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First chromosome Characterization in the Neotropical Eel, *Gymnothorax ocellatus* (Pisces, Muraenidae)

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Summary Cytogenetics studies in 12 specimens of *Gymnothorax ocellatus* reveled a diploid chromosome number of $2n=42$ (16 metacentrics, 18 submetacentrics and 8 acrocentrics). The nucleolar organizer regions were located in a terminal position on the long arm of the chromosome pair number fifteen. Conspicuous blocks of constitutive heterochromatin were observed in the centromeric and pericentromeric regions of some chromosome pairs. The results obtained are similar to those previously described for others species of this family. However, the cytogenetic informations may be useful in the identification of a possible variety of this species in Brazilian coast and contribute to the understanding of relationships among the species and the process of diversification which occurred in this group.

Key words Fish cytogenetics, Chromosome, Eels, *Gymnothorax ocellatus*.

Among the fish species found in the Brazilian oceanic waters, about 44 have been investigated under cytogenetics perspective (Brum *et al.* 1992), and this number reaches now to 70 species, including representatives of 6 orders, 23 families and 38 genus, belonging to the class Osteichthyes and the infraclass Teleostei (Oliveira, C. personal communication).

The Order Anguiliformes presents a large number of species (Nelson 1994), with the diploid chromosome number varying from $2n=26$ to $2n=54$ (Klinkhardt *et al.* 1995). Among the eleven genus described in the family Muraenidae, only representatives of three genus were studied cytogenetically until now, with diploid numbers ranging from $2n=36$ to $2n=42$, being $2n=42$ chromosomes the most frequent diploid number observed. According to the same authors the genus *Gymnothorax* is the best studied in this family with four species cytogenetically analyzed (Table 1).

Specific techniques have been used recently, early and late replication bandings have been obtained by *in vitro* BrdU incorporation in the Mediterranean Muraenidae species *Muraena helena* and *Gymnothorax unicolor* (Salvadori *et al.* 2003).

Gymnothorax ocellatus is a quite common species in the Brazilian coast, and presents a wide distribution. However, Carvalho-Filho (1992) points that a taxonomic revision in this species is necessary, since some discussions concerning the number of valid species still remain unresolved. The objective of the present work was to characterize the karyotype of *Gymnothorax ocellatus*, captured in the Ubatuba Bay, São Paulo, as well as to localize the nucleolus organizer regions (Ag-NORs) and the general pattern of constitutive heterocromatin distribution, identified by C-banding technique in this species.

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Materials and methods

Cytogenetic analysis were performed in 12 specimens (8 males and 4 females) of *Gymnothorax ocellatus*, collected in the Ubatuba Bay (São Paulo), Brazil. Fishes were identified and were deposited in the fish collection of the Laboratory of Fish Biology, UNESP, Botucatu, São Paulo, Brazil. Chromosome preparations were obtained from lymphocyte culture following the technique described by Fenocchio and Bertollo (1988). C-banding was obtained following the method of Sumner (1972) and preparations for silver-staining of the nucleolus organizer regions followed the technique proposed by Howell and Black (1980). Chromosome morphology was determined on the basis of arm ratios as proposed by Levan *et al.* (1964) and the chromosomes were classified as metacentrics (M), submetacentrics (SM), subtelocentrics (ST) and acrocentrics (A).

Results and discussion

The specimens of *Gymnothorax ocellatus* analyzed presented $2n=42$ chromosomes, being 8 pairs of metacentrics, 9 pairs of submetacentrics and 4 pairs of acrocentrics (Fig. 1). No chromosomal differences related to the sex were observed. The same and very conservative diploid number of chromosomes was also observed in other species of family Muraenidae analyzed so far; thus, *Gymnothorax eurostus* (Takai and Ojima 1986), *Gymnothorax kidako* (Nogusa 1960), *Gymnothorax reevesi* (Shoubai *et al.* 1991), *Gymnothorax unicolor* (Deiana *et al.* 1990), *Muraena helena* (Cau *et al.* 1988), *Muraena paradalis* (Takai and Ojima 1986), and *Sideria picta* (Ojima 1985, Rishi 1973) all have $2n=42$ chromosomes. However, differences in the karyotypic formula were observed for those species (Table 1).

