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A comparative study of two marine catfish (Siluriformes, Ariidae): Cytogenetic tools for determining cytotaxonomy and karyotype evolution

Thaís Saad Sczepanski [a], Rafael Bueno Noleto [a], Marta Margarete Cestari [a], Roberto Ferreira Artoni [b,*]

[a] Universidade Federal do Paraná, Programa de Pós-Graduação em Genética, Centro Politécnico, Jardim das Américas, 81531-990 Curitiba - PR, Brazil
[b] Universidade Estadual de Ponta Grossa, Departamento de Biologia Estrutural, Molecular e Genética, Av. Carlos Cavalcanti, 4748, 84030-900 Ponta Grossa - PR, Brazil

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ABSTRACT

The family Ariidae comprises approximately 130 catfish species on both warm-temperate and tropical continental shelves around the world. The systematics of the group is problematic, with several misidentification problems. In order to better understand the evolutionary relationships in the family, the present study used a cytogenetic approach to characterize two populations of *Genidens genidens* and two populations of *Aspistor luniscutis* from the southern coast of Brazil using conventional techniques and fluorescent *in situ* hybridization with 18S rDNA probes. The two species had the same diploid number ($2n = 56$), high fundamental numbers and similar banding patterns, thereby corroborating the karyotypic homogeneity proposed for the group. Single nucleolus organizer regions (NORs) were found in the genus *Genidens* and multiple NORs were found in *Aspistor*, which are considered an important cytotaxonomic marker for this genus. Karyotypic evolution trends were hypothesized, providing a better understanding of the karyotype diversity and chromosome evolution processes.

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1. Introduction

The order Siluriformes is composed of 37 recognized families of catfish that are widely distributed and highly diversified in freshwaters (Sullivan et al., 2006). Among these, only two apomorphic families became adapted to saltwater: Plotosidae from the Indo-West Pacific and Ariidae. Ariidae, or sea catfish, comprise approximately 130 species (Marceniuk and Menezes, 2007) that inhabit marine, brackish and freshwater environments along the tropical and subtropical continental shelves (Betancur-R et al., 2007). The monophyly of Ariidae is well-supported by morphological and molecular characters (Diogo, 2004; Kailola, 2004; Sullivan et al., 2006; Betancur-R et al., 2007). However, the systematics of its species is complex and has many nomenclatural problems (Marceniuk and Menezes, 2007). Thus, karyotype analyses for this group may be an important tool for the identification of each species.

The knowledge of the Ariidae karyotype organization is rather preliminary. Thus far, the few cytogenetic studies available have been restricted to chromosome and fundamental numbers. These data have demonstrated an apparent conservation of the chromosome macrostructure among species, with a predominance of $2n = 54 \pm 2$, a fundamental number (NF) greater than 100 and few acrocentric chromosomes.

The aim of this paper is to update the karyotype information on *Genidens genidens* (Cuvier, 1829) and *Aspistor luniscutis* (Valenciennes, 1840), using different staining methods and fluorescence *in situ* hybridization (FISH) to provide cytotaxonomic information for the understanding of the evolution of Ariidae.

2. Materials and methods

A total of 21 specimens of *G. genidens* were analyzed: nine (six males and three females) from Antonina Bay (25°25′S and 48°40′W) and 12 (five males and seven females) from Pontal do Paraná (25°33′S and 48°21′W) (both sites located in the state of Paraná, Brazil). Fourteen specimens of *A. luniscutis* were analyzed: four males from Pontal do Paraná (25°33′S and 48°21′W) and 10 (two males and eight females) from Guaratuba Bay (25°51′S and 48°3′W) (in the State of Paraná, Brazil). Species identification followed the diagnostic characters described by Marceniuk (2005). Voucher specimens are available at the fish collection of Capão da Imbuia Natural History Museum (MHNCI) (Curitiba - PR, Brazil): *G. genidens* (MHNCI 851) and *A. luniscutis* (MHNCI 8217).

