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Preliminary data on the age structure of *Phrynocephalus horvathi* in Mount Ararat (Northeastern Anatolia, Turkey)

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Abstract. In this study, the age structure, growth and longevity of 27 individuals (8 juveniles, 8 males and 11 females) from the Mount Ararat (Iğdır, Turkey) population of *Phrynocephalus horvathi* were examined with the method of skeletochronology. According to the obtained data, the median age was 3.5 (range= 2-5) for males and 4 (2-5) for females. Both sexes reach sexual maturity after their first hibernation, and no statistically significant difference in age composition was observed between the sexes. According to von Bertalanffy growth curves, asymptotic body length was calculated as 51.29 mm and growth coefficient k - 0.60.

Key words: Skeletochronology, growth, longevity, *Phrynocephalus horvathi*, Northeastern Anatolia.

Introduction

The genus *Phrynocephalus* is a core of the Palearctic desert herpetofauna together with the species from genus *Eremias* (Ananjeva et al. 2006). The Horváth's toad-headed agama, *Phrynocephalus horvathi* inhabits the Araks River Valley of Armenia, Turkey, Nakhichevan (Azerbaijan), and northwestern Iran (Melnikov et al. 2008) and is found at up to 1,000 m a.s.l. The species generally lives in the open desert landscapes and saltwort and wormwood semi-desert with sparse xerophytic herbaceous vegetation and takyr-like (clay desert) soils (Başoğlu & Baran 1977, Ananjeva & Agasyan 2009). The main threats on the species is habitat fragmentation and loss due to land conversion, agriculture and urbanization in the Armenian part of its distribution (Ananjeva & Agasyan 2009). On the other hand, there is no visible anthropogenic pressure on the population in Anatolia (Çiçek et al. 2011).

Skeletochronology is a widely used method for age estimates regarding amphibians and reptiles depending on the annual growth rings developed in bones (Castanet & Smirina 1990, Castanet et al. 1993, Smirina 1994). This technique is quite successful in determining the age compositions of lizards (Castanet et al. 1988, Castanet & Baez 1991, El Mouden et al. 1997). Although there are studies on the distribution (Ananjeva et al. 2006), population dynamics and ecology (e.g. Shenbrot 1987, Rustamov & Shammakov 1967), age structure and life history (Smirina & Ananjeva 2001) of *Phrynocephalus helioscopus* s.l. (include *P. horvathi*), there is still lack of information about the age composition of the Anatolian population. The aim of this paper is to examine, through the skeletochronological study of humerus bones of *P. horvathi*, and to estimate age structure, some growth parameters, longevity and size at maturity.

Materials and Methods

In this study, 27 preserved specimens of *P. horvathi* (8 males, 11 females and 8 juveniles) were collected between 25 June and 27 August from the sand dunes in Aralık, Iğdır Province of Turkey [Lat.: 39.863483 °N, Long.: 44.505245 °E, 826 m a.s.l.]. The specimens were primarily collected to determine the herpetofauna of the vicinity of Iğdır and secondarily evaluated in this study. The individuals were separated by sex and their body length (SVL) and total length (TL) were measured using dial calipers to the nearest 0.01 mm and recorded.

Humerus bones were dissected from specimens, fixed in 70% alcohol and then washed with distilled water. After fixation, decalcification was performed in 5% nitric acid for 3-5 hours according to the size of bone. Tissue samples were embedded in paraffin, stained with Ehrlich's hematoxylin and then examined under light microscope. For each humerus, we selected at least three cross sections at the mid-diaphyseal level, with the smallest marrow cavity. The lines of arrested growth (LAG) were counted by two observers (K.Ç. and M.K.). In all cases, the observers were blind to the identification of the individuals. All mount preparations were photographed with an Olympus LC-20 Soft-Imaging System.

As our data was not normally distributed, the non-parametric Mann-Whitney U test was used to compare medians, and Spearman's rank correlation was used to estimate the relationship between SVL and age. Growth was estimated by von Bertalanffy's equation (1983) previously used in several studies on saurians (e.g. Wabstra et al. 2011, Roitberg & Smirina 2006, Guarino et al. 2010, Tomašević Kolarov et al. 2010). The modified growth formula is $SVL_t = SVL_{max}-(SVL_{max} - SVL_0)e^{-k(t)}$, where *k* is the Brody growth rate coefficient (units are yr^{-1}), t is the number of growing seasons experienced (age), SVL is the average length in the age groups calculated, SVL_{max} is the average length at the maximum age, and SVL_0 is the average SVL of hatchling. The SVL at hatchling, approximately 20 mm (N= 6), was estimated by measuring newly hatched individuals at the end of June. SVL_{max}, *K*, and their 95% confidence intervals were estimated through the nonlinear least-square regression with R version 2.15.1 (R Development Core Team 2012). The alpha was set at 0.05.

