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Age Structure and Body Size of the Endemic Lizard Species *Anatololacerta danfordi* (Günther, 1876) (Reptilia: Lacertidae) in a Highland Turkish Population

Ufuk Bülbül[1], Halime Koç-Gür[1*], Hatice Özkan[1] & Engin Zaman[2]

[1]Department of Biology, Faculty of Science, Karadeniz Technical University, 61080 Trabzon, Turkey
[2]Elvanpazarcık District, Zonguldak, Turkey

Abstract: Some life-history traits (e.g. body size, SSD and age) of the Danford's lizard *Anatololacerta danfordi* were studied applying skeletochronology techniques to the phalangeal bones (n = 33). The mean of the snout-vent-length (SVL) was 59.02 ± 1.07 mm in females and 62.17 ± 1.38 mm in males. The mean SVL was not significantly different between sexes. Sexual size dimorphism was weakly expressed in the slightly bigger SVL of the males (SDI = -0.053). Sexual maturity was attained between the second and the fourth year of life in both sexes. The age ranged from 2 to 6 years in females and from 3 to 8 years in males. The means of age were 4.29 ± 0.31 LAGs in female specimens and 4.63 ± 0.37 LAGs in male specimens. The mean age was not significantly different between the sexes.

Key words: body length; skeletochronology; endosteal resorption; sexual maturity

Introduction

The Danford's lizard *Anatololacerta danfordi* (Günther, 1876) is an endemic lizard species in Turkey. It is distributed in the Mediterranean and Central Anatolia regions of the country (Baran et al. 2012, Bellati et al. 2015, Mert & Kiraç 2017, Beşer et al. 2020). The IUCN Red List of Threatened Species classifies this lizard in the LC (Least Concern) category since 2009 (Tok et al. 2009). The studies on *A. danfordi* are generally focused on distribution (Afsar & Tok 2011, Cihan & Tok 2014, Ege et al. 2015, Kucharzewski 2015, Yavuz & Tunç 2015, Sarikaya et al. 2017, Gidiş & Başkale 2020), habitat suitability (Mert & Kiraç 2017, Kiraç & Mert 2019) and phylogeny (Bellati et al. 2015, Candan et al. 2016). Knowledge on the age structure of *A. danfordi* is limited to the study of Beşer et al. (2020) that compared body size and age structure of the species at different altitudes in Turkey. The age of vertebrates could be estimated by observing the cyclic growth patterns in their bones, i.e. using skeletochronology (Castanet & Smirina 1990, Castanet 1994). Ectotherm vertebrates show chromophilic lines in their bones formed during growing periods and lines of arrested growth (LAGs) corresponding to the resting periods (Castanet & Smirina 1990). The information of the age is obtained by counting the LAGs and it makes skeletochronology a useful method for determining age (Castanet 1994).

There is an increasing interest on age structure and lifespan of reptilian species (Cabezas-Cartes

*Corresponding author: koc.halime@gmail.com

et al. 2018, Cayuela et al. 2019). Data on the age structures (Gül et al. 2015, Yakin & Tok 2015, Bülbül et al. 2016a, Eroğlu et al. 2017), body length (Üzüm et al. 2015, 2018, Gül et al. 2017, Beşer et al. 2019, Kalayci-Ergül et al. 2020) and maturity age (Üzüm et al. 2014, Bülbül et al. 2016b, Odabaş et al. 2019, Yildirim et al. 2019) were reported for several lizard species in Turkey.

The present study aimed to assess some life-history traits (age structure, longevity, body length and SSD) through skeletochronology in a Turkish population of *A. danfordi*.

Materials and Methods

Totally, 33 individuals of *A. danfordi* (16 ♂♂ and 17 ♀♀), were caught from the Aşağı Kırıntı population located in Sütçüler District, Isparta Province, on 26–30 June 2019. The field studies were carried out between 9.00 am and 18.00 pm. The Aşağı Kırıntı population (37°31'729"N, 31°15'008"E) is located in a highland area at an altitude of 841 m a.s.l. The habitat of the population consists of rocky areas along the creeks and walls of houses close to groves. The active period of *A. danfordi* in the studied population lasts from April to October. The average air temperature in daytime was 27°C during the sampling period. The mean annual temperature and precipitation over the past 80 years at the Aşağı Kırıntı site were 12.2°C and 47.51 mm, respectively, according to data of the 4th Meteorology Regional Directorate, Antalya. During the active period of the lizards, the mean temperature and precipitation were 18.5°C and 32.01 mm, respectively, according to data of the meteorological station.