The chromosome preparation was obtained from the anterior portion of the kidney using short-time culture and air-drying preparation (Fenocchio et al., 1991), followed by conventional staining for analysis. Chromosome morphology was determined based on arm ratio, as proposed by Levan et al. (1964). Nucleolus

* Corresponding author. Tel.: +55 42 3220 3739; fax: +55 42 3220 3102.
E-mail address: rfartoni@pq.cnpq.br (R.F. Artoni).

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organizer regions (NORs) were visualized using silver nitrate impregnation (Ag-NOR), as proposed by Howell and Black (1980). Combined staining with 4′-6-diamin-2-phenylindole (DAPI) and Chromomycin A_3 (CMA_3) was employed to obtain fluorescent bands (Schweizer, 1976). C-banding was performed as described by Sumner (1972).

Fluorescent *in situ* hybridization (FISH) was used to detect the major rDNA sites in the chromosomes. 18S rDNA probes from *Prochilodus argenteus* (Hatanaka and Galetti, 2004) were labeled with biotin-14-dATP by nick translation, following the manufacturer's instructions (BioNick™ Labeling System—Invitrogen). The overall hybridization procedure followed the protocol described by Pinkel et al. (1986), under high stringency conditions (2.5 ng/μL probes, 2 μg/μL salmon sperm DNA, 50% deionized formamide, 10% dextran sulphate, 2×SSC at 37 °C overnight). After hybridization, the slides were washed in 15% formamide/0.2×SSC at 42 °C for 20 min, 0.1×SSC at 60 °C for 15 min, and 4×SSC/0.05% Tween at room temperature for 10 min; the latter consisting of two 5-min washes. The hybridization mark was detected using conjugated streptavidin–fluorescein isothiocyanate (FITC). The chromosomes were counterstained with propidium iodide (25 μg/mL) and analyzed afterwards with a Zeiss Axiophot epifluorescence microscope. Chromosome images were captured using the Case Data Manager Expo 4.0 software program (Applied Spectral Imaging).

3. Results and discussion

Both populations of *G. genidens* specimens analyzed had $2n = 56$ chromosomes, with a karyotype formula of 14m + 22sm + 16st + 04a and a fundamental number (FN) of 108 (Fig. 1a). The two populations of *A. luniscutis* also had the same diploid chromosome number ($2n = 56$), karyotypic formula and fundamental number (14m + 22sm + 20st, FN = 112) (Fig. 1b). Gomes et al. (1994) describe similar results; however, the *A. luniscutis* population from Cananéia, São Paulo, Brazil, had a different fundamental number (FN = 110) from the one found described here. This divergence may be attributed to differences in the karyotype macrostructure, reflecting a real geographical variation common to widespread species. No heteromorphic elements indicating sex chromosomes were detected in *G. genidens* or *A. luniscutis*, which was similar to most of the Siluriformes studied thus far.

While the low vagility and specialized reproductive strategies (i.e. male mouthbrooding) (Betancur-R et al., 2007) of the Ariidae could favor chromosome variability, the results of the present study support the conservation of the karyotype macrostructure within the group, especially regarding the diploid chromosome number ($2n = 56$) (Table 1). As the diploid number $2n = 52$ (Arreguin, 1983) was found in the basal Ariidae lineage Galeichthinae (Marceniuk, 2003) and the ancestor of all Siluriformes probably had $2n = 56$

