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ORIGINAL ARTICLE

First report of the karyotype of *Bufo dhufarensis* from Saudi Arabia

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Abstract We report for the first time the description of the karyotype of the Dhufar toad *Bufo dhufarensis* from Saudi Arabia. This karyotype consists of 22 chromosomes and could be classified into two categories; eight pairs of metacentric and three pairs of submetacentric chromosomes. The 10th pair of this karyotype has a secondary constriction on the long arms of both male and female chromosomes. The fundamental number of this species is 44.

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1. Introduction

It is of a surprise to find several amphibian species abundant to a harsh environment such as in the Arabian Peninsula. The work of Balletto et al., 1985 is considered as the more precise surveys dealing with the morphological and classification of Arabian anura. However, there are few research on the distribution of frogs and toads in kingdom of Saudi Arabia and neighboring country such as Yemen (Schmidt, 1953; Hass, 1957, 1961; Briggs, 1980, 1981).

According to the literatures, *Bufo arabicus* is the first toad to be classified from the region (Heyden, 1827) but recent re-

search on the amphibian chromosomes of the Arabian Peninsula has just started, and few paper have been published (Al-Shehri and Al-Saleh, 2005a,b, 2008).

Therefore, it is very important to study and protect such creatures because they are under grave threat not only due to general habitat alteration but also to climate change, pollutant and the emergence of deadly and infectious diseases such as fungal disease which has been due to global warming. Amphibian and reptile populations have declined by 75% since 1970, which indicate to the extinction danger that amphibian might face in the near future (Whitfield et al., 2007).

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

2. Materials and methods

Samples of males and females of *B. dhufarensis* Parker were collected from Al-Derayya village, Riyadh province of Kingdom of Saudi Arabia. Each sample was injected interaperitoneal with 0.2 ml of colchicine solution (1 mg/ml)

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for 24 h before being killed. Chromosome preparations followed the method described by Al-Shehri and Al-Saleh (2008) and well-spread metaphases chromosomes were analyzed and photographed. The nomenclature proposed by Levan et al. (1964) was followed for the classification of each homologous pair.

3. Results

For the first time we report and describe the karyotype of *B. dhufarensis*, which has been collected from Riyadh region, Kingdom of Saudi Arabia. The karyotype consists of 22 chromosomes, eight pairs of metacentric and three pairs of submetacentric chromosomes (Figs. 1 and 2).

The measurement of the length of the chromosomes is calculated for seven different karyotypes and the relative length is represented in Table 1. Metacentric chromosomes are classified according to their length to three types:

1. Chromosomes 1–3 are large size chromosomes.
2. Chromosome 4 is medium size chromosomes.
3. Chromosomes 5–8 are small size chromosomes

