Age structure and body size in three populations of Darevskia rudis (BEDRIAGA, 1886) from different altitudes

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4 authors:

Serkan Gül
Recep Tayyip Erdoğan Üniversitesi

Nurhayat Özdemir
Recep Tayyip Erdoğan Üniversitesi

Yusuf Kumlutaş
Dokuz Eylul University

Çetin Ilgaz
Dokuz Eylul University

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Age structure and body size in three populations of *Darevskia rudis* (BEDRIAGA, 1886) from different altitudes
(Squamata: Sauria: Lacertidae)

Altersstruktur und Körpergröße in drei Populationen von *Darevskia rudis* (BEDRIAGA, 1886) aus unterschiedlichen Höhenlagen
(Squamata: Sauria: Lacertidae)

SERKAN GÜL & NURHAYAT ÖZDEMIR
& YUSUF KUMLUTAŞ & ÇETIN ILGAZ

KURZFASSUNG

Darevskia rudis (BEDRIAGA, 1886) zeigt im Gebiet der türkischen Schwarzmeerregion eine weite vertikale Verbreitung. Insgesamt wurden 62 Individuen dieser Eidechse von drei Populationen/Fundorten aus unterschiedlichen Höhenlagen (Provinz Artvin: Ardanuç, 2137 m ü. M., Borçka, 1277 m ü. M., Çermik, 700 m ü. M.) skeletochronologisch altersbestimmt und hinsichtlich ihrer Kopf-Rumpf-Länge vermessen. Im Mittel waren Kopf-Rumpf-Länge und Alter in beiden Geschlechtern bei der höchst gelegenen Population geringer als bei den beiden tiefer gelegenen Populationen. Ein signifikant positiver Zusammenhang fand sich zwischen Alter und Kopf-Rumpf-Länge bei den Weibchen der beiden hoch gelegenen Populationen, jedoch nicht für die aus Çermik und die Männchen aller drei Populationen. Der Geschlechtsdimorphismus in der Körpergröße war in der tiefstgelegenen (Çermik) Population durch größere Männchen und in den höher gelegenen Populationen (Borçka and Ardanuç) durch größere Weibchen gekennzeichnet. Die skeletochronologischen Untersuchungen zeigten deutliche Unterschiede in der Körpergröße und Altersstruktur zwischen tiefer und höher gelegenen Populationen von *D. rudis* auf.

ABSTRACT

Darevskia rudis (BEDRIAGA, 1886) occupies a wide range of altitudes in the Black Sea region of Turkey. A total of 62 individuals of this lizard from three populations/locations at different altitudes (Province of Artvin: Ardanuç, 2137 m a.s.l., Borçka, 1277 m a.s.l., Çermik, 700 m a.s.l.) were studied for body size and age using skeletochronology. In both sexes, mean body size and age were smaller/lower in the highest altitude population than in the lower altitude populations. A significant positive correlation was found between age and snout-vent length in the females of the high altitude populations, however, not for Çermik females and the males of all three populations. Sexual size dimorphism was male-biased in the lowest (Çermik) population, but female-biased in the higher altitude (Borçka and Ardanuç) populations. The skeletochronological analyses revealed distinct differences in body size and age structure between low and high altitude populations of *D. rudis*.

KEY WORDS

Reptilia: Squamata: Lacertidae: *Darevskia rudis*, age structure, population ecology, geographic variation, skeletochronology, Turkey

INTRODUCTION

Age determination is very important in ecological studies. A population's age structure is directly related to the life-history of individuals. If a shift occurs in life-span, age of reproduction, or growth rate, all of which are important life-history traits, it would be clearly evident in the age structure of the population in that the average age and distribution of individuals in age classes would be altered (CHAMBERLAIN 2011). Many environmental factors influencing populations have been examined for their correlation to variation in age structure and other life-history traits. These environmental factors include elevation, drought, and climate (SAGOR et al. 1998; ESTEBAN & SANCHIZ 2000; FRANKLIN et al. 2000; MIAUD et al. 2000; JAKOB et al. 2002; MORRISON et al. 2004; ROITBERG &

Smirina 2006a, 2006b; Roitberg 2007; Gül et al. 2011; Tarkhnishvili 2012; Özdemir et al. 2012).

