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Reproductive characteristics of the Batuecan Lizard, *Iberolacerta martinezricai* (ARRIBAS, 1996)

(Squamata: Sauria: Lacertidae)

Zur Fortpflanzungsbiologie von *Iberolacerta martinezricai* (ARRIBAS, 1996)
(Squamata: Sauria: Lacertidae)

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KURZFASSUNG

Der Autor untersuchte das Fortpflanzungsgeschehen von *Iberolacerta martinezricai* (ARRIBAS, 1996). Kopulationen begannen Ende April. Im Juli trugen alle Weibchen zahlreiche frische Paarungsnarben auf Bauch und Schwanzbasis. *Iberolacerta martinezricai* ist monoestrisch. Die Eiablage erfolgte im Juli und Anfang August, am häufigsten gegen Ende Juli. Das Eiablageverhalten wird beschrieben. Eier wurden zwischen 9:00 und 15:30 Uhr (GMT) gelegt, wobei der ganze Ablagevorgang, 33 - 42 Minuten (Durchschnitt: 36.57 Minuten) dauerte. Die Kopf-Rumpf-Länge reproduktiver Weibchen betrug 56.7 - 69.71 mm. Die Gelege umfaßten zwei bis sechs Eier (Median und Modalwert: 4, Mittel: 4,23). Die abgelegten Eier klebten nicht aneinander. Biometrische Daten von 49 Eiern (darunter 12 Laboraufzuchten) werden angegeben. Während des Großteils der Inkubationsphase nahmen Eivolumen und Eimasse durch Wasseraufnahme kontinuierlich zu. Zu Beginn der Inkubation erfolgte ein rascher Anstieg des Ei-Querdurchmessers (dieser wurde während der Ablage durch die Enge des Geburtskanales verringert). Die Inkubation dauerte 33 - 42 Tage (im Mittel 37.75 Tage). Das Schlüpfen erfolgte zwischen 3:00 und 22:00 Uhr (GMT) (im Mittel 9.40 Uhr) und dauerte 4.10 - 12 Stunden (im Mittel 6.9 Stunden). Die Merkmale des Schlüpflings werden beschrieben. Der Eizahn bestand für acht bis 36 Stunden (im Mittel 23.6 Stunden) nach Schlupfbeginn. Schlüpflinge konnen von Geburt an mit ihrem leuchtend blauen Schwanz wedeln und diesen autotomieren. Diese auffällige Schwanzfärbung verschwand im Laufe des zweiten Kalender-Lebensjahres.

ABSTRACT

The author studied reproductive traits of *Iberolacerta martinezricai* (ARRIBAS, 1996). Copulation began at the end of April. In July all the females presented numerous recent mating scars on the belly and proximal part of the tail. *Iberolacerta martinezricai* is monestrous. Oviposition occurred during July and beginning of August, mostly towards the end of July. The female's behavior during the egg-laying process is described. Eggs were laid from 9:00 to 15:30 h (GMT), the whole process lasting 33 - 42 minutes (average 36.57). Snout-vent-length of reproductive females was between 56.7 - 69.71 mm. Clutches comprised two to six eggs (median and mode: 4, average: 4.23). Eggs were not glued together but remained separated from each other. Biometric data of 49 eggs (12 lab-controlled until their eclosion) are given. During most of the incubation period, the eggs increased their volume and mass continuously by water absorption. There was a rapid increase of egg width at the beginning of the incubation (during oviposition egg width is constricted by the tightness of the birth canal). Incubation lasted between 33 - 42 days (average 37.75 days). Eclosions occurred from 3:00 to 22:00 h (GMT) (average 9.40) and lasted 4.10 - 12 hours (average 6.9). Hatchling characteristics are described. The egg-tooth persisted during eight to 36 hours (average 23.6) after the beginning of the hatching process. Hatchlings could autotomize and wave their bright blue tails from birth; this conspicuous caudal coloration was reduced and lost during the second calendar year of life.

