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Age structure and growth in a Turkish population of the Crimean Wall Lizard, *Podarcis tauricus* (PALLAS, 1814)
(Squamata: Sauria: Lacertidae)

Altersstruktur und Wachstum in einer türkischen Population der Taurischen Eidechse, *Podarcis tauricus* (PALLAS, 1814) (Squamata: Sauria: Lacertidae)

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KURZFASSUNG

Wachstums- und Entwicklungsmerkmale von *Podarcis tauricus* (PALLAS, 1814) wurden mit skelettochronologischen Arbeitsmethoden untersucht. Das Alter in der adulten Stichprobe (20 Männchen, 20 Weibchen aus Sergen,Westtürkei) betrug für beide Geschlechter gemeinsam betrachtet 4-10 ($\bar{x}$ = 6,68), für Männchen 5-10 ($\bar{x}$ = 7,2) und Weibchen 4-10 ($\bar{x}$ = 6,15) Jahre und differierte zwischen den Geschlechtern signifikant. Aus der Anordnung der Linien verlangsamten Wachstums (LAGs) ergab sich ein Eintrittsalter der Geschlechtsreife von 2 bis 3 Jahren. Die Kopf-Rumpflänge der Echsen war positiv mit der Anzahl der LAGs in den Zehenknochen korreliert. Der geschlechtsbedingte Größendimorphismus zugunsten der Männchen war gering (SDI = -0.005), der Wachstumskoeffizient (k) bei Männchen kleiner als bei Weibchen (k ± Konfidenzintervall; Männchen: 0.37 ± 0.16; Weibchen: 0.67 ± 0.22). Die Wachstumsraten der Geschlechter unterschieden sich nicht voneinander.

ABSTRACT

Life-history traits of a Turkish population of *Podarcis tauricus* (PALLAS, 1814) were studied using the methods of skeletochronology. In the adult sample (20 males and 20 females from the population of the village of Sergen), the age ranged from 4-10 ($\bar{x}$ = 6.68) years for both sexes collectively, 5-10 ($\bar{x}$ = 7.2) years in males, 4-10 ($\bar{x}$ = 6.15) years in females and differed significantly between sexes. Derived from the LAG (Lines of Arrested Growth) configuration, the age at sexual maturity was 2-3 years in both males and females. There was a positive correlation between the lizards' body size (SVL) and number of LAGs counted in the toe bones. Sexual size dimorphism was weakly expressed by the slightly bigger snout-vent-length of the males (SDI = -0.005). The growth coefficient (k) was lower in males than in females (k ± Confidence Interval; males: 0.37 ± 0.16; females: 0.67 ± 0.22). There was no difference in growth rate between sexes.

KEY WORDS

Reptilia: Squamata: Sauria; Lacertidae; *Podarcis tauricus*; skeletochronology, LAG, Lines of Arrested Growth, von Bertalanffy's growth model, age structure, growth, longevity, body size, population ecology, Turkey

INTRODUCTION

The Crimean Wall Lizard, *Podarcis tauricus* (PALLAS, 1814), is found at altitudes of 0 to 2,350 m a.s.l., from the Balkan Peninsula in the west to the Crimean Peninsula in the east, with its Turkish range area restricted to the extreme northwest of the country (KABISCH 1986; BÖHME et al. 2009). The IUCN Red List of Threatened Animals classifies the species at the LC (Least Concern) category since 2009 (BÖHME et al. 2009).

Skeletochronology is a widely recognized tool for the study of the age structure within reptile populations (JAMES 1991; CASTANET 1994; GUARINO et al. 2010; KIM et al. 2010; ARAKELYAN et al. 2013; KURITA & TODA 2013) and various growth parameters (CASTANET & BAEZ 1988; HALLIDAY & VERRELL 1988; ROITBERG & SMIRINA 2006; KIM et al. 2010). Skeletochronological age estimates in lizards are more accurate and timesaving as compared to age estimates using a mark-recapture approach (SMIRINA & TSELLARIUS 1996). This is why this method was applied here to study life-history traits such as age and size structure, longevity and age at maturation in a Turkish population of *Podarcis tauricus* (PALLAS, 1814).

