

# Karyotypic diversity and evolution of Loricariidae (Pisces, Siluriformes)

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We present cytogenetic analyses of four fish species, belonging to four Loricariidae subfamilies: *Neoplecostomus microps* (Neoplecostominae) with  $2n=54$  chromosomes, *Harttia loricariformis* (Loricariinae) with  $2n=56$  chromosomes, *Hypostomus affinis* (Hypostominae) with  $2n=66$  chromosomes and *Upsilodus* sp. (Upsilodinae), with  $2n=96$  chromosomes. In addition to karyotypes, data on the location of 18s rDNA sites are presented, derived from indirect (silver nitrate impregnation) and direct (FISH) methods. There is

only one pair of nucleolar organizing regions (NORs) per species, except in *H. affinis*. Diversity and NOR macrokaryotypic evolution in the species analyzed are discussed in relation to the evolution of the Loricariidae as a whole. In addition, a revision of the cytogenetic data available for this family is presented.

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## Introduction

The Serra da Bocaina region shelters the springs of the Paraitinga river, one of the main affluents of the Paraíba do Sul river, which is one of the most significant and important rivers of the East Brazilian drainage system. This area has suffered intense tectonic phenomena, facilitating the formation of population isolates, which constitute good models for cytogenetical and evolutionary studies. In the ichthyofauna of the Paraitinga river springs, small loricariid fishes belonging to the genera *Neoplecostomus*, *Harttia*, *Hypostomus* and *Upsilodus* are found.

The family Loricariidae contains about 600 species distributed into 70 genera, and is probably one of the families with the greatest taxonomic complexity among the Siluriformes (Isbrücker, 1980). The phylogenetic relationships of the family Loricariidae are relatively well defined, despite the great morphological diversity observed (Pina, 1998). Schaefer (1987) hypothesized that loricariids can be grouped in six subfamilies (Lithogeninae, Neoplecostominae, Hypoptopomatinae, Loricariinae, Ancistrinae and Hypostominae), constituting a monophyletic group. However, through the study of morphological characters (osteology and external and gastrointestinal anatomy), Armbruster (1997) proposed two new subfamilies, Hemipsilichthiinae and Upsilodinae, the latter being considered as the most basal among the loricariids, with only one monotypic genus, *Upsilodus*.

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Considering the number of species in this family, cytogenetic analyses are still scarce (Alves, 2000; Artoni and Bertollo, 2001), and nonexistent in the subfamilies Upsilodinae and Lithogeninae. The data available indicate that the smallest diploid number is  $2n=36$  chromosomes for *Rineloricaria latirostris* (Giuliano-Caetano, 1998), and the highest is  $2n=80$  chromosomes in *Hypostomus* sp E (Artoni and Bertollo, 1996), showing the great numeric variability in this group. Additionally, there is also a general structural diversity, where members of the same nominal species may possess differentiated karyotypic formulae, as observed in *Rineloricaria latirostris* populations (Giuliano-Caetano, 1998). More detailed data on Loricariidae karyotypes are shown in Table 1.

Karyotypic variability analysis can be complemented by the chromosomal localization of specific genes, such as the nucleolar organizing regions (NORs). In fish, the location of the 45S rDNA (18S + 5.8S + 28S) is an important cytogenetic marker, with some groups having only one pair of NORs (Curimatidae, Anastomidae, Parodontidae, Prochilodontidae, Cichlidae), and others showing multiple NORs (Characidae, Lebiasinidae, Loricariidae, Erythrinidae and Callichthyidae), including one located on a sex chromosome (Bertollo and Cavallaro, 1992; Born and Bertollo, 2000; Artoni and Bertollo, 2002). NOR localization can be carried out directly with fluorescent *in situ* hybridization (FISH) with specific probes, or indirectly with the use of silver nitrate (Ag-NOR). The latter detects the transcriptional activity of the ribosomal genes during the preceding interphase (Howell, 1977; Hubbel, 1985), since the silver binds to the nucleolar proteins and not directly to the rDNA (Miller *et al.*, 1976).

