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Male reproduction cycle of Kulzer's Rock Lizard, *Phoenicolacerta kulzeri* (Müller & Wettstein, 1932), in Lebanon (Reptilia: Lacertidae)

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The reproduction cycle of male Kulzer's Rock Lizard, *Phoenicolacerta kulzeri*, was studied in a mountain population living at 2000 m a.s.l. on Mount Sannine, Lebanon. Males showed active spermiogenesis in spring, following the renewal of the post hibernation activity, and in autumn, from September until they enter into hibernation in November. About 40% of males exhibited a short testicular regression period during the hottest months, in July and August. Relative testicular volume was correlated with male body size and varied seasonally. Males of *P. kulzeri* showed a more distinct reproductive pattern than the common reproductive pattern of most lacertid lizards in the Mediterranean region.

Keywords: Spermiogenesis; epididymis; histology; *Phoenicolacerta laevis*; mixed-type spermatogenesis

Introduction

The genus *Phoenicolacerta* belongs to the family Lacertidae and includes four species (Arnold, Arribas, & Carranza, 2007) distributed in the Middle East region (Sindaco & Jeremčenko, 2008). *Phoenicolacerta troodica* (Werner, 1936) is endemic to Cyprus, *P. cyanisparsa* (Schmidtler & Bischoff, 1999) is endemic to Syria and Turkey, *P. laevis* (Gray, 1838) is widespread with a distribution extending from Turkey to Jordan, and, finally, our studied species *P. kulzeri* (Müller & Wettstein, 1932) is found at high altitudes in Jordan, Syria and Lebanon (Hraoui-Bloquet, Sadek, Sindaco, & Venchi, 2002; Sindaco & Jeremčenko, 2008). Several studies have focused on molecular and phylogenetic analyses in order to elucidate the relationships within and among *Phoenicolacerta* species (Beyerlein & Mayer, 1999; In den Bosch et al., 2003; Modrý et al., 2013; Tamar et al., 2015). However, little is known about their reproductive biology, even though the reproduction pattern of other Mediterranean Lacertidae species is well documented (Carretero, 2006). In the present study, we investigate the male reproductive cycle of *P. kulzeri* and provide the first data on the reproductive strategy of this Middle Eastern lacertid lizard.

Material and Methods

The population studied is located in Mahrouka - Mount Sannine, Lebanon at an altitude of around 2000 m (34°00'N, 35°52'E). The habitat is predominantly rocky with sparse vegetation, with a mean annual temperature of 14.9°C and a total annual precipitation of 44.8 mm (10 years mean for the period 2007-2011). Monthly variations of mean precipitation and temperature are shown in Figure 1. Climatic data were obtained from Tal Amara meteorological station (33°51'N, 35°59'E) located in the Bekaa Valley at about 18 km from the study area.

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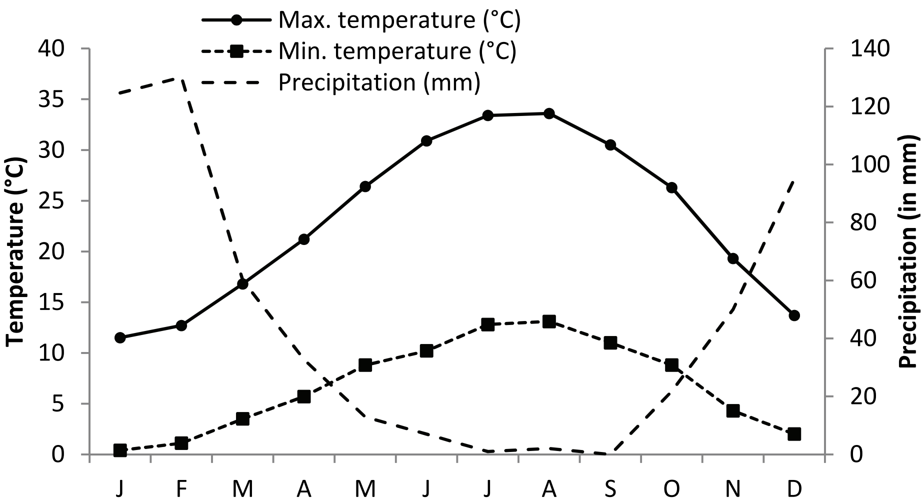

Figure 1. Annual variation of mean minimum and maximum air temperatures (left axis) and mean precipitation (right axis), for the period 1998-2007 (10 years mean).

