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The life-history traits in a breeding population of *Darevskia valentini* from Turkey

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Abstract. We investigated the age structure, body size, longevity and growth in a breeding population of *Darevskia valentini* inhabiting highland altitude in Balahor, Turkey. According to the skeletochronological analysis (n= 25; 14 ♂♂, 11 ♀♀), the estimated ages ranged from 3 to 9 years (from 4 to 7 in males and from 3 to 9 in females). The maximum life span was 7 years in males and 9 years in females. The age at maturity was found to be 3 years in both sexes. The mean age and SVL were not statistically different between sexes. For both sexes, we found a significant positive correlation between body size and the number of LAGs. The growth coefficient (k) was lower in females (0.30) than in males (0.76) while asymptotic SVL was higher in females (70.06) than in males (60.55). Growth rates were found to be significantly different between both sexes (females grew faster than males). However, a low level of female-biased sexual size dimorphism (SSD) was observed in the population.

Keywords. Skeletochronology, longevity, von Bertalanffy model, growth rate, SSD.

INTRODUCTION

The Valentin's Lizard, *Darevskia valentini* (Boettger, 1892) inhabits highland areas above 1800 m (Baran and Atatür, 1998; Eiselt et al., 1992). It has three subspecies (*D. v. valentini*, *D. v. lantzicyreni* and *D. v. spitzenbergerae*) distributed in a large area including most of Anatolia. Many studies about systematic, distribution and ecology were performed on this species (Darevsky, 1972; Franzen, 1990; Eiselt et al., 1992; Mulder, 1995; Sindaco et al., 2000; Tarkhnishvili et al., 2013), but to date, there is no study related to age determination in Turkey.

Age determination yields important data for ecological studies of the reptilian species. One of the most commonly used techniques to determine the individual age in reptiles is skeletochronology that many studies have proven to be a very successful and reliable method (Castilla and Castanet, 1986; Girons et al., 1989; Piantoni et al., 2006; Nayak et al., 2008; Kim et al., 2010; Guarino, 2010). Skeletochronology estimates the age using lines of arrested growth layers in the bone tissue (Castanet and Baez, 1991; Castanet, 1994). Due to its reliability and the fact that it is less time-consuming than other age-determination methods such as marking-recapture (Halliday and Verrell, 1988), the number of the studies using skeletochronology method has increased recently (Luís et al., 2003; Altunışık et al., 2013; Tok et al., 2013; Gül et al., 2014; Üzüm et al., 2014; Yakın and Tok, 2015; Kanat and Tok, 2015; Gül et al., 2015; Üzüm et al., 2015; Comas et al., 2016).

Since there are no detailed studies based on age and growth parameters of *Darevskia valentini*, here we present data on life-history traits (age structure, body size and some growth parameters) of the species. In order to assess population dynamics of *D. valentini* living at altitudes between 1300-3000 m a.s.l. (Tok et al., 2009),

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we chose a mountain population (2400 m a.s.l) living at mean altitude regarded as typical for the species.

MATERIALS AND METHODS

Field Study

A total of 25 individuals of Valentin's Lizard, *Darevskia valentini* (14 ♂♂, 11 ♀♀) were caught during breeding season from Balahor Plateau (near Gümüşhane), Turkey. The Balahor population is located in a highland area (40°29'51"N, 39°56'24"E) at an altitude of 2400 m a.s.l. The habitat consists of rocky areas and open ground. The active period for lizards varies from early May to the middle of September. During the sampling period (16-20 August 2015), the average air temperature in daytime was recorded as 27 °C. Lizards were caught by hand. The sex of each individual was determined by direct examination of the sexual organs and secondary sex characters (e.g., dark blue spots on the margins of ventral plates and dorsal coloration of males). Snout-vent length (SVL) was measured to the nearest 0.01 mm using a digital caliper. We quantified Sexual Size Dimorphism (SSD) with the Lovich and 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, we used -1 because females were larger than males and defined as positive.

