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Age Structure and Body Size Variation in Populations of *Darevskia bithynica* (Méhely, 1909) (Reptilia: Lacertidae) from Different Altitudes in North-western Turkey

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Abstract: We present the first data of body size and age structure (based on the skeletochronological examination) of *Darevskia bithynica* from two localities characterised with different altitudes, i.e. Zonguldak (50 m) and Bolu (1200 m). Males from both lowland and highland populations were slightly larger than females. This difference was not significant for the lowland population. The maximum lifespan was found to be six years for both populations. Intersexual differences in snout-vent length were male-biased and this may be linked to the climatic conditions.

Key words: Age structure**,** *Darevskia bithynica*, sexual size dimorphism, skeletochronology, Turkey

Introduction

The lacertid genus *Darevskia* Arribas, 1997 includes 31 species. It has a wide distribution: from Armenia, Azerbaijan, Georgia, Iran and Turkey in Western Asia, to Bulgaria, Greece, Romania, Russia, Serbia and Turkmenistan in Eastern, South-eastern and Central Europe (Arnold *et al.* 2007, Grechko *et al.* 2007, Ahmadzadeh *et al.* 2013). Thirteen species of the genus *Darevskia* occur in Turkey: *D. armeniaca* (Méhely, 1909), *D. bendimahiensis* (Schmidtler, Eiselt and Darevsky, 1994), *D. bithynica* (Méhely, 1909), *D. clarkorum* (Darevsky and Vedmederja, 1977), *D. derjugini* (Nikolskij, 1898), *D. nairensis* (Darevsky, 1967), *D. parvula* (Lantz and Cyrén, 1913), *D. praticola* (Eversmann, 1843), *D. raddei* (Boettger, 1892), *D. rudis* (Bedriaga, 1886), *D. sapphirina* (Schmidtler, Eiselt and Darevsky, 1994), *D. unisexualis* (Darevsky, 1966), *D. uzzelli* (Darevsky and Danielyan, 1977) and *D. valentini* (Boettger, 1892) (see Ananjeva *et al.* 2006; Baran *et al.* 2012; Arribas *et al.* 2013). *Darevskia bithynica* has two subspecies, the nominotypical *D. b. bithynica* (Méhely 1909) and *D. b. tristis* (Lantz and Cyrén, 1936), inhabiting north-west of Anatolia (Arribas *et al.* 2013). It is a small lacertid with length approximately up to 75 mm. It lives mostly on rock exposures, uses crevices as refuges, and prefers habitats in and around herbaceous vegetation (Arribas *et al.* 2013). Since *Darevskia bithynica* was raised to the species level by Arribas *et al.* (2013), This should be changed as "It has not yet been assessed for the IUCN Red List"

The determinations of age, growth rates and stage duration in amphibians and reptiles have relied largely on the skeletochronological method. It uses growth marks in bone tissue or lines of arrested growth (LAG, see Castanet *et al.* 1993). Numerous scientists have applied this technique to lizards (e.g. Arakelyan 2002, Roitberg and Smirina 2006a, 2006b, Roitberg 2007; Kuo *et al.* 2009, Guarino *et al.* 2010, Guarino 2010, Kim *et al.* 2010, Altunişik *et al.* 2013, Arakelyan *et al.* 2013, Gharzi and Yari

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2013, Gül *et al.* 2014, Üzüm *et al.* 2014, Gül *et al.* 2015, Üzüm *et al.* 2015, Yakin and Tok 2015).

In this study, we present the first information on the age structure, body size and lifespan of individuals of two breeding populations of *D. bithynica* from the north-west of Anatolia. We also examine altitudinal differences between populations of this species.

Material and Methods

Seventy-four specimens of *D. bithynica* (37♂♂ and 37♀♀), deposited in the Fauna and Flora Research and Application Centre of Dokuz Eylül University (Buca-İzmir), were examined. They were from two populations (Bolu and Zonguldak) in the north-west of Anatolia, with 54 of these specimens being used only for skeletochronological analysis. Specimens were captured in mid April 2009 at altitudes of 1200 m a.s.l. (Bolu, 40°33'13.21''N, 31°15'52.68''E) and 50 m a.s.l. (Zonguldak, 41°5'58.18''N, 31°15'17.61''E).

The climate of Zonguldak varies from the coastal areas to the inland areas due to the mountains that run parallel to the coast. Summers are warm and humid, and the average temperature is around 11°C in April. Winters are cool and damp. Precipitation is the heaviest in autumn and early winter, and minimal in spring, the average precipitation is around 60.9 mm in April (MGM 2015). Bolu has cold and snowy winters, and warm summers with cool nights. The average temperature is around 9°C in April, and the average precipitation is around 51 mm in April (MGM 2015).

