

Age and growth in two populations of Danford's lizard, *Anatololacerta danfordi* (Günther, 1876), from the eastern Mediterranean

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Abstract: The age and growth in two breeding populations of *A. danfordi*, inhabiting altitudes ranging from 678 m a.s.l. to 1200 m a.s.l. in Turkey, were investigated. The age differences between sexes were not statistically significant in either population. The mean age was calculated as 8.73 ± 2.12 and 8.33 ± 1.8 years in Kozan and 7.25 ± 1.58 and 5.78 ± 1.64 years in Saimbeyli for males and females, respectively. The age distributions of males did not statistically differ between populations, but females in Kozan were significantly older than those in Saimbeyli. The mean snout-vent length (SVL) difference between sexes was not significant in the Kozan population, whereas males were significantly longer than females in Saimbeyli. However, SVL was similar between the populations. Intersexual differences in body size were found to be male-biased for both populations. There was a positive and statistically significant relationship between SVL and age in both populations. This study examines the feasibility of the use of skeletochronology for *A. danfordi*. It also shows that these populations are getting older and this may endanger the future of *A. danfordi* in a warming world. Some life history parameters and population structures of *A. danfordi* are provided in this study but some questions still remain.

Key words: Skeletochronology, sexual size dimorphism, lizards, endemic species, Turkey

1. Introduction

The Mediterranean Basin occupies a position in a shifting zone between midlatitude and subtropical atmospheric circulation regimes (Cramer et al., 2018). This region already suffers from water insufficiency and episodes of drought (Chenini, 2010), but more is yet to come. The average temperature in the Mediterranean region will exceed the global rate by 25%, accompanied by high-temperature events (Lionello and Scarascia, 2018). A total 2 °C temperature increase of the globe will probably result in 10%–15% decreased rainfall during summer in southern France, northwestern Spain, and the Balkans, and that may reach 30% in Turkey and Portugal (Vautard et al., 2014). Danford's lizard from Turkey, *Anatololacerta danfordi* (Günther, 1876), is one of the many species that are living at the frontier of climate change in the Mediterranean basin and will suffer the consequences of many factors. All population clusters occupy an area within the eastern part of the southern Anatolian peninsula, from Abanoz to the Bolkar Mountains (Bellati et al., 2015), reaching the northern parts of Adana province (Baran et al., 2012). Their natural environment preference comprises rocky

surfaces in forested or wooded areas and Mediterranean-type shrubby vegetation (Tok et al., 2009). *A. danfordi* is the type species for the genus *Anatololacerta* that can only be found in western and southern Asiatic Turkey and some neighboring Greek islands (Arnold et al., 2007). It is endemic to Turkey and categorized as "Least Concern" in the IUCN Red List of Threatened Animals (Tok et al., 2009). This distribution area was considered to have a crucial role for diversification, especially of some reptile taxa, by Bellati et al. (2015); however, its future is uncertain owing to climate change.

In spite of all these circumstances, there is no investigation about any life history traits of *A. danfordi* populations. Life history includes the growth, development, and reproduction model of an organism throughout its life (Sarasola-Puente et al., 2011) and can help to create conservation strategies. Environmental selection pressures such as the time span of the breeding season, altitude, and temperature cause variations in the aforementioned characteristics. Individuals principally must decide how to allocate their energy between growth and reproduction (Kozłowski, 1996; Kolarov et al., 2010).

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For instance, species with high mortality should mature early (Kozłowski, 1996), because energy intended for growth would be wasted. Consequently, this results in small body size. Conversely, having a larger body might be a product of low mortality (along with delayed maturity) (Kozłowski, 1996), size-dependent fecundity success (Wikelski and Romero, 2003; Kolarov et al., 2010), defending a breeding territory (Tinkle, 1970; Kolarov et al., 2010), and so on. Animals possess their own optimal strategy that is modulated by the forenamed factors in a given environment. The vicissitude in life history traits between generations or populations occurs with age patterns as evidence (Gül et al., 2014).