For each lizard, the second phalange from the longest finger of the hind limb was clipped and preserved in 10% formalin solution for subsequent histologic analyses. After registration and toe-clipping, the lizards were released back into their natural habitats. The animals were treated in accordance with the guidelines of the local ethics committee.

Skeletochronological Analyses

The procedure of skeletochronology is based on the calculation of the lines of arrested growth (LAGs) in transverse sections of the middle part of phalangeal diaphyses using a portion of the second phalanx from the third toe (Gül et al., 2014).

After removing the skin, toes were treated for 2.5 hours with 5% nitric acid for decalcification of bone tissue. All samples were then loaded in a tissue processor (Leica Tissue Processor TP1020, Germany) and all tissue samples were embedded in paraffin by a tissue embedding device (Thermo Shandon B64100010, USA). Cross-sections (15 µm) were obtained with a rotary microtome and thereafter were stained using the haematoxylin procedure. The stained sections were mounted using Entellan and observed under a light microscope.

Age determination was estimated using skeletochronology analysis (Castanet and Smirina, 1990; Smirina, 1994). The numbers of LAGs in each section were independently calculated by three observers (M. Kurnaz, A.İ. Eroğlu and U. Bülbül) and results were compared. Possible double lines were counted as a single LAG. A double line is two adjacent age rings formed during a winter season. As previously stated in the study of Özdemir et al. (2012), we assessed endosteal resorption of the first LAG by comparing the diameters of eroded marrow cavities with the diameters of non-eroded marrow cavities in sections from the youngest specimens. Endosteal resorption did not interfere with age estimation procedures because the resorption zone never reached the first LAG. The distance between two adjoining LAGs is a good indicator of individual growth in a given year (Kleinenberg and Smirina, 1969; Özdemir et al., 2012). Where we observed an obvious decrease in spacing between two subsequent LAGs, we took it to mark the age when sexual maturity was achieved (Ryser, 1998; Yılmaz et al., 2005; Özdemir et al., 2012). Uncountable cross section samples were not incorporated into our study.

Statistical Analyses

Normality of the SVL and age distribution for the males and females was tested with the One-Sample Kolmogorov-Smirnov test ($P \geq 0.05$). Being that the variables normally distributed, we used parametric independent sample t-test to estimate significant differences ($P < 0.05$). Pearson's correlation was used to estimate the relationship between SVL and age ($P < 0.05$). All statistical tests were processed with IBM SPSS 21.0 for Windows.

Growth Pattern Estimating

The growth patterns were estimated by using the von Bertalanffy equation model between body size and age according to previous studies (James, 1991; Wapstra et al., 2001; Roitberg and Smirina, 2006; Guarino et al., 2010). The general form of the von Bertalanffy equation used is $L_t = L_\infty (1 - e^{-k (t-t_0)})$ where L_t is 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 hatching, which is the starting point of the growth interval under the present study. As applied in the study of Guarino et al. (2010), we assumed the mean value provided by Tayhan et al. (2011) as size at hatching (L_{t0} = 25.3 mm) because of the lack of incontrovertible data on the size at hatching of the populations studied, due to lack of young lizards collected during the field studies. The parameters L_∞ (asymptotic SVL) and k, and their asymptotic confidence intervals (CI) were estimated using the non-linear regression procedure by means of the IBM SPSS 21.0 software program. Then, the growth rates were calculated as $R = k (L_\infty - L_t)$. Growth curves were considered to be significantly different if the 95% confidence intervals did not overlap (James, 1991; Wapstra et al., 2001).

