

Distribution and Cytogenetics of Amphibians from the Occupied Palestinian territories (West Bank of Jordan)

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ABSTRACT

The distribution of four species of amphibians (*Bufo variabilis*, *Pelophylax bedriagae*, *Hyla savignyi* / *H. felixarabica*, and *Pelobates syriacus*) are given in the occupied Palestinian territories of the West Bank are recorded. Karyotypic data on *Bufo variabilis* (2n=22) and *Pelophylax bedriagae* (2n=26) are reported

Keywords: Amphibians, Palestinian Territories, Karyotype.

INTRODUCTION

The order Anura is a group of amphibians with some 6000 described species which has been subject to significant taxonomic revision (Frost et al. 2006). Amphibians are an important component of ecosystems but increasingly threatened by human activities including global warming (Blaustein et al. 2010). Amphibians can also provide evidence of environmental health and resilience. Decline or loss of species can act as an early warning of global change (Gardner 2001). Thus environmental management of fragile habitats should be directed towards monitoring the diversity of amphibians to assist in their protection or recovery especially in wetlands (Storfer 2003). One species, the Hula painted frog, originally described by Mendelssohn and Steinitz in 1943 initially disappeared when the Hula lake and surrounding wetlands were first drained (Honnegger 1981) but has recently been rediscovered and assigned to the genus *Latonina* based on molecular and morphological data (Biton et al. 2013).

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Amphibian species in the Eastern Mediterranean region were studied by various authors via standard taxonomic, morphological and ecological methods (e.g. Werner 1988, Disi & Amr 2010, Degani & Didi 1999). However more modern methods now available were used by Gvoždík et al. (2010) to study the mitochondrial DNA of some species of Hylidae and a new cryptic species *Hyla felixarabica* was identified. This species is currently found in Palestine, Jordan and Yemen. Some species of Ranidae (Schneider & Sinsch 2001, Plötner et al. 2010) can also be differentiated by acoustic methods, but care must be taken in the interpretation of calls because of the effects of temperature (Huble & Schneider 1979). The aim of this study is to provide an initial assessment of anurans present in the Occupied Palestinian territories and their distribution, and to examine their karyotype.

MATERIALS AND METHODS

We surveyed several of locations in the occupied West Bank to survey location of permanent or seasonal bodies of water that could harbor amphibians. Table 1 shows localities from which material were collected. All animals caught were photographed in the field and in some cases preserved and deposited in the Palestine Museum of Natural History (PMNH), Bethlehem.

Table 1: localities from which materials were collected.

Locality	N	E
Al-Auja	31° 56'	35°27'
Artas	31° 41'	35° 11'
Beit Sahour	31° 70'	35° 22'
Ein Arik	31° 54'	35° 08'
Ein Fara	31° 49'	35° 18'
Ein Sinya	31° 58'	35° 13'
Fasayil	32° 01'	35° 26'
Husan	31° 42'	35° 07'
Wadi Quf	31° 33'	34° 58'
Jenin	32° 26'	35° 17'
Jericho	31° 51'	35° 27'

Jiftlik	32° 08'	35° 29'
Jinsafut	32° 10'	35° 07'
Kufr Dan	32° 28'	35° 15'
Mar Saba	31° 42'	35° 19'
Mikhmas	31° 52'	35° 16'
Nebi Saleh	32° 00'	35° 07'
Nuba	31° 36'	35° 02'
Salfit	32° 05'	35° 10'
Taffouh	31° 32'	35° 03'
Wadi Al-Qelt	31° 50'	35° 24'
Wadi Fukeen	31° 71'	35° 10'
Wadi Zarqa	31° 59'	35° 06'

Laboratory analysis

A number of those animals collected from the field (n=64) were also used for chromosomal analysis. Animals were first injected with 0.2 ml colchicine solution (1 mg/ml) for a 5-14 hours before bone marrow was removed for cytogenetic study. Blood was taken from the cardiac area and cultured in PHA medium (Al-Shehri & Al-Saleh 2005a,b; Amor et al. 2007) . A minimum of five metaphases were analyzed for each specimen and pictures taken using an Olympus MX41 microscope fitted with a digital camera.

RESULTS AND DISCUSSION

Three species of amphibians were collected from the study area. *Pelobates syriacus* was not collected during the present study. Figure 1 shows the current distribution of known amphibians in the Occupied Palestinian Territories. Karyotypic data was given for two species; *Bufo variabilis* and *Pelophylax bedriagae*.