The present work pursued the karyotypic characterization, in particular of the NORs of four Loricariidae

Table 1 Cytogenetic data compilation for the family Loricariidae

Subfamily	Species	Sample locality	2n	Bs	Chromosomal types				Ref.
					M	SM	ST	A	
Hypoptopomatinae	<i>Hisonotus gibbosos</i>	Betari River (SP)	58	—	—	—	—	—	1
	<i>Microlepidogaster depressicauda</i>	Santo Inácio River (SP)	54	—	14	28	2	10	2
	<i>Microlepidogaster leucomaculatus<sup>a</sup></i>	Poço Grande River (SP)	54	1 to 2	—	48	8	—	3
	<i>Microlepidogaster leucomaculatus<sup>a</sup></i>	Marumbi River (PR)	54	1 to 2	22	24	4	2	2
	<i>Microlepidogaster</i> sp 1	Alambari River (SP)	54	—	30	20	4	—	2
	<i>Microlepidogaster</i> sp 2	Moia Stream (SP)	54	—	22	28	4	—	2
	<i>Microlepidogaster</i> sp	Jacutinga Stream (SP)	54	—	—	—	—	—	4
	<i>Microlepidogaster</i> sp	Quinta Stream (SP)	54	—	—	—	—	—	5
	<i>Otocinclus</i> aff. <i>vestitus</i>	Livramento River (PA)	72	—	22	12	4	34	2
	<i>Otocinclus</i> affinis	Biguá River (SP)	54	—	46	8	—	—	2
	<i>Otocinclus</i> affinis	Bonito River (RJ)	54	—	40	12	2	—	2
	<i>Pseudocinclus maculicauda</i>	Poço Grande River (SP)	54	—	20	32	2	—	6
	<i>Pseudocinclus tietensis<sup>b</sup></i>	Grande River (SP)	54	—	26	20	6	—	7
	<i>Pseudotothiris obtusa</i>	Itanhaém River (SP)	54	—	26	18	4	6	2
Loricariinae	<i>Harttia kronei</i>	Betari River (SP)	58	—	42	—	16	—	8
	<i>Harttia loricariformis</i>	Grande Stream (SP)	52	—	32	—	20	—	8
	<i>Harttia loricariformis</i>	Paraitinga River (SP)	56	—	16	22	10	8	25
	<i>Loricaria</i> sp	Solimões River (AM)	62	—	—	—	—	—	9
	<i>Loricaria</i> sp	Paraná River (PR)	64	1 to 5	10	6	4	44	10
	<i>Loricaria</i> sp	Guaíba River (RS)	66	—	2	2	—	62	8
Loricariinae	<i>Loricaria macrodon</i>	—	58	—	18	2	—	38	11
	<i>Loricaria parva</i>	—	48	—	—	—	—	—	12
	<i>Loricaria prolixa</i>	Paraná River (PR)	62	1 to 5	20	4	—	38	10
	<i>Loricariichthys</i> sp	Paraná River (ARG)	54	—	6	26	4	18	13
	<i>Loricariichthys platymetopon<sup>a</sup></i>	Paraná River (PR)	54	—	6	20	4	24	14, 22
	<i>Rineloricaria</i> sp	Betari River (SP)	70	—	2	—	68	—	8
	<i>Rineloricaria kronei</i>	Cavallo Stream (RS)	64	—	6	—	58	—	8
	<i>Rineloricaria kronei</i>	Itapocu River (SC)	64	—	6	—	58	—	8
	<i>Rineloricaria latirostris</i>	Passa Cinco River (SP)	44	—	12	4	—	28	15
	<i>Rineloricaria latirostris</i>	—	44	—	10	4	—	30	15
Loricariinae	<i>Rineloricaria latirostris</i>	—	44	—	13	2	—	29	15
