

Comparison of the body size and age structure of Lebanon lizard, *Phoenicolacerta laevis* (GRAY, 1838) at different altitudes in Turkey

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Abstract

In this study, comparison of a life history traits (e.g. body size, age at maturity, longevity) of two populations of *Phoenicolacerta laevis* from different altitudes is being carried out (Anamur, 22 m a.s.l.; Andırın, 1.083 m a.s.l.) for the first time. We applied phalangeal skeletochronology to obtain the age of juveniles and adults. Age was determined by counting the lines of arrested growth (LAGs) in cross-sections. Males in both populations were the larger of the two sexes and a male biased sexual dimorphism was determined for both populations. Body size (SVL) was similar in both sexes and populations. Age of maturity was calculated to be 3 years of age for males and females in both populations. The average age of males and females was calculated as 6.62 ± 0.37 (Mean \pm SE) and 6.11 ± 0.26 years in Anamur, and 6.15 ± 0.51 and 5.26 ± 0.24 years in Andırın. There was statistically significant variation between sexes, but no significant difference in populations was found in relation to age. For both populations, a significant positive correlation was found between age and SVL in males and females.

Key words

Lacertidae, *Phoenicolacerta laevis*, age, size, sexual dimorphism, skeletochronology, Turkey.

Introduction

Lebanon lizard, *Phoenicolacerta laevis* (GRAY, 1838) has a widespread distribution range; it has been found in Turkey, northwestern Syria, Lebanon, northern Israel and northwestern Jordan. In Turkey, it has a sporadic distribution range as they can be found in western and southern Anatolia including Hatay, Adana, Mersin, Kahramanmaraş, Osmaniye, Antalya, Muğla and İzmir (SINDACO & JEREMCENKO, 2008; ILGAZ *et al.*, 2016). Species of large size of the genus (up to 250 mm total length), is characterized by the presence of keeled dorsal scales and without granules between anal cleft and large anal plate. It has plumped and flat head and body (BARAN *et al.*, 2012). Because of the high adaptability, it is observed in different habitat, such as rocky forested areas, meadows, gar-

dens, walls of houses and orchards (BAŞOĞLU & BARAN, 1977). Altitudinal distribution ranges from sea level up to 1.800 m a.s.l. (BARAN & ATATÜR, 1998). The IUCN Red List of threatened species lists *P. laevis* under Least Concern in view, of its wide distribution, tolerance of a degree of habitat modification, presumed large population, and because it is unlikely that its population will be declining fast enough to qualify for listing in a more threatened category. According to data obtained from two mitochondrial and two nuclear genes, *P. laevis* is paraphyletic with the Turkish populations branched within *P. cyanisparsa* (TAMAR *et al.*, 2015). It was also stated that *P. laevis* has high levels of undescribed diversity and its populations has to re-revise. However, since a taxonomic naming was

