Age determination and sexual size dimorphism in three populations of *Darevskia valentini* (Boettger, 1892) from Turkey

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Received: 18. January 2021 / Accepted: 20. February 2021 / Available online: 05 December 2021 / Printed: December 2021

Abstract. Age structure, body size and growth rate of $Darevskia\ valentini\$ inhabiting from different three localities were studied by skeletochronology. Age was determined by counting the lines of arrested growth (LAGs) in phalangeal cross-sections. A total of 73 museum specimens (24 specimens from Kayseri, 20 specimens from Van and 29 specimens from Sivas) were used for this study. The mean age of males in three populations was older than those of females. The mean age of males was found to be 4.33 ± 0.28 years in Van population, 5 ± 0.28 years in Kayseri population and 5.18 ± 0.35 years for Sivas population. The maximum lifespan in Sivas population was 7 years for both sexes whereas it was 6 years in Kayseri population and 5 years in Van population. In the three populations, ages at sexual maturity of females and males were 3 and 2 years, respectively. The sexual size dimorphism was female-biased in the three populations. The mean snout-vent length (SVL) for males was calculated as 64.30 ± 1.15 mm in Van population, 68.48 ± 1.43 mm in Sivas population and 66.96 ± 1.19 mm in Kayseri populations. The mean SVL for females was determined as 60.36 ± 1.04 mm in Van population, 64.50 ± 1.73 mm in Sivas population and 68.24 ± 0.85 mm in Kayseri population.

Key words: Age, body size, skeletochronology, Darevskia valentini, Turkey

Introduction

Rock lizards including the genus *Darevskia* (Arribas 1999, Arribas et al. 2017) are present in Armenian highlands, Iran, southern Turkmenistan, Caucasus, places of Eastern Europe (Balkans) and the Crimean Peninsula. *Darevskia valentini* (Boettger, 1982), which has been recorded from 1,300 to 3,000 m asl, is distributed in north-eastern, eastern and southeastern of Turkey at borders of Iran, Armenia, Georgia and Azerbaijan (Ananjeva et al. 2006, Baran et al. 2012).

Age structure is the part of ecological studies that provide to obtain lifespan, longevity, growth and demography of lizards (Tinkle 1967, Barbault & Mou 1988, Galoyan et al. 2019). Skeletochronology is an important method for estimate age by using the presence of growth layers in bone tissue (Castanet & Smirina 1990, Castanet 1994). The age structure of the rock lizards were investigated by Arakelyan and Danielyan (2000), the parthenogenic and bisexual rock lizards (Lacerta armeniaca, L. unisexualis, L. dahli, L. nairensis, L. raddei); Arakelyan (2002), the triploid hybrids of rock lizards (Darevskia valentini × D. unisexualis, D. valentini × D. armeniaca, D. nairensis × D. unisexualis); Danielyan et al. (2008), D. valentini, D. unisexualis and D. armenica; Arakelyan et al. (2013), the parthenogenetic lizards (D. sapphirina, D. uzzelli, D. armeniaca, D. unisexualis); Gül et al. (2014), D. rudis; Gül et al. (2015), D. bithynica; Bülbül et al. (2016a), D. clarkorum; Bülbül et al. (2016b), D. parvula; Kurnaz et al. (2017), D. valentini; Altunışık & Eksilmez (2018), D. dryada; Kurnaz et al. (2018), D. derjugini; Yıldırım et al. (2019), D. bendimahiensis. There is no study on the age structure of Valentin's Rock Lizard, except Kurnaz et al. (2017). In the study of Kurnaz et al. (2017), they studied life-history traits of the same species from Gümüşhane, which is located in the eastern Black Sea Region of Turkey. This study aimed to obtain first data on the life-history traits of Valentin's Rock Lizard from three different localities in Turkey.

