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Research Article

# Karyotype description of five species of *Trichomycterus* (Teleostei: Siluriformes: Trichomycteridae)

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## Abstract

Trichomycteridae is a family of small catfish which are widely distributed throughout Southern Central America and South America. The present study showed that the cis-Andean species *Trichomycterus florensis*, *Trichomycterus* sp. aff. *Trichomycterus itatiyae*, *Trichomycterus reinhardti*, *Trichomycterus davisi* and *Trichomycterus auroguttatus* had 2n = 54 chromosomes (42 metacentric, 10 submetacentric and 2 subtelocentric), with *T. reinhardti*, *T. auroguttatus* and *T.* sp. aff. *T.* itatiyae exhibiting only one chromosome pair with silver-stained nucleolus organizer regions (NORs). The cytogenetic data suggest the existence of at least two groups of species in the cis-Andean representatives of the genus *Trichomycterus*. In the first group 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 while in the second group the first and second metacentric pairs are about the same size and larger than the other metacentric pairs and the NORs are located in the pericentromeric position of the long arm of a large metacentric pair. The relative conservatism of the karyotype of the cis-Andean *Trichomycterus* species contrasts with the wide diversification observed in the trans-Andean species, reinforcing the hypothesis that the genus is not monophyletic.

*Key words:* chromosome evolution, NOR banding, fish.

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## Introduction

Trichomycteridae is a family of small-sized catfish which are widely distributed throughout Southern Central America and South America (Wosiacki, 2002). This family has 171 described species, distributed between 39 genera and eight subfamilies: Trichogeninae, Copionodontinae, Sarcoglanidinae, Glanapteryginae, Tridentinae, Vandelliinae, Stegophilinae, and Trichomycterinae (de Pinna and Wosiacki, 2003). According to de Pinna (1998), the Trichomycteridae is a monophyletic group because it exhibits seven synapomorphies, most of which are concentrated in the opercular suspensory arch, a highly modified structure that enables fish to anchor on either the substrate or the tissue of their hosts. The subfamily Trichomycterinae is a non-monophyletic assemblage that currently includes eight genera and about 100 species (Wosiacki, 2002). However, according to Mário C.C. de Pinna (personal communication) a large number of new species are yet to be described in this genus.

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Only a few cytogenetic studies of the family Trichomycteridae have been conducted, in spite of the large number of species and the wide distribution of this family (Table 1). The diploid chromosome number ranges from 2n = 50 in a *Trichomycterus* species to 2n = 64 in *Vandellia cirrhosa* (Table 1), although most species of this family have 2n = 54 chromosomes (Table 1).

In this paper we describe the karyotypes of five *Trichomycterus* species collected from different Brazilian hydrographic basins and discuss the evolution of chromosomes in the family Trichomycteridae.

## Materials and Methods

Five *Trichomycterus* species were analyzed, the species, collection site and number (male, female and unsexed) of specimens collected being shown in Table 2. The fish were identified and kept in the fish collection of Laboratório de Biologia de Peixes (LBP), Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil.

Chromosome preparations and staining techniques, including silver-staining for the nucleolus organizer regions (NORs), were carried out according to Foresti *et al.*

