Amphibia-Reptilia 37 (2016): 450-456

Body size and age structure of the endangered Clark's lizard (*Darevskia clarkorum*) populations from two different altitudes in Turkey

Ufuk Bülbül*, Muammer Kurnaz, Ali İhsan Eroğlu, Halime Koç, Bilal Kutrup

Abstract. We investigated age structure, body size and longevity in two breeding populations of *Darevskia clarkorum* inhabiting altitudes ranging from 450 m a.s.l. (Kamilet) to 2250 m a.s.l. (Başyayla) in Turkey by skeletochronology performed on the phalanges. The mean age was found to be 6 years in the Kamilet population and 7 years in the Başyayla population. The maximum life span was 10 years in the lowland population while it was 12 years in the highland population. Age at sexual maturity of both males and females was 1-2 years in the lowland population while it was 2-3 for both sexes in the highland population. Both age and SVL of specimens from the Kamilet population were significantly different between the sexes while age and SVL did not differ significantly between the sexes in Başyayla population. As a conclusion, we observed that the mean age, longevity and age at maturity were increased by altitude while there was a decrease based on the mean SVL in the highland population of *D. clarkorum*. Our data on body size, longevity and age at sexual maturity may contribute to conservation efforts for this endangered species.

Keywords: Black Sea, LAG, longevity, skeletochronology, SVL.

Clark's lizard, *Darevskia clarkorum* (Darevsky and Vedmederja, 1977) is distributed in the Black Sea coastal region of Turkey (between Giresun and Artvin), Georgia (Adjaria autonomous region) and Armenia (Baran and Atatür, 1998; Ilgaz, 2007). The main distribution area of the species comprises the northern spurs of the East-Pontic Mountains in the northeast of Turkey and a small isolated region within the western slopes of the Mountain Mtirala in the vicinity of the town of Batumi in southwestern Ajaria from Georgia (Tuniyev et al., 2009).

The first specimens of the species were collected by Clark and Clark (1973) from Cankurtaran Pass into Artvin province of Turkey. Later, Darevsky and Vedmederja (1977) examined specimens of Clark and Clark and their own specimens from Adjaristan, Georgia. They described all specimens in the two studies as members of *Lacerta clarkorum*. Based on his morphological, osteological and karyological data, Arribas (1999) assigned rock lizard species to a new genus (*Darevskia*) and the name of the species changed as *D. clarkorum*. Ilgaz (2007) extended the known range of the species in Turkey. Other studies related to the species were mainly based on taxonomy, systematics, ecology and breeding biology (Franzen, 1991; Darevsky and Tuniyev, 1997; Fu, Murphy and Darevsky, 1997; Murphy et al., 2000; Panner, 2001; Ilgaz, 2007). Although *D. clarkorum* lives in a large area in the eastern Black Sea Region in Turkey, there are no detailed studies including population dynamics (age structure, size, longevity and age of maturation) and life threats (habitat degradations and declining of individuals in the life area) of the species.

Darevskia clarkorum is classified as EN (Endangered) in the IUCN Red List of Threatened Animals. For the conservation of an endangered species, knowledge of their life history and population dynamics, including age structure of natural populations, is a matter of critical importance (Germano, 1992; Andreone, Guarino and Randrianirina, 2005).

The studies related to age determination have been performed to obtain some information

Karadeniz Technical University, Department of Biology, 61080 Trabzon, Turkey

*Corresponding author; e-mail: ufukb@ktu.edu.tr

 DOI:10.1163/15685381-00003073

about the current status of rare or common reptilian species (e.g. Tinkle, 1967; Barbault and Mou, 1988; Frazer, Gibbons and Greene, 1991; Dubey et al., 2013). Skeletochronology is used to calculate age using presence of growth layers in the bone tissue and counting the lines of arrested growth (Castanet and Smirina, 1990; Guarino, Gia and Sindaco, 2010). Many studies have been recently performed on the rare and common lizards using skeletochronology (Orlova and Smirina, 1983, *Darevskia derjugini*; Kim et al., 2010, *Eremias argus*; Kolarov et al., 2010, *Dinarolacerta mosorensis*; Arakelyan et al., 2013, *Darevskia armeniaca*, *D. uzzelli*, *D. sapphirina*, *D. unisexualis*; Gül et al., 2014, *Darevskia rudis*; Cabezas-Cartes, Boretto and Ibargüengoytía, 2015, *Phymaturus spectabilis*; Gül et al., 2015, *Darevskia bithynica*; Üzüm et al., 2015, *Eremias suphani*; Bülbül et al., 2016, *Darevskia parvula*).

