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Body Size and Age Structure of the Lebanon Lizard *Phoenicolacerta laevis* (Gray, 1838) (Reptilia: Lacertidae) in a Lowland Turkish Population

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Abstract: Life-history traits (e.g. body size, sexual size dimorphism and age) of a Turkish population of the Lebanon lizard *Phoenicolacerta laevis* were studied using skeletochronology techniques. We measured phalangeal bones (n = 30). The mean snout-vent length (SVL) was slightly different between the sexes. We recorded weakly expressed sexual size dimorphism: the snout-vent-length of the males was slightly bigger (SDI = -0.01). Sexual maturity was attained between the second and the fourth year of life in both sexes. The age ranged from 3 to 11 years in females and from 3 to 12 years in males. The mean SVL was 65.33 ± 3.06 mm in females and 66.59± 3.76 mm in males. The mean age was 6.67 ± 2.63 years in females and 6.47 ± 2.75 years in males. There was no statistically significant difference in mean age between the sexes.

Key words: snout-vent length, skeletochronology, longevity, sexual size dimorphism, maturity age

Introduction

Previous studies on the Lebanon lizard *Phoenicolacerta laevis* (Gray, 1838) have been focused on its distribution (Disi et al. 2014, Kariş et al. 2014, Handal et al. 2016, Ilgaz et al. 2016, Tarknishvili et al. 2017). Data on its reproduction (Frankenberg & Werner 1992, Baran & Atatür 1998, Disi et al. 2001, Bar & Haimovitch 2012) and phylogeny (Tamar et al. 2015, Tarkhnishvili et al. 2017) have also been published. *Phoenicolacerta laevis* is distributed in Israel, Jordan, Lebanon, Syria and Turkey (Crochet et al. 2009). Recently, Tarknishvili et al. (2017) recorded an isolated population of this species from the Georgian Black Sea Coast. In Turkey, it is distributed in the Aegean and Mediterranean regions (Ilgaz et al. 2016). The IUCN Red List of Threatened Species classifies it in the LC (Least Concern) category since 2009 (Crochet et al. 2009).

There is an increasing interest on age structure as it assists in studying the lifespan of reptilian species (e.g. Cabezas-Cartes et al. 2018, Cayuela et al. 2019). For some Turkish species, age structures (Yakin & Tok 2014, Üzüm et al. 2015, Bülbül et al. 2016), body length (Yakin & Tok 2014, Gül et al. 2017, Altunişik & Eksilmez 2018, Beşer et al. 2019) and age at maturity (Bülbül et al. 2016, Odabaş et al. 2019, Yildirim et al. 2019) have also been reported. Knowledge on the age structure of *P. laevis* is limited to the study of Üzüm et al. (2018) comparing body size and age structure of the species at different altitudes in Turkey.

The biological specialisations of reptiles are associated with body size and morphometric parameters that are part of life history and demography

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of an organism (Pal et al. 2010). Skeletochronology can be used to estimate the individual age (Castanet et al. 1993). It is based on the principle that the deposition of growth marks fundamentally corresponds to periodical phenomena; it is considered a powerful and suitable chronological tool to understand biology and ecodemography of populations (Castanet 1994).

The present study aimed to assess life-history traits (age structure, longevity, body length and sexual size dimorphism) by skeletochronology in a Turkish population of *P. laevis*.

Materials and Methods

Totally, 30 individuals of *P. laevis* (15 ♂♂ and 15 ♀♀) were caught on 11-12 July 2018 from the Meydan Köyü located in the Samandağ District of the Hatay Province, Turkey. Meydan Köyü (36°02'049'' N, 35°58'830'' E) is located in a lowland area at 4 m a.s.l. The habitat of the population represents orange groves and walls of houses close to the groves. For the active period of *P. laevis* (March to September), we recorded an average air temperature of 31°C in daytime. The mean annual temperature and precipitation over the past 80 years in the Meydan Köyü site were 18.3°C and 93.75 mm, respectively, according to data of the 6th Meteorology Regional Directorate, Adana. During the active period of the lizards, the mean temperature and precipitation were 22.4 °C and 57.3 mm, respectively, according to data of the meteorological station.

