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Robertsonian rearrangements and pericentric inversions in Scaridae fish (Perciformes)

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ABSTRACT. The parrotfishes (family Scaridae) are comprised of the subfamilies Sparisomatinae and Scarinae. They are important agents of marine bioerosion, which rework the substrate with their beaklike jaws. Despite their importance, there are no published cytogenetic data on this group. We made cytogenetic analyses of *Sparisoma axillare* (Sparisomatinae) and *Scarus coelestinus* (Scarinae) from the Brazilian coast. Differentiation in the diploid number in *S. axillare* compared to the basal karyotype of the Perciformes apparently occurred due to a Robertsonian fusion, combined with pericentric inversions. *S. coelestinus* presented a conserved diploid number, but showed considerable structural karyotypic changes, resulting mainly from pericentric inversions. The Ag-NOR sites were unique and located on the short arm of the 1st subtelocentric pair in both species (possibly homeologous), corresponding to the 11th pair in *S. axillare* and the 9th pair in *S. coelestinus*. The constitutive heterochromatin is reduced in these species and is distributed in centromeric and pericentromeric regions in most of the

chromosomes. The low fundamental number compared to the *Scarus* genus suggests a more basal condition for *Sparisoma.* The chromosome formula in *S. coelestinus* was more diversified, deriving from large-scale pericentric inversions. Karyotypic evolution patterns observed for these representatives of the Sparisomatinae and Scarinae subfamilies, added to new data from a larger number of species, would allow us to determine if there is a tendency among the Sparisomatinae for centric fusion events.

Key words: Sparisomatinae, Scarinae, Scaridae, Fish cytogenetics, Robertsonian rearrangements

INTRODUCTION

The Scaridae family is among the best-known groups of typical coral reef fish, with two subfamilies (Sparisomatinae and Scarinae), totaling 10 genera and 90 species, popularly known as parrotfishes (Sale, 1991; Parenti and Randall, 2000). The *Sparisoma* (Sparisomatinae) and *Scarus* (Scarinae) genera stand out from both an evolutionary and an ecological standpoint, because of their capacity to modify coral reefs by ingesting specific algae and because of complex social systems, including highly differentiated sexual stages, territoriality and defense of harems (Choat and Robertson, 1975; Bonaldo et al., 2006).

These genera are among the most representative groups of the Scaridae, aggregating almost 70% of all species. *Sparisoma*, recorded only from the Atlantic, is the most speciose scarid genus in the Atlantic (Bernardi et al., 2000; Streelman et al., 2002). The species presently know from Brazil are *S. amplum*, *S. axillare* and *S. frondosum* (Moura et al., 2001), and more recently a new species, *S. tuiupiranga* (Gasparini et al., 2003). Surveys on reef fish in the Atlantic show the Scaridae as one of the main representatives of reef environments (Floeter et al., 2001). In spite of the importance of the Scaridae, there are no karyotypic data on this group.

The phylogenetic position of the *Sparisoma* genus has been considered intermediary within the family, based on analyses of mitochondrial DNA with ribosomal gene probes 12S and 16S (Bernardi et al., 2000). To date about 8% of the species in the Perciformes have been karyotyped, revealing a modal diploid number of 2n = 48 chromosomes (Ohno, 1970; Le Grande and Fitzsimons, 1988; Affonso et al., 2001). However, karyotypes different from the typical Perciformes pattern have frequently been detected, indicating Robertsonian rearrangements as the preferential process in some groups, such as the Labridae and Pomacentridae subfamilies (Ueno and Takai, 2000; Molina and Galetti Jr., 2002).

Our objective was to obtain the first cytogenetic data for the Scaridae family by analysis of *S. axillare* (Sparisomatinae) and *Scarus coelestinus* (Scarinae), using conventional staining, Ag-nucleolar organizer regions (NORs) and C-banding.

MATERIAL AND METHODS

Specimens of *S. axillare* (three males, three females) were collected from the coast of Rio Grande do Norte State (Natal - 5º 46' S, 35º 12' W), and of *S. coelestinus* (four, sex undefined) from coastal reefs in Salvador (12º 58' S, 38º 31' W), Bahia, Brazil. The individuals were

submitted to mitotic stimulation overnight (Lee and Elder, 1980; Molina, 2002), before obtaining mitotic chromosomes (Gold et al., 1990). The heterochromatic pattern and the NORs were visualized by the methods described by Sumner (1972) and Howell and Black (1980), respectively.

