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A skeletochronological study of parthenogenetic lizards of genus *Darevskia* from Turkey

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Abstract. The skeletochronological method has been used to assess age distribution and age-related differences in body size among populations of the parthenogenetic lizards *Darevskia sapphirina, D. uzzelli, D. armeniaca* and *D. unisexualis* from Turkey and Armenia. The age distribution between *D. armeniaca* and *D. unisexualis* did not significantly differ and ranged from 1 to 8 years. Maximum age for both *D. uzzelli* and *D. sapphirina* was 6 years, and 8 years for both *D. unisexualis* and *D. armeniaca*. In all the studied species, individuals reached sexual maturity after third hibernation. According to patterns of growth marks resorption, *D. sapphirina* is distinguished from all other rock lizards of genus *Darevskia* by a narrowest periosteal bone as result of high rate of endosteal resorption resulting in complete destruction of hatchling line and line of the first hibernation.

Keywords. *Darevskia*, parthenogenesis, age, SVL, skeletochronology.

INTRODUCTION

The genus *Darevskia* includes seven parthenogenetic species of Caucasian rock lizards widely distributed throughout the inner part of the Armenian Plateau on territories of central, northwestern and northern Armenia, adjacent region of southern Georgia, western Azerbaijan and northeastern Turkey. Among parthenogenetic species, *D. armeniaca, D. dahli, D. unisexualis* and partly *D. rostombekowi* have comparatively wide areas of distribution, while *D. uzzelli*, *D. sapphirina* and *D. bendimahiensis* are endemics of eastern and northeastern Turkey with limited distribution ranges. The habitats of the cited species are similar and occur primarily in steppe zone, where they prefer rocks and conglomerates of stones at elevation above 1500 m with drastic seasonal climatic variations (Darevsky, 1967; Darevsky and Danielyan, 1977; Schmidtler et al., 1994; Ananjeva et al., 2006; Ilgaz, 2006; Arakelyan et al., 2011).

Previous studies of parthenogenetic *Darevskia* species revealed low genetic variability in comparison with their bisexual parental species, in which genome diversity may arise as a result of mutation or multiple hybridization events (Fu et al., 2000, Moritz et al., 1992, Martirosyan et al, 2002). According to results obtained with mitochondrial and allozyme markers (Fu et al., 2000; Murphy et al., 2000), parthenogenetic species *D. unisexualis, D. uzzelli*, *D. sapphirina* and *D. bendimahiensis* originated from hybridization events of the same biparental species *D. raddei* and *D. valentini* during reticulate evolution. Thus, comparative studies about age and growth of parthenogenetic lizards may provide additional information about the origin of parthenogenetic species and possibility to study the influence of enviroment on phenotypic diversity in the clonal populations of lizards. However, the data on growth, longevity and age composition of populations of *Darevskia* parthenogenetic species

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are scarce. Skeletochronological data for *D. armeniaca*, *D. dahli*, *D. unisexualis* and *D. rostombekowi* from Armenia show that parthenogenesis does not influence growth rate, longevity and maturity and are correlated with body size rather than reproduction mode (Arakelyan and Danielyan, 2000; Arakelyan, 2001; Arakelyan, 2002). Despite their conservation importance due to restricted ranges (Akarsu et al., 2009, Kaska et al., 2009), no data are currently available on the life history of endemic parthenogenetic species of genus *Darevskia* from Turkey.

The purpose of this study is to determine and compare the age and body size of parthenogenetic species of genus *Darevskia* from Turkey and analyze differences in body size for each age group among populations of unisexual lizards.

