Cytogenetic studies of *Mastacembelus armatus* (Pisces, Mastacembelidae)

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Abstract

The mitotic chromosomes, nucleolus organizer regions (NORs), C-banding pattern and nuclear DNA content of *Mastacembelus armatus* were studied. The karyotype (2n = 48; 10m + 6sm + 4st + 28a) was characterized by the presence of one chromosome pair with NORs and a small quantity of heterochromatin. The DNA content observed in erythrocyte nuclei of *M. armatus* was 1.39 ± 0.08 pg. Comparison of this karyotype with those of other Synbranchiformes revealed a strong similarity, suggesting that small chromosome rearrangements may have been maintained during its evolutionary history.

Introduction

The fish family Mastacembelidae belongs to the order Synbranchiformes and comprises a small group of freshwater fish consisting of two subfamilies, four genera, and about 67 species confined to southern Asia from Iran to Korea (Nelson, 1994).

Some cytogenetic studies have been carried out in fish from this family (Table 1), but no previous published information about chromosome banding was discovered. Thus, the objective of the present paper was to describe the karyotype of *M. armatus* obtained by standard cytogenetic analysis, as well as the location of the nucleolus organizer regions, C-banding, and the nuclear DNA content, and to compare the results with the information available about other Synbranchiformes.

Materials and methods

Three specimens (2 females and 1 male) of *Mastacembelus armatus* obtained from a tropical fish dealer were analysed. Specimens were identified and deposited in the Zoology Museum of the Universidade de São Paulo, São Paulo, Brazil.

Chromosome spreads and staining were carried out as described by Foresti *et al.* (1993). For the DNA content determination, blood was collected by caudal vein puncture, spread over the slides and air dried.

The DNA content of each individual was determined according to the technique described by Gold and Price (1985) with some minor modifications. Absorbancy values of fish nuclei from each slide were standardized as a percentage of the mean absorbancy value of three controls, namely chicken erythrocytes, common carp erythrocytes, and rainbow trout erythrocytes. Microdensitometry analysis was performed in a Zeiss microscope using a x100 oil-immersion objective, and OPTIMAS software version 4.1. For each individual fish, 150 nuclei were measured from each of two slides (300 nuclei per individual).

Results and discussion

Mastacembelus armatus has 2n = 48 chromosomes, with ten metacentrics, six submetacentrics, four subtelocentrics, and 28 acrocentrics (Figure 1). The NORs are present in the largest submetacentric pair in the terminal region of the long arm and revealed extensive size polymorphism (Figure 1). Heterochromatin distribution was investigated in two specimens, a male and a female. The results showed the presence of a small quantity of heterochromatin near the centromeric region of almost all chromosomes (Figure 2). A large heterochromatic segment was evident in the long arm of the largest submetacentric pair (pair 6) near the NOR region. A clear difference in the size of the heterochromatic segment in one chromosome of pair 6 was seen only in the male in all metaphases analysed. The mean DNA content observed in erythrocyte nuclei of *M. armatus* was 1.39 ± 0.08 pg.

The diploid number of 2n = 48 found in *M. armatus*, was the same as that described previously for this species and coincides with the diploid numbers found for other fish from the family Mastacembelidae (Table 1). Karyotypic analysis showed that the sample analysed in the present study manifested a larger number of submetacentric and subtelocentric chromosomes than those found by Manna and Khuda-Bukhsh (1978) for this species. Such differences in chromosome formulae may be due to technical problems with chromosome condensation or, alternatively, may be real and due to the use of fish from different local populations.