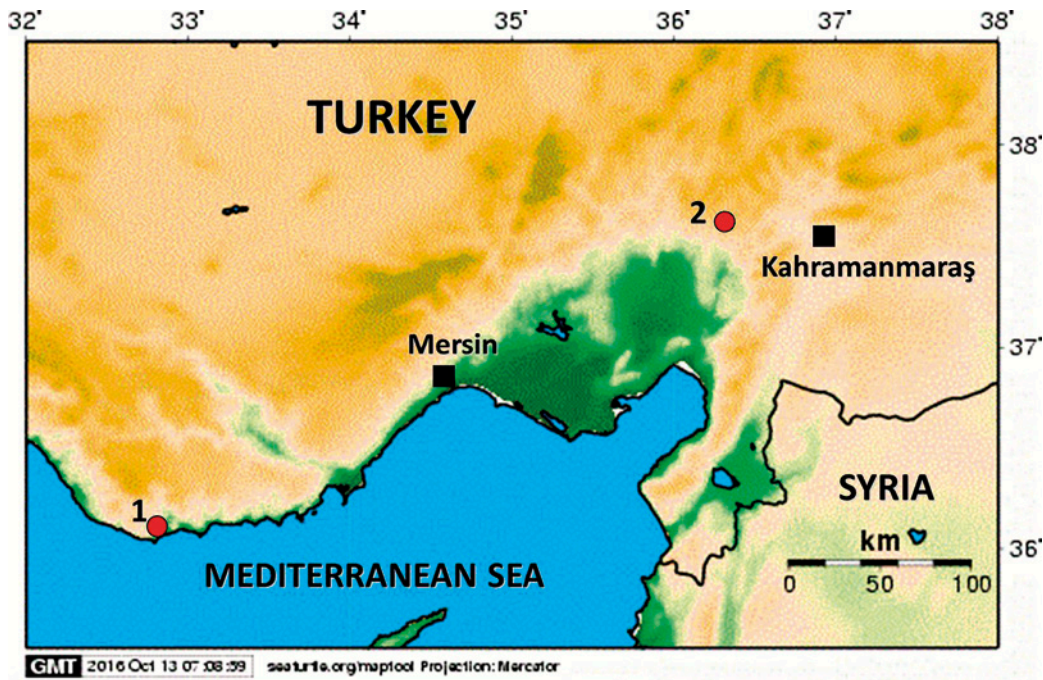


Fig. 1. Map of the sampling locations for *Phoenicolacerta laevis* (1: Anamur, Mersin, 2: Aslanboğa village, Andırın, Kahramanmaraş).

not used in the study, it is considered that both populations used in the study accepted as *P. laevis*.

There have been various studies on various parts of *P. laevis* life in literature: e.g. systematics, ARNOLD *et al.*, 2007; phylogeny and phylogeography, TAMAR *et al.*, 2015; distribution, KARIŞ & GÖÇMEN, 2014; ILGAZ *et al.*, 2016; morphology and serology, BUDAK, 1976; BUDAK & GÖÇMEN, 1995; TOSUNOĞLU *et al.*, 1999; TOSUNOĞLU *et al.*, 2001, life-history characteristics HRAOUI-BLOQUET, 1987; HRAOUI-BLOQUET & BLOQUET, 1988; VOLYNCHIK, 2014, but there has been no data available on age structure especially when assessed via skeletochronology.

Life-history of an organism is characterized by its body size, age and size at maturity, frequency of reproduction, clutch or litter size, size of eggs and hatchlings, and survivorship at different life stages (BAUWENS, 1999; PIANKA & VITT, 2003; GÜL *et al.*, 2015a). Of them, age and body size are accepted as the two standard characteristics that have been used to quantify life history traits of animals by many researchers (e.g. CHEN *et al.*, 2011; GÜL *et al.*, 2011; LOU *et al.*, 2012; ÖZDEMİR *et al.*, 2012; ALTUNIŞIK *et al.*, 2013; HUANG *et al.*, 2013; GÜL *et al.*, 2014; GÜL *et al.*, 2015a). Lizard life-history characteristics, which are often phenotypically plastic, vary in response to temperature, food availability, and other environmental factors, and also widely among species and populations (TINKLE, 1967; DUNHAM *et al.*, 1988; ADOLPH & PORTER, 1993). Lizards are found in different kinds of thermal environment, including hot tropical lowlands, temperate deserts, and cool areas, as well as highly seasonal habitats that are at high elevation or high latitudes (PEARSON & BRADFORD, 1976). As well as variability in thermal environments, the variation in activity season (HUEY, 1982) affects the life history characteristics and causes some variations among species and widespread populations of single spe-

cies (GRANT & DUNHAM, 1990). Altitudinal gradient as an environmental factor is important variable in order to test ecological and evolutionary processes in the life history of organisms (KÖRNER, 2007).

A population's age structure is directly related to the life-history of that population (GÜL *et al.*, 2014). So, for age determination, we used skeletochronological method, which is based on the presence of growth layers in bone tissue, and then counting those lines to see when the arrested growth occurred (LAGs) (CASTANET & SMIRINA, 1990).

In this study, we compare some life history traits (e.g. body size, age at maturity, longevity) of two populations of *P. laevis* for the first time, from different altitudes in Turkey. Specifically, the following questions were addressed: (i) Is there any difference between sexes in terms of age structure and body size? (ii) Do age and body size vary between the populations in relation to altitude?

Material and methods

Specimens and study sites

Lizard specimens were collected from two populations (Aslanboğa Village, Andırın, Kahramanmaraş, southern Anatolia and Anamur, Mersin, southern Anatolia) at different altitudes (Fig. 1) during the herpetological expedition, which occurred in mid May 2016. All specimens were etherized, then injected with 96% ethanol and stored in glass jars that had a solution that was 70% ethanol (BAŞOĞLU & BARAN, 1977). Afterwards, they were deposited in the Zoology Lab. of the Department of Biology at the Faculty of Science, Dokuz Eylül Uni-

Table 1. Number of sampled individuals (n), SVL (Mean ± SE and range) and age in *Phoenicolacerta laevis* populations (SE: standard error of the mean).

