

A Note on the Karyotype of the Amphibian *Pelophylax bedriagae* from Jordan

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Abstract

New data are presented on the karyotype of the Levant Water Frog, *Pelophylax bedriagae*, from Jordan. 26 chromosomes were observed for both male and female *Pelophylax bedriagae*. Seven chromosomes are metacentric (chromosomes 1, 3, 5, 6, 7, 8 and 9) and six are submetacentric (chromosomes 2, 4, 10, 11, 12 and 13). This is the first study on the karyotype of the Jordanian amphibians.

Keywords Karyotype, amphibians, *Pelophylax bedriagae*, Jordan

The diversity of the amphibians of Jordan is relatively low, consisting of three extant species (Disi & Amr, 2008). The taxonomic status of amphibians has undergone radical changes over the past 10 years. Recent molecular investigations on the amphibians of the Palaearctic region yielded new insights to the taxonomic status of the amphibians of the Middle East and Jordan in particular. For example, Plötner *et al.* (2001) showed that the Levant Water Frog, *R. bedriagae*, in Jordan and Syria is distinct from the European Water Frog, *Rana ridibunda*, and the Anatolian *R. bedriagae* differs from the southern populations by 2.2-3.4% of the analysed mitochondrial DNA. Lymberakis *et al.* (2007) compared partial mitochondrial DNA sequences for the cytochrome b and 16S rRNA genes for *Rana* populations in the Eastern Mediterranean region. They consider that within the *rindibunda/bedriagae* lineage, *R. (P.) ridibunda*, *R. (P.) epeirotica*, *R. (P.) cretensis*, *R. (P.) bedriagae*, *R. (P.) cerigensis* and *R. (P.) kurtmuelleri* were differentiated from a common ancestor through a series of vicariant and dispersal events.

In the Middle East, some studies reported on the karyotype of amphibians, including the former *Rana ridibunda* (= *Pelophylax bedriagae*). Studies were performed in Saudi Arabia (Al-Shehri & Al-Saleh, 2005), Palestine (Salman *et al.*, 2015), and Turkey (Alpagut & Falakali, 1995).

The present study is the firsts to report on the karyotype of *Pelophylax bedriagae* collected from Jordan.

Four adult *Pelophylax bedriagae* were collected from a pool at the Jordan University of Science and Technology campus. Frogs were injected with 0.2 ml colchicine solution (1 mg/ml) for 6-12 hours before the bone marrow of the femoral bones was removed for the cytogenetic study. The bone marrow was washed by using 0.9% KCl hypotonic solution into centrifuge tube, then

incubate for 15 minutes at 37⁰ C. The homogenate was centrifuged for 6-8 minutes at 1000 rpm, and then fixed using fresh fixative (3 volumes of methanol+1 volume of glacial acetic acid). The supernatant was removed by a pipette as much as possible, keeping the bone marrow pellet. More fixative was added and then centrifuged again 6-8 minutes at about 1000 rpm. The same procedure was repeated three times. The pellet was suspended in a small volume of the fixative and then dropped on clean microscopic slide. Slides were stained in giemsa solution in phosphate buffer for 12-15 minutes.

A minimum of five metaphases were analyzed for each specimen and images were taken using an Olympus MX41 microscope fitted with a digital camera. Standard karyotypes were constructed from micrographs of well spread chromosomes. Metaphase chromosomes were arranged in homologous pairs according to size and centromere position. The diploid chromosome number was determined from ten photographed spreads for each species.

Twenty-six chromosomes were observed for both male and female *Pelophylax bedriagae* (Fig. 1). The karyotype of both sexes are similar and cannot be differentiated from each others. Seven chromosomes are metacentric (chromosomes 1, 3, 5, 6, 7, 8 and 9) and six are submetacentric (chromosomes 2, 4, 10, 11, 12 and 13).

Species of the genus *Rana* in both the old and new worlds have 2n=26 (Haertel *et al.*, 1974; Nishioka *et al.*, 1987). Our results are similar to those obtained by Al-Shehri & Al-Saleh (2005) where they reported seven metacentric and six submetacentric chromosomes, and to those reported by Salman *et al.* (2015). We were unable to differentiate the sex chromosomes.

Alpagut & Falakali (1995) showed that the karyotypes of two Turkish populations of *Rana ridibunda* consisted of 26 chromosomes (2n=26), with a similar chromosomal

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morphology. However, they indicated that there were heteromorphic male sex chromosomes.

Further studies should investigate the taxonomic status of the three amphibian species known in Jordan, both for the molecular structure and morphology of their chromosomes, including G-banding.

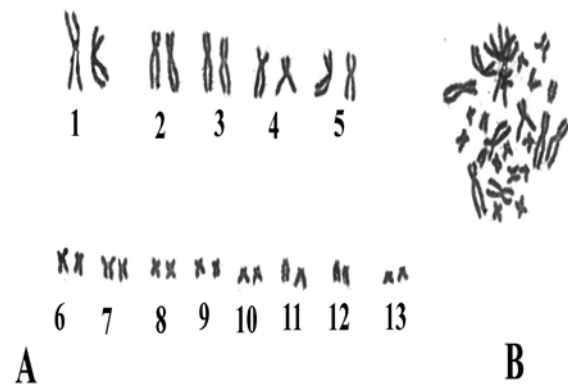


Figure 1: A. Karyotype for male *Pelophylax bedriagae*. B. Metaphase plate.

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