The diploid number of 2n = 48 chromosomes observed in *M. armatus* is the most frequent found in fish from the superorder Acanthopterygii (*sensu* Nelson, 1994) which includes the order Synbranchiformes (Klinkhardt *et al.*, 1995). The presence in *M. armatus* of some biarmed chromosomes and many uniarmed chromosomes has also been observed among several Mastacembelidae (Table 1) and in many other

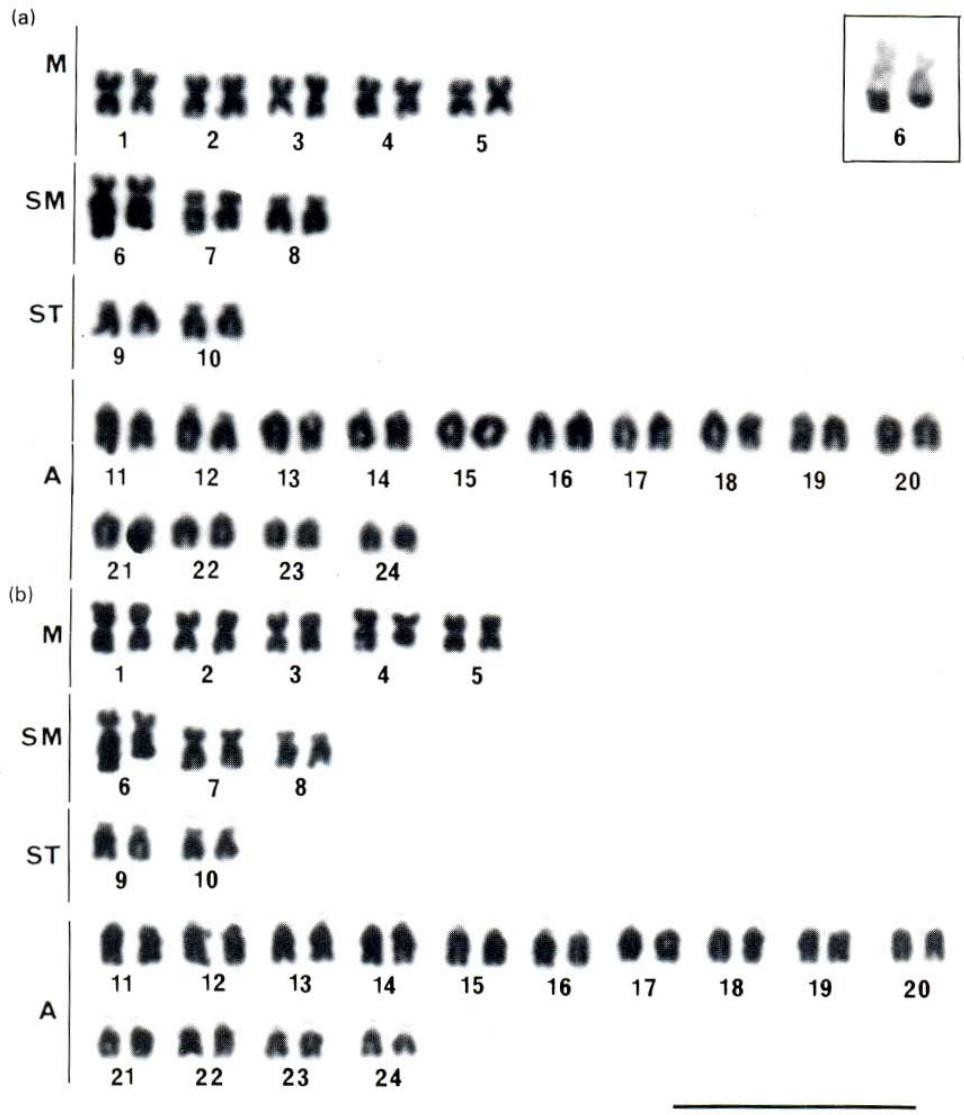

Figure 1 Karyotypes of (a) male and (b) female *M. armatus* with 2n = 48 chromosomes, and (inset) the chromosome pair with NORs. Bar scale represents 10 μm.

species of the order Synbranchiformes (Klinkhardt *et al.*, 1995). The presence among the Synbranchiformes of a similar diploid number and different chromosomal formulae reinforces the hypothesis that pericentric inversions played a significant role in the evolution of their karyotypes, as proposed by Manna and Khuda-Bukhsh (1978) for the family Mastacembelidae.

