

Age structure and growth in a Turkish population of the Balkan Green Lizard, *Lacerta trilineata* BEDRIAGA, 1886 (Squamata: Sauria: Lacertidae)

Altersstruktur und Wachstum in einer türkischen Population
der Riesensmaragdeidechse *Lacerta trilineata* BEDRIAGA, 1886
(Squamata: Sauria: Lacertidae)

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KURZFASSUNG

Die Studie bringt Informationen zum Alter und Wachstum von *Lacerta trilineata* BEDRIAGA, 1886 in der Population von Sergen, Westtürkei (420 m ü. M.). Das Alter bei Erreichen der Geschlechtsreife, geschlechtsbedingte Größenunterschiede (SSD) und Wachstumsraten werden angegeben.

Dazu wurden Querschnitte von Zehenknochen mit skeletochronologischen Methoden untersucht. In der Stichprobe aus erwachsenen Individuen (14 Männchen und 12 Weibchen) betrug das Alter für beide Geschlechter zusammen 6-13 ($\bar{x} = 8,77$) Jahre, wobei der Unterschied zwischen Männchen mit 7-13 ($\bar{x} = 9,07$) Jahren und Weibchen mit 6-10 ($\bar{x} = 8,42$) Jahren nicht signifikant war. Die Geschlechtsreife erreichen beide Geschlechter im dritten Lebensjahr. Weder bei Männchen noch Weibchen zeigte sich eine signifikante Korrelation zwischen Kopf-Rumpflänge (SVL) und Alter. Die Männchen der Sergen Stichprobe waren kaum größer als die Weibchen (SSD = 0,014). Der Wachstumskoeffizient (k) war bei Weibchen kleiner als bei Männchen ($k \pm$ Konfidenzintervall, Männchen: $0,96 \pm 0,22$; Weibchen: $0,75 \pm 0,41$). Die Wachstumsraten der Geschlechter unterschieden sich nicht voneinander.

ABSTRACT

This study provides information about age and growth of *Lacerta trilineata* BEDRIAGA, 1886, in the population from Sergen, West Turkey, at an altitude of 420 m a.s.l. Age at maturity, sexual size difference (SSD) and growth rate of the sample are presented.

Cross-sections of phalangeal bones were examined based on the skeletochronology method. In the adult sample (14 males and 12 females), the age ranged from 6-13 ($\bar{x} = 8,77$) years for both sexes collectively, the values 7-13 ($\bar{x} = 9,07$) years in males and 6-10 ($\bar{x} = 8,42$) years in females not differing significantly from each other. Sexual maturity was attained in the third year of life in both sexes. There was no significant correlation between the lizards' body size (SVL) and age for both males and females. A slightly male-biased sexual size dimorphism (SSD = 0,014, not significant) was observed. The growth coefficient (k) was lower in females than males ($k \pm$ confidence interval, males: $0,96 \pm 0,22$; females: $0,75 \pm 0,41$). There was no difference in the growth rate between sexes.

KEY WORDS

Reptilia: Squamata: Sauria: Lacertidae; *Lacerta trilineata*; ecology, life history, skeletochronology, LAGs, lines of arrested growth, von Bertalanffy growth curve, age, growth, longevity, body size, Turkey

INTRODUCTION

The Balkan Green Lizard, *Lacerta trilineata* BEDRIAGA, 1886, is the largest species of the genus. It has a wide distribution area from the coastal regions of Croatia, Bosnia and Herzegovina, Serbia, Montenegro and Romania southwards across Albania, Greece, Macedonia and Bulgaria to

western Turkey in the east (mostly according to UETZ et al. 1995-2018). It is classified as LC (Least Concern) in the IUCN Red List (BÖHME et al. 2009) due to its wide distribution, presumed large population and the unlikelihood to decline fast enough to qualify for listing in a more threatened category.