Age and body size of individuals are two standard characteristics that were used by many researchers to quantify life-history traits. However, it is shown that only two methods guarantee actual age determination in terrestrial vertebrates: mark-recapture and skeletochronology (Halliday & Verrell 1988). Mark-recapture studies require the release of already known aged individuals, typically neonates, over several years and marking them for later recapture (Lebreton et al. 1992). Skeletochronology is based on the regular (usually annual or seasonal) acceleration and deceleration of appositional bone deposition, resulting from cyclic, differential activity levels (Hemelaar & Van Gelder 1980). Skeletochronology is the most reliable method to measure age in anurans (Miaud et. al. 2007; Liao & Lu 2010; Li et. al. 2010; Gül et al. 2011; Özdemir et. al. 2012), urodeles (Olgun et. al. 2005; Üzüm & Olgun 2009; Üzüm 2009; Hasumi 2010; Üzüm et al. 2011; Wagner et. al. 2011), lizards (Roitberg & Smirina 2006a; Guarino et. al. 2010; McCoy et. al. 2010; Tomasevic et. al. 2010; Borczyk & Paśco 2011), and turtles (Guarino et al. 2004; Chinsamy & Valenzuela 2008; Casale et. al. 2011).

The objective of the present study is to examine altitudinal and sex related variation of age structure and body size in three populations of *Darevskia rudis* (Bedriaga, 1886) from northeastern Turkey, thereby representing for Turkey the first skeletochronological study on *D. rudis.*

MATERIALS AND METHODS

Darevskia rudis inhabits northern coastal Turkey, Georgia, Russia and Azerbaijan and can be found from sea level up to 2400 m a.s.l. (Baran et al. 2012) where it lives in rocky, pebbly temperate forest habitats, sometimes also in montane steppe areas (Baran & Atatür 1998). From its conservation needs, this species is categorized 'Least Concern' in view of its wide distribution, presumed large population, and because it is unlikely to be declining fast enough to qualify for listing in a more threatened category (Tok et al. 2009).

Collection sites and studied populations.- A total of 62 specimens of *Darevskia rudis* from three populations in the Province of Artvin (Ardanuç, 2137 m a.s.l., Borçka, 1277 m a.s.l., Çermik, 700 m a.s.l.) entered the skeletochronological analysis (Table 1). Only preserved specimens that had been collected earlier and kept in the Fauna and Flora Research Center of Dokuz Eylül University (Buca-İzmir) were processed.

The Province of Artvin is characterized by steep valleys carved by the Çoruh River system, surrounded by high mountains (Kaçkar, Karçal and Yalnızçam; up to 3900 m a.s.l.) and forests including the Karagöl-Sahara National Park, which contains the Lakes Şavşat and Borçka. The climate is very wet and mild at the coast, and as a result the province is heavily forested. This greenery runs from the mountain top all the way down to the Black Sea coast. The rain turns to snow at higher altitudes, and the peaks are very cold in winter (http://www.yeniansiklopedi.com/artvin).

Age Determination.- Age estimation was done applying skeletochronological methods (Castanet & Smirina 1990; Olgun et al. 2005). The Lines of Arrested Growth (LAGs - Castanet et al. 1993) were counted on transverse sections of the middle part of phalangeal diaphyses using a portion of the second phalanx from the third toe (Fig. 1). After digits were dissected, the phalangeal bones were washed in running tap water for 24 h, decalcified in 5 % nitric acid for 2 h and then washed again under running tap water for 12 h. Cross sections (20 μm) of the diaphyseal part of the phalanx were obtained using a freezing microtome and stained in Ehrlich's haematoxylin. Sections were submerged in glycerol for observation under a light microscope.

