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On the reproduction of *Lacerta trilineata* (Squamata: Lacertidae) in Bulgaria

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True lizards (family Lacertidae) include at least 385 recognised species, with almost ubiquitous distribution throughout the Old World (Uetz et al. 2024). In Europe, 88 species are recognized, i.e., almost half of the species of the order Squamata that occur on the continent (Speybroeck et al. 2020). Although the reproduction of a number of lacertid species is well studied (Bosch & Bout 1998, Amat 2008), data is still lacking for others.

The Balkan Green Lizard, *Lacerta trilineata* Bedriaga, 1886, is the largest member of Lacertidae in the Balkans, reaching up to 17.4 cm in snout–vent length (SVL) (Wettstein 1953). Both sexes become sexually mature at approximately 80 mm in SVL (Sagonas et al. 2019). According to modern concepts, four subspecies are recognised (*L. t. hansschweizeri* Müller, 1935, *L. t. major* Boulenger, 1887, *L. t. polylepidota* Wettstein, 1952, and *L. t. trilineata* Bedriaga, 1886), distributed along the Adriatic Coast and southwestern part of the Balkan Peninsula, as well as in some of the adjacent islands, incl. Crete (Kornilios et al. 2019). In Bulgaria, the nominate subspecies is found, and its national range includes only the most southwestern part of the country (the Struma Valley south of the beginning of the Kresna Gorge) up to about 550–700 m a.s.l. (Stojanov et al. 2011; authors' unpublished data from 2023–2024). Data on the clutch and eggs size of *L. trilineata* have been published only by Nettmann & Rykena (1984) (based on terrarium observations of individuals from Split (Croatia) and Sifnos Island (Greece)) and Sagonas et al. (2019) (based on dissection of dozens of specimens from Greece (museum material)). Data on hatchling size are available only for *L. t. hansschweizeri* from the Sifnos Island (Nettmann & Rykena 1984). The main goal of the present study was to derive and analyse data for the size of clutches, eggs and hatchlings, in *L. t. trilineata* from its northern range limit.

For this purpose, fieldwork was conducted in May 2024 the area between the villages of Vlahi and Stara Kresna (41°44'–41°46' N, 23°10'–23°13' E; 450–700 m a.s.l.) in the Struma Valley (SW Bulgaria). The terrain there is mountain foothills with a well-developed microrelief and heterogeneous land cover (a mosaic of low-stem forests, open stony patches with grass and shrub vegetation, bare rocks, etc.; Fig. 1A). According to a climate model for Vlahi village (https://www.meteoblue.com/en/weather/historyclimate/climatemodelled/vlahi_bulgaria_725821) the mean daily maximum of the air temperature varies between 6 °C (January) and 29 °C (July and August), the mean daily minimum respectively between -2 °C and 17 °C, and the mean monthly precipitation varies between 32 mm (August) and 79 mm (December).

Four females of *Lacerta trilineata* (thereafter as F1– F4) captured during the field trips had visible signs of pregnancy, were kept in laboratory conditions and later released at the site of capture in July. These gravid females were placed individually in transparent boxes (dimensions 40/20/20 cm) with a "warm spot" (ca. 35 °C) provided by underfloor heating active from 08:00 to 20:00 h and were given live food (crickets and cockroaches) every two days. Each female was provided with a small, covered plastic container filled with moist substrate and equipped with an access hole, serving as a nesting site.

Egg laying in these females occurred between 4–13 June, and the number of eggs they laid varied between 7 and 13 (average 9.5) (Fig. 1B, C; Table 1). The laid eggs were placed in individual plastic containers (15/8/8 cm, filled with perlite), which in turn were placed side by side in a larger box with a lid (with air holes). The box was not heated, but the temperature was automatically recorded every 30 minutes by an Elitech RC-51H data logger, and the individual egg containers were humidified every 5–6 days by spraying

with water. The mean diurnal temperature in the incubation box was 26.1 °C (Min 23.9 °C; Max 29.5 °C) with a mean diurnal amplitude of 1.5 °C. Incubation period of the individual clutches lasted from 79 to 88 days (with a mean of 84 days). The hatchlings were kept in the same conditions as adult females and were released at the capture sites of the respective females in September 2024.