Results

In the 27 individuals examined (8 males, 11 females and 8 juveniles), the mean snout-vent length was 28.26 mm±(1SD) 4.72 in juveniles, 43.71 mm±4.97 in males and 45.35 mm±5.05 in females (Table 1). No difference in SVL (t= 0.70, df= 17, P<0.491) and TL (t= 1.66, df= 17, P<0.115) values was observed between the sexes.

The median age was calculated as 1 year (SD= 0.46, range= 1-2) in juveniles, 3.5 years (SD=0.93, range= 2-5) in males and 4 years (SD= 1.12, range= 2-5) in females (Table 1, Figs 1 and 2). When compared in terms of age structure, no statistical significant difference was observed between males and females (*Mann-Whitney U test*, U= 42.50, P= 0.904). Both sexes reach sexual maturity after their first hibernation. In

Table 1. Summary statistics of snout-vent length (SVL) and total length (TL) related to each sexes and age of *P. horvathi* from Mount Ararat (Northeastern Anatolia).

Sex	Age	N	SVL			TL		
			Mean	SD	Range	Mean	SD	Range
Juveniles	I	6	26.02	2.427	22.01-28.73	54.11	5.211	45.62-59.59
	II	2	34.98	2.369	33.31-36.66	74.84	9.185	68.35-81.34
	Total	8	28.26	4.716	22.01-36.66	59.29	11.117	45.62-81.34
Males	II	1	34.33			77.23		
	III	3	43.48	4.691	38.08-46.55	91.45	12.357	77.88-102.06
	IV	3	45.19	0.996	44.04-45.81	100.02	2.212	96.32-102.06
	V	1	49.36			110.31		
	Total	8	43.71	4.966	34.33-49.36	95.24	11.888	77.23-110.31
Females	II	4	40.69	3.097	38.08 -44.86	83.42	7.089	76.81-90.65
	IV	6	47.01	3.066	44.26-51.72	95.25	4.475	89.83-100.74
	V	1	54.03			108.72		
	Total	11	45.35	5.048	38.08-54.03	92.17	9.424	76.81-108.72