MATERIALS AND METHODS

Forty adult lizards (20 females, 20 males) from the population at the Village of Sergen (41°42'20" N, 27°43'40" E, 450 m a.s.l., Province of Kırklareli, European Turkey) were collected on May 09 and 10, during the breeding season of 2015. The specimens were caught by hand and sexed by visual examination of secondary sex characters (e.g., presence of dark blue spots on the margins of ventral plates and the more conspicuous orange colored belly in males only). The active period for lizards lasts from early April to early October in the Sergen area; the average air temperature by day was about 20 °C during the sampling period. The habitat consists of rocky ground, sparsely vegetated by thorny plants, which provide refuges. The lizard species *Podarcis tauricus*, *P. muralis* (Laurenti, 1768), *Lacerta trilineata* Bedriaga, 1886 and *Lacerta viridis* (Laurenti, 1768) live in sympatry at the study locality.

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 = (mean SVL of the larger sex / mean SVL of the smaller sex) ± 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 second phalange of the longest (4th) toe was clipped and preserved in 10 % formalin solution for subsequent histological analyses. After toe-clipping, the lizards were released back to their natural habitats. The specimens were treated in accordance with the guidelines of the ethics committee of the Karadeniz Technical University (KTÜ.53488718-651/2014/56).

After peeling the skin from the toes, they were preserved in 10 % formalin for 2.5 hours and thereafter transferred into 5 % nitric acid solution for decalcification of the bone. Later, the toe samples passed a tissue processing system (Leica Tissue Processor TP1020) before they were transferred into a paraffin embedding workstation (Thermo Shandon B64100010). Phalangeal cross-sections (15 µm) cut with a rotary microtome, deparaffinized (at 60-70 °C for an hour, thereafter rinsed with xylene for 7-10 minutes) and stained with haematoxylin were put on microscope slides and closed using Entellan® for observation under a light microscope.

Age was estimated using skeletochronological methods (Castanet & Smirina 1990; Castanet 1994). The numbers of Lines of Arrested Growth (LAGs) visible on the phalangeal bone cross-sections were counted independently by all three authors; the observers' results were harmonized following discussions. In the bone, arrival at sexual maturity was assumed to be indicated by an obvious decrease in spacing between two subsequent LAGs (Ryser 1998; Yilmaz et al. 2005; Özdemir et al. 2012).

Since age classes and body measurements (SVL) were normally distributed (one-sample Kolmogorov-Smirnov test), parametric tests were used for comparison of means (independent sample t-test) and correlations (Spearman's correlations test). All tests were processed with the IBM software package SPSS 21.0 for Windows with the level of significance set at $P < 0.01$.

According to von Bertalanffy's (1938) growth model, the authors computed growth curves as was done in other studies (e. g., James 1991; Wapstra et al. 2001; Roitberg & Smirina 2006; Guarino et al. 2010). The authors used the general formula of the von Bertalanffy equation, $L_t = L_\infty (1 - e^{-k(t-t_0)})$, where L_t is the SVL at the age t, L_∞ the asymptotic maximum SVL, e the base of the natural logarithm, k a growth coefficient and t_0 the age at hatching, which is the starting point of the growth interval described in the present study. Because of lack of data on hatching size in the studied population, 26.7 mm was taken for L_{t0}, which is the mean value for the species provided by In den Bosch & Bout (1998). The parameters L_∞ (asymptotic SVL) and k, and their asymptotic confidence intervals (CI), were estimated using the non-linear regression procedure in SPSS 21.0. The growth rate (R, mm/yr) was calculated as $R = k (L_\infty - L_t)$. Growth curves were considered significantly different if the 95 % confidence intervals did not overlap (James 1991; Wapstra et al. 2001).

Table 1: Descriptive statistics of snout-vent-length [SVL, mm], age [yr] and annual growth rate [R, mm/yr] of the Sergen population of *Podarcis tauricus* (PALLAS, 1814). N – sample size, SE – standard error of the mean).

Tab. 1: Deskriptive Statistiken von Kopf-Rumpflänge [SVL, mm] Alter [a] und jährlicher Wachstumsrate [R, mm/a] von *Podarcis tauricus* (PALLAS, 1814) aus der Population von Sergen (Türkei). N – Stichprobenumfang, SE – Standardfehler des Mittelwertes.