The diploid DNA content of *M. armatus* (1.39 ± 0.08 pg DNA) is smaller than that recorded by Jianxun *et al.* (1991) for *Macrognathus aculeatus* (1.61 pg DNA), which also had 2n = 48 chromosomes.

Table 1 Cytogenetic data known for the family Mastacembelidae

Species	2n	NF	Karyotype	DNA content* (pg)	Reference
Macrognathus aculeatum	48	58	8m + 2sm + 38st,a		Khuda-Bukhsh and Barat (1987)
	48	58	8m + 2sm + 38st,a		Manna and Prasad (1977)
	48	96	48m,sm		Natarajan and Subrahmanyan (1974)
	48			1.61	Jianxun *et al.* (1991)
Mastacembelus armatus	48				Khuda-Bukhsh and Manna (1974)
	48	62	10m + 4sm + 2st + 32a		Manna and Khuda-Bukhsh (1978)
	48	64	10m + 6sm + 4st + 28a	1.39 ± 0.08	Present paper
Mastacembelus pancalus	48	74	14m + 12sm + 14st + 8a		Khuda-Bukhsh and Barat (1987)
	48	70	16m + 6 sm + 8st 18a		Manna and Prasad (1977)
Mastacembelus sinensis	48		15m + 4sm + 3st + 26t		Yajuan and Qixing (1991)
Rhynchobdella aculeata	48				Kaur and Srivastava (1965)
	48	48	48a		Kaur and Srivastava (1965)
	48				Nogusa (1960)

*Mean ± SE.