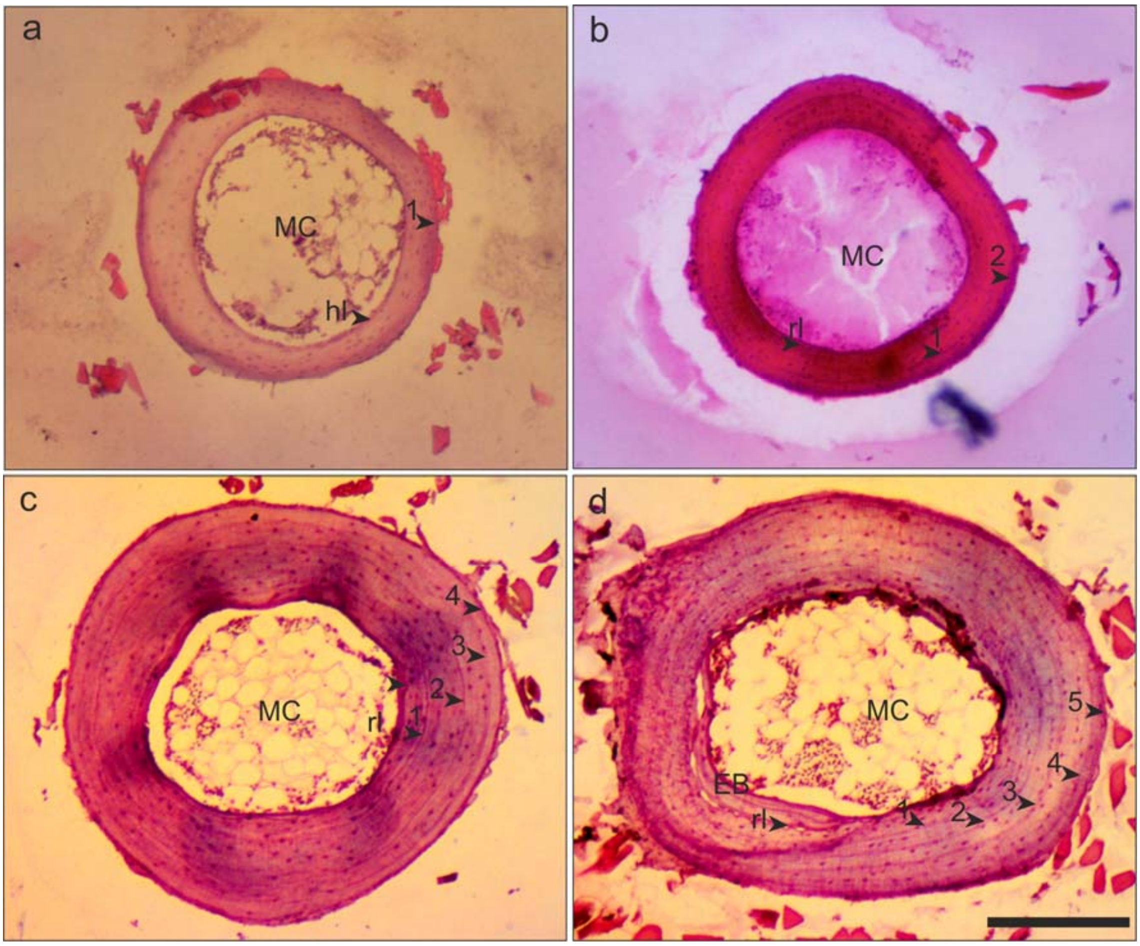

Figure 1. The cross-sections of *P. horvathi* from Mount Ararat (Northeastern Anatolia). (**a**) one year-old juvenile (SVL = 25.3 mm). (**b**) two year-old female (41.2 mm). (**c**) four year-old male (45.7 mm). (**d**) five years old female (54.0 mm). MC: marrow cavity, EB: endosteal bone, hl: hatchling line, rl: resorption line. arrows point to lines of arrested growth and resorption lines. Bar = 200 µm.

the cross sections, it was observed that the first LAG was partially resorbed by the endosteal bone but did not disappear (in 14% of the individuals). The maximum age found was 5 in both sexes.

We recorded a strong positive correlation between SVL and age (Spearman's correlation coefficient, r= 0.991, p<0.001). According to the von Bertalanffy formula, the maximum SVL was calculated as 51.29 mm (SE= 3.044, CI= 45.00-57.57) and the growth coefficient k as 0.60 (SE= 0.18, CI= 0.23-0.97) (Fig. 3).

Discussion

This study revealed that the estimated median age and longevity of *Phrynocephalus horvathi* from Mount Ararat were 3.5

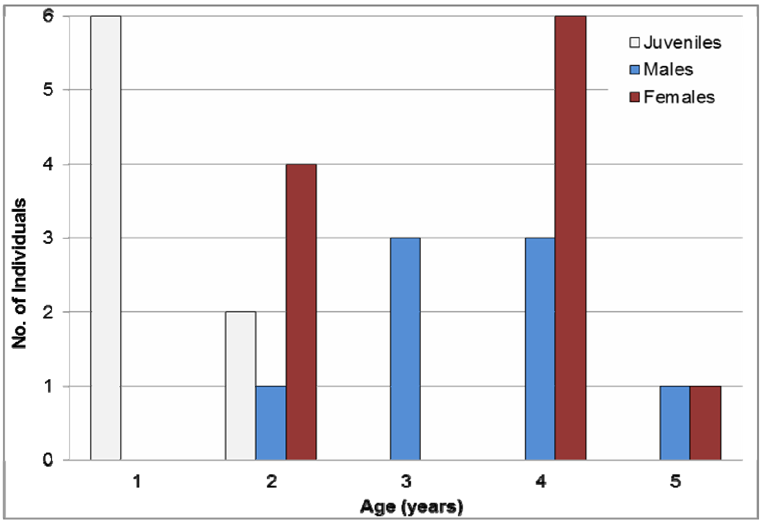

Figure 2. Age distribution of *P. horvathi* from Mount Ararat (Northeastern Anatolia).

