

Comparative cytogenetics of nine species of Hypoptopomatinae (Teleostei: Siluriformes: Loricariidae): the importance of structural rearrangements in chromosome evolution

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Abstract — Fishes of the subfamily Hypoptopomatinae are very common and found in the lowlands of cis-Andean South America from Venezuela to the north of Argentina. With the main objective of contributing for a better understanding of the importance of chromosome rearrangements in the loricariid evolution, cytogenetic analyses were conducted in nine species of Hypoptopomatinae. The results showed a marked gross karyotypic conservation with the presence of $2n=54$ chromosomes in all species analyzed. The main differences were found in the karyotypic formulae level. Most species had a single interstitial Ag-NORs, however terminal Ag-NORs were observed in three species. One species exhibited two Ag-NOR-bearing chromosome pairs. The distribution of C-band positive segments was species specific but chromosome markers were observed among the species analyzed. The gross cytogenetic characteristics observed among the Hypoptopomatinae species are similar to those observed in other primitive Loricariidae species suggesting that small changes, mainly paracentric and pericentric inversion were the main events in the karyotypic evolution of this fish group.

Key words: Ag-NORs, C-band, chromosome evolution, fish cytogenetics, karyotypes.

INTRODUCTION

The family Loricariidae, with 673 species (REIS *et al.* 2003), is the largest family of the order Siluriformes and also one of the largest fish families in the world (NELSON 1994). Traditionally, Loricariidae has been divided into six subfamilies: Hypoptopomatinae, Hypostominae, Ancistrinae, Loricariinae, Neoplecostominae and Lithogeninae (REIS *et al.* 2003), however a recent study conducted by ARMBRUSTER (2004) showed that this division should be reevaluated since Hypoptopomatinae and Loricariinae were the only subfamilies that seemed to be monophyletic. The subfamily Hypoptopomatinae presents 79 species in 16 genera (SCHAEFER 2003). Fishes of this subfamily are found in the lowlands of cis-Andean South America from Venezuela to the north of Argentina and most species are relatively small when adults, usually ranging in size from 20 to 35

mm in standard length (SCHAEFER 2003). As observed in other loricariids the species of Hypoptopomatinae are typically herbivores and live in small rivers and lakes (SCHAEFER 1998).

Although the family Loricariidae is one of the largest fish families in the world, the number of species karyotyped so far is reduced. Among the karyotyped loricariids, a wide variation in diploid numbers with values ranging from $2n=36$ in *Rineloricaria latirostris* (GIULIANO-CAETANO 1998) to $2n=80$ in *Hypostomus sp.* (ARTONI and BERTOLLO 1996) was verified. Besides this numeric variability, a karyotypic variability was also observed in several species such as *Hisonotus leucofrenatus* (cited as *Microlepidogaster leucofrenatus* by ANDREATA *et al.* 1994) and *Rineloricaria latirostris* (GIULIANO-CAETANO 1998). Cytogenetic studies conducted on ten species of Hypoptopomatinae suggested that the group is very conserved, with almost all species presenting $2n=54$ chromosomes (Table 1).

In the present study the karyotypes of nine species of the subfamily Hypoptopomatinae were analyzed for the first time, with the main objective of contributing for a better understanding of the

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importance of chromosome rearrangements in the loricariid evolution

MATERIAL AND METHODS

Cytogenetic studies were conducted with nine species collected in different Brazilian localities (Table 1). The species identified as *Pseudotocinclus n. sp.* was not named because it is new to science and will be described elsewhere. After processing, fishes were identified and stored as vouchers at the fish collection of Laboratório de Biologia e Genética de Peixes (LBP), Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil.

Chromosome preparations were carried out according to FORESTI *et al.* (1993). Silver staining of the nucleolar organizer regions was performed according to the technique proposed by HOWELL and BLACK (1981), the C-banding technique was performed as described by SUMNER (1972). 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), subtelocentric (ST) and acrocentric (A). Chromosome arm number or fundamental number (FN) was determined considering ST/A chromosomes as uniarmed and M/SM chromosomes as biarmed, respectively.

RESULTS

The genus *Corumbataia* is composed of two species, *C. cuetae* and *C. tocantinensis* (BRITSKI 1997). The karyotypes of these two species (Figs.