Family Bufonidae

Bufo variabilis (Pallas 1769)

Fig. 2

Material examined (n=13): Jericho (PMNH1856 ♂ 20.ix.2010; PMNH1870 ♂



Fig. 1: Light trap sampling location in the period of the study.

25.ix.2010; PMNH1872 ♀ 25.ix.2010; PMNH1873 ♂ 1.x.2010; PMNH1874, ♂ 25.ix.2010); **Beit Sahour** (PMNH1841 ♀ 2.xiii.2010; PMNH1848 ♂ 4.ix.2010; PMNH1850 ♂ 4.ix.2010; PMNH1851 ♀ 14.ix.2010); **Jiftlik** (PMNH1979, ♀ , 27.iii.2013; PMNH1980, ♂ 27.iii.2013); **Mikhmas** (PMNH1700 ♂ 27.iv.2013); **Idnah** (PMNH1824 ♂ 26.vi.2012). Also we recorded it via observations in **Mar Saba, Nebi Saleh, and Jenin**.

Remarks: This species is distributed widely in the Arabian Peninsula (Degani and Kaplan 1999; Disi and Amr, 2001; Odierna et al. 2004). This is a common and highly adaptable toad found in different habitats. Previous records in the West Bank include from Wadi Malha northeast of Tubas (Odierna et al. 2004). It was found in Palestine in temporary winter ponds during the rainy season and into early spring and at the edges of permanent waters year round especially common in the Jordan Valley. Hundreds of juvenile toads were noted in Mikhmas at a sewage stream. This was diluted sewage as we observed an abundance of other wetland species in the area. We



Fig. 2: *Bufo variabilis* from Beit Sahour.

also reported this species from a cave at Idnah, Hebron district. Locals recognize this species as coming out after the initial winter rains.

Small pools that last sometimes merely few weeks act as breeding habitats. The toads have a very quick turnover with eggs developing to small toads able to aestivate in 3-4 weeks. Frost et al. (2006) describe a new genus *Pseudepidalea* which was considered a junior synonym of *Bufo* (Dubois & Bour 2010; Frost 2013). The name etymology was in reference to the morphological similarity of this group to *Epidalea calamita*.

Below the genus level, the group of “*viridis*” clearly had some distinguishing clades by molecular phylogenetic methods. Stöck et al. (2006) stated that all Near Eastern and much of the northern Eurasian populations belong to a separate taxon suggested by molecular

clocks to have diverged from the nearest European *viridis sensu strictu* in Lower to Middle Pliocene. Because of the fact that the range of this clade included the type locality of *variabilis* in Lubeck, Germany, these authors tentatively suggested the use of the name *Bufo variabilis* for the populations that occur in our area (see also Stöck et al. 2001). Combined with the data of Frost et al. (2006, 2013) and Dubois and Bour (2010) we now use the name *Bufotes variabilis* (instead of the older name *Bufo viridis* in Palestine).

Karyotypic data: The diploid number of *Bufotes variabilis* we observed in Palestine is 22 with all metacentric and submetacentric chromosomes in three specimens examined (Fig. 3) and is identical to that seen in this complex elsewhere (Miura 1995, Odierna et al. 2004, 2007, Al-Shehri & Al-Saleh 2010). Stöck et al. (2013) showed that Low rates of X-Y recombination account for homomorphic sex chromosomes (difficult to distinguish because males and females have similar chromosomes).

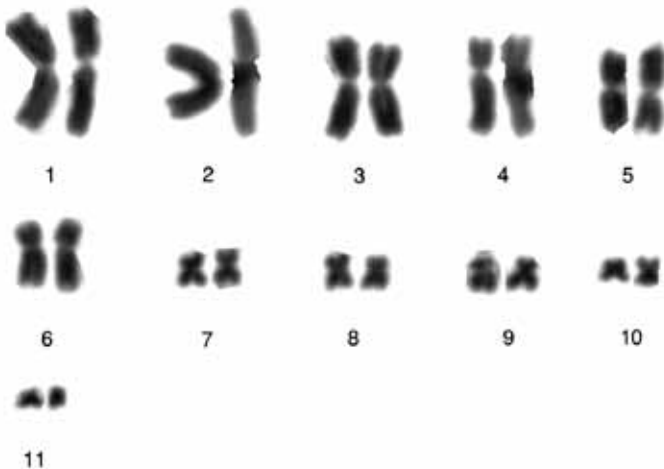


Fig. 3: karyotype of *Bufotes variabilis*

Family Pelobatidae

Pelobates syriacus (Boettger 1889)

Remarks: Munwes et al. (2010) collected this species from Jinsafut, the only known presence in the West Bank. We visited this locality in the northern part of the West Bank but not during the rainy season. Locals confirm the presence of this toad during the seasonal filling by rain of this pool. *Pelobates syriacus* prefer temporary ponds of Terra Rosa soil at high attitude according to Disi and Amr (2010). It digs in the humid sub-soil when not in the rainy season. *Pelobates syriacus* prefers open steppe-like habitats and also preys on snails following the rainy season. Cytogenetic studies of *Pelobates syriacus* showed $2n=26$ from various countries (Belcheva & Ilieva 1977; Morescalchi et al. 1977; Schmid et al. 1987; Ugurtas et al. 2001).