On each section, the number of LAGs was assessed by two observers (S. Gül, N. Özdemir) independently, then results were compared. Double lines and endosteal re-

Table 1: Origin and sample size (N) of the studied specimens of *Darevskia rudis* (BEDRIAGA, 1886).
Tab. 1: Herkunft und Stichprobengröße (N) der untersuchten Exemplare von *Darevskia rudis* (BEDRIAGA, 1886).

Population	Altitude (m a.s.l.) Seehöhe (m ü. M.)	Latitude (North) nördl. Breite	Longitude (East) östl. Länge	N males N Männchen	N females N Weibchen
Artvin (Çermik)	700	41°25'18"	42°24'38"	13	15
Artvin (Borçka)	1277	41°18'26"	41°50'19"	5	10
Artvin (Ardanuç)	2137	41°04'33"	42°27'04"	9	10

sorption did not cause any serious interpretation problems regarding age estimation, and full agreement between the observers was achieved for all samples.

Statistical Analysis.- Independent Sample t-test and one-way analysis of variance (ANOVA) were used to compare variables between sexes and populations. The relationship between snout-vent length (SVL) and age was analyzed using Pearson's correlation coefficient. Kolmogorov-Smirnov and Levene tests were applied in addition. The equation between age and SVL was based on a cubic regression. Data analysis was performed using SPSS v.16.0.

Sexual size dimorphism (SSD) was quantified with the sexual dimorphism index SDI = [(size of larger sex / size of smaller sex) – 1], arbitrarily expressed as positive if females are larger and negative if males are larger (LOVICH & GIBBONS 1992; SMITH 1999).

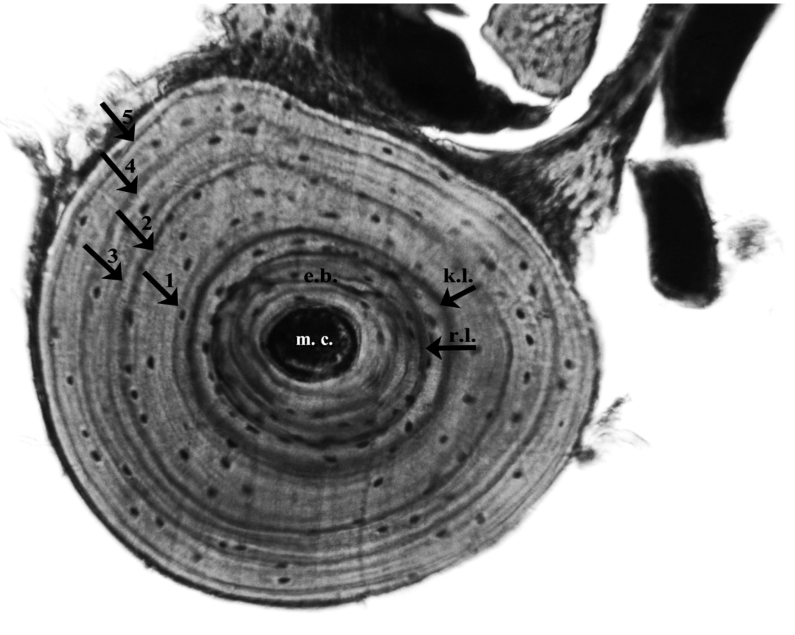

Fig. 1: Cross-section (20 μm thick) at the diaphysis level of the second phalangeal bone of the third toe of a female *Darevskia rudis* (BEDRIAGA, 1886) from the Çermik population. m.c. - marrow cavity, e.b. - endosteal bone, r.l. - resorbtion line, k.l. - Kastschenko Line (i.e., the interface between endosteal and periosteal zones). Black arrows indicate the five LAGs (Lines of Arrested Growth).

Abb. 1: Querschnitt (Dicke 20 μm) durch die Diaphysenregion der zweiten Phalange der dritten Zehe einer weiblichen *Darevskia rudis* (BEDRIAGA, 1886) der Çermik Population. m.c. - Markhöhle, e.b. - endostaler Knochen, r.l. - Resorptionslinie, k.l. - Kastschenko Linie (die Grenze zwischen endostalem und periostalem Knochen). Schwarze Pfeile zeigen auf die fünf LAGs (Linien verminderten Wachstums).