Here we present the first study about the age structure and some life history traits of *A. danfordi*. We wanted to successfully use the skeletochronological method for age determination in two *A. danfordi* populations for the first time. Skeletochronology, which is used to estimate individual age by counting growth rings, also known as lines of arrested growth (LAGs), from the cross-sections of long bone tissue, is the most expedient way as it shortens the time of the study and does not require any animals to be sacrificed (Castanet and Smirina, 1990). In addition to this, skeletochronology has yielded successful results in previous studies on lizards such as *Acanthodactylus boskianus* (Üzüm et al., 2014), *Acanthodactylus harranensis* (Beşer et al., 2019), *Anatololacerta anatolica* (Yakın and Tok, 2015), *Apathya cappadocica* (Gül et al., 2015a), *Darevskia rudis* (Gül et al., 2014), *Dinarolacerta mosorensis*

(Kolarov et al., 2010), *Eremias argus* (Kim et al., 2010), *Lacerta agilis* (Guarino et al., 2010), *Phoenicolacerta laevis* (Üzüm et al., 2018), *Podarcis lilfordi* (Rotger et al., 2016), and *Podarcis tauricus* (Eroğlu et al., 2017). We tested the correlation of the age structure and some morphological characteristics of the two *A. danfordi* populations. Finally, we discussed the similarities and differences between our species and closely related ones that have been previously studied.

2. Materials and methods

A total of 31 *A. danfordi* specimens (22 ♂♂, 9 ♀♀) from Kozan (37°35'N, 35°50'E; 678 m a.s.l.) and 17 specimens (8 ♂♂, 9 ♀♀) from Saimbeyli (38°01'N, 36°05'E; 1200 m a.s.l.) in Adana province of Turkey (Figure 1) were used in this study. The Kozan population was located around the Kilgen River and Kozan Dam. Specimens were collected from a rocky area that is not far away from a small river. The area where specimens were observed has annual herbaceous and scrub plants. Specimens were caught while they were browsing on rocks. Kozan has mild and rainy climate characteristics. The mean annual temperature and precipitation in Kozan is 14.2 °C and 620 mm, respectively (Beşer, 2015). Saimbeyli is located in a highland area. Lizard samples were collected under stones along the the Obruk waterfall. The collection area was mainly covered by Austrian pine (*Pinus nigra*), oak (*Quercus* sp.), and cedar (*Cedrus libani*) trees. The climate is hot and dry in the summer, while winter is usually mild and rainy. The