Material and Methods

A total of 73 museum specimens (29 \$3, 44 \$\pi\$) were examined in the current study. Specimens were collected from Yukarı Narlıca, Van province (9 \$3, 11 \$\pi\$; 38°07'14"N - 43°04'15"E; 2400 m asl); Erciyes, Kayseri province (9 \$3, 15 \$\pi\$, 38°36'03"N - 35°23'08"E; 2152 m asl) and Yaylacık in Sivas province (11 \$3, 18 \$\pi\$; 38°42'36"N - 37°02'57"E; 1605 m asl). After that, all specimens were stored in the Fauna Flora Applied and Research Center (FAMER) of Dokuz Eylül University, İzmir, Turkey. The snout-vent length (SVL) of specimens was measured with a digital calliper with an accuracy of 0.01 mm. The sex of each individual was determined by observing the presence of hemipenis in the cloacal opening (Baran et al. 2012).

The skeletochronology method was performed according to Castanet (1990) and Castanet & Smirina (1994). The longest of the toes (4th toe) was clipped and stored in 70% ethanol. All phalanges were decalcified by using 5% nitric acid for 3 h. Each phalange was removed from acid excess by rinsing in tap water for 12 h. The cross-sections (16µm thickness) of the diaphysis of phalanges were obtained using a rotary microtome and were stained with Erlich's haematoxylin for 20 min. Finally, sections were examined under a light microscope and photographed with a Leica DFC295 stereomicroscope with a digital camera. The numbers of the lines of arrested growth (LAGs) on the cross-sections were calculated by two observers. Sexual maturity and endosteal resorption were determined according to Özdemir et al. (2012).

We quantified Sexual Size Dimorphism (SSD) with the Lovich & Gibbons (1992) index according to the following formula: SDI = (mean length of the larger sex/mean length of the smaller sex)±1. In this formula, +1 is used if males are larger than females and defined as negative, or -1 is used if females are larger than males and defined as positive arbitrarily (Üzüm et al. 2014).

Normality of the SVL and age for both sexes was tested with the Shapiro-Wilk normality test. According to the normality test, non-parametric (Wilcoxon test) and parametric tests (Welch's t-test) were used to estimate differences. Spearman's rank correlation and linear regression (Cabezas-Cartes et al. 2018, Guarino et al. 2020) were performed to estimate the relationship between SVL and age. All statistical analyses were run using the packages 'dplyr' (Wickham et al., 2020) and 'ggplot2' (Wickham, 2016) in R (R Core Team, 2020).

Results

<u>Van population:</u> According to the Shapiro-Wilk normality test, the age data did not show the normality (P < 0.01) while the distribution of SVL data was normal (P = 0.364). Cross-section at the diaphysis of the phalange of a 5-year-old D. *valentini* is shown in Fig. 1. Age ranged from 3-5 years for both sexes (Table 1). The mean age did not differ between males and females (Wilcoxon test, W = 36, P = 0.287). Intersexual differences in terms of SVL was male-biased (SDI = -2.06). The age at maturation was 2 years for males while it was 3 years for females. The oldest male and female individuals were 5 years (Fig. 2). The endosteal resorption that was observed in 9 (45%) specimens did not reach the first LAG and did not prevent age estimation.

SVL ranged from 53.25-66.52 mm in females and 60.00-69.03 mm in males. The mean SVL was significantly different between males and females (Welch's t-test, t=2.53, df=17.17, P=0.021). There was a significant positive correlation between SVL and age for males (Spearman's rank correlation, r=0.90; P<0.01) while for females, there was no significant correlation (Spearman's rank correlation, r=0.42; P=0.18) (Fig. 3A).

<u>Kayseri population:</u> Age ranged from 4-6 years for males and 3-6 years for females (Table 1). The mean age did not differ between males and females (Wilcoxon test, W = 60, P = 0.668). Intersexual differences in terms of SVL was femalebiased (SDI = 0.01). The age at maturation was 2 years for males while it was 3 years for females. The oldest male and female individuals were 6 years old (Fig. 2). The endosteal resorption that was observed in 11 (46 %) specimens did not reach the first LAG and did not prevent age estimation.