The main purpose of the present study was to assess the age structure and some growth parameters in two populations of *Darevskia clarkorum* inhabiting different altitudes in Turkey for the first time by skeletochronology. The present paper attempted to resolve the deficiency of information on age and body size in the literature about *D. clarkorum*.

Lizards were collected from two populations (Başyayla population in Hatila Valley and Kamilet population in Kamilet Valley located in Artvin Province of Turkey) (see example in online supplementary fig. S1) during the breeding season (number of permission to capture: 72784983-488.04-113815 taken from Ministry of Forest and Water Affairs). Başyayla population (41°10′49″N, 41°38′49″E) is located in a highland area at an altitude of 2250 m a.s.l. The habitat occurs in timberline zone. The specimens were caught under medium and large sized rocks found on a steep slope. The Kamilet population (41°15′10″N, 41°21′30″E) is located in a lowland area at an altitude of 450 m a.s.l. and there is a stream which flows along the edge of the area. The habitat consists of dense vegetation. In the Kamilet site, the specimens were seen in thorn patches near the path, under dried leaves and upon the wood blocks.

The activity period for lizards varies from early May to late September in Başyayla and from early April to late October in Kamilet (the authors' observations). The average air temperatures in daytime were recorded as 19°C and 25°C, respectively during the sampling period (between 10-11 August 2015 for Başyayla population and 26-31 July 2015 for Kamilet population). At each site, the lizards were caught by hand. Snout-vent length (SVL) was measured with a digital caliper (0.01 mm precision). We quantified Sexual Size Dimorphism (SSD) with the Lovich and Gibbons (1992) index according to the following formula:

$$\text{SDI} = (\text{mean length of the larger sex} / \text{mean length of the smaller sex}) \pm 1.$$

In this formula, +1 is used if males are larger than females and defined as negative, or −1 is used if females are larger than males and defined as positive arbitrarily (Üzüm et al., 2014).

For each lizard, the second phalange from the longest finger of the hind limb was clipped and preserved in 10% formalin solution for subsequent histologic analyzes. 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 (KTÜ.53488718-648/2014/53).

A total of 89 specimens (39 ♂♂ and 46 ♀♀) were used in age determination [31 specimens (14 ♂♂ and 17 ♀♀) from Başyayla and 54 ones (25 ♂♂ and 29 ♀♀) from Kamilet].

Age determination was estimated using skeletochronology analysis (Castanet and Smirina, 1990). After skins of the toes preserved in 10% solution of formaldehyde were peeled, toes were immersed for 2.5 hours in 5% nitric acid solution for decalcification of bone tissue. Later, all samples of the toes were loaded into a tissue processing system of the Leica brand. Then, all tissue samples were embedded into paraffin with a tissue embedding device (Thermo brand). The cross-sections (15 μm) were obtained from embedded phalanges with rotary microtome, after which they were stained using the hematoxylin procedure (Bülbül et al., 2016). The stained cross-sections, which were put on the microscope slides, were closed using entellan. The numbers of LAGs on the cross-sections were independently calculated by three of the authors. The observed double lines were not taken into account for age determination. As previously stated in the study of Özdemir et al. (2012), we assessed endosteal resorption of the first LAG by comparing the diameters of eroded marrow cavities with the diameters of noneroded marrow cavities in sections from the youngest specimens. Where we observed an obvious decrease in spacing between two subsequent LAGs, we took it to mark the age when sexual maturity was achieved (Ryser, 1998; Özdemir et al., 2012).

In order to determine whether the age and SVL differed between both sexes, we employed the two-way analysis of covariance (ANCOVA) and performed Pearson's Correlations Test ($P < 0.01$) to measure the degree of relationship between the two variables. In the ANCOVA of age parameter, SVL was employed as the covariate while age was used as the covariate in the ANCOVA of the SVL parameter. All statistic tests were processed with SPSS 21.0 for Windows and the level of significance chosen was $P < 0.05$. Growth curves of both females and males of the Clark's lizard were estimated by applying the von Bertalanffy growth model using relationships of the age and SVL parameters (James, 1991; Wapstra et al., 2001; Roitberg and Smirina, 2006; Guarino et al., 2010). The general form of the von Bertalanffy growth equation used was $Lt = L_{\infty}(1 - e^{-k(t-t0)})$, where Lt is length at age t, L_∞ is a parameter representing asymptotic maximum length, e is the base of the natural logarithm, k is a growth coefficient, and t_0 is the age at hatching, which is the starting point of the growth interval in the present study. Because data on the size at hatching of the studied population were lacking, we assumed size at hatching ($L_{t0} = 27.6$ mm) as the mean value provided by In den Bosch and Bout (1998). The parameters L_∞ and k, and their asymptotic confidence intervals (*CI*), were estimated using a non-linear regression procedure by means of the IBM SPSS 21.0 software program.