	<i>Rineloricaria latirostris</i>	—	44	—	13	4	—	27	15
	<i>Rineloricaria latirostris</i>	—	44	—	13	1	—	30	15
	<i>Rineloricaria latirostris</i>	—	44	—	10	4	—	30	15
	<i>Rineloricaria latirostris</i>	Passa Cinco River (SP)	46	—	10	3	—	31	15
	<i>Rineloricaria latirostris</i>	Mogi-Guaçu River (SP)	36	—	24	—	12	—	15
	<i>Rineloricaria latirostris</i>	—	37	—	23	—	14	—	15
	<i>Rineloricaria latirostris</i>	—	38	—	22	—	16	—	15
	<i>Rineloricaria latirostris</i>	—	39	—	21	—	18	—	15
	<i>Rineloricaria latirostris</i>	—	40	—	20	—	20	—	15
Loricariinae	<i>Rineloricaria latirostris</i>	Três Bocas Stream (PR)	43	—	17	—	26	—	15
	<i>Rineloricaria latirostris</i>	—	44	—	16	—	28	—	15
	<i>Rineloricaria latirostris</i>	—	46	—	14	—	32	—	15
	<i>Rineloricaria latirostris</i>	—	47	—	13	—	34	—	15
	<i>Rineloricaria latirostris</i>	—	48	—	12	—	36	—	15
Ancistrinae	<i>Rineloricaria latirostris</i>	Passa Cinco River (SP)	44	—	16	—	28	—	15
	<i>Rineloricaria latirostris</i>	—	45	—	15	—	30	—	15
	<i>Rineloricaria latirostris</i>	—	46	—	14	—	32	—	15
	<i>Rineloricaria latirostris</i>	—	47	—	13	—	34	—	15
	<i>Rineloricaria pentamaculata</i>	Keller Stream (PR)	56	—	8	—	48	—	15, 16
	<i>Sturisoma</i> cf. <i>nigrirostrum</i>	Araguaia River (MT)	74	—	20	18	—	36	20
	<i>Ancistrus</i> sp	Paraná River (PR)	48	—	18	14	12	4	17
	<i>Ancistrus</i> sp	Betari River (SP)	52	—	—	32	—	20	8
	<i>Ancistrus</i> sp	Itapocu River (SC)	52	—	—	28	—	24	8
	<i>Ancistrus</i> sp	São Francisco Stream (AC)	38	—	30	—	8	—	8
Uspilodinae	<i>Hemiancistrus</i> sp	Rio Araguaia (MT)	52	—	20	20	—	12	20
	<i>Megalancistrus aculeatus</i>	Rio Paraná (PR)	52	—	26	26	—	—	17
	<i>Panaque</i> cf. <i>nigrolineatus</i>	Rio Araguaia (MT)	52	—	26	20	6	—	20
	<i>Upsilodus</i> sp	Paraitinga River (SP)	96	—	16	8	—	72	25
	<i>Hypostomus</i> aff. <i>affinis</i>	Jacuí Stream (SP)	66	—	14	14	12	26	25
	<i>Hypostomus</i> aff. <i>affinis</i>	Mogi-Guaçu River (SP)	74	—	10	20	—	44	18
	<i>Hypostomus</i> aff. <i>affinis</i>	Monjolinho Stream (SP)	68	—	16	18	—	34	18
	<i>Hypostomus</i> aff. <i>affinis</i>	Mogi-Guaçu River (SP)	76	—	8	30	—	38	18
	<i>Hypostomus</i> emarginatus	Araguaia River (MT)	52	—	16	30	6	—	20
	<i>Hypostomus</i> macrops <sup>b</sup>	—	68	—	10	14	—	44	11
Hypostominae	<i>Hypostomus paulinus</i>	—	74	—	10	20	—	44	11
	<i>Hypostomus plecostomus</i>	—	54	—	—	24	12	18	19