The lizards were caught by hand and sexed through a direct examination of their sexual organs (presence of a hemipenis in the cloacal opening of the male individuals). Snout-vent length (SVL) was measured to the nearest 0.01 mm using a digital calliper. We quantified Sexual Size Dimorphism (SSD) using the index of Lovich & Gibbons (1992) according to the following formula:

SDI = (mean length of the larger sex / mean length of the smaller sex) ± 1.

In this formula, +1 was used if males were larger than females and defined as negative, or -1 was used if females were larger than males and defined as positive arbitrarily.

For each lizard, the second phalange of 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 of the Karadeniz Technical University.

The procedure of skeletochronology is based on a calculation of the lines of arrested growth (LAGs) in transverse sections of the middle part of phalangeal diaphysis (in this case a portion of the second phalanx from the longest toe) (Bülbül et al. 2018). In the cross-sections of the present study, it was observed that the resorption zone did not reach the first LAG in all specimens.

We followed the experimental procedure used by Eroğlu et al. (2018). The skins of the lizards were first preserved in a 10% solution of formaldehyde, followed by peeling. Decalcification of bone tissue was performed by keeping the tissues for 2.5 hours in a 5% nitric acid solution. After decalcification, all samples were loaded into a tissue processing system (Leica TP1020 tissue processor). The skeletochronology protocol lasted 16 hours with 80-minute periods (change time of the solution) using solutions of alcohol (eight times), xylene (two times) and paraffin (two times), respectively. Later, all tissue samples were embedded in paraffin with a tissue-embedding device (Thermo brand). The cross-sections (14 µm) were obtained from embedded phalanges with a rotary microtome. Using haematoxylin (Non-Acidified type, Thermo Scientific™ Shandon™ Harris Haematoxylin), the cross-sections were stained for 2 minutes. Entellan (Merck brand "Entellan® new", rapid mounting medium for microscopy) was used for mounting the stained cross-sections on microscope slides. Finally, the cross-sections were observed under a light microscope.

We estimated the age of the lizards using skeletochronology analysis (Castanet & Smirina 1990, Smirina 1994). The numbers of LAGs on the cross-sections were calculated by two independent observers (U. Bülbül & H. Koç-Gür) and the results were compared. The observed double lines were considered as one LAG for age determination (Guarino & Erismis 2008, Bülbül et al. 2018). 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 as described in Özdemir et al. (2012). The distance between two adjoining LAGs is a good indicator of individual growth in a given year (Kleinenberg & Smirina 1969, Özdemir et al. 2012). The point where an obvious decrease in spacing between two subsequent LAGs was observed marked the age when sexual maturity was achieved (Ryser 1998, Özdemir et al. 2012, Bülbül et al. 2016).

Because age classes and body measurements (SVL) were normally distributed (one-sample

Kolmogorov-Smirnov test, $P < 0.05$) we used the parametric independent-samples *t*-test ($P > 0.05$) for comparison of means and Pearson's rank correlation test, $P < 0.01$) to analyse correlations. All statistic tests were done using SPSS 22.0 for Windows.

Results

A growth zone and thin haematoxylinophilic outer line corresponding to a winter line of arrested growth were present in cross-sections of the phalanges in 100% (n = 30) of adult individuals (Fig. 1). The resorption zone did not reach the first LAG in all specimens. The resorption zone was out of the endosteal bone in all preparations and never created difficulty for age determination. We observed double lines and endosteal resorptions in 11 (36.6%) and five (16.6%) individuals, respectively. The age at maturity was 2-4 years for both sexes. The age at maturity was 2 years (40%), 3 years (33.3%) and 4 years (26.6%) in females, while it was 2 years