1a and 1b) show biarmed chromosomes only; however *C. cuetae* has more metacentric chromosomes while *C. tocantinensis* has more submetacentric chromosomes (Table 2). The Ag-NORs are located at the terminal position on the long arm of the first M pair in *C. cuetae* species (Fig. 1a) and at the terminal position on the short arm of first SM pair in *C. tocantinensis* (Fig. 1b). The Ag-NORs were C-band negative (Figs. 3a and 3b). Both species of *Corumbataia* exhibited a few C-band positive segments distributed similarly in the karyotypes of both species (Figs. 3a and 3b). However, an interesting characteristic only found in this genus is that the Ag-NOR-bearing chromosome pairs were almost entirely C-band positive (Figs. 3a and 3b). The different morphology of the Ag-NOR-bearing chromosomes and the different position of the Ag-NORs are probably due to a pericentric inversion.

The species *Hypoptopoma guentheri* presented a high number of uniarmed chromosomes, mainly A (Fig. 1c, Table 2), which is uncommon among Hypoptopomatinae, only being found in *Otocinclus aff. vestitus* (Table 2) and in a local sample of *O. vittatus* (Fig. 2d, Table 2). The Ag-NORs occupied all the short arm of the chromosome pair 23 and a large polymorphism was observed among the specimens analyzed (Fig. 1c). Small C-band positive segments were observed in the centromeric position of almost all chromosomes (Fig. 3c). The Ag-NORs were C-bands negative (Fig. 3c).

Both samples of *O. vittatus* analyzed presented a very different karyotypic constitution. The sample of *O. vittatus* from Cuiabá River exhibited many ST/A chromosomes (Fig. 1d) while the sample from Taquari River exhibited only M/SM chromosomes (Fig. 1e). Additionally, Taquari

Table 1 — List of species analyzed, places of collection, specimens analyzed and voucher numbers. (?) unsexed fish.

Species	Locality	Specimens analyzed ♀/♂/?	Voucher Numbers
<i>Corumbataia cuetae</i>	Alambari Stream, Botucatu, São Paulo	8/3/0	LBP 876
<i>Corumbataia tocantinensis</i>	Vermelho River, Goiás, Goiás	1/1/0	LBP 1653
<i>Hypoptopoma guentheri</i>	Pirai River, Poconé, Mato Grosso	1/2/0	LBP 693
<i>Otocinclus vittatus</i>	Taquari River, Coxim, Mato Grosso do Sul	0/0/10	LBP 1463
<i>Otocinclus vittatus</i>	Cuiabá River, Santo Antônio do Leverger, Mato Grosso	1/1/0	LBP 694
<i>Otocinclus flexilis</i>	Santo Antônio da Patrulha, Rio Grande do Sul	0/3/3	LBP 877
<i>Otothyris juquiae</i>	Rio Preto Stream, Itanhaém, São Paulo	4/8/0	LBP 1770
<i>Otothyris travassosi</i>	Ribeira da Terra Firme River, Canavieiras, Bahia	8/3/0	LBP 1970
<i>Pseudotocinclus n.sp.</i>	Juquiá River, Juquitiba, São Paulo	2/8/2	LBP 616
<i>Schizolecis guentheri</i>	Parati-Mirim Stream, Parati, Rio de Janeiro	4/2/0	LBP 2123
	Sítio do Meio Stream, Mongaguá, São Paulo	16/5/0	LBP 1765
	Descoberto Stream, Guaratuba, Paraná	1/2/0	LBP 2052
	Garuva River, Garuva, Santa Catarina	1/1/0	LBP 2023