KEY WORDS

Reptilia: Squamata, Lacertidae, *Iberolacerta*, *Iberolacerta martinezricai*, biology, life history, reproduction, breeding, egg-laying, egg characteristics, incubation, eclosion, hatchling characteristics, juvenile coloration; Peña de Francia, Batuecas, Spain

INTRODUCTION

Iberolacerta martinezricai (ARRIBAS, 1996), is the most range-restricted and probably one of the rarest and most threatened reptile species in continental Europe. A recent survey of the habitat and distribution of this steno-endemic lizard confirmed that the size of its distribution area is only 12-15 km^2 located in three 10 km x 10 km UTM grid squares (29TQE48; 29TQE38; 29TQE39) with an estimated total occupied area between

20-25 km^2 and a population of 1,200 to 1,500 individuals (CARBONERO et al. 2016).

The habitat of *I. martinezricai* lies in the north of the Natural Park of Las Batuecas, at medium to high altitudes of the Sierra de Francia mountain range (840 m - 1,730 m). One subarea is centered around the peak Peña de Francia (1,723 m), extending northward to the peak Pico de los Robledos (1,611 m) plus surroundings, to the northeast as far as the Sierra del Guindo (Hastiala mountain, 1,735 m; Alto del Copero, 1,560 m), southward to the Mesa del Francés mountain (1,640 m) and towards the southeast, up to the northern slopes (and probably also the southern upper parts) of the Sierra de la Grajera (Rongiero peak, 1,627 m) with the range ending in the Extremadura region (ARRIBAS 2006, 2013; CARBONERO et al 2016). The other subarea is called Puerto El Portillo ("El Portillo Pass", from 840 m to 1,400 m) and includes the heights of the Batuecas Valley on the southern slope of the Sierra de la Alberca where populations of the Batuecan Rock Lizard were discovered in 2004 (ARRIBAS 2004a, 2009, 2013). The species was not found in other areas more to the west, as previously expected. The Batuecan Rock Lizard is considered Critically Endangered (Cr, B2ab(v); C2a(ii)) (ARRIBAS 2006, 2013; PÉREZ-MELLADO et al. 2009). Despite the fact that the entire population occurs inside the protected area of the Parque Natural de las Batuecas y Sierra de Francia and is protected by law, a specific management program or recovery plan to ensure preservation of this species is still pending, and the lizard is simply protected along with its wild habitat.

Life history parameters such as phenology, molting events, thermoregulation, activity, distribution, habitat selection, population density and sex-ratio are dealt with in ARRIBAS (2013), growth, allometry, sexual dimorphism, longevity and an estimation of predation pressure in ARRIBAS (2014a). The most serious deficit in the knowledge of this species is its reproductive traits, which are described in the present paper.

MATERIALS AND METHODS

Study sites.- Data about the reproductive state of the lizard come from all over its distribution area. First studies were made in the Peña de Francia mountaintop, later in the Batuecas valley, and finally again in the Peña de Francia slopes at lower altitudes. Fieldwork was done in sporadic visits from 1993 to 2014.

Gravid females.- Data on females were collected concerning the following aspects: i) Activity - inactive (cold and immobile under rocks), active (basking or moving). ii) Reproductive state - copulated (recent mating scars present), highly gravid (oviductal eggs palpable), or short after oviposition (lateral folds present). iii) Habitat - estimated percentages of rocks, stones, bare ground, grass or shrubs covering the site ("percent cover") in a two-meter radius around the spot of first localization of the animal. These percent covers (%) were estimated by eye, comparing with graphic surface-calculation scales (EMBERGER 1983); the inclination of the site was recorded as well.

Clutches in laboratory.- Some gravid females (lizards in the state just before egg-laying are rare and difficult to find) were transported to the laboratory (located at 1,020 m a.s.l.) to monitor the oviposition and incubation processes. Females were placed in glass or plastic terraria (base: 30 cm x 20 cm) with peat substratum and a flat stone for digging underneath. Food (small arthropods from the laboratory area captured with a light-trap) was supplied ad libitum, although gravid females when very near to oviposition do not feed at all. Lizards were maintained under natural illumination and photoperiod conditions (direct sun from sunrise to near 12:00 GMT, and shadowy but warm until sunset).

