KARYOTYPE OF *SPARISOMA CHRYSOPTERUM* (BLOCH & SCHNEIDER 1801): INSIGHTS INTO CHROMOSOMAL EVOLUTION IN SPARISOMATINAE (PERCIFORMES: SCARIDAE)

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ABSTRACT**:** This work provides the first detailed report on diploid number, karyotype formula, distribution of constitutive heterochromatin and nucleolus organizing regions (NORs) in *Sparisoma chrysopterum* (Bloch & Schneider 1801). The species has a modal diploid number 2n = 46 chromosomes composed of 8 metacentric (m), 10 submetacentric (sm), 10 subtelocentric (st) and 18 acrocentric (a) elements and a number of arms (NF) equal to 74. C-banding revealed a nonhomogeneous distribution of constitutive heterochromatin occupying pericentromeric sites on nearly all chromosomes but with terminal blocks on the long arm in the metacentric series, whereas the submetacentric series terminal exhibited blocks located preferentially on the short arms. Three pairs of chromosomes presented weak staining for heterochromatic areas. Silver nitrate impregnation revealed a single pair of NORs located in the terminal position of the short arms of chromosome pair No. 12, and their variation among *Sparisoma* species seems to indicate that it is an important chromosomal marker in the genus. The data presented here confirm that Scaridae have a conserved diploid number (46-48) with notable karyotypic variation and support that the occurrence of numerous pericentric inversion events could be responsible for modifying the karyotypic formula and that microstructural alterations, likely translocations, involving NORs have frequently occurred during diversification into the genus *Sparisoma*.

Key words: Ag-NORs; C-bands; cytogenetics; parrotfish; Robertsonian rearrangement

RESUMEN: Este trabajo proporciona el primer informe detallado sobre el número diploide, la fórmula del cariotipo, la distribución de la heterocromatina constitutiva y las regiones organizadoras del nucléolo en *Sparisoma chrysopterum* (Bloch & Schneider 1801). La especie tiene un número modal diploide 2n = 46, compuestos por 8 elementos metacéntricos (m), 10 submetacéntricos (sm), 10 subtelocéntricos (st) y 18 acrocéntricos (a) y un número de brazos (NF) igual a 74. El bandeo C reveló una distribución de heterocromatina constitutiva no homogénea que ocupa sitios pericentroméricos en casi todos los cromosomas, pero con bloques terminales en el brazo largo de la serie de metacéntricos, mientras que en la serie de submetacéntricos los bloques terminales se localizan principalmente en los brazos cortos. Tres pares de cromosomas presentaron áreas heterocromáticas débilmente teñidas. La impregnación con nitrato de plata reveló un solo par de regiones organizadoras de nucléolo (RONs) ubicadas en la posición terminal de los brazos cortos del par cromosómico No. 12. La variación de las RONs entre las especies de *Sparisoma* parece indicarlo como un marcador cromosómico importante en el género. Los datos aquí presentados confirman que la familia Scaridae posee un número diploide conservado (46-48) con una variación cariotípica notable y apoyan que la ocurrencia de numerosos eventos de inversión pericéntrica podría ser responsables de modificar la fórmula cariotípica, y que las alteraciones microestructurales, probablemente translocaciones, que involucran RONs han sido frecuentes durante la diversificación en el género *Sparisoma*.

Palabras clave: Ag-NOR; bandas C; citogenética; pez loro; reordenamiento robertsoniano

INTRODUCTION

Scaridae are popularly known as “parrotfish” and are distributed in the tropical areas of the Pacific, Indian and Atlantic oceans (Froese & Pauly 2021), where they play a key role in ecology as driving agents of reef geomorphology due to their ability to bioerode

and generate and transport sediments (Nicholson & Clements 2020). They are also important components of marine park tourism and the diving industry, and many are popular marine aquarium species, some of which are collected by the tens of thousands each year (Comeros-Raynal *et al*. 2012).

The Scaridae family is divided into two subfamilies, Scarinae and Sparisomatinae, each containing five genera. Sparisomatinae, containing the genera *Calotomus, Cryptotomus, Leptoscarus, Nicholsina* and *Sparisoma,* includes 25 valid species (Fricke *et al*. 2021), and due to their notable karyotypic variation, rarely reported in marine species, they are of interest for understanding chromosomal evolution and testing the efficacy of cytotaxonomy (Almeida *et al.* 2017).