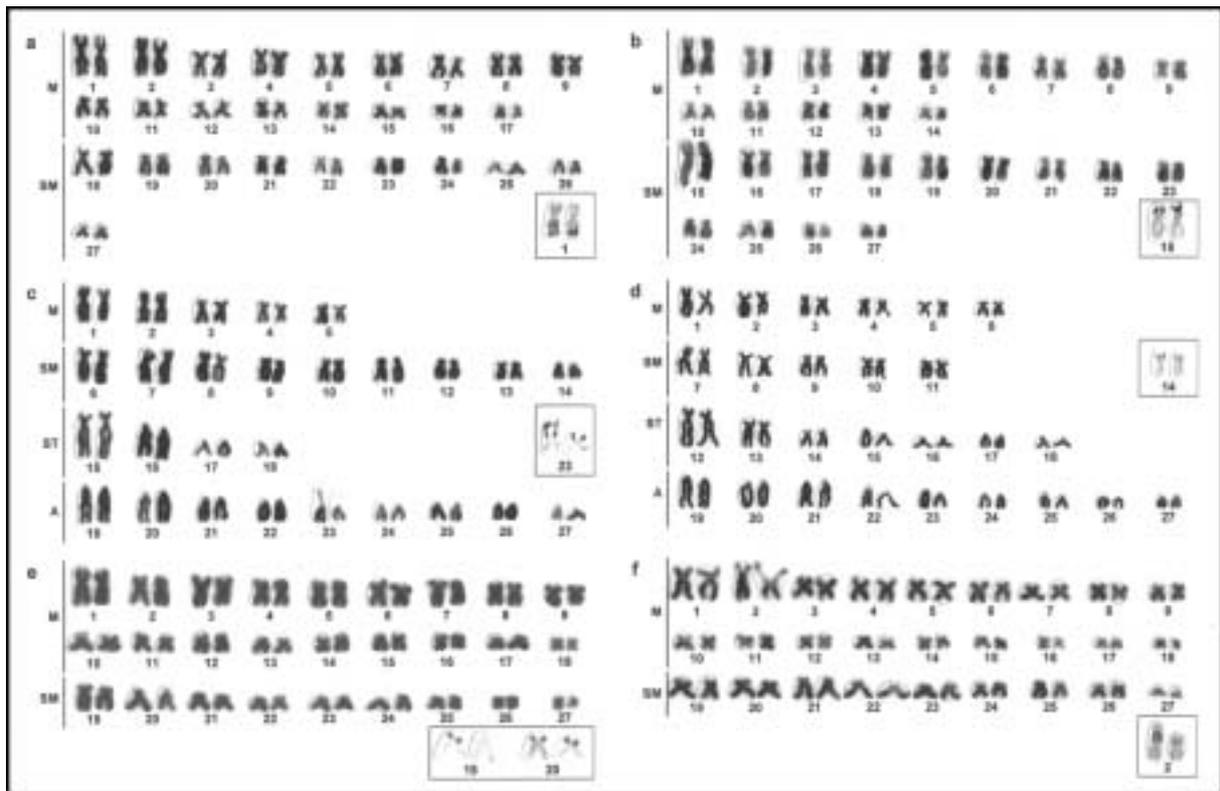


Fig. 1 — Giemsa stained karyotypes of: (a) *Corumbataia cuestae*, (b) *Corumbataia tocantinensis*, (c) *Hypoptopoma guentheri*, (d) *Otocinclus vittatus* from Cuiabá River, (e) *Otocinclus vittatus* from Taquari River and (f) *Otocinclus flexilis*. In the insets, the Ag-NOR-bearing chromosome pairs.

River displays two Ag-NOR-bearing chromosome pairs (pairs 19 and 20) (Fig. 1e), while the sample from Cuiabá River presented only one Ag-NOR-bearing chromosome pair (pair 14) (Fig. 1d). Both samples exhibited small C-band positive segments in centromeric position of almost all chromosomes and a marker chromosome with two large C-band positive segments (pair 10 in the sample from Cuiabá and pair 20 in the sample from Taquari). The sample from Cuiabá River presented large C-band positive segments in the chromosome pairs 4, 17 and 19 not found in the chromosomes of the sample from Taquari River. The rivers Cuiabá and Taquari are both tributaries of the Paraguay River and although no physical barrier separating the samples studied are verified, the expressive differences found in their karyotypes show that they are presently isolated, thus constituting cryptic species. *Otocinclus flexilis* exhibited only biarmed chromosomes (Fig. 1f, Table 2) and Ag-NORs in interstitial position on the short arm of the chromosome pair 2 (Fig. 1f). Small C-band positive segments were observed in the centromeric position of almost all chromosomes (Fig. 3f). The Ag-NORs were C-bands negative (Fig. 3f).

Previous cytogenetic studies conducted in two local samples of *Otocinclus affinis* (ANDREATA *et al.* 1994) showed that both samples presented $2n=54$ chromosomes but with a different karyotypic constitution (Table 2). *Otocinclus aff. vestitus* from Amazon Basin (ANDREATA *et al.* 1994) presented $2n=72$ chromosomes and a high number of uniarmed chromosomes (Table 2). The karyotypic variability observed in the genus *Otocinclus* is the largest found in the subfamily Hypoptopomatinae suggesting that several chromosome rearrangements were fixed in the group. While the differences found between the karyotypes of the samples with $2n=54$ were possible related to paracentric inversions, the presence of $2n=72$ in *O. aff. vestitus* (Table 2) allows suggesting that chromosome fissions played important role in the karyotypic evolution of this species.

