



ORIGINAL ARTICLE

First Karyological study of the Talysh toad (*Bufo eichwaldi*) in Mazandaran province, Iran (Anura: Bufonidae)

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ABSTRACT

Karyological characteristics of the Talysh toad (*Bufo eichwaldi*) in Mazandaran province in the northern part of Iran show that the chromosome number is $2n = 22$ and the arm number $NF=44$. The chromosomes divided in two groups: large chromosomes (pairs 1-6) and small ones (pairs 7-11) and consisted of 9 metacentric (M) and 2 submetacentric (SM) chromosome pairs. The Centromeric Index ranges from 32.88 to 48.79, the arm ratio between 1.05 and 2.04, the relative length between 3.60 and 16.47, and the length variation between 3.03 and 13.86. Average total length of the chromosomes is 84.14 μm . The largest chromosome in this species is a pair of the metacentric chromosome. This is the first study on karyotype analysis in *Bufo eichwaldi* in Iran and add basic useful information that may be used for population studies.

Keywords: Karyotype, Talysh toad, *Bufo eichwaldi*, Iran, Middle East.

Received 27.05.2014

Revised 19.07.2014

Accepted 24.07.2014

INTRODUCTION

Bufo eichwaldi (Litvinchuk, Borkin, Skorinov & Rosanov, 2008), commonly known as Eichwald's toad or the Talysh toad, is a large toad much resembling *Bufo verrucosissimus* (Pallas, 1814). *B. eichwaldi* belongs to the Class Amphibia, Order Anura, family Bufonidae. Bufonidae is global in its distribution, except for Australia, New Zealand, and Madagascar and approximately 48 genera and 558 species [10, 14] Blanford (1876) was the first who reported common toads (mentioned as *Bufo vulgaris*) for the territory of Iran and then different authors designated these toads either as *B. bufo* [3], *Bufo (Bufo) eichwaldi* [11] or *B. verrucosissimus* [9]. But in 2008, *B. eichwaldi* was described as a new species from *Bufo bufo* group based on genome size, allozyme and morphological evidences. *B. eichwaldi* is believed to occur in south east Azerbaijan and northern Iran in the Talysh and Elburz Mountains. It also occurs in Mazandaran, Gilan and Golestan provinces in Iran near the Caspian Sea [8, 9]. [8] stated that *B. eichwaldi* seem to be infrequent in nature and they are quite rarely represented in museum collections. The IUCN Red List of Threatened Species lists this species as vulnerable. It is believed to have declined by 30% over the last 24 years. Currently, the species is greatly endangered because of extensive cutting of forests for fuel and for agricultural, water pollution around settlements and industrial facilities, habitat loss and the impacts of exotic species [7]. So the biology of *B. eichwaldi* has been poorly studied.

In addition to the morphological, skeletal and biometric characteristics, karyological study is the key to the animal identification and classification [5]. Morphology of karyotype, which includes number, shape and size of chromosomes, forms one class of the phenotypic characteristics, which is usually independent of the genotype [4, 6]. Most of the amphibians do not have a large number of chromosomes. Nevertheless, their chromosomes are relatively large in size and easy to obtain, so the amphibians have an excellent position in chromosomal research [5]. In this paper we are going to describe for the first time the karyotype for *B. eichwaldi* from Iran using bone marrow cells treated with colchicine *in vivo*, with the intention of increasing knowledge of toad cytogenetics and its characterization.

MATERIAL AND METHODS

Six Eichwald's toad with a weight of 202-328 g and snout-vent length 108-110 cm were collected from

Sari in Mazandaran province in the North of Iran, during spring 2014. The toads were transported alive to the laboratory, and kept in an akvarium before analysis. The procedure used for karyotype test in samples has been described previously [13].

The chromosome pairs were classified following Levan et al. (1964) into metacentric (M), submetacentric (SM), subtelocentric (ST) and telocentric (T). The pair numbers were attributed following this classification and in decreasing length order within each class. Finally, the karyotype was constructed by first dividing the chromosome pairs into classes on the basis of centromere position, and then by arranging the homologous pairs in decreasing length order within each group. The idiogram was arranged by Adobe Photoshop CS6 software to provide the common feature of the chromosomes of *B. eichwaldi*.

