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Research Article

# Cytogenetic analysis in western Atlantic snappers (Perciformes, Lutjanidae)

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## Abstract

The Lutjanidae or snappers are a family of perciform fishes, mainly marine but with some members living in estuaries and entering fresh water to feed. Some are important food fish. Cytogenetic data for Lutjanidae are scarce. In the present work, we cytogenetically characterized through conventional Giemsa staining techniques, Ag-NOR and C-banding the species *Ocyurus chrysurus*, *Lutjanus analis*, *L. alexandrei*, *L. cyanopterus*, *L. jocu* and *L. synagris*, all found along the Brazilian coast. Karyotype analysis of all six species showed a modal value of 2n = 48 acrocentric chromosomes. Single NORs were found at pericentromeric position on the long arms of the 2nd pair in *O. chrysurus*, *L. alexandrei* and *L. cyanopterus*, on the 5th pair in *L. analis* and on the 23rd pair in *L. synagris*. The species *L. jocu* presented multiple NORs located on the 2nd pair at a pericentromeric region and on the 5th pair at a telomeric region. Heterochromatic blocks were identified at the centromeric region of all chromosomes of the studied species. These results indicate that, despite of the chromosomal stability of this family, a relative structural diversification seems to have occurred in the chromosome evolution of the group. Such diversification was evidenced by divergent number and location of ribosomal sites among species. The NOR-bearing pairs represented an efficient cytotaxonomic marker for most of the analyzed species. The data suggest that the presence of interstitially located single NORs on a large acrocentric pair should represent a basal condition for lutjanids.

*Key words:* chromosome evolution, fish cytogenetics, Lutjanidae, *Lutjanus*, *Ocyurus*.

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## Introduction

Species from the family Lutjanidae represent one of the major resources for marine fishery (Resende *et al.*, 2003). This family comprises 17 genera and nearly 105 species mainly inhabiting the marine realm, with few species living in estuarine environments. Lutjanids are widespread over reef areas of tropical and subtropical regions, throughout Atlantic, Pacific and Indian oceans (Nelson, 2006). In Brazil, they are distributed along the entire seashore, where four genera from two subfamilies (Etelinae and Lutjaninae) are reported: *Lutjanus*, comprising eight species (Moura and Lindeman, 2007), and the monotypic genera *Ocyurus*, *Rhomboplites* and *Etelis* (Cervigón, 1993). Lutjanids are represented by medium to large demersal fish species up to 1 m in length or more that are able to live at great depths, and are popularly known as snappers (Nelson, 2006). They are great predators, playing a major role on their ecosystem (España, 2003). Most species from this family grow slowly, are long-lived (20 to 30 years) (Polovina and Ralston, 1987), and are highly vulnerable to overfishing (Coleman *et al.*, 2000).

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Up to now, cytogenetic data in Lutjanidae have been published only for some species; *Lutjanus argentimaculatus* (2n = 48a) (Raghunat and Prasad, 1980), *L. kasmira* (2n = 48a) (Choudhury *et al.*, 1979), *L. sanguineus* (2n = 48a) (Rishi, 1973), and *L. quinquelineatus* (2n = 48, female; 2n = 47, male) (Ueno and Takai, 2008). Based on both ecological and economical relevance of the family Lutjanidae, coupled with the lack of genetic information about the group, the goal of the present study was to cytogenetically analyze the species *Lutjanus analis, L. alexandrei, L. cyanopterus, L. jocu, L. synagris* and *Ocyurus chrysurus*, from the northeastern coast of Brazil, through conventional Giemsa staining, Ag-NOR and C-banding.

## Material and Methods

Six Lutjanidae species from Brazilian northeastern coast were analyzed. The species *Lutjanus analis* (4 male and 6 immature), *L. cyanopterus* (2 male), and *L. jocu* (1 female, 2 male and 1 immature) were collected in the estuary from Potengi River - Natal, Rio Grande do Norte (RN)

(5°46'43" S; 35°13'5" W). Samples of *L. synagris* (5 female, 1 male and 3 immature) and *L. alexandrei* (7 female and 2 male) came from the city of Muriú (5°33'41" S; 35°14'17" W) and Potengi River, RN while *Ocyurus chrysurus* (5 female) specimens were collected at shallow reefs in the city of Maracajaú, RN (5°23' S; 35°15' W).

