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Karyotypic Studies of two Species of South American Snakes (*Boa constrictor amarali* and *Bothrops jararaca*)[1]

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Abstract. Karyotypic studies have been made of two species of South American snakes, *Boa constrictor amarali* and *Bothrops jararaca*. Both have 36 chromosomes; 16 macrochromosomes and 20 microchromosomes. While no morphological difference is discernible between the mitotic chromosome complements of the male and the female *Boa constrictor amarali*, a heteromorphic pair is evident in the female *jararaca*, in which the subterminal W is distinctly smaller than the mediocentric Z. Thus female heterogamety of the ZZ-ZW type is cytologically recognizable in *Bothrops jararaca*.

Introduction

The sex elements can be readily identified by cytological means in the chromosome complements of most mammalian and avian species. In the lower vertebrates, however, the Z and W, or X and Y, may still be so undifferentiated as to be morphologically indistinguishable.

Earlier claims by Oguma (1934), Makino and Asana (1948) and Makino and Momma (1949) that female heterogamety in reptiles could be recognized cytologically were seriously questioned by Matthey (1943), Margot (1946), Matthey and van Brink (1956) and van Brink (1959).

The present report describes the chromosome constitutions of two species of South American snakes, *Boa constrictor amarali* and *Bothrops jararaca*. In the latter, the Z and W were clearly recognized as a heteromorphic pair in the female.

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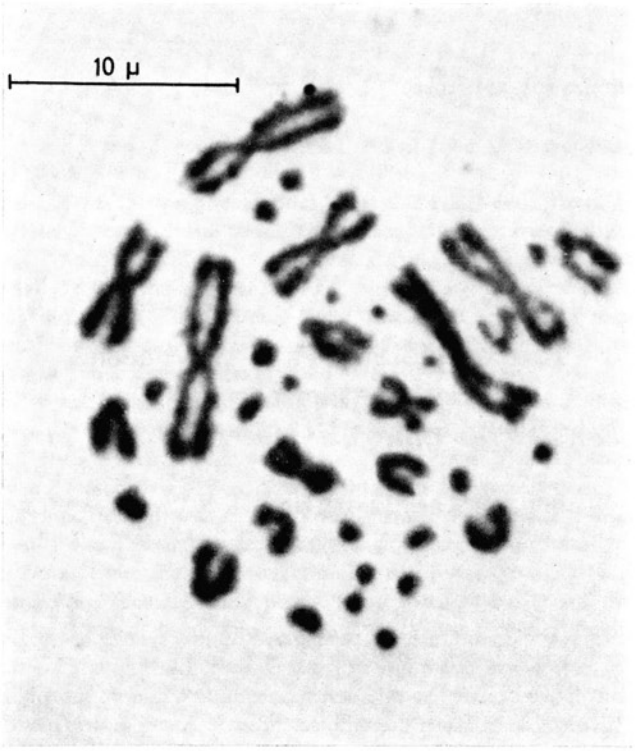
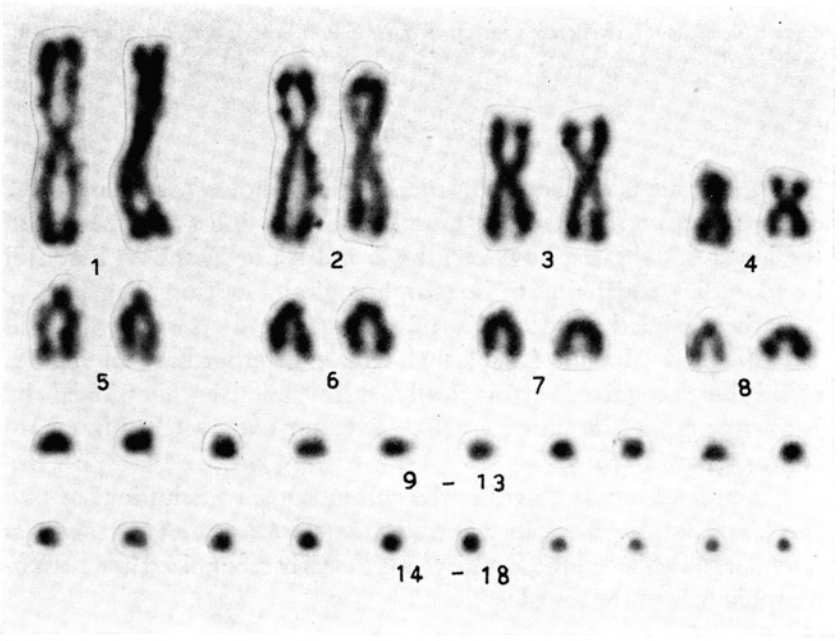