With 15 species in the genus (Fricke *et al.* 2021), only two species have been analyzed to date in *Sparisoma*, *S. axillare* (Sena & Molina 2007) and *S. radians* (Paim *et al.* 2014), although there is another report that provides the diploid number and fundamental number of *S. chrysopterum* (Nirchio *et al.* 2014).

With the aim of expanding the cytogenetic information in *Sparisoma*, this work provides the first detailed report on diploid number, karyotype formula, distribution of constitutive heterochromatin and nucleolus organizer regions in *S. chrysopterum* (Bloch & Schneider 1801). This species is distributed in the western Atlantic Caribbean Sea and is associated with marine reefs at depths between 1-20 m (IUCN 2009; Froese & Pauly 2021), presents sexual dichromatism, is a monandric protogynic hermaphrodite (all males are derived from females through sex change), and has two distinctive color phases, initial and terminal (Robertson & Warner 1978; Figuerola *et al*. 1998).

MATERIALS AND METHODS

Six specimens of *Sparisoma chrysopterum* were captured using an artisanal creel in a reef near the coast of Cubagua Island (10° 50’11.7 “N - 64° 09’06.6” W). Fish were transported to Nueva Esparta Scientific Research Institute of the Universidad de Oriente, where they were analyzed.

To obtain the chromosomal preparations, each fish was intraperitoneally injected with a 0.0125% colchicine solution (1 cc/100 g of body weight). After 50 min, the fish were euthanized by numbing in a benzocaine solution (250 mg/L) as recommended by the American Veterinary Medical Association (Leary *et al.* 2013). Vouchers are deposited in the Fish Cytogenetics Laboratory of the Escuela de Ciencias Aplicadas del Mar, UDO, Isla de Margarita, Venezuela (ECAM-400) and in the Laboratório de Biologia e Genetica de Peixes, UNESP, Botucatu, Brazil (LBP6096).

Mitotic chromosomes were obtained following the conventional air-drying method from a kidney cell suspension (Nirchio & Oliveira 2006). Diploid complement and standard chromosome morphology were determined by Giemsa staining. The nucleolus organizer regions (NORs) were revealed by impregnation with silver nitrate (Howell & Black 1980). C bands were obtained by chromatin depurinization with acid treatment (HCl), DNA denaturation with saturated solution of barium hydroxide and subsequent washing in saline solution (2xSSC) at 60°C (Sumner 1972). Metaphase cells were imaged using a Motic B 400 microscope equipped with a Moticam 5000 C digital camera using Motic Images Plus 2.0 ML software. Images were edited to optimize brightness and contrast using Photoshop (Adobe Systems, Inc. San José, CA, USA) Version 2020. Chromosomes were arranged in decreasing order and ranked according to centromere position (Levan *et al.* 1964).

RESULTS

The analysis of 186 metaphase cells revealed a modal diploid number of 2n = 46 chromosomes with a karyotype composed of 8 metacentric (m), 10 submetacentric (sm), 10 subtelocentric (st) and 18 acrocentric (a) elements and a number of arms (NF) equal to 74. The karyotype with the ordered chromosomes according to decreasing size and position of the centromere is shown in Figure 1.

C-banding revealed the presence of heterochromatic blocks on almost all chromosomes distributed in the pericentromeric regions and in the terminal portions of the long arms of chromosomes. 1, 2, 3 and 4 of the metacentric series. In the submetacentric series, pairs 5, 7, 8 and 9 presented heterochromatic blocks in the terminal portion of the short arms, with the exception of pair 6, in which an intensely stained block was evidenced in the terminal portion of the long arm. In the st/a series, all chromosomes exhibited conspicuous pericentromeric blocks with the exception of pairs 13, 14 and 20, which presented weak staining for heterochromatic areas.

Silver nitrate impregnation revealed a single pair of Ag-positive NORs located in the terminal position of the short arms of st chromosome pair No. 12.