Table 1 Continued

Subfamily	Species	Sample locality	2n	Bs	Chromosomal types				Ref.
					M	SM	ST	A	
Hypostominae	<i>Hypostomus regani</i>	Mogi-Guaçu River (SP)	72	—	10	20	42	18	
	<i>Hypostomus</i> sp A	Rincão Stream (SP)	70	—	18	14	38	18	
	<i>Hypostomus</i> sp B	Mogi-Guaçu River (SP)	72	—	12	18	42	18	
	<i>Hypostomus</i> sp B	Mogi-Guaçu River (SP)	72	—	13	18	41	21	
	<i>Hypostomus</i> sp D <sup>1</sup>	Mogi-Guaçu River (SP)	72	—	10	26	36	18	
	<i>Hypostomus</i> sp D <sup>2</sup>	Mogi-Guaçu River (SP)	72	—	14	20	38	18	
	<i>Hypostomus</i> sp E	Mogi-Guaçu River (SP)	80	—	8	16	56	18	
	<i>Hypostomus</i> sp F	São Francisco River (MG)	76	—	10	16	50	17	
	<i>Hypostomus</i> sp F	São Francisco River (MG)	75	—	10	17	48	21	
	<i>Hypostomus</i> sp G <sup>a</sup>	Araguaia River (MT)	64	—	14	24	26	24	
			64	—	15	24	25	24	
	<i>Hypostomus</i> sp 1	Quinta Stream (SP)	72	—	—	—	—	—	5
Neoplecostominae	<i>Hypostomus</i> sp 2	Alambari Stream (SP)	68	—	—	—	—	—	5
	<i>Hypostomus</i> sp 3	Paranapanema River (SP)	66	—	—	—	—	—	5
	<i>Hypostomus</i> sp 4	Hortelã Stream (SP)	76	—	—	—	—	—	5
	<i>Liposarcus anisitsi</i>	Preto River (SP)	52	—	16	24	8	4	23
	<i>Liposarcus</i> sp	Tietê River (SP)	52	—	—	—	—	—	5
	<i>Rhinelepis aspera</i>	Paraná River (PR)	54	—	20	26	8	—	20
	<i>Pogonopoma wertheimeri</i>	Macuri River (BA)	54	—	20	30	4	—	20
	<i>Pterygoplichthys multiradiatus</i>	Solimões River (AM)	52	—	—	—	—	—	9
	<i>Neoplecostomus microps</i>	Grande Stream (SP)	54	—	42	—	12	8	
	<i>Neoplecostomus microps</i>	Pindamonhangaba City							
Hemipsilichthiinae	<i>Neoplecostomus microps</i>	Grande Stream (SP)	54	—	42	—	12	8	
	<i>Neoplecostomus microps</i>	Campos do Jordão City							
	<i>Neoplecostomus microps</i>	Paraitinga River (SP)	54	—	24	20	10	—	25
	<i>Neoplecostomus paranensis</i>	Hortelã Stream (SP)	54	—	36	—	18	8	
	<i>Isbrueckerichthys alipionis</i>	Betari River (SP)	54	—	38	—	16	8	
	<i>Kronichthys heylandi</i>	Betari River (SP)	54	—	50	—	4	8	
	<i>Hemipsilichthys splendens</i>	São João River (SC)	54	—	50	—	4	8	
	<i>Hemipsilichthys steindachneri</i>	Cavalo Stream (SC)	54	—	40	—	14	8	
	<i>Pareiorhina rudolphi</i>	Grande Stream (SP)	54	—	48	—	6	8	

<sup>a</sup>Occurrence of a ZZ/ZW sex chromosome system reported. <sup>b</sup>Occurrence of an XX/XY sex chromosome system reported. Bs = presence of B chromosomes; M = metacentric; SM = submetacentric; ST = subtelocentric; A = acrocentric; Ref. = reference. AC = Acre; AM = Amazonas; BA = Bahia; MG = Minas Gerais; MT = Mato Grosso; PA = Pará; PR = Paraná; RJ = Rio de Janeiro; RS = Rio Grande do Sul; SC = Santa Catarina; SP = São Paulo – Brazilian States; ARG = Argentina; 1 – Andreata *et al* (2000); 2 – Andreata *et al* (1994); 3 – Andreata *et al* (1993); 4 – Carvalho *et al* (1998); 5 – Fenerich (1998); 6 – Andreata (1991); 7 – Andreata *et al* (1992); 8 – Alves (2000); 9 – Della-Rosa *et al* (1980); 10 – Scavone and Júlio Jr (1994); 11 – Michelle *et al* (1977); 12 – Gyldenholm and Scheel (1971); 13 – Fenocchio (1993); 14 – Scavone (1993); 15 – Giuliano-Caetano (1998); 16 – Giuliano-Caetano *et al* (1999); 17 – Artoni (1996); 18 – Artoni and Bertollo (1996); 19 – Muramoto *et al* (1968); 20 – Artoni and Bertollo (2001); 21 – Artoni and Bertollo (1999); 22 – Scavone and Júlio Jr (1995); 23 – Artoni *et al* (1999); 24 – Artoni *et al*, 1998; 25 – Present paper.

species sampled from the Paraitinga river, with a discussion of probable events related to the karyotypic evolution of these fishes, in relation to their biological and cytogenetic characteristics.

## Materials and methods

In all, 18 *Neoplecostomus microps* specimens (nine males and nine females), 22 *Harttia loricariformis* (eight males and 14 females) and three *Upsilodus* sp specimens (one male and two females) were collected from the Paraitinga river (S 22°52.225'/Wo 44°51.041'), and two *Hypostomus affinis* specimens (males) from the Jacuí stream (S 23°02.436'/Wo 44°56.103'), all belonging to the Paraíba do Sul river basin, São Paulo State (SP), Brazil. The samples were identified by the National Museum of Rio de Janeiro (Brazil), where they appear in the ichthyological collection under the OMNRJ REG 20020417 registration.

