

Life history traits of a Turkish population of the Yellow-bellied Toad, *Bombina variegata* (LINNAEUS, 1758)

(Anura: Bombinatoridae)

Wachstumsbiologische Merkmale einer türkischen Population der Gelbbauchunke

Bombina variegata (LINNAEUS, 1758)

(Anura: Bombinatoridae)

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KURZFASSUNG

Entwicklungsbiologische Merkmale einer türkischen Population (Enez, Edirne) der Gelbbauchunke *Bombina variegata* (LINNAEUS, 1758) wurden mit Hilfe skeletochronologischer Analysen an Phalangenknochen untersucht. Die erwachsenen Unken der Stichprobe (21 Männchen, 22 Weibchen) waren zwischen zwei und acht ($\bar{x} = 4,35$) Jahren alt. Das Alter bei Erreichen der sexuellen Reife betrug ein bis zwei Jahre. Es bestand eine positive Korrelation zwischen Körpergröße und Alter für beide Geschlechter. Geschlechtsbedingte Größenunterschiede waren sehr schwach (SDI = 0,04) zugunsten der Weibchen ausgeprägt. Der Wachstumskoeffizient (k) war bei Männchen größer als bei Weibchen ($k \pm$ Konfidenzintervall, Männchen: $0,47 \pm 0,05$, Weibchen: $0,34 \pm 0,02$). Es fand sich keinen Unterschied in der Wachstumsrate zwischen den Geschlechtern. Die mangelnde Signifikanz im geschlechtsbedingten Größenunterschied der Individuen der Enez-Population könnte auf die großen Übereinstimmungen der beiden Geschlechter bezüglich Lebenserwartung, Alter bei Erreichen der Geschlechtsreife und Wachstumsrate zurückzuführen sein.

ABSTRACT

Life history traits of the Yellow-bellied Toad, *Bombina variegata* (LINNAEUS, 1758), were studied in a population from Enez (Edirne, Turkey) applying skeletochronological techniques to phalangeal bones. In the adult sample (21 males and 22 females) age ranged from two to eight ($\bar{x} = 4.35$) years. The age at sexual maturity was one to two years. There was a positive correlation between body size and age for both sexes. Sexual size dimorphism was weakly expressed by the slightly bigger snout-vent-length of the females (SDI = 0.04). The growth coefficient (k) was bigger in males than females ($k \pm$ Confidence Interval; males: 0.47 ± 0.05 , females: 0.34 ± 0.02). There was no difference in growth rate between sexes. The non-significant difference in the size of male and female individuals from Enez may be related to similar longevity, age at maturity and growth rates of both sexes.

KEY WORDS

Amphibia: Anura: Bombinatoridae; *Bombina variegata*, longevity, age at maturity, sexual size difference, skeletochronology, life history, population ecology, biology, Enez, Turkey

INTRODUCTION

The Yellow-bellied Toad, *Bombina variegata* (LINNAEUS, 1758), is distributed across central and southeast Europe (ARNTZEN 1978; GOLLMANN et al. 1997; BARBIERI et al. 2004; NÜRNBERGER et al. 2016). In the territory of Turkey, it was detected only recently at Kurtkaya, municipality of Enez, Province of Edirne (BÜLBÜL et al. 2016b).

Bombina variegata is classified in the LC (Least Concern) category in the IUCN Red List (KUZMIN et al. 2009), albeit with a

general tendency towards population decline. After all, the range area of the species in this sole known Turkish locality is very limited in size, potentially putting its *B. variegata* population at risk of e.g., shrinkage or loss of habitat. The authors' field observations confirm this expectation.

To protect any species, knowledge of its life-history is critically important (GERMANO 1992; ANDREONE et al. 2005). Studies that determine the individuals' age (LECLAIR

1990; CAETANO & CASTANET 1993; SMIRINA 1994; MATTHEWS & MIAUD 2007; GUARINO & ERIŞİMİŞ 2008; ÜZÜM 2009; LIAO & LU 2010; KALAYCI et al. 2015; GÜL et al. 2017; KURNAZ et al. 2018; VERGILOV et al. 2018) are essential for understanding population dynamics and life histories of amphibian and reptile species. Since current knowl-

edge on age, growth and longevity of the Yellow-bellied Toad is largely based on the investigation of a population in Poland (PLYTYCZ & BIGAJ 1984, 1993), the present skeletochronological study was set off to obtain comparative information from Turkish individuals of the Enez population living at the edge of the species' territory.

