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# Age structure and body size of the endangered species *Darevskia bendimahiensis* (Schmidtler, Eiselt & Darevsky, 1994) from eastern Turkey (Squamata, Sauria, Lacertidae)

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## Abstract

The life-history traits of the parthenogenetic lizard *Darevskia bendimahiensis* were studied by skeletochronology in a population inhabiting highlands in Çaldıran, Van, Turkey. Endosteal resorption was observed in 18 specimens (55%). The mean age was 4.91 ± 0.19 SD years. The mean snout-vent length (SVL) was 51.11 ± 1.15 SD mm. The age at sexual maturity was estimated as 3 years. Longevity was 7 years. Snout-vent length and age were positively correlated (Spearman's correlation; $r = 0.797$, $P = 0.000$). The aim of this study is to contribute to the future conservation activities for this endangered species.

## Key Words

Bendimahi lizard, life-history traits, skeletochronology, longevity, endosteal resorption

## Introduction

The genus *Darevskia* Arribas, 1997 is distributed in Armenia, Azerbaijan, Bulgaria, Georgia, Greece, Iran, Kosovo, Romania, Russia, Serbia, Turkey, Turkmenistan and Ukraine (Ananjeva et al. 2006). This genus includes seven parthenogenetic rock lizard species. The Bendimahi Lizard, *Darevskia bendimahiensis* (Schmidtler, Eiselt & Darevsky, 1994), is an endemic to eastern Anatolia, Turkey where it inhabits the north-eastern part of Van province and the south-eastern region of Ağrı province (Baran et al. 2012). *Darevskia bendimahiensis* is a medium sized parthenogenetic rock lizard with a total length of 19 cm (Baran et al. 2012). It is one of the four parthenogenetic species originating from the hybridisation of *D. raddei* (maternal) and *D. valentini* (paternal) (Fu et al. 2000; Murphy et al. 2000). The IUCN Red List of Threatened Animals has classified the species as endangered (EN) because its extent of occurrence is less than 5000 km$^2$, all individuals are in fewer than four locations and there is a continuing decrease in the extent and quality of its habitat (Kaska et al. 2009). It ranges between 1800 and 2300 m a.s.l.

Skeletochronology is an indirect method for estimating the age of reptiles by counting the lines of arrested growth (LAGs) observed in bone sections (Castanet and Smirina 1990; Castanet 1994). This method has been successfully used for numerous studies on different lizard species (e.g. Roitberg and Smirina 2006; Üzüm et al. 2015; Bülbül et al. 2016a, b; Eroğlu et al. 2017; Gül et al. 2017; Altunışık and Eksilmez 2018; Kurnaz et al. 2018).

This study aimed to gain information on some life history traits (e.g. body size, age at maturity, longevity) of a population of *D. bendimahiensis* using skeletochronology of phalanges. To the best of our knowledge, this is the first study to investigate potential effects of climatic factors on life-history traits of *D. bendimahiensis* from eastern Turkey.

# Material and methods

## Specimens and study sites

A total of 33 adult female individuals of Bendimahi Lizard were collected from 11 km northeast of Çaldıran, Van province, eastern Anatolia during the breeding season of 2015 (Fig. 1). Çaldıran has cold climatic characteristics. Precipitation is higher in winter than in summer. According to data from the meteorological station, the annual average temperature of the Çaldıran is 7 °C and annual average precipitation is 603 mm (https://mgm.gov.tr/). *Darevskia bendimahiensis* is found in stony or rocky grassland, usually close to water. The habitat of this lizard, which is not very far from the highway road, consisted of stony and rock grassland. The altitude of the habitat where the specimens were collected is 2067 m (highland habitat). *Darevskia bendimahiensis* lives in sympatry with *D. valentini* (Boettger, 1892) and *Ablepharus bivittatus* (Menetries, 1832). Lizards were caught by hand and anaesthetised by injecting 0.7% buffered MS-222 (tricaine methanesulphonate) into their intracoelomic cavity. The lizards were later released to their habitats after snout-vent length (SVL) was measured with a digital caliper (0.01 mm precision). The longest (fourth) digit of a hind limb was taken and transferred to 96% ethanol for skeletochronological analysis.

## Skeletochronology

The age of the lizards was assessed by using phalangeal sections and by skeletochronological standard methods (e.g. Castanet and Smirina 1990; Guarino et al. 2010). The ethanol-preserved phalangeal bones were decalcified in 5% nitric acid solution for two hours and washed in tap water for 24 h. Then, each phalanx was dehydrated using a graded ethanol series and then cleared in xylene, before embedding in paraffin. Diaphyseal cross-sections of phalanges, 12 µm thick, were prepared using a rotary microtome and then were stained with Erlich's Hematoxylin for 20 min. All the sections were examined under a stereomicroscope and acquired with a Leica DFC 295 to select the good sections. Then, the selected good sections were placed in glycerine for better observation through a light-microscope. The bone sections from each individual were photographed at the same magnification setting. All photos were examined and the number of LAGs was independently calculated by two observers (E.Y. and K. C.) and the results were compared. The proportion of endosteal resorption was assessed by comparing the diameters of eroded marrow cavities with the diameters of non-eroded marrow cavities, according to Üzüm et al. (2014). The distance between two adjoining LAGs is a good indicator of individual growth in a given year (Özdemir et al. 2012; Kurnaz et al. 2018). Any obvious decrease in space between two subsequent LAGs was