**Table 1** - Cytogenetic data for members of the family Trichomycteridae.

| Species | Locality | n | 2n | Karyotype | Reference |
|---|---|---|---|---|---|
| **Data from studies by other workers** | | | | | |
| *Bullockia maldonadoi* | | | 60 | 46M,SM+14ST,T | Arratia and Campos (1997) |
| *Hatcheria macraei* | | | 52 | 30M,SM+22ST,T | Arratia and Campos (1997) |
| *Trichogenes longipinnis* | Ubatuba, São Paulo, Brazil | | 54 | 36M+12SM+6ST | Lima and Galetti Jr. (1990) |
| *Trichomycterus areolatus* | | | 56 | 56M,SM | Arratia and Campos (1997) |
| *Trichomycterus chiltoni* | | | 52 | 44M,SM+8ST,T | Arratia and Campos (1997) |
| *Trichomycterus davisi* | Três Barras, Paraná, Brazil | | 54 | 40M+12SM+2ST | Borin and Martins-Santos (1999) |
| *Trichomycterus laucaensis* | | | 58 | 42M,SM+16ST,T | Arratia and Campos (1997) |
| *Trichomycterus laucaensis* | | | 62 | 62M,SM,ST | Arratia and Veloso (1980) |
| *Trichomycterus paolence* | Botucatu, São Paulo, Brazil | | 54 | 44M+8SM+2ST | Torres *et al.* (1998) |
| *Trichomycterus paolence* | Botucatu, São Paulo, Brazil | | 54 | 40M+14SM | Torres *et al.* (1998) |
| *Trichomycterus paolence* | Itatinga, São Paulo, Brazil | | 54 | 46M+6SM+2ST | Torres *et al.* (1998) |
| *Trichomycterus spegazzini* | Calchaquí, Arenales and Mojotoro rivers, Argentina | | 54 | 42M+12SM | Gonzo *et al.* (2000) |
| *Trichomycterus stawiarski* | Três Barras, Paraná, Brazil | | 54 | 42M+8SM+4ST | Borin and Martins-Santos (1999) |
| *Trichomycterus* sp. | | | 50 | 44M,SM+6ST,T | Arratia and Campos (1997) |
| *Trichomycterus* sp. | Três Barras, Paraná, Brazil | | 54 | 42M+10SM+2ST | Borin and Martins-Santos (1999) |
| *Trichomycterus* sp. | | | 54 to 56 | 42M+10SM+2ST | Torres *et al.* (1995) |
| *Vandellia cirrhosa* | | 32 | | | Scheel (1973) |
| **Data from the present study** | | | | | |
| *Trichomycterus auroguttatus* | Desterro de Melo, Caranaíba, Capela Nova, Santa Bárbara do Tugúrio, Minas Gerais, Brazil | | 54 | 42M+10SM+2ST | Present study |
| *Trichomycterus davisi* | Lapa, Paraná, Brazil | | 54 | 42M+10SM+2ST | Present study |
| *Trichomycterus florensis* | Santa Rita de Jacutinga, Minas Gerais, Brazil | | 54 | 42M+10SM+2ST | Present study |
| *Trichomycterus* sp. aff. *T. itatiyae* | Castrolândia, Paraná, Brazil | | 54 | 42M+10SM+2ST | Present study |
| *Trichomycterus reinhardti* | Barbacena, Minas Gerais, Brazil | | 54 | 42M+10SM+2ST | Present study |

(1993). Chromosome morphology was determined on the basis of arm ratio as proposed by Levan *et al.* (1964) and chromosomes were classified as metacentric (M), submetacentric (SM) or subtelocentric (ST).

## Results and Discussion

All the species analyzed in the present study exhibited 2n = 54 chromosomes: 42 metacentric, 10 submetacentric and 2 subtelocentric (Figures 1 and 2). Numeric polymorphisms, such as those described for *Trichomycterus paolence* (Torres *et al.*, 2002) and *T. davisi* (Borin and Martins-Santos, 2000), were not detected in the species studied.

Karyotypic analysis of the samples showed that the first metacentric pair was considerably larger than the second metacentric pair in *T. florensis* (Figure 1a), *T. reinhardti* (Figure 1c) and all the *T. auroguttatus* samples (Figures 2b and 2c), this characteristic having also been observed in two cytotypes of *T. paolence* (Torres *et al.*, 1998), *Trichomycterus spegazzini* (Gonzo *et al.*, 2000), and *Trichogenes longipinnis* (Lima and Galetti Jr., 1990).

Another common characteristic of the *T. reinhardti* and *T. auroguttatus* sampled was the presence of NORs in the pericentromeric position of the short arm of a large submetacentric pair (Figures 1c and 2b, respectively), this characteristic having also been described in *T. spegazzini* (Gonzo *et al.*, 2000).

In *T.* sp. aff. *T. itatiyae* (Figure 1b) and *T. davisi* (Figure 2a) the first and the second metacentric pairs were about the same size and larger than the other metacentric pairs. This characteristic was also observed in the karyotypes of one *T. paolence* cytotype (Torres, 1998) and also in *T. davisi*, *Trichomycterus stawiarski* and *Trichomycterus* sp. (Borin and Martins-Santos, 1999).

