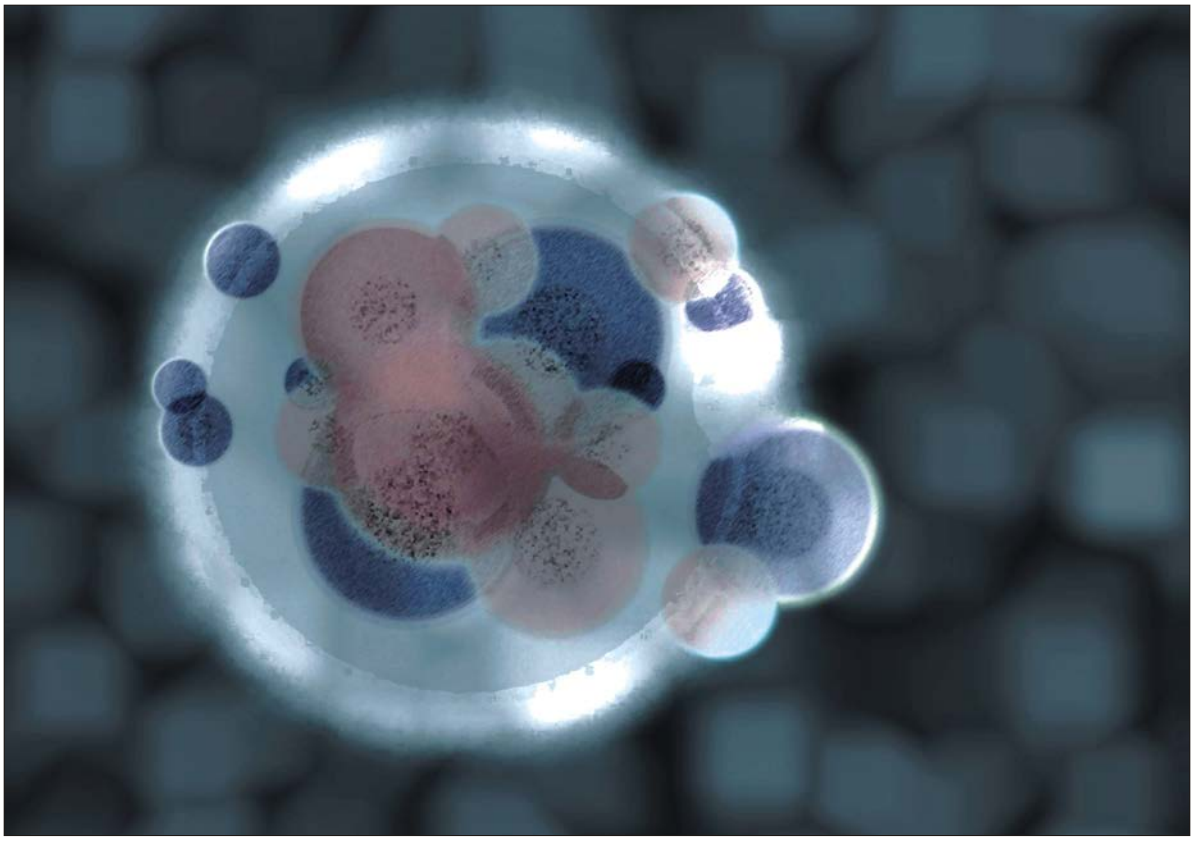

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Karyotype of Amphibians in Saudi Arabia 1: The Karyotype of *Rana ridibunda*

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Abstract: The karyotype of the marsh frog *Rana ridibunda* is consist of 26 chromosomes. These chromosomes are 7 pairs of metacentric and 6 pairs of submetacentric chromosomes. The second pair sex chromosomes, are homomorphic in females and heteromorphic in males. The fundamental number of this species is 52, while the autosomal fundamental number is 48. The tenth pair of this karyotype has a secondary constriction on the long arms of the chromosomes on both sexes.

Key words: Amphibian, frog, karyotype, chromosomes

INTRODUCTION

The first description of an Arabian amphibian was *Bufo arabicus*[1]. Since that time till late 1950's more herpetological material was made available but the recent surveys carried by Balletto *et al.*[2] is the most precise work describing the amphibian of the Arabian Peninsula.

However, more interest has concentrated on studies of amphibia from Kingdom of Saudi Arabia mainly on taxonomy and distribution. The marsh frog *Rana ridibunda* is the only one true frog reported from the oasis of Al-Hassa and Al-Qatif[3-6]. This frog has been subject of study to determine the seasonal changes in population structure, breeding behavior, tadpole development and individual growth in Saudi climate[7].