The mitotic chromosomes were obtained according to Bertollo *et al* (1978). The Ag-NOR impregnation followed the methodology of Howell and Black (1980). The

localization of ribosomal cistrons was performed according to Pinkel *et al* (1986), by the use of FISH with a specific 18S rDNA probe (kindly donated by Dr Terumi Hatanaka and Dr Pedro M Galetti Jr). The 18S rDNA probe was marked with biotinylated uridine (BdUTP) according to the protocol of the Nick Translation Bionik Labeling System Kit, Invitrogen®. The signal amplification was performed using an FITC-avidin solution and a biotin-conjugated anti-avidin solution. The slides were mounted with 25 µl Vectashield Mounting Medium antifade, Vector® with propidium iodide (1.5 µg/ml). The classification of the chromosomal types into metacentric, submetacentric, subtelocentric and acrocentric was based in their the arm ratios according to Levan *et al* (1964).

## Results

A total of 534 metaphasic cells were analyzed for the four species, as follows: *N. microps*: 191; *H. loricariformis*: 116; *H. affinis*: 148 and *Upsilodus* sp: 79.

*N. microps* showed  $2n=54$  chromosomes, a  $24M+20SM+10ST$  karyotypic formula and a fundamental number (FN) of 108 (Figure 1a). Only one NOR site was found using Ag-NOR and FISH (Figures 1a' and 2a, respectively), in an interstitial position in the long arm of the large submetacentric chromosome pair 14.

*H. loricariformis* showed  $2n=56$  chromosomes, a  $16M+22SM+10ST+8A$  karyotypic formula and a FN of 106 (Figure 1b). The NOR is also of the simple type (Figures 1b' and 2b), located in the terminal long-arm region of the large acrocentric chromosome pair 25.

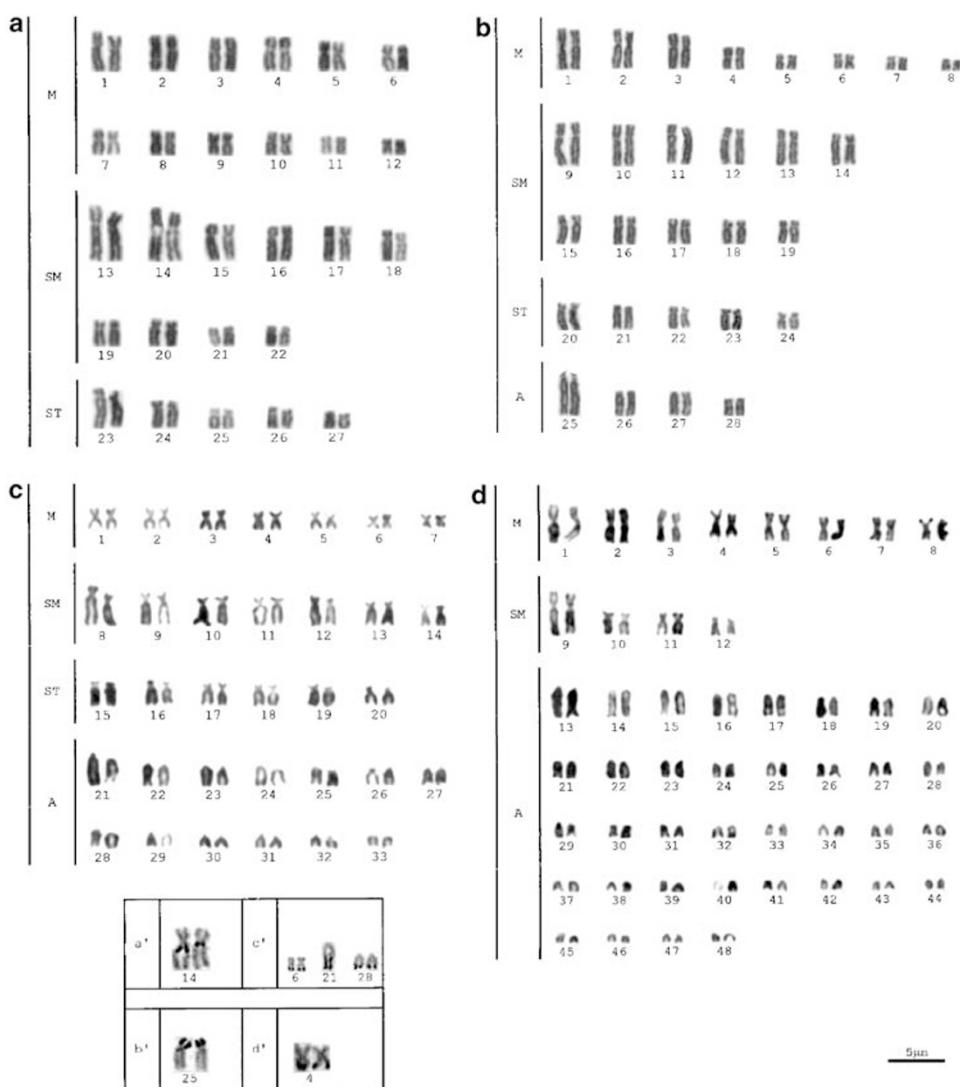
*H. affinis* showed a diploid number of  $2n=66$  chromosomes, with a  $14M+14SM+12ST+26A$  karyotypic formula and an FN of 106 (Figure 1c). Ag-NOR showed two to five NOR-bearing chromosomes (Figure 1c'), with a mode of 4; by *in situ* hybridization five 18S rDNA sites were observed (Figure 2c), which corresponds to the maximum number of Ag-NORs obtained. They occur in the terminal region of the long arm of a metacentric and an acrocentric chromosome pair of medium size, in addition to the large acrocentric pair