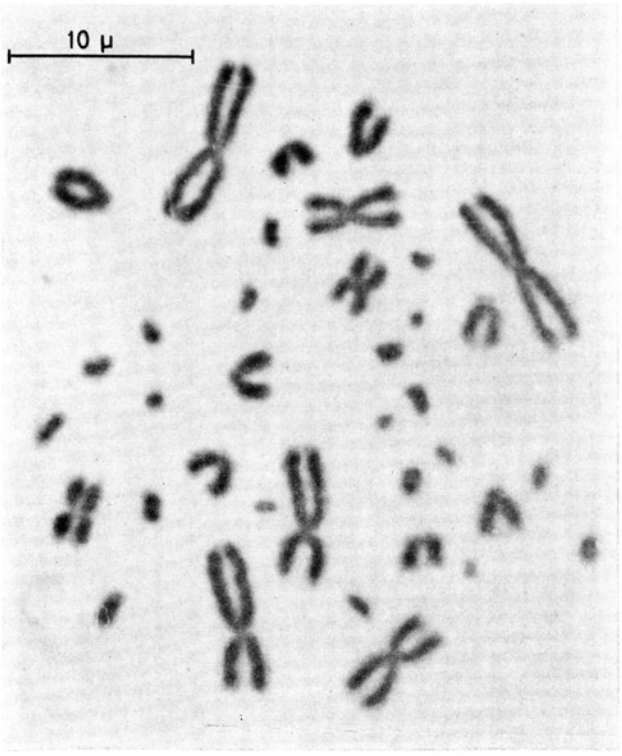
1 a



1 b

All mitotic metaphase figures are from leucocytes which had been subjected to short term culture. All slides were stained with orcein.

Fig. 1 a. Mitotic metaphase figure of a male *Boa constrictor amarali* showing 36 chromosomes. 1 b. Karyotype showing eight pairs of macrochromosomes and 20 microchromosomes.



