vandellia cirrhosa***,** but 11 species (ten cis-Andean species) have 2n=54 chromosomes (Sato *et al.*, 2004). Aiming to extend the knowledge about the karyotype constitution of this fish group, the present paper describe the karyotype of a recent discovered species, *Trichomycterus diabolus*, that occur in tributaries of the Paranapanema river. The results are compared with those available for other species of the family.

Material and Methods

The species *Trichomycterus diabolus* (Fig. 1) was recently found in tributaries of the rio Paranapanema (Bockmann *et al.*, 2004). In the present study six specimens (3 males and 3 females) of *T. diabolus* were collected in the córrego Hortelã (22°55' S 48°30' W - a small tributary of the rio Pardo, an important component of the rio Paranapanema basin), Botucatu, São Paulo, Brazil, and karyotyped. The specimens analyzed were deposited in the collection of Laboratório de Biologia e Genética de Peixes, Departamento de Morfologia, UNESP, Botucatu (LBP 143).

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Fig. 1. A specimen of *Trichomycterus diabolus* with 110 mm in total length. Photo by C. Oliveira.

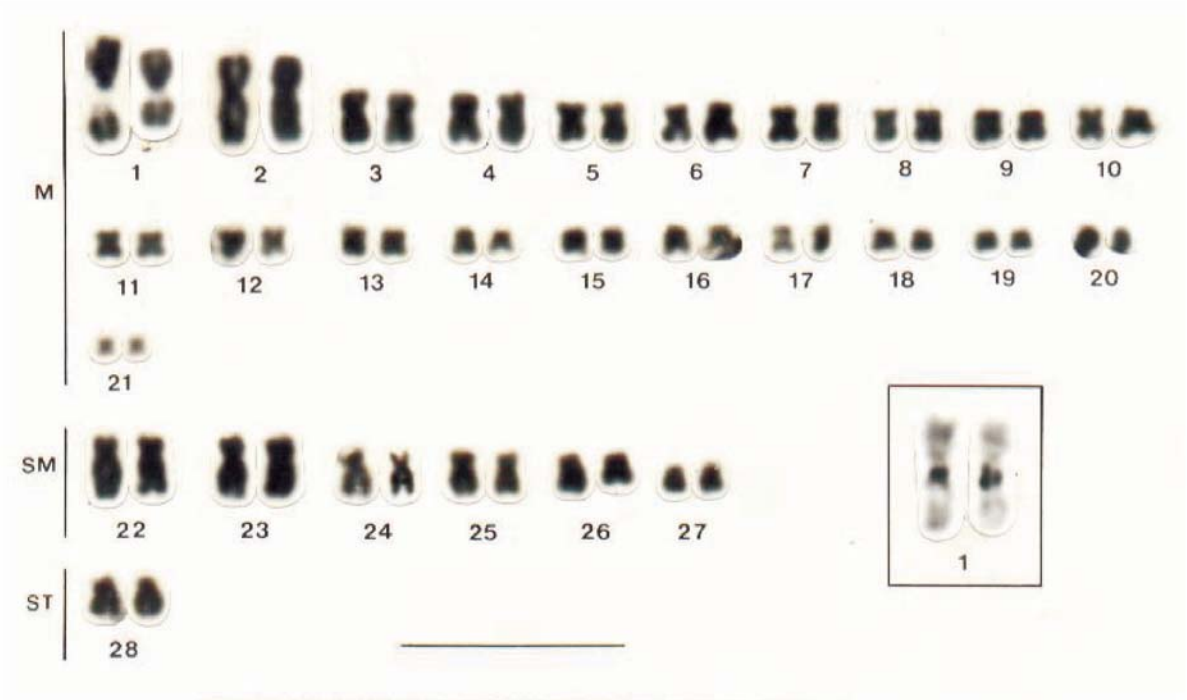

Fig. 2. Karyotypic macrostructure of *Trichomycterus diabolus*. In detail the NOR-bearing chromosome pair after silver staining. M=metacentrics; SM=submetacentrics; ST=subtelocentrics chromosomes. Bar = 10 μm.