We also identified NORs in the second metacentric pair of *T.* sp. aff. *T. itatiyae* in the pericentromeric position

**Table 2** - Specimens used in the study presented in this paper.

| Species | Collection site | Number of fish collected | | |
|---|---|---|---|---|
| | | Male | Female | Unsexed |
| *Trichomycterus florensis* | Jacutinga stream (S22°04.363' W44°08.804'), Rio Paraíba do Sul basin, Santa Rita de Jacutinga, Minas Gerais, Brazil | 3 | 2 | |
| *Trichomycterus* sp. aff. *T. itatiyae* | Small tributary of the Onça river (S25°22.674, W49° 48.322'), Rio Tibagi basin, Castrolândia, Paraná, Brazil | 1 | 1 | |
| *Trichomycterus reinhardti* | Sapateiro stream (S21°16.432', W43°38.613'), Rio Grande basin, Barbacena, Minas Gerais, Brazil | 2 | | 1 |
| *Trichomycterus davisi* | Tributary of the Patos river (S25°52.438', W49°43.404'), Rio Iguaçu basin, Lapa, Paraná, Brazil | | 1 | 1 |
| *Trichomycterus davisi* | Tributary of the Patos river (S25°50.644', W 49°43.660') Rio Iguaçu basin, Lapa, Paraná, Brazil | | | 3 |
| *Trichomycterus auroguttatus* | Xopotó river, S 21°08.947' W 43°23.973', Rio Doce basin, Desterro de Melo, Minas Gerais, Brazil | 1 | 4 | 2 |
| *Trichomycterus auroguttatus* | Piranga river, S 20°58.171' W 43°42.331', Rio Doce basin, between Caranaíba and Capela Nova, Minas Gerais, Brazil | | 2 | 2 |
| *Trichomycterus auroguttatus* | Papagaio river, S 20°51.129' W 43°43.127', Rio Doce basin, Caranaíba, Minas Gerais, Brazil | | 1 | |
| *Trichomycterus auroguttatus* | Fernandes stream, S21°14.796', W43°34.124', Rio Paraíba do Sul basin, Santa Bárbara do Tugúrio, Minas Gerais, Brazil | | 1 | |