Females were controlled about hourly during the day, and twice per night, during the entire study of the oviposition process. Eggs laid were marked, measured and placed in plastic boxes for incubation under natural temperature conditions (24-30 ºC) in the open air under the conditions described by ARRIBAS (2004b) and ARRIBAS & GALAN

(2005) for other *Iberolacerta* species. These plastic boxes were furnished with peat or vermiculite substrate and a piece of moss to cover the eggs, to provide a humid environment and facilitate easy periodic inspection. Four perforations in the boxes ensured air circulation and prevented from mold development. Excessive moisture decrease was countervailed by spraying the moss and the eggs with water). Clutches were inspected daily and egg metrics (length, width, mass) were taken weekly. Egg volume (V) was calculated based on an ellipsoid approximation ($V = 4\pi a^2 b/3$), a and b being half the width and half the length of the egg, respectively.

Length measurements were taken with digital callipers (Mitutoyo®, accuracy 0.1 mm) and egg-masses with a portable digital scale (Tomopol s050®, accuracy 0.01 g).

When hatchlings perforated their eggshells, hour (GMT) and total duration of hatching process were recorded, as well as duration of egg-tooth persistence. From each newborn lizard, snout-vent-length (SVL), body mass and the extent (relative to ventral scale rows) of the umbilical scar were noted. Hatchlings were sexed mainly by the number of transversal series of ventral scales (males 27-28, females 28-30)(Arribas 2014d).

Newborn specimens were successfully fed with aphids until release at the site of their mother's origin.

Statistical calculations were performed with NCSS-2007© (Hintze 2007). Differences were tested using t-tests or One-Way ANOVA, with Tukey-Kramer post-hoc tests for pairwise comparisons among the group means (Sokal & Rohlf 1969; online help in Hintze 2007).

RESULTS AND DISCUSSION

Sexual maturity and dimorphism were studied by Arribas (2014a). The former is most probably reached when lizards are three years old, i.e., in their fourth calendar year (4th CY). The smallest gravid female studied was 56.7 mm in SVL and probably reproducing in the spring of its 5th CY, i.e., at the age of four years. Some male and female specimens may reach maturity in their third CY, as occurs in *I. monticola* (Boulenger, 1905) (Arribas 2014a). The biggest male (SVL 68.15 mm) and female (SVL 68.86 mm) conserved had seven growth rings (corresponding to two strong and five weak growth periods) in the femur cross sections. Both specimens were most likely eight years old (in their ninth CY: the birth year + seven seasons of growth + the year of capture) (Arribas 2014a).

Comparable results are known from *I. monticola* which reaches the age of 10 years in the Serra da Estrela (Portugal) (Moreira et al. 1999) and one among hundreds the exceptional age of 15 years (Galán 2011a). Similarly, a male *I. aurelioi* (Arribas, 1994) was at least 16 and a female possibly 14 years old (Arribas 2004b, 2007).

As to sexual dimorphism, male and female *I. martinezricai* differ at $p < 0.001$ in the number of transversal ventral scale rows (M < F, males less than females) and femoral scales (M > F), forelimb length (M > F), hindlimb length (M > F) and pileus length (M > F), and at $p < 0.05$ in the number of collar scales (M > F) and longitudinal dorsal scale rows ["dorsalia"] (M > F) (Arribas 2014a).

Sex ratio calculated from the author's morphological database was 0.74:1 (29 males : 39 females), which does not differ significantly from 1:1 (Multinomial test: $Chi^2 = 1.47$, NS), whereas from the clearly higher number of field observations (repetitions included) was (1.27 : 1), significantly favoring males (260 males : 204 females) (Multinomial test: $Chi^2 = 6.75$, P = 0.009) (Arribas 2013). However, recalculation based on new field data (357 observations of males and 337 of females) resulted in the absence of significant differences, showing a balanced sex ratio (1.06:1) (Multinomial test: $Chi^2 = 0.57$, P = 0.44, NS). From 12 hatchlings of lab-controlled eggs, six were females and six males.

Phenology (largely based on Arribas 2013).- The lizard's annual activity begins at the end of March or, more frequently, during April and lasts until the end of September or beginning of October. A general pre-oviposition molt is observed in gravid females during the first half of July.