A growth zone and thin basophilic outer line corresponding to a winter line of arrested growth were present in cross sections of the phalanges in all individuals of both populations (see example in online supplementary fig. S2). The resorption zone reached the first LAG in 2 specimens (6.25%) of the Başyayla population and in 4 ones (1.75%) in the Kamilet population, but never created difficulty for age determination. We observed double lines in 17 (54.83%) specimens in the Başyayla population and in 10 (18.51%) in the Kamilet population. The oldest females and males in the Başyayla population were 12 and 9 years old, respectively while maximum ages for both sexes were 10 years old, in Kamilet population (fig. 1). The age at maturity was 2 years in 47 (77.42%) specimens, 3 years in 7 (22.58%) specimens in the Başyayla population while it was 1 year in 6 (11.11%) specimens, 2 years in 48 (88.89%) specimens of the Kamilet population.

The means of SVL and age values were 58.8 ± 0.7 mm and 7 ± 0.4 for the Başyayla population (58.3 ± 0.7 mm; 6.6 ± 0.4; in male specimens and 59.2 ± 1.2 mm; 7.3 ± 0.6; in female specimens), respectively. In the Kamilet population, the means of SVL and age values were 61.2 ± 0.5 mm and 6 ± 0.2 (62.5 ± 0.4 mm; 6.7 ± 0.4; in male specimens and 60.1 ± 0.8 mm; 5.6 ± 0.3; in female specimens), respectively. Descriptive statistics of the Başyayla and Kamilet populations were given in table 1.

In the Başyayla population, age was ranged from 3-12 years in females and 5-9 years for males. The mean age of the specimens did not differ significantly between the sexes (ANCOVA; $F = 0.475$, df = 1, $P = 0.496$). Intersexual differences in body size (length) was female-biased (SDI = 0.01). However, SVL did not differ significantly ($F = 0.000$, df = 1, $P = 0.994$) between sexes. There was a positive correlation between SVL and age for males (Pearson's correlation $r = 0.686$, $P < 0.01$) and females ($r = 0.623$, $P < 0.01$). The growth pattern estimated by von Bertalanffy showed a well fit to the relation between age and SVL (fig. 1). For both sexes, the estimated asymptotic SVL was smaller than the maximum SVL recorded ($SVL_{asym} \pm CI$, males: 60.33 ± 15.08 mm; females: 60.15 ± 15.55 mm). The growth coefficient was higher in males than in females ($k \pm CI$, males: 0.25 ± 0.26; females: 0.94 ± 0.39). The growth curve of males was significantly different from that of females.

In the Kamilet population, age was ranged from 3-10 years in both sexes. Age (ANCOVA; $F = 2.043$, df = 1, $P = 0.159$) and SVL ($F = 2.238$, df = 1, $P = 0.141$) of the specimens did not differ significantly between the sexes. Intersexual differences in body size (length) was male-biased (SDI = −0.039). There was no correlation between age and SVL for males (Pearson's correlation $r = 0.314$, $P = 0.127$) while there was a positive correlation between age and SVL for females ($r = 0.486$, $P < 0.01$). There was a positive correlation between SVL and age for males (Pearson's correlation $r = 0.686$, $P < 0.01$) and females ($r = 0.623$, $P < 0.01$). The growth pattern estimated by von Bertalanffy showed a well fit to the relation

Figure 1. (A) Age distributions for male and female *Darevskia clarkorum* from Başyayla (highland) and Kamilet (lowland) populations. (B) The von Bertalanffy growth curves for Başyayla (highland: open circle, grey line for males and solid circle, solid line for females) and Kamilet (lowland: open circle, solid line for males and solid circle, grey line for females) populations of *D. clarkorum*. The solid square shows SVL mean (27.6 mm) of the lizards at hatching as reported by In den Bosch and Bout (1998). Growth parameters are given in the text.

