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Karyotype variability in eight species of the subfamilies Loricariinae and Ancistrinae (Teleostei, Siluriformes, Loricariidae)

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Abstract - Loricariidae is one of the largest fish families of the world, with about 650 species separated into six subfamilies. To date, cytogenetic data on only 56 species of this family are available. In the present study, the karyotypes of three Ancistrinae species and five Loricariinae species were studied. The lowest diploid number, 2*n*=38, was observed in *Ancistrus* n.sp. 1 (Ancistrinae) and the highest diploid number, 2*n*=70, was observed in *Rineloricaria* n.sp. (Loricariinae). The nucleolar organizer regions (NORs) were seen at a terminal position in six species and at an interstitial position in two. The karyotypic analysis of Loricariinae and Ancistrinae species revealed that these groups exhibit a large diversity of diploid numbers, suggesting the occurrence of intense karyotypic evolution during their evolutionary history.

Key words: Chromosome, karyotypic variability, NOR banding, Loricariidae, Siluriformes.

INTRODUCTION

The family Loricariidae, one of the largest fish families of the world, has about 650 species distributed in the Neotropical region (ISBRÜCKER 1980; NELSON 1994; FERRARIS 1998). The family is divided into six subfamilies: Hypostominae, Ancistrinae, Loricariinae, Hypoptopomatinae, Neoplecostominae, and Lithogeninae (SCHAEFER 1987) and in spite of numerous studies recently developed (ARMBRUSTER *et al*. 2000; ARMBRUSTER 2002); the identification of many species groups is still a very difficult task, which show that further studies, taxonomic reviews and species descriptions are necessary.

Cytogenetic studies were conducted in only about 56 species of Loricariidae (OLIVEIRA and GOSZTONYI 2000). In this family, diploid numbers range from 2*n*=36 in *Rineloricaria latirostris* (GIULIANO-CAETANO 1998) to 2*n*=80 in *Hypostomus* sp. (ARTONI and BERTOLLO 1996). Most of the cytogenetic studies so far conducted focused on species of the subfamily Hypostominae which exhibits the greatest diversity of diploid numbers, from 2*n*=52 to 2*n*=80 chromosomes (ALVES 2000). Among the karyotyped Ancistrinae species, diploid numbers range from 2*n*=48 to 2*n*=52 (Table 1) and, in Loricariinae, they range from 2*n*=36 to 2*n*=74 (Table 1). In contrast to these subfamilies that show a great variation in diploid number and karyotype, fish of the subfamilies Hypoptopomatinae and Neoplecostominae are characterized by the presence of 2*n*=54 chromosomes in almost all species (ALVES 2000). In the present study, the karyotypes of three Ancistrinae species and five Loricariinae species were investigated with the main purpose of characterizing these species and analyzing the chromosomal relationships between these subfamilies.

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MATERIAL AND METHODS

Cytogenetic analyses were performed on chromosome preparations from three Ancistrinae species and five Loricariinae species, collected from streams of five Brazilian hydrographic basins (Table 1). The specimens were studied by taxonomists that provided the species identification, when possible. Two species of *Ancistrus* (here identified as *Ancistrus* n.sp. 1 and *Ancistrus* n.sp.2) and one species of *Rineloricaria* (here identified as *Rineloricaria* n.sp) were not named because they are new to science. The description of these species will be done elsewhere. The fish were deposited in the fish collection of Laboratório de Biologia de Peixes (LBP), UNESP, Botucatu, São Paulo, Brazil or in the Laboratório de Ictiologia, Museu de Ciências e Tecnologia, PUCRS (MCP), Porto Alegre, Brazil.

Chromosome preparations were obtained from gill and kidney tissues using the technique described by FORESTI *et al.* (1993). Silver-staining of the nucleolar organizer regions was performed according to the technique proposed by HOWELL and BLACK (1980). 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).

RESULTS

Our results showed that *Ancistrus* n.sp. 1 exhibited $2n$=38 chromosomes (30 M/SM, 8 ST) and one metacentric pair with interstitial NORs in the short arms (Fig. 1a). *Ancistrus* n.sp. 2 displayed $2n$=52 chromosomes (32 M/SM, 20 ST/A) and one subtelocentric pair with terminal NORs in the short arms (Fig. 1b). *Ancistrus multispinnis* had $2n$=52 chromosomes (28 M/SM, 24 ST/A) and one subtelocentric pair with terminal NORs in the short arms (Fig. 1c).