Age determination studies are carried out by researchers to reveal the life history traits of reptile species (ESTEBAN et al. 2004; ROITBERG & SMIRINA 2006; GUARINO et al. 2010). Skeletochronology is the most appropriate and reliable method for determining the age of lizards. Many studies on lizards in Turkey used skeletochronological methods (ARAKELYAN et al. 2013; ALTUNIŞIK et al. 2013; TOK et al. 2013; GÜL et al. 2014; ÜZÜM et al. 2014; YAKIN & TOK 2015; KANAT & TOK 2015; GÜL et al. 2015; ÜZÜM et al. 2015). This method is based on counting of the lines of arrested growth (LAGs) that appear in long bone sections of individuals with strongly reduced activity during the hibernation, estivation or starvation periods (HEMELAAR 1981; CASTANET & SMIRINA 1990; SMIRINA 1994). During their periods of surface activity, climate and envi-

ronmental conditions affect the lizards' life history traits such as age at maturity, longevity, reproductive phenology, survival rates and growth rates (ADOLPH & PORTER 1996).

ÜSTEL (2010) reported on longevity and the relationship between age and SVL in the *L. trilineata* population from the Gelibolu and Biga Peninsulas, Province of Çanakkale, Turkey, and the paper by KALAYCI et al. (2018) dealt with life-history traits such as average and maximum age, age upon arrival at maturity and various morphometric parameters relative to age, comparing Turkish lowland (Edirne, 17 m a.s.l.) and high altitude (Bolu, 1,250 m a.s.l.) populations. The aim of this study is to add life-history information on NW Turkish *Lacerta trilineata* based on skeletochronological methods.

MATERIALS AND METHODS

A total of 26 adult specimens (14 males and 12 females) were caught from a population in Sergen, Province of Kırklareli, European Turkey, during the breeding season (capture permission no. 72784983-488.04-42844 issued by the Turkish Ministry of Forest and Water Affairs). The Sergen population (41°42'31" N, 27°42'27" E) is located at an altitude of 427 m a.s.l. The lizards were sexed by examination of the secondary sexual characters (presence of light blue color at the ventral side of the head in adult males) and by sounding for the presence or absence of hemipenis pockets.

At the collecting site, the lizard surface-active period lasts from early March to November. The lizards were captured on 12-16 August 2017 between 11.00 a.m. and 4.00 p.m. The average temperature at the time of sampling was 29 °C according to climate data provided by 1st Edirne Meteorology Regional Directorate.

Snout-vent length (SVL) was measured to the nearest 0.01 mm using a digital caliper; sexual size dimorphism was quantified by the Sexual Dimorphism Index (SDI) as described by the formula: $SDI = (\text{mean SVL of the larger sex} / \text{mean SVL of the smaller sex}) \pm 1$. The value +1 is used if

males are larger than females and -1 if the opposite is true. The result is arbitrarily defined as positive if the females are larger and negative if the males are larger (LOVICH & GIBBONS 1992).

From each lizard, the longest (4th) toe was clipped at the second phalange and preserved in 10 % formalin solution for subsequent histological analyses. After toe-clipping, the lizards were released back into their original habitats. The specimens were treated in accordance with the guidelines of the ethics committee of the Karadeniz Technical University (KTÜ.53488718-417/2016/38).

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 (10 µm, rotary microtome) of the phalanges were stained with hematoxylin using the procedure described by BÜLBÜL et al. (2016), then mounted on microscope slides, closed using Entellan® and observed under a light microscope.