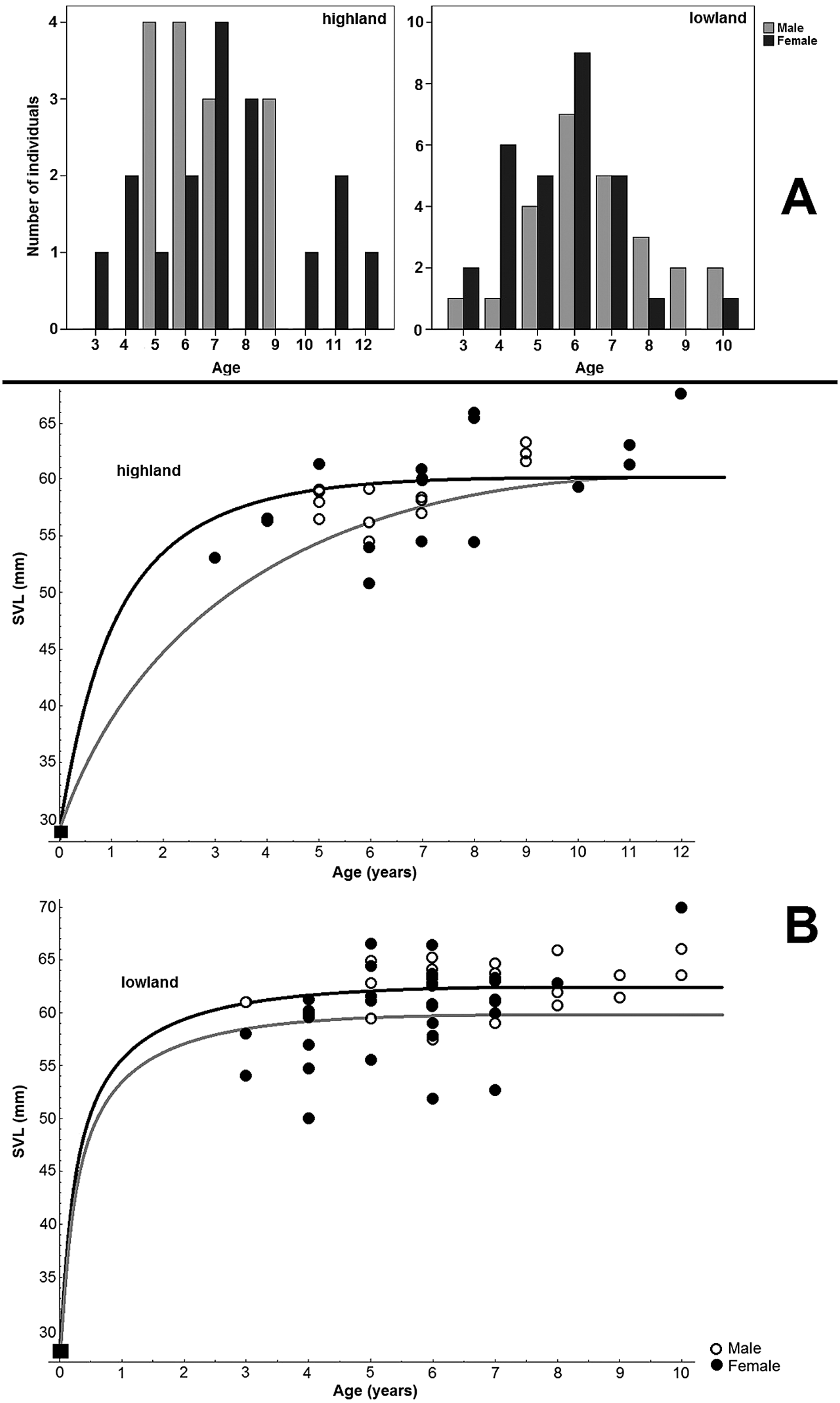
highland
lowland
Male
Female
A
Number of individuals
Age
SVL (mm)
Age (years)
B
Male
Female

Table 1. Descriptive statistics of age and SVL for both populations. For abbreviations, see text (*n*: number of samples; Range: maximum and minimum values and SE: standard error).

Characters	Sex	Başyayla (highland)				Kamilet (lowland)			
		n	Mean	Range	SE	*n*	Mean	Range	SE
SVL	♂♂	14	58.31	54.12-63.45	0.74	25	62.46	57-54-66-15	0.43
Age		14	6.57	5-9	0.40	25	6.46	3-10	0.35
SVL	♀♀	17	59.18	50.67-67.74	1.17	29	60.09	50.12-70.06	0.84
Age		17	7.29	3-12	0.63	29	5.58	3-10	0.29
SVL	♂♂	31	58.79	50.67-67.74	0.72	54	61.19	50.12-70.06	0.51
Age	♀♀	31	6.97	3-12	0.39	54	6.07	3-10	0.23

between age and SVL (fig. 1). For both sexes, the estimated asymptotic SVL was smaller than the maximum SVL recorded ($SVL_{asym} \pm CI$, males: 62.52 ± 14.11 mm; females: 60.55 ± 12.03 mm). The growth coefficient was higher in males than in females ($k \pm CI$, males: 3.03 ± 0.23; females: 1.20 ± 0.20). The growth curve of males was significantly different from that of females.