The following measurements were taken on the laid eggs (on the day of laying): maximum length and width (using a digital calliper with an accuracy of 0.01 mm), and weight (using a digital scale with an accuracy of 0.01 g). Egg volume was calculated using the standard formula for

Table 1. Measurements of the captured gravid females of *Lacerta t. trilineata*: snout–vent length in mm (SVL), clutch size (CS = number of eggs), clutch mass in grams (CM), and the dates of egg laying (D1) and hatching (D2). * Two of the eggs were not weighted, i,e. the sum refers to 11 eggs.

ID	SVL	CS	CM	D1	D2
F1	127	8	–	4 June	28–29 August
F2	120	10	–	4 June	21–25 August
F3	126	7	7.77	9 June	n/a
F4	136	13	11.9*	10–13 June	5–6 September

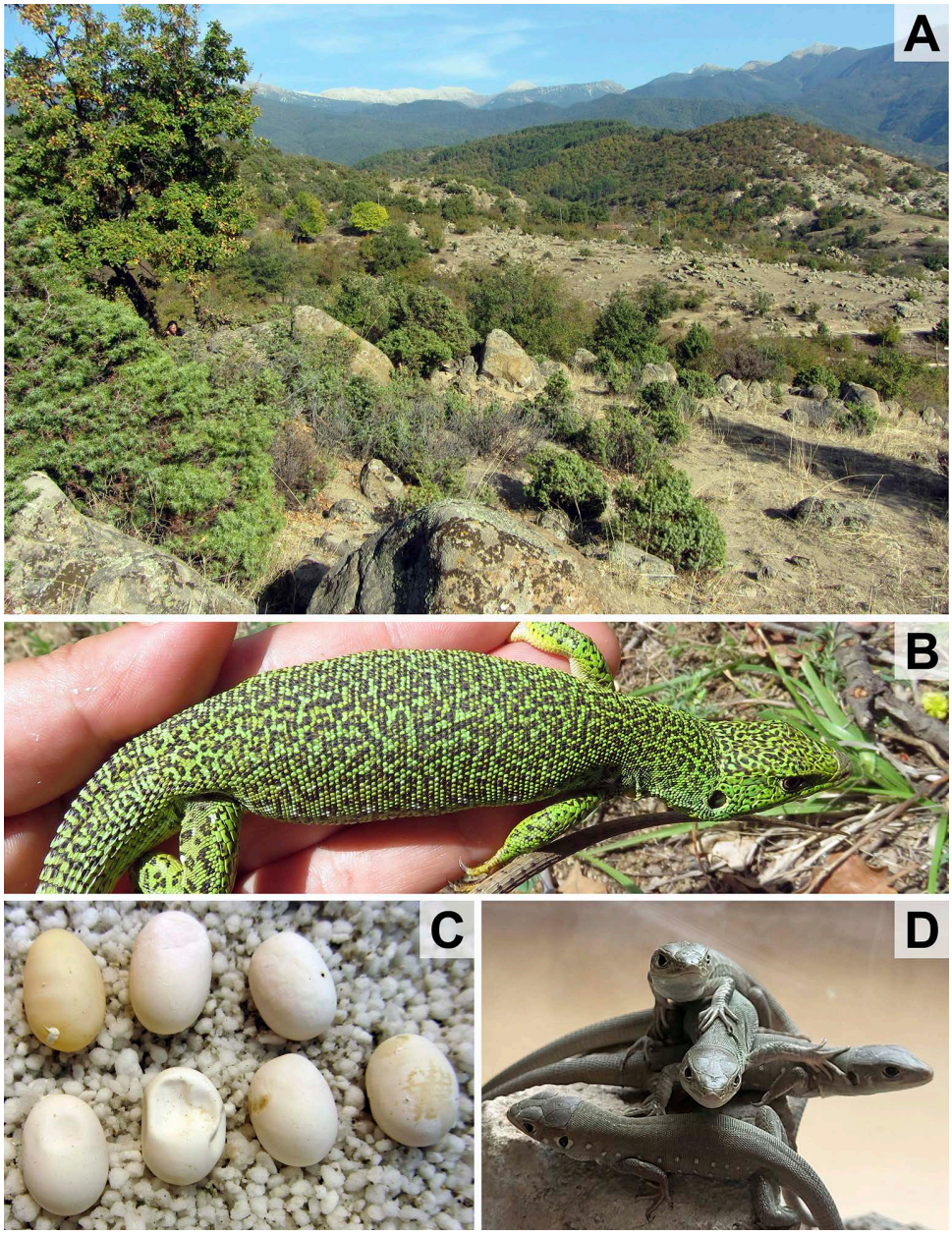

Figure 1. (A) a view of the study area (October 2023); (B) one of the gravid females of *L. t. trilineata* (22 May 2024); (C) one of the clutches (June 9, 2024); (D) hatchlings (31 August 2024).