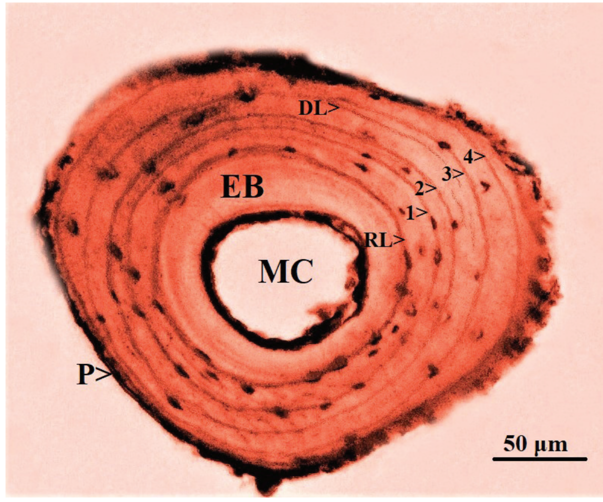

Fig. 1. A cross-section (14 µm thick) of a phalange of a four-year-old male (72.66 mm SVL) *P. laevis* from the Meydan Köyü population. For abbreviations, see text (MC, Marrow Cavity; EB, Endosteal Bone; RL, Resorption Line; DL, Double Line; P, Periphery). Periphery was not regarded as a LAG.