The cytogenetic study of two species of *Otothyris*, *O. juquiaie* and *O. travassosi*, showed that both species presented similar karyotypes but *O. juquiaie* has more M chromosomes while *O. travassosi* has more SM chromosomes (Figs. 2a and 2b, Table 2). Another difference observed was that in *O. juquiaie* the Ag-NORs were interstitial on the short arm of the chromosome pair 17 (Fig.

Table 2 — Summary of the cytogenetic data available on the subfamily Hypoptopomatinae. 2n = diploid number; FN = fundamental number; M = metacentric; SM = submetacentric; ST = subtelocentric; A = acrocentric; NORs = nucleolus organizer regions.

Species	Locality	2n	Karyotypic formulae	FN	Pairs with NORs	Sex chromosomes	References
<i>Corumbataia cuetae</i>	Alambari River, Botucatu, São Paulo	54	34M+20SM	108	1		Present paper
<i>Corumbataia tocantinensis</i>	Vermelho River, Goiás, Goiás	54	28M+26SM	108	1		Present paper
<i>Hisonotus depressicauda</i>	Santo Inácio River, São Paulo	54	14M+28SM+2ST+10A	96	1		ANDREATA <i>et al.</i> (1994)
<i>Hisonotus leucofrenatus*</i>	Poço Grande River, São Paulo	54-56	♀ 24M+25SM+5ST ♂ 24M+26SM+4ST	103 104	1	ZW	ANDREATA <i>et al.</i> (1993)
	Marumbi River, Paraná	54-56	♀ 24M+25SM+5ST ♂ 24M+26SM+4ST	103 104	1	ZW	ANDREATA <i>et al.</i> (1993)
<i>Hisonotus sp. A</i>	Alambari River, São Paulo	54	30M+20SM+4ST	104	2		ANDREATA <i>et al.</i> (1993)
<i>Hisonotus sp. B</i>	Moia Stream, São Paulo	54	22M+28SM+4ST	104	1		ANDREATA <i>et al.</i> (1993)
<i>Hypoptopoma guentheri</i>	Pirai River, Poconé, Mato Grosso	54	10M+18SM+8ST+18A	82	1		Present paper
<i>Otocinclus affinis</i>	Biguá River, São Paulo	54	46M+8SM	108	1		ANDREATA <i>et al.</i> (1994)
	Bonito River, Rio de Janeiro	54	40M+12SM+2ST	106	1		ANDREATA <i>et al.</i> (1994)
<i>Otocinclus flexilis</i>	Santo Antônio da Patrulha, Rio Grande do Sul	54	36M+18SM	108	1		Present paper
<i>Otocinclus aff. vestitus</i>	Livramento River, Pará	72	22M+12SM+4ST+34A	106	1	ZW	ANDREATA <i>et al.</i> (1994)
<i>Otocinclus vittatus</i>	Cuiabá River, Santo Antônio do Leverger, Mato Grosso	54	12M+10SM+14ST+18A	76	1		Present paper
	Taquari River, Coxim, Mato Grosso do Sul	54	36M+18SM	108	2		Present paper
<i>Otothyris juquiae</i>	Rio Preto Stream, Itanhaém, São Paulo	54	32M+10SM+12ST	96	1		Present paper
<i>Otothyris travassosi</i>	Ribeira da Terra Firme River, Canavieiras, Bahia	54	26M+16SM+12ST	96	1		Present paper
<i>Parotocinclus maculicauda</i>	Poço Grande Stream, São Paulo	54	20M+32SM+2ST	106	1		ANDREATA <i>et al.</i> (1994)
<i>Pseudotocinclus tietensis</i>	Paranapiacaba, São Paulo	54	♀ 28M+20SM+6ST ♂ 27M+21SM+6ST	102 102	1	XY	ANDREATA <i>et al.</i> (1992)
<i>Pseudotocinclus n.sp.</i>	Juquiá River, Juquitiba, São Paulo	54	22M+24SM+8ST		1		Present paper
<i>Pseudotothyris obtuse</i>	Itanhaém River, São Paulo	54	26M+18SM+4ST+6A	102	1		ANDREATA <i>et al.</i> (1994)
<i>Schizolecis guentheri</i>	Parati-Mirim Stream, Parati, Rio de Janeiro	54	30M+18SM+6A	102	1		Present paper
	Sítio do Meio Stream, Mongaguá, São Paulo	54	30M+18SM+6A	102	1		Present paper
	Descoberto Stream, Guaratuba, Paraná	54	30M+18SM+6A	102	1		Present paper
	Garuva Stream, Garuva, Santa Catarina	54	30M+18SM+6A	102	1		Present paper

* species with supernumerary chromosomes.