Table 2. Descriptive statistics for measurements of the eggs and hatchlings of *Lacerta t. trilineata* (SVL = snout–vent length; TL = total body length). * One of the two hatchlings had an underdeveloped tail (24 mm).

			N	Mean	SD	Min	Max
F1	Eggs	Length (mm)	8	18.20	0.60	17.0	19.0
		Breadth (mm)	8	11.84	0.33	11.2	12.2
		Mass (g)	–	–	–	–	–
		Volume (mm^3)	8	901.77	33.16	839.8	939.8
	Hatchlings	SVL (mm)	8	42.0	1.3	40	44
		TL (mm)	8	121.6	1.8	119	124
		Mass (g)	8	1.616	0.090	1.51	1.78
F2	Eggs	Length (mm)	10	18.54	0.30	18.1	19.0
		Breadth (mm)	10	11.84	0.21	11.5	12.1
		Mass (g)	–	–	–	–	–
		Volume (mm^3)	10	918.94	17.41	894.2	944.5
	Hatchlings	SVL (mm)	7	34.6	1.3	33	36
		TL (mm)	7	94.9	2.5	91	97
		Mass (g)	7	1.106	0.130	0.95	1.25
F3	Eggs	Length (mm)	7	16.84	1.13	16.0	19.0
		Breadth (mm)	7	11.56	0.39	11.1	12.3
		Mass (g)	7	1.110	0.186	0.82	1.43
		Volume (mm^3)	7	816.33	81.78	743.6	978.4
	Hatchlings	SVL (mm)	–	–	–	–	–
		TL (mm)	–	–	–	–	–
		Mass (g)	–	–	–	–	–
F4	Eggs	Length (mm)	11	16.25	0.63	14.5	16.7
		Breadth (mm)	11	11.21	0.74	9.4	11.9
		Mass (g)	11	1.082	0.147	0.80	1.24
		Volume (mm^3)	11	764.22	72.82	570.6	827.0
	Hatchlings	SVL (mm)	2	38.5	n/a	38	39
		TL (mm)	2	81.0*	n/a	63*	99
		Mass (g)	2	1.245*	n/a	1.23*	1.26
Overall	Eggs	Length (mm)	36	17.44	1.19	14.5	19.0
		Breadth (mm)	36	11.59	0.54	9.4	12.3
		Mass (g)	18	1.093	0.159	0.80	1.43
		Volume (mm^3)	36	847.90	86.40	570.6	978.4
	Hatchlings	SVL (mm)	17	38.5	3.8	33	44
		TL (mm)	16	108.5	13.7	91	124
		Mass (g)	16	1.369	0.277	0.95	1.78

ellipsoid volume. Egg and hatchling sizes were described by arithmetic mean (Mean), standard deviation (SD), minimum (Min) and maximum (Max).

The hatch success (as percentage) varied as follows: F1 – 100%; F2 – 80% (including one case of death when the hatchling emerged from the egg); F3 – 0% (the eggs looked normal on the day of laying, but died soon after); F4 – 15% (two of the eggs were severely deformed when laid, and the rest looked normal, but most died a few days later).

The egg size (Table 2) varied between 14.5 and 19.0 mm in length, between 0.80 and 1.43 g in weight, and between 570.6 and 978.4 mm^3 in volume The size of the hatchlings ranged between 33 and 44 mm in SVL, between 91 and 124 mm in total length, and between 0.95 and 1.78 g in weight.

The few available data concerning the breeding cycle of *Lacerta trilineata* shows regional similarities for the time of the year in which egg laying occurs. For a population near Split, Croatia (resp. *L. t. major*), Nettmann & Rykena (1984) report freshly hatched young in October, which according to the authors suggests that the eggs were laid in June. Our observations coincide with this, since egg laying in the individuals studied occurred in the first half of June. However, more research is needed to prove this assumption.