RESULTS

Age ranged from 4-7 years in males and 3-9 years in females (Table 1). The mean age of the specimens was not different between the sexes (independent sample t-test: t = -0.802; df = 23; P = 0.431). Intersexual differences in body size (length) was female-biased (SDI = 0.052). The mean SVL (t = -1.143; df = 23; P = 0.431) did not differ between sexes. There was a significant positive correlation between SVL and age for both males (Pearson's correlation r = 0.797; P < 0.01) and females (r = 0.836; P < 0.01). The growth pattern estimated by von Bertalanffy's showed a best fit to the relation between age and SVL (Fig. 1). For males, the estimated asymptotic SVL was lower than the maximum SVL record (SVL_{asym} ± CI: 63.89 ± 5.80 mm) while for females, it was higher than the maximum SVL record (SVL_{asym} ± CI, females: 73.71 ± 5.30 mm). The growth coefficient was higher in males than in females (k ± CI, males: 0.27 ± 0.11; females: 0.19 ± 0.10). For all populations (males + females), the asymptotic SVL, growth coefficient (k) and mean growth rate were calculated as 64.60 ± 4.33 mm, 0.24 ± 0.08 and 4.11 ± 0.79 mm per year, respectively. The growth curve of males was significantly different from that of females. In this population, females grow faster than males (the average growth rate was 0.97 ± 0.43 in males and 4.12 ± 0.98 in females) (Fig. 2). The mean growth rates of males and females were significantly different within the population (Independent Sample t-test: t = -2.323; df = 9; P < 0.05). Descriptive statistics of the growth rates for the Balahor population are given in Table 2.

A growth zone and thin hematoxylinophilic outer line most likely corresponding to a winter line of arrested growth were present in sections of the phalanges in 100% of both male (n = 14) and female (n = 11) adult specimens (Fig. 3). The resorption zone never reached the first LAG and did not interfere with age determination. We observed double lines in 18 (72%) specimens. The oldest females and males were 9 and 7 years old, respectively (Fig. 4). The age at maturation was 3 years for both sexes in the population.

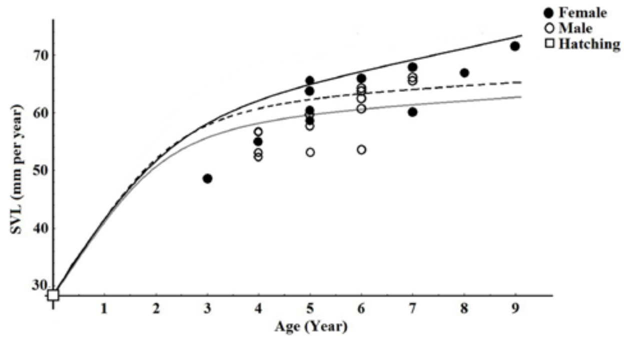

Fig. 1. The von Bertalanffy growth curves for males (open circle, solid line), females (solid circle, grey line) and all specimens (dot line) of *D. valentini*. Open square shows SVL mean of the lizards at hatching (25.3 mm) as reported by In den Bosch and Bouth (1998). Growth parameters are given in the text.

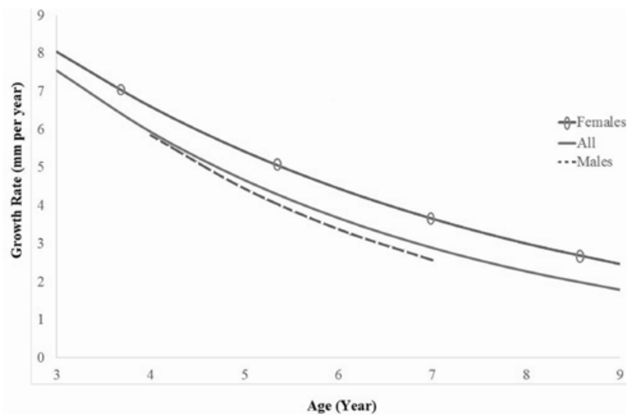

Fig. 2. Relationships among the growth rates of age groups belonged to individuals of *D. valentini* from Balahor population.

Table 1. Descriptive statistics of age and SVL of the Balahor population. For abbreviations, see text (n: number of samples; Range: maximum and minimum values and SE: standard error).

