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# KARYOTYPIC ANALYSIS AND CHROMOSOME BIOMETRY OF CELL CULTURES OF THE YELLOW THROATED ALLIGATOR (*Caiman latirostris* DAUDIN)

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## ABSTRACT

Blood from eight specimens of both sexes of the alligator *Caiman latirostris* was collected and incubated in culture medium. Conventional as well as chromosomal banding (C and NOR) techniques were used.

The diploid number was determined as 42, being 24 telocentric, 12 metacentric and six submetacentric, with real lengths varying from 1.49 to 6.08, 1.63 to 3.71, and 2.41 to 3.19 μm, respectively. The fundamental number was 60. About 81% of the chromosomes were small and 19% medium in size. NOR-banding was presented for the first time for this species and it was verified that only one submetacentric pair (no. 20) was marked on arm q, and under conventional staining it presented a secondary constriction. There was no association between NOR marked chromosomes.

## INTRODUCTION

According to Buffetaut (1979), the first creatures analogous to modern crocodilians appeared approximately 200 million years ago. They have changed little in terms of morphology, physical structure and at the molecular level (Densmore, 1983). The singularites of this order include sex determination modulated by environmental temperature, low number of polymorphic loci, low percentage of heterozygosity and an apparent sex chromosome homomorphism (Menzies and Kushlan, 1991).

Studies involving crocodilian cytogenetics were initiated by Matthey (1945), Matthey and Van Brink (1957) and Cohen and Clark (1967). It was determined that the diploid chromosome number of this order varies from 30 to 42 and the fundamental number from 56 to 62. All chromosomes have been classified as macrochromosomes without sexual heteromorphism, and with karyotype evolution probably being by centric fusions/fissions, pericentric inversions and reciprocal translocations (Cohen and Gans, 1970). These authors also found a fundamental chromosome number for yellow-throated alligators of 60, with 42 being the diploid number. Twelve chromosomes were classified as small matacentric, six submetacentric and 24 telocentric.

The purpose of this project was to make a cytogenetical analysis of the yellow-throated alligator, using chromosome biometry.

## MATERIAL AND METHODS

Eight yellow-throated alligators (*Caiman latirostris*) kept at the Ribeirão Preto (SP) Zoological Garden were utilized (five males and three females).

For cell culture, the modified technique of Moorhead *et al.* (1960) was employed; 0.5 ml of leucocitary ring was collected and transferred to culture medium (HAM F-10, GIBCO USA), containing 16% inactivated equine serum along with the mitogenic agent phytohemagglutinin, both laboratory-made. The material was incubated for 48 hours at 30°C. At 45 hours 0.03 ml of colchicine (0.0016%) was added. Afterwards, the material was treated with hypotonic solution (0.075M KCl) and fixed, two to three times, with a 3:1 methanol-acetic acid solution.

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Microscope slides containing the material to be analysed were stained with 3% Giemsa for conventional and biometrical analysis or prepared for C and NOR-banding. Chromosome classification was based on data obtained from the relative length of short and long arms, as well as total and real lengths (Giannoni, 1986). The chromosomes were classified as metacentric, sub-metacentric or telocentric, considering the centromeric position, based on their arms ratio (AR) and centromeric index (CI) (Guerra, 1988).

The technique adapted for NOR-banding was based on Howell and Black (1980) and for C-banding Sumner (1972) and King (1980).

## RESULTS AND DISCUSSION

The alligator karyotype has a diploid chromosome number of 42, with a fundamental number of 60 and the chromosomes can be arranged into three morphological groups (Figure 1). In the first group, twelve pairs of telocentric chromosomes ordered by decreasing size can be distinguished, while the second group is characterized by six metacentric pairs, also in decreasing order. The last pairs of these two groups are much smaller and are distinct from the rest. The third group is formed by three submetacentric pairs. Chu and Bender (1961), found that more primitive crocodilian species have a high percentage of telocentric chromosomes. The form and size of the chromosomes of the males and females did not vary. Many authors, like Cohen and Gans (1970) and Janzen and Paukistis (1991) also did not find sexual chromosome heteromorphism in this group.

