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Karyotype of Amphibians in Saudi Arabia. 3. The Karyotype of *Bufo regularis*

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Abstract: The karyotype of the maculated toad *Bufo regularis* is consist of 20 chromosomes. These chromosomes are nine pairs of metacentric and one pairs of submetacentric chromosomes. According to the size the metacentric chromosomes, are of three types, chromosomes 1-4 large, chromosomes 5-6 medium and chromosomes 7-9 small size, while the submetacentric chromosomes are small size. The third pair of this karyotype has a secondary constriction on the short arms of both male and female chromosomes. The fundamental number of this species is 40.

Key words: Amphibian, toad, karyotype, chromosomes

INTRODUCTION

Bufo arabicus is the first toad to be classified from Arabian Peninsula (Reuss, 1833). Furthermore, Arabian anura have been studied from the morphological, classification, ecological point of view and more information has been accumulated and several papers also published (Schmidt, 1953; Hass, 1957, 1961; Briggs, 1980, 1981). The recent surveys carried by Balletto *et al*. (1985) are the most precise work describing the amphibian of the Arabian Peninsula (Balletto *et al*., 1985). They found nine nominal Anuran species, six of which are endemic to the peninsula (*Bufo arabicus*, *Bufo hadramautinus*, *Bufo scorteccii*, *Bufo dhufarensis*, *Bufo tihamicus* and *Euphlyctis ebrenbergii*) and three of which occur in the Palaearctic region (*Bufo viridis*, *Hyla savignyi*, *Rana ridibunda*).

Studying the Amphibian chromosomes started as early as 1932, when Makino described the existence of 22 chromosomes in the genus *Bufo sachalinensis* by histological sectioning of tissue which were undergoing mitosis (Makino, 1932). In Witschi (1933) described the diploid number of male *Bufo americanus* as 22 and Bianchi and Laguens (1964) confirmed the diploid number of Bufo arenarum as 22 by treating toad embryo with colchicines. Manna and Bhunya (1966) have mentioned that the chromosome number of an Indian toad, *Bufo stomatictus* studied not only from the germ cells of males but from the various tissues like liver, spleen and follicular epithelium of ovary of adult specimens as well as from the tail and intestine of the larval tadpoles. However somatic chromosomes of toad have very rarely been studied with success.

Goin and Goin (1962) listed the diploid number for *Bufo regularis* as 22 chromosomes and this encourage Manna and Bhunya (1966) to conclude that the diploid chromosome number for almost all reported Bufo species has not deviated from that of 22 chromosomes. In applying short-term tissue culture methods Beckert and Doyle (1968) to study of Anuran karyotypes, the diploid number of chromosomes for *Bufo regularis* has been determined to be 20.

In this study we are going to describe for the first time the diploid number of chromosomes for *Bufo regularis* from Kingdom of Saudi Arabia using bone marrow cells treated with colchicine *in vivo*.

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MATERIALS AND METHODS

Samples of males and females of *Bufo regularis* Reuss 1833 were collected from Bani Saad's village, Taif region, Makah province of Kingdom of Saudi Arabia 2005. 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 10% Giemsa stain in phosphate buffer pH 6.8. More than 100 metaphase chromosomes spreads from 10 males and 10 females were examined under bright field illumination, using 100X oil immersion objective and 10X eyepiece. by ordinary Zeiss microscope.

RESULTS AND DISCUSSION

The present study deals with karyotype *Bufo regularis* Reuss 1833, which has been collected from Taif region, Kingdom of Saudi Arabia. The diploid number of this species is found to be 20 chromosomes and the karyotype has been classify into two major groups (Fig. 1, 2). Following the nomenclature proposed by Levan *et al.* (1964). Group 1 consists of 9 pairs of metacentric chromosomes, 1-4 are large chromosomes, 5-6 are medium size and 7-9 are small chromosomes. Group 2 consists of 1 pair of small size submetacentric chromosomes (Table 1).

