

## MULTIPLE SEX CHROMOSOMES IN SOUTH ATLANTIC FISH, *Brevoortia aurea*, CLUPEIDAE

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### ABSTRACT

Sex chromosomes in Clupeidae fish are described for the first time. An  $X_1X_2Y$  multiple sex chromosome system is related for *Brevoortia aurea* of the South Atlantic. Mitotic preparations showed  $2n=46$  in female specimens and  $2n=45$  in all males analyzed. Chromosome arms were constant in both sexes ( $NF=50$ ). A centric fusion between two median acrocentric chromosomes seems to have developed a large metacentric in the males, which is absent in the female. Testicular analyses showed a typical trivalent and two different haploid numbers ( $n=22$  and  $n=23$ ) in the meiotic products.

### INTRODUCTION

Until some years ago, fish were considered a group with no differentiated sex chromosomes or having an incipient degree of differentiation (Ohno, 1974). However, even though most of the fish species seems to be homogametic, at least at a cytological level, several occurrences of sex chromosomes have already been described (see Ojima, 1982 for a revision). As opposed to mammals and birds where XY and ZW systems are, respectively, shared by most of the species of these groups, among fishes sex chromosome systems are more variable. XO, XY and ZW, as well as multiple sex chromosomes appear to have evolved several times. Among Clupeiformes, differences in chromosome number between sexes were already detected in just one Engraulidae species, *Colia brachygnathus* (Zhou, 1984). Before this study, no sex chromosomes had been described

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in the Clupeidae. This paper is part of a cytogenetical study on South Atlantic clupeidae species and we describe an  $X_1X_2Y$  multiple sex chromosome system in *Brevoortia aurea*.

## MATERIALS AND METHODS

*B. aurea* specimens were collected in the Rodrigo de Freitas Lagoon, township of Rio de Janeiro (RJ, Brazil). Mitotic chromosomes were obtained from kidney cells either by the direct air drying method (Bertollo, 1978) or from short term culture cells (Fenocchio *et al.*, 1991). In the first method, a 0.025% colchicine solution was intraperitoneally injected (1 ml/100 g). The animals were sacrificed after 50-60 minutes and the cephalic portion of the kidney was dissociated and incubated in a saline solution (0.075M KCL) at 37°C, during 30 minutes. In the short term culture cells, the cephalic portion of the kidney was initially dissociated and incubated in TC-199 culture medium plus 20% human serum, during 24-25 hours at 25°-30°C. Colchicine solution (0.025%) was added (4-5 drops) during the last 40 minutes of incubation and the cells were then transferred to 0.075M KCL solution (30 minutes - 37°C). Cell suspensions were fixed three times in fresh methanol-acetic acid (3:1) fixative, dropped on clean warm slides (38°-40°C) and stained by 5% phosphate Giemsa solution. Testicular meiotic preparations were basically obtained according to Klingerman and Bloom (1977). Small pieces of testis were hypotonized in 0.075M KCL solution by 35 minutes and fixed twice in fresh methanol-acetic acid (3:1) fixative. Cell suspensions were obtained in fresh 50% acetic acid solution, dropped on clean warm slides (38°-40°C) and stained in 5% phosphate Giemsa solution. Specimens from this study have been deposited in the Museu Nacional, of Universidade Federal do Rio de Janeiro, series MNRJ 12101-12126.

## RESULTS

The mitotic metaphases obtained from 14 males and 12 females showed a different diploid number for the sexes (Table I). Females had  $2n=46$  while males had  $2n=45$  chromosomes. The female karyotype presented two large metacentric, two submetacentric and forty-two acrocentric chromosomes. On the other hand, the male karyotype presented three large metacentric chromosomes, one of them absent in the females, plus two submetacentrics and forty acrocentric chromosomes (Figure 1). Chromosome arms were equal in both sexes,  $NF=50$ . Testicular meiotic preparations showed the occurrence of one trivalent and twenty-one bivalents in late prophase and metaphase I cells (Figure 2). Spermatocytes in metaphase II with 22 chromosomes showed two large metacentrics. On the other hand, those with 23 chromosomes presented only one large metacentric (Figure 2).