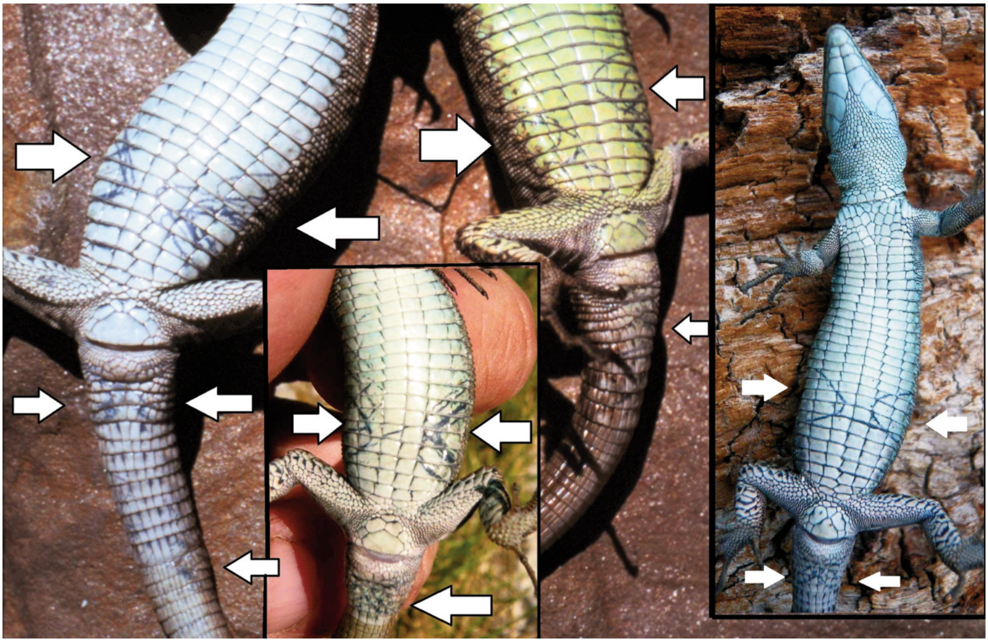

Fig 1: *Iberolacerta martinezricai* (ARRIBAS, 1996) from Peña de Francia and Batuecas Valley (Salamanca, Spain). Females showing recent mating scars on belly and tail base. Arrows indicate the areas worst affected.

Abb. 1: *Iberolacerta martinezricai* (ARRIBAS, 1996) von der Peña de Francia und dem Batuecas Tal (Salamanca, Spanien). Die Weibchen zeigen frische Paarungsnarben auf Bauch und Schwanzbasis. Pfeile weisen auf die am stärksten betroffenen Stellen.

Places occupied by the species (in particular the lower altitudinal and therefore usually milder sites) are surrounded and protected from direct insolation by mountain spurs that condition the lizard's activity and phenology. In some locations the sun does not reach the ground during the cold periods of the year. In specimens living under such conditions, the yearly and daily activity periods are considerably shortened.

Fig. 2 (opposite page) / Abb. 2 (gegenüberliegende Seite)

A - *Iberolacerta martinezricai* (ARRIBAS, 1996) from Peña de Francia (Salamanca, Spain). Gravid female of the blue morph. Note that contrary to gravid female lizards, the body does not appear very bulky in this species with small clutch size. B - Gravid female of the green morph. C - Two gravid females of the green morph, one reticulated and the other with paravertebral rows of spots. As in A and B, gravid females are not very voluminose. D - Comparison between gravid adult females of *Iberolacerta martinezricai* (ARRIBAS, 1996) from the Sierra de Francia, Salamanca, Spain and *Iberolacerta cyreni* (MÜLLER & HELLMICH, 1937) from the neighboring Sierra de Bejar (Salamanca, Spain). Note the differences in size and bulkiness.

A - *Iberolacerta martinezricai* (ARRIBAS, 1996) von der Peña de Francia (Salamanca, Spanien). Trächtiges Weibchen der blauen Morphe. Man beachte die im Unterschied zu anderen trächtigen Eidechsen geringe Massigkeit des Rumpfes bei dieser durch geringe Gelegegröße gekennzeichneten Art. B – Trächtiges Weibchen der grünen Morphe. C - Zwei trächtige Weibchen der grünen Morphe, eines mit Netzzeichnung, das andere mit paravertebralen Punktreihen. Wie bei A und B, erscheinen die trächtigen Weibchen nicht sehr füllig. D – Vergleich zwischen adulten trächtigen Weibchen von *Iberolacerta martinezricai* (ARRIBAS, 1996) der Sierra de Francia, Salamanca, Spanien, und *Iberolacerta cyreni* (MÜLLER & HELLMICH, 1937), aus der benachbarten Sierra de Bejar (Salamanca, Spain). Man beachte die Unterschiede in Größe und Körperfülle.