All of the authors worked full time in the field studies for each of the studied days. The lizards were caught by hand and sexed through direct examination of sexual organs (presence of a hemipenis in the cloacal opening of the male individuals). During the breeding season, the neck varies from red to white in adults. The neck is brick-red, especially in mature males. Adult males usually have dark spots in the middle 4 rows or all 6 ventral longitudinal rows. We used these secondary sexual characteristics for identifying the maturity of the lizards. Snout-vent length (SVL) was measured to the nearest 0.01 mm using a digital calliper. We quantified Sexual Size Dimorphism (SSD) using the Lovich & Gibbons (1992) index according to the following formula:

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

For each lizard, the second phalange of the longest finger of the hind limb was clipped and preserved in 10 % solution of formaldehyde for subsequent histological 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 in transverse sections of the middle part of phalangeal diaphyses (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 in the study of Eroğlu et al. (2018). The toes 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 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 h, with periods for changing solutions of 80 min.: ethanol (eight times), xylene (two times) and paraffin (two times). 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 min. Entellan (Merck brand Entellan®new, rapid mounting medium for microscopy) was employed for mounting the stained cross-sections on microscope slides. The cross-sections were examined 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 independently calculated by two observers (H. Özkan & U. Bülbül) and the results were compared. Not always the double lines represented real two LAGs due to the species having two arrested growth periods in the same year. However, most of the environmental influences affecting growth of a LAG occur within one year and the observed double lines were considered as one LAG in the age determination (Aragón & Fitze 2014). We considered the double line as one LAG in our study as proposed by Guarino & Erismis (2008), Aragón & Fitze (2014) and Bülbül et al. (2018). 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 observed was taken to mark the age when sexual maturity had been achieved (Ryser 1998, Özdemir et al. 2012, Bülbül et al. 2016a).

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 processed with IBM SPSS 22.0 for Windows.

Results

A growth zone and a thin haematoxylinophilic outer line corresponding to a winter line of arrested growth were present in cross sections of the phalanges in 100 % (n = 33) of adult individuals (Fig. 1). The resorption zone did not reach the first LAG in all specimens. The resorption zone clearly seemed out of the endosteal bone in all preparations and never created difficulty for age determination. We observed double line and endosteal resorption in six (18 %) and four (12 %) individuals, respectively. The age at maturity was 2–4 years for both sexes. The age at maturity was two years (47.1 %), three years (47.1 %) and four years (5.9 %) in females, while it was two years (50.0 %), three years (43.8 %) and four years (6.2 %) in males.

The means of SVL and age were, 60.55 ± 0.90 mm and 4.45 ± 0.24 for all individuals of *A. danfordi* (59.02 ± 1.07 mm and 4.29 ± 0.31 in females and 62.17 ± 1.38 mm and 4.63 ± 0.37 in males), respectively (Table 1).

Age ranged from 2–6 years in females and 3–8 years in males. The mean age of the specimens was not significantly different between the sexes (Independent Sample T-Test; t = -0.676, df = 31, P = 0.50). Intersexual differences in body size (length) were slightly male-biased (SSD = -0.05). The mean SVL (t = -1.806, df = 31, P = 0.08) was not significantly different between the sexes. There was a

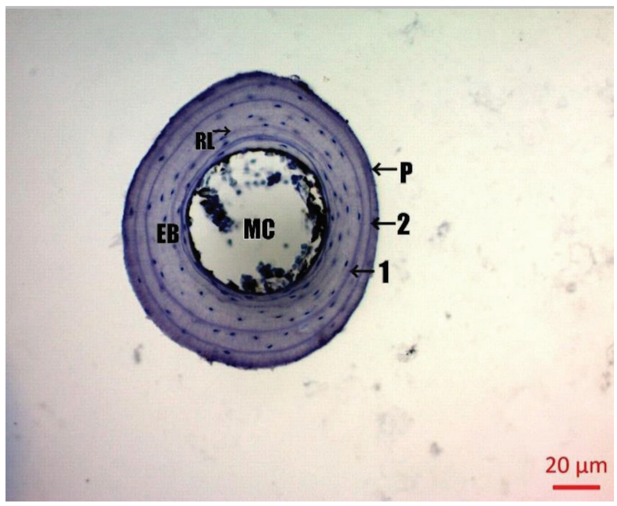

Fig. 1. Cross-section (10 µm thick) of a phalange of a two-year-old female (50.57 mm SVL) of *Anatololacerta danfordi* from the Aşağı Kırıntı population. Abbreviations: MC, marrow cavity; EB, endosteal bone; RL, resorption line; P, periphery. Periphery was not counted as a LAG.

