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On the age structure of two samples of *Lacerta trilineata* BEDRIAGA, 1886, from different altitudes in Turkey

(Squamata: Sauria: Lacertidae)

Zur Altersstruktur zweier Stichproben von *Lacerta trilineata* BEDRIAGA, 1886 aus unterschiedlichen Höhenlagen der Türkei

(Squamata: Sauria: Lacertidae)

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KURZFASSUNG

Die Autoren untersuchten die Altersstruktur zweier Stichproben (Edirne: 17 m a.s.l. und Bolu: 1,250 m a.s.l.) von *Lacerta trilineata* BEDRIAGA, 1886 in der Türkei. Die Altersbestimmung mittels Skeletochronologie ergab Höchstalter von etwa sieben Jahren in der Stichprobe von Bolu und fünf in der von Edirne. Die Überlebensrate bei Eintritt in die Geschlechtsreife betrug 0,59 % bei den Männchen und 0,24 % bei den Weibchen von Bolu. Die entsprechende Lebenserwartung belief sich in dieser Stichprobe auf 2,94 Jahre für die Männchen und 1,82 für die Weibchen. Der Eintritt in die Geschlechtsreife erfolgte in beiden Stichproben im Alter von zwei Jahren. Obwohl das mittlere Alter in der Stichprobe der Hochlandpopulation höher war als in der des Tieflandes, war die mittlere Kopf-Rumpf-Länge in letzterer größer.

ABSTRACT

The authors studied the age composition in two samples (Edirne: 17 m a.s.l. and Bolu: 1,250 m a.s.l.) of *Lacerta trilineata* BEDRIAGA, 1886, in Turkey. Aging by skeletochronology showed that the maximum age of the lizards was about seven years in the Bolu and five in the Edirne sample. The adult survival rate was 0.59 % in males and 0.24 % in females of Bolu. The adult life expectancy was calculated as 2.94 years for males and 1.82 for females in the Bolu sample. Age at sexual maturity was two years in both samples. Although the mean age of the high altitude sample was older than of the low altitude sample, the mean snout-vent-length of the latter was longer.

KEY WORDS

Reptilia: Squamata: Sauria: Lacertidae; *Lacerta trilineata*, population ecology, age structure, survival rate, life expectancy, maximum age, skeletochronology, Turkey

INTRODUCTION

The Balkan Green Lizard, *Lacerta trilineata* BEDRIAGA, 1886, is distributed from coastal Croatia, Bosnia-Herzegovina, Serbia, Montenegro, east Bulgaria, southeastern Romania, Albania, Macedonia and Greece, including the Ionian and several Aegean Islands such as Crete, Lesvos and Rhodes, to western and central Turkey. This large, bright green and thus easily observed (VALAKOS et al. 2008) species prefers dry habitats with Mediterranean climate (BÖHME et al. 2009). In Turkey, it is found in the regions of Thrace, Marmara, the Aegean, western Mediterranean and western Black Sea (BARAN et al. 2012).

Reptilian life history (e.g., age structure of populations and longevity) is influenced by biotic (e.g., inter- and intraspecific competition, predation pressure) and abiotic factors (e.g., food availability, nutrients, temperature, photoperiod and availability of shelters) (ANGILLETTA 2009). Counting the dark circular lines of arrested growth (LAGs) in sections of long bones under a

