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Karyotype of Amphibians in Saudi Arabia 2: The Karyotype of *Hyla savignyi*

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Abstract: The karyotype of the tree frog *Hyla savignyi* consists of 24 chromosomes. These chromosomes include 5 pairs of metacentric, 5 pairs of submetacentric and 2 pairs of acrocentric chromosomes. The ninth pair sex chromosomes are homomorphic in females and heteromorphic in males. The fundamental number of this species is 44, while the autosomal fundamental number is 40. The ninth pair of this karyotype has a secondary constriction only on the long arms of females chromosomes.

Key words: Amphibian, tree frog, karyotype, chromosomes, *Hyla savignyi*

INTRODUCTION

Arabian anura have been studied from the morphological, classification and ecological point of view and the recent surveys carried by Balletto *et al.*^[1] describing the amphibians of the Arabian Peninsula. *Bufo arabicus* is the first toad to be classified from Arabian Peninsula^[2]. However, more research mainly on taxonomy and distribution of amphibian from Saud Arabia has been accumulated by many researchers^[3-7].

The tree frog *Hyla savignyi* is one of the most endemic to Arabian Peninsula. This frog inhabits the areas between 400 to 1000 mm isohyetal lines, where there are perennial or semi-perennial water bodies, lower temperature and relatively good vegetation^[1].

It is well known that anurans are abundant, varied, widespread and the diversity of their karyotypes make them particularly suited to cytogenetic research^[8]. Therefore, the main objective of this study was to elucidate the chromosome structure of this tree frog and confirm its karyotype.

MATERIALS AND METHODS

Samples of male and female *Hyla savignyi* Audouin 1827 were collected from Aseer province of Kingdom of Saudi Arabia, Abha region. Each sample was injected intraperitoneal with 0.2 mL of colchicine solution (1 mg mL⁻¹) for 24 h before being killed. The

bone marrow of the femur was flushed with 5 mL of 0.075 M KCL into centrifuge tube. The cell suspension was kept at room temperature for 15 min and a few drops of 1:3 glacial acetic acid and absolute methanol, freshly prepared fixative, were added. The cells were pelleted by centrifugation and the supernatant was discarded. A fresh fixative was added to the cells, suspended very well, left for 30 min and centrifuged again. The suspension, fixation and centrifugation of the cells were repeated three times. The slides were prepared by placing two drops of cell suspension on clean very cold slide and air-dried. The chromosomes were stained with Giemsa stain. More than 100 metaphase chromosomes spreads from 10 males and 10 females were examined.

RESULTS AND DISCUSSION

The present study deals with karyotype of *Hyla savignyi* Audouin 1827, were collected from Abha region, Kingdom of Saudi Arabia. The diploid number of this species found to be 24 chromosomes and the karyotype was classify into three major groups (Figs. 1 and 2). Following the nomenclature proposed by Levan *et al.*^[9]. Group 1 consists of 5 pairs of metacentric chromosomes, 1-2 , large chromosomes and 3-5, small chromosomes. Group 2 consists of 5 pairs of submetacentric chromosomes, chromosome 6, large chromosome, 7-8, medium chromosomes and the rest 9-10 as smaller ones. Group 3 consists of 2 pairs of acrocentric chromosomes; 11, large chromosome and 12, medium chromosome

Table 1: Arm ratios and type of centromeres of *Hyla savignyi*

Chrom. No.	Short arm P (µm)	Long arm Q (µm)	Total length q + p (µm)	Arm ratio q/p	Type of centromere
1	4.68	6.24	10.92	1.3	M
2	3.90	5.07	8.97	1.3	M
3	1.95	2.34	4.29	1.2	M
4	1.56	2.34	3.90	1.5	M
5	1.17	1.95	3.12	1.6	M
6	3.12	5.46	8.58	1.7	SM
7	2.33	4.67	7.00	2.0	SM
8	1.95	3.51	5.46	1.8	SM
9 X	1.56	3.12	4.68	2.0	SM
Y	1.17	2.73	3.90	2.3	SM
10	0.78	1.56	2.34	2.0	SM
11	1.56	5.85	7.41	3.7	A
12	1.17	4.29	5.46	3.6	A

Table 2: The Haploid, diploid and fundamental chromosome numbers of *Hyla savignyi* chromosomes

Centromere Type	Haploid No. (n)	Diploid No. (2n)	Fundamental No. (FN)	Autosome fundamental No. (Fn ^a)
M	5	10	20	20
SM	5	10	20	16*
A	2	4	4	4
Total	12	24	44	40