The specimens of *G. ocellatus* analyzed presented a maximum number of two chromosomes with NORs (pair 15) located in the terminal position on the long arm (Fig. 1). The NORs showed to be similar to those found in other species analyzed until now in this family, in which only one chromosomes pair was found to be involved with the nucleolar organization (Takai and Ojima 1986, Cau *et al.* 1988, Deiana *et al.* 1990). The differences found were due to the chromosomes involved

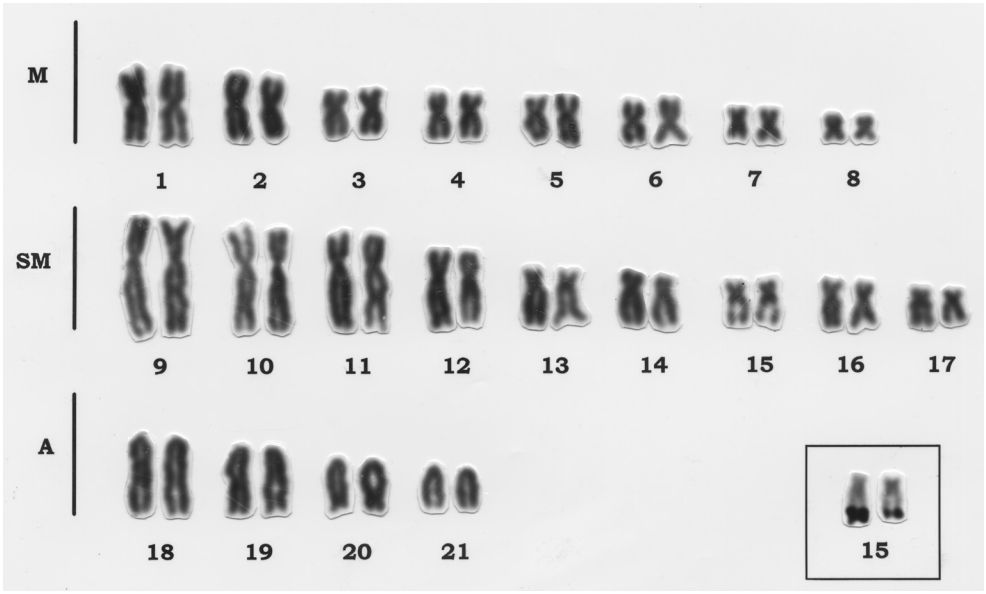

Fig. 1. Karyotype of *Gymnothorax ocellatus*, stained with Giemsa. In the inset, the NOR-bearing chromosome pair.

Table 1. Karyotypic characterization of species of the family Muraenidae.

Species	2*n*	Karyotype	NOR	C-Band	Replication banding	References
Gymnothorax eurostus	42	–	2	–	–	Takai and Ojima 1986
Gymnothorax kidako	42	–	–	–	–	Nogusa 1960
Gymnothorax kidako	36	–	2	–	–	Takai and Ojima 1986
Gymnothorax reevesi	42	–	–	–	–	Shoubai *et al.* 1991
Gymnothorax unicolor	42	12M/SM+30A	2	+	+	Deiana *et al.* 1990, Salvadori *et al.* 2003
Gymnothorax ocellatus	42	16M+18SM+8A	2	+	–	Present study
Muraena helena	42	–	–	–	+	Cau *et al.* 1988, Salvadori *et al.* 2003
Muraena paradalis	40	–	–	–	–	Nogusa, 1960
Muraena paradalis	42	–	2	–	–	Takai and Ojima 1986
Sideria picta	42	–	–	–	–	Ojima 1985
Sideria picta	42	42A	–	–	–	Rishi 1973