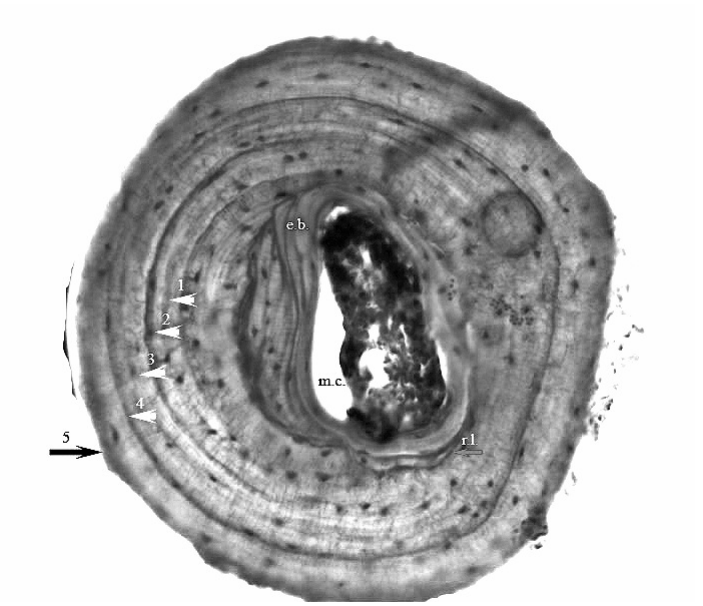

Fig. 1: Cross-section (17 µm thick) at the diaphysis level of a terminal phalanx bone of the fourth toe of *Lacerta trilineata* BEDRIAGA, 1882, from Bolu showing five Lines of Arrested Growth highlighted by arrows. m.c. - marrow cavity, e.b. - endosteal bone, r.l. - resorption line.

Abb. 1: Der Querschnitt (17 µm stark) im Diaphysenbereich einer Endphalange einer vierten Zehe von *Lacerta trilineata* BEDRIAGA, 1882 aus Bolu zeigt fünf Linien verminderten Wachstums (durch Pfeile gekennzeichnet). m.c. - Markhöhle, e.b. - endostaler Knochen, r.l. - Resorptionslinie.

light microscope is a common method of aging ectothermic animals (WASKOWA & MATEUS 2017).

Knowledge of life history traits in *L. trilineata* is largely based on a publication by NETTMANN & RYKENA (1984) and an unpublished master thesis (ÜSTEL 2010). Due to lack of appropriate published information from Turkish populations, the authors conducted skeletochronological studies on native *L. trilineata* samples from two localities at different altitudes from Edirne (Thrace) and Bolu (western Black Sea Region).

The present paper reports first data on the age structure, longevity, adult survival rate and adult life expectancy of low (Edirne) and high (Bolu) altitude populations of *L. trilineata* from Turkey.

MATERIALS AND METHODS

Sampling.- Twenty eight *L. trilineata* specimens stored in the Herpetological Collection of the Section of Zoology, Department of Biology, Çanakkale Onsekiz Mart University, Turkey (COMU-ZM) were studied. The collection numbers are specified in BAYCAN & TOSUNOĞLU (2017). The materials included 2 females, 5 males and 5 immatures from Edirne (17 m a.s.l., 40.411725° N, 26.053262° E) and 9 females and 7 males from Bolu (1,250 m a.s.l., 40.410359° N, 32.024764° E).

Each individual's snout-vent length (SVL), head width (HW) and head length (HL) were measured to the nearest 0.01 mm with a digital caliper (Mitutoyo Corp.,

Table 1: Age composition in Turkish samples of *Lacerta trilineata* BEDRIAGA, 1886, originating from two samples (Edirne and Bolu) including descriptive statistics of the morphometric characters studied. SD - Standard deviation.

Tab. 1: Alterszusammensetzung in türkischen Stichproben von *Lacerta trilineata* BEDRIAGA, 1886 aus zwei Populationen (Edirne und Bolu) mit beschreibenden Statistiken morphometrischer Merkmale. Males - Männchen, Females - Weibchen, Immatures - Jungtiere, SVL - Kopf-Rumpf-Länge, SD - Standardabweichung.