MATERIALS AND METHODS

A total of 44 specimens (21 males, 22 females and 1 subadult) of *B. variegata* were caught from a population in Enez (Edirne) on August 13, 2016. The Enez population (40°45'104" N, 26°13'508" E), generally active from early April to October, is located in a lowland area at an altitude of 181 m a.s.l. The specimens were found at temperatures of about 27-34 °C during an excursion between 11:30 and 15:00. The habitat is comprised of forests of broad-leaved trees (*Quercus ilex*, *Fraxinus* sp., *Tilia* sp., *Paliurus aculeatus* and *Acacia* sp.) intermixed with small puddles on rocky and sandy soil where, *B. variegata* lives in syntopy with *Pelophylax ridibundus* (PALLAS, 1771), *Bufo bufo* (LINNAEUS, 1758), *Bufoles variabilis* PALLAS, 1769) and *Lissotriton vulgaris* (LINNAEUS, 1758).

The toads were caught by hand and sexed by visual examination of the secondary sex characteristics (presence of nuptial pads in males). Snout-vent length (SVL) was measured with a digital caliper (0.01 mm precision). Sexual Size Dimorphism (SSD) was quantified using the LOVICH & GIBBONS (1992) index (SDI) according to the formula: $SDI = (\text{mean length of the larger sex} / \text{mean length of the smaller sex}) \pm 1$. This formula, arbitrarily uses +1 if males are larger than females by defining the result as negative, or -1 if females are larger than males defining the result as positive.

From each toad, the second phalange of the longest (fourth) toe was clipped and preserved in 10 % formaldehyde solution for subsequent histological analyses. After registration and toe-clipping, the toads were released back to their natural habitats.

Age estimation using skeletochronology (CASTANET & SMIRINA 1990) is based on counts of the lines of arrested growth

(LAGs) in cross sections of long bones. After ablation of the skin, the toes were put in 5 % nitric acid solution for 2.5 hours to decalcify the bone tissue. After the toe samples had passed a tissue processing system (Leica® Tissue processor), they were embedded in paraffin with a tissue embedding device (Thermo®). Deparaffinized cross-sections (9 µm, rotary microtome) of the phalanges were stained with hematoxylin using the procedure described by BÜLBÜL et al. (2016a), then mounted on microscope slides, closed using Entellan® and observed under a light microscope.

The numbers of the LAGs on about ten cross-sections per adult individual studied were independently counted by three observers (A. İ. Eroğlu, M. Kurnaz and Y. Odabaş). Double lines in cross sections were counted as single lines. Following BÜLBÜL et al. (2016a), endosteal resorption of the first (innermost) LAG was assessed by comparing the diameters of eroded marrow cavities with the diameters of non-eroded marrow cavities in sections from the youngest specimens. The distance between two adjoining LAGs is a good indicator of individual growth in a given year (KLEINENBERG & SMIRINA 1969; ÖZDEMİR et al. 2012). An obvious decrease in spacing between two subsequent LAGs was taken to mark the age when sexual maturity was achieved (RYSER 1998; YILMAZ et al. 2005; ÖZDEMİR et al. 2012). In the present study, endosteal resorption never caused serious problems in age estimation.

Because age classes and body measurements (SVL) were normally distributed (One-Sample Kolmogorov-Smirnov Test) parametric tests were used for comparison of means (Independent Sample T-Test) and correlations (Pearson's Correlations Test).

Table 1: Descriptive statistics of snout-vent-length [SVL, mm] and age [yrs] of the studied specimens of the Enez population of *Bombina variegata* (LINNAEUS, 1758). N – sample size, SE – standard error of the mean).

Tab. 1: Deskriptive Statistiken von Kopf-Rumpflänge [SVL, mm] und Alter [Age, a] der untersuchten Individuen von *Bombina variegata* (LINNAEUS, 1758) aus der Population von Enez (Türkei). N – Stichprobenumfang, SE – Standardfehler des Mittelwertes.