In order to identify sex, we used the presence/absence of hemipenes that are retracted in hemipenial sacks at the base of the tail. The snout-vent length (SVL) of all specimens was measured using a digital calliper with an accuracy of 0.01 mm. Snout-vent length was used to study sexual size dimorphism (SSD) between sexes and age groups, and to present relationship between SVL and age of *D. bithynica* specimens. Lovich and Gibbons (1992) proposed that SSD was quantified with the sexual dimorphism index (SDI). According to Lovich and Gibbons (1992), SDI = (mean size of larger sex / mean size of smaller sex) ± 1. In this formula ± 1 is arbitrarily expressed as +1 if males are larger than females and defined as negative, or –1 if females are larger than males.

According to the standard protocol, LAGs were counted on transverse sections of the middle part of phalangeal diaphyses using a portion of the second phalanx from the third toe of the hind foot. After digits were dissected, the phalangeal bones were kept in water for 24 h, and then were decalcified with 5% nitric acid for 2 h. Then they were kept in water for 12 h. Cross sections (18 μm) of the middle part of phalangeal diaphysis were prepared using a freezing microtome and stained with Ehrlich's haematoxylin. Cross sections were treated with glycerol for observation under a light microscope.

Age data were not normally distributed (Shapiro-Wilk test, $P<0.05$), whereas SVL data were normally distributed (Shapiro-Wilk test, $P>0.05$). Therefore, Mann-Whitney U test and Independent sample t-test were used to compare relationship between sexes with respect to age and SVL, correspondingly. Pearson's correlation coefficient was used to infer the pattern of relationships between age and SVL. All statistic analyses were performed using SPSS version 22 (IBM SPSS Statistics for Windows, Armonk, NY).

Results

Cross sections of the phalangeal bones of adults showed that LAGs were pronounced enough to be counted easily (Fig. 1). Endosteal resorption appeared in almost all cross sections of adult specimens. Cross sections in which the oldest lines were difficult to distinguish because of the endosteal resorption were not included in the analyses.

Males from both lowland and highland populations were slightly larger than females (Table 1). Nevertheless, no significant difference was observed between the sexes in the lowland population (independent samples t-test, $t=0.777$, $df=16$, $P=0.449$, SDI:–0.025). However, there was a significant dif-

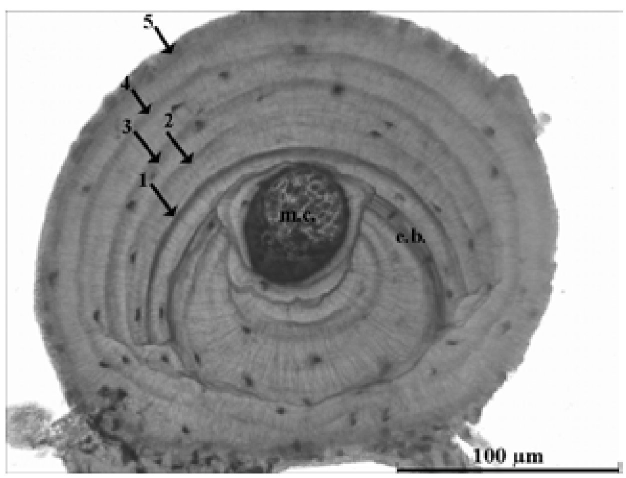

Fig. 1. Cross section (18 μm) of the middle part of phalangeal diaphysis of a male specimen of *Darevskia bithynica* from the highland population (SVL: 67.3 mm). The LAGs are indicated by black arrows (m.c. = marrow cavity, e.b. = endosteal bone)

ference between the sexes in the highland population (independent samples t-test, t=3.861, df=34, P=0.000, SDI:–0.082). Snout-vent length ranged from 59.2 to 67.92 mm (average 63.67±0.62) for males and from 50.94 to 66.2 mm (average 58.87±1.15) for females of the highland population, and from 55.24 to 69.56 mm for males and from 54.62 to 66.12 mm for females of the lowland population. There were not significant differences between the populations for both sexes in terms of SVL (independent samples t-test, for females: t=1.444, df=22, P=0.163; for males: t=–0.368, df=28, P=0.716; Table 2).

In the lowland population, age ranged between four and six years in both males and females (mean: 5.2±0.44; 4.87±0.44, respectively). In the highland population, it ranged between four and six years (mean: 5.0±0.21) for males and three and six years (mean: 4.68 ± 0.35) for females. However, there was no significant difference in terms of average age between males and females both for the lowland population (Mann-Whitney U-test, U=32.5, z=–0.722, P=0.470) and for the highland population (Mann-Whitney U-test, U=127.5, z=–1.124, P=0.261). There were not significant differences between the populations in terms of age (Mann-Whitney U-test, for females: U=59, z=–0.325, P=0.745; for males: U=85, z=–0.703, P=0.482) for both sexes (Table 2). Both SVL and age were combined to determine sexual maturity of *D. bithynica* specimens. Thus, individuals of both populations were determined to have reached sexual maturity at the age of two. A correlation was not found between age and SVL in both males (P=0.883, r=0.035) and females (P=0.371, r=0.24) for the two populations.