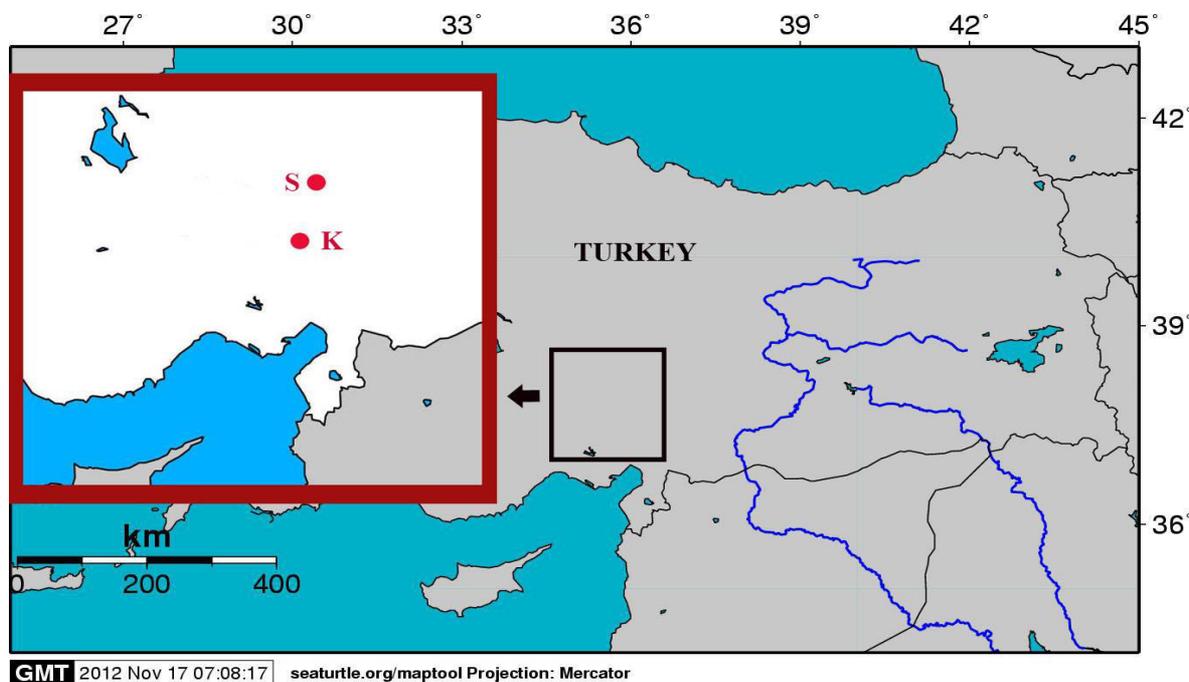


Figure 1. Map showing localities of studied *A. danfordi* populations. K: Kozan population, S: Saimbeyli population.

precipitation is concentrated in the winter season. The annual mean temperature and precipitation of Saimbeyli is 11.7 °C and 592 mm, respectively (Beşer, 2015). August is the driest month with 7 mm of precipitation with a mean of 88 mm precipitation. The maximum rainfall is recorded in December. The activity period for lizards varies from early May to late September in Saimbeyli and from early April to late October in Kozan (authors' observations). The temperature was recorded as approximately 20 °C and 26 °C, respectively, for Saimbeyli and Kozan during the sampling period in May.

No animals were specifically sacrificed for this study. They were already collected owing to a scientific project overseen by TÜBİTAK (Scientific and Technological Research Council of Turkey) (number of permission to capture: B.23.0.DMP.0.15.01-510.02-2943, from the Ministry of Forest and Water Affairs). The animals were treated in accordance with the guidelines of the local ethics committee (DEU.02/2012) and deposited in the Zoology Laboratory of Dokuz Eylül University (İzmir, Turkey). The lizards were captured by hand. The presence/absence of a hemipenis retracted in the hemipenial sack at the base of the tail and direct examination of the secondary sexual characteristics (e.g., larger abdomen width in females, larger head length and bright coloration during the field work in males especially at reproduction time) were used to assess individuals' sex. The snout-vent length (SVL) of specimens was measured in the laboratory using a digital caliper compass with an accuracy of 0.02 mm. SVL was used to study sexual size dimorphism (SSD) between sexes and age groups, and to present relationships between body length and age in *A. danfordi* specimens.

Sexual dimorphism index (SDI) was quantified with the index of Lovich and Gibbons (1992) according to the following formula:

$$SDI = [(mean\ length\ of\ the\ larger\ sex / mean\ length\ of\ the\ smaller\ sex) \pm 1],$$

with +1 if males are larger or -1 if females are larger. It is defined as positive whenever females are larger than males and negative in the converse case.

Age was determined by using the skeletochronological approach. The LAGs were counted on cross-sections of the

middle part of the phalangeal diaphysis taken from the middle phalanx of the longest (middle) finger of the right hind foot. The skeletochronological analysis was adapted from its standard procedure as previously used by Üzümlü et al. (2014, 2015): phalanges, which had been preserved in 70% ethanol solution, were washed in tap water for 24 h and then decalcified in 5% nitric acid solution for 2 h. Later, the washing procedure was repeated again for another 12 h. Cross-sections (18 µm) from the diaphyseal region of the phalanx were obtained by using a freezing microtome and were dyed with Ehrlich's hematoxylin. Then all the sections were examined under a stereomicroscope. The considerably good sections were placed in glycerin in order to conveniently observe them by light microscope. Bone sections from each individual lizard were photographed at the same magnification setting. All photographs were examined and the analysis of LAGs was performed by N.B. and N.Ü. according to a previous technique that has become standard. The proportion of endosteal resorption (remodeling bone process that can reabsorb parts of or entire LAGs) was assessed by comparing the diameters of eroded marrow cavities with the diameters of noneroded ones (e.g., Üzümlü et al., 2014).

All numerical data were analyzed using STATISTICAL ANALYSIS SYSTEM (Stat Soft Inc., USA) with the probability level of $P \leq 0.05$ considered significant. Normal distribution of data was controlled according to skewness and kurtosis values and the Shapiro-Wilk test. Age and size were compared using parametric tests (t-test). The Spearman correlation coefficient was calculated to understand the relationship between age and SVL. The most suitable distribution curves were drawn according to R^2 values.