2a) and in *O. travassosi* the Ag-NORs were interstitial on the long arm of the chromosome pair 23 (Fig. 2b). The species *O. juquiae* exhibited small centromeric C-band positive segments in almost all chromosomes and interstitial C-band positive segments in the pair 1 (Fig. 4a) while several interstitial large C-band positive segments were observed in the chromosomes of *O. travassosi* (Fig. 4b). The Ag-NORs were C-band negative (Figs. 4a and 4b).

The specimens of *Pseudotocinclus n. sp.* from Ribeira de Iguape River basin, showed 2n=54 chromosomes (Fig. 2c). The Ag-NORs were localized in the pair number 25 in the interstitial posi-

tion on the long arm (Fig. 2c). The presence of C-band positive segments was mainly observed in the long arms of the pairs 1 and 21 (Fig. 4c) but other chromosome pairs exhibited C-band positive segments weakly stained. The Ag-NORs were C-band positive (Fig. 4c). Previous cytogenetic studies conducted with a local sample of *P. tietensis* showed that this species presented XX/XY-type sex chromosome system where the Y chromosome could be differentiated from the X chromosomes by the presence of an additional large C-band positive segment (ANDREATA *et al.* 1992). The absence of sex chromosomes in *Pseudotocinclus n. sp.* reinforce the hypothesis that this sample

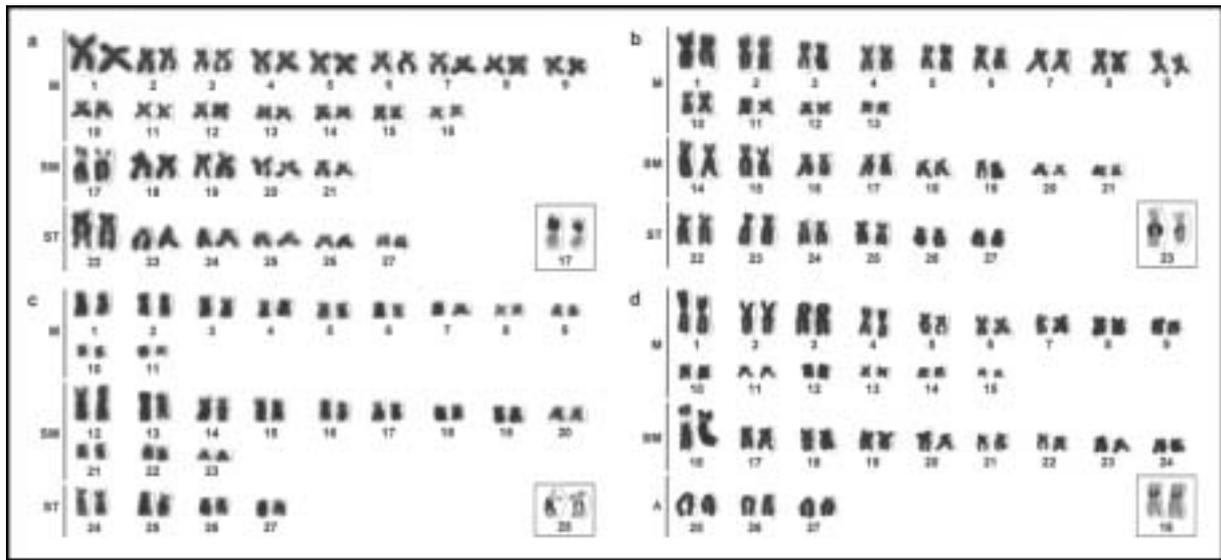


Fig. 2 — Giemsa stained karyotypes of: (a) *Otothyris juquiae*, (b) *Otothyris travassosi*, (c) *Pseudotocinclus n. sp.* and (d) *Schizolecis guentheri*. In the insets, the Ag-NOR-bearing chromosome pairs.