Table I - Diploid and haploid numbers and chromosomes type of each sex in *Brevoortia aurea*.

Sex	Diploid cells		Haploid cells	
	2n	NF	n	Sex chromosomes
♀	46 (2M+2SM+42A)	50	23	X <sub>1</sub> , X <sub>2</sub>
♂	45(3M+2SM+40A)	50	23	X <sub>1</sub> , X <sub>2</sub>
			22	Y

## DISCUSSION

The different diploid numbers between males and females of *B. aurea* may indicate a multiple sex chromosome system of the X<sub>1</sub>X<sub>1</sub>X<sub>2</sub>X<sub>2</sub>/X<sub>1</sub>X<sub>2</sub>Y type. The meiotic trivalent formed by one large metacentric and two acrocentric chromosomes paired end-to-end, probably results from the synapses of the Y chromosome with both X chromosomes. Size differences between the X chromosomes were not observed and it was not possible to identify these chromosomes among all acrocentrics of the karyotype. Homologous chromosomes were tentatively grouped. However, except for the large metacentrics and submetacentrics, exact identification of homologous chromosomes would require some banding techniques (e.g. G-banding) which, unfortunately, still are not well developed for fish chromosomes.

Multiple sex chromosomes have been described in several fish groups (Uyeno and Miller, 1971, 1972; Levin and Foster, 1972; Thorgaard, 1978; Murofush *et al.*, 1980; Moreira-Filho *et al.*, 1980; Bertollo *et al.*, 1983; Pezold, 1984; Almeida Toledo *et al.*, 1984; Ewulonu *et al.*, 1985). In *Apareiodon affinis* the heterogamety is associated with the females and a ZW<sub>1</sub>W<sub>2</sub> multiple sex chromosome system appears to have evolved by a fission procedure (Moreira-Filho *et al.*, op. cit.). In all remaining cases, males are the heterogametic and it has been assumed that these systems evolved by a Robertsonian fusion of a Y chromosome and an autosome. This assumption presumes an earlier existence of a Y chromosome even though it may be cytologically cryptic in some groups (Ewulonu *et al.*, op.cit.). Thus, although the X<sub>1</sub>X<sub>2</sub>Y system in *B. aurea* represents the first description of sex chromosomes in Clupeidae, a Y chromosome probably occurs in other species of this genus even they are not cytologically distinguishable. About 190

