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Chromosomes of *Gymnothorax funebris* and the karyotypical differentiation within *Gymnothorax* (Anguilliformes: Muraenidae)

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ABSTRACT. Cytogenetic studies in *Gymnothorax funebris* revealed a diploid chromosome number 2n = 42 (6 metacentrics, 4 submetacentrics, and 32 acrocentrics, FN = 52). The results obtained are novel and similar to those previously described for species belonging to Muraenidae family. The conventional karyotype is also novel and divergent from other species of the genus *Gymnothorax*, where a higher proportion of metacentric chromosomes predominate. The data are reported and discussed considering the cytotaxonomy of the genus. These results strongly support the current view that chromosomal alterations such as centric fusion and Robertsonian's translocations have an important role in the evolution of this group.

Key words: Chromosome number; Cytotaxonomy; Evolution; Anguilliformes

INTRODUCTION

The order Anguilliformes is taxonomically complex, including 15 families, 141 genera, and 791 species (Nelson, 2006). Chromosome studies among anguilliform fish are limited to a few taxa, with chromosome numbers ranging from 2n = 26 to 54 (Vasconcelos and Molina, 2009). The order attracts much interest because its karyotypic traits are highly specific among teleosts and reveal a unique position in the context of fish karyotype evolution (Amores et al., 1995a,b).

The basic chromosome number among Anguilliformes is considered to be 2n = 38, based on the remarkable equivalence between the karyotypes of the two phylogenetically distant species *Astroconger myriaster* and *Anguilla japonica* (Park and Kang, 1979). The ancestral chromosome formula for the modern teleosts is considered to be 2n = 48 with one arm (Brum and Galetti, 1997); therefore, the basic karyotype for Anguilliformes is likely to have suffered various centric fusions between primitive acrocentric chromosomes, resulting in an increased number of large two-armed chromosomes. Chromosome number reductions and fundamental number (FN) increases are considered a trend towards a symmetric karyotype in many fish groups.

Chromosome data are available for 13 Muraenidae species, a small fraction of the family's 197 species (Vasconcelos and Molina, 2009; Smith, 2012). Detailed chromosome morphology data are available for four genera, *Gymnothorax* (8 species), *Muraena* (2 species), *Echelycore* (1 species), and *Sideria* (1 species). The chromosome number reported for all 13 species is 2n = 42, with the exception of *Gymnothorax kidako*, which presents 2n = 36 (Takai and Ojima, 1985). However, FN seems to be widely variable, ranging from 42, in *Sideria picta*, where the whole complement is acrocentric, to 84 in *Gymonothorax miliaris* (Vasconcelos and Molina, 2009). The genus *Gymnothorax* is likely to be a polyphyletic assemblage and many of its species have been moved to different genera over time (Smith, 2012). The aim of the present study was to describe the karyotypic parameters of the cosmopolitan species *Gymnothorax funebris* from the Brazillian northeastern coast. In addition, karyotype differentiation in the genus *Gymnothorax* is discussed.

MATERIAL AND METHODS

Sampling

Twelve green morays (*G. funebris*) were captured at Sabiaguaba beach (3°47'00"S, 38°25'37"W), Ceará, Brazil. The fish were collected during the day, at low tide, through free diving. After collection, the animals were immediately transported to the laboratory in 20-L aerated tanks, where they were individually kept until chromosome analysis.

Mitotic stimulation and cell culture

A mixed antigens complex (NIKKHO-VAC® and MUNOLAN®; 1 mL per 100 g weight) was used for mitotic stimulation through intraperitoneal injection. Twenty-four hours after the mitotic stimulation, a 0.025% colchicine solution (1 mL per 100 g body weight) was intraperitoneally injected, after which the animals were returned to the tanks for one hour. The animals were then anesthetized for removal of spleen, liver, and blood. The tissues were ground and cultured indirectly, by following the solid tissue culture technique adapted from

Fenocchio et al. (1991). Details were as follows: 1) a RPMI 1640 medium (Life Technologies) containing 4 µg/mL penicillin, 124 µg/mL streptomycin and 20% bovine fetal serum was filtered through a 0.22-µm Millipore membrane; 2) ground tissue was incubated at 29°C for 6-8 h, followed by centrifugation at 800 rpm; 3) the cell pellet was re-suspended in 8 mL hypotonic solution (0.075 M KCl) and incubated at 37°C for 40 minutes; 4) the cell suspension was pre-fixed with 10 drops of a mixture of methanol:acetic acid (3:1), and then centrifuged at 800 rpm for 10 min, at room temperature; 5) the cell pellet was carefully re-suspended in 5 mL methanol:acetic acid (3:1) fixative mixture. The best results were obtained from spleen.