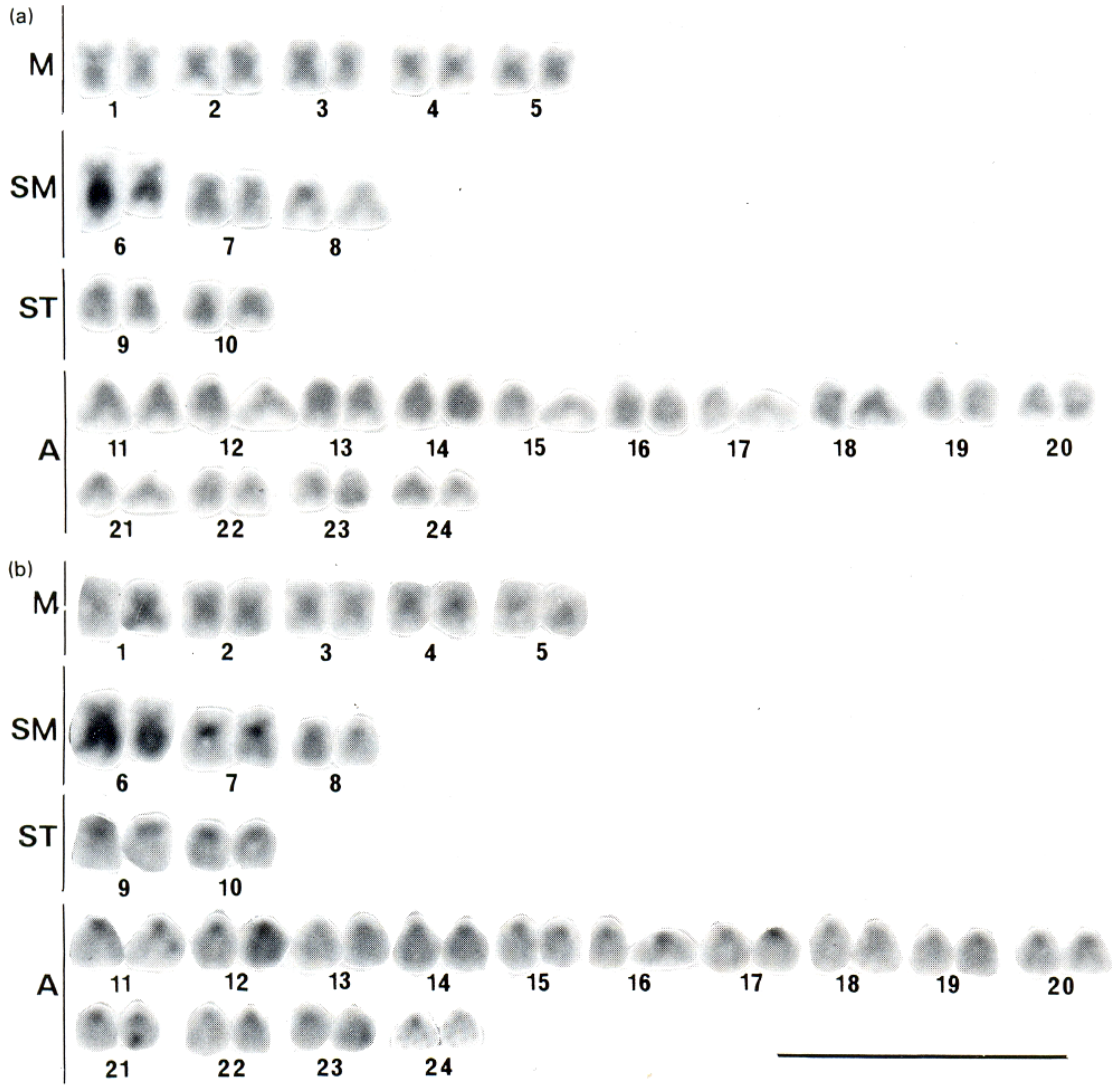

Figure 2 Karyotype of *M. armatus* treated by the C-banding technique. (a) Male; (b) female. Bar scale indicates 10 μm.

However, it is similar to that described by the same authors for another species of Synbranchiformes, *Monopterus albus* (1.24 pg DNA), which has only 2n = 24 chromosomes. Although the available data about the DNA content of Synbranchiformes are scarce, it is apparent that only a few changes in the DNA content occurred during the evolutionary history of the group. This was not so in the genus *Synbranchus* since their chromosomes are larger than those in other fish genera from the order Synbranchiformes. Consequently, data on the nuclear DNA content of species are needed before conclusions can be reached.

The presence of only one NOR-bearing chromosome pair, as in *M. armatus*, was the most common feature found among the species under consideration (Almeida-Toledo and Foresti, 1985). However, since fish from the genus *Synbranchus* also have only one NOR-bearing chromosome pair (Foresti *et al.*, 1992) the order Synbranchiformes may be classified as having single NORs. The presence of a small quantity of heterochromatin in the chromosomes of *M. armatus* resembles the data obtained for *S. marmoratus* (Foresti *et al.*, 1992). In this case the presence of a small quantity of heterochromatin was the most common feature observed.

Size heteromorphism which occurred in the heterochromatic segment present in pair 6 (submetacentric) of one male of *M. armatus* and its absence in the female may indicate the occurrence of an XX/XY sexual chromosome system in this species. Since only one male and one female were analysed in the present study it is necessary to investigate this characteristic in more specimens before any view can be expressed. However, the data about the synaptonemal complex obtained by Yajuan and Qixing (1991) for *Mastacembelus sinensis* showed that the species presents an XX/XY sex chromosome system in which the X chromosome is the largest metacentric chromosome and the Y chromosome is a subtelocentric identified as the second in size in the karyotype. Thus, although the sex chromosomes found in *M. sinensis* are not the same putative sex chromosomes in *M. armatus*, these results show that new studies are necessary to elucidate effectively the sex chromosomes in this group.

The absence of more detailed karyotypic data from other Mastacembelidae prevents a comparison of the karyotype of *M. armatus* with those of other species. In spite of this, our results show that there is a considerable karyotypic similarity between fish from the family Mastacembelidae and the family Synbranchidae. Nevertheless, more detailed chromosomal data from other species of these groups will be necessary in order to understand the patterns of karyotypic relationship among species of the order Synbranchiformes.

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