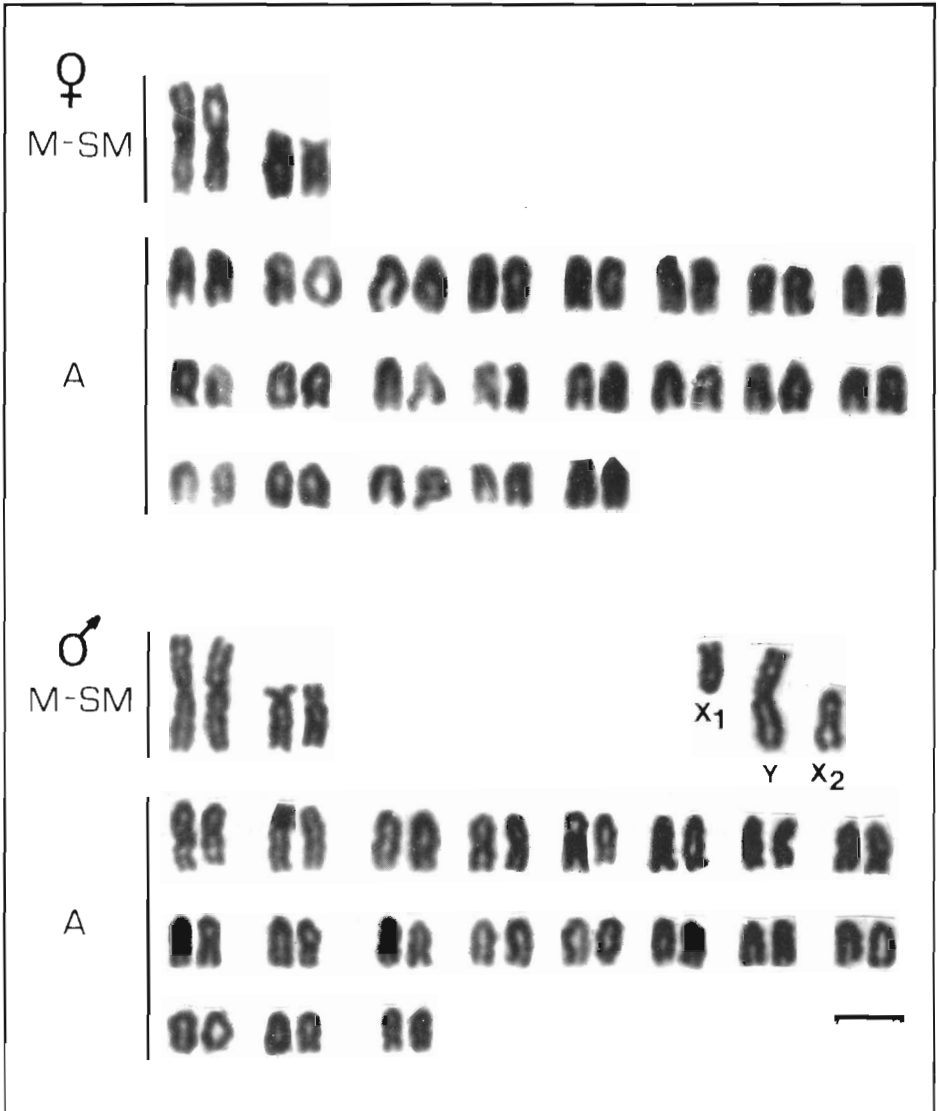


Figure 1 - Female and male karyotypes of *Brevoortia aurea* (sex chromosomes are paired suggesting homology). The bar equals 4  $\mu$ m.

clupeid species are known (Nelson, 1984), and only 12 of these (including the present paper) have been chromosomically studied (see Doucette and Fitzsimons, 1988 for revision). Further studies could show sex chromosomes in other species of this group.