In spite of the phenotypic similarity among related species, karyological studies can be used to identify each species unequivocally[8]. Therefore, the main objective of this study was to elucidate the chromosome structure of this marsh frog and confirm its karyotype.

MATERIALS AND METHODS

Samples of males and females of *Rana ridibunda* Pallas 1771 were collected from eastern province of Kingdom of Saudi Arabia, Al-Hassa region. Each sample was injected interaperitoneal 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

The present study deals with karyotype of Rana ridibunda Pallas 1771, which has been collected from Al-Hassa region, Kingdom of Saudi Arabia. The diploid number of this species is found to be 26 chromosomes and the karyotype has been classify into two major groups (Fig. 1 and 2). Following the nomenclature proposed by Levan *et al.*[9]. Group1 consists of 7 pairs metacentric chromosomes, 1-4 are large chromosomes, 5-6 are medium chromosomes and 7 is small chromosome. Group 2 consists of 6 pairs of submetacentric chromosomes, chromosome 8 is large chromosomes and the rest 9-13 are small chromosomes (Table 1).

Chromosome number 2 represents metacentric sex chromosomes of the type xy in the males and xx in the females. The length of sex chromosomes is clearly differ between x and y chromosomes. The x chromosome is longer than y chromosomes by nearly 4 micrometer (Table 1). The 10th chromosome has a secondary constriction on its long arms and could be considered as a marker chromosome for this species.

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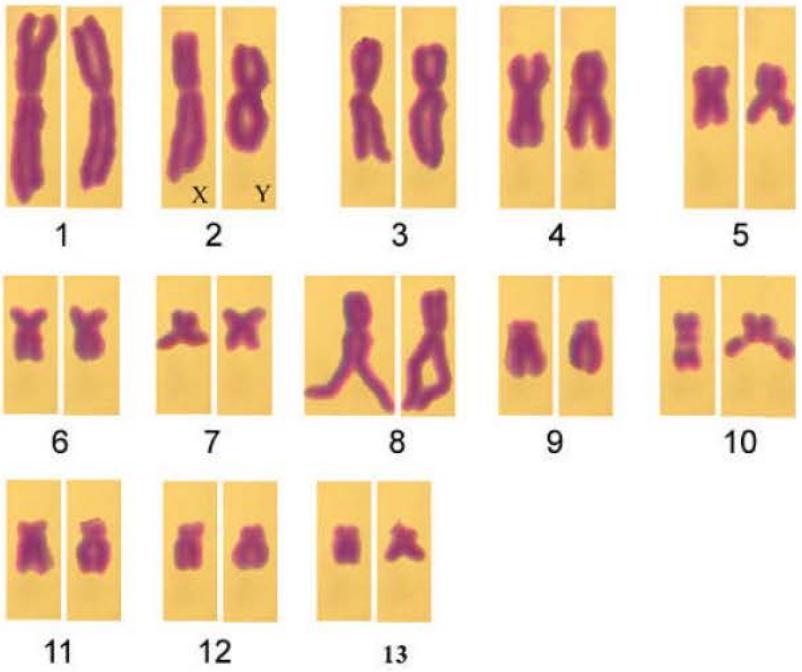