2A

2B

2C

2D

By this, the total activity cycle of *I. martinezricai* is only slightly longer (six months in total) than in true high mountain species, such as other *Iberolacerta* (*Pyrenesaura*), whose annual activity period covers slightly less than five months (ARRIBAS & GALAN 2005).

In the lizard's habitats, not only the annual but also the daily insolation cycles are relatively short, just a few hours of direct sunlight per day. Due to the relief of the mountains and the orientation and inclination of the slopes, the sun reaches the habitats late in the morning, especially the lowermost portions of the slopes. Hence, and paradoxically for a lizard living at the edge of the Mediterranean realm, its activity period seems to be shorter and reproduction activities seem to be delayed in comparison with the neighboring high mountain (Oro and Crioromediterranean) dweller *I. cyreni* (MÜLLER & HELLMICH, 1937), from the Sierra de Bejar (ARRIBAS, pers. obs.).

Mating and egg-laying period.- In the first days of April, females have not yet copulated (recent mating scars are absent). Copulations begin at the end of April (three females with mating scars and five without it in April 30, 1995). There is no information concerning the period of May and June, but it is reasonable to assume that, depending on the site characteristics and weather, territorial behavior and copulation occur mainly during these months. In the first week of July, all the observed females presented numerous recent mating scars (very dark, almost black) at the belly sides and proximal part of the tail, corresponding to copulatory bite marks received during the previous courtship (Fig. 1).

Copulation in the field was observed in the first week of July. Seven out of 21 females with copulation scars on the belly had also big marks at the tail base (Fig. 1). Females were observed basking even under suboptimal weather conditions during the first fortnight of July (ARRIBAS unpublished), probably to promote vitellogenesis.

There was no significant difference in body temperature (BT) between gravid females of the "blue" and "green" morphs (BT green gravid females: 33.24 ± 0.18 ºC, 29.2-36.4, N = 85 observations; BT blue gravid females: 32.71 ± 0.47 ºC, 18.5-36.8, N = 50 observations; $t = 1.21$, $P = 0.22$) (Fig. 2A - 2C). Also, there was no significant difference in BT between gravid (reproductive) and non-gravid (post-reproductive) females (BT gravid females: 32.98 ± 2.45 º C, 18.5-36.8, N = 143 observations; BT non-gravid females: 33.27 ± 1.84 ºC, 26.2-36.7, N = 105 observations; $t = 1.02$, $P = 0.30$).

Oviposition occurred during July and in the first days of August. The egg-laying period spanned from calendar week # 25 (first week of July) to calendar week # 29 (first week of August) (average: week # 25.5 ± 0.4). From 13 clutches controlled, two were laid in the first week of July (calendar week # 25), two during the second (# 26), none in the third, five during the last week of July (# 28), and four in the first week of August (# 29). The earliest post-oviposition female was found in the first week of July (July 6, 2007).

Surprisingly for a mountain lizard in an almost Mediterranean environment, the phenology of *I. martinezricai* does not precede the timing of the seasonal life cycle events of other *Iberolacerta*. However, as detailed above, the species occupies high and particularly harsh climate habitats in its range area. The shading capacity of the slope that influences the phenology, the weather in late spring and perhaps the age of the females (maybe younger females lay their eggs later, as occurs in other small la-

Fig. 3 (opposite page) / Abb. 3 (gegenüberliegende Seite)

Iberolacerta martinezricai (ARRIBAS, 1996) from the Peña de Francia (Salamanca, Spain). A - Freshly laid eggs. B - Eggs during incubation, close to the eclosion. C and D - Two moments of the eclosion in the laboratory.

Iberolacerta martinezricai (ARRIBAS, 1996) von der Peña de Francia (Salamanca, Spanien).
A – Frisch gelegte Eier. B – Eier während der Bebrütung, nahe dem Schlupftermin.
C und D – Zwei Zeitpunkte des Schlupfes im Labor.