Chromosome and FN and karyotype description

Cell suspensions were transferred onto a microscopy glass slide and covered with a 60°C water film, set to dry at room temperature, and then stained with pH 6.8 phosphate-buffered Giemsa (5%) for 20 to 25 min. The metaphases resulting in ideal spreading and chromosome contraction were photomicrographed. Diploid chromosome number (2n) and FN were determined through direct count of chromosomes and their arms, respectively. Chromosome types (metacentric, submetacentric, acrocentric, and telocentric) were identified following the method of Levan et al. (1964). Constitutive heterochromatin was studied with the C-banding protocol of Sumner (1972).

The chromosome data for *G. funebris* were compared with available data from other *Gymonothorax* species (Table 1). A karyotypic parameter matrix was built including the somatic chromosome number (2n), the FN, and chromosomal formula. This matrix was analyzed through PC-ORD 6.0 (McCune and Mefford, 2011), to produce a UPGMA cluster based on the Bray-Curtis similarity index.

Table 1. Chromosome numbers for *Gymnothorax* species (Muraenidae, Anguilliformes).

Species	2n	FN	Karyotype	Reference
G. eurostus (C. C. Abbott, 1860)	42	54	12SM/SM + 30A	Manna, 1989
G. funebris Ranzani, 1840	42	52	6M + 4SM + 32A	Present study
G. kidako (Temminck & Schlegel, 1846)	36	60	16M + 8SM + 12A	Takai and Ojima, 1985
G. miliaris (Kaup, 1856)	42	84	14M + 18SM + 10 ST	Vasconcelos and Molina, 2009
G. ocellatus Agassiz, 1831	42	76	16M + 18SM + 8A	Porto-Foresti et al., 2005
G. tile (F. Hamilton, 1822)	42	76	16M + 18SM + 8A	Colluccia et al., 2010
G. unicolor (Delaroche, 1809)	42	54	12M/SM + 30A	Deiana et al., 1990
G. pictus (J. N. Ahl, 1789)	42	42	42A	Rishi, 1973
G. vicinus (Castelnau, 1855)	42	56	8M + 6SM + 28A	Vasconcelos and Molina, 2009

2n: diploid chromosome number, FN: fundamental number.

RESULTS AND DISCUSSION

As shown in Figure 1A, the chromosome number for *G. funebris* was 2n = 42. This number agrees with those previously reported for the genus *Gymnothorax*, with the exception of *G. kidako*, that presents 2n = 36 (Takai and Ojima, 1985; Table 1). The chromosome number in *Gymnothorax* is a reduction from the synapomorphic 2n = 48 present in modern teleosts (Brum and Galetti, 1997; Accioly and Molina, 2008). This reduction is likely the result of chromosomal rearrangements such as centric or in tandem fusion, followed by pericentric inversions (Vasconcelos and Molina, 2009).