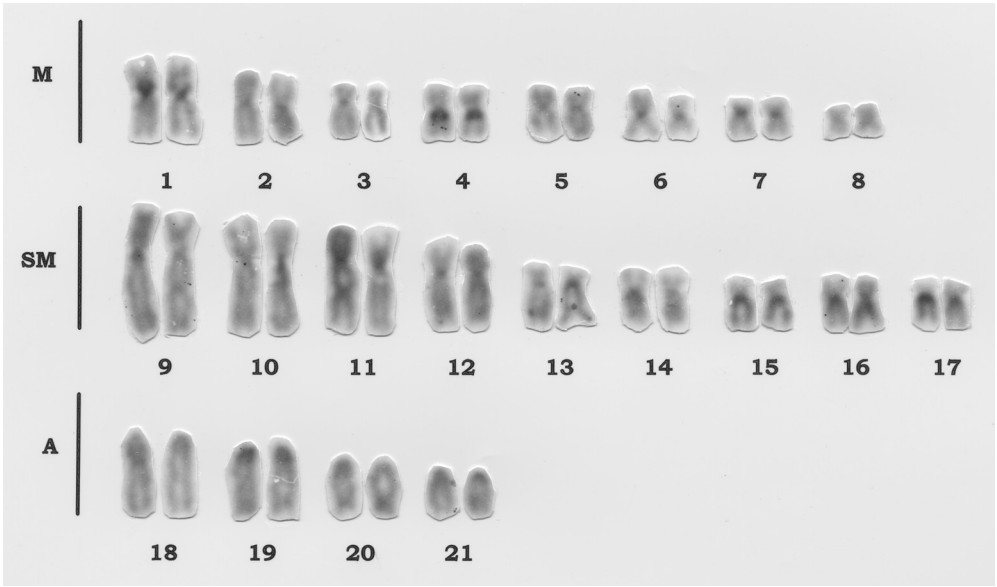

Fig. 2. C-banding karyotype of *Gymnothorax ocellatus*.

with the NORs. It is considered that the NORs can constitute a quite useful cytological marker and a decisive element for the identification of the species of this group.

The identification of constitutive heterochromatin by C-banding revealed the occurrence of small heterochromatic segments in *G. ocellatus* (Fig. 2). Small differences were observed in the patterns obtained by Deiana *et al.* (1990) for *G. unicolor*. The presence of small heterocromatic segment located in the pericentromeric area of a metacentric chromosome pair in *G. unicolor* was also observed in *G. ocellatus*.

It is considered that further studies involving other samples and other species of this genus should be very important for a better understanding of the taxonomic and phylogenetic relationships in this group.

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References

Brum, M. J. I., Galetti Jr., P. M., Correa, M. M. O. and Aguilar, C. T. 1992. Multiple sex chromosome in South Atlantic fish, Brevoortia aurea, Clupeidae. Rev. Brasil. Genet. **15**: 547–553.

Carvalho-Filho, A. 1992. Peixes—Costa Brasileira. São Paulo, Marca D'Água, pp. 304.

Cau, A., Salvadori, S., Deiana, A. M., Bella, J. L. and Mezzanotte, R. 1988. The characterization of *Muraena helena* L. mitotic chromosome: Karyotype, C-banding, nucleolar organizer regions and *in situ* digestion with restriction endonucleases. Cytogenetic. Cell Genet. **47**: 223–226.

Deiana, A. M., Salvadori, S. and Cau, A. 1990. The characterization of somatic chromosomes of *Gymnothorax unicolor* (Delaroche, 1809) by C-banding and NOR staining (Osteichthyes, Anguiliformes). Genetica **81**: 17–20.

Fenocchio, A. S. and Bertollo, L. A. C. 1988. A simple method for fresh-water fish lymphocyte culture. Rev. Brasil. Genet. **11**: 847–852.

Howell, W. M. and Black, D. A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia **36**: 1014–1015.

Klinhkhardt, M., Tesche, M. and Greven, H. 1995. Database of Fish Chromosomes. Magdeburg, Westarp-Wiss, pp. 237.

Levan, A., Fredga, K. and Sandberg, A. A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201–220.

Nelson, J. S. 1994. Fishes of the World. 3ª edition, John Wiley & Sons, Inc., New York, USA.

Nogusa, S. 1960. A comparative study of the chromosome in fishes with particular consideration on taxonomy and evolution. Mem. Hyogo Univ. Agric. **3**: 1–62.

Ojima, Y. 1985. Fish CDR List. Dep of Biol. Fac. Sci., Kwansei Gakuin Univ.

Rishi, K. K. 1973. A preliminary report on the karyotypes of eighteen marine fishes. Res. Bull. Panjab Univ. **24**: 161–162.

Shoubai, R., Tianguo, Z., Wenhui, Z. and Jiedong, C. 1991. The karyotype pf *Gymnothorax reevesi*. Tropic. Oceanol. **10**: 98–101.

Salvadori, S., Coluccia, E., Cannas, R., Cau, A. and Deiana, A. M. 2003. Replication banding in two Mediterranean moray eels: Chromosomal characterization and comparison. Genetica, **119**: 253–258.

Sumner, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. Expl. Cell Res. **75**: 304–306.

Takai, A. and Ojima, Y. 1986. Some features on nucleolus organizer regions in fish chromosomes.—In: Uyeno, T., Arai, R., Taniuchi, T. and Matsuura, K. (eds.), Indo Pacific Fish Biology. Ichthyol. Soc. of Japan, Tokyo. p. 899–909.