Among Loricariinae species, *Harttia loricariformis* had $2n$=52 chromosomes (32 M/SM, 20 ST/A) and one acrocentric pair with interstitial NORs in the long arms (Fig. 2a); *Harttia kronei* had $2n$=58 chromosomes (40 M/SM, 18 ST/A)

Table 1 – A summary of the cytogenetic data available on the subfamilies Loricariinae and Ancistrinae.

Species	Locality	$2n$	Karyotypic formulae	Reference
Ancistrinae				
Ancistrus n.sp. 1	São Francisco river, Acre, Brazil	38	30M/SM, 8ST	Present study
Ancistrus sp.	Iguaçú river, Paraná, Brazil	48	18M, 14SM, 12ST, 4A	LARA and JÚLIO JR. (1994)
Ancistrus sp.	Alto Alegre river, Paraná, Brazil	50	12M, 14SM, 14T, 10A	TCHAICKA and MARGARIDO (1999)
Ancistrus n.sp. 2	Betari river, São Paulo, Brazil	52	32M/SM, 20ST/A	Present study
Ancistrus multispinnis	Itapocu river, Santa Catarina, Brazil	52	28M/SM, 24ST/A	Present study
Megalancistrus aculeatus	Paraná river, Paraná, Brazil	52	26M, 26SM	LARA and JÚLIO JR. (1994)
Hemiancistrus sp.	Araguaia river, Mato Grosso, Brazil	52	20M, 20SM, 8ST, 4A	ARTONI (1996)
Panaque cf. *nigrolineatus*	Araguaia river, Mato Grosso, Brazil	52	26M, 20SM, 6ST	ARTONI (1996)
Loricariinae				
Harttia sp.	Itabapoana river, Minas Gerais, Brazil	56	14SM, 42A	CARNEIRO *et al.* (1998)
Harttia kronei	Betari river, São Paulo, Brazil	58	40M/SM, 18ST	Present study
Harttia loricariformis	Grande river, São Paulo, Brazil	52	32M/SM, 20ST/A	Present study
Loricaria carinata	Paraná river, Argentina	64	-	RONCATI *et al.* (1999)
Loricaria macrodon	-	58	18M, 2SM, 38A	MICHELLE *et al.* (1977)
Loricaria parva	-	48	-	GYLDENHOLM and SCHEEL (1971)
Loricaria prolixa	Paraná river, Paraná, Brazil	62	20M, 4SM, 38A	SCAVONE and JÚLIO JR. (1995)
Loricaria sp.	Paraná river, Paraná, Brazil	64	10M, 6SM, 4ST, 44A	SCAVONE and JÚLIO JR. (1995)
Loricaria sp.	Solimões river, Amazonas, Brazil	62	-	DELLA-ROSA *et al.* (1980)
Loricaria sp.	Jari river, Pará, Brazil	52	-	OLIVEIRA *et al.* (1998)
Loricariichthys sp.	Paraná river, Argentina	54	6M, 26SM, 4ST, 18A	FENOCCHIO (1993)
Loricariichthys sp.	Itabapoana river, Minas Gerais, Brazil	54	28M, 26A	CARNEIRO *et al.* (1998)
L. platymetopom	Paraná river, Paraná, Brazil	54	7M, 20SM, 4ST, 23A	SCAVONE (1993)
L. platymetopom	Paraná river, Argentina	54	-	RONCATI *et al.* (1999)
L. maculata	Paraná river, Argentina	56	-	RONCATI *et al.* (1999)
Rineloricaria latirostris	Passa-Cinco river, São Paulo, Brazil	44-47	-	GIULIANO-CAETANO (1998)
R. latirostris	Mogi-Guaçu river, São Paulo, Brazil	36-40	-	GIULIANO-CAETANO (1998)
R. latirostris	Três Bocas river, Paraná, Brazil	43-48	-	GIULIANO-CAETANO (1998)
R. pentamaculata	Keller river, Paraná, Brazil	56	8M/SM, 48ST/A	GIULIANO-CAETANO *et al.* (1999)
R. kronei	Itapocu river, Santa Catarina, Brazil	64	6M/SM, 58ST/A	Present study
R. cadeae	Guaíba river, Rio Grande do Sul, Brazil	66	2M, 64ST/A	Present study
Rineloricaria n.sp.	Betari river, São Paulo, Brazil	70	2SM, 68A	Present study
Sturisoma cf. *nigrirostrum*	Araguaia river, Mato Grosso, Brazil	74	20M, 18SM, 18ST/A	ARTONI (1996)