Localitiy Fundort	Sex (total number of individuals) Geschlecht (Gesamtzahl der Individuen)	Number of individuals (age in years) Anzahl der Individuen (Alter in Jahren)	Mean SVL ± SD (mm) Mittlere SVL ± SD (mm)	Range SVL (mm) Spannweite der SVL (mm)	Mean Head Width ± SD (mm) Mittlere Kopfbreite ± SD (mm)	Range Head Width (mm) Spannweite der Kopfbreite (mm)	Mean Head Length ± SD (mm) Mittlere Kopflänge ± SD (mm)	Range Head Length (mm) Spannweite der Kopflänge (mm)
Edirne	Males (5)		109.57±22.35	84.64-133.88	13.21±3.13	10.05-16.53	25.62±5.21	20.54-31.24
17 m a.s.l.		2 (4)	86.75±2.98	84.64-88.85				
		3 (5)	124.79±11.25	112.21-133.88				
	Females (2)		108.49±32.94	85.2-131.78	13.13±4.83	9.72-16.55	24.26±7.78	18.76-29.76
		1 (3)	85.2	85.2				
		1 (4)	131.78	131.78				
	Immatures (5)		46.79±18.13	35.09-78.74	6.13±1.64	4.82-8.94	12.35±3.11	10.13-17.78
		2 (1)	35.78±0.98	35.09-36.47				
		3 (2)	54.14±21.32	41.14-78.74				
Bolu	Males (7)		102.50±10.54	89.53-118.00	12.38±1.22	10.92-14.17	23.54±1.60	21.80-25.83
1,250 m a.s.l.		3 (4)	92.77±4.21	89.53-97.53				
		2 (5)	110.04±11.26	102.07-118.00				
		2 (6)	109.56±2.74	107.62-111.5				
	Females (9)		98.33±15.80	61.63-114.97	11.99±1.05	10.55-13.55	21.56±0.87	20.27-22.62
		1 (3)	61.63	61.63				
		2 (4)	91.80±4.30	88.76-94.84				
		1 (5)	107.16	107.16				
		2 (6)	106.99±4.26	103.97-110.00				
		3 (7)	106.20±7.60	101.65-114.97				

Kawasaki, Japan); sex determination and assignment to immatures or adults were based on the examination of the gonad condition. The longest (fourth) digit of a hind limb was taken and transferred to 96 % ethanol for skeletochronological analysis.

Age determination.- Standard skeletochronological procedures (CASTANET & SMIRNA 1990) were applied to determine the age of the individuals. Phalangeal bones were decalcified in 5 % nitric acid for 1.5 h and then washed in tap water. Cross-sections of the diaphyseal part of the terminal phalanges, about 17 µm wide, were obtained using a freezing microtome (Shandon, Thermo) and stained in Ehrlich's hematoxylin (Fig. 1). The sections with the smallest medullar cavity were selected and mounted in aqueous synthetic resin (Aqua-mount, ADR).

On each section, the number of Lines of Arrested Growth (LAGs) was assessed by two observers (T. Ergül Kalaycı and N. Özdemir) independently; there was full agreement among the observers regarding their numbers in all samples. An obvious decrease in space between two subsequent LAGs (not clearly expressed in Fig. 1) was taken to mark the age when sexual maturity was achieved (RYSER 1988).

The annual adult survival rate (Sr, %) was calculated using the formula introduced by ROBSON & CHAPMAN (1961): $Sr = (T/R) + T - 1$, where $T = N_1 + 2N_2 + 3N_3 + \ldots$, $R = \Sigma N_i$ and N_i = the number of individuals in the age group *i*. The adult life expectancy (ESP, yrs), i.e., the expected life span of lizards that have attained maturity, was determined according to SEBER's (1973) formula: $ESP = 0.5 + (1/(1 - Sr))$.

Statistical analysis.- Due to small sample size, normality was tested using the Shapiro-Wilk test. SVL and age were normally distributed ($P > 0.05$). Because of inadequate size of the Edirne sample, differentiation between sexes in terms of morphological measurements and age was restricted to the Bolu population. The Independent Samples t-test was applied to compute statistical differences between sexes in Bolu. Pearson's correlation coefficients (r) were calculated to show relationships between the variables. The statistical analyses were done using SPSS 21 (IBM SPSS Statistics for Windows).