Skeletochronology is widely applied to gain information about the life histories

of individuals with the aim to describe age structures, growth rates and maturity ages within the studied population (CASTANET et al. 1993; ÜZÜM & OLGUN 2009). There is agreement among researchers that this method produces reliable results when clipped phalangeal bones are analyzed, so that even endangered populations can be studied since the individuals are not detached from their habitat (CASTANET & SMIRINA 1990; GUARINO et al. 2010). Studying cross-sections of long bones, the age of individuals is estimated counting the lines of arrested growth (LAGs) that are formed during the hibernation period. Summer heat can lead to estivation which results in an accessory LAG preceding the following hibernation-caused LAG, both appearing as double line (CABEZAS-CARTES et al. 2015; CASTANET et al. 1993; YAKIN & TOK 2015). To minimize the subjective error margin, three of the authors (A. İ. Eroğlu, Y. Odabaş H. Koç) independently counted the LAGs on the cross-sections and their results were compared and harmonized. Double lines were counted as single lines. Arrival at sexual maturity was assumed where any obvious decrease in space between two subsequent LAGs was observed (RYSER 1988; YILMAZ et al. 2005; ÖZDEMİR et al. 2012).

Since age classes and SVL measurements were normally distributed (One-

Sample Kolmogorov-Smirnov Test, $P > 0.05$) within the sample, the parametric Independent Samples T-Test was used for comparison of means and Spearman's Rank Correlation Test ($P < 0.01$) to analyze correlations. All statistic tests were processed with SPSS 21.0 for Windows and the level of significance set at 0.05.

Using the von Bertalanffy's model, growth curves were formed as described in the literature (JAMES 1991; WAPSTRA et al. 2001; ROITBERG & SMIRINA 2006; GUARINO et al. 2010). The authors used the general form of the von Bertalanffy equation, $L_t = L_\infty (1 - e^{-k(t-t_0)})$, where L_t is SVL at the age t , L_∞ is the asymptotic maximum length, e is the base of the natural logarithm, k is a growth coefficient, and t_0 is the age at hatching. Due to the unavailability of data on the hatching size in the studied population $L_{t_0} = 27.0$ mm was used, which is the mean value provided by JAMES & SHINE (1988). The parameters L_∞ (asymptotic maximum SVL) and k , and their asymptotic confidence intervals (CI), were estimated using a non-linear regression procedure. Growth rates (R) were calculated as $R = k(L_\infty - L_t)$. Growth curves were considered to be significantly different if their 95 % confidence intervals did not overlap (JAMES 1991; WAPSTRA et al. 2001).

RESULTS

A growth zone and thin hematoxylinophilic outermost line corresponding to a winter line of arrested growth were present in the phalangeal cross sections of all 26 individuals (Fig. 1). In several cases, the first (innermost) LAG was not completely decomposed by endosteal resorption or the resorption zone did not even reach the first LAG. In all cross-sections, the extension of the resorption zone was clearly restricted to endosteal bone and never a difficulty for age determination. Double lines and endosteal resorption were observed in 17 (65.4 %) and 4 (15.4 %) individuals, respectively. The oldest females were 10, the oldest males 13 years old (Fig. 2). The age upon arrival at maturity was three years

(i.e., after their third hibernation) for both sexes in all 26 specimens.

The means of the adult SVL, age and growth rate values were, 109.37 ± 5.27 mm; 8.77 ± 1.6 yrs; 0.07 ± 0.1 mm/yr for all individuals of *L. trilineata* (110.12 ± 6.09 mm; 9.07 ± 1.6 yrs; 0.037 ± 0.05 mm/yr in the male and 108.51 ± 4.21 mm; 8.42 ± 1.37 yrs; 0.32 ± 0.34 mm/yr in the female specimens) (Table 1).

Age ranged from 7-13 years in males and 6-10 years in females. The mean age of the specimens was not significantly different between the sexes (Independent Samples T-Test; $t = 1.020$, $df = 24$, $P = 0.318$). Intersexual differences in body size (SVL) were slightly male-biased ($SSD = 0.014$).