and one submetacentric pair with terminal NORs in the short arms (Fig. 2b); *Rineloricaria kronei* had 2*n*=64 (6 M/SM, 58 ST/A) and one subtelocentric pair with terminal NORs in the short arms presenting a large size polymorphism (Fig. 3a); *Rineloricaria cadeae* had 2*n*=66 chromosomes (2 M, 64 ST/A) and one subtelocentric pair with terminal NORs in the short arms (Fig. 3b); and *Rineloricaria* n.sp. had 2*n*=70 chromosomes (2 SM, 68 A) and one submetacentric pair with terminal NORs in the short arms (Fig. 3c).

DISCUSSION

The subfamily Ancistrinae is believed to be a monophyletic group (SCHAEFER 1987; DE PINNA 1998). Although this subfamily has about 190 species (ISBRÜCKER 1980), it is the Loricariidae subfamily that has the lowest number of species karyotyped (Table 1). The results obtained in the present study and those described in the literature show that 2*n*=52 chromosomes is a common characteristic in the group, being present in two species of *Ancistrus* and in three species of other genera (Table 1). Among the Ancistrinae species analyzed, most chromosomes are metacentric and submetacentric, as observed in most Loricariidae species (ARTONI and BERTOLLO 1996; ALVES 2000).

The occurrence of a single chromosome pair with terminal NORs, observed in the majority of the Ancistrinae species, is also a common characteristic in Loricariidae (ARTONI and BERTOLLO 1996; ALVES 2000). In the present study, the species *Ancistrus* n.sp. 1 showed the most differentiated karyotype as it presents one of the lowest diploid number found in the family Loricariidae (2*n*=38) and interstitial NORs. To our knowledge, this is the first time that this characteristic is described in members of the subfamily Ancistrinae.

In Ancistrinae species, pericentric and paracentric inversions were probably the main chromosome rearrangements involved in the process of karyotypic evolution. This resulted in the different karyotypic formulae found in the different species. Moreover, centric fusions might have occurred and thus be responsible for the reduced