The minimum and maximum SVL of species were 61.00-71.68 mm in females and 59.60-71.50 mm in males. The mean



Figure 1. Cross-section of a phalanx of *Darevskia valentini* with an age of 5 years. Abbreviations: eb, endosteal bone; rl, resorption line.

SVL was not significantly different between males and females (Welch's t-test, t = -0.87 df = 15.91, P = 0.324). There was no significant positive correlation between SVL and age for males (Spearman's rank correlation, r = 0.21; P = 0.586) while for females, there was a significant correlation (Spearman's rank correlation, r = 0.78; P < 0.01) (Fig. 3B).

<u>Sivas population:</u> Age ranged from 3-7 years in males and from 2-7 years in female specimens (Table 1). The mean age differs between males and females (Wilcoxon test, W = 53.5, P = 0.034). Intersexual differences in terms of SVL was female-biased (SDI = 1.94). The age at maturation was 2 years for males while it was 3 years for females. Among the three populations, the oldest male and female individuals were found in Sivas population, and have an age of 7 years

Table 1. Descriptive statistic of age and SVL for both sexes of *Darevskia valentini* from Van (Yukarı Narlıca), Kayseri (Erciyes) and Sivas (Yaylacık) (n: number of specimens; Range: maximum and minimum values; SE: standard error).

		Van (Yukarı Narlıca)				Kayseri (Erciyes)				Sivas (Yaylacık)			
Characters	Sex	n	Mean	Range	SE	n	Mean	Range	SE	n	Mean	Range	SE
SVL	33	9	64.30	60.00-69.03	3.47	9	66.96	59.60-71.50	1.19	11	68.48	59.78-74.80	1.43
Age		9	4.33	3.00-5.00	0.86	9	5.00	4.00-6.00	0.28	11	5.18	3.00-7.00	0.35
SVL	22	11	60.36	53.25-66.52	3.45	15	68.24	61.00-71.68	0.85	18	64.50	54.82-77.94	3.45
Age		11	4	3.00-5.00	0.63	15	4.73	3.00-6.00	0.28	18	4.28	2.00-7.00	0.24

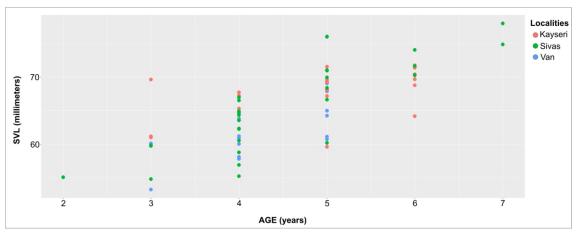


Figure 2. Age and SVL distributions in the three populations of Darevskia valentini were studied.

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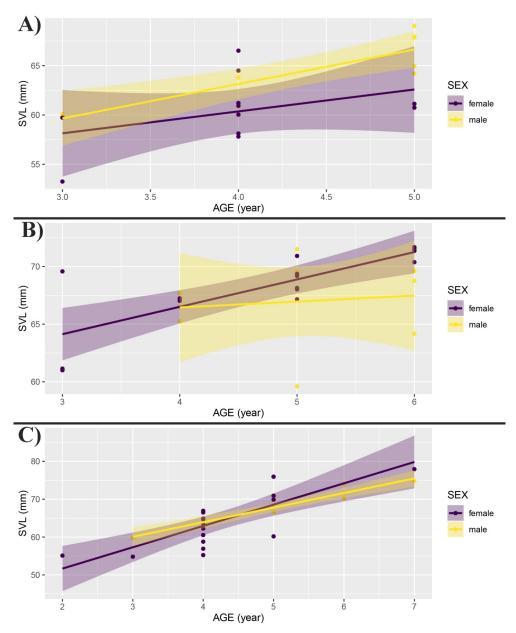


Figure 3. Relationship between age and SVL in A) Van, B) Kayseri, and C) Sivas populations of Darevskia valentini.