DISCUSSION

From the existing data, it is possible to generalize that a diploid number 2n = 46 is a conserved characteristic in the genus *Sparisoma*, although with marked variation in the karyotypic formula and fundamental number (NF = 70-84; TABLE 1). This variation would be closely linked to chromosomal mutation events, such as pericentric inversions, which would explain conserved diploid numbers but with variation in the arms number and, therefore, in the karyotype formula (Yoshida & Kitano 2021).

Pericentric inversions have been recognized as the primary factor of divergence among Perciformes (Molina 2007), and it has been indicated that although this type of structural change is a rare event, it constitutes an important source of karyotypic diversification in Labridae (Sena & Molina 2007; Sena & Molina 2008). Indeed, the nature of the inversions makes this type of mutation critical for accumulating differences and positively selected genes that cause incompatibility between species (Navarro & Barton 2003), explaining the appearance of pre- and postzygotic reproductive isolation mechanisms among closely related species (Kirkpatrick 2010).

In a cytogenetic study performed in *Sparisoma axillare*, it was observed that the first pair of the metacentric series was the largest and has a length of approximately twice that of the second pair in decreasing order (Sena & Molina 2007). This characteristic is shared by *S. radians* (Paim *et al*. 2014) and *S. chrysopterus* (this

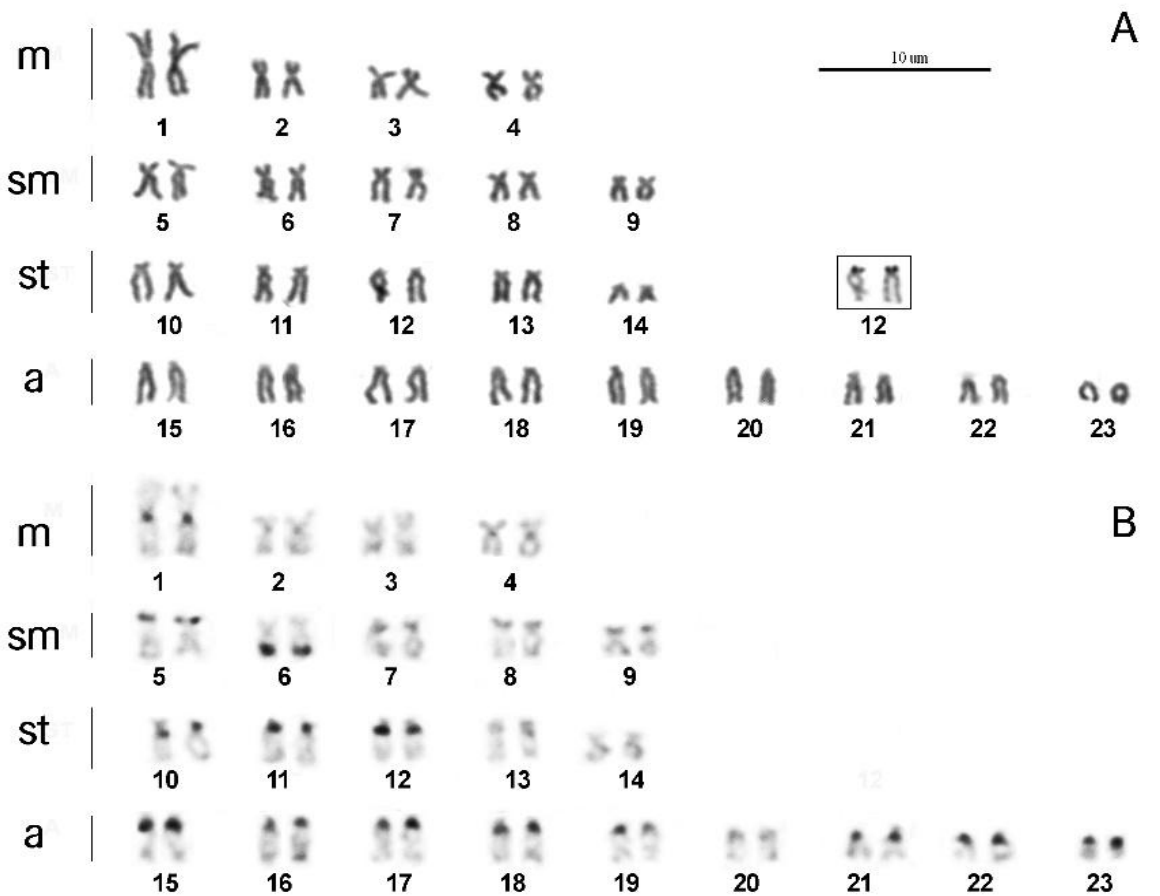

Figure 1. Conventional Giemsa stained (A) and C-banded (B) karyotype of *Sparisoma crysopterum*. m, metacentric; sm, submetacentric; st, subtelocentric; a, acrocentric. Inset shows the NOR-bearing chromosomes after sequential silver staining.

report), suggesting that it represents a plesiomorphic trait in the genus.

As observed in species of Scaridae analyzed to date for constitutive heterochromatin distribution, all species exhibit centromeric and pericentromeric blocks (Table 1). To date, terminal heterochromatic blocks have only been reported in S. radians (PAIM *et al*. 2014) and *S. chrysopterum* (present study).

The nucleolus organizer regions (NORs) contain the ribosomal genes (rRNA) 5.8S, 18S and 28S and have turned out to be important cytogenetic markers for studies of chromosomal evolution in fish (NIRCHIO & OLIVEIRA 2014). The presence of a single site (a pair of loci) for each rDNA group is a common feature in most teleost fishes (GORNUNG 2013; SOCHOROVÁ *et al.* 2018) and ancient nonteleost actinopterygians (MAJTÁNOVÁ *et al.* 2017, SYMONOVÁ *et al.* 2017), which has been interpreted as a primitive condition (plesiomorphic), while multiple NORs are usually a derived (apomorphic) trait (HSU *et al*. 1975).