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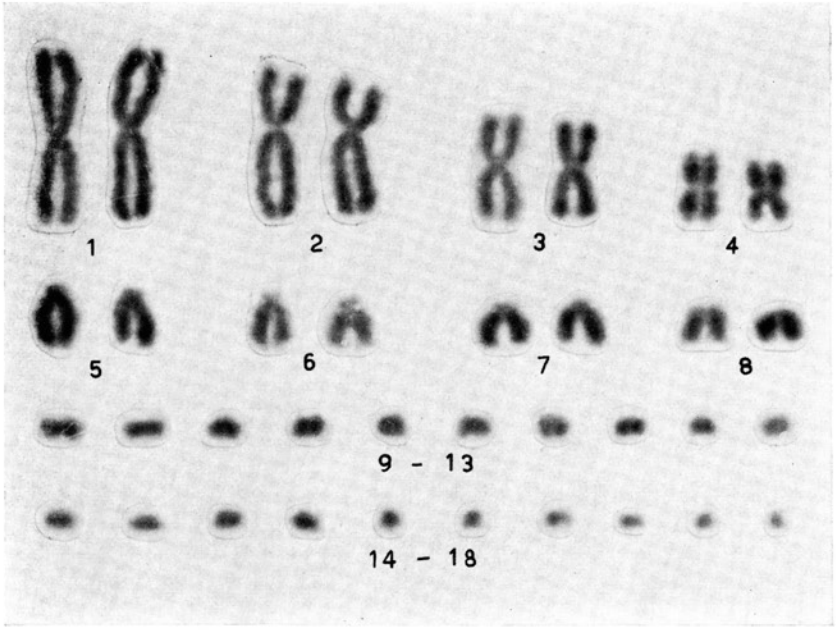
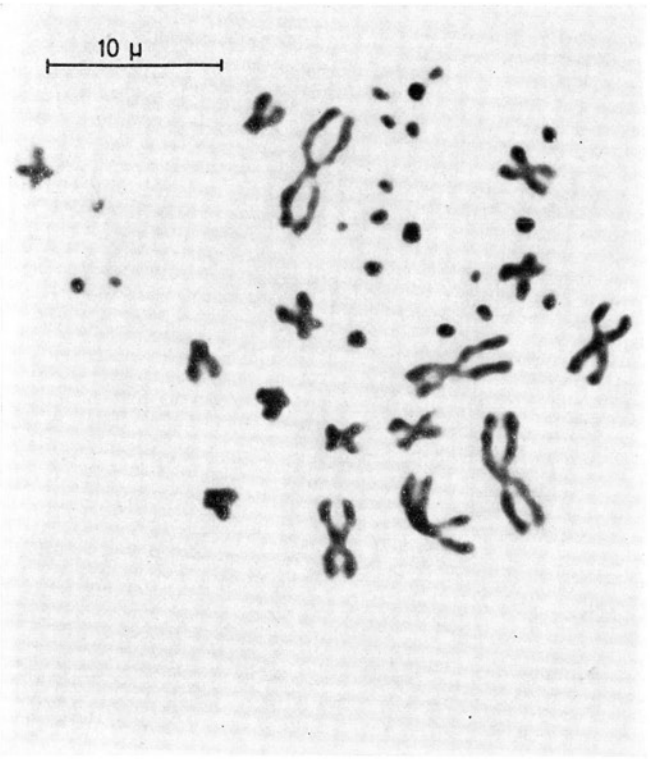
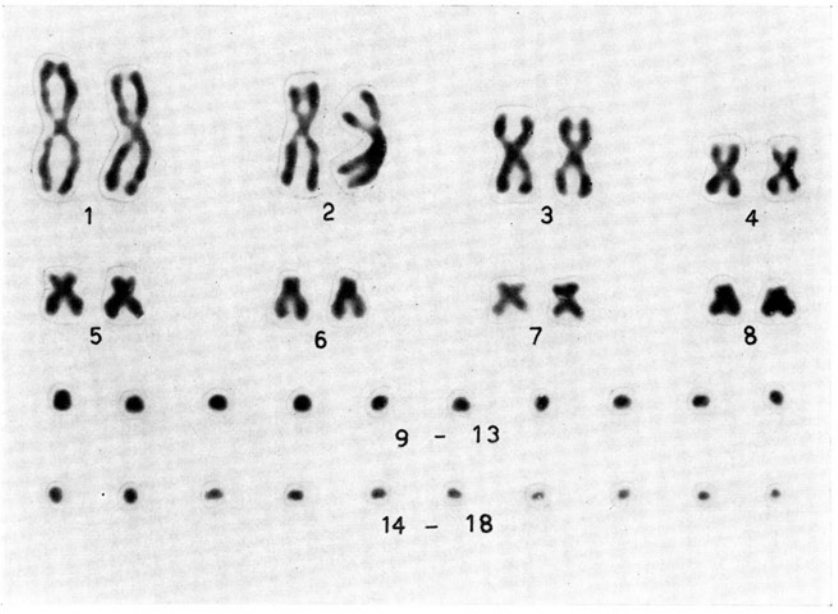


2 b

Fig. 2a. Mitotic metaphase figure of a female *Boa constrictor amarali*. 2b. Karyotype showing eight pairs of macrochromosomes and 20 microchromosomes.