3. Results

Descriptive statistics of SVL and age are summarized in Table 1. SVL measurements for both populations were normally distributed. In the Kozan population, the mean SVL differences between sexes were not statistically significant ($t = 0.659$, $df = 20.83$, $P = 0.571$), but in the Saimbeyli population males were significantly longer than females ($t = 2.487$, $df = 14.964$, $P = 0.025$). Meanwhile, a male-biased sexual size dimorphism was found in both

Table 1. Descriptive statistics for SVL (mm) and age (number of hibernations) (N: number of specimens; SE: standard error).

Population	Sex	N	Mean SVL ± SE	Range SVL	Mean age ± SE	Age range
Kozan	Male	22	57.71 ± 6.21	44.62–68.06	8.73 ± 2.12	5–13
	Female	9	56.2 ± 4.44	49.72–62.36	8.33 ± 1.8	5–11
Saimbeyli	Male	8	59.27 ± 5.75	48.58–67.50	7.25 ± 1.58	4–9
	Female	9	52.05 ± 6.21	43.66–59.60	5.78 ± 1.64	3–8

populations ($SDI_{Kozan} = -0.02$, $SDI_{Saimbeyli} = -0.13$). SVL differences between populations were not statistically significant either in males ($t = 0.728$, $df = 13.396$, $P = 0.479$) or females ($t = -1.627$, $df = 14.494$, $P = 0.125$).

The aestivation line and endosteal resorption were observable from the cross-sections (Figure 2) and caused difficulties in counting LAGs. Endosteal resorption was observed in almost all cross-sections. We determined that endosteal resorption completely destroyed only the first LAG [3 males (13.64%) and 2 females (22.22%) from Kozan population, only 2 males (25%) from Saimbeyli population] or partially [17 males (77.27%) and 5 females (55.56%) from Kozan population, 3 males (37.5%) and 2 females (22.22%) from Saimbeyli population]. The age differences between sexes were not statistically significant in either Kozan ($t = 0.524$, $df = 17.479$, $P = 0.607$) or Saimbeyli ($t = 1.882$, $df = 14.884$, $P = 0.08$) populations. Mean ages of Kozan and Saimbeyli males were not statistically different from each other ($t = -2.055$, $df = 16.755$, $P = 0.056$). Kozan females were significantly older than Saimbeyli females ($t = -3.144$, $df = 15.861$, $P = 0.006$). When we tested age differences between populations without considering sexual dimorphism, the Kozan population was found to be older than the Saimbeyli population ($t = -3.861$, $df = 37.408$, $P < 0.001$). Age-dependent SVL measurements are given in Table 2.

The Spearman correlation test was used to determine how age and body length were correlated (Figure 3). It was found to be statistically significant in both populations ($r = 0.942$, $P < 0.001$ and $r = 0.953$, $P < 0.001$ for Kozan males and females respectively; $r = 0.894$, $P < 0.001$ and $r = 0.953$, $P < 0.001$ for Saimbeyli males and females respectively).

4. Discussion

Reptiles continue growing after they have fully matured (Kozłowski, 1996). Body length, growth rates, age at maturity, and longevity of animals can widely vary between populations (Guarino et al., 2010). The life style and strategies of animals and the habitat conditions may impact these traits during an animal's lifetime. Within this framework, we revealed and tested some quantitative characteristics of *A. danfordi* populations and compared our results with previous studies.

Male-biased SSD was found in both populations. An inconsistency in results can be observed in previous research. While predominantly male-biased results were obtained in previous studies (Kim et al., 2010; Kolarov et al., 2010; Üzümlü et al., 2014, 2015; Gül et al., 2015a, 2015b; Bülbül et al., 2016a), female-biased results were also found in some research (Gül et al., 2014; Bülbül et al., 2016b). SSD is thought to be a complex matter that is the consequence of several interacting factors (Roitberg and Smirina, 2006). Particularly, male-biased SSD has been frequently correlated with male-male competition for intercourse pairing success (Hews, 1990; Üzümlü et al., 2014). However, Cox et al. (2007) stated that across the lizard families, the mean SSD is strongly male-biased and Rensch's rule is evident in territorial species. Our data about initial characteristics of populations such as sexual selection pressure, courtship behavior, and competition between males is fairly limited. Thus, these data are insufficient to understand the main reason for this particular male-biased SSD. Apart from different possible causes, this could be a result of continued growth as males and females spend their energy on reproducing more than growing after maturation (Kolarov et al., 2010). However, Yakın and