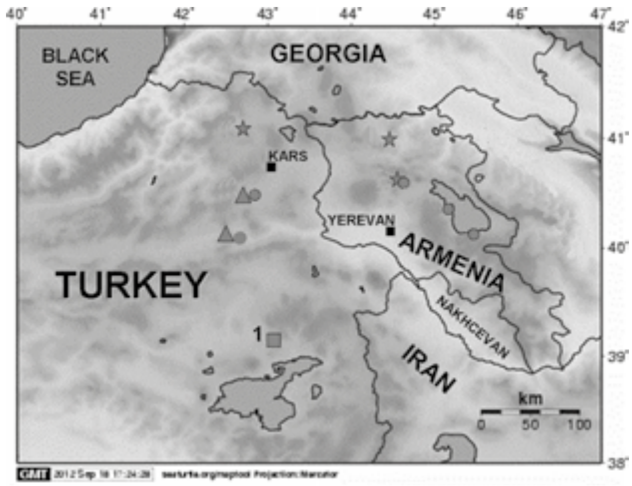

Fig. 1. Sampling localities of parthenogenetic species (square: *Darevskia sapphirina*, triangle: *Darevskia uzzelli*, circle: *Darevskia unisexualis*, star: *Darevskia armeniaca*).

MATERIALS AND METHODS

A total of 255 individuals of partenogenetic species *D. armeniaca* (Mehely, 1909), *D. sapphirina* (Schmidtler et al, 1994), *D. unisexualis* (Darevsky, 1966) and *D. uzzelli* (Darevsky and Danielyan, 1977) were collected from different localities from northeastern and eastern part of Turkey and compared with populations of *D. armeniaca* and *D. unisexualis* from Armenia (Table 1 and Figure 1). We used 70% ethanol preserved specimens that had already been collected and incorporated into the collections of ZDEU (Zoology Department Ege University, Turkey) and Yerevan State University, Armenia. Lizard snout-vent length (SVL) was measured from the tip of snout to anal cleft by using a dial caliper with an accuracy of 0.01 mm.

The age of individuals was determined by skeletochronological method (Smirina, 1974; Castanet and Smirina, 1990) which is based on counting of numbers of arrested growth lines (LAG) formed in the bones of reptiles as a result of seasonal growth processes. Cross-sections of femur bones were used for counting of concentric arrested growth lines. The bones were decalcified in 5% nitric acid solution to one hour and rinsed in tap water for over 12 hours. The cross sections (10 mm thickness) of diaphysis of femur were prepared using a freezing microtome and then were stained with Erlich's haematoxylin for 20 min. The sections were mounted on the slide with a drop of glycerin and a cover slip was placed over the slide. The cross sections were examined under a light microscope and recorded with a digital Canon A100 camera. LAGs were counted and verified independently by two authors. The cross-sections were processed by image analysis. For each individual at least three selected cross-sections from the middle of diaphysis were used for measurement. Measurements were made on images of bone sections using the morphometric software tpsDig (Rohlf, 2004). The square roots of mean values of longest and shortest axes of diameters of bone sections and diameters of marrow cavity were calculated. The periosteal bone width was expressed as difference between periosteal diameter (bone width) and the marrow diameters of bones. The hatchling line (neonatal line) and the first hibernation resting line suggests that the first

Table 1. Data on the specimens of parthenogenetic rock lizard used for skeletochronological analysis (N – number of samples)

Species	N	Locality	Date	Altitude	Coordinates
D. armeniaca	76	Ardahan, Turkey	09.07.2003	1750	41°04'N, 42°50'E
		Artavazd vil., Kotayk Province, Armenia	20.07.1999	1870	40°37'N, 44°33'E
		5 km of Gyulagarak vil., Lori Province, Armenia	30.06.1999	1400	40.59'N, 44°77'E
D. uzzelli	52	28 km SE of Horasan, Erzurum, Turkey	03.09.2002	2000	39°08'N, 43°06'E
		7 km W of Selim, Kars, Turkey	29.06.2001	1950	39°08'N, 43°06'E
D. unisexualis	112	28 km SE of Horasan, Erzurum, Turkey	03.09.2002	2000	39°08'N, 43°06'E
		7 km W of Selim, Kars, Turkey	29.06.2001	1950	39°08'N, 43°06'E
		5 km of Noratuz vil., Gegharkunik Province, Armenia	07.06.2004	1930	40°23'N, 45°12'E
		2 km of Tcovinar vil., Gegharkunik Province, Armenia	08.07.2000	1920	40°09'N, 45°27'E
		Artavazd vil., Kotayk Province, Armenia	20.07.1999	1870	40°37'N, 44°33'E
D. sapphirina	15	26 km NW of Erciş, Van, Turkey	19.06.2002	1950	39°08'N, 43°06'E

