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

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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.

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

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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 $2n = 56$

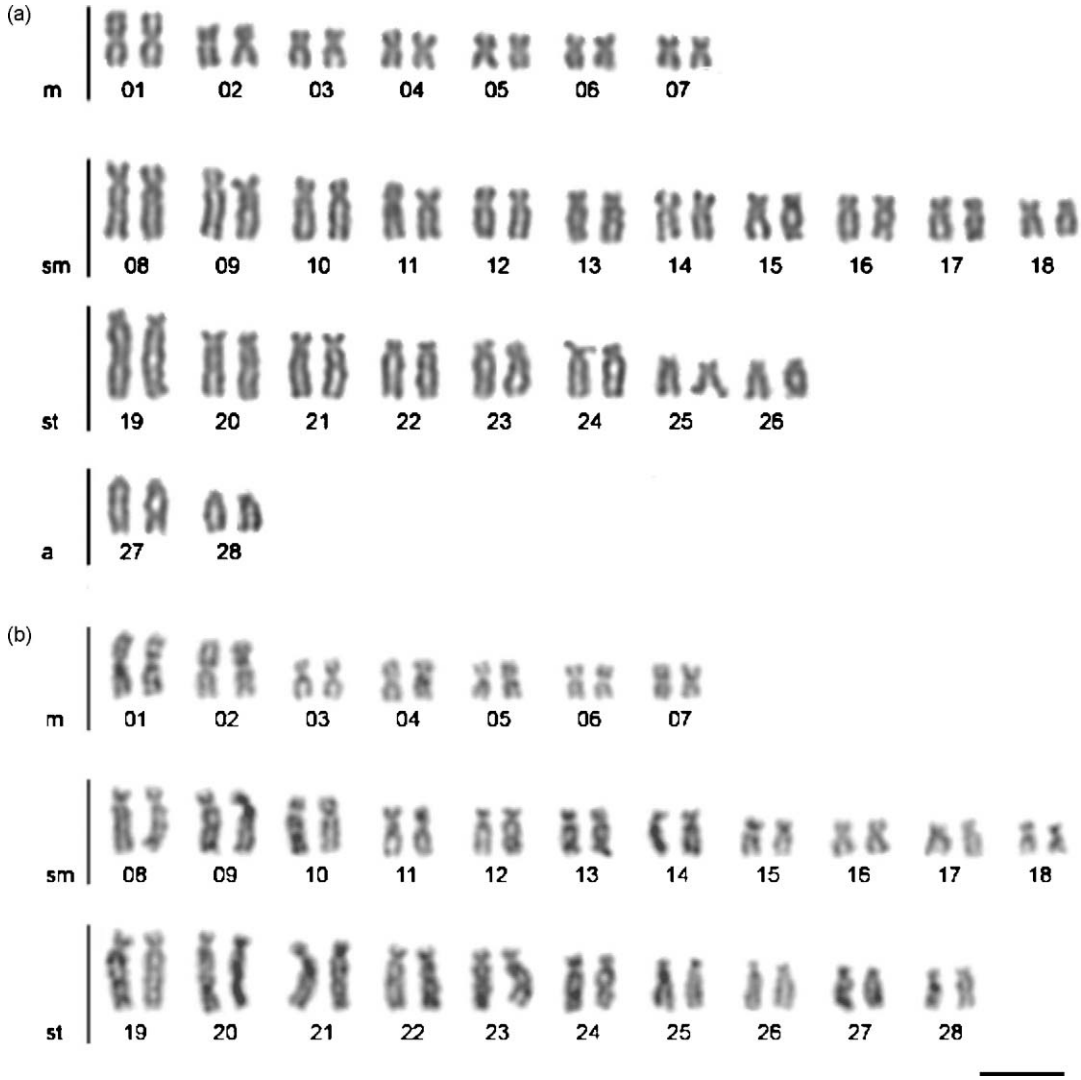

Fig. 1. Karyotypes of (a) *Genidens genidens* and (b) *Aspistor luniscutis* with conventional Giemsa staining. Bar = 5 μm.