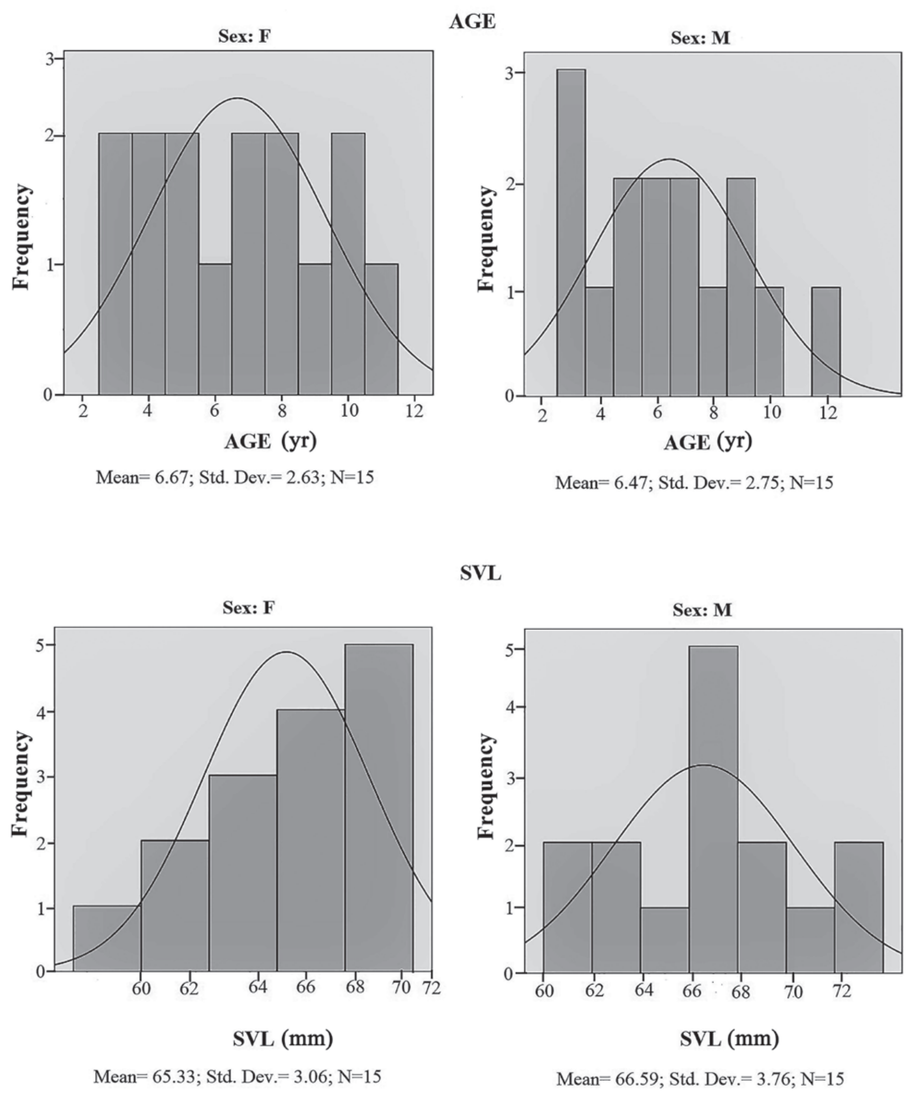

Fig. 2. Age and SVL distributions for both females and males of *P. laevis* from the Meydan Köyü population (Std. dev. = Standard deviation; N = Number of individuals; yr = year; mm = milimeter).

Table 1. Snout-vent length (SVL) and age of the examined specimens of *Phoenicolacerta laevis* (Gray, 1838) from Meydan Köyü, Turkey.

Characters	*n*	Mean	Range	SE
Males				
SVL	15	65.33	57.92-68.54	0.79
Age	15	6.67	3-11	0.68
Females				
SVL	15	66.59	60.14-72.66	0.97
Age	15	6.47	3-12	0.71
Both sexes				
SVL	30	65.96	57.92-72.66	0.62
Age	30	6.57	3-12	0.48

(40.0%), 3 years (53.3%) and 4 years (6.6%) in males.

The means of SVL and age were 65.96 ± 3.43 mm and 6.57 ± 2.65 years, respectively, for all individuals of *P. laevis* (65.33 ± 3.06 mm and 6.67 ± 2.63 years for females and 66.59± 3.76 mm and 6.47 ± 2.75 years for males; Table 1). For results of the Frequency Analyses, see Fig. 2.

Age ranged 3-11 years in females and 3-12 years in males. There was no significant mean age difference between the sexes (independent-samples *t*-test; $t = 0.203$, df = 28, $P = 0.840$). Intersexual differences in body size (length) were slightly male-biased (SDI = -0.01). The mean SVL ($t = -1.009$, df = 28, $P = 0.321$) was slightly different between the sexes. There was a negative correlation between SVL and age (r = -0.671, P = 0.006) for females, while there was no correlation between SVL and age for males (Pearson's correlation r = 0.002, P = 0.995).

Discussion

Environmental factors (e.g. climate conditions, length of the active period, type of the locality, predation, altitude and latitude) affect population demography by generating differences in age structure and longevity (Wapstra et al. 2001, Roitberg & Smirina 2006, Roitberg 2007). We have found the mean age as 6.47 years (3-12 years in range) for males and 6.67 years (3-11 years in range) for females of the Meydan Köyü population of *P. laevis*. Similarly to our results, Üzüm et al. (2018) have found that the average age of males and females is 6.62 (3-10 years in range) and 6.11 years (4-9 years in range), respectively, in the Anamur population (located at 22 m a.s.l.). For the Andırın population (1083 m a.s.l.) of the same species, the age of females is 6.15 years (range 3-10) and that of males is 5.26 years (range 3-7 years). The population examined in the present study (Meydan Köyü) and the Anamur population (Üzüm et al. 2018) are located in lowlands. Therefore, a similar average age and longevity may be considered expected. As a general phenomenon, lizards from high elevations and northern latitudes live longer than from lower elevations and southern latitudes (Wapstra et al. 2001, Roitberg & Smirina 2006). However, Üzüm et al. (2018) have reported a slightly lower mean age and longevity in the highland population of the species. In line with those findings, Gül et al. (2014) have found that individuals of the spiny tailed-lizard *Darevskia rudis* from lower altitudes exhibit higher mean ages and longevity than those from higher altitudes. A similar result has been reported by GUARINO et al. (2010) for the sand lizard *Lacerta agilis*.