RESULTS

Both SVL and age showed normal distribution (Kolmogorov-Smirnov test, $P > 0.05$) and homogeneity of variance (Levene test, $P > 0.05$). Descriptive statistics of age and body length are summarized in Table 2. The age distributions and body size observed in the three populations are separately shown for the two sexes in Fig. 2. Endosteal resorption, which creates partial erosion of the periosteal bone on the edge of the marrow cavity, was observed in almost all individuals.

Intersexual differences in body size were male-biased in Çermik population (SDI = - 0.02), whereas it was female-biased in Borçka (SDI = 0.03) and Ardanuç (SDI = 0.08) populations. A significant difference was found between the males of the studied populations in terms of both age (one-way ANOVA, $F = 7.275$, $P < 0.01$) and SVL (one-way ANOVA, $F = 11.389$, $P < 0.01$). According to the Tukey test, Ardanuç and Çermik males were different from each other in both age and SVL (Tukey test, $P < 0.05$) with Çermik males being larger and older. Also in females, there was a significant difference between the three populations related to age (one-way ANOVA, $F = 4.740$, $P < 0.05$) and SVL (one-way ANOVA, $F = 5.609$, $P < 0.05$). In addition, Ardanuç and Çermik females were distinct from each other by both age and SVL (Tukey test, $P < 0.05$) with Çermik females being larger and older. Ardanuç and Borçka females were significantly different from each other in terms of age (Tukey test, $P < 0.05$), but not SVL.

In the Ardanuç population, a significant difference between sexes was expressed in terms of SVL (Independent *t*-test $t = -2.189$, $P < 0.05$), but not age (Independent *t*-test $t = -1.23$, $P = 0.236$). In Çermik and Borçka, differences between sexes in terms of age and SVL were not significant (Çermik, age: Independent *t*-test $t = -0.44$, $P = 0.664$, SVL: Independent *t*-test $t = 0.565$, $P = 0.577$; Borçka, age: Independent *t*-test $t = -1.759$, $P = 0.102$, SVL: Independent *t*-test $t = -0.656$, $P = 0.523$).

No significant positive correlation was found between age and SVL in males (Borçka: $n = 5$, $r = 0.663$, $P = 0.222$; Ardanuç: $n = 9$, $r = 0.605$, $P = 0.084$; Çermik : $n = 13$, $r = 0.551$, $P = 0.051$) contrary to the females from Borçka ($n = 10$, $r = 0.773$, $P < 0.01$) and Ardanuç ($n = 10$, $r = 0.874$, $P < 0.01$) but not Çermik ($n = 15$, $r = 0.343$, $P = 0.211$). Simple cubic regressions fitted the correlation between age (years: x-axis) and body size (mm: y-axis) in the females of the populations of Borçka ($y = 62.902 - 2.287.x + 0.579.x^2 + 0.000.x^3$, $R^2 = 0.607$, $P < 0.05$) and Ardanuç ($y = 4.927 + 20.928.x - 1.655.x^2 + 0.000.x^3$, $R^2 = 0.786$, $P < 0.01$).

Table 2: Statistical data of male and female *Darevskia rudis* (BEDRIAGA, 1886) from the study localities in the Province of Artvin (Turkey). SVL - Snout-Vent Length, SD - Standard Deviation, SSD - Sexual Size Dimorphism, m – male, f – female.

Tab. 2: Beschreibende Statistiken zu Kopf-Rumpf-Länge (in mm) und Alter (in Jahren) bei Männchen und Weibchen von *Darevskia rudis* (BEDRIAGA, 1886) aus drei Untersuchungsstellen in der Provinz Artvin (Türkei). SVL – Kopf-Rumpf-Länge, SD - Standardabweichung, SSD – Geschlechtsspezifische Größenunterschiede, Mean – Mittelwert, Range – Spannweite, m – Männchen, f – Weibchen.

