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CLASSICAL CYTOGENETIC CHARACTERIZATION OF *OPISTOGNATHUS MACROGNATHUS* (PERCIFORMES: OPISTOGNATHIDAE)

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ABSTRACT: Cytogenetic analysis of *Opistognathus macrognathus* by conventional Giemsa staining, AgNO3-impregnation to highlight Nucleolus Organizer Regions (NOR), and C-banding revealed a diploid modal chromosome complement of 2n=40 comprising 2 metacentric, 6 submetacentric, 4 subtelocentric, and 28 acrocentric elements. Sequential silver nitrate impregnation of selected metaphase spreads carried out to identify congruity of NOR-bearing chromosomes with the same chromosomes previously stained with Giemsa allowed the identification of a pair of active NOR-bearing chromosomes having positive signals at the terminal end of the short arm of the largest subtelocentric pair (number 5). C-banding showed small heterochromatic blocks in the centromeric regions of almost all chromosomes. These results prove that karyotypes differing from the 2n=48 acrocentric arrangement are not uncommon to marine fishes.

Kewords: Karyotype, C-banding, NOR, *Opistognathus macrognathus.*

RESUMEN: El análisis citogenético mediante tinción convencional con colorante de Giemsa, impregnación con AgNO3 y bandeo-C en *Opistognathus macrognathus* reveló un complemento cromosómico diploide modal de 2n = 40 compuesto por 2 elementos metacéntricos, 6 submetacéntricos, 4 subtelocéntricos y 28 acrocéntricos. La impregnación secuencial de metafases seleccionadas, con nitrato de plata, para identificar la correspondencia entre los cromosomas portadores de las Regiones Organizadoras del Nucléolo (RON) con los mismos cromosomas previamente teñidos con Giemsa permitió la identificación de un par de cromosomas portadores de RONs con señales positivas situadas en posición terminal del brazo corto del par subtelocéntrico más grande (Par N° 5). El bandeo-C mostró pequeños bloques heterocromáticos localizados en las regiones centroméricas de casi todos los cromosomas. Estos resultados indican que cariotipos diferentes a la condición 2n=48 A no son poco frecuentes en peces marinos.

Palabras clave: Cariotipo, Bandeo-C, RON, *Opistognathus macrognathus*.

INTRODUCTION

Fishes are the most diverse group of vertebrates with more than 32,000 recognized species characterized by the great diversity of their morphology, physiology, ecology, life history and behavior (NELSON 2006). Because of the basal position that they occupy in the phylogeny of vertebrates, studies on fish chromosomes have provided valuable information for the understanding aspects such as the genetic divergence between conspecific species, relations among groups, occurrences of cryptic species and species complexes, mechanisms of sex determination and evolution of sex chromosomes, distribution of the Nucleolus Organizers Regions, existence of supernumerary chromosomes and the relationship between polyploidy and evolution (OLIVEIRA *et al*. 2009).

The more comprehensive revision of fish cytogenetic (ARAI 2011) reveals that the available data cover 3,425 species/subspecies of agnathans, cartilaginous fish, actinopterygians (ray-finned fish) and sarcopterygians (lobe-finned fish), which represent barely 10,7% of extant species at the global level.

In Venezuela, the Fishbase (http://www.fishbase.org/) registers at least 930 freshwater and 805 marine species (FROESE & PAULY 2011) but cytogenetic studies in this group are scantly (NIRCHIO 2009).

The family Opistognathidae (jawfishes), composed of three genera (*Opistognathus*, *Lonchopisthus*, and *Stalix)* with about 78 species, is a group of marine demersal fishes generally of small size distributed in the western and central

Atlantic, Indian and eastern Pacific Oceans from the Gulf of California to Panama (NELSON 2006). The family is represented in Venezuela by two genera (*Opistognathus* and *Lonchopisthus*) (CERVIGÓN 1994).

This work provide the first karyotype analysis of the jawfish *Opistognathus macrognathus* using classic cytogenetic methods (Giemsa staining, C-banding, and Ag-NOR).