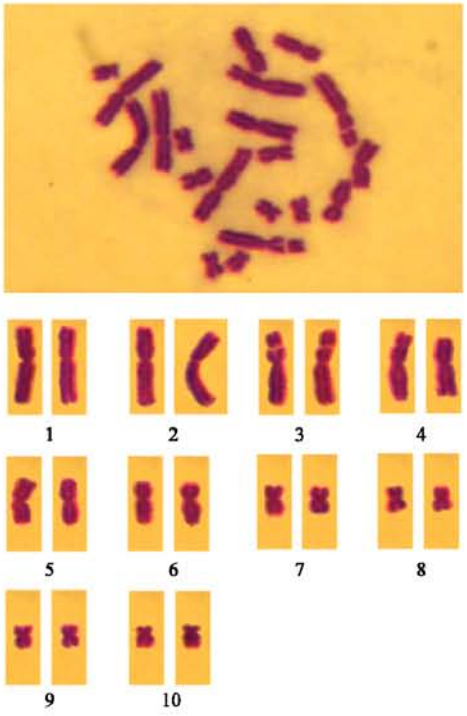

Fig. 1: Karyotype of male *Bufo regularis*

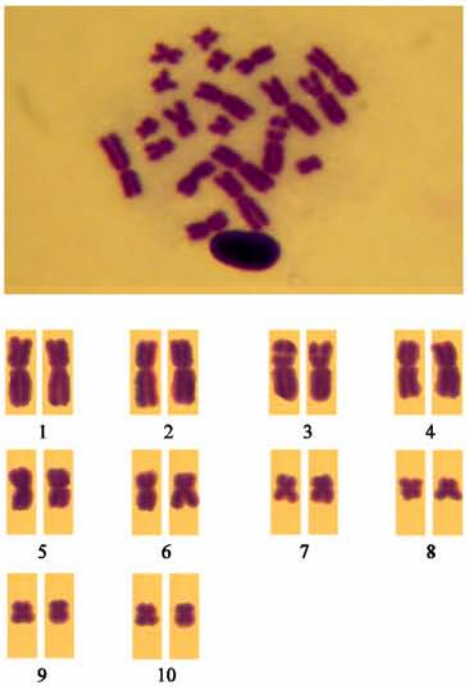

Fig. 2: Karyotype of female *Bufo regularis*

Table 1: Arm ratios and type of centromeres of *Bufo regularis*

Chrom. No.	Short arm p	Long arm q	Total length q+p	Arm ratio q/p	Type of centromere
1	4.8	6.4	11.2	1.3	M
2	4.4	6.0	10.4	1.4	M
3	4.4	5.2	9.6	1.2	M
4	2.8	4.4	7.2	1.6	M
5	2.8	3.6	6.4	1.3	M
6	2.4	3.6	6.0	1.5	M
7	2.0	2.8	4.8	1.4	M
8	2.0	2.4	4.4	1.2	M
9	1.6	2.0	3.6	1.3	M
10	1.2	2.8	4.0	2.3	SM

Table 2: The fundamental number for *Bufo regularis*

Type centromere	Haploid No. N	Diploid No. 2 N	Fundamental No. FN
M	9	18	36
SM	1	2	4
Total	10	20	40

The third pair of this karyotype has a secondary constriction on its short arms and could be considered as a marker chromosome for this species. The Fundamental Number (FN) has been calculated to be 40 (Table 2). The type of centromeres has been determined according to the formula of Matthey and the finding is represented in Fig. 3.

Unlike the Hylidae whose diploid complements are known to vary (Duellman and Cole, 1965), the Bufonidae have been reported as maintaining chromosomal stability with un wavering diploid constitution of twenty two chromosomes. Some authors extended this concept to imply that the karyotype of most Bufo uniform with respect to each other (Volpe and Gebahrdt, 1968; Robinson and

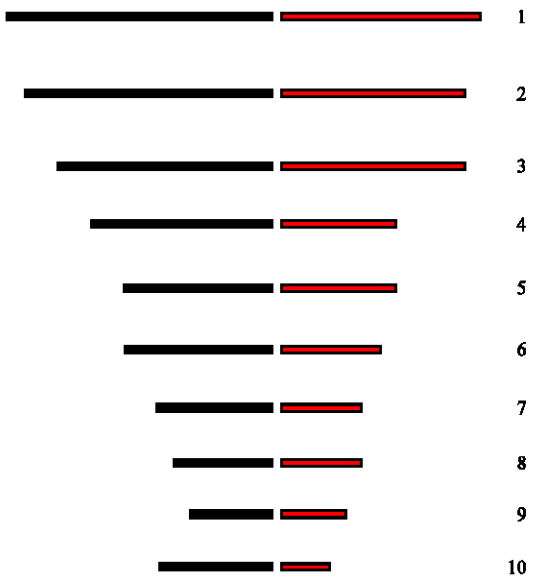

Fig. 3: An ideogram of *Bufo regularis* constructed on the basis of chromosome numbers and the position of the centromere

Stephenson, 1967). The discovery and confirmation that *Bufo regularis* has a diploid complement of only twenty chromosomes has changed the picture (Beckert and Doyle, 1968; Bogart, 1968; Doyle and Beckert, 1970). This study not only reconfirm that the diploid number is twenty but confirms for the first time *Bufo regularis* is endemic to the Arabian Peninsula.

In conclusion, the present results have revealed in details the karyotype of *Bufo regularis* 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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