Parameters	Anamur			Andırın		
	n	Mean±SE	Range	n	Mean±SE	Range
Males						
Age	24	6.62±0.37	3–10	20	6.15±0.51	3–10
SVL	25	62.42 ±0.81	57.22–71.60	21	60.78±1.42	52.98–70.00
Females						
Age	26	6.11±0.26	4–9	23	5.26±0.24	3–7
SVL	28	59.21±0.89	50.00–69.47	24	59.81±1.43	50.50–71.61

versity (Turkey). The snout–vent length (SVL) of lizard specimens was measured using Mitutuyo digital calipers that a 0.01 mm precision. The sex of each individual was determined by observing if the lizard had the presence of a hemipenis in the cloacal opening.

Anamur (22 m a.s.l., 36°05'00.9"N, 32°50'48.10"E, low altitude site) is in a lowland area. Specimens were collected from the cemetery on the edge of the highway. Specimens were caught while there were browsing on a wall or under a small stones in the cemetery where there were dense annual herbaceous plants. Anamur has the typical Mediterranean climate where the winters are mild and rainy, and summers are hot and dry. The mean annual temperature and precipitation of Anamur is 19°C and 921.6 mm respectively. In addition, the mean annual humidity is 71.9% (<http://www.meteor.gov.tr/>).

Aslanboğa village is located in a highland area that is 5 km N of Andırın (1083 m, 37°37'14.5"N, 36°20'38.1"E, high altitude site). Lizard samples were collected under the stones along a small river. The collection area was mainly made up of Turkish pine (*Pinus brutia*), juniper (*Juniperus communis*) and cedar (*Cedrus libani*) trees. The climate is hot and dry in the summer, while the winter is usually mild and rainy. The precipitation is concentrated in the winter months. The annual mean temperature and precipitation of Andırın is 13°C and 729 mm respectively (<http://www.meteor.gov.tr/>).

Skeletochronology

We used a total of 102 preserved phalanx samples (25♂♂, 28♀♀, and 3 juveniles from Anamur/Mersin and 21♂♂, 24♀♀, and 1 subadult male from Aslanboğa Village/Andırın/Kahramanmaraş) in order to assess the age of individuals. Age was determined by using the skeletochronological method that has been applied before on various lizard species in the literature (e.g. GUARINO *et al.*, 2010; TOMASEVIC *et al.*, 2010; GÜL *et al.*, 2014; ÜZÜM *et al.*, 2014; GÜL *et al.*, 2015a, b; ÜZÜM *et al.*, 2015). The LAGs were counted on transverse sections of the middle part of the phalangeal diaphyses using a portion of the second phalanx from the third toe of the hind foot (GÜL *et al.*, 2014). The skeletochronological analysis was adapted from its standard procedures of ÜZÜM *et al.*

(2014) and ÜZÜM *et al.* (2015). Phalanges were preserved in a 70% ethanol solution and dissected; the bone of the phalanx was washed in tap water for 24 h and decalcified in a 5% nitric acid solution for 2 h. Later, phalanges were washed again in tap water for about 12 h. Cross-sections (18 µm) from the diaphyseal region of the phalanx were obtained using a freezing microtome and were stained with an Ehrlich's hematoxylin. Then, all the sections were examined under a stereomicroscope. The good sections were placed in glycerin in order to be observable though a light microscope. Bone sections from each individual lizard were photographed at the same magnification setting. All photos were examined and the analysis of LAGs was performed by Nazan ÜzüM according to previous technique that have become standards. The rate of endosteal resorption (the remodeling bone process that can reabsorbed part of or entire LAGs) was assessed by comparing the diameters of eroded marrow cavities with the diameters of non-eroded marrow cavities of juveniles (e.g. ÜZÜM *et al.*, 2014).

Data analysis

Variations in body size (SVL) and age between populations and sexes were analyzed by using Factorial ANOVA. To investigate age dependent size variations, the homogeneity of slope models between populations and sexes was tested using ANCOVA. The Spearman's correlation coefficient was computed to infer what kind of pattern was there between SVL and age. The logarithmic regression model was selected to show the relation between SVL and age. All tests were processed using STATISTICA 7.0 (StatSoft Inc., USA) and Excel (Microsoft) and were interpreted at $\alpha=0.05$. The sexual dimorphism index (SDI) of LOVICH & GIBBONS (1992) was calculated to estimate sexual size dimorphism (SSD).

