INTRODUCTION

The family Serranidae is one of the most important marine fish group, as many species are of commercial value and present particular biological traits. Serranids present a great size, shape and color variation, since species no longer than 3,5 centimeters until others with more than 2 meters and 300 kilograms. Despite of such variation, there are some examples of cryptic species, which external differences are restrained to color patterns, and most of them lack morphological specializations currently used to identify individual species (RANDALL 1995). Sex determination is also peculiar; Serraninae species are synchronous hermaphrodites (genera Serranus and Hypoplectrus), while groupers and allies - Epinephelinae (genera Alphestes, Epinephelus, Mycteroperca, and Cephalopholis) present asynchronous hermaphroditism (OHNO 1974). Cirrrotropical, they are typical of rocky and coral bottoms (FIGUEIREDO and MENEZES 1980).

Considered as a monophyletic group, the family Serranidae is divided into three subfamilies, nearly 60 genera and 449 species, half of them belonging to the subfamily Epinephelinae (NELSON 1994). From this total, less than 0,5% was karyotyped (KLINKHARDT 1998). In contrast to species diversification, the cytogenetical reports on Serranidae have revealed conserved chromosomal patterns (2n and NF equal or close to 48).
The processes involved to the maintenance of stable karyotypes in morphologically diverse species are still unknown.

In the present work, cytogenetical analyses by conventional staining, Ag-NOR and C-banding were carried out on three species of wide geographical distribution at Western Atlantic (MENEZES and FIGUEIREDO 1980; HUMANN 1994), *Epinephelus adscensionis* and *Alphestes afer* from subfamily Epinephelinae and *Serranus flavigventris* from Serraninae.

**MATERIAL AND METHODS**

Six individuals of *Epinephelus adscensionis*, five of *Alphestes afer* and one of *Serranus flavigventris* were collected along the coastline of Rio Grande do Norte and Bahia States, at Northeastern region of Brazil. Mitotic stimulation method (LEE and ELDER 1980) was performed prior to *in vitro* chromosomal preparations (GOLD et al. 1990). Heterochromatin patterns were obtained by C-banding (SUMNER 1972). The nucleolar organizer regions (NOR) were identified by silver nitrate staining (HOWELL and BLACK 1980).

**RESULTS**

The cytogenetical surveys in *A. afer*, *E. adscensionis* and *S. flavigventris* showed that such Serranidae species share a similar karyotype, composed by 48 acrocentric chromosomes, characterized by slight size differences amongst chromosomal pairs (Figs. 1, 2, and 3). In several metaphases from *E. adscensionis* and *A. afer*, interstitial secondary constrictions on the smallest chromosomal pair (24th) were present.

The constitutive heterochromatin is located on centromeric regions of all chromosomes, without any difference between each species (Figs. 1, 2, and 3).

In *A. afer*, single Ag-NOR sites, equivalent to the secondary constrictions, were observed on the 24th pair (Fig. 1, box). Single NORs were also detected in *S. flavigventris*, but located on a medium size chromosomal pair (16th) (Fig. 3, box). In *E. adscensionis*, besides interstitial signals on 24th pair, an additional NOR site was sporadically seen at telomeric position of a large chromosomal pair (2nd) (Fig. 2, box). This NOR polymorphic condition was quantified over 38 metaphases, revealing that active interstitial NORs on 24th pair were the most common NOR phenotype (94.74%) (for details, see Fig. 4 A-H).

**DISCUSSION**

Previous cytogenetical reports on the genera *Cromileptis* (TAKAI and OJIMA 1986), *Epinephelus* (MARTINEZ et al. 1989; SOLA et al. 2000, among others), *Mycteroperca*, and other Serranidae species (AGUILAR and GALETTI 1997) have showed a remarkable numerical (*2n*=48) and structural chromosome homogeneity with several acrocentric chromosomes and a common heterochronomic distribution at centromeric or pericentromeric position.

NORs in Serranidae are, most frequently, single and located at interstitial position (AGUILAR and GALETTI 1997). The presence of ribosomal sites on the smallest chromosomal pair (24th) is conserved within the genus *Epinephelus* (SOLA et al. 2000, present study). The same NOR-bearing pair is also found in *Alphestes* (present study) and *Mycteroperca* species (AGUILAR and GALETTI 1997), revealing phylogenetic affinities among the three groups. This evidence supports the paraphyletic hypothesis of the genus *Epinephelus* as revealed by mtDNA analyses (CRAIG et al. 2001).