Characters	Sex	n	Mean	Range	SE
Age	♂♂	14	5.36	4-7	0.29
SVL		14	59.24	53.03-67.40	1.33
Age	♀♀	11	5.82	3-9	0.54
SVL		11	62.31	49.05-71.65	1.95
Age	♂♂	25	5.56	3-9	0.28
SVL	♀♀	25	60.59	49.05-71.65	1.15

Table 2. Descriptive statistic of growth rate and growth coefficient (k) of the Balahor population. For abbreviations, see text (n: number of samples; Range: maximum and minimum values and SE: standard error).

Characters	Sex	n	Mean	Range	SE
Growth rate	♂♂	4	4.06	2.57-5.85	0.71
k		14	0.27	-	0.11
Growth rate	♀♀	6	4.81	2.47-8.04	0.76
k		11	0.20	-	0.10
Growth rate	♂♂	7	4.11	1.79-7.55	0.79
k	♀♀	25	0.24	-	0.08

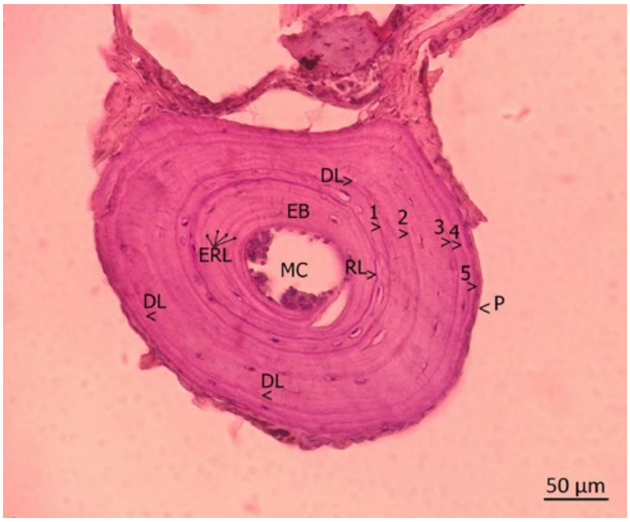

Fig. 3. A cross section (15 µm thick) through phalange of a five-year-old female (63.87 mm SVL) *D. valentini* from Balahor population. For abbreviations, see text (MC: Marrov Cavity; EB: Endosteal bone; RL: Resorption Line; DL: Double Line; ERL: Endosteal Resting Line; P: Periosteal Bone).

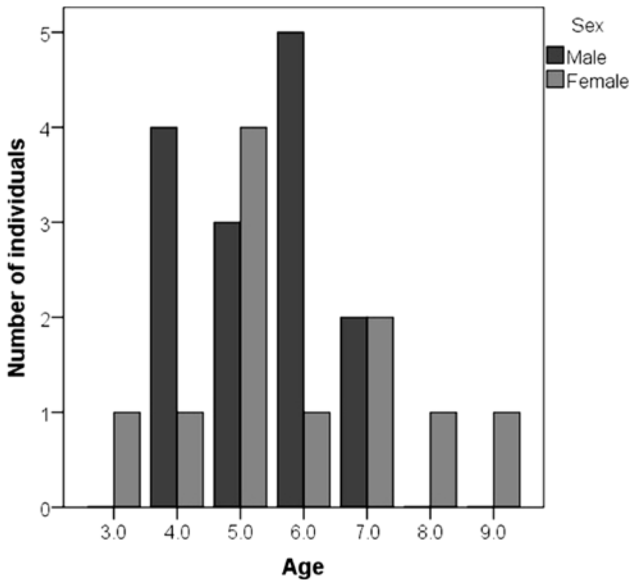

Fig. 4. Age distributions for both males and females of *D. valentini* from Balahor population.

DISCUSSION

The present study provides data on the age structure and growth patterns of the Valentin's Lizard from a Turkish population. Life history traits (e.g., mean age, age at sexual maturity and maximum longevity) could depend on genetic characteristics and be species-specific. In the present study, the mean age of the *Darevskia valentini* was found to be 5.36 years in males and 5.82 years in females of the Balahor population located in a high elevation site (2400 m). However, Gül et al. (2014) reported a mean age about 1 year lower (4.3 in males and 4.8 in females) in a high altitude population (inhabiting 2137 m a.s.l.) of another rock lizard species (*D. rudis*). Conformably, Arakelyan et al. (2013) reported a lower mean age for the four parthenogenetic rock lizards (4.46 years for *D. armeniaca*, 4.44 years for *D. unisexualis*, 4.44 years for *D. sapphirina* and 4.06 years for *D. uzzelli*) living in highland habitats (between 1400 and 2000 m a.s.l.).

