Cytogenetics of *Trichomycterus brasiliensis* (Siluriformes: Trichomycteridae) from the Upper São Francisco River Basin (MG)

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Summary  Trichomycteridae is a family of small catfish which is widely distributed throughout Southern Central America and South America. Specimens of *Trichomycterus brasiliensis* collected in the upper São Francisco River Basin in Brasil were studied cytogenetically. All individuals presented 2n=54 chromosomes, including 34 metacentric, 18 submetacentric and 2 subtelocentric chromosomes. A secondary constriction was observed in the interstitial region of the short arm of the chromosome pair number 20, coinciding with the NOR and 18S rDNA. The first metacentric pair is considerably larger than the second metacentric pair and the NORs occur in the pericentromeric position of the short arm of a large submetacentric pair, allowing to place this species in one of the cis-Andean groups already identified of Trichomycterus.

Key words  C-banding, NOR-banding, Fish, 18S, *Trichomycterus brasiliensis*.

Trichomycteridae is a monophyletic group composed of eight subfamilies, 41 genera and approximately 207 species (Ferraris 2007). Seven subfamilies are demonstrably monophyletic groups, but Trichomycterinae is the largest and clearly a polyphyletic group of Trichomycteridae (Wosiacki 2002). The *Trichomycterus* genus, touted as not monophyletic (de Pinna 1989), is the richest and most widely distributed of Trichomycteridae family, with 113 valid species spread throughout South and Central America, on both sides of the Andes (de Pinna and Wosiacki 2003, Ferraris 2007). A considerable diversity of Trichomycterus species occurs in the hydrographic basins of South and Southeast of Brazil. In these regions 34 species (Bockman *et al.* 2004) have been described.

The diploid chromosome number ranges from 2n=50 in *Trichomycterus* sp. to 2n=64 in *Vandellia cirrhosa*, although most species of this family have 2n=54 chromosomes (Sato *et al.* 2004). About 20 species of the Trichomycteridae family were studied cytogenetically until now, and represent only a small fraction of the total species of the group. In order to increase knowledge about this family, *Trichomycterus brasiliensis* from the headwaters of the upper São Francisco River Basin (MG) was cytogenetically analyzed and the results were compared with other species of the family.

Materials and methods

Nine specimens (five males and four females) collected in the Ribeirão das Araras (20°26’16.4″S; 045°55’39.8″W), a small tributary from the upper São Francisco River Basin (MG), were cytogenetically studied in this work. Voucher specimens are deposited in the ichthyological
Collection of the National Museum under number MNRJ 29331. The mitotic metaphases were obtained according to Bertollo et al. (1978). Chromosome morphology was determined according to the arm size relation proposed by Levan et al. (1964). The fundamental number (FN) was established through the sum of the number of chromosome arms, counting two arms for metacentric (m), submetacentric (sm), and subtelocentric (st) chromosomes, and one arm for acrocentric (a) chromosomes. The constitutive heterochromatin was identified using the barium hydroxide method (Sumner 1972), and the nucleolar organizing regions were detected through silver nitrate staining (Howell and Black 1980). Each preparation was stained in conventional Giemsa staining, and subsequently submitted to C-banding.

The localization of the 18S DNA sites in the chromosomes was performed using the Fluorescence In Situ Hybridization (FISH) technique (Pinkel et al. 1986), with 77% stringency and probes obtained from Prochilodus argenteus Spix & Agassiz, 1829 (Hatanaka and Galetti 2004). The probes were marked with 14-dATP-biotin by nick translation according to the manufacturer’s instructions (Bionick Labelling System-Invitrogen). The chromosomes were counterstained with DAPI (0.2 mg/mL) and analyzed in an Olympus BX50 epifluorescence microscope. The software Image-Pro Plus (Media Cybernetics) was used for image capture.

Results and discussion

The samples presented 2n=54 chromosomes with 34m+18sm+2st (Fig. 1a) and a fundamental number of 108. No gender-related chromosomal differences were observed. A conspicuous secondary constriction coinciding with the Ag-NOR was observed in the interstitial region of the short arm of the chromosome pair number 20 (Fig. 1a).

Phylogenetic analysis using sequences of nuclear genes (Sullivan et al. 2006) put Trichomycteridae and Nematogenyidae in the base of the phylogeny of Siluriformes. Most species of Trichomycteridae family has 2n=54 (Sato et al. 2004), very close to the presumed ancestral karyotype of the Order Siluriformes suggested by Oliveira and Gosztonyi (2000), confirming the ancestry of the family. Moreover, among karyotyped species of the family, Trichogenes longipinnis with 2n=54 is considered the most ancestral (Lima and Galetti 1990).