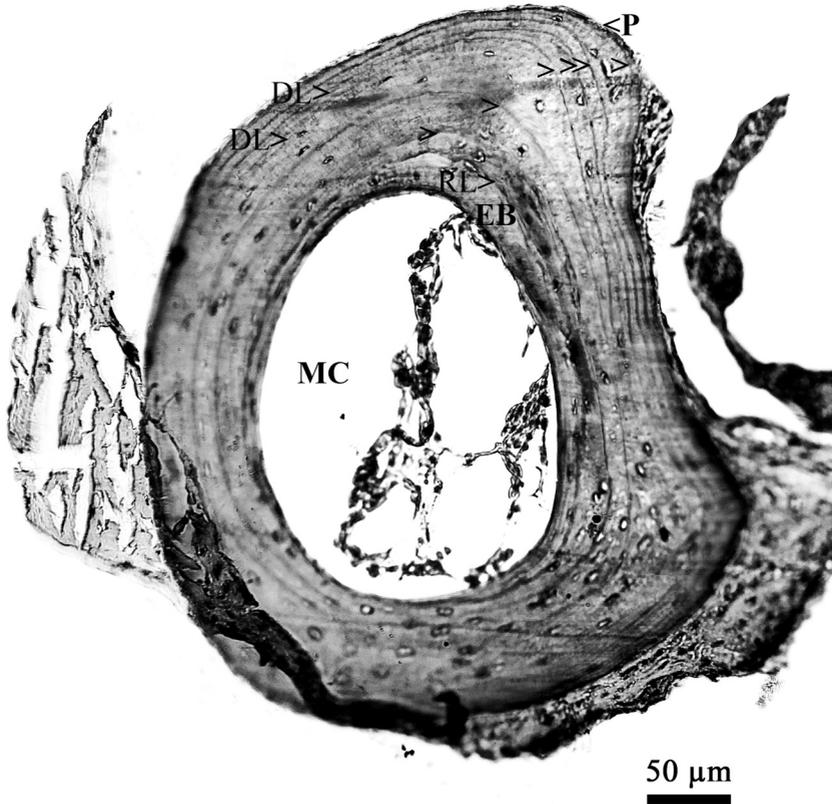


Fig. 1: Cross section (10 μm) of a toe bone of a six-year-old female (117.23 mm SVL) of *Lacerta trilineata* BEDRIAGA, 1886 from the Sergen population in Turkey. The age was derived from the presence of six lines of arrested growth (marked with six > symbols in the upper portion of the picture) surrounding the resorption line (RL). MC – marrow cavity; EB – endosteal bone; RL – resorption line; DL – double line; P – periphery.

Abb. 1: Querschnitt (10 μm) eines Zehenknochens eines sechs Jahre alten Weibchens (117.23 mm Kopf-Rumpflänge) von *Lacerta trilineata* BEDRIAGA, 1886 aus der Population von Sergen (Türkei). Das Alter wurde aus der Anzahl von sechs Linien verlangsamten Wachstums (markiert durch sechs > Symbole im rechten oberen Bildbereich) außerhalb der Resorptionslinie (RL) abgeleitet. MC – Markhöhle; EB – endostaler Knochen; RL – Resorptionslinie, DL – Doppellinie, P – Peripherie.

The mean SVL was not significantly different between sexes ($t = 0.771$, $df = 24$, $P = 0.448$). The correlation between SVL and age was weak for both males (Spearman's rank correlation: $r = 0.318$, $P = 0.268$) and females ($r = -0.675$, $P = 0.016$). The growth curves estimated by von Bertalanffy's equation are shown in Fig. 3. For both sexes, the estimated asymptotic SVL based on $L_{t_0} =$

27.0 mm was slightly shorter than the maximum SVL recorded ($SVL_{\text{asym}} \pm \text{CI}$, males: 108.40 ± 25.68 mm; females: 109.21 ± 44.21 mm). The growth coefficient was higher in males than females ($k \pm \text{CI}$, males: 0.96 ± 0.22 ; females: 0.75 ± 0.41). There was no significant difference in growth rate between the sexes (Independent Samples T-Test; $t = -1.85$, $df = 8$, $P = 0.100$).