In lacertid lizards, females typically lay one or two clutches per season, depending on species and environmental conditions. While sperm storage allows fertilization of multiple clutches from a single mating (Zotos et al. 2013), mating between clutches may still occur, particularly in populations with extended breeding seasons or high male activity. This can provide adaptive advantages, as genetically diverse sperm may enhance offspring fitness by allowing female choice among sperm from different males (Olsson et al. 1997). Two clutches per year are known for *L. trilineata* from ex-Yugoslavia (probably *L. t. major* from Split in view of the other data in the cited publication). Also, for *L. t. hansschweizeri*, kept in terrariums, there was an interval of four to five weeks between the two clutches (Nettmann & Rykena 1984). Additionally, Sagonas et al. (2019) claim that the larger females (SVL 125 ± 14.18 mm) of *L. trilineata* from Greece may produce more than one clutch per year compared to smaller ones (SVL 105 ± 20.05 mm). In our case, there was no second egg-laying during the stay of the four females kept under laboratory conditions (30–37 days after the initial recorded egg-laying), and at the time of release none of them had visible signs for the presence of eggs in the oviducts. There was no way to determine whether these individuals had laid eggs before capture (May 21–22), but this seems very unlikely. In fact, these females were relatively large (SVL 113.5 ± 10.39 mm), so probably a lack of a second pregnancy during the year could not be due to body size. In view of these, it can be assumed that in the study area, *L. trilineata* lays eggs only once a year. This could be explained by differences in climatic conditions, as our study population is located at the northern limit of the species range, while the data for two clutches per year refer to populations from the Adriatic coast and Greece, where the warmer climate probably allows for a longer activity period of the lizards during the year.

In terms of clutch size, our results showed an average of 9.5 eggs per clutch, which corresponds to what Sagonas et al. (2019) found for *L. trilineata* in mainland Greece: an average of 9.4 eggs in the oviducts (via dissection of 33 females). Nettmann & Rykena (1984) reported an average of 7.5 eggs in four clutches of Mediterranean *L. trilineata* from the first year of sexual maturity, but an average of 12.5 eggs in 17 clutches of older animals. However, it should be noted that "Mediterranean" probably also includes taxa no longer considered subspecies of *L. trilineata*. Although our sample size was modest, it is interesting to note that the largest female (SVL 136 mm) laid the highest number of eggs (13), which to some extent confirms the positive correlation between the number of eggs and maternal body size found by Sagonas et al. (2019).We also have to point out that the largest clutch had the smallest eggs (Table 2), which corresponds to the negative correlation between clutch size and egg volume found by Sagonas et al. (2019).

All available data (including this study) for the period of egg incubation of *L. trilineata* originate only from observations in artificial environments, and it is not clear to what extent they reflect the natural length of this period. Our results reported a total of 79–88 days of incubation, with an average 84 days at an average daily temperature of 26 °C. On this matter, Nettmann & Rykena (1984) registered an incubation time of 79–81 days at about 27 °C for *L. trilineata* from ex-Yugoslavia (resp. *L. t. major* and/or *L. t. trilineata*), and 89–96 days at 29 °C, 90–96 days at 29 °C, and 85–93 days at 30 °C for *L. t. hansschweizeri*. Although it is unclear whether these data have been obtained under constant temperatures. In our case, the temperature was not controlled, but the average value of the diurnal amplitude was small (1.5 °C). Therefore, it seems that at lower temperatures the incubation period is shorter. It is possible that this reflects ecological adaptations of the taxa to local climatic conditions. Nettmann & Rykena (1984) have registered an incubation period of 150 days at 30 °C for *Lacerta media israelica* Peters, 1964, and have interpreted this as an adaptation to the climatic conditions, in which hatching is avoided in the dry summer months.

Regarding the hatchlings, it seems that in *Lacerta t. trilineata* (this study: mean SVL 38.5 mm; n = 17) they are smaller than in *L. t. hansschweizeri* (Nettmann & Rykena (1984): mean SVL 43.3 mm; n = 16). It should be noted that both samples are small, so it is possible that they reflect features of local populations rather than characterizing the two taxa as a whole. This data is not yet available for the other two subspecies at least to our knowledge. Thus, for a complete morphological characterization of *L. trilineata* hatchlings further research across other parts of the species' range are needed.

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