We examined the reproductive organs of 59 males of *P. kulzeri* collected between April 2000 and September 2001 in Mahrouka by S. Hraoui-Bloquet and deposited at the Natural History Museum of the Lebanese University. The snout-vent length (SVL) of each individual was taken immediately after capture with a calliper to the nearest 0.1 mm. The male reproductive organs were excised and fixed in Bouin's solution. Later, in the laboratory, they were dehydrated in increasing concentrations of ethanol and kept in butanol until paraffin embedding using standard protocol. The right testis and epididymis were sectioned with a rotary microtome at 5µm and stained with hematoxylin and eosin. Testes slides were examined to determine the stage of the spermatogenic cycle. For each male, we recorded its reproductive condition according to three stages: (1) Spermiogenesis: spermatozoa line the lumina of the seminiferous tubules; several rows of metamorphosing spermatids, spermatocytes and spermatogonia are present. (2) Recrudescence: marked by the presence of spermatogonia and spermatocytes with several rows of metamorphosing spermatids with no spermatozoa. (3) Regression: seminiferous tubules contain spermatogonia, spermatocytes and Sertoli cells, sparse spermatozoa may be present in the lumen. Epididymides were examined for the presence of spermatozoa and secretions in their lumen. The smallest reproductively active male was determined by the presence of spermatozoa in the testis and in the epididymis. An estimate of testicular volume was obtained using the ellipsoid formula $v = 4/3\pi ab^2$ where v is the volume, and a= ½ the longest diameter and b= ½ the shortest diameter at the widest part of the testis.

For statistical analysis, we used the statistical software SPSS 20.0®. All variables were log transformed. Analysis of variance by means of ANCOVA was run using the logarithm of SVL as a covariate, to test the differences in spermatogenic stages in both years and the differences in testicular volume between months. Pearson correlation was used to test the significance of the relationship between testicular volume and body size, and to test if large lizards possessed relatively large testes.

Results

The smallest reproductively active male of *P. kulzeri* measured 42 mm SVL and was collected in May. Therefore, only males with an SVL of ≥42 mm were considered to be adults. Our sample consisted of 55 adult males (43 adult males from 2000 and 12 from 2001). No differences were found in male adult stages between the two years with

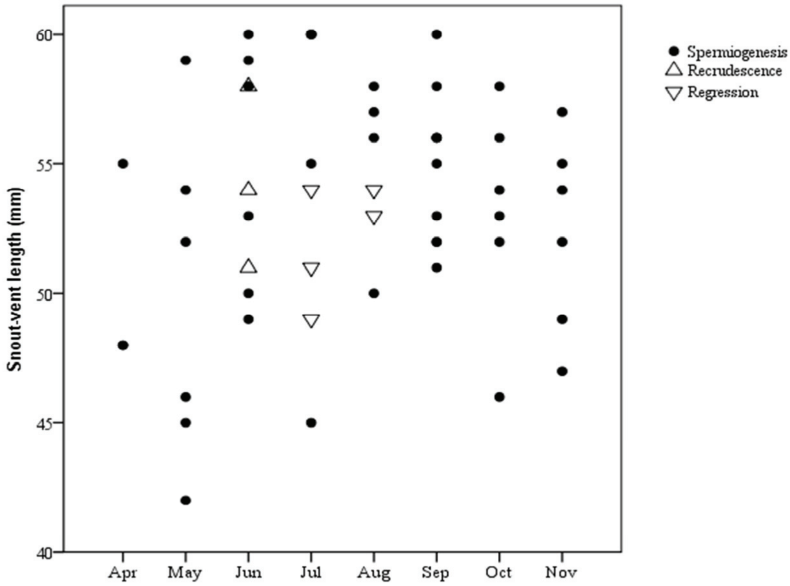

Figure 2. Male reproductive cycle of *Phoenicolacerta kulzeri* in Lebanon.

respect to body size (ANCOVA, $F_{(1,52)}$=0.43, P>0.5, n=55). Hence, the data from both years were pooled for subsequent analysis. Their mean SVL was 53.50±4.55 mm, ranging from 42 to 60 mm.

Males of *P. kulzeri* spend four months in hibernation and emerge from hibernation in April. Their testes showed active spermiogenesis in all histological sections from April and May and from September to November (Figure 2). During this period, the testicular volumes were at their maximum (Figure 3). Epididymides were hypertrophied with spermatozoa and secretion granules in their lumen. About 40% of males examined in July and August exhibited a short testicular regression (Figure 2). Their epididymides were involuted with no spermatozoa in their lumen. This period corresponds to the hottest months (Figure 1).

Monthly variations in the testicular volume were detected (ANCOVA $F_{(7,46)}$=3.06, p<0.05) and a significant correlation was found between relative testicular volume and body size (R=0.81, p<0.001). However, large animals did not have relatively large testes (R=0.0, p>0.5).