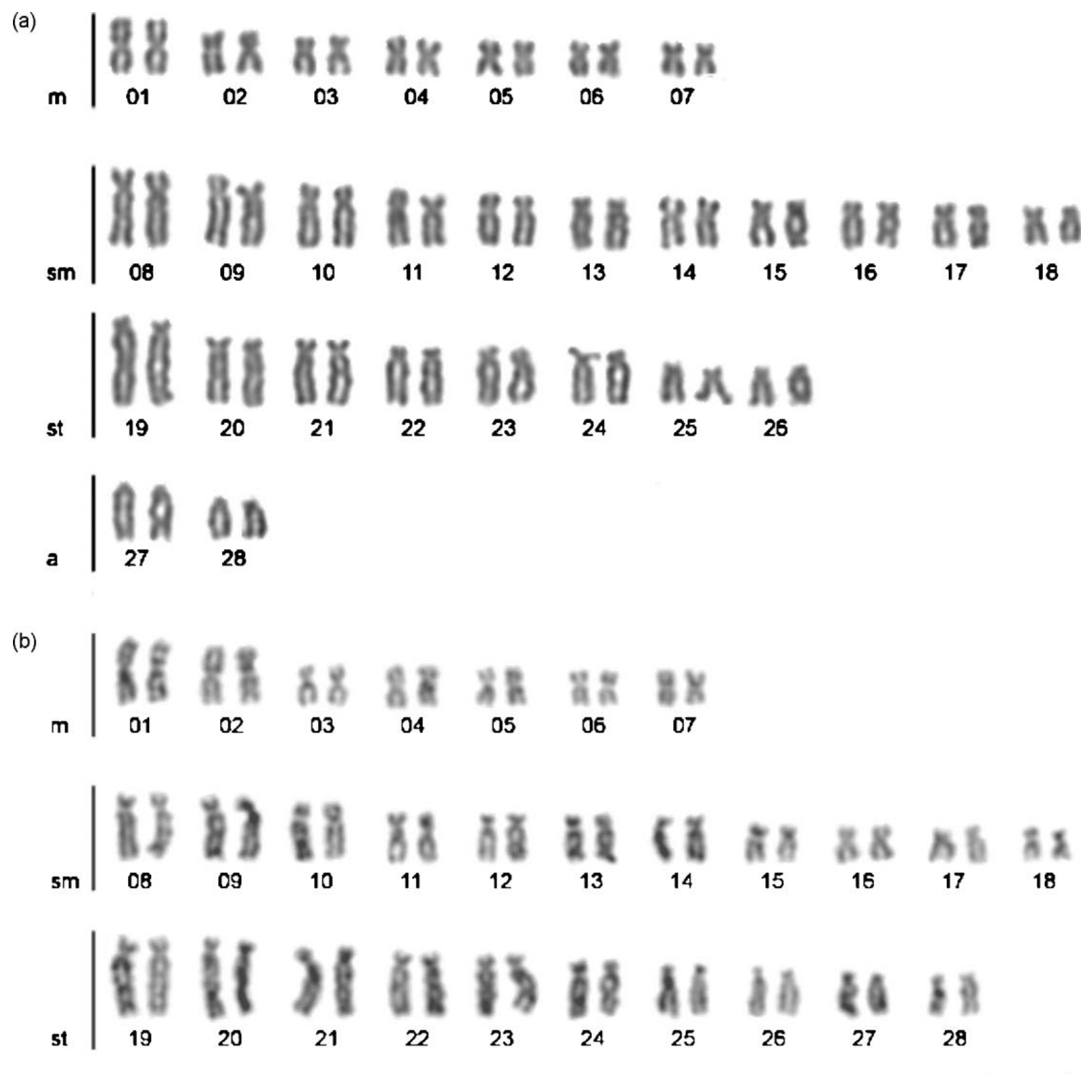

Fig. 1. Karyotypes of (a) *Genidens genidens* and (b) *Aspistor luniscutis* with conventional Giemsa staining. Bar = 5 μm.

Table 1
Summary of the chromosome findings of the species of Ariidae.