MATERIALS AND METHODS

Six males and three females of *Opistognathus macrognathus* collected in the near shore of Cubagua Island, Venezuela were analyzed. Their sex was established by external sexual dimorphism (Fig. 1) and confirmed by the presence of testes or ovaries. Voucher specimens are deposited in the Fish Collection of the Escuela de Ciencias Aplicadas del Mar at Universidad de Oriente.

Mitotic chromosomes were obtained by injecting a solution of colchicine 0.0125% (1cc/g). Exposure to the alkaloid during 50 min, obtaining cell suspension from kidney tissue, hypotonic treatment for 20 min and centrifugation-fixation (3 times) with Carnoy (NIRCHIO & OLIVEIRA 2006) previous stimulation of mitotic activity (LEE & ELDER 1980). Diploid complement and chromosome standard morphology were determined by Giemsa staining at pH 6.88. The plates were sequentially silver-stained (HOWELL & BLACK 1980) for accuracy in identifying Nucleolus Organizer Regions (Ag-NORs) bearing chromosome. C-banding was performed following the method of SUMNER (1972). Metaphases were photographed using a Motic camera and images were digitally processed using the Adobe Photoshop CS5 software. The chromosomes were classified as metacentric (M), submetacentric (SM), subtelocentric (ST), and acrocentric(A), according to the arms ratio (LEVAN *et al*. 1964), and arranged in descending order by size. The chromosome formula and FN (fundamental number or number of chromosomal arms) were established considering acrocentric chromosomes with a single arm and the remaining chromosomes with two arms.

Fig. 1. Adult *Opistognathus macrognathus*. Males (A) can be distinguished from females (B) by their longer jaw and dorsal fin.

RESULTS AND DISCUSSION

Counts of 238 diploid metaphasic cells from 6 males and three females revealed a modal chromosome complement 2n=40 composed of 2 metacentric, 6 submetacentric, 4 subtelocentric and 28 acrocentric chromosomes (Fig. 2) with an arm number (NF) of 52. No karyotype differentiation was observed between males and females indicating the absence of chromosoma heterogamety.

The karyotype displayed by *O. macrognathus* is far from the 48 uniarmed complement shared by approximately 60% of marine Perciformes, a condition originally proposed by OHNO (1970) as the primitive karyotype for all Teleostei but considered by BRUMM (1995) as an evolutionary novelty of the Clupeocephala shared by the Euteleostei.

Cytogenetic data of Neotropical fishes show that marine fishes has conserved karyotypes with 48 uniarmed chromosomes whereas freshwater fishes display karyotypes with a higher diploid number and more bi-armed chromosome (NIRCHIO 2009). These differences in chromosome and arm number (NF) between marine and freshwater fishes has been explained taking into account topographic barriers common to freshwater environments that would hamper the gene flow between populations, leading to fixation of macro-structural alterations in chromosomes, whereas the absence of well-defined geographical barriers, the occurrence of large populations and therefore intense gene flow due to the large capacity of dispersion in the marine environment would contribute