(Fig. 2). The endosteal resorption was observed in 13 (45 %) specimens.

SVL ranged from 54.82-77.94 mm in females and 59.78-74.80 mm in males. The mean SVL was not significantly different between males and females (Welch's t-test, t = 1.76, df = 26.85, P = 0.089). There was a significant positive correlation between SVL and age for males and females (Spearman's rank correlation, r = 0.89; P < 0.01; r = 0.77; P < 0.01, respectively) (Fig. 3C).

Discussion

Although *D. valentini* has wide distribution sites in Turkey, there is only one study based on age and growth parameters of the species (Kurnaz et al. 2017). The present study is the first to study the age, body size, longevity, age at sexual maturity and growth rate of Valentin's Rock Lizard of three dif-

 $ferent\ populations\ (Van,\ Kayseri\ and\ Sivas)\ in\ Turkey.$

Life history traits such as mean age, age at sexual maturity, lifespan, the growth rate were studied for Valentin's Rock Lizard, D. valentini, from three different populations. The mean age in Van population was found to be 4 years for females and 4.33 years for males. The mean age in Kayseri population was found to be 4.73 years for females and 5 years for males whereas 4.27 years for females and 5.18 years for males in Sivas population. In the study of Kurnaz et al. (2017), the mean age was found to be 5.82 years for females and 5.36 for males. Darevskia valentini inhabiting both Van and Gümüşhane provinces is present at the same elevations (about 2400 m). In our study, specimens have shorter lifespan (5 years for Van population, 6 years for Kayseri and 7 years for Sivas) than specimen from Gümüşhane (up to 9 years). This difference may be caused by the climatic condition, predation, food availability and activity period (Roitberg & Smirina 2006). The range of ages of some species of

the genus *Darevskia*, was found to be between 1-12 years (Arakelyan & Danielyan 2000, Danielyan et al. 2008, Arakelyan et al. 2013, Gül et al. 2014, 2015, Bülbül et al. 2016a, 2016b, Kurnaz et al. 2017, 2018, Altunışık & Eksilmez 2018, Yıldırım et al. 2019).

The length of activity period, which depends on the climatic condition (e.g. temperature) is related to the reach of sexual maturity, affecting the growth rate of lizards. In general, lizards at high altitudes and latitudes had a restrict growth season and shorten active period (Adoph & Porter 1993). In the current study, age at sexual maturity of females and males of *D. valentini* was 3 and 2 years, respectively. Similar results were indicated for the other members of the genus *Darevskia* when located at high altitude populations (Bülbül et al. 2016b, Kurnaz et al. 2018).

Life history traits may relate to the variation in sexual size dimorphism. According to Fitch (1981), males in lizards are larger than females and it is caused by competition for food among specimens. In our study, sexual size dimorphism was male-biased in the Van population, but femalebiased in the Kayseri and Sivas populations. Sex dimorphism from the populations of, D. clarkorum (Bülbül et al. 2016a), D. valentini (Kurnaz et al. 2017) and D. derjugini (Kurnaz et al. 2018) in Turkey were determined as femalebiased. On the other hand, the male-biased (SSD) results were reported for the populations of *D. bithynica* (Gül et al. 2015), D. parvula (Bülbül et al. 2016b) and D. dryada (Altunışık & Eksilmez 2018) in Turkey. As we mentioned before, environmental factors (e.g. temperature, precipitation, food ability) affects the growth rate (Adolph & Porter 1993). The climate of the current study area, which has a low predator density and food availability, is a harsh environment and these factors may have effect SSD discovered in the cur-

In conclusion, life-history traits have been studied in *D. valentini* from three different populations. Data about life-history traits provide more information about intraspecific variation in rock lizards inhabiting in different populations.

Acknowledgement. This study was supported by Dokuz Eylül University BAP under grant number 2019.KB.FEN.003.

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