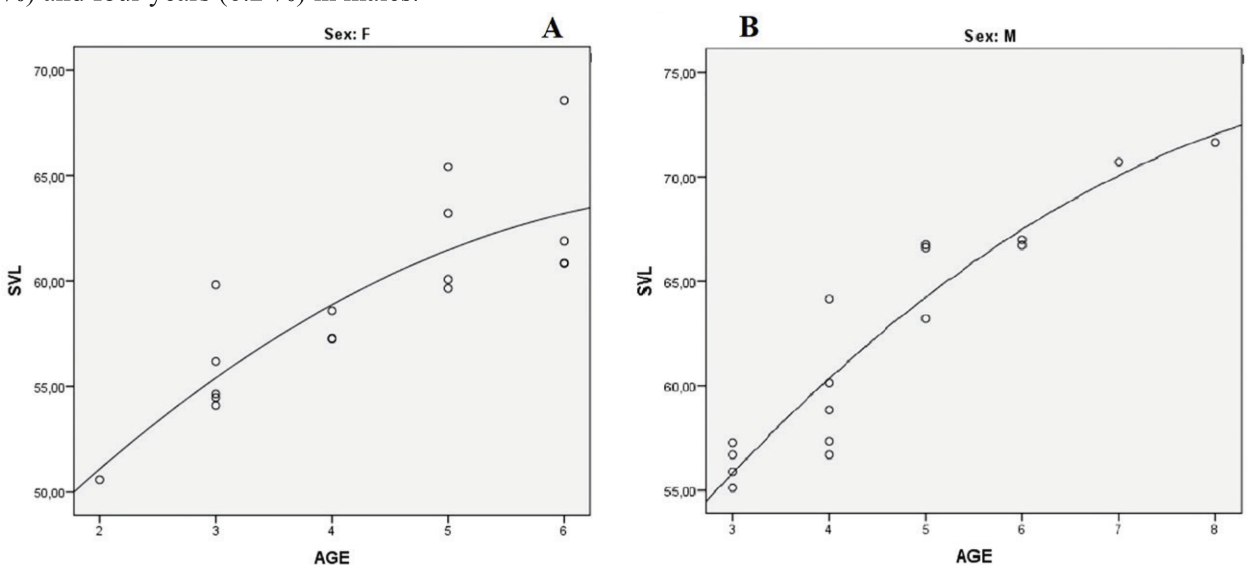

Fig. 2. The correlation charts between age and SVL of *Anatololacerta danfordi* from the Aşağı Kırıntı population (SVL: mm).

Table 1. Descriptive statistics of snout-vent length (SVL, mm) and age (years) of the Aşağı Kırıntı population of *Anatololacerta danfordi* (Günther, 1876). Abbreviations: *n*, number of samples; SE, standard error.

Charac-ters	Sex	*n*	Mean	Range	SE
SVL	♀♀	17	59.02	50.57-68.56	1.07
Age		17	4.29	2-6	0.31
SVL	♂♂	16	62.17	55.12-71.62	1.38
Age		16	4.63	3-8	0.37
SVL	♀♀+♂♂	33	60.55	50.57-71.62	0.90
Age		33	4.45	2-8	0.24

strong correlation between SVL and age for female (Pearson's correlation coefficient ($r = 0.827$, $P \leq 0.01$) and male ($r = 0.927$, $P \leq 0.01$) individuals of the species (Fig. 2).

Discussion

The lizards inhabiting different habitats are affected by environmental factors (e.g. climate conditions, length of the active period) determined by altitude and latitude; these factors affect lizard populations by generating differences in age structure and longevity (Wapstra et al. 2001, Roitberg & Smirina 2006, Roitberg 2007).

Double lines are irregularities in bone deposition caused by unpredictable ecological factors, such as dry period, hot climate, food availability and other conditions (Jakob et al. 2002, Guarino & Erismis 2008, Özdemir et al. 2012). The individuals of *A. danfordi* in Aşağı Kırıntı population were not exposed to hot climate during their activity season and we found a low rate of double lines in six (18 %) specimens. Endosteal resorption in bone specimens also may be related to environmental conditions (Smirina 1972). We found a low rate of endosteal resorption in four (12 %) specimens in the Aşağı Kırıntı population. Due to hot climate in Kozan and Saimbeyli populations, Beşer et al. (2020) reported that that endosteal resorption completely or partially destroyed the first LAG of almost all cross-sections. On the other hand, Caetano & Castanet (1993) reported fewer endosteal resorptions in lowland populations than in highland populations. Contrary to their findings, Beşer et al. (2020) found higher endosteal resorption rate in the lowland population of *A. danfordi*. The lower endosteal resorption rates were also reported in the highland areas where other lizard species were located (Arakelyan et al. 2013, Gül et al. 2014). All these results show that daily and annual activity and climate conditions are the probable determinant of bone resorption of long bones in animals as mentioned in the studies of Hemelaar (1988), Esteban (1990), Leclair (1990), Augert (1992) and Esteban et al. (1999).