21, which presented a consistent size heteromorphism in the two males studied (Figure 1c).

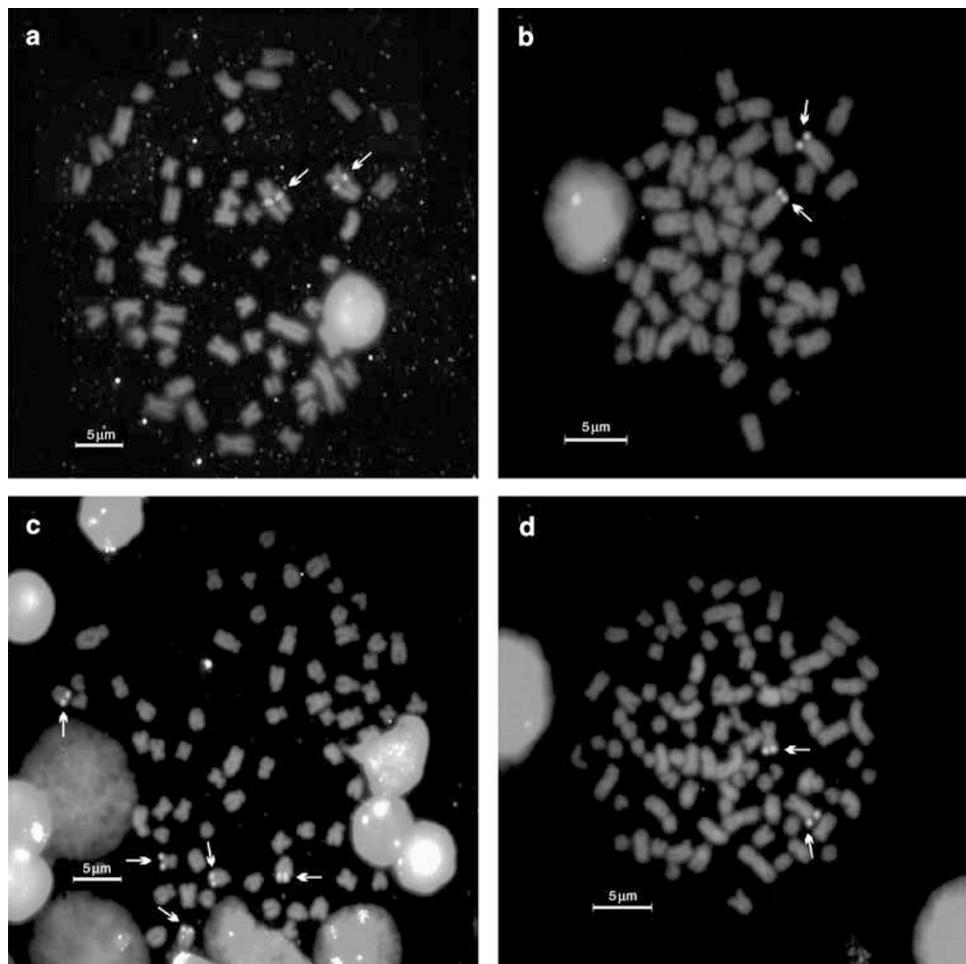
*Upsilodus* sp possesses the highest diploid number ever observed in loricariids,  $2n=96$  chromosomes, with a  $16SM+8SM+72A$  karyotypic formula and an FN of 120 (Figure 1d). The Ag-NOR staining and 18S-FISH indicate the presence of a single NOR, located in the medium-sized metacentric chromosome pair 4, in the terminal region of the long arm (Figures 1d' and 2d, respectively).