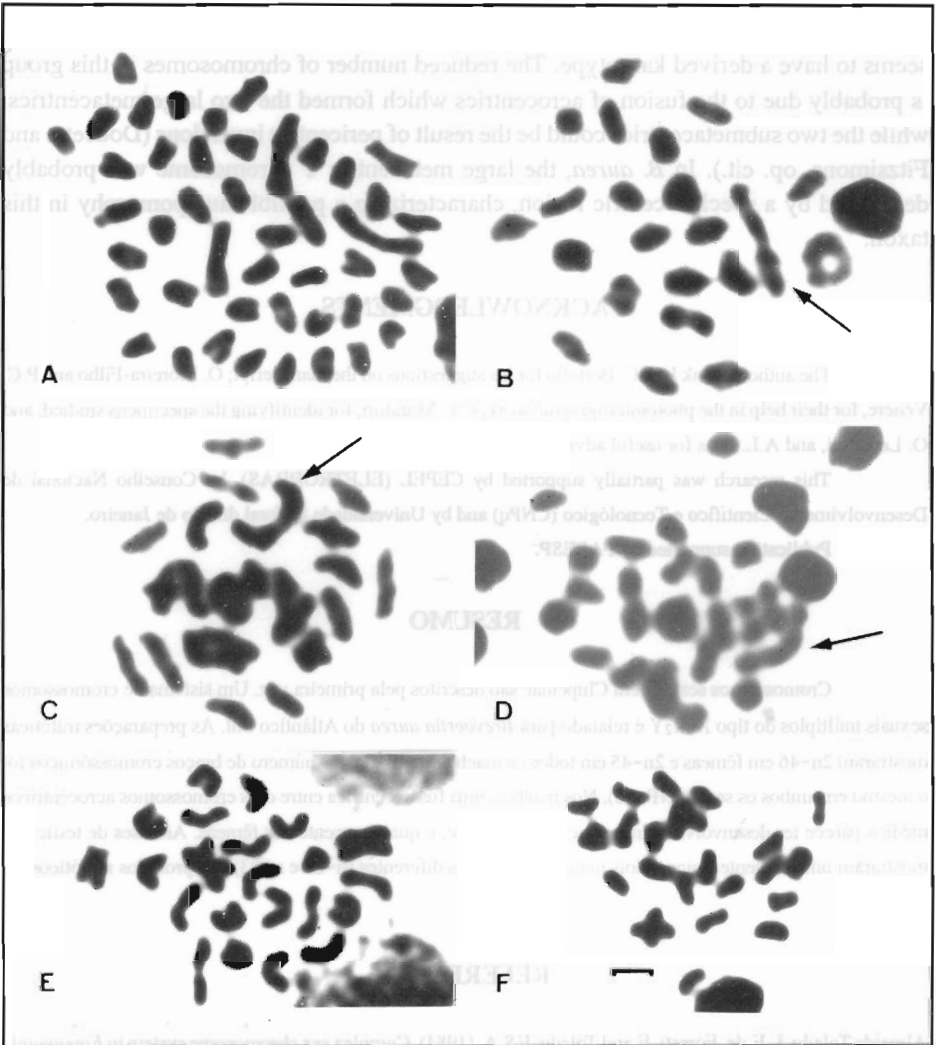


Figure 2 - Male meiotic chromosomes of *Brevoortia aurea*: spermatogonial metaphase (A) with  $2n=45$  chromosomes; late prophases (B, C) and metaphase I (D) with 21 bivalents and one trivalent (arrows); metaphase II with 22 (E) and 23 (F) chromosomes, respectively. The bar equals 4  $\mu\text{m}$ .

Despite the occurrence of sex chromosomes in *B. aurea*, a common trend in chromosome evolution is observed in this genus. *B. patronus*, *B. smithi* and *B. tyrannus* (Doucette and Fitzsimons, op. cit.) and *B. aurea* females show the same karyotypic structure with two large metacentrics, two median submetacentrics and forty-two

acrocentrics. Since most Clupeiformes has  $2n=48$  acrocentric chromosomes, *Brevoortia* seems to have a derived karyotype. The reduced number of chromosomes in this group is probably due to the fusion of acrocentrics which formed the two large metacentrics, while the two submetacentrics could be the result of pericentric inversions (Doucette and Fitzsimons, op. cit.). In *B. aurea*, the large metacentric Y chromosome was probably developed by a specific centric fusion, characterizing a possible autapomorphy in this taxon.

## ACKNOWLEDGMENTS

The authors thank L.A.C. Bertollo for his suggestions on the manuscript; O. Moreira-Filho and P.C. Vêner, for their help in the photomicrographic work; C.F. Muratori, for identifying the specimens studied; and O. Leoncini, and A.L. Dias for useful advices.

This research was partially supported by CEPEL (ELETROBRÁS), by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and by Universidade Federal do Rio de Janeiro.

Publication supported by FAPESP.

## RESUMO

Cromossomos sexuais em Clupeidae são descritos pela primeira vez. Um sistema de cromossomos sexuais múltiplos do tipo  $X_1X_2Y$  é relatado para *Brevoortia aurea* do Atlântico Sul. As preparações mitóticas mostraram  $2n=46$  em fêmeas e  $2n=45$  em todos os machos analisados. O número de braços cromossômicos foi o mesmo em ambos os sexos (NF-50). Nos machos, uma fusão cêntrica entre dois cromossomos acrocêntricos médios parece ter desenvolvido um metacêntrico grande, o qual é ausente nas fêmeas. Análises de testículos mostraram um trivalente típico e dois números haplóides diferentes ( $n=22$  e  $n=23$ ) nos produtos meióticos.

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(Received September 20, 1991)