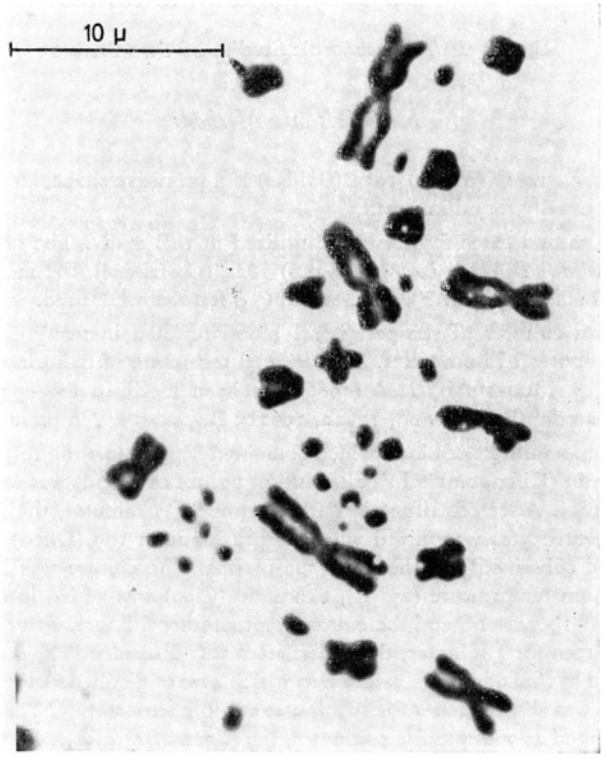


3 a

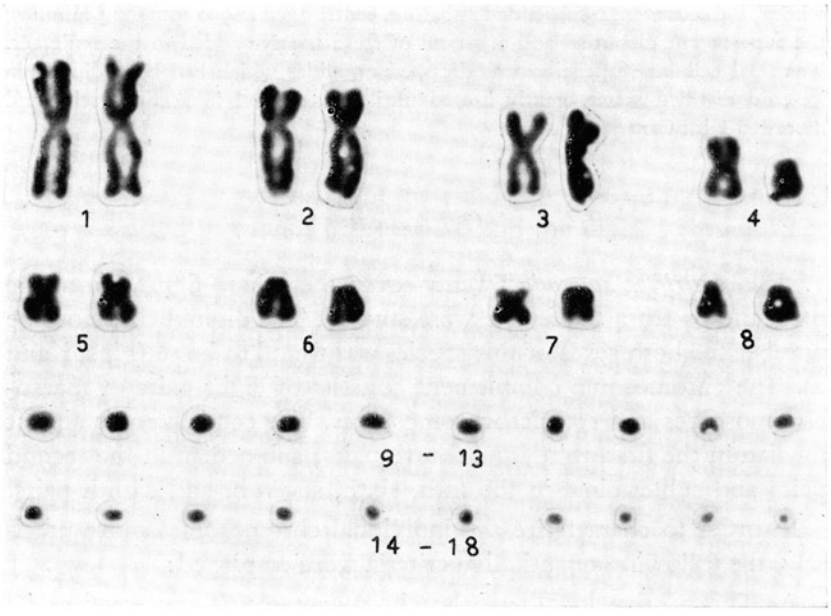


3 b

Fig. 3 a. Mitotic metaphase figure of a male *Bothrops jararaca* with 36 chromosomes. 3 b. Karyotype showing eight pairs of macrochromosomes and 20 microchromosomes.



4 a



4 b

Fig. 4a. Mitotic metaphase figure of a female *Bothrops jararaca*. 4b. Karyotype showing seven pairs of homomorphic chromosomes, one pair of heteromorphic chromosomes (presumably the ZW pair), and 20 microchromosomes.

Materials and Methods

Boa constrictor amarali (STULL) 1932 (Boidae) is a primitive snake, large and non-poisonous, usually found in Brasil.

Two males and three females were utilized in this study. The other species investigated was *Bothrops jararaca* (WIED) 1824 (Crotalidae), the most common poisonous snake of Brasil. Two males and two females were used.

Short-term cultures of the peripheral blood resulted in many clear somatic metaphase figures of both sexes. The original technique of culturing leucocytes developed by MOORHEAD *et al.* (1960) has been modified for application to ophidian material (BEÇAK *et al.*, 1962a, 1962b; BEÇAK *et al.*, in press).

Five to ten milliliters of blood which contained heparin (0.1 mg/ml) and Phytohemagglutinin (Difco, 0.01 ml stock solution per ml of blood) was kept ice-cold for 30 minutes. After centrifugation at 400 rpm for 5 minutes, the supernatant containing leucocytes was mixed with Culture Medium 199 (Difco) so that the final culture contained 30% homologous plasma. The culture was kept for 72 hours at room temperature (25° C), Colcemid (Ciba) was added in a final concentration of 1×10^{-6} M, and the culture kept another 6 hours. After centrifugation at 900 rpm for 5 minutes, the supernatant was discarded. The sediment was resuspended in 1 ml of culture medium to which 4 ml of distilled water was added; the mixture was allowed to set for 10 minutes at room temperature. The suspension was centrifuged at 700 rpm for 5 minutes, the supernatant discarded, and fixative (acetic acid:methanol = 1:3) added to the sediment. After fixation for at least 1 hour, the cells were resuspended as before, centrifuged at 900 rpm for 5 minutes, the supernatant discarded and 0.5–1 ml of fresh fixative added to the sediment. The final cellular suspension was dropped on slides which had been dipped in ice-cold distilled water, gently heated until dry, stained in acetic orcein, and mounted with Canada balsam.