Species	Karyotype					NF	Sex system	Locality	Ref.
	2*n*	m	sm	st	a				
Galeichthys caerulescens	52	16	24	10	02	102	–	Guerrero, Mexico	Arreguin (1983)
Plicofolis dussumieri (cited with *Arius dussumieriy*)	54	12	18	12	12	96	–	Indian Ocean	Rishi et al. (1983)
Cathorops melanopus (cited with *Arius melanopus*)	52	16	30	06	–	104	–	Gulf of Mexico	Ramírez (1985)
Ariopsis felis (cited with *Arius felis*)	54	–	26	28	–	108	–	Gulf of Mexico	LeGrande (1980)
	54	16	12	20	06	102	–	Campeche, Mexico	García-Molina and Uribe-Alcocer (1988)
Bagre marinus	54	12	08	34	–	108	–	Gulf of Mexico	Fitzsimons et al. (1988)
Bagre bagre	56	24	26	06	–	112	–	Cananeia, Brazil	Gomes et al. (1990)
Cathorops sp.	54	13	13	28	–	108	–	Cananeia, Brazil	Gomes et al. (1992)
Nemapteryx nenga (cited with *Arius nenga*)	54	16	36	02	–	108	–	Gopalpur Sea, India	Choudhury et al. (1993)
Netuma thalassina (cited with *Arius serratus*)	56	08	24	24	–	112	–	Gopalpur Sea, India	Choudhury et al. (1993)
Genidens genidens	56	12	20	20	04	108	–	Cananeia, Brazil	Gomes et al. (1994)
	56	14	22	16	04	108	–	Paraná, Brazil	Present study
Netuma barba (*Genidens barbus*)	56	18	18	18	02	110	XY	Cananeia, Brazil	Gomes et al. (1994)
Aspistor parkery (cited with *Aspistor luniscutis*)	56	16	16	22	02	110	–	Cananeia, Brazil	Gomes et al. (1994)
	56	14	22	20	–	112	–	Paraná, Brazil	Present study
Sciades herzbergii (cited with *Hexanematichthys*) *herbergii*	56	24	24	06	02	110	–	Maracaibo, Venezuela	Molina et al. (2004)

chromosomes (Oliveira and Gosztonyi, 2000), an increase in the diploid number occurred throughout the evolution of the family, indicating that centric fission played an important role in the karyotype definition of this group, maintained putative cell homeostasis and achieved more stable karyotypes.

Despite the conservation of chromosome number, a wide variability in chromosome morphology is found between species, which may indicate a prevalence of non-Robertsonian rearrangements (Kirpichnikov, 1981). The difference in karyotype formula between *Genidens* and *Aspistor* caused by the presence of acrocentric chromosomes may also suggest that pericentric inversions have played a substantial role during the evolutionary pathway of these fish. Moreover, differences in the karyotype may be limited to cryptic chromosome rearrangements, such as those involving the heterochromatin segments and/or the nucleolus organizer regions. The fixation of such rearrangements in the populations was probably intensified by the effects of inbreeding in small populations.

The distribution pattern of heterochromatin in *G. genidens* and *A. luniscutis* is similar to that of many Siluriformes, in which weak centromeric and telomeric bands occur in a large number of chromosomes. As this pattern is seen in many other species, it may represent a symplesiomorphic condition for teleosts (Oliveira and Gosztonyi, 2000). Conspicuous C-bands were also observed in the pericentromeric position of pairs 11 and 17 in *G. genidens* and pair 1 in *A. luniscutis* (Fig. 2a and b, respectively). Ag-NORs coincided with positive C-band labeling (Fig. 3). The association between constitutive heterochromatin and rDNA *cistrons* has been frequently reported in fish (Pendás et al., 1993a,b; Galetti, 1998; Fujiwara et al., 1998; Molina et al., 1998; Born and Bertollo, 2000). In some cases, the nucleolus organizer regions seem to be adjacent to the heterochromatic bands (Artoni and Bertollo, 1999), whereas, in others, both regions overlap or are interspersed in the chromosome (Pendás et al., 1993a,b).

Chromosome staining with CMA_3 revealed the occurrence of bright labeling in the terminal position of the short arms in pair 13 in *G. genidens* and in pairs 15, 25 and 27 in *A. luniscutis*, corresponding to Ag-NOR sites (Fig. 3a and b, respectively). This correspondence between the two types of staining procedures has been reported for a large number of species (Amemiya and Gold, 1986; Rab et al., 1996; Margarido and Galetti, 2000). However, this may not be the rule among fish (Artoni et al., 1999). In *A. luniscutis*, besides the NOR-bearing pairs of chromosomes, other chromosomes of the complement were also observed on the telomeres with fluorescent staining, suggesting that a primordial GC-rich heterochromatin (probably the NOR-associated heterochromatin) spread to some chromosomes through a process of dispersion (Schweizer and Loidl, 1987). Treatment with the AT-specific fluorochrome DAPI revealed uniform staining in both species, indicating that chromatin is not compartmentalized in isochores, which is commonly observed in fish. Consequently, the ratio between AT and GC base pairs is equally interspersed in the genomic DNA.