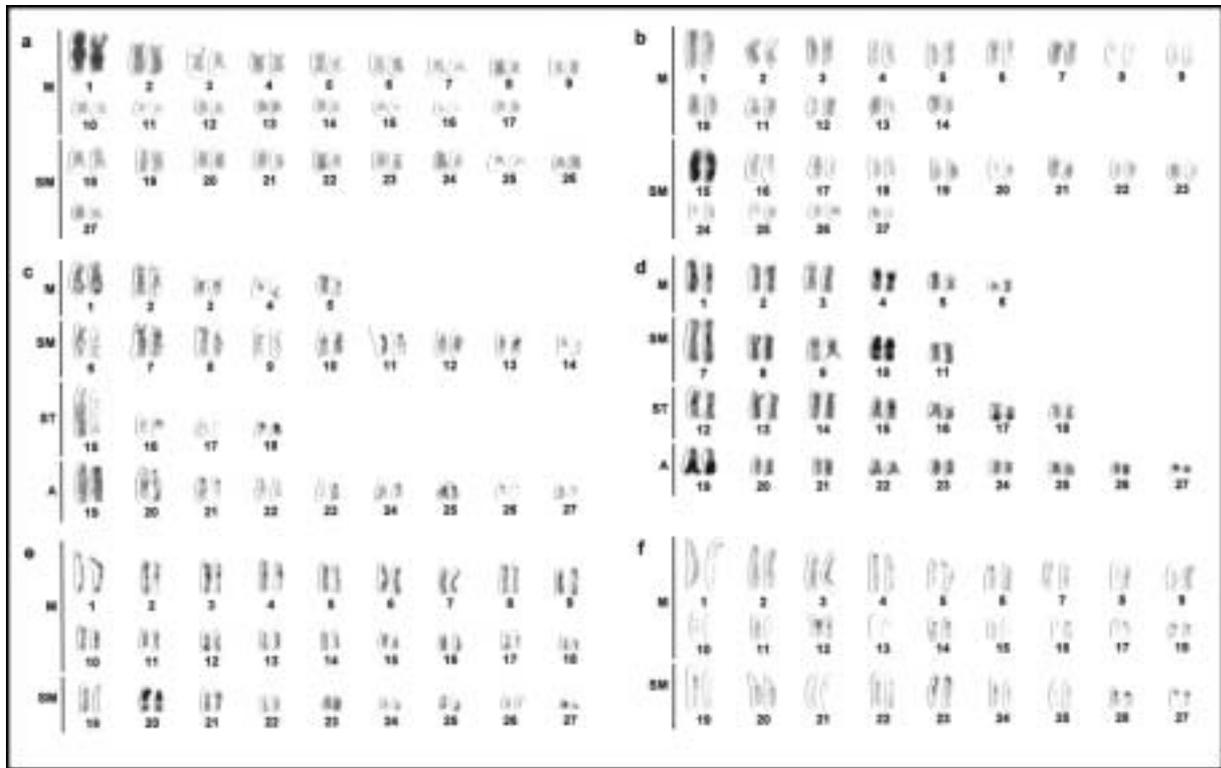


Fig. 3 — C-band stained karyotypes of: (a) *Corumbataia cuestae*, (b) *Corumbataia tocantinensis*, (c) *Hypoptopoma guentheri*, (d) *Otocinclus vittatus* from Cuiabá River, (e) *Otocinclus vittatus* from Taquari River and (f) *Otocinclus flexilis*.