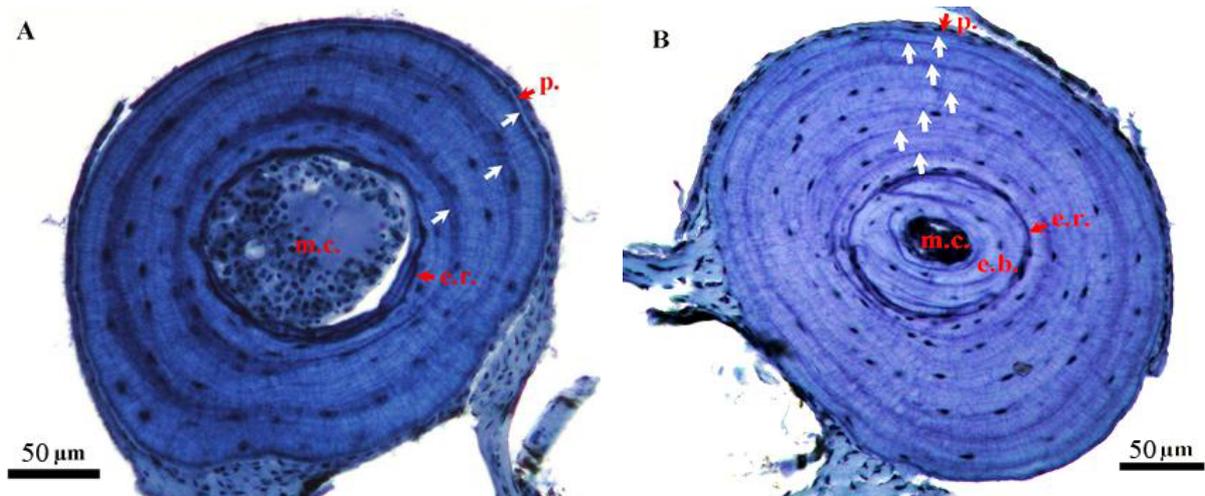


Figure 2. Cross-sections from *A. danfordi* phalanges. A) Female lizard with 3 hibernations and B) male lizard with 3 hibernations. e.b.: Endosteal bone, e.r.: endosteal resorption, m.c.: medullary cavity, p.: peripheral, white arrows: LAGs.

Table 2. SVL (mm) measurements of age classes (N: number of specimens; Min: minimum; Max: maximum; SE: standard error).

	Age class	Population	N	SVL		
				Mean	Min–Max	SE
♂♂	4	Saimbeyli	1	48.58		
	5	Kozan	1	48.40		
	6	Kozan	3	48.42	44.62–52.88	4.16
	7	Kozan	3	52.34	50.50–53.34	1.6
		Saimbeyli	4	57.83	55.88–58.78	1.33
	8	Kozan	3	57.59	56.16–58.90	1.37
		Saimbeyli	1	64.40		
	9	Kozan	3	58.29	56.38–60.34	1.98
		Saimbeyli	2	64.96	62.42–67.50	3.59
	10	Kozan	5	61.47	58.54–65.26	2.65
	11	Kozan	2	62.93	61.26–64.60	2.36
	12	Kozan	1	65.58		
	13	Kozan	1	68.06		
♀♀	3	Saimbeyli	1	43.66		
	4	Saimbeyli	1	45.90		
	5	Kozan	1	49.72		
		Saimbeyli	2	46.89	45.88–47.90	1.42
	6	Saimbeyli	1	53.54		
	7	Kozan	1	51.70		
		Saimbeyli	3	57.34	56.26–58.04	0.95
	8	Kozan	4	55.14	52.18–56.84	2.05
		Saimbeyli	1	59.60		
10	Kozan	2	60.72	60.64–60.80	0.11	
11	Kozan	1	62.36			

Tok (2015) reported that females were larger than males in a study of *A. anatolica* from Çanakkale, Turkey, based on age structure. Even though both species belong to the same genus and *A. danfordi* is located at a higher altitude, the average SVL of female *A. anatolica* is bigger than that of *A. danfordi* while it is still similar in males (Table 3). Kim et al. (2010) reported that SVL did not differ between female and male Mongolian racerunners (*Eremias argus* Peters, 1869). *Darevskia clarkorum* males, collected from Başyayla, (Artvin, Turkey), displayed lower mean SVL than females (Bülbul et al., 2016b). On the other hand, *Apathya cappadocica* males were reported to be larger than females with male-biased SSD in three populations (Gül et al., 2015a). SVL difference between sexes was also observed in various studies about different lizard species previously (see Table 3).