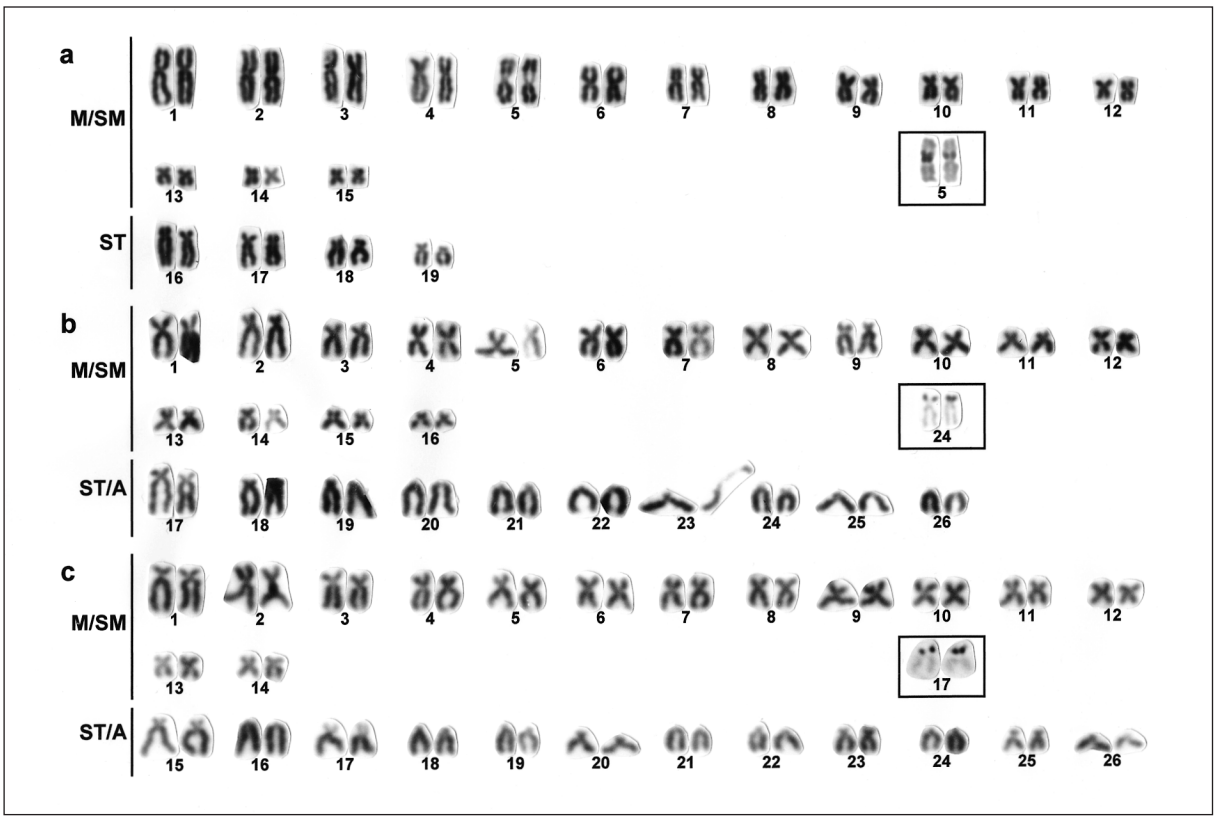

Fig. 1 – Giemsa stained karyotypes of fish from the subfamily Ancistrinae. (a) *Ancistrus* n.sp. 1 with 2*n*=38 chromosomes; (b) *Ancistrus* n.sp. 2 with 2*n*=52 chromosomes; (c) *Ancistrus multispinnis* with 2*n*=52 chromosomes. In the insets, the NOR-bearing pairs.

diploid number found in some species such as *Ancistrus* n.sp. 1 (2*n*=38, present study) and *Ancistrus* sp. (2*n*=48, LARA and JÚLIO JR. 1994) (Table 1). Therefore, the results obtained suggest that the species of the subfamily Ancistrinae had a divergent karyotypic evolution.

In spite of recent taxonomic studies in the subfamily Ancistrinae (e.g. ARMBRUSTER 2002), many taxonomic problems are still present, which make species identification a very difficult task. Thus, further studies are necessary for a better understanding of the karyotypic diversity of this subfamily as well as the relationship pattern between the members of this group.

The subfamily Loricariinae has been considered a well defined monophyletic group, the sister-group of the subfamilies Ancistrinae+Hypostominae (SCHAEFER 1987; DE PINNA 1998). With about 200 species this is the largest Loricariidae subfamily in species number. Although cytogenetic descriptions are restricted to the karyotypes of five genera, they show a great karyotypic complexity (Table 1). In the present study, diploid number varied from 2*n*=52 in *Harttia loricariformis* to 2*n*=70 in *Rineloricaria* n.sp., these numbers are within the range observed in the subfamily Loricariinae (Table 1).

The only *Harttia* species cytogenetically studied so far exhibit 2*n*=56 chromosomes (Table 1). The diploid numbers found in *Harttia loricariformis* (2*n*=52) and *Harttia kronei* (2*n*=58), suggest that the species in this genus have a high karyotypic variability. In the two species analyzed, most chromosomes were metacentric and submetacentric but, in *Harttia* sp. most chromosomes were acrocentric (Table 1). These results suggest that chromosome rearrangements occurred in the evolutionary history of this group and changed diploid number as well as chromosomal structure. This assumption is supported by the fact that, in *Harttia kronei,* NORs were at a terminal position and at an interstitial position in *Harttia loricariformis*.

Two *Rineloricaria* species had their karyotype described: *Rineloricaria pentamaculata*, with 2*n*=56 chromosomes (GIULIANO-CAETANO *et al.* 1999) and several local samples of *Rineloricaria latirostris*, whose diploid numbers ranged from 2*n*=36 to 2*n*=48 (GIULIANO-CAETANO 1998). In the present study, *Rineloricaria* n.sp. exhibited the higher diploid number in the genus, 2*n*=70, *Rineloricaria cadeae* showed 2*n*=66 and *Rineloricaria kronei* had 2*n*=64 chromosomes. Moreover, in the three species analyzed the karyotypes were different from those of *R. pentamaculata* and *R. latirostris* due the presence of a higher number of acrocentric chromosomes, mainly in *Rineloricaria* n.sp. (2*n*=70). The difference in diploid