RESULTS

The maximum age found was five years in the Edirne and seven in the Bolu sample. The average age of males was 4.60 ± 0.55 (standard deviation) and 4.86 ± 0.90 years for Edirne and Bolu, respectively, and 4.00 ± 1.41 and 5.44 ± 1.51 years for females of Edirne and Bolu, respectively. The age varied from three to five years in the Edirne and from three to seven years in the Bolu sample (Table 1, Fig. 2). The age of immatures from Edirne was one to two years. In the Bolu sample, the adult survival rates of males and females were 0.59 % and 0.24 %, respectively, adult life expectancy was 2.94 and 1.82 years for males and females, respectively. Age at sexual maturity was two years for both samples.

The mean body size (SVL) was 109.57 ± 22.35 (standard deviation) mm and 102.50 ± 10.54 mm in males and 108.49 ± 32.94 mm and 98.33 ± 15.80 mm in females of the Edirne and Bolu samples, respectively (Table 1).

Descriptive statistics of head length and head width of males, females and juveniles are given in Table 1. Snout-vent-length and head width were not differentiated between sexes of the Bolu sample (Student test; SVL: $t = -0.60$, $df = 14$; HW: $t = -0.69$, $df = 14$, $P > 0.05$), whereas, head length was (Student t-test - $t = -2.95$, $df = 8.76$, $P < 0.05$).

In the Bolu sample, body size and age were significantly and positively correlated (males: $r = 0.80$, females: $r = 0.93$, $P < 0.05$). The positive correlations between the parameters SVL and HL, SVL and HW and HL and HW were significant only in the males of this population ($r_{SVL\text{-}HL} = 0.92$, $r_{SVL\text{-}HW} = 0.82$, $r_{HL\text{-}HW} = 0.85$, $P < 0.01$). In the combined sample of the Edirne population, the parameter pairs SVL-age, SVL-HL, SVL-HW and HL-HW were significantly correlated ($r_{SVL\text{-}age} = 0.94$, $r_{SVL\text{-}HL} = 0.99$, $r_{SVL\text{-}HW} = 0.99$, $r_{HL\text{-}HW} = 0.99$, $P < 0.01$).

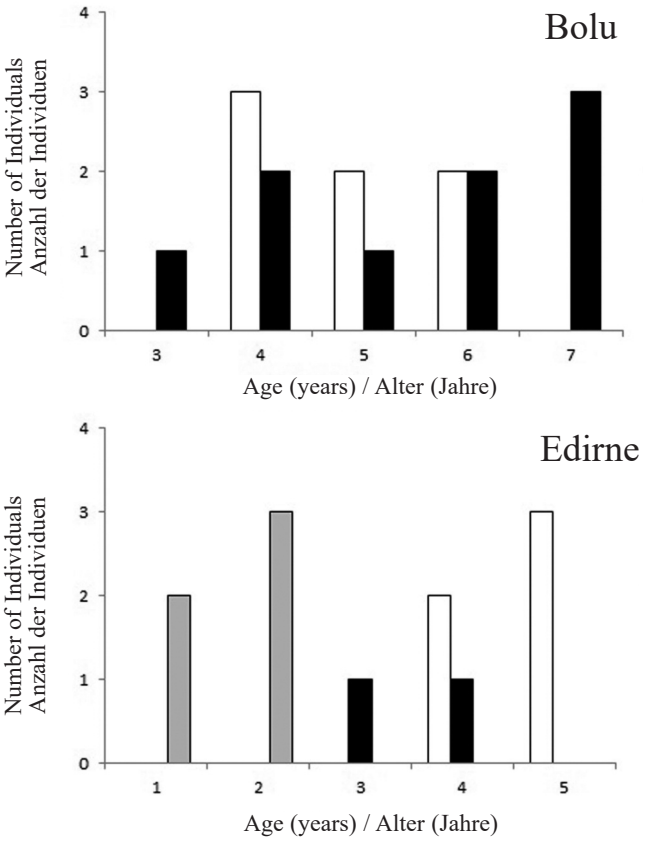

Fig. 2 : Frequency distribution of age classes in the samples of *Lacerta trilineata* BEDRIAGA, 1882, from Bolu and Edirne. White bars - males, black bars - females, gray bars - immatures.