Results

Descriptive statistics of body size and age for both populations in both sexes is given in the Table 1. Mean body size (SVL) was larger in males than in females in both

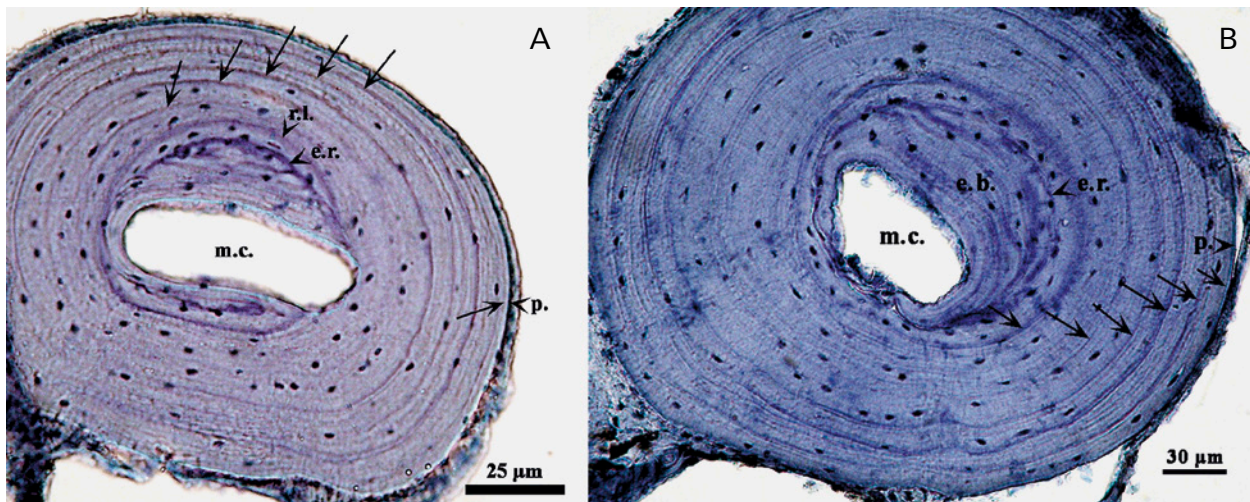


Fig. 2. Cross-sections (18 µm thick) at the diaphysal level of a phalanx of a male (A) and a female (B) of *Phoenicolacerta laevis*. A) Six-year-old male from Anamur (lowland site) B) Six-year-old female from Andırın (highland site). Arrows show the LAGs and arrowheads show the endosteal resorption, reversal line and the periphery. Periphery was not regarded as a LAG (e.r. = endosteal resorption, e.b. = endosteal bone, m.c. = marrow cavity, r.l. = reversal line).

populations. The highest SVL was recorded in Anamur for males (SVL=71.60 mm), but in Andırın for females (SVL=71.61). The ANOVA procedure, that used SVL as the dependent variable and along with population, sex and population × sex interaction as factors, revealed statistically non-significant variation in SVL between populations and sexes: $F=0.21$, $p=0.645$ (factor population) and $F=3.35$, $p=0.070$ (factor sex). Also, non-significant population × sex interaction ($F=0.96$, $p=0.329$) indicated that SVL does not differ in the SSD level between populations. SDI was calculated to be -0.05 and -0.02 indicating a male bias for Anamur and Andırın population respectively.

LAGs were evident and easily counted almost in all individuals (Fig. 2A and B). Age was determined in 94.64% ($n=53$) and 95.56% ($n=43$) of the available phalanges for Anamur and Andırın populations, respectively. All cross sections from the phalanges of adult *P. laevis* exhibit endosteal resorption and endosteal bone formation as well (Fig. 2A and B). By comparing the diameters of eroded marrow cavities of adults with the diameters of noneroded marrow cavities of juveniles, we determined that endosteal resorption did not destroy any LAGs and could not cause any problem in determining the age of a lizard.

Minimum age was determined to be 3 years for both sexes in Andırın population whereas 3 years for males and 4 years for females in Anamur population. Maximum age was determined to be 10 years for males in both populations, but 9 years for Anamur and 7 years for Andırın females (Table 1, Fig. 3A and B). Maturity age was calculated to be 3 years for both sexes in both populations. The average age of males and females was 6.62 ± 0.37 (Mean \pm SE) and 6.11 ± 0.26 years in Anamur, and 6.15 ± 0.51 and 5.26 ± 0.24 years in Andırın (Table 1). To analyze the variations in age, a factorial ANOVA was performed using population, sex and population × sex

interaction as factors. There was a non-significant variation between populations ($F=3.635$, $p=0.060$) and marginally significant variation between sexes ($F=4.024$, $p=0.048$) was determined. The population × sex interaction was also found not to be significant ($F=0.296$, $p=0.588$), which indicated that the age changes between sexes did not differ between populations.