Discussion

Males of *Phoenicolacerta kulzeri* showed a more distinct reproductive pattern than the common reproductive pattern of lacertid lizards of the Mediterranean region. Indeed, two types of spermatogenesis occur in Mediterranean lacertids (Saint-Girons, 1984; Carretero, 2006): the vernal or prenuptial type, in which active spermatogenesis begins immediately before or even during breeding, and the mixed type, in which active spermiogenesis occurs in spring, followed by summer regression and subsequent autumnal recrudescence. In autumnal testicular recrudescence, the presence of spermatocytes (Hraoui-Bloquet, Sadek, & Sabeh, 1999; Nassar & Hraoui-Bloquet, 2014), and spermatids (Carretero & LIorente, 1997) and even spermatozoa (Angelini, Picariello, &

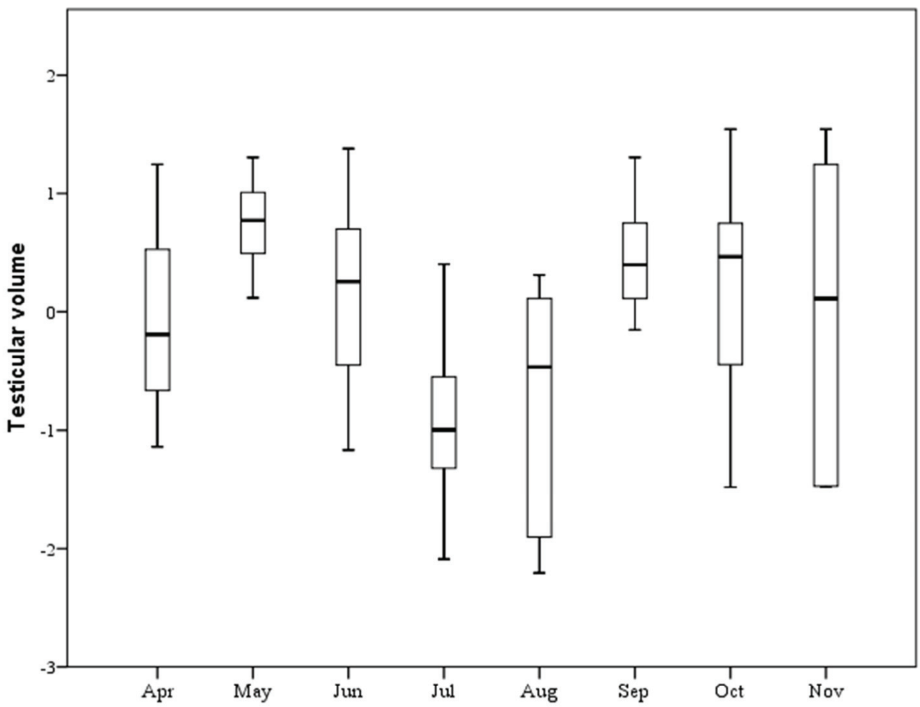

Figure 3. Annual variation in the testicular volume of *Phoenicolacerta kulzeri* males. Abscissa shows the regression residuals between testicular volume and the SVL (both log-transformed). Box lengths represent the interquartile range; Horizontal lines within the boxes represent the median values and the whiskers represent the smallest and largest sample values (minimum-maximum).

Botte, 1976; Roig, Carretero, & Llorente, 2000; Saint Girons & Duguy, 1970; Angelini, Brizzi, & Barone, 1979; Carretero, Ribeiro, Barbosa, Sá-Sousa, & Harris, 2006) indicates a mixed-type spermatogenesis (Saint-Girons, 1963, 1984; Carretero, 2006). However, this process of autumnal spermiogenesis has no reproductive function since it is not followed by any release of spermatozoa in the epididymis nor is there appropriate development of other secondary sex characters (Angelini et al., 1976, 1979; Roig et al., 2000). Males of *P. kulzeri* exhibited spermiogenesis in early autumn, from September until they enter into hibernation in November, and in spring following the renewal of the post-hibernation activity, with the presence of spermatozoa and secretory granules in their epididymis. Similar observations were reported in *P. laevis* with secondary sexual characters well developed and showing secretory activity in both autumn and spring (Haroui-Bloquet, 1985, Haroui-Bloquet & Bloquet, 1988). Molecular and phylogenetic analysis clearly shows a close relation between *P. laevis* and *P. kulzeri* but they are considered distinct species (Beyerlein & Mayer, 1999; In den Bosch, 2003; Modrý et al., 2013; Tamar et al., 2015). The presence of sperm in the epididymis is functionally an important event. Males of *P. kulzeri* are fertile, and autumnal spermiogenesis is not abortive or non-functional. This reproductive pattern is uncommon in lacertid lizards. However, the presence of spermatozoa in the testes and epididymis in spring and autumn was reported in *Liolaemus* species from the cold temperate climate of the Patagonian steppe (Medina & Ibargüengoytía, 2010).

The reproductive activity in lizards is under the control of complex interactions among phylogenetic, physiological and environmental factors (Angelini et al., 1976; Angelini, D'Uva, Picariello, & Ciarcia, 1978; Saint-Girons, 1984; Carretero, 2006; Van Dyke, 2014). Our results showed that the male reproductive pattern of *P. kulzeri* reflects the phylogenetic or/and physiological constraints of this species rather than an adaptive response to its environmental conditions.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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