The diploid number (2n=54) reported in Trichomycterus brasiliensis of the Araras River was observed in virtually all congeners distributed in the cis-Andean region of South America (Sato et al. 2004); only T. diabolus, with 2n=56 (Torres et al. 2004), presented another diploid number. Chromosomal stability observed in these taxa was not expected due to the biological characteristics of the species of this genus, which are usually highly adapted to specific habitats (Caramaschi 1986).

Comparing the relative sizes of the first pair of metacentric chromosomes and the location of the NOR, Sato et al. (2004) grouped the cis-Andean species of Trichomycterus into two groups. T. brasiliensis belongs to the first group which consists of species that have the first pair of metacentric considerably larger than the second pair and NORs located in a pericentromeric position of the short arm of a large submetacentric chromosome. In the species of the second group, the first two pairs of metacentric chromosomes are approximately equal in size and pericentromeric NORs are present on the long arms of metacentric chromosomes. The species T. reinhardtii (Sato et al. 2004), T. auroguttatus (Sato et al. 2004) and T. Spegazzini (Gonzo et al. 2000) also have the characteristics of the first group, indicating a possible relationship between these taxa and T. brasiliensis.

Based on the presence of apomorphic characters Bockmann and Sazima (2004) reported that Trichomycterus maracaya, T. brasiliensis, T. iheringi, T. mimonha, T. potschi, T. vermiculatus are members of the Trichomycterus brasiliensis species-complex.

The data available in the literature for T. brasiliensis (this work) and T. iheringi (Sato 2007)
indicate some chromosomal similarities, such as the presence of only one chromosome pair of subtelocentric type, first chromosome pair substantially greater than the second and interstitial RON in a submetacentric chromosome pair. However, it is important to note that the RON is located on the short arm in *T. brasiliensis* and in the long arm in *T. iheringi*. This difference is
caused by a possible pericentric inversion. Future cytogenetic studies with other species of the complex may aid in the taxonomy of this group.

*Trichogenes longipinnis* (Lima and Galetti 1990) also has a first pair of chromosome substantially greater than the second, resembling the first group proposed by Sato et al. (2004). However, the NOR of *Trichogenes longipinnis* is terminal, unlike the two patterns suggested for *cis*-Andean *Trichomycterus*. The *Trichogenes* genus is considered one of the most basal of the *Trichomycteridae* family (de Pinna 1998), and thus the difference size between the first two pairs of metacentric would be an ancestral character. *Copionodon orthiocarinatus* (Sato 2007), another species of a genus also considered basal in the family, has the first two pairs of metacentric chromosomes of very different sizes. Chromosomal rearrangements as pericentric inversions may have been responsible for karyotypic changes between the two *Trichomycterus* groups.

In general the chromosomes of *T. brasiliensis* showed no large amount of constitutive heterochromatin. Only the pair of NOR and the pair number 4 have conspicuous brands (Fig. 1b).

The C-banding observed in the present study differs from results obtained in *T. davisi*, *T. stawiarski* and *Trichomycterus* sp. from Iguacu River (Borin and Martins-Santos 1999). In these, there are larger amounts of heterochromatin. Two other species, *Trichomycterus paolence* (Torres et al. 1998) and *Trichomycterus spegazzini* (Gonzo et al. 2000) have heterochromatic pattern more similar to that obtained in the present study. These results support groups created by Sato et al. (2004) since *T. davisi*, *T. stawiarski* and *Trichomycterus* sp. belong to the second group while *T. paolence*, *T. spegazzini* and *T. brasiliensis* (present study) belong to the first group.

Data with FISH technique with species of the genus *Trichomycterus* are nonexistent in the literature. The application of 18S rDNA probe confirmed the data obtained with impregnation by silver nitrate; there is only one chromosome pair carrying NORs in *T. brasiliensis* (Fig. 2). The detection of only one locus in this species coincides with the position adopted by Lima and Galetti (1990), which postulates that the location of the nucleolus organizer regions in only one chromosome pair would be a symplesiomorphic condition of *Trichomycteridae* family. Data from *in situ* hybridization should be evaluated in a comparative way, and so, as more FISH results are obtained in other species of this family, more conclusive analysis can be obtained.

References


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