Chromosome preparations and staining techniques were conducted according to Foresti *et al.* (1993). Chromosome morphology was determined on the basis of arm ratio, as proposed by Levan *et al.* (1964), and chromosomes were classified as metacentrics (M), submetacentrics (SM) and subtelocentrics (ST).

Results and Discussion

The chromosomal analyses performed in *T. diabolus* revealed the occurrence of 2n=56 chromosomes and the karyotypic formula: 42M+12SM+2ST (Figure 2). The Ag-NOR is located near to the centromere region on the long arm of the first metacentric chromosome pair (Fig. 2).

The presence of 2n=56 chromosomes in *T. diabolus* is an interesting feature since until now, only *T. areolatus* from Chile (Arratia & Veloso, 1980) was known as having the same diploid number. However, the absence of additional information about the karyotype of *T. areolatus* makes difficult a comparative analysis with *T. diabolus*. On the other hand, the karyotype of *T. diabolus* has two marked characteristics: (1) the two largest M pairs have similar sizes and are considerably larger than the other M pairs; and (2) the Ag-NOR is interstitially located in a large M pair. These characteristics were already found in some other *Trichomycterus* species, as *T. paolence* (Torres *et al.*, 1998), *T. davisi*, *T. stawiarski* and *Trichomycterus* sp. (Borin & Martins-Santos, 1999), and *Trichomycterus* sp. *aff. T. itatiayae* (Sato *et al.*, 2004) suggesting that *T. diabolus* may be related with these species. Indeed, the karyotype of *T. diabolus* only differ from that of *Trichomycterus* sp. *aff. T. itatiayae* (Sato *et al.*, 2004) by the presence of an additional SM pair.

T. diabolus is morphologically similar to *T. castroi*, which

is distributed in small streams of the rio Iguaçu basin (Bockmann *et al.*, 2004). Phylogenetic studies conducted by Wosiacki (2002), using morphological data, showed that *T. davisi*, *T. castroi*, *T. stawiarski* and two new species of *Trichomycterus* are, in this order, the most derived clades of Trichomycterinae, but are not related to *T. areolatus*, which is a basal clade. In addition, Sato (2003) obtained similar results using molecular data, showing that *T. diabolus* and *T. davisi* belong to the most derived monophyletic group of Trichomycteridae, reinforcing that these species are not related to *T. areolatus*.

Considering the karyotypic and phylogenetic data, it is possible to suggest that the karyotype of *T. diabolus* was originated from an ancestral with 2n=54 chromosomes. However, the available data are not informative about the origin of the additional SM pair found in this species since there is no evidence of chromosome rearrangements such as centric fissions. It is interesting to note that *T. davisi*, a species related to *T. diabolus*, showed an additional supernumerary microchromosome (Borin & Martins-Santos, 1999).

Caramaschi (1986), in an wide ecological and ichthyofaunistic study, recognized five morphotypes of *Trichomycterus* in the Cuesta of Botucatu. These morphotypes are easily distinguishable by their particular color patterns, presence or absence of ventral fins, position of anal and dorsal fins, eye size and shape of the caudal fin boundary. In three of those morphotypes it was found 2n=54 chromosomes, although differing by particular karyotypic features (Torres *et al*., 1998). The data obtained in the present study show that the fourth morphotype, actually identified as *T. diabolus*, also has particular karyotypic characteristics that distinguish it from the previous three forms, being the presence of a different diploid number (2n=56) the most striking feature.

Until now, all cis-Andean species of *Trichomycterus* karyotyped presented 2n=54 chromosomes while all the trans-Andean species presented different diploid numbers (Sato *et al.*, 2004). Thus, the presence of 2n=56 chromosomes in *T. diabolus* is an exception for that general rule. Considering that *T. diabolus* is not related with any trans-Andean *Trichomycterus* species, the presence of 2n=56 chromosomes in this species may indicate that changes in chromosome number among the cis-Andean species may not be so rare as previously supposed (Sato *et al.*, 2004). Further chromosome analysis of additional *Trichomycterus* species will permit to better understand the extension of chromosome rearrangements in this genus.

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