3A

3B

3C

3D

Table 1: Measurements (arithmetic mean ± standard error; minimum-maximum) of all the studied eggs of *Iberolacerta martinezricai* (ARRIBAS, 1996) ["All recently laid clutches known"], and data of the lab-controlled eggs ["incubated and hatched clutches"] during the weeks of incubation. Data in the left part of the table represent all available data from eggs at oviposition including fully lab-controlled as well as occasionally observed clutches, in total, 49 eggs from 11 clutches. Column three and the columns to its right, present information of three controlled clutches comprising 12 eggs from oviposition to eclosion. See text for interpretation of the parameters and their changes. (∗) - Only to be compared with the data of the column "Oviposition" under "Three incubated and hatched clutches".

Tab. 1: Meßwerte (arithmetisches Mittel ± Standardfehler des Mittelwertes; Minimum-Maximum) aller untersuchten Eier von *Iberolacerta martinezricai* (ARRIBAS, 1996) ["All recently laid clutches"], und der im Labor überwachten Gelege ["incubated and hatched clutches"] während der Wochen der Inkubation. Die Angaben auf der linken Tabellenseite repräsentieren alle verfügbaren Informationen über Eier zum Zeitpunkt ihrer Ablage, einschließlich jener von laborkontrollierten und nur gelegentlich überwachten Gelegen, insgesamt 49 Eier von 11 Gelegen. Ab Tabellenspalte drei beziehen sich die Angaben auf drei Gelege mit insgesamt 12 Eiern, deren Zeitigung von der Ablage bis zum Schlupf überwacht wurde. Im Text werden die Meßwerte und ihre Veränderung interpretiert. (∗) - Nur zum Vergleich mit den Daten in der Spalte "Oviposition" unter "Three incubated and hatchel clutches".

All recently laid clutches known(∗)		Three incubated and hatched clutches (12 eggs in total)						
(both fully lab-controlled and occasionally observed)			Oviposition	One week	Two weeks	Three weeks	Four weeks	Five weeks
					after	oviposition		
			(N = 12 eggs)	(N = 12 eggs)	(N = 12 eggs)	(N = 12 eggs)	(N = 12 eggs)	(N = 7eggs)
Egg length (N = 49 eggs from 11 clutches)	12.57±0.18 9.0-14.93	Egg length (mm)	11.58±0.2 10.73-12.65	12.7±0.28 11.58-14.48	13.20±0.31 11.88-15.34	14.00±0.34 12.56-16.54	14.63±0.35 13.21-17.31	15.72±0.4 14.65-17.83
Egg width (N = 49 eggs from 11 clutches)	7.31±0.1 6.0-11.2	Egg width (mm)	7.5±0.09 6.94-7.98	8.46±0.06 8.14-8.78	9.04±0.11 8.37-9.65	9.59±0.1 9.21-10.12	10.09±0.12 9.31-10.66	10.38±0.21 9.78-11.26
Egg mass (N = 49 eggs from 11 clutches)	0.34±0.01 0.17-1.43	Egg mass (g)	0.36±0.007 0.33-0.41	0.54±0.01 0.48-0.65	0.64±0.02 0.54-0.79	0.76±0.02 0.64-0.96	0.87±0.03 0.69-1.08	0.97±0.06 0.77-1.205
Egg volume (N = 49 eggs from 11 clutches)	356.48±13.35 194.15-886.68	Egg volume (mm^3)	341.69±8.84 295.31-405.45	476.21±10.89 417.4-538.8	567.61±22.09 449.35-709.7	677.96±25.5 571.3-852.2	784.16±30.69 633.58-1006.8	892.86±53.32 736.69-1064.1
Total clutch volume (N = 11 clutches [2 of which hatched]	1587.9±102.06 1036.6-1957.9 [1717±169.7 1382.5-1931.3]	Gain of egg length (mm)	0.0	1.12±0.2 0.3-2.49	0.5±0.01 -0.1-1.0	0.79±0.06 0.46-1.2	0.63±0.06 0.32-1.04	0.67±0.19 0.31-1.53
		Gain of egg width (mm)	0.0	0.95±0.06 0.68-1.34	8.49±0.1 7.89-9.05	0.55±0.07 0.11-1.05	0.49±0.07 0.1-0.96	0.25±0.09 -0.05-0.6
Reproductive investment *sensu* ARRIBAS & GALAN (2005) (N = 11 clutches), [2 of which hatched]	19.03±0.005 16.01-21.4 [19.80±0.001 19.64-20.04]	Gain of egg mass (g)	0.0	0.18±0.008 0.14-0.24	0.09±0.007 0.05-0.14	0.12±0.007 0.095-0.165	0.01±0.008 0.05-0.15	0.08±0.02 0.007-0.205
Relative clutch mass (N = 10 clutches)	0.4903±0.04 0.2697-0.6489	Gain of egg volume (mm^3)	0.0	134.52±6.73 92.25-160.22	91.39±14.72 8.85-170.9	110.35±10.42 44.88-179.09	106.19±13.65 33.44-175.27	81.11±25.46 9.15-186.54