Apart from species variation, age structure may also differ among different populations of the same species. The age structure of lizards may fluctuate according to environmental parameters (e.g., climate, altitude, latitude and predation) and other conditions such as proportion and energetic costs of reproduction, activity season and hibernation period (Roitberg and Smirina, 2006). A different climatic condition might affect population demography by generating differences in age structure and longevity (Özdemir et al., 2012). For instance, an increase in mean age with higher altitude has been reported for various lizard species in colder environments (Roitberg and Smirina, 2006). Apart climatic conditions, other factors such as different rates of predation might affect the demography of the lizards studied.

Like mean age, longevity is dependent on the active period, which is in turn related to altitude, latitude and other climatic and environmental factors. Since the climatic conditions in Balahor were not favourable for lizard activity due to the presence of snow for at least 6.5 months, the active period of the studied population was found to be lower than other species inhabiting lowland habitats. In general, individuals from high-elevation sites have higher longevity than those from low-elevation sites (Wapstra et al. 2001; Roitberg and Smirina, 2006; Guarino et al., 2010). Congruently, we found a high longevity

(7 years in males and 9 years in females) in the Balahor population. Similar to our results, the maximum longevity was found to be 8 years in *D. armeniaca*, *D. unisexualis* (Arakelyan et al., 2013) and *D. rudis* (Gül et. al., 2014) while it was found to be 6 years in *D. sapphrina* and *D. uzzelli* (Arakelyan et al., 2013).

In the present study, the age of both sexes was significantly correlated with their body size (SVL). The adult body size depends on many factors including age at maturity and longevity (Özdemir et al., 2012). In some species, the male lizards mature earlier than females (Beebee and Griffiths, 2000; Olsson and Madsen, 2001). However, age at maturity and SVL were not found to be significantly different between both sexes in our study.

A low level of female-biased sexual size dimorphism (SSD) was observed in the Balahor population. Longevity and age at first reproduction have been identified as the main determinants of SSD at an intra-specific level (Liao and Lu, 2010; Lyapkov et al., 2010; Liao et al., 2013; Liao et al. 2015). Congruently, age at maturity was found to be similar in both sexes. Moreover, SSD in many adult lizards arises due to sexual differences in the growth rates, and the larger sex grows faster than the smaller (John-Adler and Cox, 2007; Kolarov et al., 2010; Üzüm et al., 2014). Although there was not a high-level of female-biased SSD, we found significant differences between the growth rates of both sexes. Our data show that females grew faster than males and that the growth trajectories were different between sexes. The lower value of k in the von Bertalanffy equation in females suggests that they attain the asymptotic body length slower than males. When considering the overall population, we found higher growth rates in our individuals. This is in accordance with the general prediction that lizards grow faster at high elevation sites.

Double lines are found in higher percentages in some unsuitable ecological conditions (e.g., hot climate and dry period) (Jakob et al., 2002; Guarino and Erişmiş, 2008; Özdemir et al., 2012). On the other hand, food availability may negatively affect the number of double lines (Bülbül et al., 2016). Since the Balahor population has a cold climate and less food sources, the high percentage of double lines (72 %) observed in this steppe region suggests that the unfavorable food supply has a greater effect than climatic conditions.

In conclusion, our data on the body size, age structure, longevity, and growth of *D. valentini* may contribute to the knowledge of the life-history traits of this species. Our results show different growth rates between sexes in the Balahor population, having different longevity, despite having a similar age at sexual maturity. However, long-term studies including different populations under various environmental conditions are neeeded to reveal a more comprehensive picture.

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