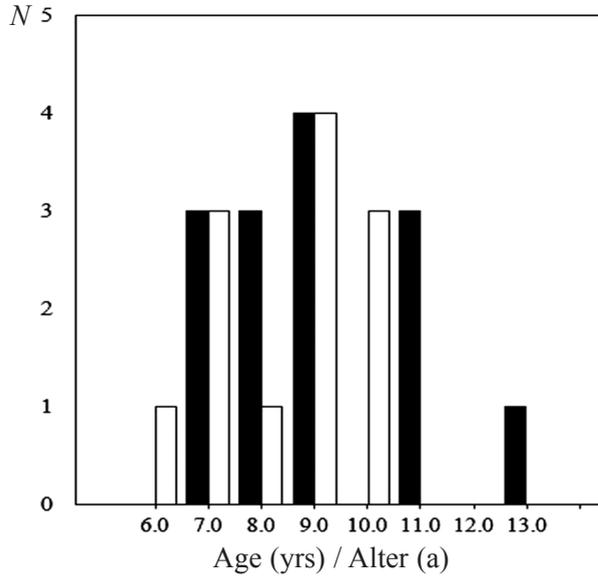


Fig. 2: Frequency distribution of the age in 14 male (black) and 12 female (white) *Lacerta trilineata* BEDRIAGA, 1886, from the Sergen population (Turkey). *N* – Number of individuals.

Abb. 2: Häufigkeitsverteilung des Alters bei 14 Männchen (schwarz) und 12 Weibchen (weiß) von *Lacerta trilineata* BEDRIAGA, 1886 in der Population von Sergen (Türkei). *N* – Anzahl der Individuen.

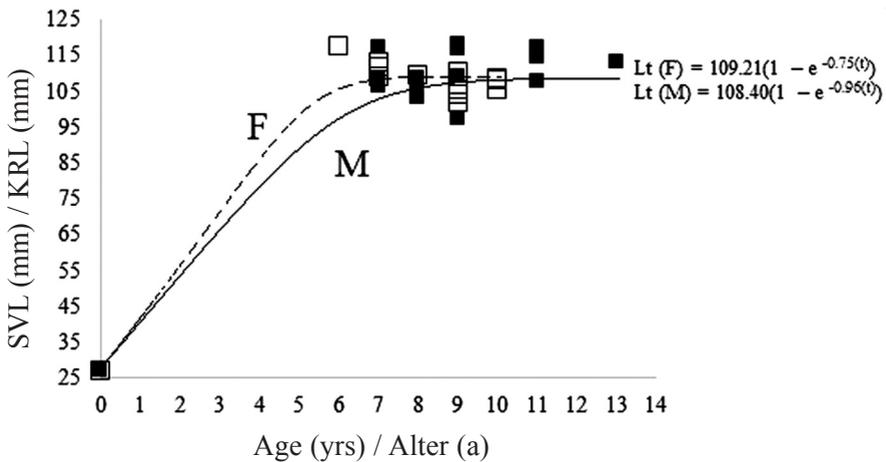


Fig. 3: Von Bertalanffy growth curves for females (open square, dashed F-line) and males (solid square, solid M-line) of *Lacerta trilineata* BEDRIAGA, 1886. For the mean SVL of the lizards at hatching 27.0 mm was taken according to JAMES & SHINE (1988).

Abb. 3: Von Bertalanffy Wachstumskurven für Weibchen (offenes Quadrat, gestrichelte F-Linie) und Männchen (gefülltes Quadrat, durchgezogene M-Linie) von *Lacerta trilineata* BEDRIAGA, 1886. Die mittlere Kopf-Rumpflänge (KRL) der Eidechsen beim Schlupf wurde nach JAMES & SHINE (1988) mit 27,0 mm angenommen.

Table 1. Descriptive statistics of snout-vent-length [SVL, mm], age [yrs] and annual growth rate [R, mm/yr] of the Sergen population of *Lacerta trilineata* BEDRIAGA, 1886. N – sample size, SE – standard error of the mean.