The analysis of nucleolus organizer regions by silver impregnation revealed the presence of single labels in the terminal region of the short arms of chromosome pair 13 in *G. genidens*, demonstrating size heteromorphism between homologous chromosomes (Fig. 3a). A single Ag-NOR is the most common condition in Siluriformes (Oliveira and Gosztonyi, 2000), Teleostei (Klinkhardt, 1998) and most vertebrate species (Amemiya and Gold, 1986). On the other hand, *A. luniscutis* exhibited multiple Ag-NORs in the short arms of pairs 15, 25 and 27 as well as size heteromorphism between homologous chromosomes (Fig. 3b). The occurrence of more than one chromosome pair with NORs can be considered an apomorphic characteristic (Hsu et al., 1975) and, in the present study, has proven to be useful for evaluating the mechanisms of chromosome differentiation within the family. The variation in the location of NOR sites may constitute a strong cytotaxonomic character between *G. genidens* and *A. luniscutis*.

In both species, labeling from fluorescent *in situ* hybridization with the 18S rDNA-specific probe (Fig. 3) corresponded to NOR-bearing pairs evidenced by silver nitrate (Ag-NORs) and CMA_3 staining, demonstrating that the size heteromorphism between homologues may be caused by a variety of mechanisms: unequal crossing over, transposition, tandem amplification and other rearrangements involving homologous segments causing structural modifications in the NORs (Viñas et al., 1996; Vicari et al., 2006). The dispersion of ribosomal *cistrons* in the genome through events of transposition could be facilitated by the fact that these regions are heterochromatic and commonly associated to the terminal position in chromosomes (Moreira-Filho et al., 1984). This location is propitious to the transference of genetic material due to the proximity domain within the interphase nucleus (Schweizer and Loidl, 1987).

Despite the apparently conserved diploid number of $2n = 54 \pm 2$ chromosomes composing different karyotype formulae within the family Ariidae, very little is known regarding the microstructural variability of these karyotypes. The data of the present study on the composition and distribution of nucleolus organizer regions and heterochromatin contribute toward clarifying the karyotype evolu-