Fig. 1: Karyotype of male *Rana ridibunda*

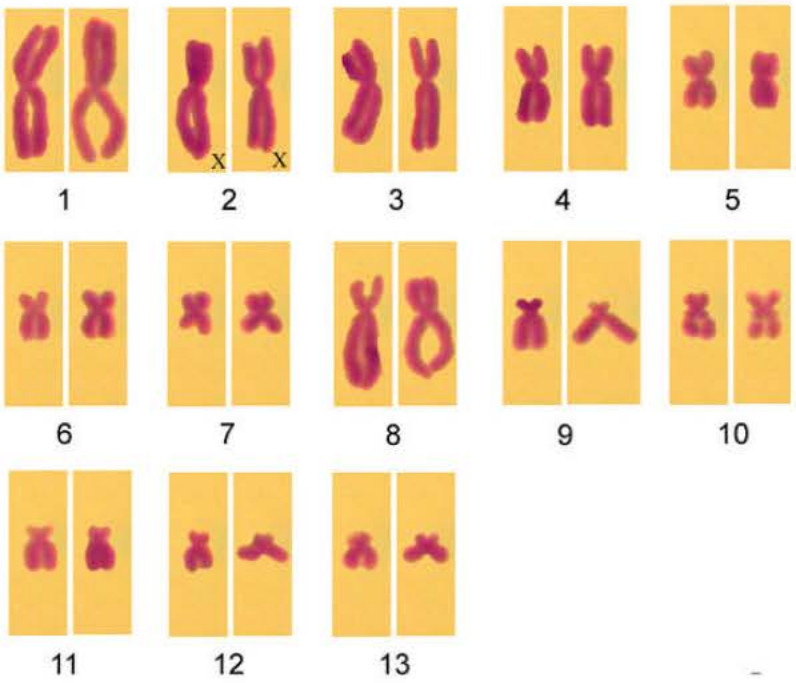

Fig. 2: Karyotype of female *Rana ridibunda*

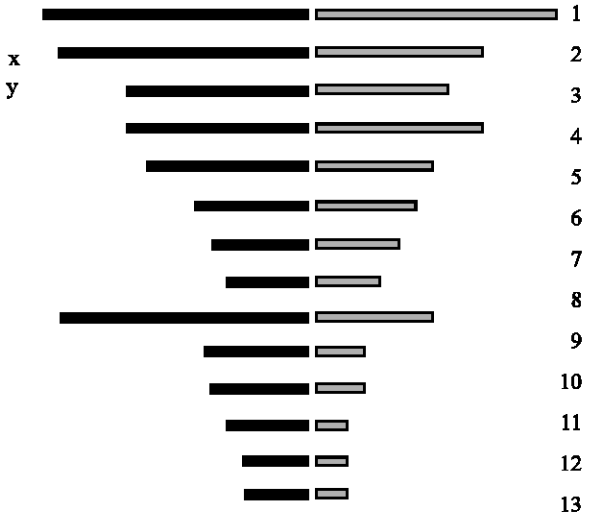

Fig. 3: An idiogram of *Rana ridibunda* constructed on the basis of chromosome numbers and the position of the centromere

Table 1: Arm ratios and type of centromeres of *Rana ridibunda*

Chrom. No	Short arm p	Long arm q	Total length q + p	Arm ratio q/p	Type of centromere
1	6.72	7.68	14.40	1.1	M
2 X	4.80	7.20	12.00	1.5	M
Y	3.84	5.28	9.12	1.4	M
3	4.80	5.28	10.08	1.1	M
4	3.36	4.80	8.16	1.4	M
5	2.88	3.36	6.24	1.2	M
6	2.40	2.88	5.28	1.2	M
7	1.92	2.40	4.32	1.3	M
8	3.36	7.20	10.56	2.1	SM
9	1.44	2.88	4.32	2.0	SM
10	0.96	2.40	3.36	2.5	SM
11	0.96	1.92	2.88	2.0	SM
12	0.96	1.92	2.88	2.0	SM
13	1.44	3.36	4.80	2.3	SM

Table 2: The Haploid, diploid and fundamental chromosome numbers of Rana ridibunda chromosomes

Centromere type	Haploid No. n	Diploid No. 2n	Fundamental No. FN	Autosome fundamental No. FN[a]
M	7	14	28	24*
SM	6	12	24	24
Total	13	26	52	48

*. The sex chromosome has not been counted.

The Fundamental Number (FN) has been calculated to be 52 and the Fundamental Number of autosomes (FNa) 48 (Table 2). The type of centromeres has been determined according to the formula of Matthey and the finding are represented in Fig. 3.

DISCUSSION

It is well known that most of the frogs of the genus *Rana* have 26 metacentric and submetacentric chromosomes[10-20]. Therefore, this study was reconfirmed that the diploid number of *Rana ridibunda* is 26 chromosomes as established by several investigators[21-26]. Not only has this, but a fine measurements and careful classification and clear picture of the morphology of the chromosomes has been presented. The sex chromosomes and the secondary constriction location have been determined too.

One of the earliest study dealing with chromosome banding of the genus *Rana* is the work of Schmid[27]. Schmid[27] examined 5 species belonging to the genus *Rana* and *Rana* ridibunda is one of them and failed to identify sex chromosomes by this technique. In the case of *R. ridibuda* in particular he examined only 4 males, therefore, he failed to identify the sex chromosomes in this species. However, the existence of sex chromosomes among amphibian of the order Anura, especially genus *Rana* has been recently reported[28-31].

The existence of the secondary constriction on the long arms of chromosome 10 is well established on most *Rana* species which support the finding of this study[21-24]. There is one study carried on 7 species of *Rana* by Miura using banding technique indicated that the secondary constriction is located on chromosome number 11 instead of chromosome number 10[25]. By looking carefully to the figure representing the karyotype of those species, it is very easy to recognize that chromosome No. 11 is longer than chromosome No.10 . If this is so, then we can conclude that the secondary constriction is located on the chromosome No.10, which support present finding.

In conclusion, the present results have revealed in details the karyotype of *Rana ridibunda* in order to contribute more cytological knowledge, especially if we considered this is the first report for this species in Saudi Arabia.

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