Prior to *in vitro* preparation of mitotic chromosomes (Gold *et al.*, 1990), the animals were submitted to mitotic stimulation (Lee and Elder, 1980). The nucleolar organizer regions (NORs) were detected following the technique described by Howell and Black (1980). Heterochromatin regions were identified through C-banding according to Sumner (1972). The chromosomal preparations were photographed using a photomicroscope (Olympus BX42) equipped with a digital imaging system DP72. The chromosomes were arranged in a decreasing size order and classified according to Levan *et al.* (1964).

## Results

The cytogenetic analyses of *Lutjanus analis*, *L. alexandrei*, *L. cyanopterus*, *L. jocu*, *L. synagris* and *Ocyurus chrysurus* revealed a modal value of 2n = 48 acrocentric chromosomes (FN = 48) (Figures 1-6A).

C-banding showed reduced heterochromatic blocks, located on centromeres of the species analyzed (Figures 1-6B), except in *L. analis* and *O. chrysurus* which presented large and sharp heterochromatic regions at centromeric position in all chromosomes.

Interstitial single NORs were identified in five from the six species studied (Figure 7), equivalent to the secondary constrictions. Active ribosomal sites were located at pericentromeric region, of the 2nd pair in *L. alexandrei*, *L. cyanopterus* and *O. chrysurus*, of the 5th pair in *L. analis*, and of the 23rd pair in *L. synagris*. Multiple NORs were found at pericentromeric region on the 2nd pair and at telomeric position on the 5th chromosomal pair in *L. jocu* (Figure 3A, detail). A numerical polymorphism in NOR sites (one to two positively stained homologous chromosomes) was observed in most of analyzed species (data not shown).

## Discussion

Conservative karyotypes bearing a diploid value of 2n = 48 (FN = 48) are usually regarded as a typical condition of a great number of perch-like species, putatively associated with decreased levels of genetic variation among marine populations (Molina and Galetti, 2004). Therefore, the occurrence of a large number of migratory or dispersive individuals (long-phase larval planktonic stage), coupled with weak geographical barriers, may be responsible for an increased gene flow, thus leading to a genetic homogeneity in marine fish populations (Brum, 1995; Molina *et al.*, 2002), reflected as unchanged karyotypes.

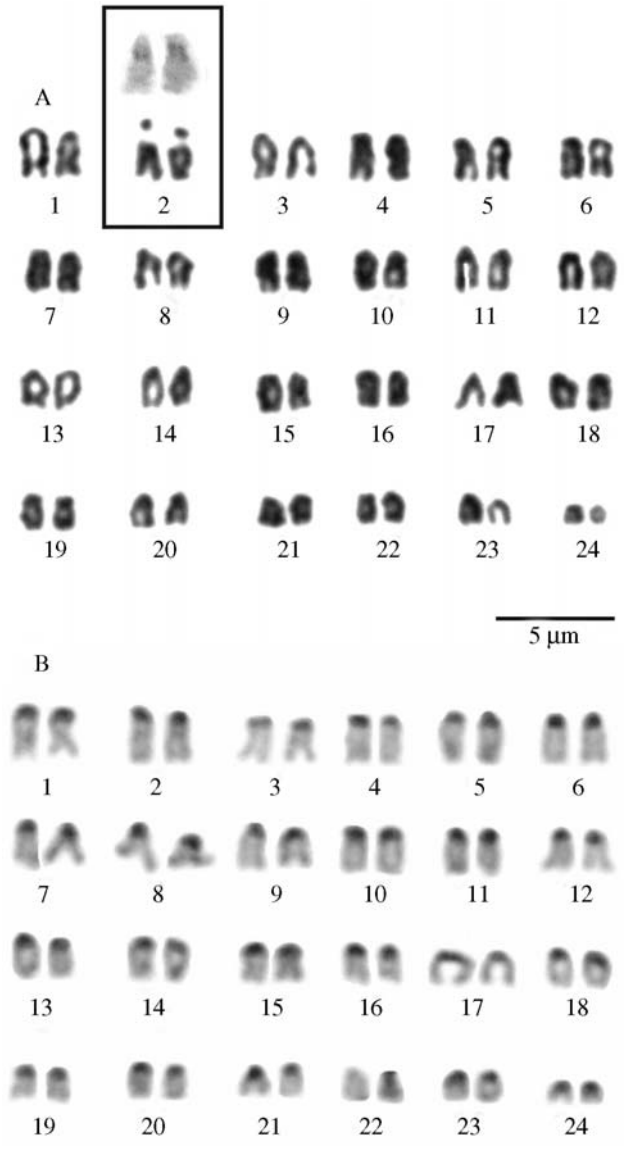


**Figure 1** - Karyotype of *Ocyurus chrysurus*. (A) Conventional Giemsa staining. In detail, the secondary constrictions equivalent to NORs. (B) C-banding in *Ocyurus chrysurus*.