NOR-banding is presented for the first time for this species. The NORs are located in a single pair of chromosomes, and no chromosomal heteromorphism between males and females was observed (Figure 2, A and B). The sequential analysis under conventional Giemsa staining, C- and NOR-bands demonstrates that pair 20 has a slight secondary constriction in the q arm, just below the centromere, which corresponds to the NOR location and it is C-band positive (Figure 3). Cohen and Gans (1970) found a similar constriction on the p arm, in *Caiman latirostris* kept at the Crandon Park Zoological Gardens, Florida (USA).

Few chromosomes are marked by C-banding (Figure 3-B) as also found by King (1980). The centromeric region was more intensely marked in only pair of chromosomes.

An NOR size polymorphism between the chromosomes of the same or different chromosome pairs is presented in Figure 4.

Table I shows the relative mean lengths of q, p and q+p arms of chromosomes of males and females. Table II indicates the arm ratio (AR) values, centromeric indexes (CI) and real length (RL) expressed in micrometers.

The real length (RL) ranged from about 1.5 to 6 μm (Table II). The biometrical analysis also showed that amongst the 21 pairs of chromosomes, four were of

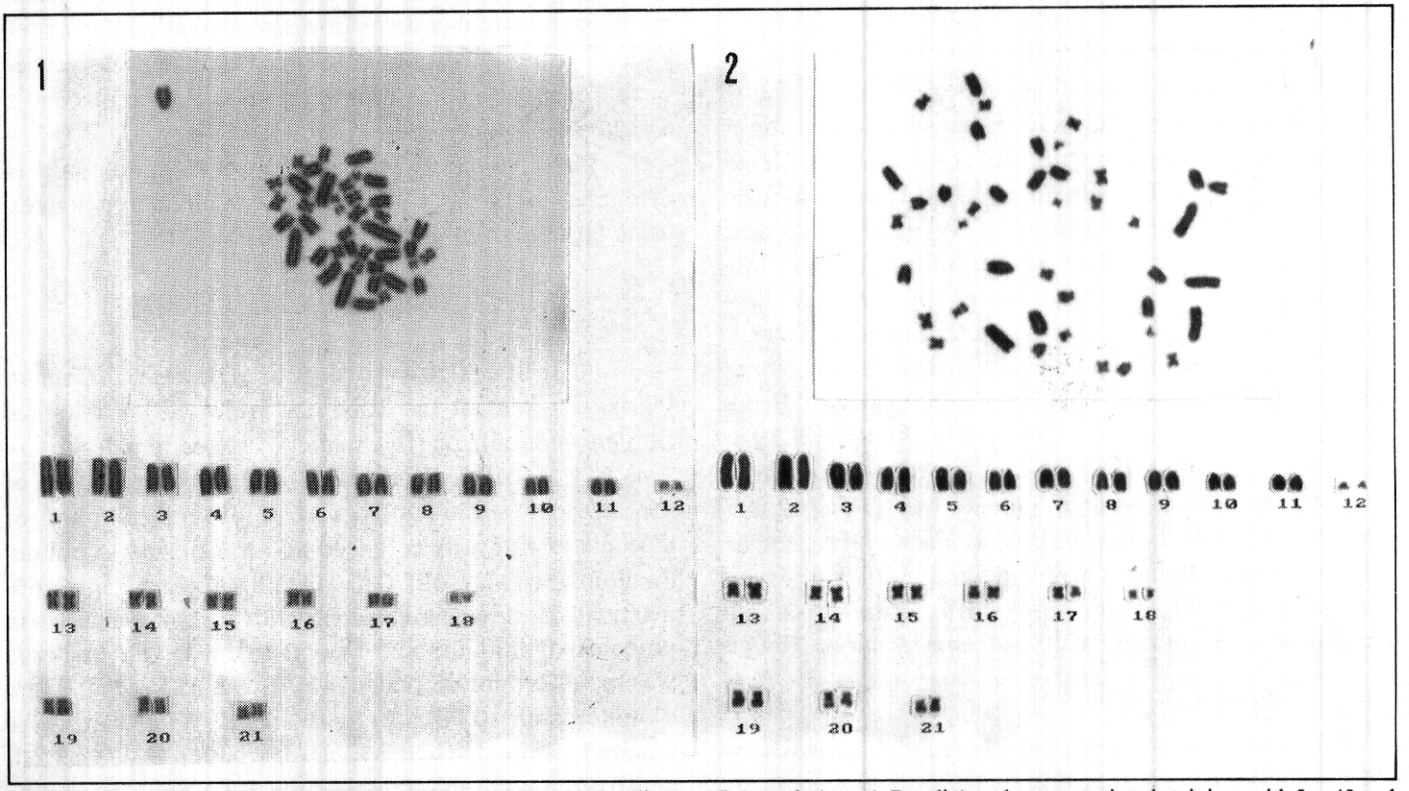


Figure 1 - Metaphase and karyotype of a male (1) and female (2) alligator (*Caiman latirostris* Daudin) under conventional staining, with 2n=42 and NF=60.

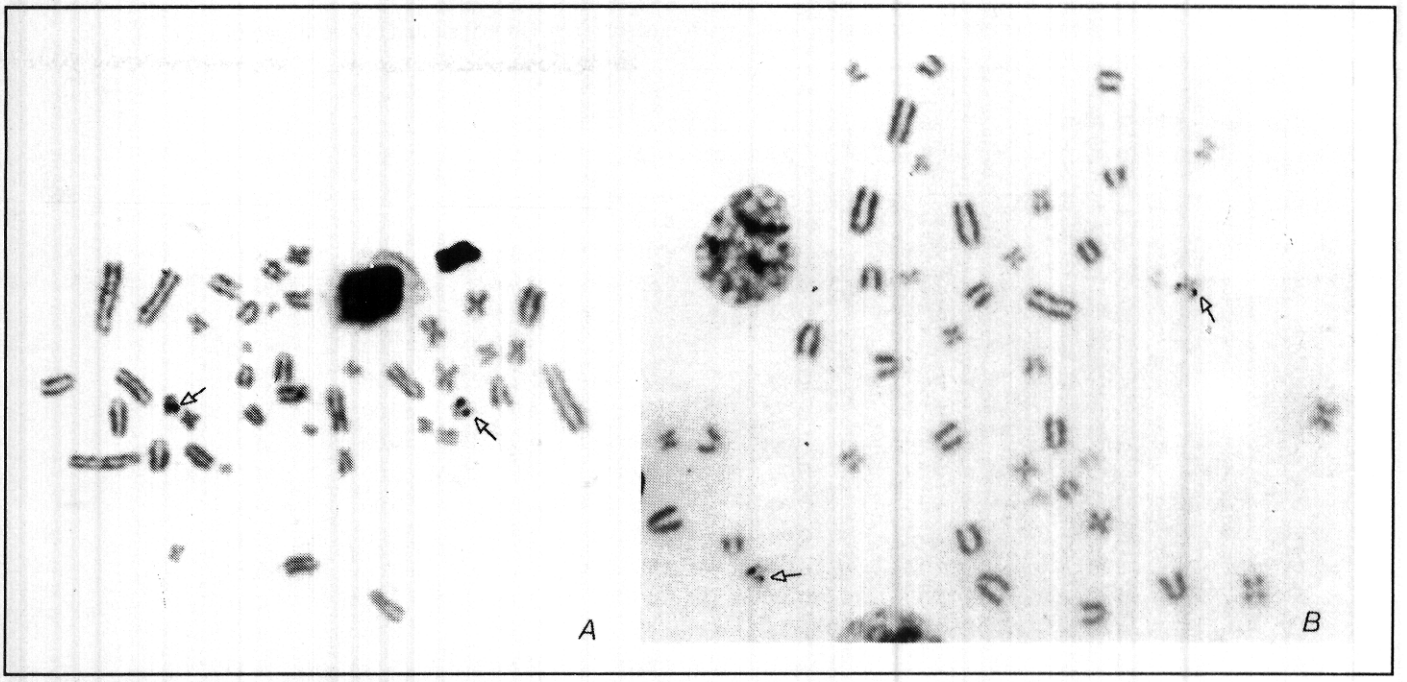


Figure 2 - Metaphase of a male alligator (A) and female (B) under NOR-banding. The arrows indicate the NOR bearing chromosomes.