The ANCOVA analyses of size, with size variable as dependent variable, population and sex as factors and age as a covariate revealed clearly significant age × population ($F=14.683$, $p=0.000$) and age × sex ($F=12.060$, $p=0.000$) interactions. These results indicated that age dependent SVL differs between populations and sexes.

For both populations, a significant positive correlation was found between age and SVL in males ($r=0.707$, $p < 0.05$ for Anamur and $r=0.935$, $p < 0.05$ for Andırın) and females ($r=0.665$, $p < 0.05$ for Anamur and $r=0.857$, $p < 0.05$ for Andırın). Logarithmic regression fitted the correlation between age (years: x-axis) and SVL (mm: y-axis) in males and females of Anamur ($y=8.990\ln(x) + 45.813$, $R^2=0.457$ for males and $y=15.347\ln(x) + 31.971$, $R^2=0.465$ for females) and Andırın population ($y=15.476\ln(x) + 33.332$, $R^2=0.877$ for males and $y=23.489\ln(x) + 21.519$, $R^2=0.602$ for females).

Discussion

In the present study, we compared the body size and age structure of two *P. laevis* populations from different altitudes as well as eventual intersexual differences, in terms of these characteristics. Skeletochronology was successfully used here to estimate the age of *P. laevis* individuals, moreover their growth is cyclical, and, as in other reptiles, it undergoes alternating periods of rapid and slow growth (CASTANET *et al.*, 1993).

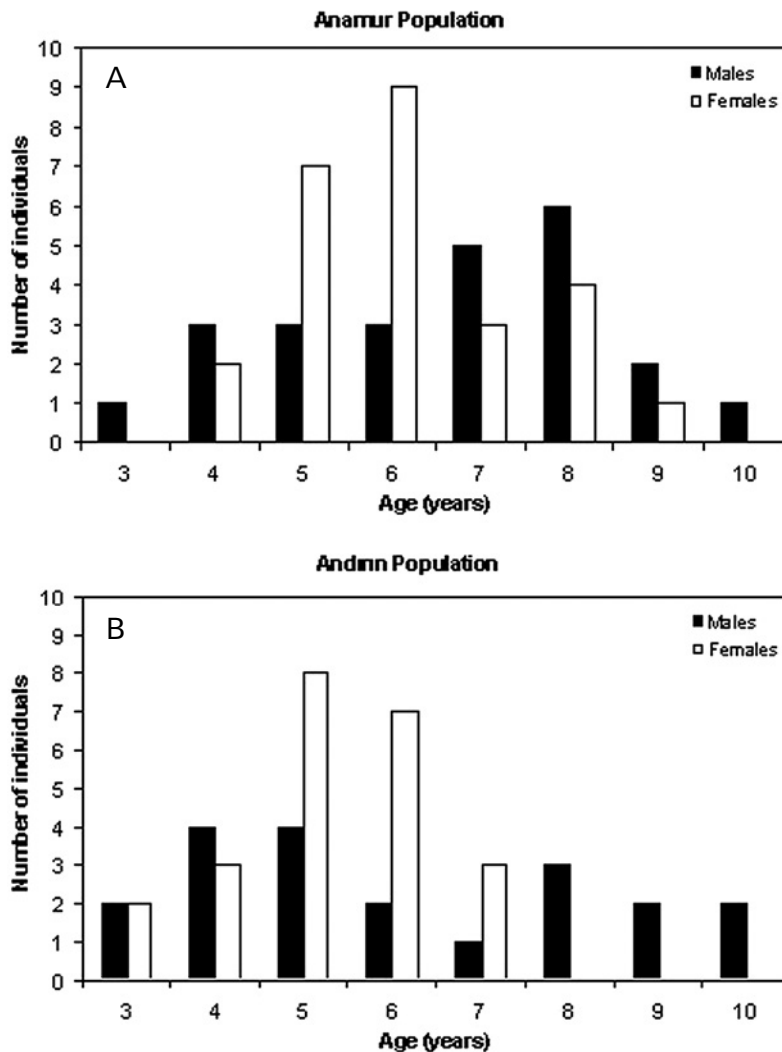


Fig. 3. Age distribution in *Phoenicolacerta laevis* populations from two different altitudes; A: Anamur population (low altitude population, B: Andrinn population (high altitude population)