Previous studies have shown that there is a close relationship between karyotypic homogeneity and duration of the larval period. Species exhibiting a long-term larval phase tend to present more stable karyotypes and vice versa (Molina and Galetti, 2004). Information about the larval dispersal phase (LDP) are available for some Lutjanidae species, ranging from 25 to 40 days (Allman and Grimes, 2002; Sponaugle *et al.*, 2003; Domeier, 2004). Such a larval pelagic period might be considered as relatively long, likely favoring karyotypic homogeneity observed in the analyzed species, which share the conserved karyotype pattern of Perciformes, with 48 acrocentric chromosomes (Molina *et al.*, 2002).

Another fact that should be taken into account for the maintenance or diversification of Perciformes karyotypes

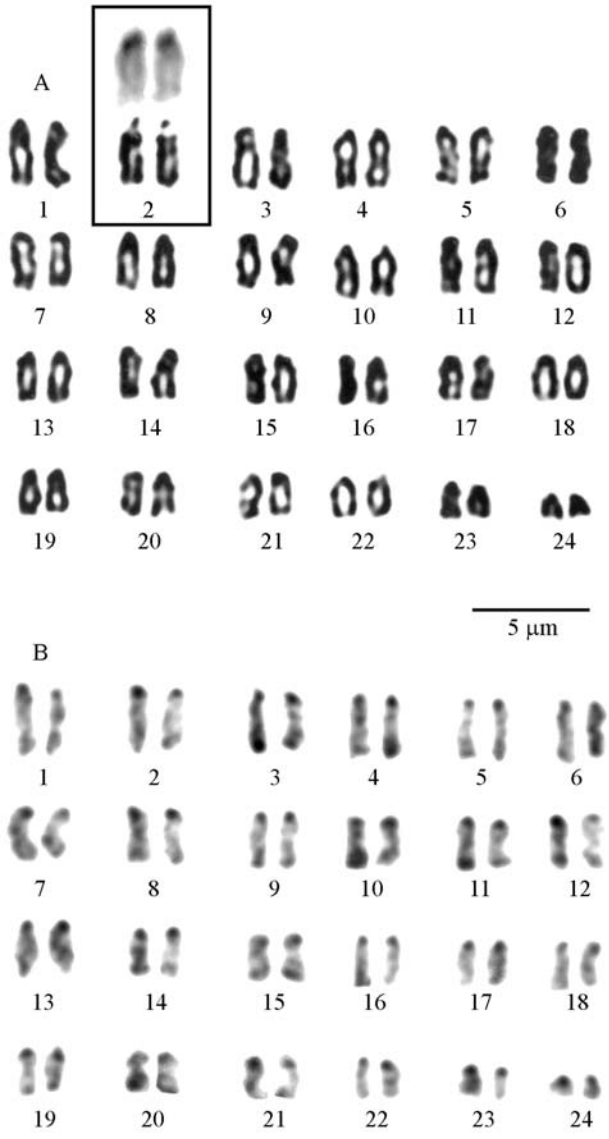


**Figure 2** - Karyotype of *Lutjanus alexandrei.* (A) Conventional Giemsa staining. In detail, the secondary constrictions equivalent to NORs. (B) C-banding in *L. apodus*.