RESULTS

The counts of chromosome ranged from 20 to 23 per metaphases with a mode at 22, representing 75% of the metaphases. In 20 metaphases from the bone marrow cells of 6 *B. eichwaldi* specimens, the diploid chromosome number were $2n=22$ (Figure 1), which is valid over 70% of metaphases cells. Cells lacking a normal number of chromosome values ($2n=20-23$) were probably caused by losses during preparation or additions from nearby cells. All chromosomes in the karyotype have a homologous pair. Homologous pairs of chromosomes were arranged in decreasing size and centromeric indexes. The sex chromosomes could not be distinguished. The representative karyotype for *B. eichwaldi* is shown in Figure 2. It has 9 metacentric and 2 submetacentric chromosome pairs. The number of chromosome arms was determined $NF=44$ and chromosome formula can be expressed as $2n=9m+2sm$. The morphological and numerical data are summarized in Table 1. The centromeric index, arm ratio, relative length and length variation range of chromosomes are between 32.88–48.79, 1.05–2.04, 3.60–16.47 and 3.03–13.86 (μm), respectively. Total length of short arm, total length of long arm and total length of haploid chromosomes were 36.49, 47.65 and 84.14 (μm) respectively. The largest chromosome is a pair of metacentric chromosome. The idiogram of the *B. eichwaldi* was made on the basis of the karyotype (Figure 3).

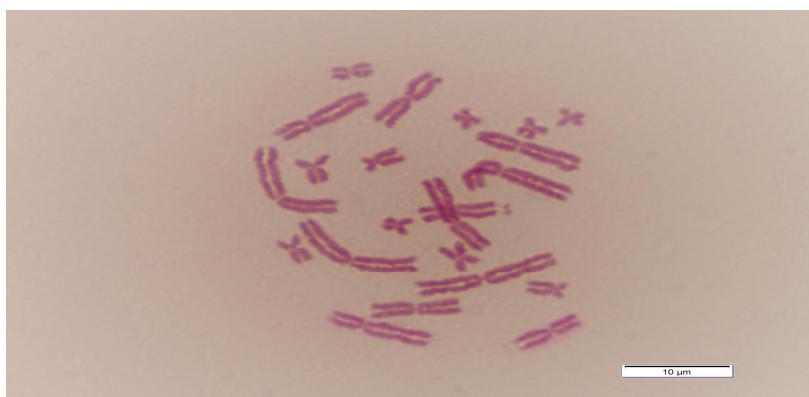


Figure 1. Metaphase spread from bone marrow of *Bufo eichwaldi* from the North of Iran, $2n=22$ (photo by R. Yadollahvand).

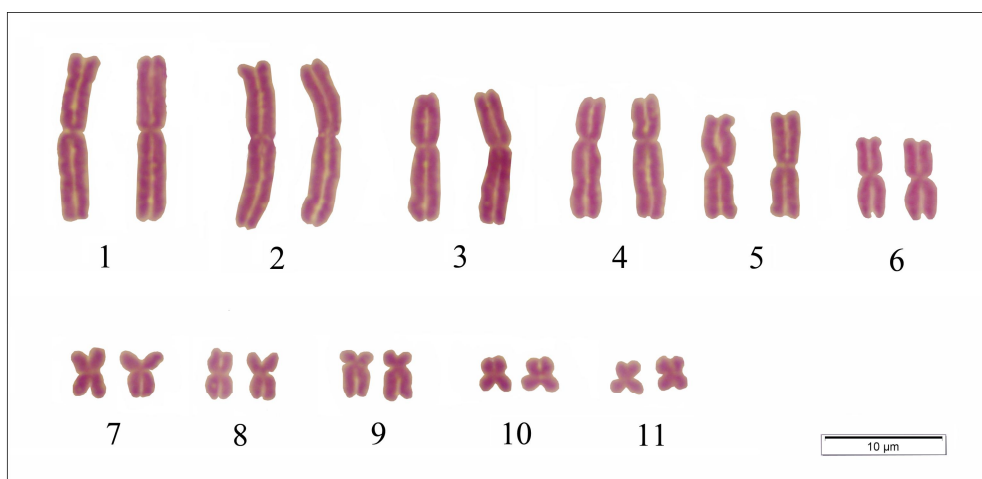


Figure 2. Karyotype of *Bufo eichwaldi* from the North of Iran, $2n=22$.