1) The value of N in growth rate (R) denotes the number of age classes (males: 7, 8, 9, 11 and 13 years; females: 6, 7, 8, 9 and 10 years).
 Tab. 1: Deskriptive Statistiken von Kopf-Rumpflänge [SVL, mm] Alter [a] und jährlicher Wachstumsrate [R, mm/a] von *Lacerta trilineata* BEDRIAGA, 1886 aus der Population von Sergen (Türkei). N – Stichprobenumfang, SE – Standardfehler des Mittelwertes.
 1) Bei der Wachstumsrate (R) bezeichnet der Wert von N die Zahl der Altersklassen (7, 8, 9, 11 und 13 Jahre bei Männchen; 6, 7, 8, 9 und 10 Jahre bei Weibchen).

Parameter	Males		Females		Males + Females	
	SVL	Age / Alter	SVL	Age / Alter	SVL	Age / Alter
N ¹⁾	14	14	12	12	26	7
Mean / Mittelwert	110.1236	9.07	108.5117	8.42	109.3796	8.77
Range / Spannweite	97.35-117.88	7-13	101.48-117.23	6-10	97.35-117.88	6-13
SE	1.62785	0.486	1.21720	0.398	1.03377	0.320
		R	R	R	R	R
		5	5	5	5	7
		0.037	0.328	0.328	0.328	0.328
		0.0003-0.12	0.043-0.891	0.043-0.891	0.0003-0.89	0.0003-0.89
		0.022	0.154	0.154	0.154	0.154

DISCUSSION

Estimating age and growth parameters of *L. trilineata* in individuals from Sergen (NW Turkey), the authors found the oldest age observed (females 10, males 13 years) to exceed the maximum values reported by ÜSTEL (2010; four and five years) and KALAYCI et al. (2018; seven and five years).

Significant differences in longevity and age upon arrival at sexual maturity are species-specific and influenced by environmental factors. GUARINO et al. (2010) found life history characteristics such as maximum age, age at sexual maturity, longevity and growth rates to vary widely between the subpopulations of one and the same species. In the study of ÜSTEL (2010), the *L. trilineata* specimens came from nineteen different populations (nine from Gallipoli Peninsula and 10 from Biga Peninsula) and the number of specimens per population (1 – 4) was low. Moreover, the number of juveniles was higher than the number of adult specimens in his study, which may be an explanation for shorter longevities than found in the Sergen population. Small sample size and the large proportion of juveniles may be the reason for the young maximum age of five years found by KALAYCI et al. (2018) in the population from Edirne (17 m a.s.l.), which is about 100 km from Sergen. Short longevity was also reported for other lacertids. For example, GUARINO et al. (2010) found three to four years in a high Alpine population of another green lizard, *Lacerta agilis* LINNAEUS, 1758, from Italy. Their results were below those reported by ROITBERG & SMIRINA (2006) for populations of the same species in the Republic of Dagestan in the Russian Federation. These authors determined maximum longevities of six years for males and five for females in populations from lowland and submontane regions (up to 600 m. a.s.l.) and seven years for males and six for females in high altitude populations (above 960 m. a.s.l.). However, OLSSON & SHINE (1996) reported higher longevity (11 years for males and 12 for females) in *L. agilis* from Sweden. As a general rule, individuals from northern latitudes and high altitudes live longer than individuals who live in southern latitudes and low altitudes (WAPSTRA et al. 2001;