Family Ranidae

Pelophylax bedriagae (Camerano 1882)

Fig. 4

Material examined (n=30): Wadi Zarqa, Beitullo (PMNH1517 ♂ 30.v.2012; PMNH1518 ♀ 30.v.2012; PMNH1519 ♂ 30.v.2012; PMNH 1529 ♀ 30.v.2012; PMNH1530 ♀ 30.v.2012; PMNH 1533 ♂ 30.v.2012); Nebi Saleh (PMNH1725 ♂ 5.v.2013); Fasayil, (PMNH1577 ♂ 27.vi.2012); Jiftlik; (PMNH1981 ♂ 27.iii.2013; PMNH1982 ♂ 27.iii.2013); Wadi Fukeen (PMNH1832 ♂ 3.iv.2009; PMNH1853 ♂ 6.viii.2011; PMNH1860, ♀ ,8.viii.2011; PMNH1865 ♂ 29.ix.2010; PMNH1943, ♀ 6.viii.2011; PMNH1945 ♂ 6.viii.2011; PMNH1948 ♂ 8.viii.2011; PMNH1949 ♂ 8.viii.2011; PMNH1984 ♀ 6.viii.2011); Husan (PMNH1875 ♂ 28.ix.2010; PMNH1963, ♀ , 29.ix.2010); Artas including Solomon's pools (PMNH1883, ♂ 28.ix.2010; PMNH1937 ♀ 1.x.2010); Salfit (PMNH1844 ♀ 3.viii.2010); Jericho (PMNH1933 ♂ 11.v.2011; PMNH1934 ♂ 11.v.2011); Wadi Al Qelt (PMNH1764 ♂ 20.xii.2012; PMNH1968 ♂ 18.ix.2011; PMNH1969 ♂ 18.ix.2011); Kufr Dan (PMNH1798 ♂ 19.vi.2013). We also recorded it in Ein Sinya, Al-Auja, and Mikhmas.

Remarks: The name *Rana ridibunda* was used for Palestinian populations for a long time. Nevo and Filippucci (1988) suggested that the populations in our area are distinct from European populations. This



Fig.4: Adult male of *Pelophylax bedriagae* from Wad Qana.

prompted Schneider et al. (1992) to describe *Rana levantina* but this is a junior synonym of *Rana bedriagae* Camerano (1882) (the type from Syria). Morphometric studies confirmed the designation of this species stretching from Turkey to Palestine and Jordan (Sinsch & Schneider 1999). Frost et al. (2006) showed that the valid generic name is *Pelophylax* and hence the proper name for our frog is *P. bedriagae*.

However, there is still some mitochondrial DNA data that suggest that some populations especially in Syria and Anatolia and possibly entering our region maybe of more than one species (Plötner et al. 2001, 2010).

Previous records of *P. bedriagae* from the occupied West Bank include Jericho (Sinsch & Schneider 1999). We found it in total 11 localities in the occupied West Bank. It is the most common species found in Palestine as in Jordan (Disi & Amr 2010). We collected 30 specimens of *P. bedriagae* or more than half of all amphibian specimens collected in the West Bank. We found this species almost everywhere near springs, agriculture ponds, and even seasonal pools.

Karyotypic data: Chromosomes analysis revealed the diploid number of this species in Palestine to be $2n=26$ all metacentric and submetacentric chromosomes (Fig. 5) in four specimens examined (5 metaphases each) similar to other parts of the range of this species and even related species of *Pelophylax* (*Rana*) (Schmid 1978, Miura 1995, Al-Shehri & Al-Saleh 2005a).