Characters Merkmal	Sex Geschlecht	N	Mean Mittelwert	Range Spannweite	SE
SVL	Males	21	41.70	33.90-46.81	0.76
Age	Männchen	21	4.33	2-7	0.28
SVL	Females	22	43.48	33.46-50.83	1.18
Age	Weibchen	22	4.36	2-8	0.30
SVL	Males+Females	43	42.61	33.46-50.83	0.71
Age	Männchen+Weibchen	43	4.35	2-8	0.20

All above tests were processed with SPSS ver. 21.0 for Windows with the level of significance set at $P < 0.05$.

Using the von Bertalanffy's model, growth curves were calculated as described for anurans (e.g., COGĂLNICEANU & MIAUD 2003; ESTEBAN et al. 2004; GUARINO et al. 2011; KUTRUP et al. 2011a). The general form of the von Bertalanffy growth equation used is $L_t = L_\infty (1 - e^{-k(t-t_0)})$, where L_t is the snout-vent-length length at age t , L_∞ is a parameter depicting asymptotic maximum length, e is the base of the natural logarithm, k is a growth coefficient, and t_0 is the age at the end of metamorphosis (stage 46 accord-

ing to GOSNER 1960), which is the starting point of the growth interval studied here. Because data on SVL at that stage was not available for the studied population, the mean value ($L_{t_0} = 15$ mm) provided by DI CERBO & BIANCARDI (2010) was taken. The parameters L_∞ (asymptotic SVL) and k and their asymptotic confidence intervals (CI), were estimated using a non-linear regression procedure. Then, the growth rates were calculated as $R = k(L_\infty - L_t)$. Male and female growth curves were considered significantly different if the 95 % confidence intervals did not overlap (JAMES 1991; WAPSTRA et al. 2001).

RESULTS

Descriptive statistics of the SVL and age values for *B. variegata* of the Enez population are given in Table 1. The corresponding ranges were 33.46-50.83 mm and 2-8 years.

Wide growth zones and narrow hematoxylinophilic lines of arrested growth (LAGs) corresponding to were present in the phalangeal cross sections of all *B. variegata* specimens studied (Fig. 1). The resorption zone reached the first LAG in 19 specimens (44.2 %) which never caused ambiguity in age determination. Double lines were observed in seven (16.3 %) specimens. The oldest studied females and males were eight and seven years old, respectively (Fig. 2). The age when maturity was achieved was one year in 27 (62.8 %; 15 males, 12 females) specimens and two

years in 16 (37.2 %; 6 males, 10 females) specimens of the *B. variegata*.

Age was ranged from 2-8 years in the females and 2-7 years in the males studied. Age in the Enez population did not differ significantly between sexes (Independent Sample T-Test; $t = -0.074$, $df = 41$ $P = 0.941$). Intersexual difference in body size did not differ significantly between sexes (SVL: $t = -1.264$, $df = 41$, $P = 0.213$; SDI = 0.04). There was a strong positive correlation between SVL and age for males (Pearson's correlation: $r = 0.817$, $P < 0.01$) and females ($r = 0.826$, $P < 0.01$).

The growth curves based on von Bertalanffy's formula showed good fit to the correlations between estimated age and measured SVL (Fig. 3). For both sexes, the estimated asymptotic SVL was smaller than