	Males			Females			Males+Females		
	SVL	Age / Alter	R	SVL	Age / Alter	R	SVL	Age / Alter	R
N	20	20	6	20	20	6	40	40	7
Mean / Mittelwert	61.81	7.20	1.78	60.50	6.15	0.96	61.16	6.68	2.48
Range / Spannweite	54.64-68.97	5-10	0.58-3.71	51.70-67.12	4-10	0.05-2.83	51.70-68.97	4-10	0.05-3.71
SE	0.75	0.34	1.18	0.81	0.34	1.05	0.56	0.25	1.70
Median		7			6			6	
Mode / Modalwert		6			5			6	

RESULTS

A series of growth zones, each followed by a thin hematoxylinophilic line corresponding to a winter line of arrested growth were present in the phalangeal cross sections of all 40 (100 %) adult individuals (Fig. 1). The resorption zone did not reach the first (innermost) LAG in all specimens of *P. tauricus*, clearly seemed to be out of endosteal bone in all preparations and never caused difficulties for age determination. Double lines were observed in 13 (32.5 %) specimens. The oldest female and male were 10 years old each (Fig. 2). Age at maturity was not correlated with sex, two years in 32 specimens (17 males, 15 females; 80 %) while it was three years in 8 (3 males, 5 females; 20 %) specimens.

The means of SVL [mm], age [yr] and growth rate [mm/yr] were 61.16 ± 0.56, 6.68 ± 0.25 and 2.48 ± 1.70, respectively for all individuals of *P. tauricus* (61.81 ± 0.75; 7.20 ± 0.34; 1.78 ± 1.18 in males and 60.50 ± 0.81; 6.15 ± 0.34; 0.96 ± 1.05 in females, respectively) (Table 1).

Age was ranged from 5-10 years in the male and 4-10 years in the female sample. The mean age was significantly higher in males (independent samples t-test; t = 2.210, df= 38, P = 0.033). Intersexual difference in body size (snout-vent-length) was slightly male-biased (SDI = -0.005). The mean SVL (t = 1.811, df = 38, P = 0.245), did however not differ significantly between sexes. There was a significant positive correlation between age and SVL for males (Spearman's correlation coefficient r = 0.599, P < 0.01), females (r = 0.499, P < 0.05) and all individuals together (r = 0.576, P < 0.01). Growth pattern estimated by von Bertalanffy's growth equation was in good fit with the data plot of age versus SVL obtained from the individuals of the study sample (Fig. 3). For both sexes, the estimated asymptotic SVL was lower than the maximum SVL recorded (SVL_{asym} ± CI; males: 63.77 ± 10.05 mm; females: 61.16 ± 13.40 mm). Growth coefficient was lower in males than in females (k ± CI, males: 0.37 ± 0.16; females: 0.67 ± 0.22). There was no difference in growth rate between sexes (independent samples t-test; t = 1.300, df = 10, P = 0.223) (Fig. 4).