When compared both populations, males of the Kamilet population had significantly larger SVL (ANCOVA; $F = 32.309$, df = 1, $P < 0.05$) than males of the Başyayla population while age did not differ significantly ($F = 3.370$, df = 1, $P = 0.075$). There were significant differences in females between two populations for age ($F = 2.807$, df = 1, $P < 0.05$). The females of Başyayla population were older than the females of Kamilet population. There were significant differences in females between the two populations for SVL ($F = -5.471$, df = 1, $P < 0.05$). When all individuals were compared, it was found that Kamilet population had significantly bigger SVL ($F = 19.685$, df = 1, $P < 0.05$) while specimens of the Başyayla population were significantly older ($F = 16.126$, df = 1, $P < 0.05$).

The estimated mean age of the highland population was found to be significantly higher than the lowland population (7 in Başyayla population and 6 in Kamilet population; $F = 16.126$, df = 1, $P < 0.05$). The length of the active period is not similar in both sites (5 months in Başyayla population and 7 months in Kamilet population). The short active period may be the reason of the higher mean age in the highland population. However, Gül et al. (2014) found higher mean age in a lowland (700 m a.s.l.) population of *Darevskia rudis* when compared with two highland (1277 and 2137 m a.s.l.) populations. A similar trend was observed in the study of Roitberg and Smirina (2006) for the populations of *Lacerta agilis* and *Lacerta strigiata*.

While we found a maximum longevity 12 years in the Başyayla population, it was found to be 10 years in Kamilet population. These findings are the highest values reported for the lizards in *Darevskia* genus until now. The longevity varied according to altitude, latitude and other climatic and environmental factors. The active period of the lizards in the highland population is short. Thus, the lizards mature later in the cold climate and their longevity is higher than those living in the hot climate. For some parthenogenetic species, maximum longevity was found to be 8 years in *D. armeniaca* and *D. unisexualis*, 6 years in *D. sapphrina* and *D. uzzelli* (Arakelyan et al., 2013) while it was recorded as 8 years in a bisexual species, *D. rudis* (Gül et al., 2014).

Smaller body size for lizards in colder climates may be the result of selection towards more rapid heating abilities (Pianka and Vitt, 2003). Conformably, a significantly smaller SVL was found in the highland population of the present study.

Correlation between SVL and age is an important factor in age determination studies. The age of both sexes is significantly correlated with their SVL in the highland population while it is significantly correlated only in females of the lowland population in the present study. Contrary to our findings, Gül et al. (2014) found a significant correlation between SVL and age only in males of *D. rudis* while there was no significant correlation in females.

Longevity and age at first reproduction have been identified as the main determinants of SSD at an intra-specific level (Castanet and Baez, 1991; Bülbül et al., 2016). Moreover, climate can directly affect SSD (Roitberg et al., 2015). More female-biased SSD in colder climates could arise as an adaptive compensation of a decrease in reproduction frequency (Tinkle, Wilbur and Tilley, 1970; Fitch, 1981; Cox, Skelly and John-Alder, 2003). The results of our study showed a low level of female-biased SSD in the highland population while this difference was not statistically significant. On the other hand, Gül et al. (2014) reported a strong level of female-biased SSD in a highland population of *D. rudis*.

The adult body size depends on many factors including age at maturity and longevity (Özdemir et al., 2012). According to the studies of Beebee and Griffiths (2000) and Olsson and Madsen (2001), male lizards mature earlier than females in some species. However; age at maturity in both populations was found to be similar for both sexes (2-3 years in Başyayla and 1-2 years in Kamilet) in our study. Conformably, Guarino, Gia and Sindaco (2010) stated that age at maturity and SVL can vary between populations of the same species.

In conclusion, our presented data on body size, longevity and age at sexual maturity of *D. clarkorum* may contribute to conservation efforts for this endangered species. The early age of maturity in the lowland population and the high maximum longevity result in the highland population are considerable data for an endangered species. These results showed the need to perform further long-term studies with specimens of *D. clarkorum* from different climate areas.

Acknowledgements. Language revision was accomplished by Duncan Gullick Lien who is an Instructor at Karadeniz Technical University, School of Foreign Languages in Turkey. The study was carried out by permissions of Ministry of Forest and Water Affairs and Karadeniz Technical University Animal Care and Ethics Committee.

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Submitted: November 20, 2015. Final revision received: November 7, 2016. Accepted: November 7, 2016.
Associate Editor: Sylvain Dubey.