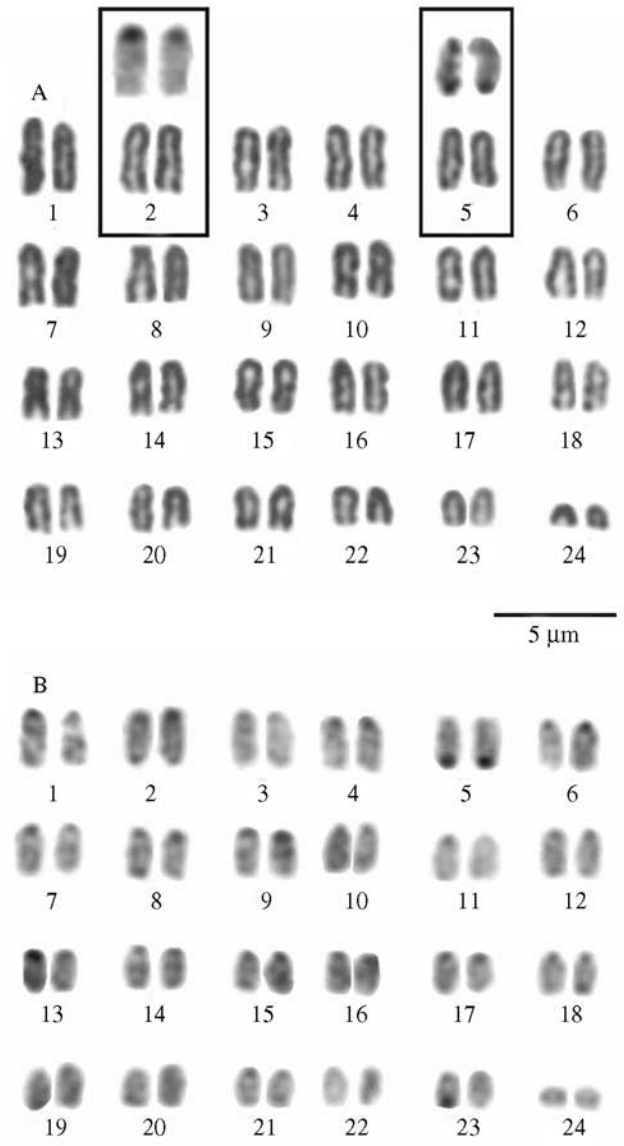


**Figure 3** - Karyotype of *Lutjanus jocu*. (A) Conventional Giemsa staining. In detail, the NOR-bearing pairs. (B) C-banding in *L. jocu*.

is the role of heterochromatin in chromosomal dynamics (Molina, 2006). Such segments can be amplified or accumulated by unequal crossovers, transpositions and/or duplications, as suggested for the marine pigmy angelfish, *Centropyge aurantonotus* (Affonso and Galetti, 2005). Evidence of karyotypic differentiation related to variation in number, size and location of heterochromatic segments has been extensively provided in fish species (Moreira-Filho *et al.*, 1984; Galetti *et al.*, 1991; Bertollo, 1996; Margarido and Galetti, 1996 among others). Cases of heterochromatin polymorphism might range from subtle to hypervariable in fish. They can be related to the size of the heterochromatic blocks (Martinez *et al.*, 1991; Jankun *et al.*, 1998) or to associations between heterochromatin and NORs (Hartley, 1988; Amores *et al.*, 1993). Some groups, particularly the Characiformes, present a remarkable karyotype variability influenced by heterochromatinization processes, as extensively seen in the species complex *Astyanax scabripinnis* (Souza *et al.*, 1996; Mantovani *et al.*, 2000; Mantovani *et al.*, 2004).

The reduced heterochromatin content over centromeric regions in chromosomes of the four lutjanids herein studied *(Lutjanus alexandrei, L. cyanopterus, L. synagris* and *L. jocu*) reveals a similar feature to that observed in several species of the families Haemulidae, Serranidae and Pomacentridae (Molina, 2006). Such a condition seems to be frequent among lutjanids and apparently has a considerable bearing on the stabilization of karyotypic differentiation processes, mainly numerical ones (Molina and Galetti, 2004). However, the chromosomal conservativeness in