## Discussion

Nearly 70 Loricariidae species have already been submitted to some cytogenetic study (Table 1). The diploid number of 54 chromosomes seems to be a plesiomorphic condition in this family. In Neoplecostominae and Hemipsilichthiinae, all the populations studied (four and five, respectively) have 54 chromosomes. Among 14 Hypoptopomatinae species, 12 possess this diploid number. For this reason, the subfamilies Neoplecostominae, Hemipsilichthiinae and Hypoptopomatinae are



**Figure 1** Giemsa-stained karyotypes of (a) *N. microps*; (b) *H. loricariformis*; (c) *H. affinis* and (d) *Upsilodus* sp. In detail, Ag-NORs of (a') *N. microps*; (b') *H. loricariformis*; (c') *H. affinis*; and (d') *Upsilodus* sp.



**Figure 2** Fluorescent *in situ* hybridization with 18S rDNA probe. The arrows indicate the sites in (a) *N. microps*; (b) *H. loricariformis*; (c) *H. affinis* and (d) *Upsilodus* sp.

considered basal, since they present a predominance of species with  $2n=54$  chromosomes (Alves, 2000). The karyotypes in these three subfamilies are made up of many biarmed chromosomes and, consequently, show high FNs.

It is interesting to observe that there are many acrocentric chromosomes (Andreata *et al.*, 1994) in *Otocinclus vestitus*, which possesses the highest diploid number among the Hypoptopomatinae ( $2n=72$ ). This seems to be one of the rare cases of karyotypic evolution by means of centric fissions in this subfamily, while the other species maintain the  $2n=54$  diploid number, and here rearrangements such as pericentric inversions seem to have a predominant role in the process of evolutionary divergence. Among the Neoplecostominae and Hemipsilichthiinae, this trend can also be observed in their karyotypic formulae (Table 1). *N. microps*, analyzed here, maintains this tendency with minor differentiations in the karyotype (Figure 1a).

Karyotypic evolution by means of centric fusions and centric fissions is, however, a common feature among other loricariids. This is seen in the subfamily Hypostominae, in particular the genus *Hypostomus*, where species with a lower diploid number have a higher number of biarmed chromosomes, and, in contrast, species with a higher diploid number have many uniaxed chromo-

somes (Artoni and Bertollo, 1996, 2001). Considering  $2n=54$  chromosomes as the ancestral condition for loricariids, Artoni and Bertollo (2001) state that this second group represents more derived karyotypic forms. In addition to their very variable diploid numbers and chromosomal types, the genus *Hypostomus* also shows karyotypic differentiation within species, as is observed among *H. affinis* populations (Carneiro *et al.*, 1998 and the present study), where the diploid number is maintained but the chromosomal formula is variable (Figure 1c). The available data for the genera *Hypostomus*, *Lioposarcus*, *Rhinelepis*, *Pognopoma* and *Pterygoplichthys* (Table 1) indicate that Robertsonian rearrangements, as well as pericentric inversions, were the main rearrangements related with the karyotypic diversification of the Hypostominae. On the other hand, this chromosomal diversity may also corroborate a probable polyphyletic origin of the Hypostominae species, as proposed by Schaefer (1987) on the basis of morphological studies.

Data on chromosomal evolution in the subfamily Ancistrinae are still very scarce, since this is a little-studied group. The diploid number comprises  $50 \pm 2$  chromosomes, with a predominance of  $2n=52$  and the constant presence of meta-, sub-meta, sub-telo and acrocentric chromosomes (Table 1). *Ancistrus* sp shows only  $2n=38$  chromosomes (Alves, 2000) and Robertsonian

rearrangements appear to have raised the number of metacentric chromosomes and lowered the diploid number.

In the subfamily Loricariinae, both centric fissions, centric fusions and pericentric inversions emerge again as common karyotypic rearrangements. In *Rineloricaria latirostris*, for example, a Robertsonian polymorphism was observed, with a variation of  $2n=36$  to  $2n=48$  chromosomes (Giuliano-Caetano, 1998). The great variety of diploid numbers among species of Loricariinae, in addition to the small number of species analyzed, makes it difficult to discern an evolutionary trend in this group. There are, however, a dozen species in this subfamily that have  $2n=54\pm 2$  chromosomes, pointing to the same karyotypic trend observed in Hypoptopomatinae, Neoplecostominae and Hemipsilichthiinae.