In most animal groups, sexual dimorphism (sexual differences in morphological characters), particularly in body size, is a common phenomenon (VIDAL *et al.*, 2002), and males are typically the larger sex in vertebrates (SHINE, 1986; ANDERSON, 1994; FAIRBAIRN, 1997; VIDAL *et al.*, 2002). The SVL which is the most important distinguishing characteristic for many species of the Lacertidae family (ARRIBAS, 1996) was larger in males than in females for both populations. But, we did not find any statistically significant variation in SVL between sexes and populations. Most species of lacertid lizards are male-biased in terms of body size and head dimensions (KALIONTZOPOULOU *et al.*, 2007; GÜL *et al.*, 2015a), but this is not always the rule. Females are bigger than males in species in several genera (*Lacerta*, *Podarcis*, *Iberolacerta*, *Zootoca*) (BRAÑA, 1996; ROITBERG, 2007; ŽAGAR *et al.*, 2012).

The non-significant population \times sex interaction indicated that SVL does not differ on the SSD level between the populations. Both in the lowland (Anamur) and highland (Andrinn) populations male-biased SSD was found. These results do not agree with the findings by ROITBERG (2007) who reported that geographic variation in SSD is male-biased in warmer climates for *Lacerta agilis exigua*

and *L. a. boemica*, and GÜL *et al.* (2014) since the researcher found that in the lowland populations of *Darevskia rudis*, SSD appeared male-biased whereas in the high altitude population it was a strong female-biased SSD. However, GÜL *et al.* (2015a) showed similar results to the current finding that the males of *Apathya cappadoccica* were larger than females in 3 different populations (male-biased SSD) that were from different altitudes.

In our study, the highest SVL was recorded in lowland for males, but in highland for females. Similar to our results, GÜL *et al.* (2014) showed that individuals of *D. rudis* from lower altitude exhibited higher mean SVL and ages than from higher altitude ones. GÜL *et al.* (2015a) also found that female individuals of *A. cappadoccica* from a low altitude area were larger than individuals from a high altitude area. In contrast to our results, BÜLBÜL *et al.* (2016) found higher SVL in the highland than the lowland population for the individuals of *D. parvula*. Males from both lowland and highland populations of *D. bithynica* were slightly larger than females (GÜL *et al.*, 2015b). As a general rule, animals from high altitudes and northern latitudes live longer than those from low altitudes and southern latitudes (WAPSTRA *et al.* 2001; ROITBERG & SMIRINA 2006a) and they also have larger

bodies in cooler climates (SEARS & ANGILLETTA, 2004). Furthermore, lizards in low altitudes are expected to have a smaller mean body size than individuals in high altitudes (ROITBERG & SMIRINA, 2006b). However, ROITBERG & SMIRINA (2006a) did not find any clear altitudinal trend in adult SVL for *L. a. boemica* and *L. strigata*.

Age distributions of male and female individuals were significantly differed in both populations, but not enough to be statistically significant. The average age of males and females was higher in lowland population than in highland population (6.62 ± 0.37 for males and 6.11 ± 0.26 years for females in Anamur, and 6.15 ± 0.51 years for males and 5.26 ± 0.24 years for females in Andırın). Moreover, maximum longevity was ascertained to be 10 years for males in both populations, but 9 years for females in the population from Anamur (22 m) and 7 years for females in the population from Andırın (1.083 m). As mentioned above, individuals from high-elevation sites and northern latitude usually live longer than those from low-elevations sites and southern latitudes (WAPSTRA *et al.* 2001; ROITBERG & SMIRINA, 2006a; SEARS & ANGILLETTA, 2004), however our results are in line with those findings by GÜL *et al.* (2014) who showed that *D. rudis* individuals from lower altitudes exhibited higher mean ages and longevity than those from higher altitudes. In addition, GÜL *et al.* (2015a) reported similar results for *A. cappadocica* and GUARINO *et al.* (2010) which found that longevity of *L. agilis* (4 years for males and 3 for females) from a high Alpine population was lower than those from lowland populations of the same species.

In conclusion, we compared some life history traits (e.g. body size, age at maturity, longevity) of two populations of *P. laevis* from different altitudes for the first time. However, further studies are needed to obtain more comprehensive data about the life history characteristics and altitudinal variations regarding age and body size of this species.

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