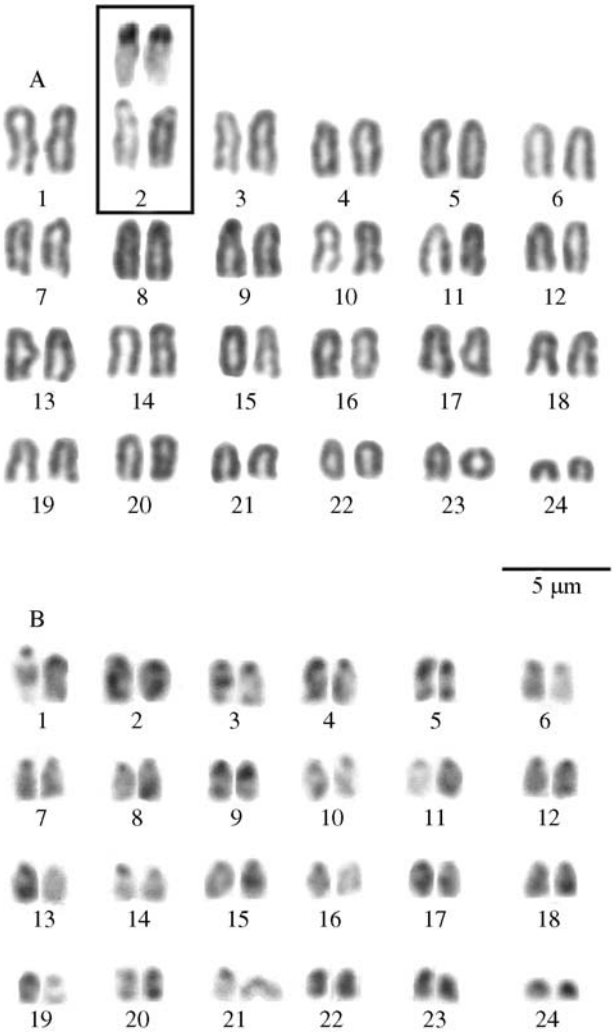


**Figure 4** - Karyotype of *Lutjanus cyanopterus*. (A) Conventional Giemsa staining. In detail, the secondary constrictions equivalent to NORs. (B) C-banding in *Lutjanus cyanopterus*.

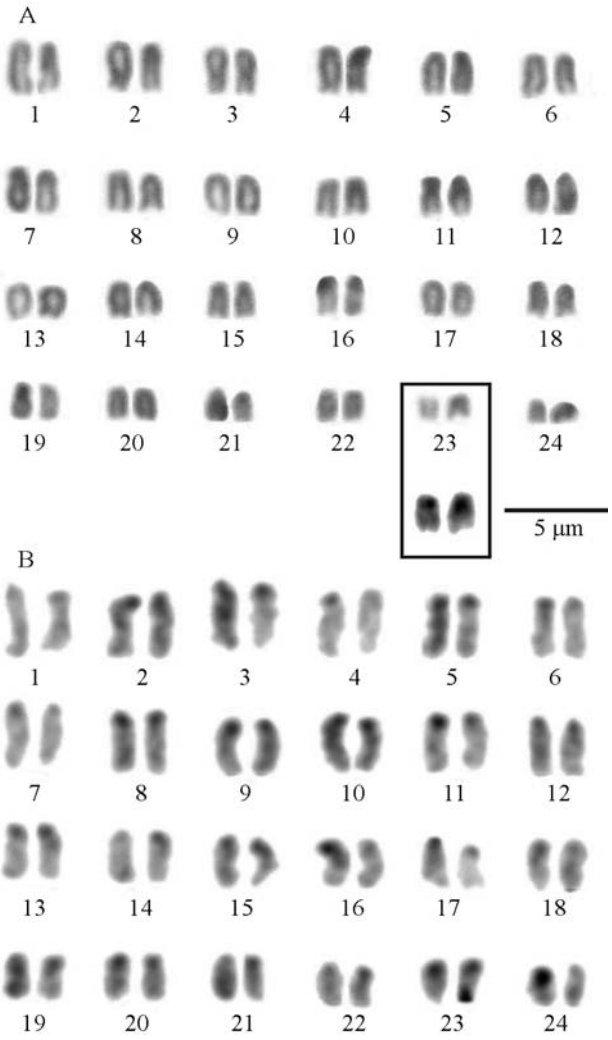


**Figure 5** - Karyotype of *Lutjanus synagris*. (A) Conventional Giemsa staining. In detail, the secondary constrictions equivalent to NORs. (B) C-banding in *Lutjanus synagris*.

snappers does not exclude the presence of some degree of structural diversification, presently undetected.

Conserved karyotypes, concerning both diploid number and chromosomal types, have been extensively identified within the family Haemulidae, phylogenetically related to Lutjanidae. Several genera, such as *Haemulon*, *Conodon*, *Pomadasys* and *Anisotremus* have presented a typical perch-like karyotype (2n = 48a). These results reveal a basal condition shared by both families. Possibly, the lack of major rearrangements in these groups was replaced by internal changes in linkage clusters, apparently as effective as the former in establishing post-zygotic barriers during speciation processes (Molina, 2006).