**Figure 1.** Sampling site of the animals used in this study (red circle).

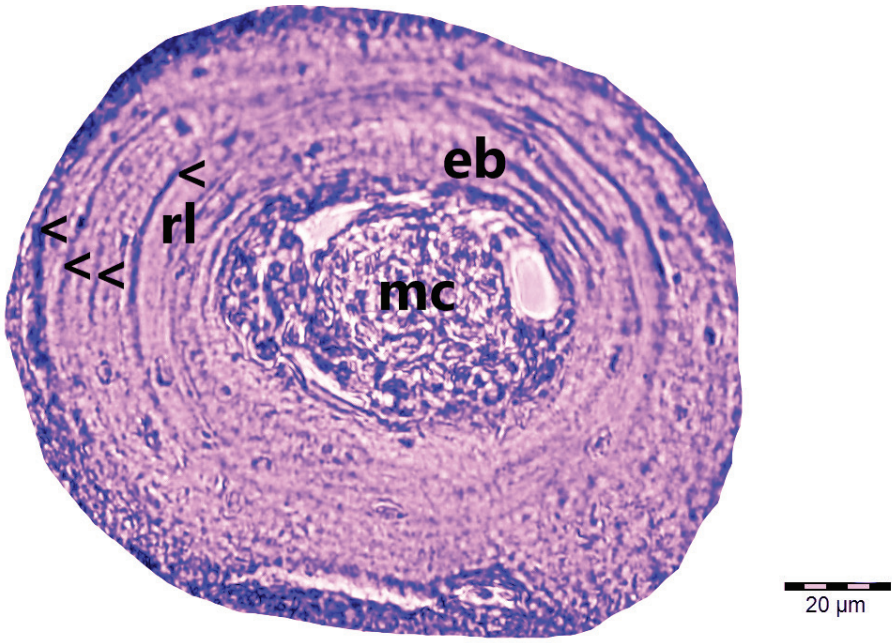


**Figure 2.** A diaphyseal cross-section of phalanx of *Darevskia bendimahiensis*. Arrows show the LAGs (4 years). Eb: endosteal bone; mc: marrow cavity, rl: resorption line.

taken as a marker for the age at sexual maturity (Ryser 1988; Guarino et al. 2008; Altunışık and Eksilmez 2018). Ethical permission for collecting of lizard specimens was obtained from the Dokuz Eylül University Animal Experiments Ethics Committee (permit no. 68/2016).

## Data analysis

All statistical analysis was performed with STATISTICA 12 (Stat Soft Inc., USA); the p-value ≤ 0.05 was considered as significant. Continuous variables were tested for normality (Shapiro-Wilk test) and for homogeneity for variances (Levene test). Age and SVL data were not normally distributed. Therefore, Spearman's rank order correlation was used to show the relationships between age and SVL.

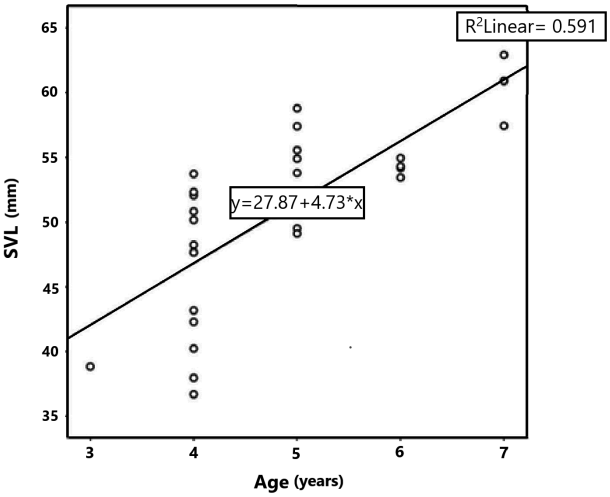


**Figure 3.** Correlation between age and SVL of *Darevskia bendimahiensis*.

# Results

A representative phalangeal cross-section of *D. bendimahiensis* is given in Fig. 2. The phalanges at the level of mid-diaphysis showed a cavity of bone marrow. The diaphyseal cortex was formed by two bone layers: the outer layer of periosteal bone and the inner layer of endosteal bone. Stained cross sections of phalanges showed that endosteal resorption is present. The first LAG growth was partly destroyed in 18 specimens (55%).

