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Report of *Bufo tihamicus* karyotype from Saudi Arabia

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This study gives a description of the Karyotype of the Tihama toad *Bufo tihamicus* from Saudi Arabia. Samples of males and females of *Bufo tihamicus* Parker were collected from Gazan Province of Kingdom of Saudi Arabia. The karyotype consists of a diploid number 2n = 22 and the fundamental number NF = 44 in both of the sexes. No heteromorphism in relation to sex chromosome pair was found.

Key words: Amphibian, karyotype, chromosome, *Bufo tihamicus.*

INTRODUCTION

There are few research on the distribution of frogs and toads in the kingdom of Saudi Arabia and neighboring country such as Yemen (Schmidt, 1953; Hass, 1957; 1961; Briggs, 1980,1981), but the work of Balletto et al. (1985) is considered a more precise survey dealing with the morphological and classification of Arabian anura. Nine nominal Anuran species were found, 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*).

Recently, we started to investigate the amphibian chromosomes of the Arabian Peninsula and few paper have been published (Al-Shehri and Al-Saleh, 2005; 2008). In this paper we are going to describe the karyotype and for the first time, *B. tihamicus* from Kingdom of Saudi Arabia using bone marrow cells treated with colchicine *in vivo*.

MATERIALS AND METHODS

Samples of males and females of *B. tihamicus* Parker were collected from Gazan Province of Kingdom of Saudi Arabia. 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 and very cold slide then, air-dried. The chromosomes were stained with 10% Giemsa stain in phosphate buffer pH 6.8. More than 20 metaphase chromosomes spreads from 5 males and 5 females were examined under bright field illumination, using 100X oil immersion objective and 10X eyepiece by ordinary Zeiss microscope. The nomenclature proposed by Levan et al. (1964) was followed for the classification of each homologous pair.

RESULTS

This study being the first of its kind, describe the karyotype of *B. tihamicus;* which has been collected from Gazan region, Kingdom of Saudi Arabia. The karyotype consists of a diploid number (2n) = 22 chromosomes. These chromosomes are nine pairs of meta-centric and two pairs of submeta-centric chromosomes (Figures 1 and 2).

The measurement of the length of the chromosomes was calculated for ten different karyotypes and the

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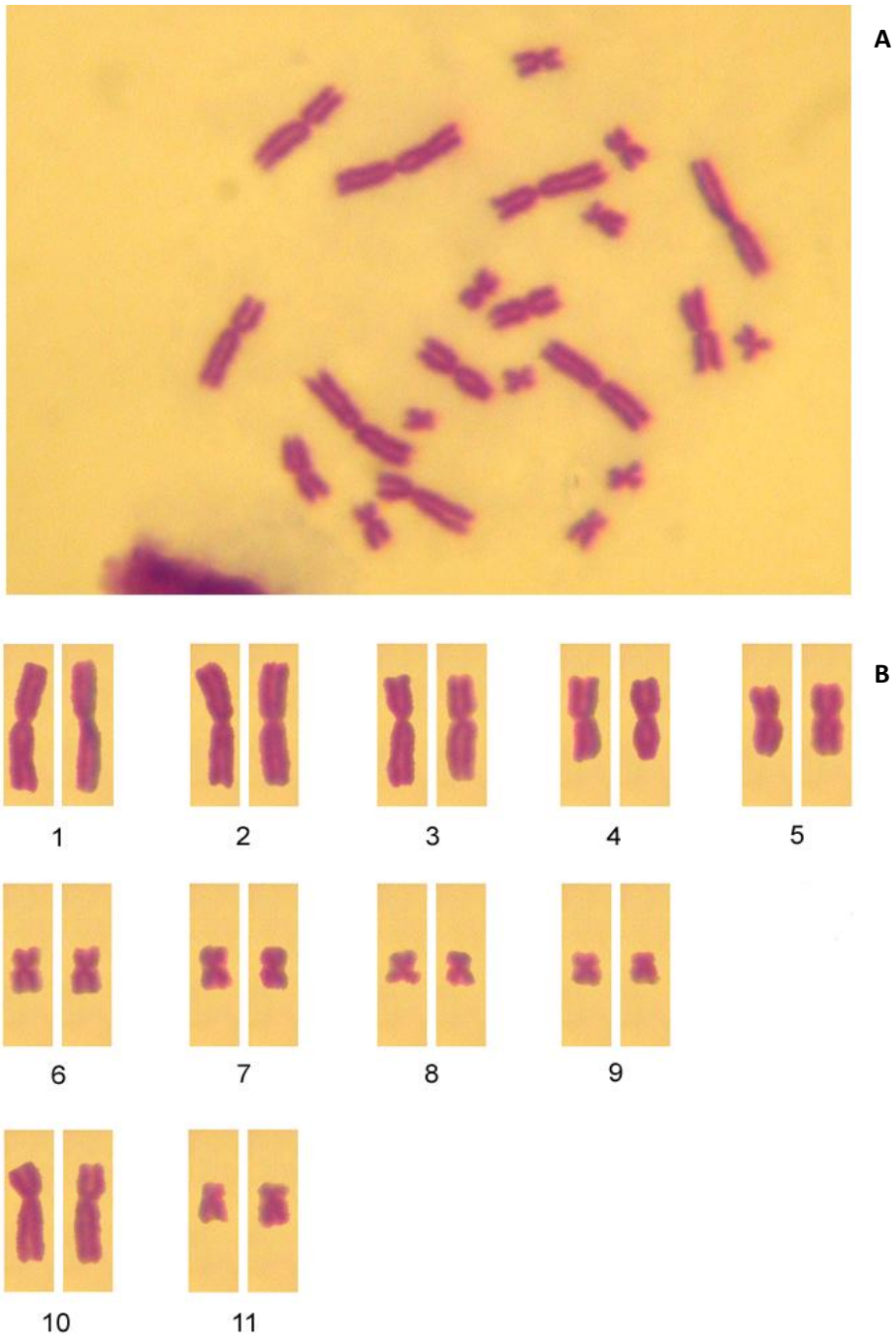