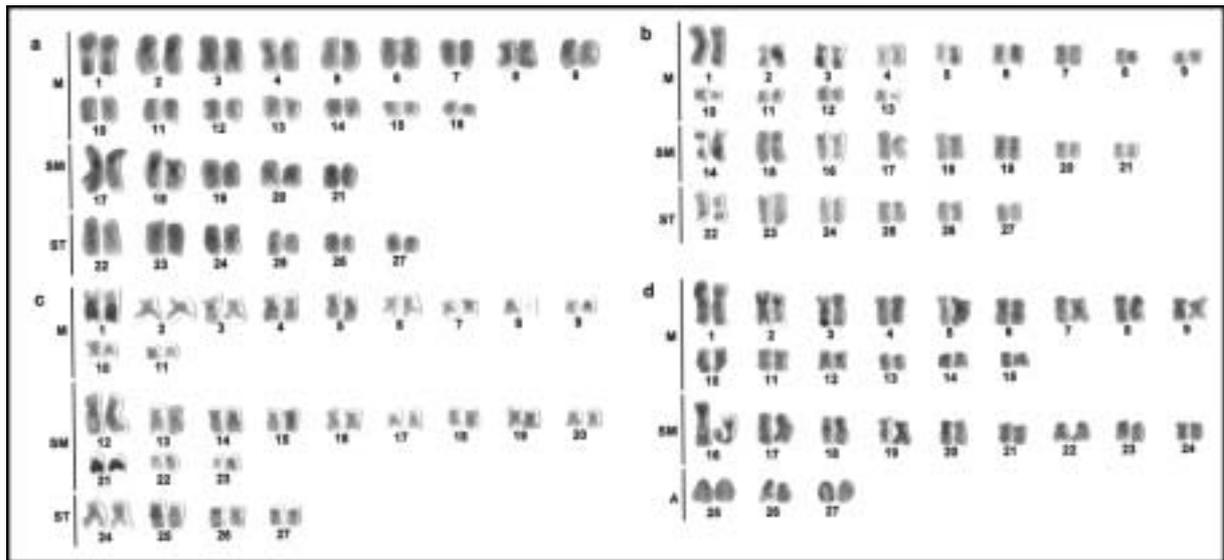


Fig. 4 — C-band stained karyotypes of: (a) *Otothyris juquiae*, (b) *Otothyris travassosi*, (c) *Pseudotocinclus n. sp.* and (d) *Schizolecis guentheri*.

from Ribeira de Iguape River basin constitutes a different species as suggested by morphological and molecular studies (A. K. TAKAKO unpublished data).

The cytogenetic analysis conducted in the species *Schizolecis guentheri* showed that all samples analyzed presented $2n=54$ and the same karyotypic constitution (Fig. 2d, Table 2). The Ag-NOR was observed in interstitial position on the short arm of the chromosome pair 16 (Fig. 2d). This species presented a very small amount of C-band positive segments in centromeric position of almost all chromosomes (Fig. 4d). The Ag-NORs were C-band negative (Fig. 4d).

DISCUSSION

All species investigated in the present study exhibit $2n=54$ chromosomes, that is the most usual feature among fishes of the subfamily Hypoptopomatinae (Table 2). The only exception is the species *Otocinclus aff. vestitus* from Amazon Basin that presented $2n=72$ chromosomes (ANDREATA *et al.* 1993). Although most species have the same diploid number, they have different chromosome formulae (Table 2), suggesting that several chromosome rearrangements such as pericentric and paracentric inversions occurred in the karyotypic evolution of the group.

A general analysis of the Ag-NORs distribution shows that the species of the genera *Otocinclus*, *Otothyris*, *Schizolecis* and *Pseudotocinclus* ex-

hibited interstitial Ag-NORs while the species of *Corumbataia*, *Hypoptopoma* and the specimens of *Otocinclus vittatus* from Taquari River exhibited terminal Ag-NORs (Figs. 1 and 2). Interstitial Ag-NORs are very common among Hypoptopomatinae being also found in species of the genera *Hisonotus*, *Otocinclus*, *Parotocinclus*, *Pseudotocinclus* and *Pseudotothyris* studied by ANDREATA *et al.* (1992; 1993 and 1994). Considering that the sister group of Hypoptopomatinae are the genus *Kronichthys* and one species of *Hemipsilichthys* (ARMBRUSTER 2004), which also have interstitial Ag-NORs (ALVES *et al.* 2005), it is possible to conclude that interstitial Ag-NOR is a plesiomorphic characteristic for this subfamily. The terminal Ag-NORs found in the genera *Corumbataia* and *Hypoptopoma* and in the sample of *Otocinclus vittatus* from Taquari River are apomorphic characteristics originated independently since these genera and species are not closely related (SCHAEFER 1998). Additionally, the presence of two chromosome pairs with Ag-NORs in the sample of *Otocinclus vittatus* from Taquari River is also a characteristic found exclusively in this sample. Although changes in Ag-NOR position are very common between different genera and species of fishes (VÈNERE and GALETTI JR. 1989; ARTONI and BERTOLLO 1996; ALMEIDA-TOLEDO *et al.* 2000; ALVES *et al.* 2003; SHIMABUKURO-DIAS *et al.* 2004) the Ag-NOR distribution in Hypoptopomatinae may be useful in the species identification.