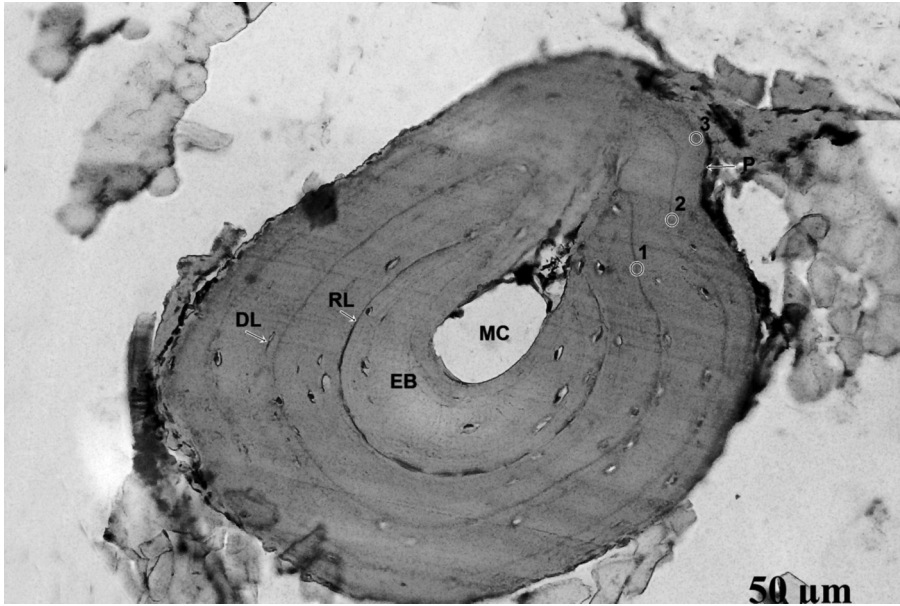


Fig. 1: Cross section (9 μm thick) of a toe bone of a three-year-old male (SVL 37.06 mm) of *Bombina variegata* (LINNAEUS, 1758) from the Enez population in Turkey. The age was derived from the presence of three Lines of Arrested Growth (LAGs 1-3) surrounding the resorption line. MC – marrow cavity; EB – endosteal bone; RL – resorption line; DL – double line; P – periphery.

Abb. 1: Querschnitt (9 μm stark) eines Zehenknochens eines drei Jahre alten Männchens (37,06 mm Kopf-Rumpflänge) von *Bombina variegata* (LINNAEUS, 1758) aus der Population von Enez (Türkei). Das Alter wurde aus der Anzahl von drei Linien verlangsamten Wachstums (LAGs 1-3) abgeleitet. MC – Markhöhle; EB – endostaler Knochen; RL – Resorptionslinie, DL – Doppellinie, P – Peripherie.

the maximum SVL recorded ($\text{SVL}_{\text{asym}} \pm \text{CI}$, males: 43.44 ± 1.88 mm; females: 46.58 ± 2.61 mm). The growth coefficient was higher in males than in females ($k \pm \text{CI}$, males: 0.47 ± 0.05 ; females: 0.34 ± 0.02) resulting in slightly different growth curves

of males [$L_t = 43.44 (1 - e^{-0.47(t-t_0)})$] and females [$L_t = 46.58 (1 - e^{-0.34(t-t_0)})$]. Growth rates of the males and females were not significantly different within the population (Independent Sample T-Test; $t = 0.059$, $df = 13$, $P = 0.954$).

DISCUSSION

PLYTYCZ & BIGAJ (1993) studied the age structure of a population of *B. variegata* from Poland. The present study adds information on the age and growth of Yellow-bellied Toads from the southeastern edge of its range in Turkey.

In anurans, body size is expected to increase in colder areas, being inversely related to the climatic factors such as temperature and sunlight intensity, which direct-

ly condition the individual thermoregulation (HUTCHINSON & DUPRÉ 1992). In addition, local habitat characteristics may affect body size and mortality (TESTER 1990; FRIEDL & KLUMP 1997). Moreover, amphibian adult body size depends on many factors, including tadpole growth rates, time of and size at metamorphosis, juvenile and adult growth rates, age at maturity and longevity (ÖZDEMİR et al. 2012).

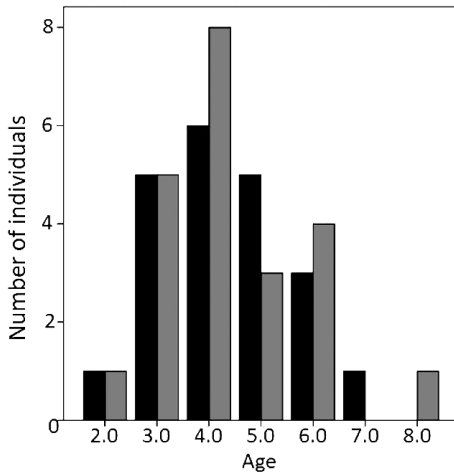


Fig. 2: Age distribution of adult specimens of *Bombina variegata* (LINNAEUS, 1758), from the Enez population in Turkey. Males – black columns, females – gray columns.

Abb. 2: Altersverteilung der erwachsenen Exemplare von *Bombina variegata* (LINNAEUS, 1758) aus der Population von Enez (Türkei). Männchen – schwarze Säulen, Weibchen – graue Säulen.