Figure 1. A, Photomicrograph of metaphase spreading from male *B. tihamicus*; B, Karyotype of male *Bufo tihamicus.*

relative length represented in Table 1. Meta-centric chromosomes are classified according to their length into three types: 1) Chromosomes 1-3 are large size chromosomes; 2) Chromosomes 4 and 5 are medium size chromosomes; 3) Chromosomes 6 to 9 are small size chromosomes.

The submeta-centric chromosomes are classified according to the length into 2 types: 1) Chromosome 10 is large size chromosomes; 2) Chromosomes 11 is small size chromosomes

The type of centromeres was determined according to the formula of Matthey and his findings is represented in Figure 3 and the fundamental number (FN) was calculated to be 44 (Table 2). We could not confirm the sex chro-

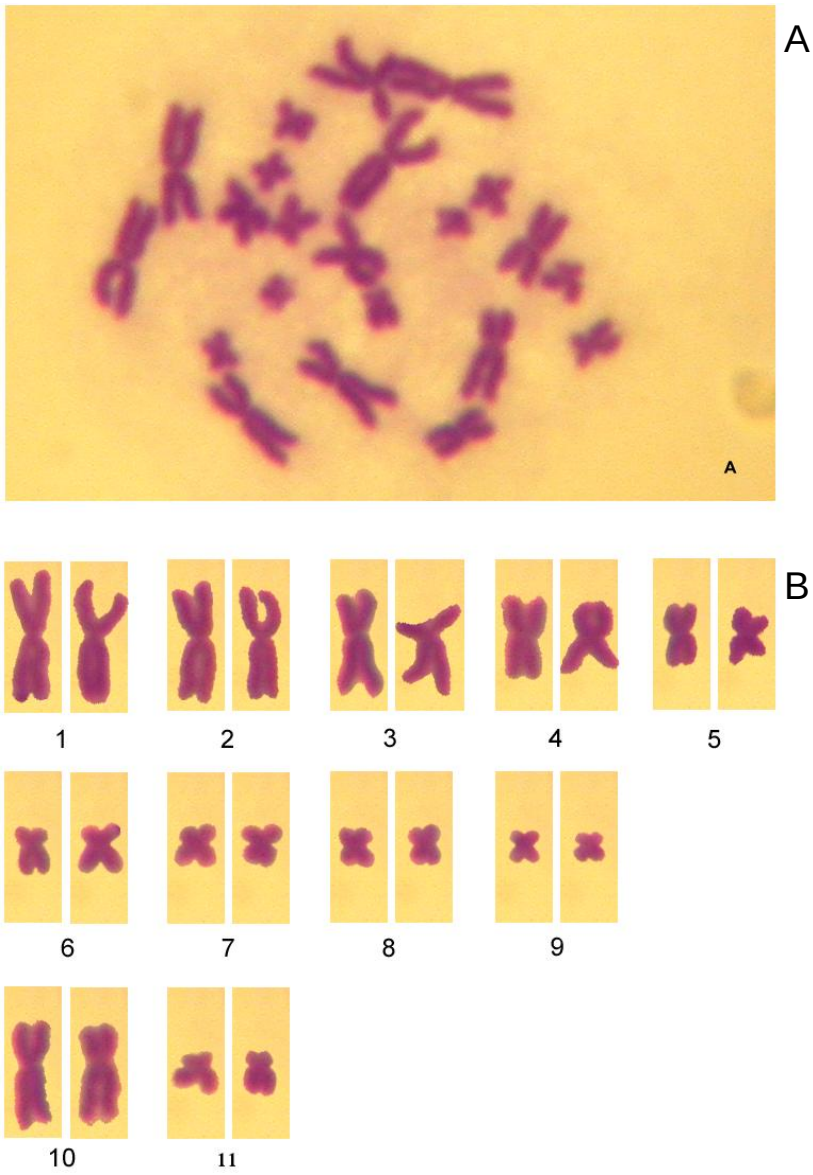

Figure 2. A, Photomicrograph of metaphase spreading from female *B tihamicus*; B, Karyotype of female *Bufo tihamicus.*