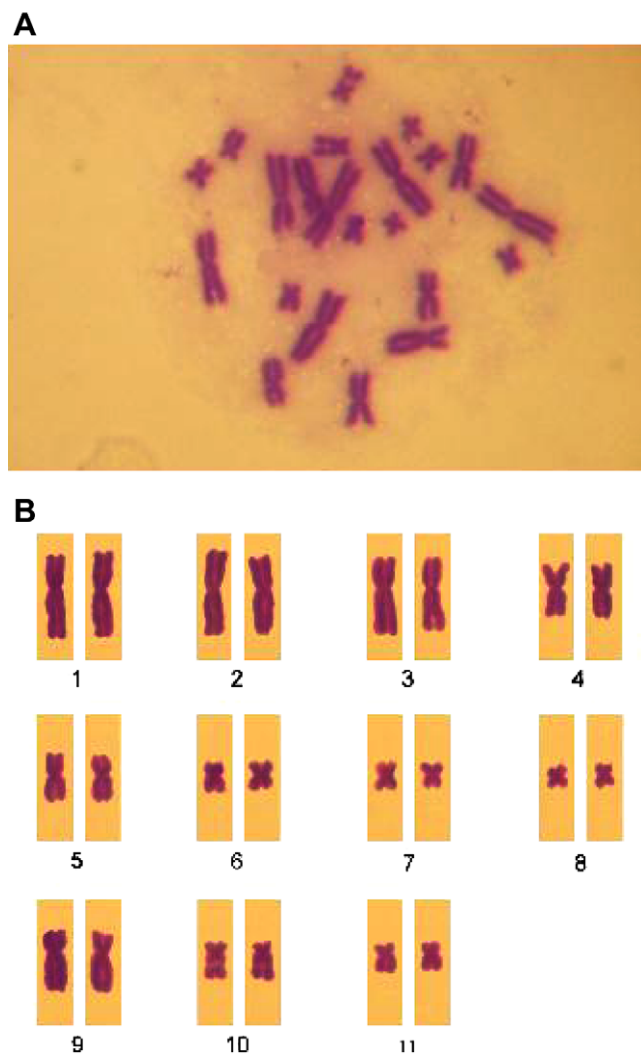


Figure 1 (A) Photomicrograph of metaphase spreading from male *B. dhufarensis*. (B) Karyotype of male *B. dhufarensis*.

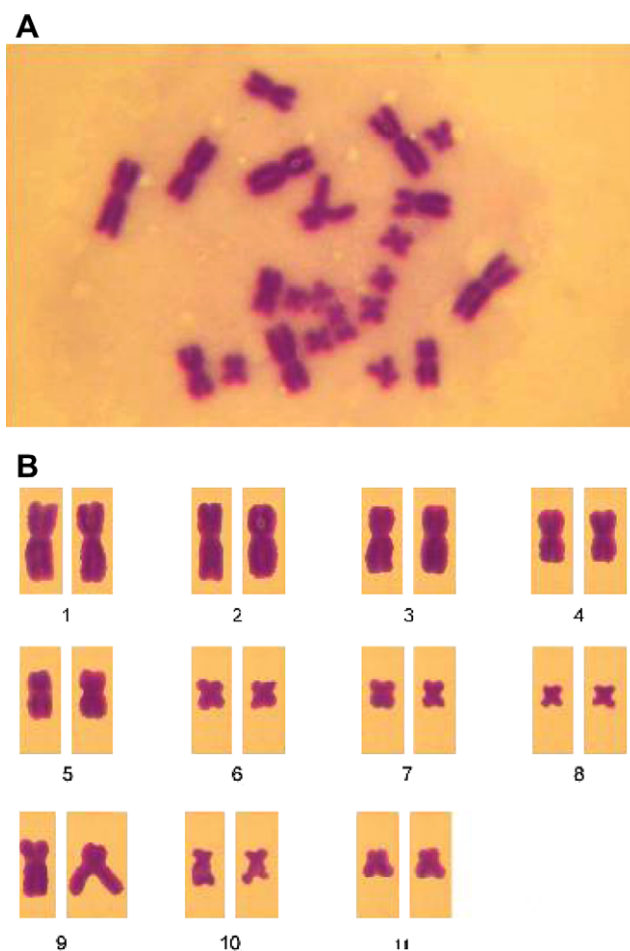


Figure 2 (A) Photomicrograph of metaphase spreading from female *B. dhufarensis*. (B) Karyotype of female *B. dhufarensis*.

Table 1 Arm ratios and type of centromeres of *B. dhufarensis*.

Chrom. no.	Short arm <i>p</i>	Long arm <i>q</i>	Total length <i>q + p</i>	Arm ratio <i>q/p</i>	Type of centromere
1	4.36	5.23	9.59	1.2	M
2	4.36	4.8	9.16	1.1	M
3	3.49	3.92	7.41	1.1	M
4	3.05	3.05	6.1	1.0	M
5	2.18	2.62	4.80	1.2	M
6	1.31	1.74	3.05	1.3	M
7	1.31	1.31	2.62	1	M
8	0.87	0.87	1.74	1	M
9	2.18	4.36	6.54	2	SM
10	1.31	2.62	3.93	2	SM
11	0.87	2.18	3.05	2.5	SM

The submetacentric chromosomes are classified according to the length to two types:

1. Chromosome 9 is medium size chromosome.
2. Chromosomes 10–11 are small size chromosomes.

The 10th pair of this karyotype has a secondary constriction on its long arms and could be considered as a marker

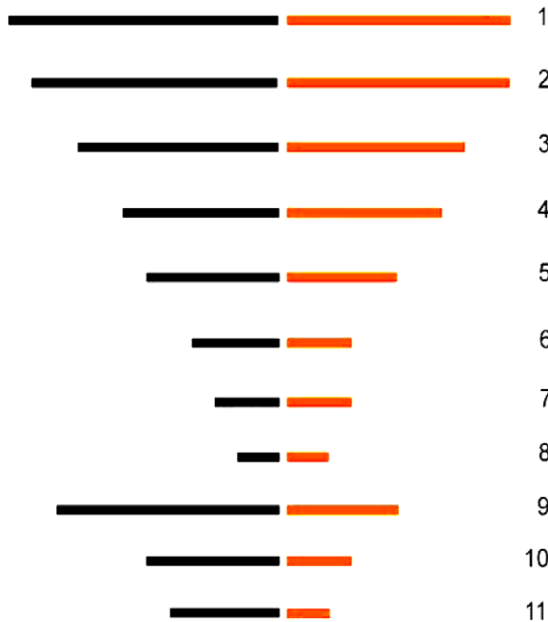


Figure 3 An ideogram of *B. dhufarensis* constructed on the basis of chromosome numbers and the position of the centromere.

Table 2 The fundamental number for *Bufo dhufarensis*.

Type centromere	Haploid no. N	Diploid no. 2N	Fundamental no. FN
M	8	16	32
SM	3	6	12
Total	11	22	44

M = Metacentric chromosome.

SM = Submetacentric chromosome.

chromosome for this species. The type of centromeres has been determined according to the formula of Matthey and the finding is represented in Fig. 3 and the fundamental number (FN) has been calculated to be 44 (Table 2). Our observations confirm the lack of heteromorphic sex chromosomes in this species.

4. Discussion

The karyotype of *B. dhufarensis* is asymmetric chromosomes consisting of diploid number of 22 chromosomes. This report is representing for the first time the karyotype of the toad *B. dhufarensis*. The finding of 22 chromosome diploid number is in consequence with the bufonidae diploid chromosomes number which has been found to have karyotypic uniformity and consist of 22 chromosomes (Ullerich, 1966; Volpe and Gerhardt, 1968; Doyle and Beckert, 1970; Azevedo et al., 2003; Al-Shehri and Al-Saleh, 2008).

The heterogametic sex is male in all mammals and female in almost all birds but in amphibians the case is different (Nakamura, 2009). The pioneer authors had discussed the occurrence of sex chromosomes in Amphibians and some assumed heterogamety in Anura while other did not accept heterogamety in this group of animals (Stohler, 1928; Witschi,

1933; Saez et al., 1935; Galgano, 1941; WickBom 1945; Yosida, 1957; Saez and Brum, 1960; Becak, 1968; Rabello, 1970). A review by Schmid and Steinlein (2001) indicated that sex chromosomes among amphibians can be divided into three categories, male hetero, female hetero and homogamety. This species has been found to be of the type of homogametic sex chromosomes.

A recent report confirms that karyotyping analyses of the cane toad *Bufo marinus* have so far failed to identify heteromorphic sex chromosomes (Abramyan et al., 2009). The uniformity of sex chromosomes among toads seems to be accepted, and there are several papers indicating such uniformity (Miura, 1995; Kasahara et al., 1996; Cavallo et al., 2002; Azevedo et al., 2003; Siripivasing et al., 2008).

An interesting finding by Hayes (1998) stated that most amphibians lack morphologically distinguished sex chromosomes and suggested that environment influences sex determination. Bergero and Charlesworth (2009) confirm recently that heteromorphic sex chromosomes are a homologous pair in origin evolved from normal pair of homologous chromosomes.

5. Conclusion

We can stress the fact that this study not only confirm for the first time that the diploid number is 22 but confirms that *B. dhufarensis* is endemic to the Arabian Peninsula.

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