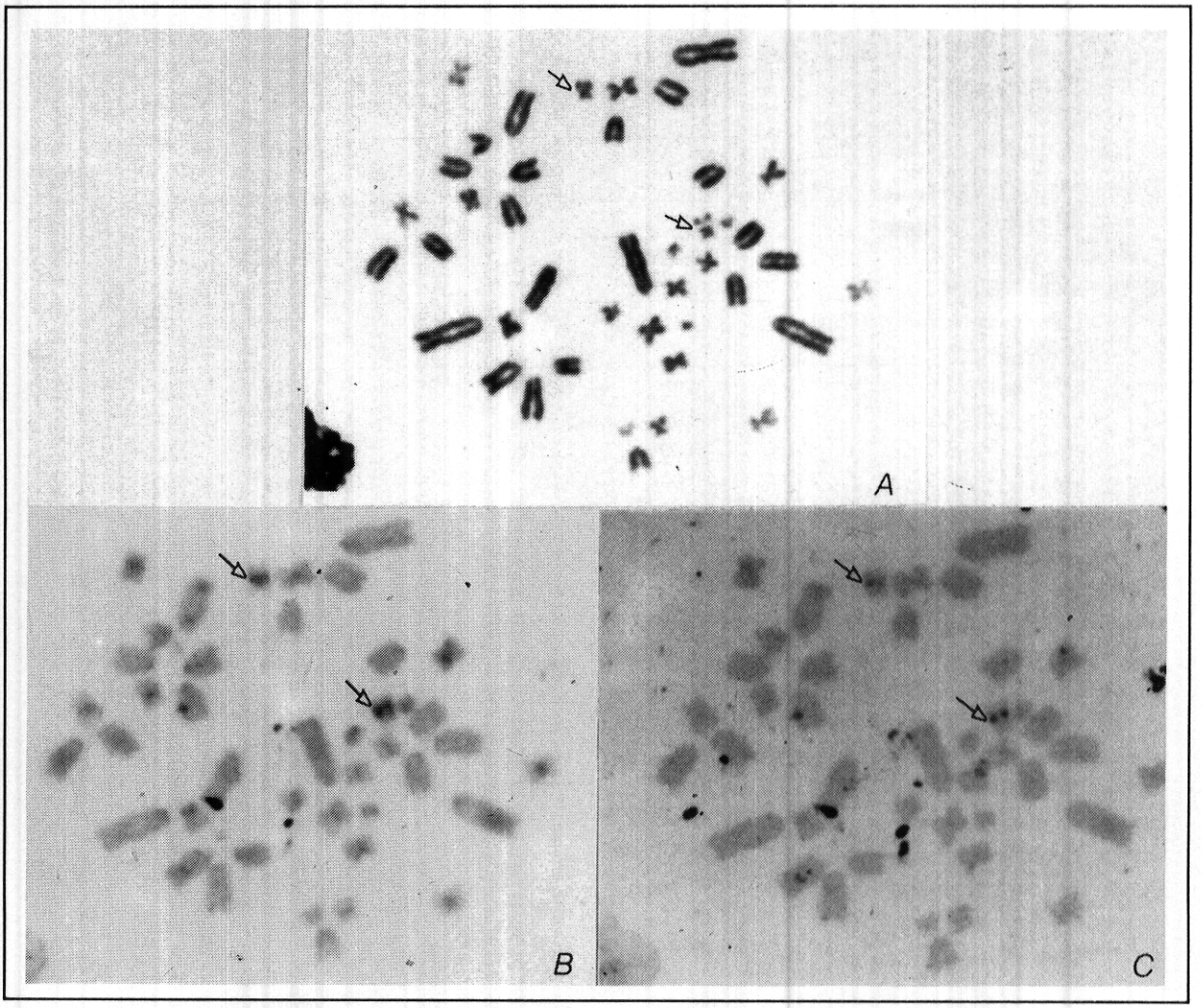


Figure 3 - Sequentional analysis with Giemsa conventional staining (A) C-band (B) and NOR-band (C) of alligator. The arrows indicate the chromosomes with marked NOR and C-bands.