RESULTS

A diploid number of 2n = 46 chromosomes (6m + 14sm + 4st + 22a, fundamental number, FN = 70) (Figure 1A) was observed in *S. axillare*. The first pair of the karyotype consisted of large metacentrics, twice the size of the second pair. Ag-staining showed single NOR sites located on the short arm of the 11th pair (larger subtelocentric pair, Figure 1). Heterochromatic centromeric and pericentromeric regions were observed on the long arm of various chromosome pairs.

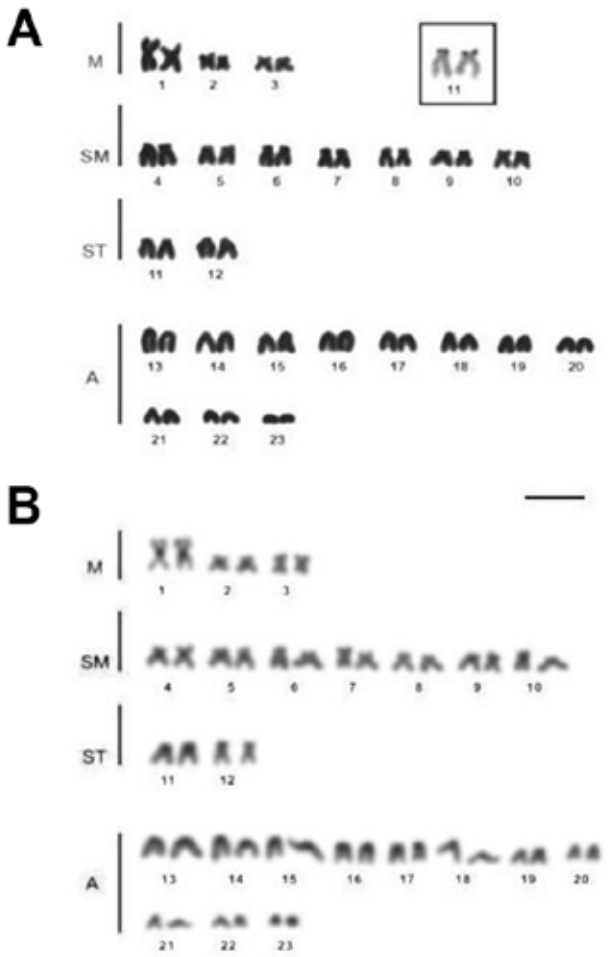

Figure 1. Karyotypes of *Sparisoma axillare*. **A.** Conventional staining by Giemsa. The Ag-NOR pair (11th) is shown in the highlighted box. **B.** C-banding pattern. M = metacentric; SM = submetacentric; ST = subtelocentric; A = acrocentric. Bar = 5 μm.

The *S. coelestinus* karyotype presented a diploid number of 2n = 48 chromosomes (6m + 10sm + 24st + 8a, FN = 88, Figure 2). NOR sites were visualized on the short arm of the 9th pair, the largest subtelocentric pair, as in *S. axillare* (Figure 2, box). Heterochromatic regions were distributed in centromeric and pericentromeric positions. The NOR regions were markedly heterochromatic in both species (Figures 1B and 2B).