Populations living at cool sites develop delayed maturity and compensate their slower growth rate with larger eggs and hatchlings (Berven 1982, Liao & Lu 2010a, 2012). According to Beebee & Griffiths (2000) and Olsson & Madsen (2001), male lizards mature earlier than females in some species. We have found the age at maturity as 2-4 years in both males and females of the Meydan Köyü population. Similarly, the maturity has been calculated three years for both sexes in lowland and highland populations (Anamur and Andırın) of *P. laevis* (Üzüm et al. 2018). From these results, it can be concluded that the average age, longevity and age at maturity of the three populations (Meydan Köyü, Anamur and Andırın) of the Lebanon lizard are dependent on the local specific conditions (e.g. predation, food availability, length of the activity period and other environmental factors) of these populations.

Double lines are irregularities in bone deposition caused by unpredictable ecological factors, such as dry periods, hot weather, food availability and other conditions (Jakob et al. 2002, Guarino & Erismis 2008, Özdemir et al. 2012). We have observed double lines in 11 (36.6%) specimens of the Meydan Köyü population. Because the climate in the Meydan Köyü, during the activity season of *P. laevis* the weather is hot and includes dry periods, which explains the high rate of double lines in bone depositions.

Endosteal resorption in bone specimens may also be related to environmental conditions (Smirina 1972). We have found a low rate of endosteal resorption in five (16.6%) specimens of the Meydan Köyü population located at a lower elevation site. Caetano & Castanet (1993) have reported fewer endosteal resorptions in lowland populations than in highland populations. In addition, Eroğlu et al. (2017) did not find any endosteal resorption in a lowland population of the Italian wall lizard *Podarcis siculus* (Rafinesque-Schmaltz, 1810). However, lower endosteal resorption rates have been reported in the highland areas where other lizard species occur (Arakelyan et al. 2013, Gül et al. 2014). Daily and annual activity and climate conditions probably have a strong effect on the intensity of bone resorption of long bones in animals (Hemelaar 1988, Esteban 1990, Leclair 1990, Augert 1992, Esteban et al. 1999).

The mean SVL of males is slightly bigger than that of females of *P. laevis* in the Meydan Köyü. In agreement with our observation, Üzüm et al. (2018) have reported non-significant variation in SVL between populations (Anamur and Andırın) and sexes of the same species. Intraspecific variation in body size along latitudinal and altitudinal gradients is common in ectothermic animals (Endler 1992, Yamahira & Conover 2002, Sears 2005).

Sexual dimorphism may evolve due to the competition between the sexes for food or other limited resource (Best & Gennaro 1984), male-male competition for mates (Vitt & Cooper 1985, Hews 1990), fecundity selection causing large female size (Halliday & Verrell 1988, Olsson et al. 2002) or other environmental factors. In the populations of *P. laevis* examined in the present study and by Üzüm et al. (2018), presence or absence of male-male combat and fecundity selection are unknown. Therefore, it is not possible to identify any kind of selection as a reason for the body-size sexual dimorphism in the studied population.

A slightly male-biased sexual size dimorphism has been observed in the Meydan Köyü population of *P. laevis.* Similar findings have been reported by Üzüm et al. (2018) for lowland and highland populations of *P. laevis*. The patterns of SSD variation may be a consequence of abiotic and biotic environmental factors (Bülbül et al. 2016). Therefore, SSD in lizards may be explained by differences in the SVL, age structure, phylogeny and climate (e.g., temperature and precipitation) between females and males (Roitberg 2007). There are no data comparing the factors, which can be more effective on SSD, in these three populations (Meydan Köyü, Andırın and Anamur).

Longevity and age at first reproduction have been identified as the main determinants of SSD at an intra-specific or inter-specific level (Liao & Lu 2010b, Lyapkov et al. 2010, Liao et al. 2013, 2015). Longevity and age at sexual maturity are similar between both sexes in the present study. Consistent with this, the SSD in the Meydan Köyü population is not statistically supported.

In conclusion, our data on *P. laevis* in a Turkish lowland population may contribute to the knowledge on life history traits of the species. Our study shows that individuals of the Lebanon lizard may live 12 years or more in their natural habitats. Further long-term and detailed studies on the populations may reveal the environmental factors affecting mean age, longevity and age at maturity of *P. laevis*.

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