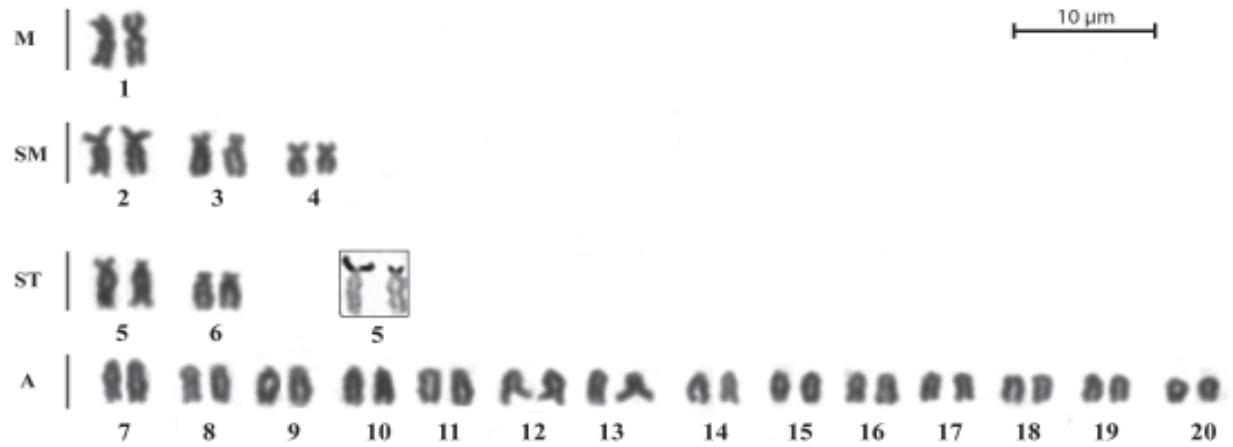

Fig. 2. Karyotype and NOR bearing chromosomes (inset) of *Opistognathus macrognathus.*

to homogenize populations and it would reducing karyotype diversification (MOLINA 2007). In fact, in marine fish, groups that have high mobility (eggs, larvae, or adults), like Haemulidae, Mugilidae, Sciaenidae, Lutjanidae and Serranidae show a conserved karyotype with 48 acrocentric chromosomes and low frequency of chromosomal macro-structural reorganizations whereas fishes with benthonic habits, that not form schools, with smaller populations and more limited spatial locomotion such as Muraenidae, Batrachoididae, and Scorpaenidae display a more extensive chromosomal diversity (NIRCHIO 2009). Since jawfish are secretive animals, preferring to live in burrows in sand which they construct themselves and vigorously defend and so spending his life in a limited territory (SMITH-VANIZ 2002), the karyotype of *O. macrognathus* presenting 2n=40 chromosomes with 12 biarmed and 28 uniarmed elements, NF=52 is a configuration that depart from the 2n=48 all-acrocentric karyotype prevailing in marine fishes and reveals the accumulation of a series of macro-structural chromosome reorganizations consistent with those displayed by marine fishes with low capacity of dispersion.

Sequential silver impregnation after Giemsa staining in *O. macrognatus* (Fig. 2) allowed the identification of one pair of NORs located in terminal position on the short arm of the largest subtelocentric pair number 5 (Figs. 2 and 3). Perciformes commonly have a single chromosome pair with active NORs (GALETTI *et al.* 2000) a characteristic presumed as a plesiomorphic (or primitive) condition whereas multiple NOR pairs the apomorphic (or derived) one (AMEMIYA & GOLD 1988).

In non-extremely condensed chromosomes a pronounced length variation of this NOR-bearing arm between homologous chromosomes both in males and females was evident, being one of them approximately twofold the size of the other. This variation was consistent with nucleoli size of interphasic cells (Fig. 2).

Nucleolar Organizer Regions (NORs), in higher eukaryotes, correspond with specific chromosomal sites in which there are clustered multiple tandem copies of a repeating unit of the major 45S rDNAs which consist of a transcriptional unit that codes for the 18S, 5.8S and 28S rRNAs and an intergenic spacer (LONG & DAVID 1980). The expression of this cistrons are visualized by the silver staining procedure (HOWEEL & BLACK 1980; HOWELL 1982) that reveals the residues of Ag-stainable-proteins complex synthetized only by active NORs in the preceding interphase. Size heteromorphism of the Ag-NORs also seen in other species of freshwater and marine fish has been explained as a product of differential transcriptional activity of rDNA genes (BRUM *et al*. 1996; ROSSI *et al*. 1997), or due to accumulation of rDNA genes in one of the two homologous (CARVALHO & DIAS 2007).