An additional feature shared by Lutjanidae and Haemulidae is the presence of single NORs located at an interstitial position on acrocentric chromosomes. Such a condition was observed in *L. alexandrei, L. analis*, *L. cyanopterus*, *L. synagris* and *O. chrysurus* and it is regarded as a basal feature in several Perciformes families, such as Serranidae (Aguilar and Galetti, 1997), Pomacanthidae (Affonso *et al.*, 2001), among others, thus corroborating the presupposition that this should be a basic pattern for the order (Galetti *et al.*, 2000; Affonso and Galetti, 2005).

The karyotypes of the species *O. chrysurus* and *L. analis*, which bear elevated heterochromatin contents, suggest that heterochromatinization processes have occurred independently and represent a derived character for this family. However, despite the macrostructural similarity among karyotypes, the lutjanids presented a relative diversification in the location of ribosomal sites. Thus, interstitial Ag-NORs on large chromosomal pairs have been detected in *L. alexandrei, L. cyanopterus* and *O. chrysurus* ($2^{nd}$ pair), on middle-sized chromosomes in *L. analis* ($5^{th}$ pair), and on small ones in *L. synagris* ($23^{rd}$ pair), or even as multiple sites in *L. jocu* ($2^{nd}$ and $5^{th}$ pairs).

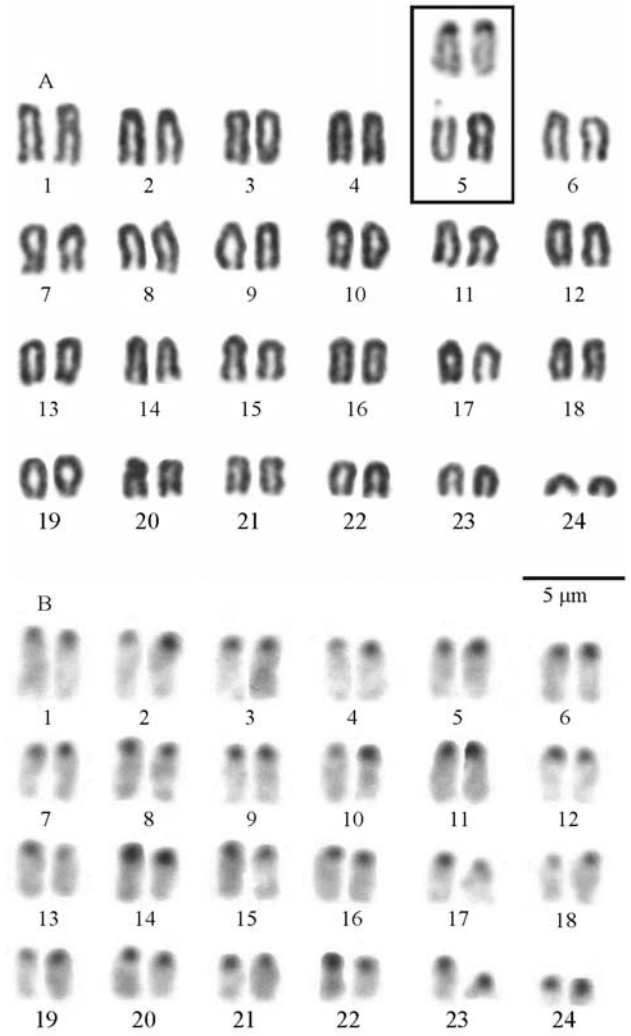


**Figure 6** - Karyotype of *Lutjanus analis*. (A) Conventional Giemsa staining. In detail, the secondary constrictions equivalent to NORs. (B) C-banding in *Lutjanus analis*.