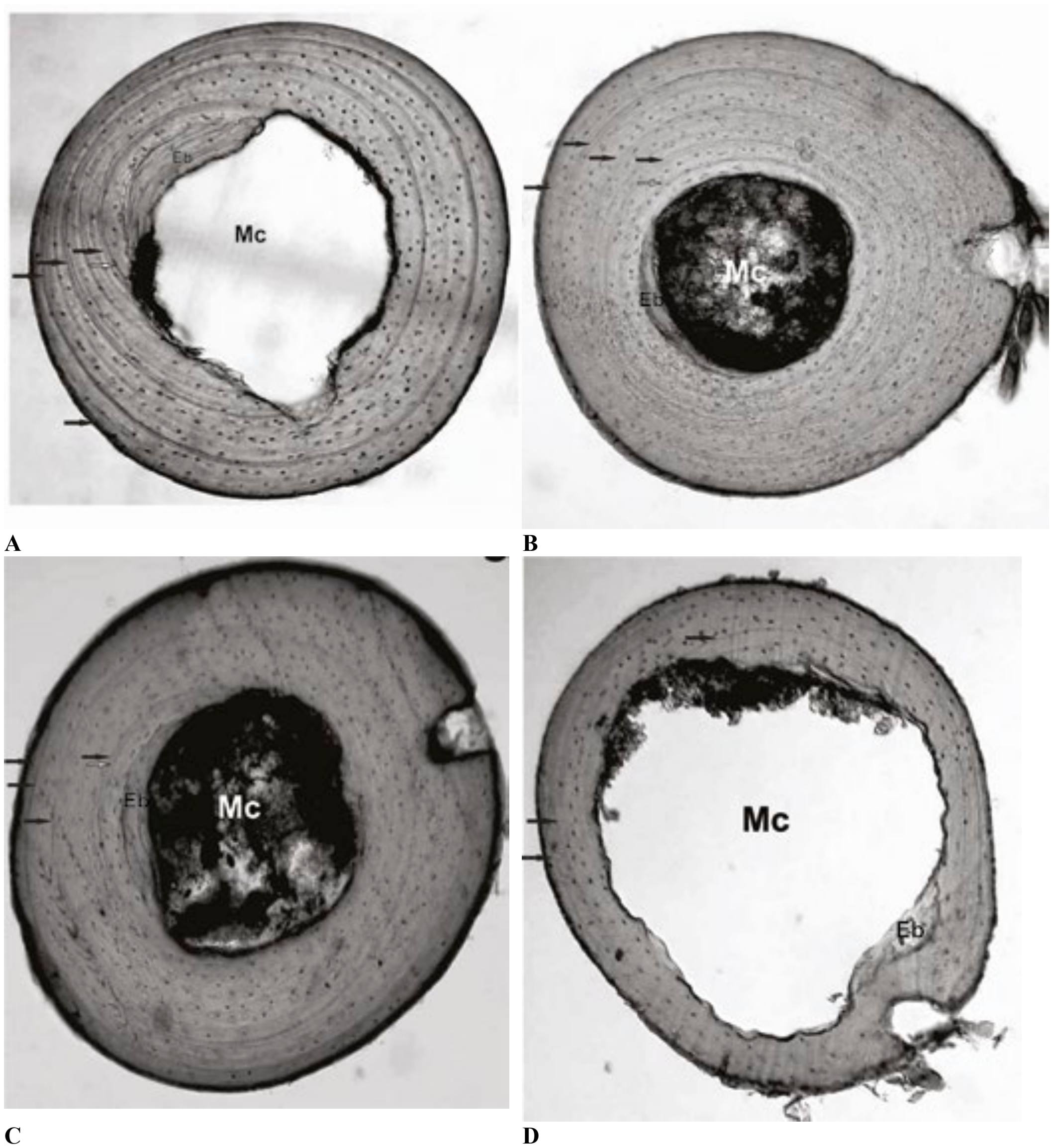

Fig. 2. Femur bone cross–sections of four-year old parthenogenetic lizards of four species (A - *D. armeniaca*, B - *D. unisexualis*, C - *D. uzzelli*, and D - *D. sapphirina*). Arrows indicate LAGs, Mc - marrow cavity, Eb - endosteal bone.