The estimated mean age in the Enez population was 4.35 years. PLYTYCZ & BIGAJ (1993) did not provide this information but reported that they mostly found 6-8 year-old individuals among the Polish Yellow-bellied Toads. Since the lines of arrested growth mirror a genetically controlled growth cycle depending on seasonal factors (CASTANET et al. 1992; ESTEBAN et al. 1996; ALCOBENDAS & CASTANET 2000), the patterns of LAG deposition can vary among populations which are subjected to different environmental conditions (OLGUN et al. 2005), with a tendency to increased mean age for amphibians in colder environments. As the annual mean temperature of Poland is lower than in the Turkey study area, the higher age in the study of PLYTYCZ & BIGAJ (1993) is compatible with this explanation.

In a similar way, longevity varies within species according to altitude, latitude and other climatic and environmental factors (BERVEN 1982; MIAUD et al. 1999). Accordingly, maximum longevity observed was

higher (9-11 years - PLYTYCZ & BIGAJ 1993) in the Poland than Enez population (8 years for females and 7 years for males). PLYTYCZ & BIGAJ (1993) supposed that some individuals of their study population probably survive more than 20 years. The active periods of the toads in cold regions are relatively short. Mean age and longevity may also be affected by factors such as food availability, predation and human-induced stress. According to observations in the Enez study area, the individuals of the *B. variegata* population live in a strongly confined area where cattle shepherds add a pinworm drug (mixture including 2.5 % Albendazole and 0.5 % Ivermectin) to the water supplies of the toads to protect the cattle from pinworm (*Cooperia* sp.) cyst infection. One must expect this substance to have a detrimental effect on *B. variegata* and its longevity.

The SVL of adult males varied between 33.90-46.81 mm, while it was 33.46-50.83 mm in females of the Enez population. PLYTYCZ & BIGAJ (1993) distinguished nine body length classes from the smallest toadlets (11 mm) to the largest adults (55 mm) but did not discriminate between sexes, juveniles and adults. The comparatively smaller maximum size of *Bombina* from the Enez population is explained by their younger mean age and shorter longevity.

In the present study, a strong positive correlation between SVL and age was shown for males and females of *B. variegata*. Similarly, COGĂLNICEANU & MIAUD (2003) found a significant positive correlation between SVL and age of *Bombina bombina* (LINNAEUS, 1761) in a population from Romania.

Male anurans are frequently observed to mature earlier than the corresponding females (MONNET & CHERY 2002; MATTHEWS & MIAUD 2007; KUTRUP et al. 2011b). However, both age at maturity and SVL did not differ significantly between the sexes of the Enez population. This may be due to similarities in their growth. Age at sexual maturity or growth rate can determine sexual size dimorphism (SSD) at the adult phase (HALLIDAY & VERREL 1988; HOWARD 1981; HEMELEAR 1988). Thus, there is no SSD in species in which males and females attain the same asymptotic body size (SVL) and age at maturity (HALLIDAY & TEJEDO 1995).

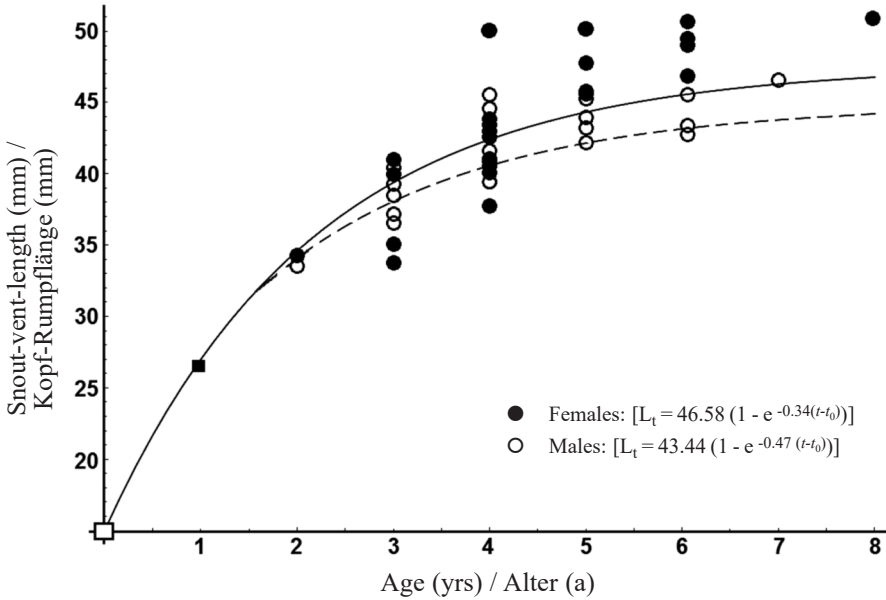


Fig. 3: The von Bertalanffy growth curves for *Bombina variegata* (LINNAEUS, 1758), from the Enez population in Turkey. Males – open circles, dotted line; females – solid circles, solid line. The solid square in the lower left represents the values of a subadult individual and the empty square at the origin of the coordinates the mean SVL (15 mm) of juvenile toads at hatching as reported by DI CERBO & BIANCARDI (2010). Growth parameters are given in the text.