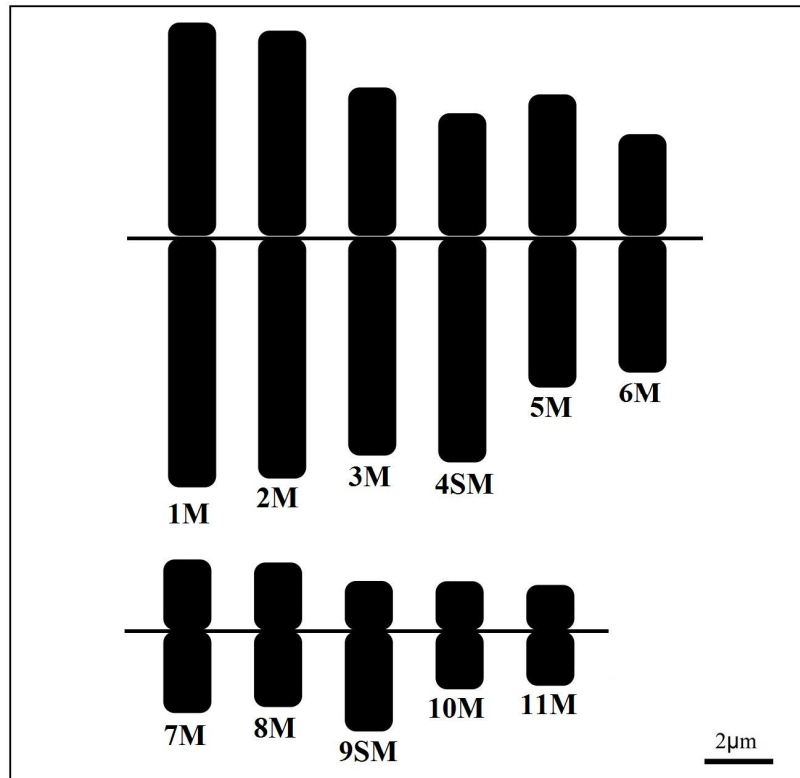


Figure 3. Idiogram of *Bufo eichwaldi* from the North of Iran, n= 11.

Tab. 1. Summarized morphological and numerical data of *Bufo eichwaldi*

NF*	2n	Total length short arm (µm)	Total length long arm (µm)	Total length of haploid chromosome (µm)
44	22	36.49	47.65	84.14

* Arm number

Tab. 2. Numeral characteristics of the karyotype of *Bufo eichwaldi* showing the mean values of measurements from ten best mitotic metaphases

Classification	Arm ratio	Centromeric index	Relative length (%)	Short arm (µm)	Long arm (µm)	Total length (µm)	Chromosome pair no.
Metacentric	1.17	46.03	16.47	6.38	7.48	13.86	1
Metacentric	1.17	45.93	15.93	6.16	7.25	13.41	2
Metacentric	1.46	40.52	13.11	4.47	6.56	11.03	3
Submetacentric	1.82	35.44	12.40	3.70	6.74	10.44	4
Metacentric	1.05	48.79	10.35	4.25	4.46	8.71	5
Metacentric	1.30	43.35	8.49	3.10	4.05	7.15	6
Metacentric	1.17	45.91	5.38	2.08	2.45	4.53	7
Metacentric	1.12	46.97	5.11	2.02	2.28	4.30	8
Submetacentric	2.04	32.88	5.27	1.46	2.98	4.44	9
Metacentric	1.17	45.98	3.85	1.49	1.75	3.24	10
Metacentric	1.19	45.54	3.60	1.38	1.65	3.03	11

DISCUSSION

The karyotype of *B. eichwaldi* is asymmetric chromosomes consisting of diploid number of 22 chromosomes and do not have any secondary constrictions on the chromosomes. This report is representing for the first time the karyotype of the toad *B. eichwaldi*. 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. All the chromosomes were biarmed and no acrocentric or sub-telocentric chromosomes were observed.

Although the diploid chromosome number were $2n=22$ in this study, some incomplete metaphases were encountered in the preparation, but this may be the result of hypotonic overtreatment. Differences in the number and types of chromosomes in other studies may have various reasons. For example differences in measurements techniques may led to differences in the type, number of chromosome arms and even number of chromosomes. High concentrations of colchicines or the longer periods of application can also be the cause of conflict as concentrations or periods of non-compliance can led to a shortening of chromosomes and therefore the difference between the measured and the arms of the chromosomes [13]. The retention of the diploid chromosome number 22 is a characteristic of the bufonid species. They have maintained chromosomal stability with unwavering constitution of twenty two chromosomes with a few exceptions like *Bufo regularis* showing the diploid number of twenty chromosomes [12]. No sex chromosome heteromorphism was observed same as *Bufo dhufarensis*. The uniformity of sex chromosomes among toads seems to be accepted, and there are several papers indicating such uniformity [1, 2].

In conclusion, karyotype of all samples of *B. eichwaldi* showed a set of $2n = 22$ chromosomes. Toads had only Metacentric and Submetacentric chromosomes. However, the present study is only preliminary to investigate the basic number and morphology of chromosomes for *B. eichwaldi*. These conclusions can be later used to contribute for the further cytological perspectives, especially if this is considered to be the first report from Mazandaran province, Iran.

ACKNOWLEDGMENT

We wish to thank Mr. A. Pischevari and Dr. S. Khodabandeh from Tarbiat Modares University, and Mrs. N. Okhli from Golestan University for scientific and technical supports.

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CITATION OF THIS ARTICLE

Haji G K, Reza Y. First Karyological study of the Talysh toad (*Bufo eichwaldi*) in Mazandaran province, Iran (Anura: Bufonidae). Bull. Env. Pharmacol. Life Sci., Vol 3 [9] August 2014: 75-78