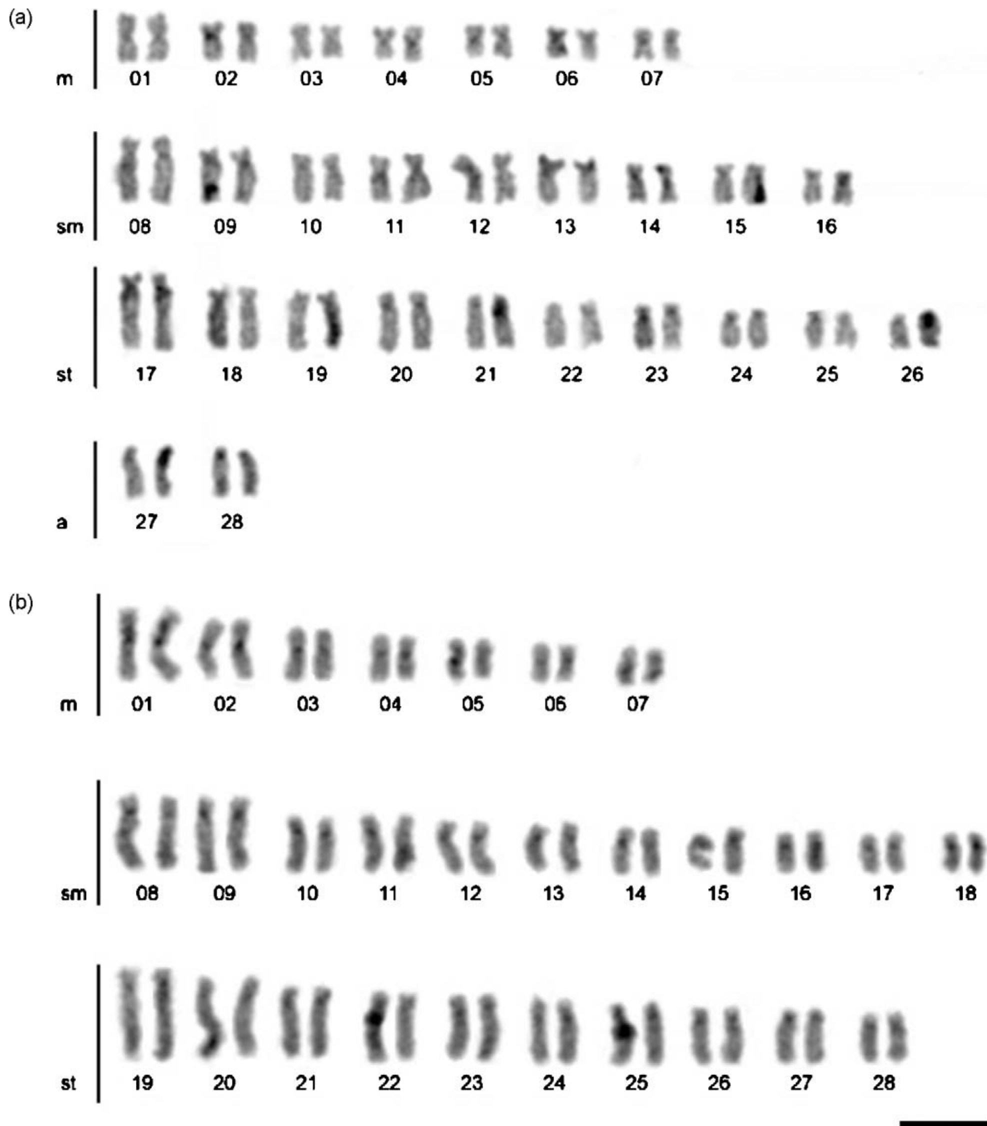

Fig. 2. Karyotypes of (a) *Genidens genidens* and (b) *Aspistor luniscutis* submitted to C-banding. Bar = 5 μm.

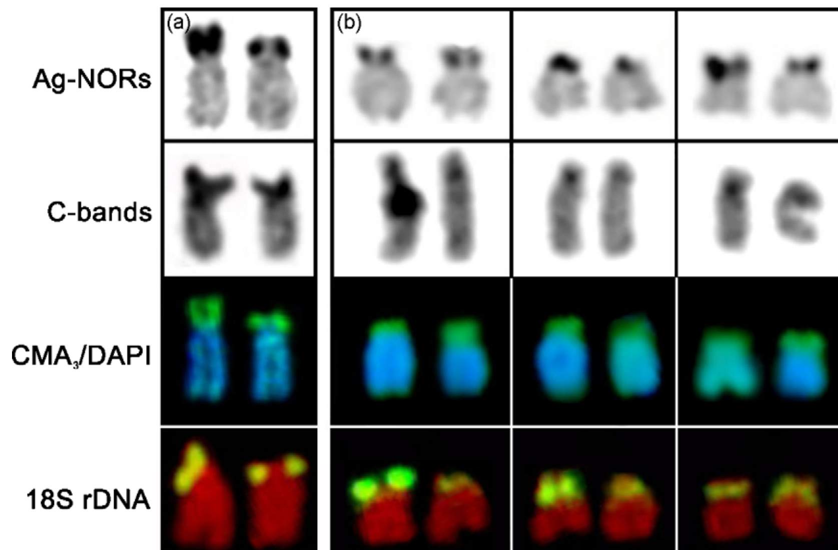

Fig. 3. NOR-bearing chromosome pairs in (a) *Genidens genidens* and (b) *Aspistor luniscutis*, showing GC-rich heterochromatin/NOR association.

tion and phylogenetic relationships in this group. Further analysis including additional species of Ariidae and different staining techniques should provide a better understanding of the chromosome evolution in the group and confirm the conservative nature of the diploid number in this fish family.Acknowledgements

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