LAGs deposited in juveniles are retained into later life time of lizards (Smirina, 1974; Castanet, 1994). Both of these closely spaced lines were completely or partly present in all samples of *D. armeniaca, D. unisexualis* and *D. uzzelli*, which allowed a correct assessment of the number of their LAGs (Fig. 2). In order to account for the number of resorbed LAGs in process of endosteal resorption in some samples of D. *sapphirina*, "back calculation" was performed by comparing the diameter of the marrow cavity of the adult lizards with that of the first LAG and outer edge of a bone of the subadult lizards (Smirina, 1974; Castanet and Smirina, 1990).

All data were tested for normality (Shapiro-Wilk) and for homogeneity of variances (Levene test). We used one-way analysis of variance (ANOVA) and Scheffé's post hoc test to test for differences in snout-vent length (SVL) or bone width within each age class in species or populations. Significance was defined as $P < 0.05$. Statistica software version 7.0 was used for data treatment (StatSoft, 2004).

RESULTS

Age structure of all studied species is characterized by a maximum age of 6-8 year and predominance of 4-5 year-olds among adult individuals (Fig. 3). The maximum age estimated by skeletochronological method for *D. armeniaca* and *D. unisexualis* was 8 years, while the old-

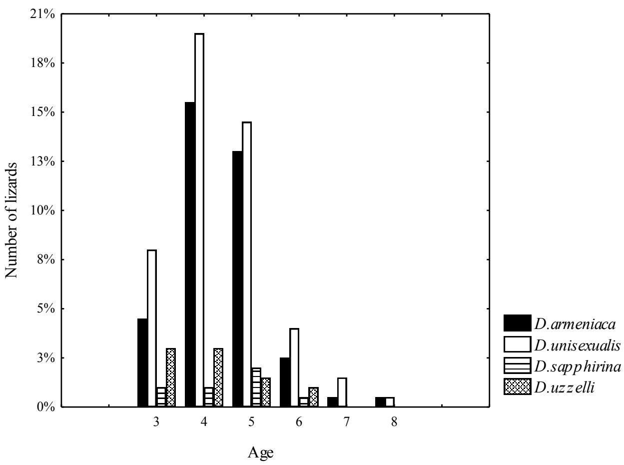

Fig. 3. Distribution of adult age groups of parthenogenetic species.

est lizards of *D. uzzelli*, *D. sapphirina* in our samples were 6 years. According to the presence of vitellogenic eggs, individuals reach sexual maturity after third hibernation in all studied species.

The average age of adults in our samples was similar among species (Mean ± SE - *D. armeniaca*: 4.46 ± 0.11 n = 73; *D. unisexualis*: 4.44 ± 0.11 n = 95; *D. sapphirina*: 4.44 ± 0.33 n = 10; *D. uzzelli*: 4.06 ± 0.25 n = 17) and not differed significantly ($F_{3, 195}$= 1.02, P = 0.39) from one another.

Significant differences in SVL between four parthenogenetic species in each age class (Table 2) were found only between 4 years-old *D. uzzelli* and *D. unisexualis* (*P* < 0.05) and between 4 years-old *D. uzzelli* and *D. armeniaca* (*P* < 0.01) according to Scheffé's post-hoc test for one-way ANOVA. The maximum SVL of 71 mm was recorded for a 6 years-old *D. armeniaca,* 70 mm in a 6 years-old *D. unisexualis,* 67 mm in a 6 years-old *D. uzzelli,* 59 mm in a 5 years-old *D. sapphirina.*

No differences in body size (SVL) for each age class of *D. unisexualis* parthenogenetic lizards were detected