We found the mean age was 4.29 years (2–6 years in range) in females and 4.63 years (3–8 years in range) in males of the Aşağı Kırıntı population (located at 841 a.s.l.) of *A. danfordi*. However, Beşer et al. (2020) found higher mean age for the Kozan population (678 m a.s.l.) – 8.33 years in females (ranging 5–11 years) and 8.73 years in males (5–13 years) as well as for the Saimbeyli population (1200 m a.s.l.) – 5.78 years in females (3–8 years) and 7.25 years in males (4–9 years). The adult lizards could survive longer and were older in the high-altitude population as compared to the low-altitude population (Lu et al. 2016). However, Beşer et al. (2020) reported a lower mean age and longevity in the highland population of the species. This result may be associated with the fewer analysed specimens (only 9 females and 8 males) in the highland population than in the lowland population (9 females and 22 males). Although the Aşağı Kırıntı population studied by us occupies a relatively close altitude with the Kozan population, we found lower mean age and longevity in 17 females and 16 males of *A. danfordi*. Kozan and Saimbeyli populations are located in the Adana Province of Turkey, thus the individuals of *A. danfordi* live in warmer climate than those of the Aşağı Kırıntı population. These results suggest that ambient temperature is more important than altitude for the average age of these lizards. There are some studies showing that high altitude alone is not a predictor of higher average age and the lizards living at high altitudes may have a lower average age (Guarino et al. 2010, Gül et al. 2014, Üzüm et al. 2018). In fact, the local specific conditions (e.g. predation, food availability, length of the activity period and other environmental factors) may also be determinant of the average age and longevity in those populations (Aşağı Kırıntı, Kozan and Saimbeyli) of *A. danfordi*.

The mean SVL of males was slightly bigger than that of females in the Aşağı Kırıntı population of *A. danfordi*. Similar to our findings, Beşer et al. (2020) reported statistically non-significant variation in SVL between sexes in the Kozan population (located at 678 m a.s.l.). However, males were significantly longer than females in the Saimbeyli population (located at 1200 m a.s.l.). Intraspecific variation in body size along with latitudinal and altitudinal gradients is common in ectothermic animals (Endler 1992, Yamahira & Conover 2002, Sears 2005), with lizards from northern latitudes being larger than those from southern latitudes, and lizards from lower elevations being smaller than those at higher elevation. Although having less opportunity for activity on both daily and annual basis, lizards in colder environments grow larger (Sears & Angiletta 2004). However, this was not the case for Kırıntı, Kozan and Saimbeyli populations of *A. danfordi* based on the mean SVL in these populations.

Sexual dimorphism may evolve because of competition between the sexes for food or any 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 *A. danfordi* observed in the present study and by Beşer et al. (2020), presence or absence of male-male combat and fecundity selection is unknown. Therefore, it is not possible to say that similar body size differences between the sexes is due to any kind of selection.

The patterns of sexual size dimorphism (SSD) variation may occur due to abiotic and biotic factors (Bülbül et al. 2016b). 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). The larger sex also tends to have higher survival, which can contribute to SSD as well (Roitberg 2007). There are no data comparing the factors that can be more effective on SSD in the three studied populations of *A. danfordi*.

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 2010, Lyapkov et al. 2010, Liao et al. 2013, 2015). Longevity was slightly higher in males and age at sexual maturity was similar between both sexes in the present study. Consistent with this, there was not statistically different the SSD in the Aşağı Kırıntı population. Similar to our findings, Beşer et al. (2020) found a slightly higher longevity in males and reported that the mean SVL differences between sexes were not statistically significant in the Kozan population. However, they found a slightly higher longevity in males but statistically significant male-biased SSD in the Saimbeyli population.

We performed the Pearson correlation test to determine how age and body length were correlated. The values were found to be statistically significant in both sexes ($r = 0.827$, $P \leq 0.01$ for females) and ($r = 0.927$, $P \leq 0.01$ for males) in the studied population (Fig. 2).

In conclusion, our data on an *A. danfordi* population may contribute to the knowledge on life-history traits of the species. Results of our study and findings of Beşer et al. (2020) indicate that the age structure of the Danford's lizard may change according to the environmental conditions of the natural habitats where the populations live. Further long-term and detailed studies on populations of this species may reveal the environmental factors affecting mean age, SVL, longevity and age at maturity of *A. danfordi*.

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