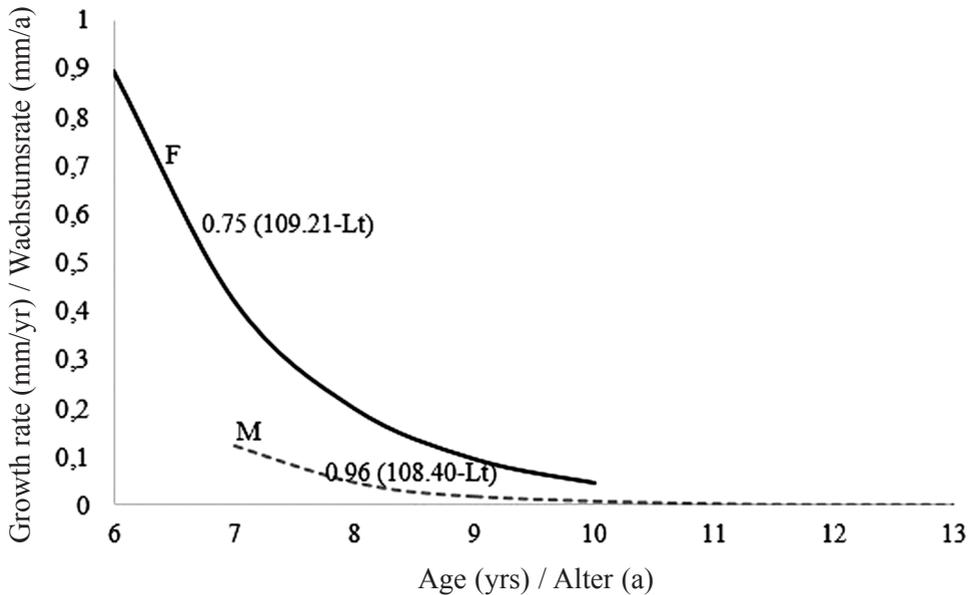


Fig. 4: Male (M) and female (F) annual growth rates (mm/yr) of *Lacerta trilineata* BEDRIAGA, 1886, from the Sergen population (Turkey).

Abb. 4: Die Wachstumsraten (mm/a) bei Männchen (M) und Weibchen (F) von *Lacerta trilineata* BEDRIAGA, 1886 der Population von Sergen (Türkei).

ROITBERG & SMIRINA 2006; GUARINO et al. 2010; SEARS & ANGILLETTA 2004). This was shown in the study by KALAYCI et al. (2018) who found seven year-old individuals in Turkish *L. trilineata* from Bolu (1,250 m a.s.l.). Other longevity studies on lizards of the genus *Lacerta* reported five to six years for *Lacerta bilineata* DAUDIN, 1802 (SAINT GIRONS et al. 1989) and five years for *Lacerta strigata* EICHWALD, 1831 (ROITBERG & SMIRINA 2006), which is also clearly below the present maximum age results for *L. trilineata*.

In the present study, the mean age was determined as 9.07 years in males and 8.42 in females of *L. trilineata*. KALAYCI et al. (2018) reported the much lower values of 4.60 and 4.86 years for the males and 4.00 and 5.44 years for the females of Edirne and Bolu specimens, respectively. In the study by GUARINO et al. (2010), the mean age was found as 2.4 years in males and 2.5 years in females for *L. agilis*. ROITBERG & SMIRINA (2006) reported that most individuals of *L.*

strigata were two to three years old. As observed by GUARINO et al. (2010) for *L. agilis* and most likely by KALAYCI et al. (2018) for *L. trilineata*, there was no significant difference in mean age of the sexes in the *L. trilineata* sample of the present study.

In the specimens of the Sergen population, the age when maturity was achieved was three years (i.e., after their third hibernation) in both males and females of *L. trilineata*, which is in accordance with KALAYCI et al. (2018) who found immatures to be one or two and the youngest matures three years old. ROITBERG & SMIRINA (2006) reported that males of *L. agilis* attain maturity after the second, females after the third year of life. An age of maturity at 22 to 23 months was previously reported for *L. agilis* and *L. strigata* (MUSKHELISHVILI 1970; KHONYAKINA 1970, 1972; DAREVSKIJ 1984; TERTYSHNIKOV 2002), except by SHAMMAKOV (1981) who demonstrated the reproduction of yearlings in *L. strigata* from south-western Turkmenistan. These results

indicate that the age upon arrival at sexual maturity may vary among species, as well as their sub-populations according to latitude and altitude of their geographical range area.