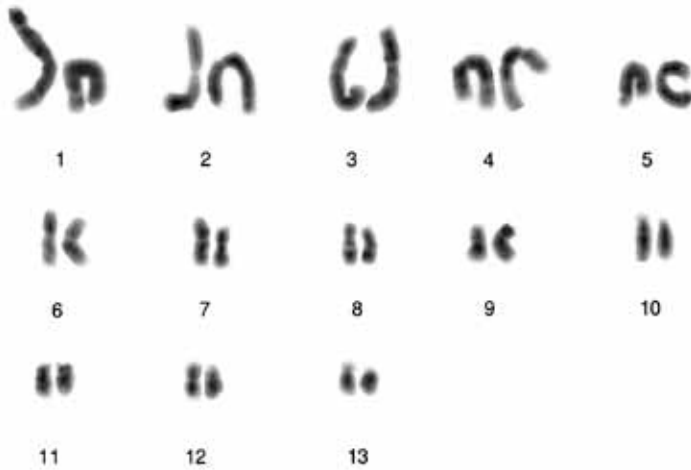


Fig. 5: Karyotype of *Pelophylax bedriagae*

Family Hylidae

Hyla sp.

Fig. 6

Material examined (n=21): Husan (PMNH1835 ♂ 24.iv.2013; PMNH1845 ♂ 17.viii.2010; PMNH1846 ♀ 17.viii.2010; PMNH1866 ♂ 1.x.2010; PMNH1877 ♀ 1.x.2010; PMNH1935 ♂ 21.xiii.2010; PMNH1965 ♂ 1.x.2010; PMNH1964 ♂ 1.x.2010; PMNH1985 ♂ 21.viii.2010); Wadi Fukeen (PMNH1501 ♀ 27.iii.2013; PMNH1502 ♀ 27.iii.2012; PMNH1830 ♀ 3.iv.2009; PMNH1946, ♂ 6.viii.2011; PMNH1967 ♂ 1/10/2010); Nuba (PMNH1744 ♂ 18.iii.2013); Salfit (PMNH1833 ♂, 3.iv.2009); Ein Arik (PMNH1987 ♂ 7.vii.2013; PMNH1988 ♀ 7.vii.2013); Mikhmas (PMNH1999 ♂ 27.iv.2013);- Rammalah district :Wadi zarqa -

Beitullo (PMNH1524 ♂ 30.v.2012; PMNH1525 ♂ 30.v.2012). We also observed it in **Ein Fara** (also reported but under a misnomer by Grach et al. 2007). Also observed at **Solomon's pools at Artas**.



Fig. 6: *Hyla cf. savignyi* from Idhna.

Remarks: Grach et al. (2007) described a *Hyla* from Jerusalem with distinct vocalization and morphology and called it a new sibling species of what was known as *Hyla savignyi* from Palestine and Jordan.

They named it *Hyla heinzsteinitzi*. However, Stöck et al. (2006; 2008) evaluated mitochondrial and nuclear DNA on samples referred to “*Hyla heinzsteinitzi*” and found them indistinguishable from *Hyla japonica* (also in vocalizations etc). It is also likely according to that work that a “paratype” from Ein Fara in the occupied areas also belongs to the common species.

The description of an introduced species as a new species emphasizes the need for larger studies perhaps with the use of mitochondrial and nuclear studies in evaluating phylogeny of amphibian species.

Based on mtDNA, rhodopsin, and tyrosinase sequences from specimens throughout the range of *H. savignyi*, Gvoždík et al. (2010) found that in our region two fairly distinct populations exist: 1) a traditional *H. savignyi* found in Lebanon, Syria, and northern Jordan (perhaps also parts of Palestine), and 2) a cluster that is similar to Yemeni specimens which he designated as a new taxon *H. felixarabica* found in areas of Palestine and Jordan near the rift valley (excluding the introduced *H. japonica*). This means that in our area of Jordan and Palestine, *Hyla* belong to two local (*H. savignyi* and *H. felixarabica*) and one introduced species, *H. japonica* (synonym *heinzsteinitzi*). But these authors found a mixed genome (*H. savignyi* and *H. felixarabica*) from a locality “Karkom, Israel” and suggested hybridization between the taxa. Until this is sorted out with further studies we use *Hyla* sp. for the taxon found in this area of Palestine and extending perhaps to Yemen.

The tree frog in this area was more common than we had expected for a threatened or even possibly endangered species. In fact, all the amphibians mentioned which were recorded in the literature from very few localities in the West Bank of Jordan were more widely distributed than we had anticipated. However, most of the permanent water sources have been depleted and springs dried in many places. As an example, Israeli authorities pumped water from the head stream of Al-Auja area which resulted in significant habitat changes in the area and we did not find tree frogs there. Karyotype of *Hyla felixarabica* from Saudi Arabia showed that it consists of diploid number of chromosomes $2n=24$ which is conserved karyotype (Al-Sheri & Al-Saleh 2005b).

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