Table 1. Descriptive statistics of age, SVL, and SSD indexes of *Darevskia bithynica* populations

	SVL (mm)		Age (Years)	
Population	Males	Females	Males	Females
Zonguldak				
n	10	8	10	8
Min-Max	55.24-69.56	54.62-66.12	4-6	4-6
Mean	63.20	61.66	5.2	4.87
SE	1.34	1.45	0.44	0.44
SSD	-0.025			
Bolu				
n	20	16	20	16
Min-Max	59.2-67.92	50.94-66.2	4-6	3-6
Mean	63.67	58.87	5	4.68
SE	0.62	1.15	0.21	0.35
SSD	-0.082			

Table 2. Statistical differences between females and males in terms of SVL and age based on parametric and nonparametric tests

	SVL			Age		
Sexes	t	df	P	u	z	P
Female	1.444	22	0.163	59	-0.325	0.745
Male	-0.368	28	0.716	85	-0.703	0.482

Discussion

Altitudinal gradients offer a natural experiment by providing divergent conditions at relatively close geographical proximity. At the highland, activity seasons are relatively short, a phenomenon especially relevant to ectotherms such as lizards (Perry 2007). In fact, animals in the highland population and northern latitudes live longer than animals in the lowland population and southern latitudes. Moreover, animals in cooler climates from highlands and northern latitudes have larger bodies (Sears, Angilletta 2004). In addition, lizards from the lowland populations are expected to have a smaller mean body size than specimens in the highland population (Roitberg, Smirina 2006b). However, our results show that female specimens from the lowland population were larger than females from the highland population, but this was not statistically significant. Male specimens from both populations had equal sizes (Table 1). We observed endosteal resorption in all cross sections. According to some studies (Caetano, Castanet 1993; Esteban *et al.* 1999), this could be related to changes in the altitudinal gradient because populations living in the highland exhibit less resorption than lowland populations.

Our study points out the life history traits of *D. bithynica* across two altitudes from two different populations in the north-west of Anatolia. Our results show that the males of *D. bithynica* are larger than the females from the two populations (male-biased SSD). These results are concordant with the studies of *Lacerta agilis exigua* and *L. a. boemica* (Roitberg 2007). Recently, it has been found that the mean SVL of *Acanthodactylus boskianus* males that inhabit South-eastern Anatolia is significantly greater than that of females (Üzüm *et al.*, 2014). Similarly, Altunişik *et al.* (2013) have found that intersexual differences in body size of *Eremias strauchi strauchi* were male-biased; they have shown that males live longer than females. In addition, Gül *et al*. (2014) have reported that males of *Darevskia rudis* from lowlands were larger than females but the difference in the highlands is not similar to our results. SSD may arise from differences in behaviour, demography, life history, physiology, ecology, age at sexual maturity, patterns of energy use, and evolution of males and females within a population (Cox et al., 2003). Besides, intersexual variation in SSD correlates with population differences in latitude, altitude, climate, and geophysical features (Cox, 2006; Cox *et al*., 2007). For instance, Sears and Angilletta (2004) have reported that cold adapted *Sceloporus gracious* show larger SVL in individuals from populations at low altitude that undergo longer periods activity than specimens in populations at high altitude that undergo shorter periods of annual activity; they have also stated that *S. graciosus* at high altitude exhibits faster rates of growth in a relatively cool environment than lizards in lower altitudes and warmer environmental. Thus, environmental conditions in lowlands and highlands can have a significant effect on life history of *D. bithynica*. Similarly, *D. rudis* individuals from low altitude display distinctly larger mean SVL and age than from high altitudes (Gül *et al*., 2014). Life history traits of *D. bithynica* across altitudinal gradients may be affected by climatic conditions because females from the highland population in Bolu (annual mean temperature 10.6°C; average amount of precipitation 45.7 mm) living in a colder and more arid environment would tend to be younger than females from the lowland population in Zonguldak (annual mean temperature 13.7°C; average amount of precipitation 101.7 mm). However, this altitudinal gradient is not statistically significant.

In conclusion, our results based on skeletochronological analysis indicated that the mean lifespan of specimens from the lowland population in both sexes is longer and intersexual differences in SVL are male-biased. However, neither differences in mean life span nor differences in mean SVL for different altitudes are statistically significant.

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