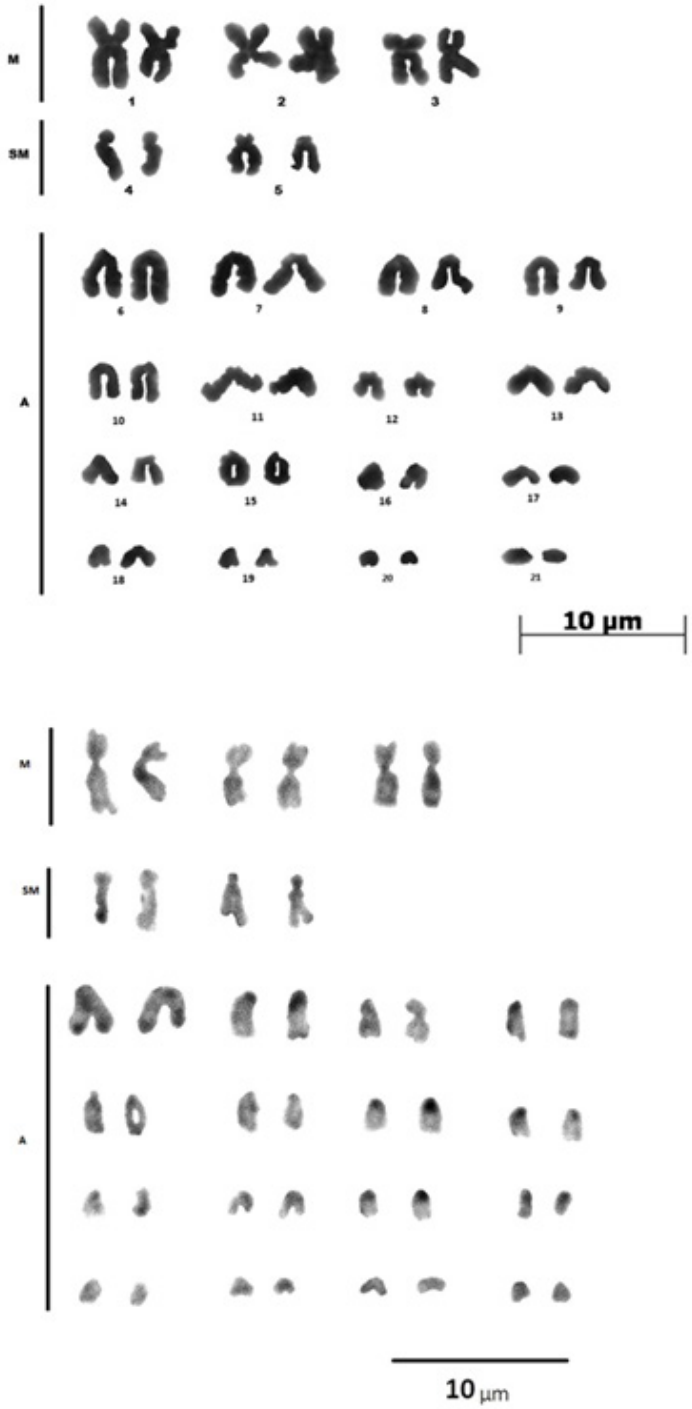

Figure 1. Karyotype of *Gymnothorax funebris* after Giemsa staining (**A**) and C-banding (**B**).

The *G. funebris* karyotype included 3 pairs of metacentric, 2 pairs of submetacentric, and 16 pairs of acrocentric chromosomes. The resulting FN of 52 is among the lowest recorded for this genus (Table 1). The most frequent FN in this genus is 76, present in *G. ocellatus* (Porto-Foresti et al., 2005) and *G. tile* (Collucia et al., 2010). However, reported FN values for *Gymnothorax* range from 42 in *G. pictus*, which presents an entirely acrocentric complement (Rishi, 1973), to 84 in *G. miliaris*, which lacks acrocentric chromosomes (Vasconcelos and Molina, 2009). Considering the previously reported chromosome-type distributions, *G. funebris* presents a prominently asymmetric karyotype, in contrast to the primarily symmetric karyotype observed for other species of the genus (Table 1). Among the Muraenidae, a strong karyotype asymmetry seems to indicate a basal condition (Vasconcelos and Molina, 2009).

The constitutive heterochromatin distribution pattern, revealed by C-banding (Figure 1B), showed large paracentric blocks of heterochromatin in all metacentric and submetacentric chromosomes (1st to 5th pairs). Among the acrocentric chromosomes (6th to 21st pairs), the 6th pair showed heterochromatic blocks at the end of both short and long arms, while the remaining pairs presented smaller telomeric bands at the end of the short arms. The observed patterns of longitudinal differentiation are similar to those presented by Muraenidae in general, among which pericentric inversions and chromosomic fusions are likely to play a significant evolutionary role (Vasconcelos and Molina, 2009).

The similarity analysis of nine *Gymnothorax* species based on karyotypic parameters (Figure 2) revealed two distinct groups with similarities above 50 percent. Group A, including *G. kidako*, *G. miliaris*, *G. ocellatus*, and *G. tile*, presented higher FN, due to a higher proportion of metacentric and submetacentric chromosomes, therefore representing a trend towards symmetry. In group B, which includes *G. eurostus*, *G. unicolor*, *G. funebris*, and *G. pictus*, the karyotypes are primarily asymmetric because of the presence of higher number of acrocentric chromosomes. Within group A, *G. kidako* is the most distant species for its unique chromosome number, whereas in group B, *G. pictus* stands out because of its entirely asymmetric karyotype (Table 1). The observation that the clustering based on similarities in karyotype parameters (Figure 2) is in strong disagreement with a recent Muraenidae molecular phylogeny (Reece et al., 2010) seems to support the distinct importance of chromosomal rearrangements in speciation within this group.

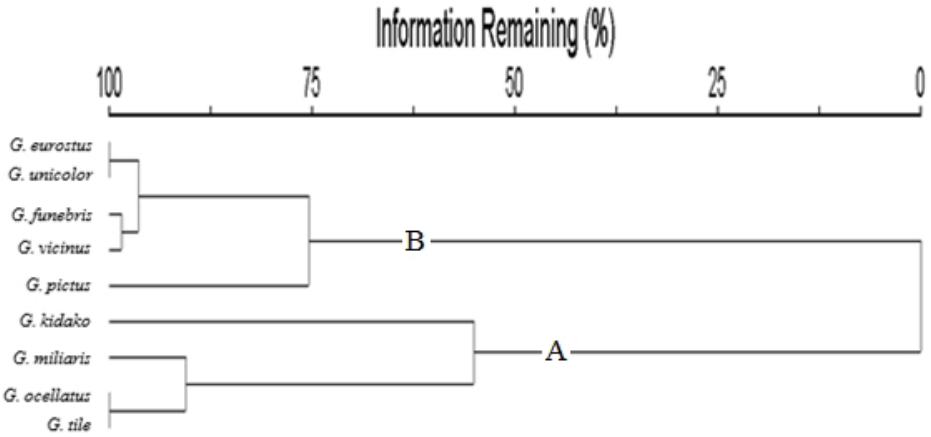

Figure 2. Bray-Curtis similarity dendrogram based on a matrix of karyotypical data. **A.** Group with predominantly symmetric karyotypes; **B.** Group with asymmetric karyotypes.

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