Several studies have discussed the role of the C-band positive segments in the differentiation process of fish species (GALETTI JR. and FORESTI 1986; GALETTI JR. *et al.* 1991; MARGARIDO and GALETTI JR. 2000; CAPUTO *et al.* 1997; ARTONI and BERTOLLO 1999; MANTOVANI *et al.*, 2000; SHIMABUKURO-DIAS *et al.*, 2004; among others); however, the authors have not come to any conclusion until the present moment. The analysis of the distribution of C-band positive segments in the species of the subfamily Hypoptopomatinae shows that species of this group, as observed in many other fish groups, have in general a very small amount of C-band positive segments. However, some particular characteristics may be very useful in the identification of gross karyotypic changes, as observed in *Hisonotus leucofrenatus* (cited as *Microlepidogaster leucofrenatus* by ANDREATA *et al.*, 1993), in the identification of XX/XY and ZZ/ZW sex chromosomes systems (ANDREATA *et al.* 1992; ANDREATA *et al.* 1993; ANDREATA *et al.* 1994). In the present study several chromosome markers were detected such as the Ag-NOR-bearing pair of both species of *Corumbataia* (Figs. 3a and 3b), the presence of a marker chromosome with two large C-band positive segments in both samples of *O. vittatus* (Figs. 3d and 3e), and the presence of large C-band positive segments in some chromosomes of *O. vittatus* from Cuiabá River (Fig. 3d) and *Pseudotocinclus n. sp.* (Fig. 4c).

Morphological studies (SCHAEFER 1998; ARMBRUSTER 2004) showed that the subfamily Hypoptopomatinae is a monophyletic group that, according to ARMBRUSTER (2004), is the sister group of the genera *Neoplecostomus*, *Kronichthys*, *Isbrueckerichthys*, *Pareiorhina* and *Hemipsilichthys*. The available cytogenetic data are in agreement with this hypothesis since studies conducted in these five genera showed that almost all species investigated exhibited $2n=54$, NF around 100, and one chromosome pair with interstitial Ag-NORs (ALVES *et al.* 2005) as found in Hypoptopomatinae.

The importance of chromosome changes in the evolution of both plants and animals have been exhaustively debated during the last 50 years and several researches have proposed interesting hypotheses about this subject (QUMSIYEH 1994; RIESENBERG 2001; LIVINGSTONE and RIESENBERG 2003; NAVARRO and BARTON 2003; among others). QUMSIYEH (1994) proposed that the selection for increases or decreases in genetic recombination, through the increase or decrease on the diploid and/or fundamental number, could explain some aspects of mammalian chromosome evolution. A

review of the chromosome evolution in salmonids, which are composed of species with very different diploid numbers, showed that the hypothesis of QUMSIYEH (1994) agreed with the chromosome data available for this group (PHILLIPS and RÁB 2001). An analysis of the cytogenetic data available for Hypoptopomatinae shows that almost all species present $2n=54$ and NF around 100 (Table 2) and thus changes in these parameters were probably not very important in the evolution of the group. Only one species presents a high diploid number, $2n=72$, but with $FN=106$ and only two species with $2n=54$ present a low fundamental number, $FN=76$ and $FN=82$ (Table 2). The lack of data about the lifestyle of particular species of Hypoptopomatinae hinders the discussion about the evolutionary importance of the chromosome rearrangements in the evolution of these three particular species. On the other hand, it is not possible to refute the hypothesis that Hypoptopomatinae species are very adapted to their habitats and thus gross changes in the karyotypes (mainly increasing $2n$ or FN) are negatively selected.

An additional aspect related to the chromosome evolution in Hypoptopomatinae is the occurrence of chromosome inversions in several species. Recent studies have pointed out that this kind of chromosome rearrangement may be very important in the karyotypic evolution and even in species differentiation (LIVINGSTONE and RIESENBERG 2003; NAVARRO and BARTON 2003). Thus, although changes on the numeric chromosome have not been frequently fixed in the evolution of Hypoptopomatinae, the structural rearrangements may have played important role in the speciation of this group.

The present study corroborates the hypothesis that a basal lineage of loricariids possibly presented $2n=54$ and this diploid number was only consistently changed in the genus *Hypostomus* (sensu ARMBRUSTER 2004). This fast chromosome evolution in *Hypostomus* may be followed by some event of polyploidy as suggested by FENERICH *et al.* (2004). Interestingly, other loricariid group with extensive chromosome diversification is the genus *Corydoras* that also experience some events of polyploidy (SHIMABUKURO-DIAS *et al.* 2004). Thus, although further studies with more primitive Loricariidae species of the subfamily Lithogeninae will be necessary for a better understanding of the chromosome evolution in the family, the data available suggest that small chromosome rearrangements as paracentric and pericentric inversions were very important in the karyo-

typic evolution of Neoplecostominae and Hypoptopomatinae as expected according to the model of chromosome speciation through rearrangements that alter the recombination rates.

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