The constitutive heterochromatin distribution as identified by C-banding showed centromeric heterochromatic blocks in almost all chromosomes, excepting a pericentromeric block corresponding with the Ag-NORs on the short arms of the chromosomes pair number 5 and a pair showing two large weakly heterochromatic blocks, one proximal to centromere and the other from telomeres to the middle of the chromosome (Fig. 4). Conserved C-positive heterochromatin distribution, occurs in several species of Perciformes

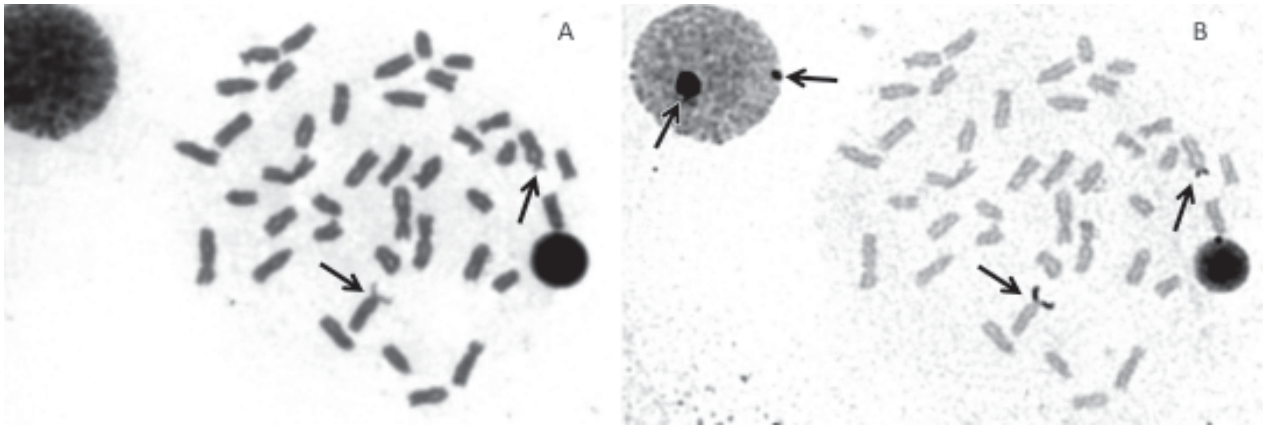

Fig. 3. Metaphase plate of *Opistognathus macrognathus* after Giemsa staining (A) and sequentially $AgNO_3$ impregnation (B). Arrows indicate the NOR bearing chromosomes.

where discrete blocks are preferentially located in the centromeric/pericentromeric regions of chromosomes (MOLINA 2007).

Although it is difficult to make comparisons with other species of jawfish due that the present description is the first one for the family Opistognathidae and additional information is required in order to infer the evolution of karyotype in this group, the information here provided contributes with the general knowledge on fish cytogenetic and reveals that as further researches are done, it is more frequent finding in marine fishes karyotypes dissimilar to the condition 2n=48 acrocentric elements.

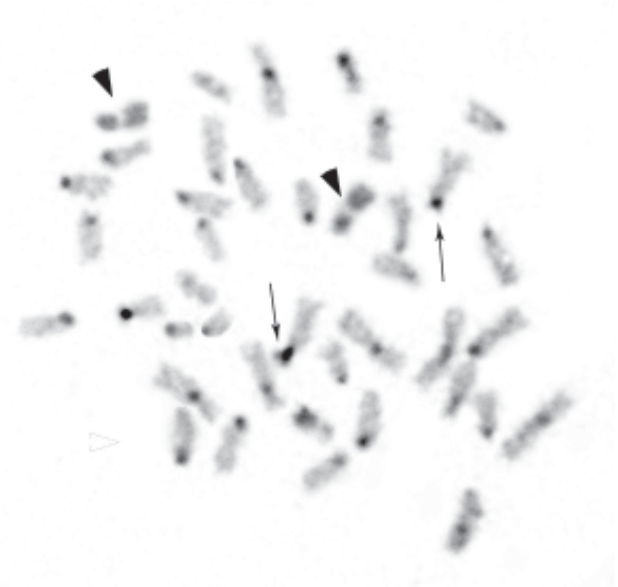

Fig. 4. Metaphase plate of *Opistognathus macrognathus* after C-banding. Arrows indicates pericentromeric block corresponding with the Ag-NORs on the short arms of the chromosomes pair number 5. Arrowheads show weakly heterochromatic blocks, one proximal to centromere and the other from telomeres to the middle of the chromosomes.

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