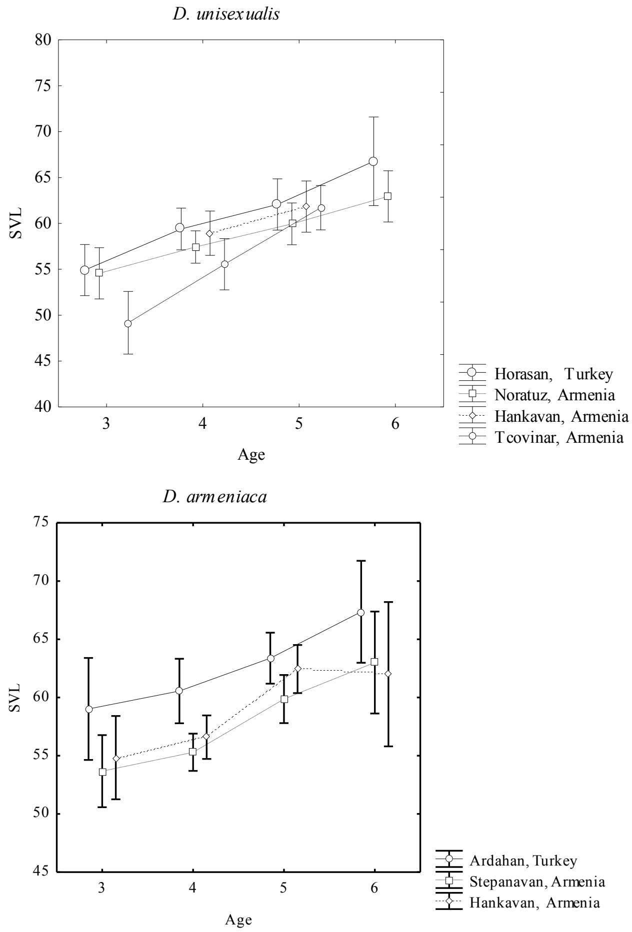

Fig. 4. Differences in body size (SVL) between populations of *D. unisexualis* and *D. armeniaca* within each age group (yr.). Vertical bars show0 .95 confidence intervals.

among seven localities while among three populations of *D. armeniaca* significant differences were found only between populations from Stepanavan in Armenia and Ardahan in Turkey (*P* < 0.05) in 4-years old lizards (Fig. 4).

Table 2. Snout-vent length of adults of parthenogenetic species in each age group (N: Number of samples, SE: Standard error of the mean, SD: Standard deviation)

Species	Age group											
	3			4			5			6		
	N	Mean ± SE	SD	N	Mean ± SE	SD	N	Mean ± SE	SD	N	Mean ± SE	SD
D. armeniaca	9	55.25 ± 1.24	3.45	31	56.60 ± 0.67	3.42	26	61.84 ± 0.73	3.33	5	64.54 ± 1.67	4.13
D. unisexualis	16	53.35 ± 0.93	4.54	38	57.92 ± 0.61	3.85	29	61.27 ± 0.69	2.73	8	63.91 ± 1.32	2.86
D. uzzelli	6	58.66 ± 1.52	4.98	6	59.24 ± 1.52	7.01	3	60.41 ± 2.15	1.78	2	63.73 ± 2.64	3.90
D. sapphirina	2	48.64 ± 2.64	0.22	4	54.62 ± 2.64	2.67	4	57.85 ± 1.87	1.20	1	59.47	

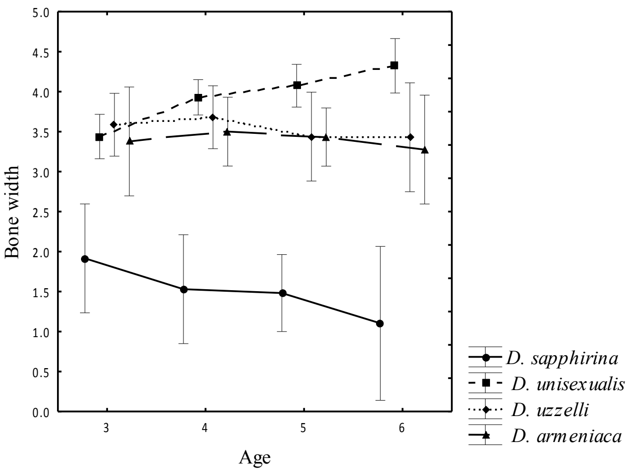

Fig. 5. The periosteal bone width (in µm) of four parthenogenetic species within each age group (yr.). Vertical bars show 0.95 confidence intervals.