Table I - Relative medium lengths of q, p and q+p chromosome arms from male and female alligators.

| Chromosome pairs | Males | | | Females | | |
|---|---|---|---|---|---|---|
| | q | p | q+p | q | p | q+p |
| 1 | 8.69 | 0.00 | 8.69 | 8.57 | 0.00 | 8.57 |
| 2 | 8.48 | 0.00 | 8.48 | 8.34 | 0.00 | 8.34 |
| 3 | 5.93 | 0.00 | 5.93 | 6.00 | 0.00 | 6.00 |
| 4 | 5.77 | 0.00 | 5.77 | 5.81 | 0.00 | 5.81 |
| 5 | 5.13 | 0.00 | 5.13 | 5.35 | 0.00 | 5.35 |
| 6 | 5.07 | 0.00 | 5.07 | 5.21 | 0.00 | 5.21 |
| 7 | 5.00 | 0.00 | 5.00 | 4.96 | 0.00 | 4.96 |
| 8 | 4.99 | 0.00 | 4.99 | 4.95 | 0.00 | 4.95 |
| 9 | 4.79 | 0.00 | 4.79 | 4.72 | 0.00 | 4.72 |
| 10 | 4.20 | 0.00 | 4.20 | 4.22 | 0.00 | 4.22 |
| 11 | 3.96 | 0.00 | 3.96 | 3.85 | 0.00 | 3.85 |
| 12 | 2.13 | 0.00 | 2.13 | 2.17 | 0.00 | 2.17 |
| 13 | 2.70 | 2.60 | 5.30 | 2.32 | 2.29 | 4.61 |
| 14 | 2.31 | 2.21 | 4.52 | 2.32 | 2.19 | 4.51 |
| 15 | 2.31 | 2.21 | 4.52 | 2.26 | 2.19 | 4.45 |
| 16 | 2.18 | 2.06 | 4.24 | 2.06 | 2.01 | 4.07 |
| 17 | 1.75 | 1.62 | 3.37 | 1.96 | 1.76 | 3.72 |
| 18 | 1.19 | 1.14 | 2.33 | 1.27 | 1.21 | 2.48 |
| 19 | 2.65 | 1.73 | 4.38 | 2.75 | 1.82 | 4.57 |
| 20 | 2.35 | 1.54 | 3.89 | 2.39 | 1.56 | 3.95 |
| 21 | 2.09 | 1.36 | 3.45 | 2.22 | 1.42 | 3.64 |

Table II - Arm ratio (AR), centromeric index (CI) and Real length (RL) of alligator chromosomes.

| Chromosome pairs | Males | | | Females | | |
|---|---|---|---|---|---|---|
| | AR | IC | RL | AR | IC | RL |
| 1 | * | 0.00 | 6.08 | * | 0.00 | 5.99 |
| 2 | * | 0.00 | 5.93 | * | 0.00 | 5.83 |
| 3 | * | 0.00 | 4.15 | * | 0.00 | 4.20 |
| 4 | * | 0.00 | 4.03 | * | 0.00 | 4.06 |
| 5 | * | 0.00 | 3.59 | * | 0.00 | 3.74 |
| 6 | * | 0.00 | 3.54 | * | 0.00 | 3.67 |
| 7 | * | 0.00 | 3.50 | * | 0.00 | 3.47 |
| 8 | * | 0.00 | 3.50 | * | 0.00 | 3.46 |
| 9 | * | 0.00 | 3.33 | * | 0.00 | 3.30 |
| 10 | * | 0.00 | 2.94 | * | 0.00 | 2.95 |
| 11 | * | 0.00 | 2.77 | * | 0.00 | 2.69 |
| 12 | * | 0.00 | 1.49 | * | 0.00 | 1.52 |
| 13 | 1.03 | 49.05 | 3.71 | 1.01 | 49.67 | 3.22 |
| 14 | 1.04 | 48.89 | 3.16 | 1.05 | 48.55 | 3.15 |
| 15 | 1.04 | 48.89 | 3.16 | 1.03 | 49.21 | 2.99 |
| 16 | 1.04 | 48.58 | 2.96 | 1.02 | 49.38 | 2.84 |
| 17 | 1.08 | 48.07 | 2.35 | 1.11 | 47.31 | 2.60 |
| 18 | 1.05 | 48.92 | 1.63 | 1.04 | 48.79 | 1.73 |
| 19 | 1.53 | 39.49 | 3.06 | 1.51 | 39.82 | 3.19 |
| 20 | 1.52 | 39.58 | 2.72 | 1.53 | 39.49 | 2.76 |
| 21 | 1.53 | 39.42 | 2.41 | 1.56 | 39.01 | 2.47 |

(*) = Infinite.