The general expectation for reptile body size, considering altitude, is that they have smaller body size than the ones from higher altitudes (Roitberg and Smirina, 2006). This situation was not observed in the *A. danfordi* populations. However, in ectotherms, neither the generality nor the possible adaptive significance of body size is clear under particular climatic conditions. Previous studies about lizards, either within or between species, have produced conflicting results (Oufiero et al., 2011). *A. danfordi* can be referred to as having smaller body size than other lizard species from different altitudes (Table 3).

Duellman and Trueb (1986) referred to many factors that can be effective on the age at maturity. Females from Kozan were found to be older than Saimbeyli females without any significant body length difference. With more thorough investigation, Kozan females had smaller

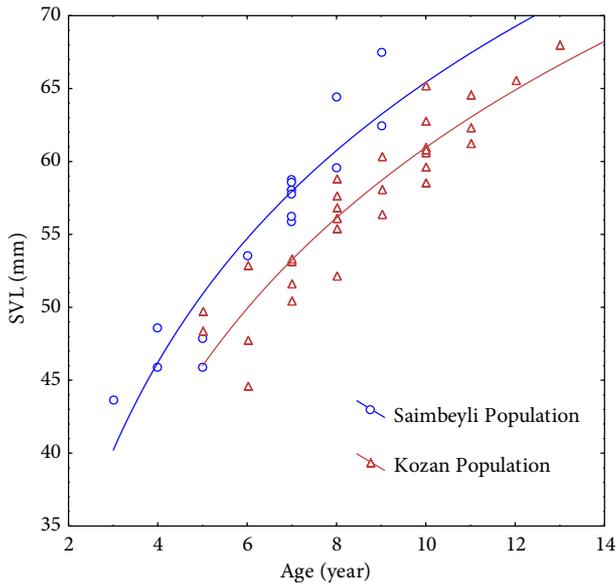


Figure 3. Age and SVL correlation in *A. danfordi* populations. Age: Number of hibernations, SVL: mm.

body size than Saimbeyli females especially at the ages of 7 and 8 (see Table 2), while there was a reverse situation in class age 5. More energy may be allocated to reproduction after age 5 in Kozan females. Juveniles are particularly needed to determine the certain age of maturity and to measure the asymptotic size in order to obtain precise results about this subject. Yakın and Tok (2015) reported that *A. anatolica* individuals need 3 years to mature. Each of our populations had different environmental conditions such as water resources. The Saimbeyli population was captured on a water front and only two other reptile species [*Mediodactylus kotschy* (Steindachner, 1870) and *Phoenicolacerta laevis* (Gray, 1838)] were observed during field work. In comparison to the Saimbeyli population, the Kozan population needs to cover much more distance in order to reach water resources. Also, more amphibian and reptile species [*Bufo variabilis* (Pallas, 1769); *Testudo graeca* Linnaeus, 1758; *Mediodactylus orientalis* (Stepanek, 1937); *Blanus alexandri* Sindaco, Kornilios, Sacchi & Lymberakis, 2014; *Stellagama stellio* (Linnaeus, 1758); and

Table 3. The mean SVL/body size (mm) of *A. danfordi* and some other lizards from different altitudes (N: number of specimens).