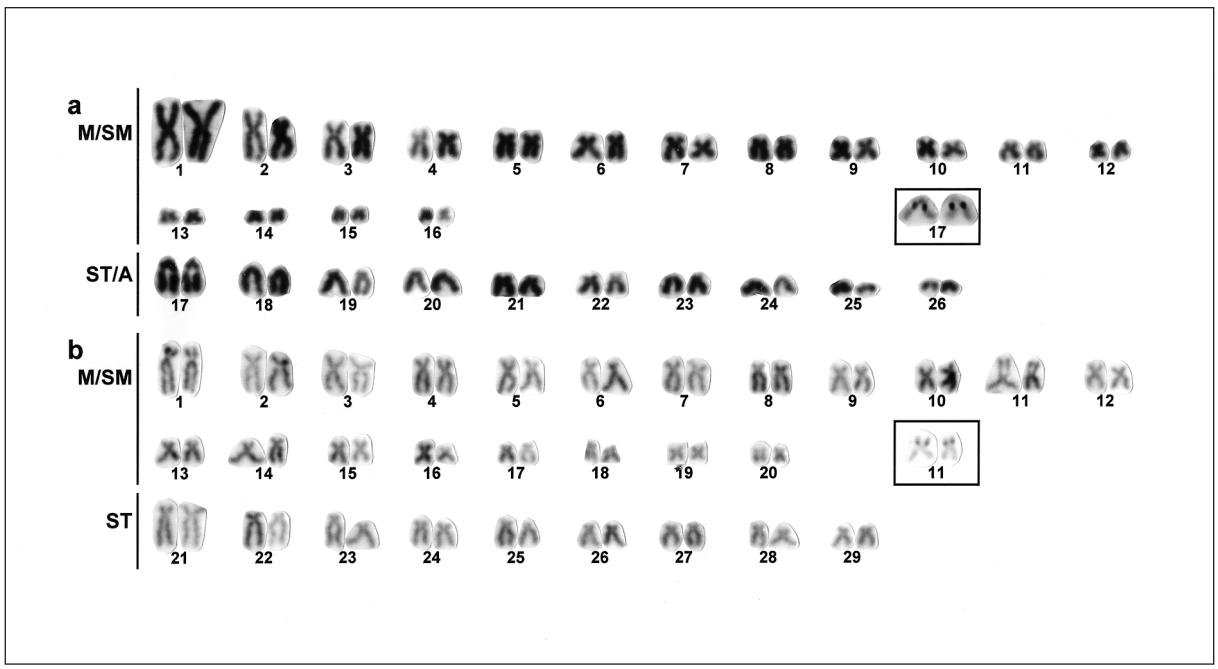

Fig. 2 – Giemsa stained karyotypes of fish from the subfamily Loricariinae. (a) *Harttia loricariformis* with 2*n*=52 chromosomes; (b) *Harttia kronei* with 2*n*=58 chromosomes. In the insets, the NOR-bearing pairs.

number probably indicates the presence of several natural groups or a high rate of karyotypic evolution in the genus *Rineloricaria*.

The high diploid number observed in some Loricariinae species is accompanied by a high number of acrocentric chromosomes (Table 1), suggesting the occurrence of centric fissions. The presence of terminal NORs in the short arms is a conservative characteristic seen in the three species of *Rineloricaria* studied. Additionally, *Rineloricaria* species exhibited evident NOR size polymorphisms, specially in *R. kronei*.

The presence of terminal NORs is probably an ancestral characteristic in the order Siluriformes (OLIVEIRA and GOSZTONYI 2000) and a common characteristic in Loricariidae species (ALVES 2000). To our knowledge, this is the first time that interstitial NORs are reported in *Ancistrus* n.sp. 1 (Ancistrinae) and *Harttia loricariformis* (Loricariinae). However, this phenotype is very common in Neoplecostominae and Hypoptopomatinae (ALVES 2000) and has also been described in some Hypostominae species (ARTONI and BERTOLLO 1996). Considering the high frequency of interstitial NORs in Loricariidae and the presence of interstitial NORs in some related groups such as Trichomycteridae and Callichthyidae (OLIVEIRA and GOSZTONYI 2000), it is possible to suggest that the presence of interstitial NORs is a primitive characteristic in Loricariidae.

The size polymorphism observed in the NORs, as evidenced in *Rineloricaria kronei*, is generated by unequal crossing-over or gene duplication, very common in Neotropical fish (FORESTI *et al.* 1981; ALMEIDA-TOLEDO *et al.* 2000), and already described in several Loricariidae species (ANDREATA *et al.* 1994; ARTONI and BERTOLLO 1996; GIULIANO-CAETANO 1998; ALVES 2000).