Abb. 3: Die von Bertalanffy-Wachstumskurven für *Bombina variegata* (LINNAEUS, 1758) aus der Population von Enez (Türkei). Männchen - Offene Kreise und Punktlinie; Weibchen – volle Kreise, durchgezogene Linie. Das volle Quadrat unten links repräsentiert die Werte eines subadulten Individuums, das leere Quadrat am Koordinatenursprung die mittlere Kopf-Rumpflänge (15 mm) frisch verwandelter Tiere nach DI CERBO & BIANCARDI (2010). Wachstums-Parameter sind im Text angegeben.

Corresponding to the bigger female asymptotic body size, a slight female-biased SSD was observed in the Enez population of *B. variegata*. That the SSD was non-significant may be due to the sexes' similarities in age at maturation and longevity rather than difference in asymptotic body size. This is in agreement with studies in which longevity and age at maturity were found to constitute the main factors of SSD at an intra- or inter-specific level (LIAO & LU 2010; LYAPKOV et al. 2010).

Although the growth curves of males and females differed from each other, the difference in their growth rates was not significant. The non-significant difference in SSD of the individuals from Enez population may also be related to the similar growth rates of the sexes.

Since the slope (k value) of a growth curve is a measure for the time required to reach the final asymptotic size, the lower k value found in the females of the Enez population visualizes their later arrival at that stage as compared to the males.

Double lines are irregularities in bone deposition caused by unpredictable ecological factors, such as abnormal drought, heat or food shortage (JAKOB et al. 2002; GUARINO & ERIŞMIŞ 2008; ÖZDEMİR et al. 2012). In the present study, double lines were observed in seven specimens (16.3 %) (Table 2). Since the individuals studied were never exposed to unusually arid conditions (mean annual temperature and precipitation over the last six years were 13.8 °C and 50.18 mm, respectively - First Edirne Meteorology Regional Directorate), drought

Table 2: Double lines of arrested growth in cross-sections of the second phalange of the fourth toe as observed in seven specimens out of 21 male and 22 female *Bombina variegata* (LINNAEUS, 1758) from Enez (Edirne, Turkey). SVL - Snout-vent-length.

Tab. 2: Doppellinien verlangsamten Wachstums in Querschnitten des zweiten Gliedes der vierten Zehe wie sie bei sieben von 21 männlichen und 22 weiblichen Individuen von *Bombina variegata* (LINNAEUS, 1758) aus Enez (Edirne, Türkei) festgestellt wurden. KRL - Kopf-Rumpf-Länge.

SVL (mm) KRL (mm)	Sex Geschlecht	Age (yrs) Alter (a)
37.06	Male	3
40.12	Male	3
41.69	Male	4
42.01	Male	5
44.02	Male	5
42.70	Female	4
46.92	Female	6

was obviously not the trigger for the double lines observed.

Presence and degree of endosteal resorption in bone specimens can be influenced by weather conditions as well as daily and annual activity patterns (SMIRINA 1972; HEMELAAR 1988; ESTEBAN 1990; LECLAIR 1990; AUGERT 1992; ESTEBAN et al. 1999). For example, CAETANO & CASTANET (1993) reported that fewer cases of resorption (13 %) were observed in lowland populations of

Triturus marmoratus (LATREILLE, 1800) than in populations living at high altitudes (16 %). Although the Enez population is located in a lowland area (181 m a.s.l), the percentage of endosteal resorption was comparatively high (44.2 %). Similar high percentages in lowland populations were reported e.g., in *Pelophylax perezii* (LÓPEZ-SEOANE, 1885) and *Pelophylax saharicus* (BOULENGER, 1913) (ESTEBAN et al. 1996; ESTEBAN et al. 1999).

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