Observations

1. *Boa constrictor amarali*. Twenty-seven metaphase figures from the male and 67 from the female were studied. The diploid chromosome number for both sexes of this species was found to be 36 (Figs. 1 and 2). The chromosome complement consists of eight pairs of macrochromosomes and 20 microchromosomes. The centromere is almost median in the first, third, and fourth pairs; submedian in the second pair; and subterminal in the fifth, sixth, seventh, and eighth pairs.

In order to characterize the individual chromosomes more precisely, the following quantitative criteria were employed:

A. The relative length of the chromosome was expressed as its percentage of the sum of the lengths of the eight macrochromosomes of the haploid set.

B. Arm ratio was determined by measuring the long and short arms of each chromosome.

C. The centromere index was obtained by finding how much of the total length of each chromosome was comprised of the short arm.

Averages of the values obtained from 10 metaphase figures of both sexes are presented in Table I. Neither the quantitative evaluation described above nor direct observation of the karyotypes revealed the presence of a heteromorphic sex pair in either the male or the female of *Boa constrictor amarali*.

TABLE I

Boa constrictor amarali

Chromosome pairs	Nº 1	Nº 2	Nº 3	Nº 4	Nº 5	Nº 6	Nº 7	Nº 8
Relative length of chromosome in percentage	26	21	16	10	9	8	6	6
Arm ratio	1.1	1.4	1.2	1.0	4.5	6.8	7.4	6.6
Centromere index	0.49	0.42	0.46	0.49	0.18	0.14	0.12	0.14

2. *Bothrops jararaca*. Forty-one male and 46 female metaphase figures were subjected to careful observation, and the diploid chromosome number for this species was also found to be 36 (Figs. 3 and 4). In the male, the macrochromosomes are easily sorted into eight homomorphic pairs. The first, third, and fourth pairs are mediocentric; the second, fifth, and seventh pairs have submedian centromeres, and the sixth and eighth pairs have subterminal centromeres.

In the female, however, the fourth pair is distinctly heteromorphic. One element is much smaller than the other and has a subterminal centromere as well. Thus in this species the fourth largest pair of chromosomes appears to be the sex pair; the female is the heterogametic sex, and the sex-determining mechanism is the ZZ–ZW type. The fact that the W chromosome appearing only in the heterogametic female is seven-tenths the size of the Z (Table II) permits cytological identification of the sex chromosomes in *Bothrops jararaca*.

TABLE II

Bothrops jararaca

Chromosome pairs	N° 1	N° 2	N° 3	N° 4 Z	N° 4 W	N° 5	N° 6	N° 7	N° 8
Relative length of chromosome in percentage	26	21	15	10	7	8	7	7	6
Arm ratio	1.1	1.5	1.2	1.1	3.5	1.6	2.7	1.5	2.3
Centromere index	0.48	0.40	0.47	0.48	0.23	0.39	0.29	0.41	0.31

Discussion

Aside from VAN BRINK's recent cytological investigation (1959) of a few species of snakes, earlier reports on ophidian chromosomes were based on observations of sectioned testicular material from the male which in reptiles is likely to be the homogametic sex (THATCHER 1922; NAKAMURA 1927, 1928, 1936; MATTHEY 1931; MAKINO and MOMMA 1949; BHATNAGAR 1960).

Thus although a heteromorphic sex pair could not be recognized in *Boa constrictor amarali* during the present study, nor in those snakes studied by VAN BRINK (1959), homomorphism between the Z and W, or X and Y, need not prevail throughout the entire ophidian group. At the conclusion of our study of *Bothrops jararaca*, we read the report by KOBEL (1962) who identified a heteromorphic sex pair in the female of *Vipera berus* L. (Viperidae). Continuing cytological investigations may well show that the sex elements of snakes are morphologically distinguishable as often as not.

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