Detection of active NORs thus far performed in species of *Sparisoma* has revealed only one pair of NORs located in the small arm of subtelocentric pair No. 11 in *S. chrysopterum*, on pair No. 14 in *S. radians* and in pair No. 14 in *S. axillare;* therefore, it is an important chromosomal marker in the genus. On the other hand, the presence of a single NOR variable position was also observed in Scarinae, which seems to be a characteristic of the Scaridae family (TABLE 1). Although these species represent 20% of the species in the group, the idea that microstructural changes involving ribosomal clusters may also have played an important role in the evolution of the karyotype within the genus cannot be ruled out.

The data presented here confirm that a) Scaridae have a conserved diploid number (46-48) but notable karyotypic variation and support that the occurrence of numerous pericentric inversion events could be responsible for modifying the karyotypic formula and b) microstructural alterations, likely translocations involving NORs, are frequent. Nonetheless, it is necessary to extend cytogenetic studies to a greater number of species to obtain a systematic view of chromosomal evolution in this group.

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TABLE 1. Available cytogenetic data for the Scaridae. Diploid number (2n), Fundamental Number (FN). subtelocentric (st), p = short arm = p

Species	2n	Karyotype	FN	C-banding*	Ag-NOR	Reference
Sparisomatinae						
Sparisoma axillare	46	6 m+14sm+4st+22a	70	cen, per	11st-p	SENA & MOLINA 2007
Sparisoma radians	46	24 m/sm+22st/a	84	cen, per, ter	14st-p	PAIM *et al*. 2014
Sparisoma chrysopterum	46	8 m+10sm+10st+18a	74	cen, per, ter	12st-p	This report
Calotomus japonicus	48	8 m+10sm+30st/a	66			ARAI & KOIKE 1980
Scarinae						
Chlorurus sordidus	48	18m+8sm+30st/a	66			ARAI & KOIKE, 1980
Scarus quoyi	48	4m+4sm+40st/a	56		6 a-p	KAEWSRI et al. 2014
Scarus trispinosus	48	6m+10sm+24st+8a	88		9-st-p	SENA & MOLINA 2007
Scarus coelestinus	48	6m+10sm+24st+8a	88	cen, per	9 st-p	SENA & MOLINA, 2007

*cen = centromeric, per = pericentromeric, ter = terminal.

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