The detection of extra NOR sites on large chromosomes (2nd) in *Epinephelus adscensionis* is similar to the pattern described in *E. marginatus* (SOLA et al. 2000), what indicates a simple-sisomorphic condition. Further, if we assume that the occurrence of single NORs is an ancestral trait of Teleostei (FORESTI et al. 1981; AFFONSO 2000), the multiple NOR sites in *Epinephelus*, the most species-rich Epinephelinae genus, would indicate that this genus is more derived than *Alphestes afer*.

In contrast to the presence of small NOR-bearing pairs, as observed in Epinephelinae, the NOR sites in Serraninae are rather situated at interstitial position on large chromosomes (VITTURI et al. 1993, present study), indicating low affinities between these subfamilies.

Cytogenetical analyses of 10 Epinephelinae species (KLINKHARDT 1998) revealed a common chromosomal pattern that supports the monophyly of the group (CRAIG et al. 2001). This asynchrony between exophenotype (morphological traits) and endophenotype (karyotypical pattern) evolutionary rates suggests that the speciation process in some (if not most) marine groups was...
Fig. 1 – Karyotypes on *Alphestes afer*. (A) Conventional staining, (B) C-banding showing conspicuous heterochromatic blocks at pericentromeric regions. In the box, the single nucleolar organizer chromosomal pair (24th) after silver nitrate staining.
Fig. 2 – *Epinephelus adscensionis*. (A) Karyotype by conventional staining, and (B) by C-banding. On inset, it is displayed a numerical polymorphism involving NOR-bearing chromosomes from 24th and 2nd pairs.
Fig. 3 – Karyotypes of *Serranus flaviventris* by (A) conventional staining and (B) C-banding. NOR sites are located on a medium size chromosomal pair (16th), as shown in the box.
mainly established by pre-zygotic mechanisms of reproductive isolation.

According to Arkhipchuk and Berdyshev (KLINKHARDT 1998), a greater karyotype diversification is found in higher taxonomical levels, such as Orders, while in lower categories it is reduced (families, genera and species). This is to be expected, as high taxonomical levels have a longer time of divergence. However, sometimes, drastic karyotypical modifications can be found even in species complex, as such as *Astyanax scabripinnis* (Characidae) (MOREIRA-FILHO and BERTOLLO 1991). In others groups, as Serranidae, the karyotype structure have resisted to several speciation pathways, occasionally associated to morphological changes. The fixation of such diversity (radiation) seems to be enhanced by the relatively long period since the appearance of modern coral reefs, about 50 million years ago in the early Tertiary (PAXTON 1995).

On the other hand, a stable chromosomal trend runs parallel to the low levels of genetic variation between populations of several marine fishes. This seems to be associated to the population structure of marine species, where a large number of individuals, dispersal (larval stage) and migratory abilities (adults), coupled with poorly defined physical barriers, should be responsible for a high gene flow, leading to genetical homogeneity (SMITH et al. 1990; LACSON 1992; WARD et al. 1994, among others). Indeed, some Serranidae species (e.g. *Epinephelus striatus*) form large aggregations at particular localities during the spawning time (BOLDEN 2000). Most of the individuals have to migrate great distances to these sites, and so, the fixation of interpopulational genetic differences would be avoided.

Divergent karyotypical patterns in Perciformes are often described in freshwater species, mostly represented by Cichlidae and Percidae (OHNO 1974), or in sedentary marine groups such as Gobiidae and Blenniidae (BRUM and GALETI 1997). This evidence seems to corroborate the role of geographic isolation and small populations on the origin and fixation of chromosomal rearrangements (GALETI et al. 2000).

Some theories have been used to explain the “enigma of 48 acrocentric chromosomes” in Perciformes (OHNO 1974). According to ÁLVAREZ et al. (1983), it possibly reflects a neutral karyotype in the speciation process and phyletic evolution. We believe that such simplesiomorphic pattern could be useful to elucidate phylogenetic aspects within Perciformes, a putative polyphyletic group (NELSON 1994).

It is possible that the karyotype structure seen in Serranidae (reduced heterochromatin amounts, interstitial NORs), associated to peculiar behav-
ioral and biological features, such as hermaphroditism and huge spawning aggregations, could be constraining chromosomal changes. These factors, not acting over the morphological diversification, would contribute for the observed asynchrony between karyotype (endophenotype) and morphology (exophenotype) of Serranidae species.

REFERENCES


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