Abb. 2: Häufigkeitsverteilung der Altersklassen in den Stichproben von *Lacerta trilineata* BEDRIAGA, 1882 aus Bolu and Edirne. Weiße Balken - Männchen, schwarze Balken - Weibchen, graue Balken - Jungtiere.

DISCUSSION

Even though *L. trilineata* shows a wide distribution across southeastern Europe, there is only little information about the age structure of the species. According to NETTMANN & RYKENA (1984) hibernation is important in the life cycle of *L. trilineata*, especially in mountain populations exposed to cold winter temperatures. These authors reported the longevity of captive *L. trilineata* to be 10-20 years. BULAKHOVA et al. (2007) found a maximum age of eight years for females and six years for males in the related green lizard *Lacerta agilis* LINNAEUS, 1758, from West Siberia, where sexually immature individuals were one to two years old. On the contrary, GUARINO et. al. (2010) detected comparatively short life spans of two to four years for *L. agilis* from the western Italian Alps but could not explain this result. KIDOV et al. (2014) found the life span of *L. agilis* from North Ossetia to last eight years for both males and females. The maximum age found for *L. trilineata* in the present study was five

years in the low altitude population of Edirne and seven in the mountain population of Bolu. Similar to the above result in the low altitude sample, Üstel (2010) found maximum ages of four and five years in *L. trilineata* lowland populations from Gelibolu (Çanakkale) and Biga (Çanakkale), respectively.

Bergmann's Rule implies the hypothesis that individuals of highland populations mature later and live longer than their lowland conspecifics. The present study supports this prediction in that the mean age was older in individuals from Bolu (1,250 m a.s.l.) than Edirne (17 m a.s.l.). However, mean SVL of adult individuals of Edirne was bigger than of Bolu specimens, which is not in line with Bergmann's Rule. Lu et al. (2017) hypothesized that reduced oxygen concentration could lead to smaller adult body size in high altitude environments.

Nettmann & Rykena (1984) summarized that *L. trilineata* become sexually mature in their second spring, which is in full agreement with the results gained from the studied populations.

Adult survival rate and adult life expectancy vary from species to species, and differ within a given species among sexes and depending on the environment inhabited. As for other Turkish lacertid species, adult survival rate and adult life expectancy were 0.51 % and 2.54 years, respectively, for female individuals of *Podarcis tauricus ionicus* (Lehrs, 1902) from Saros Bay, Turkey (Altunışık et al. 2016). In *Acanthodactylus boskianus* (Daudin, 1802) from Birecik/Şanlıurfa the corresponding values were 0.62 % and 3.13 years for males and 0.56 % and 2.77 years for females, respectively (Üzüm et al. 2014). The present study found male and female survival rates of 0.59 % and 0.24 %, respectively, in the Bolu population with male and female life expectancies of 2.94 and 1.82 years. Remarkable are the comparatively low values for females.

A clear sexual size dimorphism is reported for *L. trilineata* with males being larger than females (Herrel et al. 1996; Scharf & Meiri 2013). In the Bolu sample, the mean SVL was insignificantly longer in males than females.

Lizard head size is commonly bigger in males than females due to its role in intra and inter-sexual interactions (Anderson & Vitt 1990; Bull & Pamula 1996; Herrel et al. 1996). Head width did, however, not differ significantly between the sexes of the Bolu population, whereas, significant differences between sexes were found in terms of head length.

Age and body size are generally positively correlated in reptiles which can be used to estimate the age of an animal from its size (Szafrańska 1978; Gvozdik 2000; Castoe 2002). Corroborating this common knowledge, a significant positive correlation between age and size was observed both in the samples of Edirne and Bolu.

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