Population	Sex	N	Mean SVL ± SD (mm)	Range SVL (mm)	Mean Age ± SD (years)	Range Age (years)	SSD
Çermik	m	13	75.6 ± 7.75	55.3-83.7	5.8 ± 0.90	5 - 8	absent
Çermik	f	15	74.0 ± 6.62	58.4-82.5	5.9 ± 1.03	5 - 8	nicht vorhanden
Borçka	m	5	68.2 ± 8.12	59.5-80.3	5.0 ± 1.00	4 - 6	absent
Borçka	f	10	70.6 ± 5.88	60.7-79.5	6.0 ± 1.05	4 - 7	nicht vorhanden
Ardanuç	m	9	60.9 ± 5.35	50.8-68.1	4.3 ± 0.70	3 - 5	present
Ardanuç	f	10	66.0 ± 4.69	58.7-73.6	4.8 ± 0.90	4 - 6	vorhanden

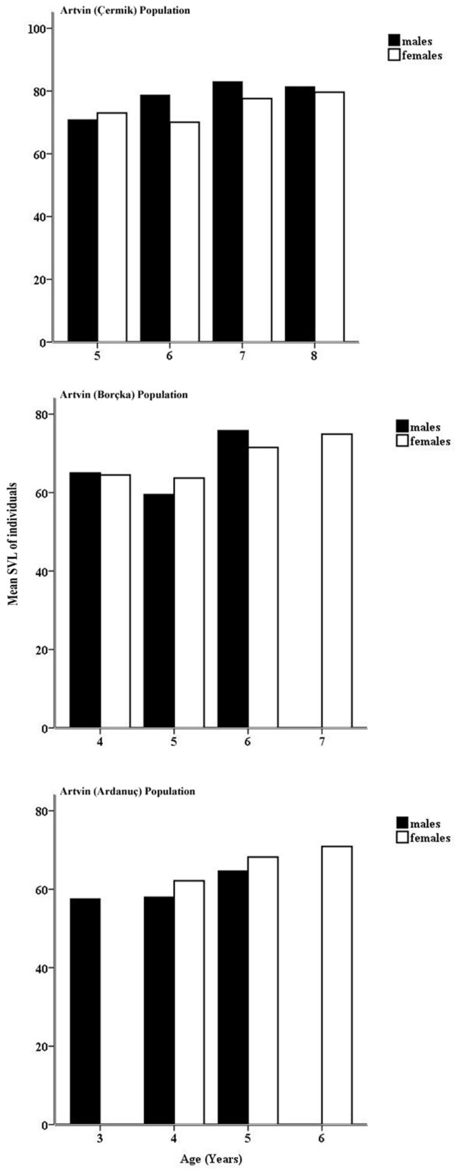

Fig. 2: Mean SVL (mm) in four age classes of male and female *Darevskia rudis* (BEDRIAGA, 1886) from Çermik (5-8 yrs), Borçka (4-7 yrs) and Ardanuç (3-6 yrs). For the number of specimens see Table 2.

Abb. 2: Mittlere Kopf-Rumpflänge (SVL, mm) in vier Altersklassen männlicher und weiblicher *Darevskia rudis* (BEDRIAGA, 1886) aus Çermik (5-8 Jahre), Borçka (4-7 Jahre) und Ardanuç (3-6 Jahre). Die Anzahl der Exemplare ist aus Tabelle 2 ersichtlich.