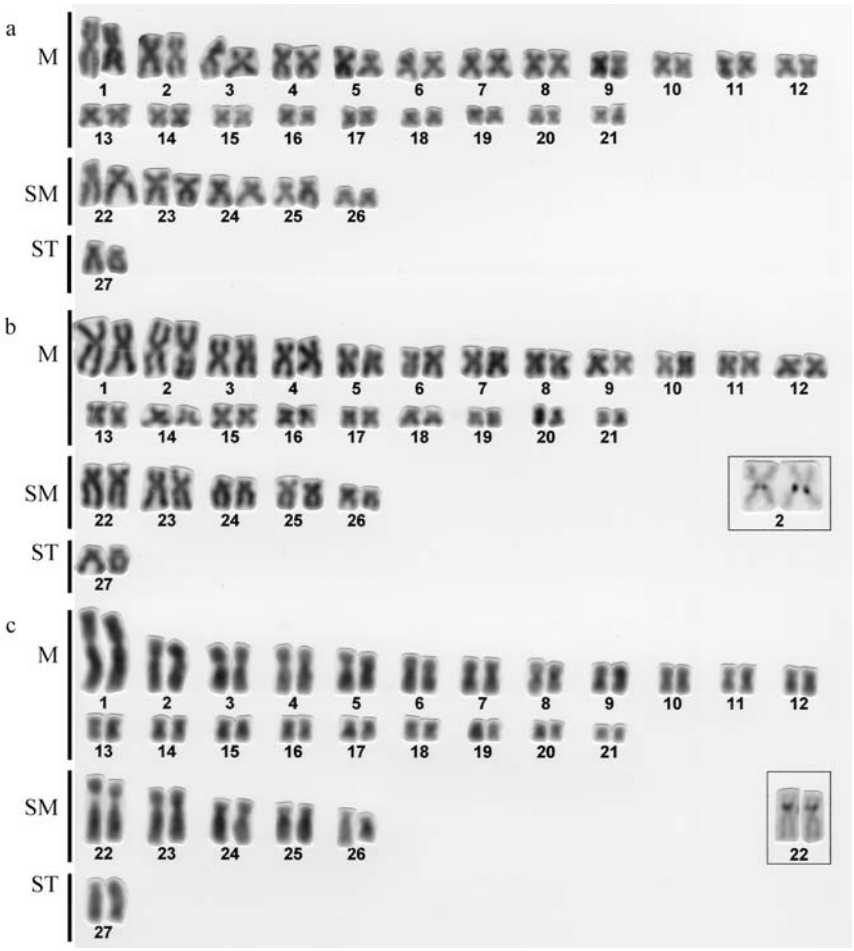


**Figure 1** - Karyotypes of: a) *Trichomycterus florensis*; b) *Trichomycterus* sp. aff. *T. itatiyae*; c) *Trichomycterus reinhardti*. The NOR-bearing chromosome pairs are shown in the insets.

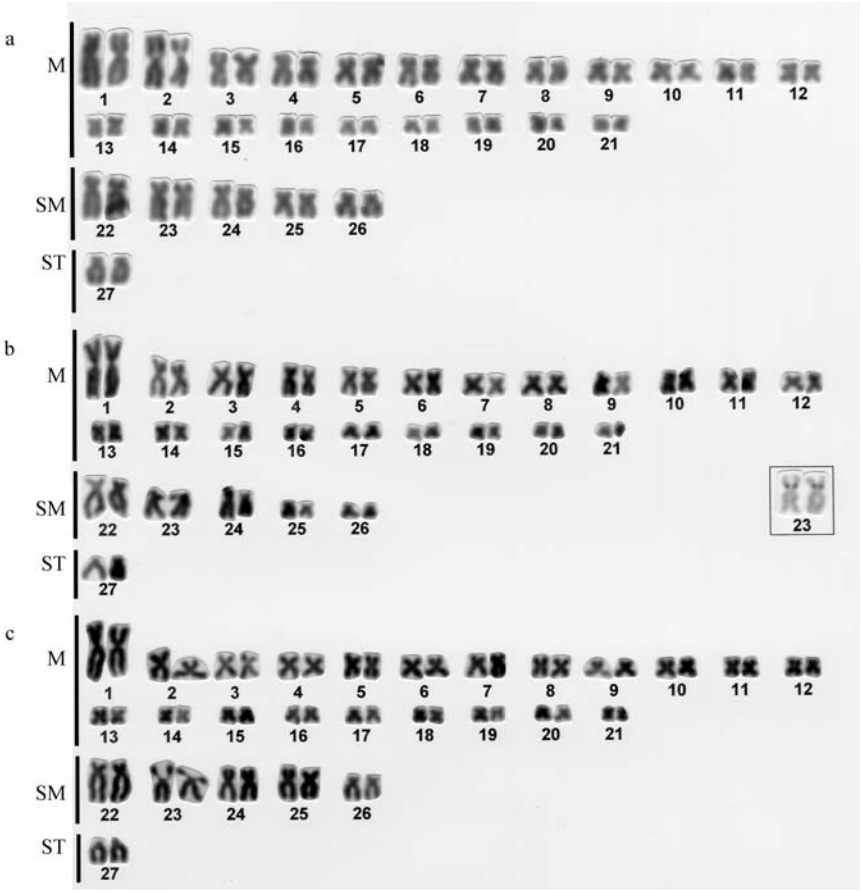


**Figure 2** - Karyotypes of: a) *Trichomycterus davisi*; b) *Trichomycterus auroguttatus* from the Rio Doce basin, the NOR-bearing chromosome pairs being shown in the insets; c) *Trichomycterus auroguttatus* from the Rio Paraíba do Sul basin.

of the long arm (Figure 1b), the same characteristic having been observed in *Trichogenes longipinnis* (Lima and Galetti Jr., 1990). Torres *et al.* (1998) found NORs in the pericentromeric position of the long arm of a large submetacentric pair of two different samples of *T. paolence* and a third specimen showed NORs in the pericentromeric position of the long arm of a large metacentric pair.

Even though silver-staining did not produce good results for the *T. davisi* metaphasic chromosomes the presence of a secondary constriction in the short arm of the second metacentric pair (Figure 2a) suggests that this is the NOR-bearing pair in this species, NORs in the same chromosome pair having also been reported by Borin and Martins-Santos (1999) in *T. davisi*, *T. stawiarski* and *Trichomycterus* sp.