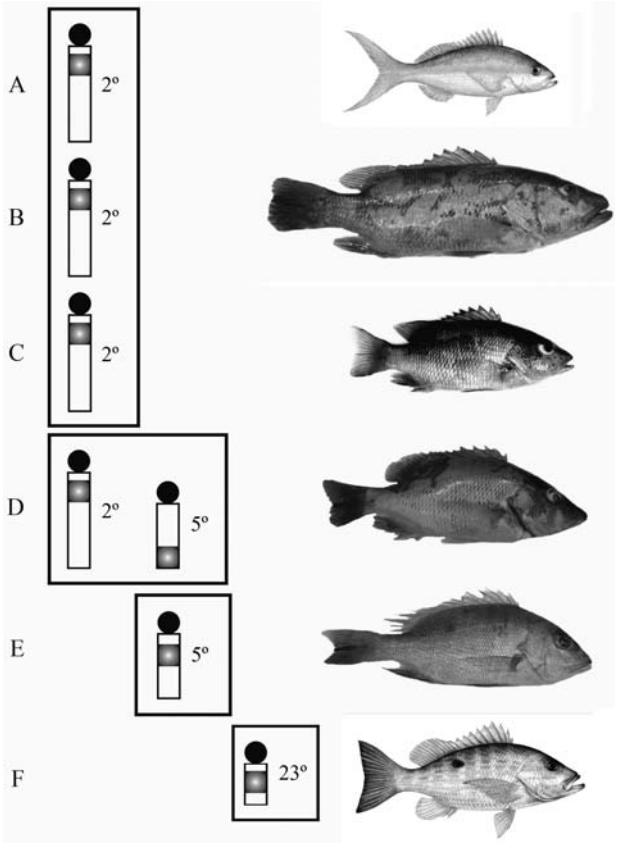


**Figure 7** - Representative picture of the number and location of ribosomal sites on NOR-bearing chromosomes of *O. chrysurus* (A), *L. cyanopterus* (B), *L. alexandrei* (C), *L. jocu* (D), *L. analis* (E), and *L. synagris* (F).

Multiple NORs have rarely been reported in Perciformes (Galetti *et al.*, 2000), although they are common in Characiformes and Siluriformes (Mantovani *et al.*, 2000; Paintner-Marques *et al.*, 2002). Mostly, such variation results from chromosomal rearrangements, but transposition events have also been indicated as one major factor for the numerical variability of NORs in fishes (Galetti *et al.*, 1995; Almeida-Toledo *et al.*, 1996; Castro *et al.*, 1996; Vitturi *et al.*, 1996).

The nucleolar organizer regions are effective cytotaxonomic markers in Lutjanidae and allowed us to distinguish most of the analyzed species, except for *O. chrysurus*, *L. alexandrei* and *L. cyanopterus*, in which the ribosomal sites were similarly located on the same chromosomal pair (2nd pair). Cytogenetic studies in other groups with conserved karyotypes have also demonstrated the same discriminatory ability of NORs, such as in Anostomidae (Galetti *et al.*, 1984) and in some Cichlidae (Brinn *et al.*, 2004). However, the efficiency of ribosomal sites as a cytotaxonomic marker is not applicable to all situations, since these regions might remain unchanged on homeologous chromosomes of several species (Feldberg and Bertollo, 1985; Molina and Galetti, 2004).

Some phylogenetic inferences have been drawn from morphometric features (Rivas, 1966; Vergara 1980; Chow and Walsh, 1992), allozymes (Chow and Walsh, 1992) and mtDNA sequences (Sarver *et al.*, 1996) for the family Lutjanidae. From these inferences a consensus of closer phylogenetic relationships among *L. apodus*, *L. griseus*, *L. synagris* and *L. analis* emerged.

The presence of ribosomal sites on a large acrocentric pair has been considered a plesiomorphic characteristic amongst Perciformes, being typical of Serranidae (Molina *et al.*, 2002), Pomacanthidae and Chaetodontidae (Affonso *et al.*, 2001), and Cichlidae (Feldberg and Bertollo, 1985). Analyzing the karyotypes of Lutjanidae species considered by phylogenetic studies as some of the most derived ones (such as *L. jocu*, *L. analis* and *L. synagris*) we observed that NORs were significantly more variable in these species (pairs 5/2, 5 and 23, respectively).

Phylogenetic data in Lutjanidae, based on mitochondrial sequences, indicated that *Ocyurus* is the most closely

related genus to *Lutjanus* and that *L. cyanopterus* would represent the most basal form in this clade (Sarver *et al.*, 1996). These data are corroborated by the present cytogenetic analyses. These species present conserved karyotypes with respect to their macrostructure and bear ribosomal sites at an interstitial position on the second chromosomal pair, a plesiomorphic condition for the family that is also found in the species *L. alexandrei*.

Up to now, there are no conclusive theories to explain the chromosomal conservativeness observed in several Perciformes families, such as the Lutjanidae, although some hypotheses have been proposed, mainly those involving karyotypic orthoselection (Molina *et al.*, 2002; Molina and Galetti, 2002; Molina, 2006). Studies utilizing methods of higher banding frequency and localization of specific sequences in the karyotype might help to elucidate the actual degree of chromosomal conservativeness for this group and for other Perciformes representatives, further contributing to our understanding of their evolutionary patterns.

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