In the Sergen sample, the correlation between age and SVL was weak for both females and males ($r = -0.675$ and 0.318 , respectively), whereas KALAYCI et al. (2018) found significant positive correlations ($r = 0.94$ and 0.8) in their Bolu and Edirne samples. Also, GUARINO et al. (2010) reported a statistically significant positive relationship between age and SVL in *L. agilis*. BAUWENS (1999) pointed to inconsistencies between increase in body size and age which may explain the above differences.

Although the mean SVL was found slightly higher in males (110.12 mm) than females (108.51 mm) in the Sergen sample, this difference was not significantly different between sexes. Similarly, KALAYCI et al. (2018) observed mean SVLs of 109.57 mm and 102.5 mm in the males and 108.49 mm and 98.33 mm in the females from Edirne and Bolu respectively. Comparative data from Greece by MEIRI (2007) indicated higher mean SVL values in an island (Spetsai) and a mainland (Peloponesus) population of *L. trilineata* (129.7 mm and 155.2 mm for males, 122.8 mm and 117.0 mm for females, respectively).

Sexual size dimorphism (SSD) is an important feature in the lacertid family (ARRIBAS 1996). That the success of large males over small ones in combats favors the evolution of large males may be a prevalent reason for male-biased SSD (ANDERSON & VITT 1990; COX et al. 2003). Most species of the Lacertidae have a male-biased sexual size dimorphism (KALIONTZOPOULOU et al. 2007). Although a clear male biased sexual size dimorphism is reported for *L. trilineata* in the literature (HERREL et al. 1996; SCHARF & MEIRI 2013), it was insignificantly expressed (SSD = 0.014) in the sample from Sergen, which is in accordance with KALAYCI et al. (2018) who reported insignificantly longer SVLs in males than females. ROITBERG & SMIRINA (2006) stated that the inhibitory effect of reproduction on growth is stronger in females than males. Also, the smaller body size of females could be the effect of

their earlier maturation as compared to males, and the male bias in SSD appears more pronounced in low-altitude populations (ROITBERG 2007).

ADOLPH & PORTER (1996) suggested that the phase of slower body growth in females (associated with the first reproduction) relative to males is shifted to younger ages in the lowland populations, because the warmer climate accelerates growth and maturation. Accordingly, the growth rate may be related to the age when sexual maturity is attained. Equal age at maturity (three years) of males and females might explain similar growth rates in both sexes of the Sergen population. As reported in various studies, sexual size dimorphism in many adult lizards arises from sexual differences in the growth rates (JOHN-ALDER & COX 2007; TOMAŠEVIĆ-KOLAROV et al. 2010). Since any bias in SSD was not significant, growth rates were not different between the sexes of *L. trilineata* from Sergen.

SMIRINA (1972) suggested that endosteal resorption may be affected by environmental conditions so that elevation may influence endosteal resorption. Although ESTEBAN et al. (1999) found that high-altitude populations of *Pelophylax saharicus* (BOULENGER, 1913), exhibited less resorption than lowland populations, CAETANO & CASTANET (1993) observed less resorption in low-altitude populations. Similar to the latter findings, *L. trilineata* from the rather low altitude site at Sergen showed a low endosteal resorption rate (15.4 %).

Unpredictable environmental events of ecological relevance (e.g., abnormal drought, humidity or changes in food availability) may cause unexpected double lines (JAKOB et al. 2002; GUARINO & ERIŞİMİŞ 2008; ÖZDEMİR et al. 2012). Although the Sergen population studied lives under stable and moderate climate conditions and can utilize ample food resources, a high rate (65.4 %) of double line formation was observed. However, EROĞLU et al. (2017) reported 32.5 % of individuals with double lines in *Podarcis tauricus* (PALLAS, 1814) from the Sergen population. It is not known what factor caused the formation of double lines in the *L. trilineata* individuals of the Sergen population.

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