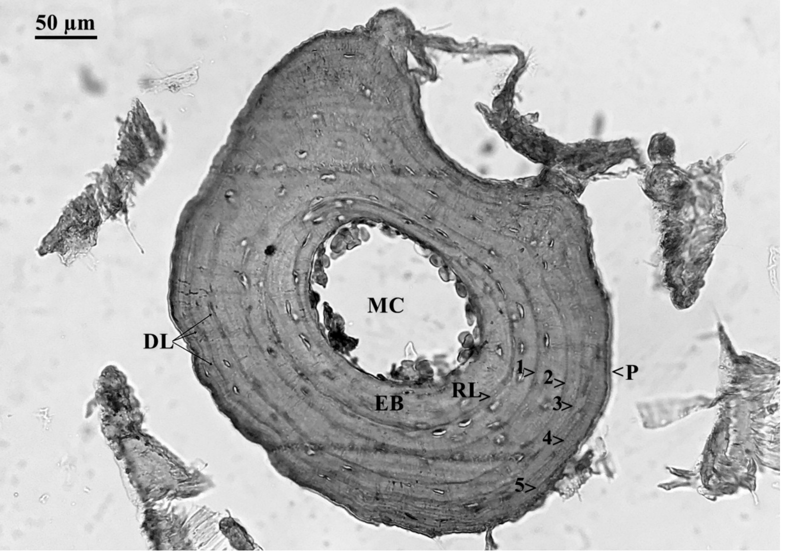

Fig. 1: Cross section (15µm thick) of a toe bone of a five-year-old female (58.98 mm SVL) of *Podarcis tauricus* (Pallas, 1814) from the Sergen population in Turkey. The age was derived from the presence of five Lines of Arrested Growth (LAGs 1-5) surrounding the resorption line. MC – marrow cavity; EB – endosteal bone; RL – resorption line; DL – double line; P – periphery.

Abb. 1: Querschnitt (15 µm dick) eines Zehenknochens eines fünf Jahre alten Weibchens (58,98 mm Kopf-Rumpflänge) von *Podarcis tauricus* (Pallas, 1814) aus der Population von Sergen (Türkei). Das Alter wurde aus der Anzahl von fünf Linien verlangsamten Wachstums (1-5) abgeleitet. MC – Markhöhle; EB – endostaler Knochen; RL – Resorptionslinie, DL – Doppelline, P – Peripherie.

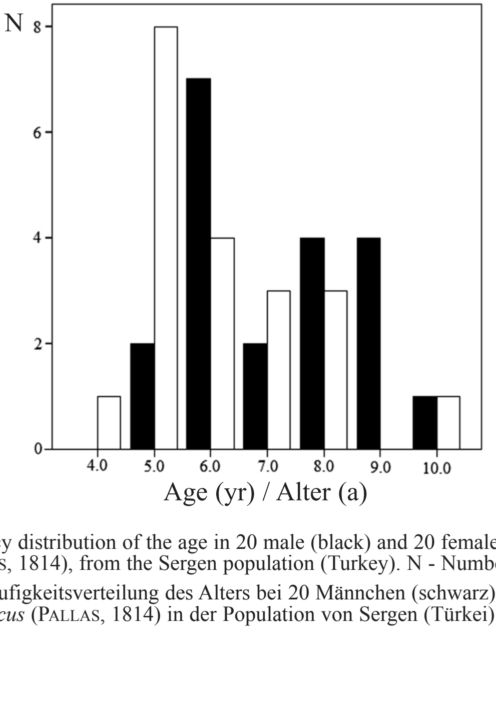

Fig. 2: Frequency distribution of the age in 20 male (black) and 20 female (white) *Podarcis tauricus* (Pallas, 1814), from the Sergen population (Turkey). N - Number of individuals.

Abb. 2: Die Häufigkeitsverteilung des Alters bei 20 Männchen (schwarz) und 20 Weibchen (white) von *Podarcis tauricus* (Pallas, 1814) in der Population von Sergen (Türkei). N - Anzahl der Individuen.

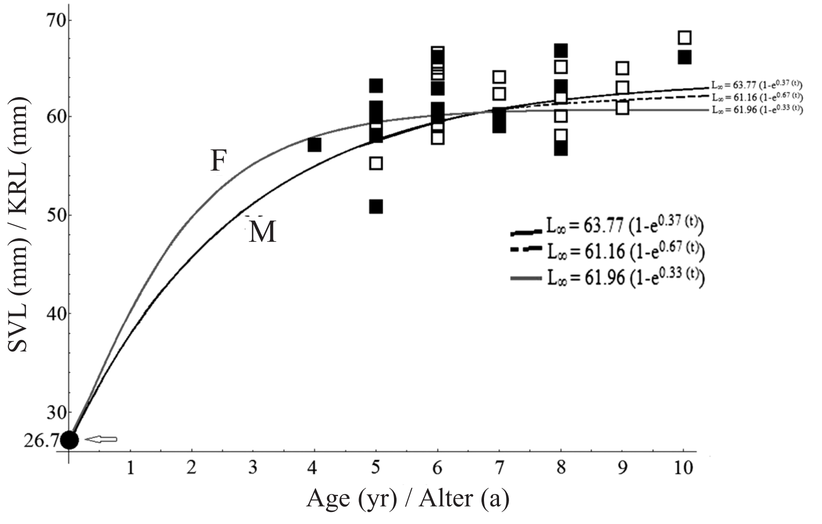

Fig. 3: Von Bertalanffy growth curves for males (open square, solid M-line), females (solid square, solid F-line) and all specimens (dotted line) of *Podarcis tauricus* (PALLAS, 1814). Solid circle and arrow point to the mean SVL of the lizards at hatching (26.7 mm acording to IN DEN BOSCH & BOUT 1998).