We also found differences between the sizes of the two last submetacentric pairs and the other submetacentric chromosomes in *T. auroguttatus* from the Rio Doce basin and the Rio Paraíba do Sul basin. In the *T. auroguttatus* specimens from the Rio Doce basin the last two submetacentric pairs were smaller than the other submetacentric pairs, while in the Rio Paraíba do Sul *T. auroguttatus* specimens all the submetacentric pairs had almost the same size.

The data discussed above suggest the existence of at least two groups of species in the cis-Andean species of the genus *Trichomycterus* that exhibit the same diploid number as well as a different karyotype structure. In the first group the first metacentric pair is considerably larger than the second metacentric pair and the species presented silver-staining NORs in the pericentromeric position on the short arm of a large submetacentric pair; in the second group the first and second metacentric pairs were about the same size and larger than the other metacentric pairs and the NORs were located in the pericentromeric position on the long arm of a large metacentric pair.

Our data corroborates the morphological studies conducted by Wosiacki (2002) who suggested that *T. davisi* and *T. stawiarski* belong to a natural group, although Wosiacki's (2002) study does not support our cytogenetic analyses indicating that *T. reinhardti* and *T. auroguttatus* belong to a natural group. New morphological and cytogenetic studies involving a wider sample of *Trichomycterus* are needed so that the karyotypic units of this group can be better defined.

Although many groups of Neotropical fish (*e.g.* the Callichthyidae and Loricariidae), exhibit high chromosome diversity at both the interspecific and intraspecific level,

several groups (*e.g.* the Anostomidae and Curimatidae) have a very conservative karyotypic macrostructure as has been shown by Oliveira *et al.* (1988), who suggested that these karyotypic characteristics could be associated with the type of population structure of each group because species with small populations isolated in headwaters usually have different karyotypes while species belonging to groups with large populations inhabiting large river systems usually have very little karyotypic diversity.

The cytogenetic study of *Trichomycterus* species has shown that in spite of the fact that many species of this genus are usually found isolated in the headwaters of small rivers most species, such as those cytogenetically analyzed by us, exhibit the same diploid number and karyotypic macrostructure (Table 1). In all cis-Andean *Trichomycterus* species studied 2n = 54 chromosomes were found (Table 1) and different karyotypes have not been found even in widely distributed species such as *T. auroguttatus* (Table 1) and *T. spegazzini* (Gonzo *et al.*, 2000). On the other hand, all trans-Andean *Trichomycterus* species studied have shown different diploid numbers, *i.e.* 2n = 56 for *Trichomycterus areolatus*, 2n = 52 for *Trichomycterus chiltoni*, 2n = 58 and 2n = 62 for *Trichomycterus laucaensis*, and 2n = 50 for *Trichomycterus* sp. (Table 1). The phylogenetic study conducted by Wosiacki (2002) showed that the trans-Andean species *T. areolatus* and *T. chiltoni* belong to a new unnamed clade, composed of these species and *Bullockia maldonadoi* (2n = 60) and *Hatcheria macraei* (2n = 52) (Arratia and Campos, 1997). Thus, at least some of the karyotypic variability presently assigned to the genus *Trichomycterus* is probably a karyotypic characteristic of Wosiacki's (2002) unnamed clade.

The most primitive species of Trichomycteridae karyotyped to date, *Trichogenes longipinnis* (Lima and Galetti Jr., 1990), exhibits 2n = 54 chromosomes and is found in coastal streams in Southeastern Brazil. Since this same diploid number is found in all cis-Andean *Trichomycterus* species it is reasonable to suppose that these species are exhibiting the primitive diploid number, although, at present, the reason for the conservation of this diploid number cannot be explained. Future studies of other Trichomycteridae species will be very important to elucidate this and other questions related to chromosome evolution in this group.

The family Trichomycteridae is a sister-group of the family Nematogenyidae (de Pinna, 1998) of which only one species, *Nematogenys inermis*, exhibits 2n = 94 chromosomes (Arratia and Veloso, 1980). In a review study of chromosome number in Siluriformes, Oliveira and Gosztonyi (2000) suggested that the putative primitive diploid number of this order could be 2n = 56. The information currently available suggests that the diploid number increased considerably in Nematogenyidae but diminished in Trichomycteridae, although further cytogenetic and phylogenetic data are needed to allow a better understanding of chromosome evolution in these families.

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