We found differences between patterns of growth marks resorption among studied species. Examination of stained cross sections of femur bones of *D. unisexualis* and *D. uzzelli* revealed the low endosteal resorption which did not destroy any part of first resting lines in 53% and 34% of cases respectively, whereas in others cases it locally destroyed up to 3/4 of the innermost rings. *D. armeniaca* had intermediate rate of resorption where the sections with intact first lines comprised 8%, and there were no sections with completely destroyed hatchling and first hibernation lines. *D. sapphirina* had considerably high resorption with 98% of cases completely destroyed hatching and line of first hibernation (Fig. 2, 5). Consequently, among compared four species, *D. sapphirina* had a significantly narrower periosteal bone ($F_{3,\,115} = 41.94$, $P < 0.0001$) in comparison with *D. unisexualis* (Scheffé's post-hoc test $P < 0.001$), *D. uzzelli* ($P < 0.001$), and *D. armeniaca* ($P < 0.001$) (Fig. 5).

DISCUSSION

Although skeletochronology has been applied to four parthenogenetic *Darevskia* species in Armenia (Arakelyan and Danielyan, 2000), no studies have been conducted in *D. sapphirina* and *D. uzzelli* in Turkey. The range of ages of *D. uzzelli* and *D. sapphirina* in our samples did not differ from other species of *Darevskia*, where maximum age was 6 years, similar to *D. rostombekowi* and *D. dahli* (Arakelyan and Danielyan, 2000) and lower than in *D. armeniaca* and *D. unisexualis*. However, the oldest lizards of *D. armeniaca* (8 years) identified by skeletochronological methods in different populations of Armenia and Turkey did not represent the oldest individuals documented under natural conditions estimated by mark-and-recapture method. The long term observation of *D. armeniaca* in Armenia showed that the maximum longevity is up to 10 year (Galoyan, 2011). Thus, the actual longevity of parthenogenetic lizards may exceed the maximum recorded by skeletochronological estimated age, especially for larger species, probably due to outer LAGs distances in older individuals that are very narrow or missing.

There were no significant differences in the snout-vent length (SVL) of parthenogenetic species among age groups, however *D. rostombekowi* had the smallest SVL in each age group while *D. uzzelli*, *D. unisexualis* and *D. armeniaca* had larger sizes. Among studied species *D. unisexualis*, *D. uzzelli* and *D. sapphirina* apparently originated from hybridization of the same parent species: *D. raddei* and *D. valentini* (Murphy et al., 2000). The comparison of their SVL indicated that the species *D. unisexualis* and *D. uzzelli* are close to each other, whereas *D. sapphirina* has a smaller size. It is possible that body size between parthenogenetic species originating from same parents does not change. The comparisons of body size of the same unisexual species will allow studies of influence of the environment on clonal organisms. We did not find significant differences in body size between populations of parthenogenetic species, with the exception of 4 years-old *D. armeniaca*. However, we found some population-specific variation in body size in each age group of *D. armeniaca* and *D. unisexualis* from Armenia and Turkey, where body size of lizards from Turkey was larger than that of Armenian lizards. The climatic and habitat characteristics of sampling localities in Armenia and Turkey were mostly similar. Observed differences among populations could result from the temperature and the length of active periods at lower and higher elevations. Previous studies showed that body length, growth rates, age of maturity, and longevity can vary considerably among populations of the same species, where lizards inhabiting high-elevation sites and northern latitudes live longer than those from low-elevation sites and southern latitudes in the Northern Hemisphere (Arakelyan, 2000; Wapstra and Swain, 2001; Roitberg and Smirina, 2006).

Surprisingly, the periosteal bones of *D. sapphirina* were notably thinner than those of other species apparently as the result of intensive endosteal resorption. The rate of resorption in *Darevskia* rock lizards is quite slow (Arakelyan and Danielyan 2000; Arakelyan, 2002) and the first growth arrested lines are always locally present with with the exception of *D. sapphirina*. The apparently high rate of resorption may result from either a specific mutation or new allele combination following a hybridization event among bisexual species which gave rise to the parthenogenetic species.

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