Abb. 3: Von Bertalanffy Wachstumskurven für Männchen (offenes Quadrat, durchgezogene M-Linie), Weibchen (gefülltes Quadrat, durchgezogene F- Linie) und beide Geschlechter (Punktlinie) von *Podarcis tauricus* (PALLAS, 1814). Voller Kreis und Pfeil weisen auf die mittlere Kopf-Rumpflänge der Eidechsen beim Schlupf (26,7 mm nach IN DEN BOSCH & BOUT 1998).

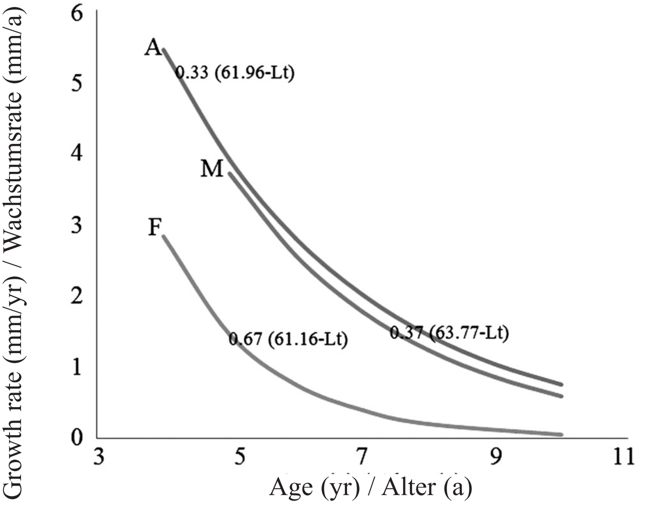

Fig. 4: Male (F), female (F) and adult (A) annual growth rates (mm/yr) of *Podarcis tauricus* (PALLAS, 1814), from the Sergen population (Turkey).

Abb. 4: Die Wachstumsraten (mm/a) von Männchen (M), Weibchen (F) und allen erwachsenen Individuen (A) von *Podarcis tauricus* (PALLAS, 1814) in der Population von Sergen (Türkei).

DISCUSSION

In populations living under harsh ecological conditions (e. g., unusual dry period, lack of food), double lines are found in an increased proportion of specimens compared to populations living under less stressful conditions. For instance, Bülbül et al. (2016) found 54.5-57.8 % in populations of *Darevskia parvula* (Lantz & Cyrén, 1913) and Altunişik et al. (2013) reported 66 % in *Eremias strauchi* Kessler, 1878. The study site at Sergen is characterized by its moderate climate and rich insect food resources, which is in accordance with the observed low percentage (32.5 %) of individuals showing double lines.

Climate conditions as well as daily and annual activity can affect endosteal resorption (Smirina 1972; Hemelaar 1988; Esteban 1990; Leclair 1990, Augert 1992; Esteban et al. 1999). Congruently, endosteal resorption was not observed in the studied population which inhabits a rather low elevation site. A converse trend was reported in *Darevskia* lizard species (Arakelyan et al. 2013; Gül et al. 2014).