*H. loricariformis*, the Loricariinae representative in the present study, showed a karyotypic difference from the other population of the same species from the Grande stream (Alves, 2000), also belonging to the Paraíba do Sul basin. This differentiation includes the diploid number ( $2n=52$  vs  $2n=56$  in the present work), the karyotypic formula, symmetry (since the karyotype of the population analyzed here shows itself more asymmetrical) and NOR localization. *H. loricariformis* from the Paraitinga river (Figure 1b) has a karyotype more similar to *H. kronei* (Table 1), showing a greater amount of biarmed chromosomes than *H. loricariformis* from the Grande stream. Thus, pericentric inversions seem to have played an important role in this genus, modifying its chromosome types. In this way, species with a certain 'karyotypic plasticity' are found among the Loricariidae. The sedentary habit of some species may contribute to these differentiations, as isolated populations are formed.

*Upsilodinae* was recently considered as the most basal Loricariidae subfamily (Armbruster, 1997), possessing only one species, *Upsilodus victori*. However, Lima (1997) considers that specimens derived from the Paraibuna river (São Paulo State, Brazil) may constitute another species, due to marked differences in morphological characters. These differences were also observed in the four specimens analyzed in the present study from the Paraitinga river, where an adult male showed a conspicuous sex dimorphism. *Upsilodus* sp has the highest diploid number among Loricariidae ( $2n=96$ ), with a karyotype made up of many acrocentric chromosomes (Figure 2d), again indicating centric fission events.

The NORs in loricariids show varied phenotypes. A trend for the maintenance of the plesiomorphic condition is observed, that is, a single NOR pair located at a terminal position on the chromosomes (Oliveira and Gosztonyi, 2000). Artoni (1996) proposed that the ancestral NOR phenotype for Loricariidae is a terminal site on the long arm of a large metacentric chromosome, since this feature is found both in Hypostominae and Hypoptopomatinae. Nonetheless, there are groups that also have multiple NOR sites, as in many Hypostominae species (Artoni and Bertollo, 2001).

Despite its specificity in the diploid number, *Upsilodus* sp maintains a single NOR pair in a terminal position on a large metacentric pair (Figures 1d' and 2d). The occurrence of this NOR phenotype in *Upsilodus* sp corroborates Artoni's (1996) proposition. So, besides basal chromosome features, *Upsilodus* sp shows an

autapomorphy in relation to its elevated diploid number, with many acrocentric chromosomes.

Other Loricariidae fishes with high diploid numbers, such as several Hypostominae species (Table 1), show variable NOR phenotypes (Artoni and Bertollo, 2001), as was also observed in *H. affinis* in the present study, which possesses five rDNA sites (Figures 1c' and 2c). The absence of a site in one homologue of the first acrocentric pair (pair 21) could be due to unequal crossovers, which would explain the size heteromorphism of this chromosome pair (Figure 1c). No homozygosity was detected for this site, possibly due to the sample size analyzed.

It is estimated that nearly 25% of the Loricariidae species show interstitial NORs (Alves, 2000). *N. microps* has this NOR state (Figures 1a' and 2a), also observed in other Neoplecostominae species. In addition, Hypoptopomatinae and Hemipsilichthiinae also show species with interstitial NORs. Therefore, the event that produced this phenotype may have occurred in a common ancestor for these three subfamilies, and, if so, would constitute a synapomorphic feature. However, among Hypoptopomatinae, only about 43% of the species have this phenotype (Alves, 2000), the remainder showing terminal NORs, which impairs a precise evolutionary interpretation for this character, without ruling out the occurrence of homoplasies.

In Loricariinae, the most frequent NOR phenotype is also a terminal location, as observed in *H. loricariformis* (Figures 1b' and 2b). The remaining *Harttia* species analyzed, however, show interstitial NORs (Alves, 2000), indicating a nonshared synapomorphy with *H. loricariformis*.

In conclusion, the data available indicate different evolutionary pathways within subfamilies as well as between different Loricariidae subfamilies. Some chromosomal features, such as diploid number and NOR phenotypes, may in fact be shared by different groups, indicating a common ancestry, or may be seen as derived and specific characters. The same occurs with heteromorphic sex chromosomes in this family. Male and female heterogamety, such as the XX/XY and ZZ/ZW systems, have already been identified for some Loricariidae (Michelle et al, 1977; Andreata et al, 1992, 1993, 1994; Scavone and Júlio Jr, 1995; Artoni et al, 1998), but represent isolated events in this group.

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