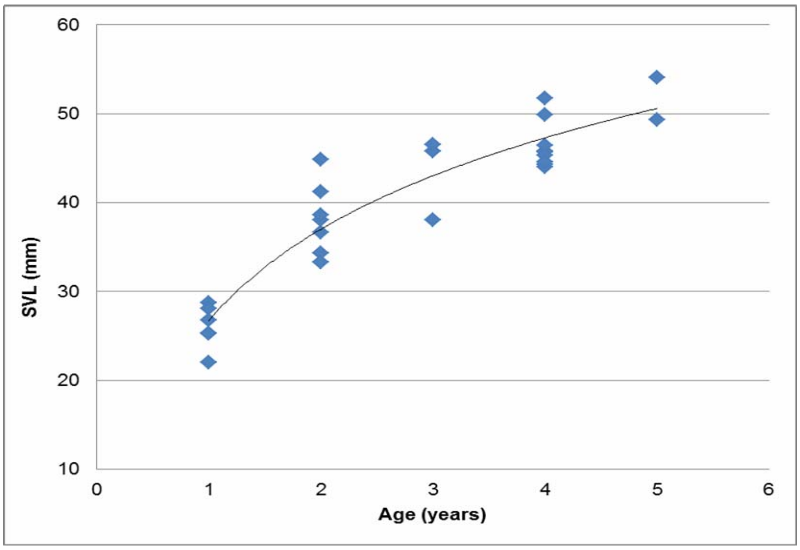

Figure 3. Growth curve of *P. horvathi* from Mount Ararat (Northeastern Anatolia). $SVL_t = 51.29 - (51.29 - 20.0)\ e^{-0.60(t)}$.

and 5 years in males and 4 and 5 years in females, respectively. The individuals reached sexual maturity after the first hibernation, and the modal age was three years for males and two years for females.

The maximum longevity was estimated in some agamid species: 4 years in *Calotes versicolor* (Lewontin 1965 in Castanet 1994), 3-4 years in *Psammophilus dorsalis* (Cole 1974 and Lewontin 1965 in Castanet 1994), 12-13 years in *Laudakia caucasia* (Panov & Zykova 2003), 9-10 years in *Laudakia stoliczkana* (Smirina & Ananjeva 2007), 5 years in *Agama impalearis* (El Mouden et al. 1997) and 6 years in *Sitana ponticeriana* (Pal et al. 2009). In the Eastern Kazakhstan population of *Phrynocephalus melanurus*, longevity was found as 5 years and a strong positive correlation was observed between SVL and age (Ananjeva et al. 2006).

The resorption rate in lizards is low and these lines are often lost even in oldest individuals (e.g. Castanet et al. 1988, Castanet 1994). The layers, formed in bones, remain throughout life because the majority of reptiles have a non-vascularized bone tissue. So, the second remodeling of the bone tissue does not occur (Smirina & Ananjeva 2007). The first LAG was partially resorbed by endosteal resorption in only 14% of the individuals.

In *Agama impalearis*, the growth rate (k) was found as 0.85 [month^{-1}] in males and 1.08 in females (El Mouden et al. 1999). In *Dinorolacerta mosorensis*, the maximum age was calculated as 9 years and the growth rate was calculated as 0.40 in males and 0.54 in females (Tomašević Kolarov et al. 2010). In the Korean population of *Eremias argus*, age ranges from 2 to 11 in females and from 2 to 8 in males, while the growth rates are 0.22 and 0.26, respectively (Kim et al. 2010). In *Phrynocephalus horvathi*, the growth rate was calculated as 0.60. Growth rates could reflect environmental conditions such as thermal environment and food availability (e.g. Jenssen & Andrews 1984; Smith & Ballinger 1994). Consistent relationships between growth rate, age at maturity and adult survival, have also been established in several lizard species (Shine & Charnov, 1992).

In reptiles, body length, growth rate, sexual maturity and age may greatly vary among populations (Guarino et al. 2010). The populations which generally live at high altitudes and in northern latitudes have higher longevity than those living at low altitudes and in southern latitudes (Wabstra et al. 2001, Roitberg & Smirina 2006, Guarino et al. 2010). Roitberg & Smirina (2006) stated that in *Lacerta agilis*, age ranged from 2 to 8 and that the maximum age was 4-6 in the populations at low altitudes (20-600m) and 7-8 in the populations at high altitudes (960-1,900m). Horváth's toad-headed agama is a species with relatively short life (5 years) and generally ranged at altitudes of up to 1,000 m.

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