An increase in mean age with increasing altitude was reported for populations of various lizard species (Roitberg & Smirina 2006). Although the altitude of the study site was comparatively low (450 m a.s.l.), mean age was high (6.68 years) in the *P. tauricus* sample studied. Similarly, the high mean age of 6.82 years was identified in a low altitude (445 m a.s.l.) population of the lacertid *Acanthodactylus boskianus* (Daudin, 1802) (Üzüm et al. 2014). However, also low altitude populations can show low mean age values: 2.21 years were found in an island population of *Podarcis siculus* (Rafinesque-Schmaltz, 1810) (Raia et al. 2010) and 4.12 years in a lowland population (50 m a.s.l) of *Eremias argus* Peters, 1869 (Kim et al. 2010). The present study revealed that the mean age in the male and female samples of *P. tauricus* differed significantly. Üzüm et al. (2014) received similar results for *A. boskianus* whereas, Gül et al. (2014) did not find such differences in *Darevskia rudis* (Bedriaga, 1886).

Longevity depends on the duration of the annual activity period, which is a function of altitude, latitude and other climatic and environmental factors (Gül et al. 2014; Bülbül et al. 2016) On the other hand, Tarkhnishvili & Gokhelashvili (1996) suggested that longevity might be related to the type of locality rather than climate. The high maximum age of ten years for males and females of the Sergen population of *P. tauricus* is very likely due to favorable conditions and paralleled by the findings of Üzüm et al. (2014) who reported the high maximum longevities of nine (males) and seven (females) years in a lowland population of *A. boskianus*.

In the *P. tauricus* lowland population studied, mean SVL did not differ significantly between sexes, which was also observed in a population of *D. rudis* at 700 m a.s.l. (Gül et al. 2014). This is obviously a species-specific trait in some small lacertids from which sexual size differences are otherwise definitely known, e.g., *A. boskianus* (Üzüm et al. 2014). The significant positive correlation between age and SVL found for male and female *P. tauricus* was also observed in the sexes of *A. boskianus* (Üzüm et al. 2014) whereas, a converse trend was reported in *D. rudis* by Gül et al. (2014).

According to studies by Beebee & Griffiths (2000) and Olsson & Madsen (2001), male lizards mature earlier than females in some species. This was, however not observed in the studied population of *P. tauricus.* Similarly, Galán (1996) and Rotger et al. (2016) reported the absence of sexual difference in age at maturity in *Podarcis bocagei* (Seoane, 1884) and *Podarcis lilfordi* (Günther, 1874). Age at the arrival of sexual maturity was the second and third calendar year of life in the studied populartion of *P. tauricus* whereas, Kabisch (1986) reported one and a half years referring to Nöllert (1983).

The adult body size depends on many factors including age at maturity and longevity (Özdemir et al. 2012). Accordingly, similar age at maturity (2-3 years) and longevity (10 years) resulted in similar adult SVL (61.81 ± 0.75 mm; 61.50 ± 0.81 mm) in both sexes of *P. tauricus*.

In many lacertid species and other saurian families, males are larger in body size than females (KALIONTZOPOULOU et al. 2007). Also, in his compilation of literature data, KABISCH (1986) reports male *P. tauricus* to attain longer SVLs than females (79.3 mm versus 70.5 mm). In the present study the intersexual differences in body size (SVL) were insignificant, albeit slightly male-biased (SDI = -0.005). Also, ÜZÜM et al. (2014) and GÜL et al. (2014) found male-biased (SDI = -0.02) intersexual differences in body size in *A. boskianus* and *D. rudis*. A similar observation was made in *P. siculus* (VOGRIN 2005), whereas, ŽAGAR et al. (2012) reported female-biased SSD in *P. muralis*.

Sexual size dimorphism (SSD) may be affected by climate (ROITBERG 2007). GÜL et al. (2014) reported increased SSD values in a highland population (2,137 m a.s.l.) of *D. rudis*, probably as a result of colder environmental temperatures at higher elevations. As expected, SSD was low in the studied lowland population of *P. tauricus*. On the other hand, longevity and age at first reproduction were identified as the main determinants of SSD at an intra-specific level (LIAO & LU 2010; LYAPKOV et al. 2010; LIAO et al. 2013, 2015). Congruently, longevity and age at sexual maturation were similar between the sexes in the present study. In many lizard species, adult SSD arises from sexual differences in the growth rates, the larger sex growing faster than the smaller sex (JOHN-ADLER & COX 2007; KOLAROV et al. 2010; ÜZÜM et al. 2014). In accordance to this, the present study found no significant difference between growth rates for both sexes and the observed low-level male-biased SSD.

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