DISCUSSION

Variation in lizards' body size (e.g., SVL) is not only common among populations, but also within populations among individuals living in different habitats (SMITH 1996 and 1998). In reptiles, body length, growth rates, age at maturity and longevity can widely vary between populations of the same species (GUARINO et al. 2010). The present study provides here the first demographic data on *Darevskia rudis* populations from different altitudes in northeast Turkey. As a rule, individuals from high-elevation sites and northern latitude live longer than those from low-elevation sites and southern latitudes (WAPSTRA et al. 2001; ROITBERG & SMIRINA 2006b). Actually, the shorter activity period in cooler climate, due to unfavorable conditions of temperature and food availability, should reduce the risk of predation (SEURS 2005; ROITBERG & SMIRINA 2006b). Cold-adapted reptiles, such as *Sphenodon punctatus* (GRAY, 1842), have low thermal requirements in terms of effective feeding performance, both at the level of physiology and behavior (BESSON & CREE 2010). In addition, CASTANET et al. (1988) showed that *S. punctatus* reach size-based sexual maturity between 11 and 13 years in cooler areas whereas between two and three years in warmer areas. However, our results showed that individuals from lower altitude (Çermik, 700 m) exhibited clearly higher mean SVL and ages than from higher altitudes (Borçka, 1277 m and Ardanuç, 2137 m, Table 2). Moreover, at different altitudes, maximum longevity ascertained was eight years for males and females in the population from Çermik (700 m); six years for males and seven for females in the population of Borçka (1277 m) and five years for males and six for females in the population of Ardanuç (2137 m).

Similar results were reported for *Lacerta agilis* (LINNAEUS, 1758), occupying the temperate zone of the Palaearctic from southern England and the Pyrenees in the west to the Baikal Lake in the east and *Lacerta strigata* EICHWALD, 1831, inhabiting the eastern Caucasus and adjacent parts of Turkey and Iran, from which five populations of different altitudes (50-1900 m a.s.l.) were studied (ROITBERG & SMIRINA 2006b).

Furthermore, Guarino et al. (2010) showed that longevity in a high altitude population of *L. agilis* (1790-1890 m a.s.l.) was only four years for males and three for females. These studies suggest that longevity may directly be affected by environmental temperatures.

In lizards, geographic-morphological variation may also derive from other climatic factors. For instance, aridity can be an important determinant for body shape and size, in that large size can represent an adaptation to arid climates (Oufiero et al. 2011). In *D. rudis*, the effect of aridity might have increased the lizard's body size. In fact, individuals from the low elevation population at Çermik living in a warmer and more arid environment, tended to be larger than individuals from Borçka and Ardanuç (Table 2). In lizards, the general pattern of smaller body sizes in colder climates may be the result of selection towards more rapid heating abilities (Pianka & Vitt 2003). Specifically, larger body size could be advantageous in a warm, dry climate as larger animals were more likely to outlast periods of food or water shortage (Oufiero et al. 2011).

Roitberg (2007) found that in lizards most of the variation in sexual size dimorphism can be explained by differences in morphology (body size), environment (climate), phylogeny, and age structure between the sexes. The present study revealed a pattern of strong geographic variation regarding SSD in *D. rudis*. In the lowland populations (Çermik and Borçka) SSD appeared male-biased, however, not statistically significant, whereas the high altitude population of Ardanuç showed a strong female-biased SSD. This divergency is probably due to colder environmental temperatures at the higher elevation site of Ardanuç (comp. e.g., Mathies & Andrews 1995). The results are in line with the findings by Roitberg (2007) who showed that geographic variation in SSD is male-biased in warmer climates for both *L. agilis exigua* Eichwald, 1831 and *L. agilis boemica* Suchov, 1929.

In conclusion, significant altitudinal variation was observed regarding mean age, longevity and body size in three populations of *D. rudis* from northeastern Turkey.

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AUTHORS: Serkan GÜL, Recep Tayyip Erdoğan University, Faculty of Science and Arts, Department of Biology, Rize, Turkey, < serkan.gul@erdogan.edu.tr >; Nurhayat ÖZDEMIR, Recep Tayyip Erdoğan University, Faculty of Science and Arts, Department of Biology, Rize, Turkey, < nurhayat61@yahoo.com >; Yusuf KUMLUTAŞ, (Corresponding author < yusuf.kumlutas@deu.edu.tr >), Dokuz Eylül University, Faculty of Science, Department of Biology, Buca-İzmir-Turkey; Çetin ILGAZ, Dokuz Eylül University, Faculty of Science, Department of Biology, Buca-İzmir-Turkey < cetin.ilgaz@deu.edu.tr >.