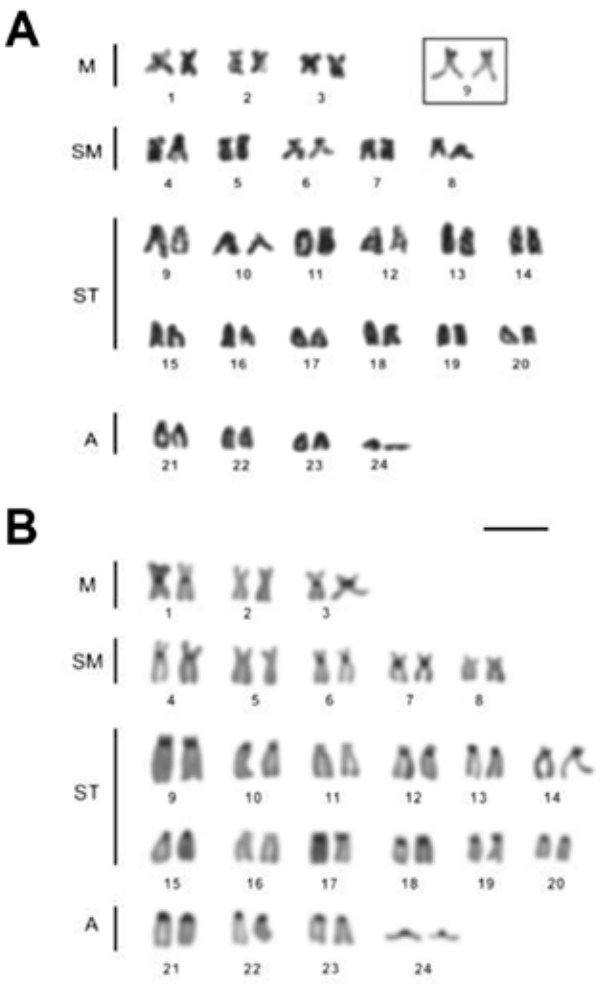

Figure 2. Karyotypes of *Scarus coelestinus*. **A.** Conventional staining and the nucleolar organizing pair (9th). **B.** Centromeric and pericentromeric heterochromatic regions evidenced by C-banding. M = metacentric; SM = submetacentric; ST = subtelocentric; A = acrocentric. Bar = 5 µm.

DISCUSSION

The behavioral, physiological and biological diversity of some reef fish has derived from events that occurred within a relatively long period since the development of modern environments, such as coral reefs; it is partially a consequence of the many historic rises and

falls in sea level (Bellwood, 1996). This evolutionary diversity is also reflected in some chromosome characteristics.

A karyotype consisting of 48 acrocentric chromosomes has been considered the basal pattern for the Perciformes (Ohno, 1974; Brum and Galetti Jr., 1997). This tendency has been observed in most of the species of this order. This karyotypic constitution seems to be more commonly found in marine than in freshwater species (Brum et al., 1995; Galetti Jr. et al., 2000; Affonso et al., 2001).

Robertsonian rearrangements, although they are rare events, have been pointed to as an important source of karyotypic diversification in some groups of fish, such as in some Labridae and Pomacentridae subfamilies (Ueno and Takai, 2000; Molina and Galetti Jr., 2002). However, a high FN indicates that pericentric inversion occurred widely in the karyotypic evolution of these species. Pericentric inversions have been considered to be the main factor responsible for the divergences among the Perciformes (Molina, 2006).

S. axillare presented a different chromosome number (2n = 46), indicating numerical reduction compared to *S. coelestinus* (2n = 48). The presence of a large metacentric pair (1st pair) in *S. axillare*, added to a reduced chromosome number (compared to the base karyotype 2n = 48a, symplesiomorphic for the Perciformes) suggests a Robertsonian fusion event, which, associated with pericentric inversion, may have molded the karyotype of this species. The distribution of the ribosomal sites apparently located on homologous pairs (1st subtelocentric pair) in *S. axillare* and *S. coelestinus*, which remained constant throughout the evolution of the family, reinforces the phylogenetic relationship between the Sparisomatinae and the Scarinae.

Characteristics such as reduced heterochromatin content located in centromeric position in both species were similar to those observed in other Perciformes, such as the Pomacentridae (Molina and Galetti Jr., 2002; Molina, 2006), as well as the Priacanthidae and the Gerreidae (Molina and Bacurau, 2006). Heterochromatin blocks coinciding with NOR sites in these species followed the pattern established previously for many groups of fish, mainly within the Perciformes.

Additional cytogenetic data for other representatives of this family will allow us to determine whether the characteristics observed in these representatives of the Sparisomatinae and Scarinae are individual and random, or reflect patterns for these subfamilies. In the face of the structural aspects of the karyotypes of these two species, a unique cause of differentiation among the groups could not be established; however, a marked prevalence of pericentric inversions was observed.

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