Species	Location	♂♂		♀♀		Altitude	Reference
		SVL	N	SVL	N		
<i>Anatololacerta danfordi</i>	Kozan, Turkey	57.71	22	56.2	9	687 m	This study
	Saimbeyli, Turkey	59.27	8	52.05	9	1200 m	
<i>Anatololacerta anatolica</i>	Çanakale, Turkey	57.39	6	63.62	29	2 m	Yakın and Tok (2015)
<i>Acanthodactylus boskianus</i>	Şanlıurfa, Turkey	79.09	9	65.9	8	445 m	Üzüm et al. (2014)
<i>Acanthodactylus harranensis</i>	Şanlıurfa, Turkey	81.66	9	76.92	11	360 m	Beşer et al. (2019)
<i>Apathya cappadocica</i>	Kilis, Turkey	72.13	6	72.01	14	697 m	Gül et al. (2015b)
	Şanlıurfa, Turkey	72.24	9	63.78	12	891 m	
	Diyarbakır, Turkey	69.97	8	65.44	7	1058 m	
<i>Darevskia bithynica</i>	Zonguldak, Turkey	63.2	10	61.66	8	50 m.	Gül et al. (2015a)
	Bolu, Turkey	63.67	20	58.87	16	1200 m.	
<i>Darevskia clarkorum</i>	Kamilet, Artvin, Turkey	62.46	25	60.09	29	450 m.	Bülbül et al. (2016b)
	Başyayla, Artvin, Turkey	58.31	14	59.18	17	2250 m.	
<i>Darevskia rudis</i>	Çermik, Artvin, Turkey	75.6	13	74	15	700 m	Gül et al. (2014)
	Borçka, Artvin, Turkey	68.2	5	70.6	10	1277 m	
	Ardanuç, Artvin, Turkey	60.9	9	66	10	2137 m	
<i>Darevskia parvula</i>	Kemalpaşa, Artvin, Turkey	52.83	28	50.3	17	17 m.	Bülbül et al. (2016a)
	Pırnallı, Artvin, Turkey	55.21	22	53.27	22	1484 m.	
<i>Eremias suphani</i>	Van, Turkey	60.88	16	58.85	7	2180 m	Üzüm et al. (2015)
<i>Lacerta agilis</i>	Northwest Italy	69.3	11	73.9	22	1790–1890 m	Guarino et al. (2010)
<i>Phoenicolacerta laevis</i>	Anamur, Mersin, Turkey	62.42	25	59.21	28	22 m	Üzüm et al. (2018)
<i>Podarcis tauricus</i>	Sergen, Kırklareli, Turkey	61.81	20	60.5	20	450 m.	Eroğlu et al. (2017)

Dolichophis jugularis (Linnaeus, 1758)] were observed during field work. The fact that the Kozan population shares its living area with many species might cause increased competition for resources and predatory pressure and finally more energy spent on reproduction.

Observed longevity of *A. danfordi* is higher for the Kozan population than Saimbeyli (see Table 2). Similarly, Gül et al. (2015a) reported that a lower altitudinal population of *Apathya cappadocica* from Kilis (697 m a.s.l.) had higher longevity than a higher altitude population from Diyarbakır, Turkey (1058 m a.s.l.). Similar results were also observed in *Darevskia rudis* by Gül et al. (2014). Additionally, a study of a *Timon lepidus* population from a hot region, where the annual activity was reduced by aestivation, showed higher average age rates than a population living in a cooler and mesic environment (Mateo and Castanet, 1994; Roitberg and Smirina, 2006). Even though the metabolism of an animal becomes lower during aestivation and hibernation, the time span of these two periods might differ. The effects of prolonged periods of nonactivity at different times of the year will produce different physiological reactions. During hibernation, cold temperature lowers metabolism and its duration is mostly dependent on the winter period. Aestivation, in general, comprises a strong metabolic diminution and methods of water retention (Storey, 2002).

As a result of climate change, fresh water availability is likely to decrease. Particularly in the south and eastern Mediterranean, where water deficiency is already being faced, river flow will be commonly reduced (Forzieri et

al., 2014). The water level in lakes is expected to follow this event. For example, the largest Mediterranean lake in Turkey, Beyşehir, which is within the *A. danfordi* distribution range (Tok et al., 2009), may dry out by the 2040s if the current outflow regime does not change (Bucak et al., 2017). Most individuals in our study cluster at older ages (Table 2). As squamate brood frequency is negatively correlated with longevity (Scharf et al., 2015), the shortage in water resources will highly risk the future of these aging populations.

In this study, the skeletochronological method was used successfully for age determination of two *A. danfordi* populations for the first time. Beside this, the body size (SVL) of animals was tested and some environmental characteristics were interpreted through age structure. Further investigation of the ecology of such populations will have crucial importance for future conservation strategies where actions are needed as the world is getting warmer. This study provided insights into some characteristics, but nevertheless, further questions remain.

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