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

Chromosome number	Short arm p	Long arm q	Total length q + p	Arm ratio q/p	Type of centromere
1	4.61	4.99	9.60	1.2	M
2	4.22	4.61	8.83	1.1	M
3	3.46	4.61	8.07	1.3	M
4	3.07	3.07	6.14	1.0	M
5	2.30	3.07	5.37	1.3	M
6	1.92	2.30	4.22	1.2	M
7	1.54	1.92	3.46	1.3	M
8	1.15	1.54	2.69	1.3	M
9	1.15	1.15	2.30	1.0	SM
10	2.69	4.61	7.30	1.7	SM
11	1.15	2.30	3.45	2.0	SM

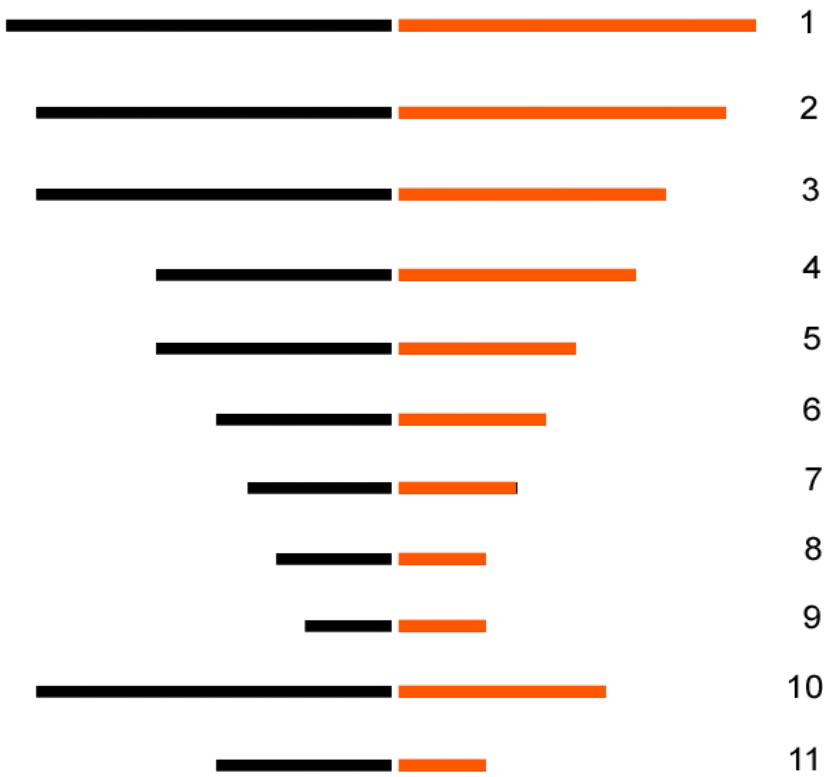

Figure 3. An idiogram of *B. tihamicus* constructed on the basis of chromosome numbers and the position of the centromeres.

Table 2. The fundamental number for *B. tihamicus.*

Type centromere	Haploid number N	Diploid number 2N	Fundamental number FN
M	9	18	32
SM	2	4	12
Total	11	22	44

M, Metacentric chromosome; SM, Submetacentric chromosome.

mosomes in this species.

DISCUSSION

The karyotype of *B. tihamicus* is asymmetric chromosomes consisting of diploid number of 22 chromosomes. This report is representing the karyotype of the toad *B. tihamicus.* The finding of 22 chromosome diploid number is in agreement 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).

B. regularis from Saudi Arabia has 20 chromosomes and has a secondary constrictions on the short arms of chromosome pairs number 3 (Al-Shehri and Al-Saleh, 2008) while *B. tihamicus* has 22 chromosomes and do not have any secondary constrictions on the karyotype. It is well accepted that mammals are male heterogametic while birds are female heterogametic; but in amphibian the case is different (Nakamura, 2009). It has been stated that all amphibians have genotypic sex determination mechanism (Hayes, 1998; Wallace et al. 1999; Eggert, 2004), and this mechanism is common in vertebrates as well (Valenzuela, 2008). The sex determination can be classified as female homogametic/male heterogametic as in mammals or female heterogametic/male homogametic as in birds. However, sex chromosomes are often not heteromorphic all the time (Schmid and Steinlein, 2001) and this species have been found to be of the type of homogametic. 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., 200). Molecular and breeding experiments indicate that many anuran genera lack heteromorphic sex chromosomes (Hillis and green, 1990; Hayes, 1998).

An interesting finding by Hayes (1998) indicates that most amphibians lack morphologically distinguished sex chromosomes and suggests that the environment influences sex determination. Bergero and Charlesworth (2009) confirmed recently that heteromorphics sex chromosomes are a homologous pair in origin evolved from normal pair of homologous chromosomes.

Conclusion

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

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