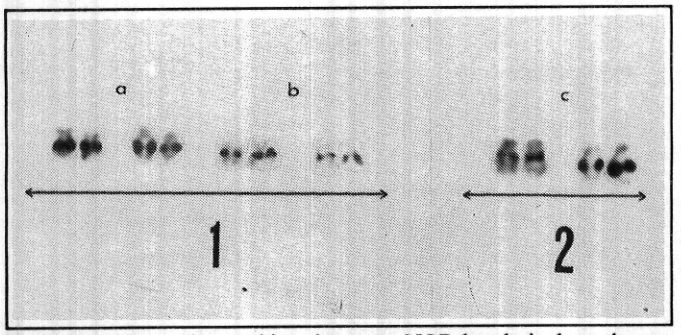


Figure 4 - Size polymorphism between NOR-bands in homologous chromosomes of alligatores. The first four pairs (1) correspond to two females (a and b), and the last two pairs (2) to a male (c).

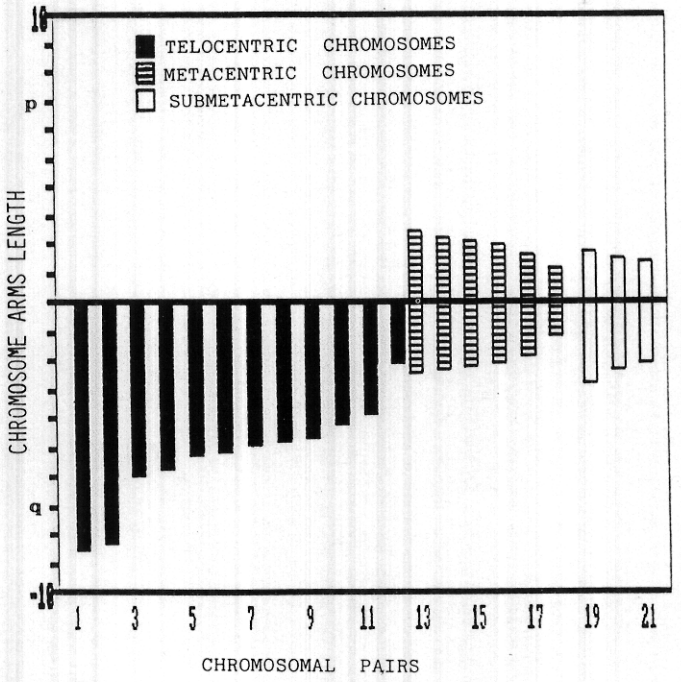


Figure 5 - Idiogram of the yellow throated alligator (*Caiman latirostris* Daudin).

medium size (the telocentrics 1 to 4) all the rest being small. A representative idiogram for male and female alligators based on their chromosome biometry is show in Figure 5.

## ACKNOWLEDGMENTS

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## RESUMO

O objetivo deste trabalho foi analisar citogeneticamente o jacaré de papo amarelo (*Caiman latirostris* Daudin) sob técnica convencional e de bandeamentos cromossômicos: C e NOR, além de biometria cromossômica visando sua classificação. Foram utilizados sangue de oito espécimes de ambos os sexos, incubado em meio de cultura.

Verificou-se que o número diplóide é 42, sendo que 24 são telocêntricos, 12 metacêntricos e seis submetacêntricos, com comprimento real que varia de 1,49 a 6,08, de 1,63 a 3,71 e de 2,41 a 3,19 micrômetros, respectivamente. O número fundamental de braços é 60 e, cerca de 80,95% dos cromossomos são pequenos e 19,04% são medianos. Foi apresentado, pela primeira vez para a espécie, o bandeamento NOR, verificando-se que apenas um par submetacêntrico (número 20) evidencia região organizadora de nucléolo no braço q, correspondendo à uma constricção secundária observada sob coloração convencional. Não houve associação cromossômica entre os cromossomos portadores de NOR.

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