Age ranged from 3–7 years. Age structure of examined individuals was characterised by a predominance of 4–5 year-old age group. The maximum and minimum SVL of examined species was 62.92 mm and 36.70 mm, respectively. The mean age and SVL of specimens were 4.91 ± 0.19 SD years and 51.10 ± 1.15 SD mm, respectively. Sexual maturity is attained at the age of three years. Age and SVL were positively correlated (Spearman's correlation; $r = 0.797$, $P = 0.000$) (Fig. 3).

## Discussion

Skeletochronology, based on phalanges, has been used to evaluate age and growth of parthenogenetic species, including *Darevskia* (Arakelyan and Danielyan 2000; Arakelyan 2001, 2002; Arakelyan et al. 2013). In the present study, for

the first time the skeletochronology method has been applied to *D. bendimahiensis* from eastern Anatolia, Turkey.

The SVL was given as 63.5 mm in the holotype of *D. bendimahiensis* (Schmidtler et al. 1994). In literature, the range of the SVL is reported as 54 - 67 mm with a mean of 60.60 (İret 2004). In our study, the average SVL for the specimens was slightly lower than specimens previously measured by Schmidtler et al. (1994) and İret (2004). Amongst reptiles, body size (SVL) may widely vary for different populations of the same species (Guarino et al. 2010; Gül et al. 2014). The relatively small maximum size of specimens in the present study could be explained by the smaller mean age and the shorter longevity (Bülbül et al. 2018a). Many factors, including age at maturity and longevity, affect the adult body size (Özdemir et al. 2012; Eroğlu et al. 2017).

The maximum age estimated for *D. bendimahiensis* (7 years) in the present study is within the range for other *Darevskia* species (Arakelyan and Danielyan 2000; Arakelyan et al. 2013; Gül et. al. 2014, 2015a; Bülbül et al. 2016a, b; Kurnaz et al. 2017, 2018; Altunışık and Eksilmez 2018).

The mean age of the specimens (4.91 years) is similar to other parthenogenetic *Darevskia* species (Arakelyan et al. 2013) living in highlands like *D. bendimahiensis*. As a general rule, lizard specimens, inhabiting high elevation sites and northern latitudes, usually live longer than specimens inhabiting low-elevations sites and southern latitudes (Roitberg and Smirina 2006; Gül et al. 2015b; Altunışık et al. 2016). The longevity is related to active period, altitude, latitude, other climatic and environmental factors, food availability, predation and human-induced stress (Bülbül et al. 2016a, 2018b).

Stained cross-sections of phalangeal bones of *D. bendimahiensis* showed that endosteal resorption was present and caused the loss of one or two innermost LAGs. Endosteal bone resorption is affected by growth processes, environmental conditions and daily and annual activity pattern of animals (Smirina 1972; Augert 1992; Esteban et al. 1999). Endosteal resorption of one or two innermost LAGs is commonly reported in lizard species in lizard species in the genus *Darevskia*: e.g. *D. unisexualis*, *D. uzzelli* (Arakelyan et al. 2013), *D. parvula* (Bülbül et al. 2016a), *D. derjugini* (Kurnaz et al. 2018). In the present study, lower endosteal resorption was observed in comparison with congeneric species (*D. sapphirina* and *D. rudis*) (Arakelyan et al. 2013; Gül et al. 2014).

The relationship between age and body size is an important life-history parameter that is frequently used in demographic surveys (Kurnaz et al. 2018). Our findings show that the age of specimens was positively correlated with their body size (SVL) as in many rock lizards (*D. clarkorum*, Bülbül et al. 2016b; *D. valentini*, Kurnaz et al. 2017; *D. dryada*, Altunışık and Eksilmez 2018; *D. derjugini*, Kurnaz et al. 2018). Based on relationships between SVL and each age group, contrary to our findings, there was no significant correlation in females of *D. rudis* (Gül et al. 2014) and both sexes of *D. bithynica* (Gül et al. 2015a) and *D. parvula* (Bülbül et al. 2016a).

The age at sexual maturity is estimated as 3 years for *D. bendimahiensis* which is in accordance with the other parthenogenetic lizard species of the genus *Darevskia* e.g. *D. uzzelli D. sapphirina*, *D. unisexualis* and *D. armeniaca* that reached sexual maturity after their third hibernation (Arakelyan et al. 2013). Previous studies reported that age at maturity was greatly influenced by local conditions (especially variation in the length of the active season for ectothermic animals at different altitudes and latitudes) (Olgun et al. 2005; Patrielle et al. 2012; Bülbül et al. 2018a). In general, lizard specimens reach sexual maturity at a higher age in cold regions (Gül et al. 2017).

In conclusion, for the first time we provided data on body size, age at maturity and longevity of *D. bendimahiensis* from Çaldıran, Van, eastern Anatolia using the skeletochronology method. We believe that detailed research is needed on the variation in life-history traits of this species. This research can help in planning of conservation measures for this endangered species that faces a number of threats along its range, such as habitat loss from tourism, overgrazing and water extraction (Eken et al. 2006).

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