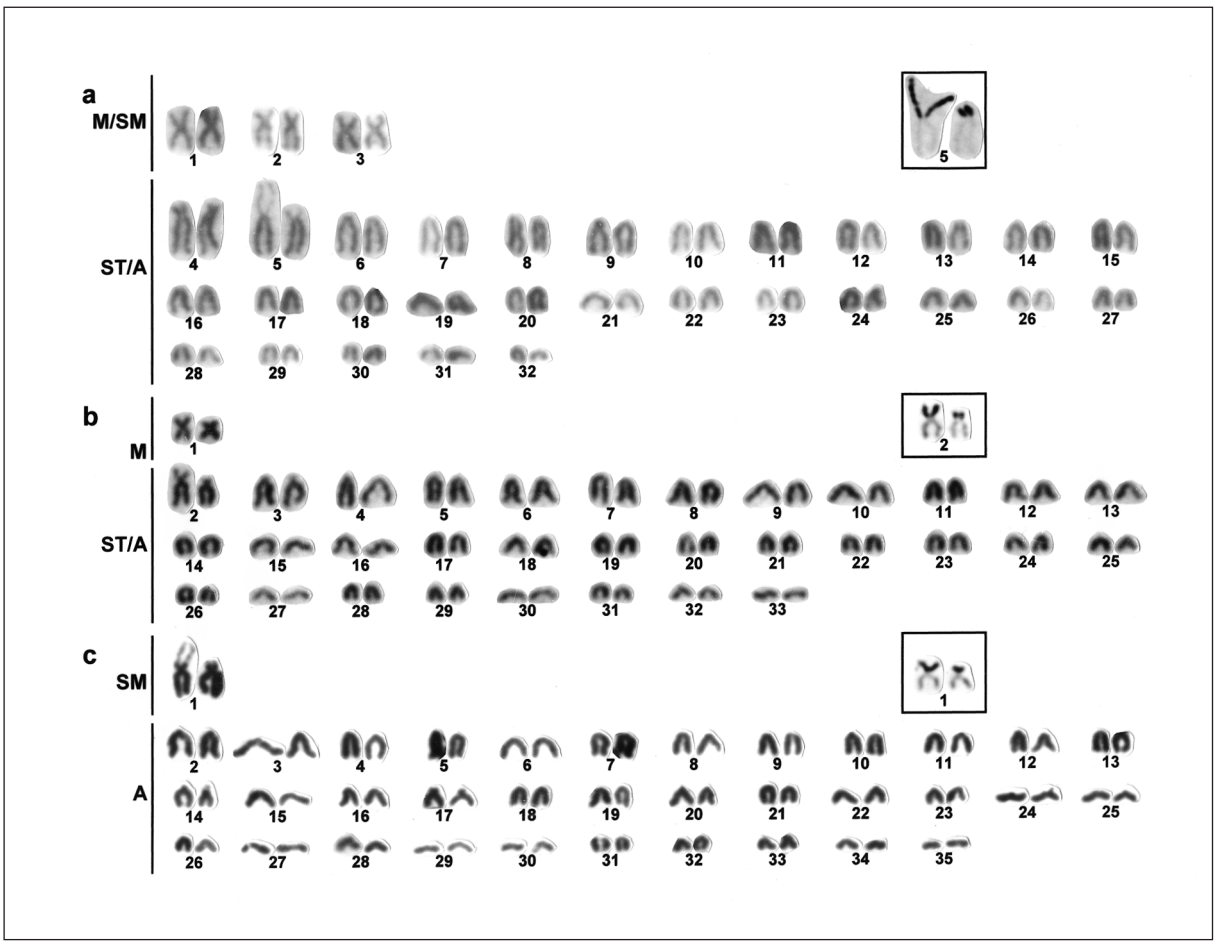

Fig. 3 – Giemsa stained karyotypes of fish from the subfamily Loricariinae. (a) *Rineloricaria kronei* with 2*n*=64 chromosomes; (b) *Rineloricaria cadeae* with 2*n*=66 chromosomes; (c) *Rineloricaria* n.sp. with 2*n*=70 chromosomes. In the insets, the NOR-bearing pairs.

The high karyotypic complexity observed in the subfamilies Ancistrinae and Loricariinae, hindered the proposition of relationship pattern between their components. Further studies on different species using other kind of information such as morphological and molecular data, will be very useful to a better understanding of the karyotypic evolution in the family Loricariidae.

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