*. The sex chromosomes not included

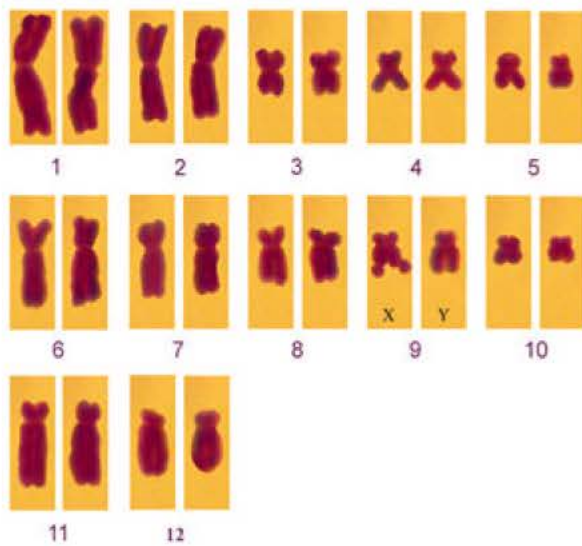


Fig. 1: Karyotype of male *Hyla savignyi*

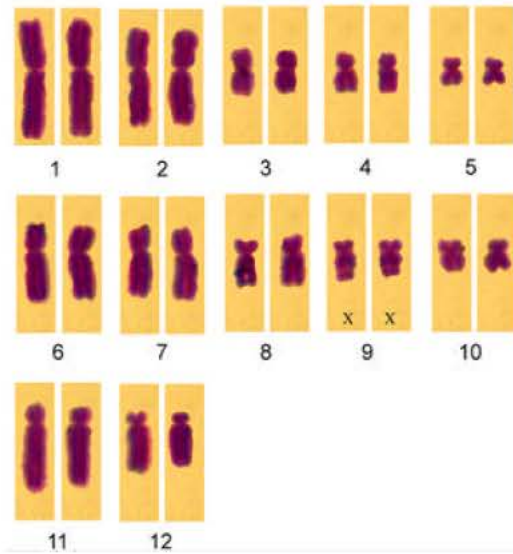


Fig. 2: Karyotype of female *Hyla savignyi*

(Table 1). Chromosome number 9 represents small submetacentric sex chromosomes of the type xy in males and xx in females. The x chromosome was longer than y chromosomes by nearly 0.8 µm (Table 1). The x chromosome also had a secondary constriction on its long arms and could be considered as a marker chromosome for this species.

The Fundamental Number (FN) were calculated to be 44 and the Fundamental Number of autosomes (FN_a) to be 40 (Table 2). The type of centromeres were determined according to the formula of Matthey and the finding are represented in Fig. 3.

It is well known that most of the Hylidae to which the tree frogs of the genus *Hyla* belong, had diploid chromosome number range between 22-34 chromosomes^[10-16]. It is also well known that all species belonging to the genus *Hyla* have 24 diploid chromosome number^[17-21]. Therefore, this study reconfirmed that the diploid number of *Hyla savignyi* is 24 chromosomes as recently established by several investigators^[22-24]. The sex chromosomes and the secondary constriction location on x chromosome were also determined.

In conclusion, the present results revealed that, the karyotype of *Hyla savignyi* and contributed more

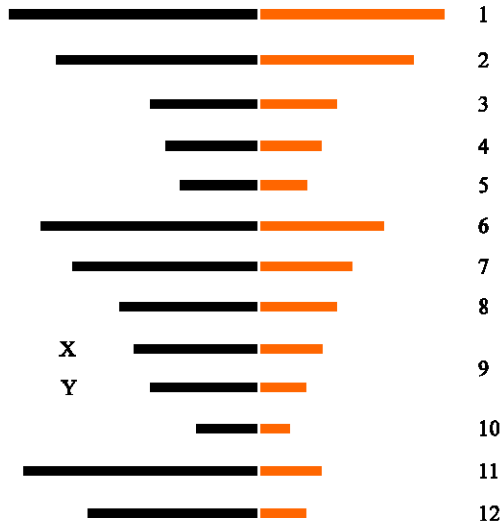


Fig. 3: An idiogram of *Hyla savignyi* constructed on the basis of chromosome number and the position of the centromere

cytological knowledge, especially if we considered this karyotype is the first report for this species in Saudi Arabia.

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