certids; GALAN 1997, 1999; RÚA & GALAN 2003) may determine the final moment of oviposition. In fact, oviposition of *I. martinezricai* is later than in other high mountain species, e.g., of the subgenus *Pyrenesaura* (ARRIBAS 2004b; ARRIBAS & GALAN 2005) or *I. cyreni* from the Sierra de Bejar (ARRIBAS unpublished). Furthermore, females of *I. martinezricai* are clearly less bulky than their congeners when gravid (Fig. 2 D).

There is no evidence of more than a single clutch per year and female, regardless of its size. Yet, due to lack of information from May and June one cannot totally dismiss that eggs observed in July could be second clutches of the year. However, the first appearance of hatchlings in the field (in September) and the lack of different cohorts of juvenile lizards corresponding to different periods of oviposition speak in favor of a unique oviposition per year and female (monestrous female sexual cycle, *sensu* GALAN 2009).

In other populations or species of small sized oviparous lizards (which are mainly polyestrous), at least the largest females are able to produce two clutches per year. But as climatic factors limit the duration of the reproductive period they seem to be responsible for the absence of a second or even multiple clutches as is the case in the mountain dwelling *Iberolacerta*, but not in lowland *I. monticola* (GALAN 2009).

Egg-laying sites.- Sites of egg-laying in nature are almost unknown. Despite the great number of stones lifted, only a few old eggshells have been found. This is in part due to the fact that egg-laying sites are inaccessible if lizards inhabit screes where there are rocks on top of each other. However, gravid females near their egg-laying date are located in the vicinity of water, a scarce resource in these places during the summer. Lizards are seen near fonts and depressions of the terrain where boulders accumulate under which water runs. Thus, suitable egg-laying sites represent a compromise between the need for sufficient solar radiation to incubate the eggs (thus, near the surface) and the presence of moisture (hence, near the bottom of the scree through which water flows). Both factors can coincide in the lower areas of screes and in the proximity of water sources.

Oviposition and egg-laying behavior.- The excavation process of the egg chamber takes several hours. This burrow is not immediately placed under a stone seated on the ground but a little bit below its lower surface, completely dug into the ground, in one case under laboratory conditions, 4 cm below the surface of the soil.

During the labor to expel an egg, the female stretches the hind legs spasmodically several times, raising the base and the proximal third of the tail for a couple of seconds. All these actions are repeatedly executed, interrupted by long periods of rest. At the same time, she twists the body and tongue-flicks excitedly while moving backwards. The body takes the shape of an “S”, squirming with the tail a bit raised.

When an egg is ejected it remains attached to the tip to the cloaca, although most of it is expelled within a few seconds. The female kicks with the feet to detach the egg that rolls to the ground. In one case, the kicks counted during the expulsion of the egg were 25, and continued increasingly spaced after the total detachment of the egg (about 18 seconds in total). These maneuvers also move the previously laid eggs and cover them with soil.

After completion of the clutch, the female continues with digging movements, scraping soil with the front legs and a few times but very strongly with the hind legs. With the front legs, she scraps the ground and pulls back earth, which is pushed toward her eggs with the hind legs.

As in other *Iberolacerta* s. str. and contrary to *Pyrenesaura* or the oviparous *Zootoca*, the eggs were not glued together but remained loose from each other (Figs. 3A-3B).

Ovipositions detected in the laboratory occurred from 9:00 h to 15:30 h (GMT) (average = 11.32 ± 0.5 h; N = 22 eggs). Total duration of the egg-laying process from the appearance of the first egg to the deposition of the last was from 33 to 42 minutes (average = 36.57 ± 1.32; N = 7 clutches).

Characteristics of egg-laying females and clutch size.- Snout-vent length of reproductive females was

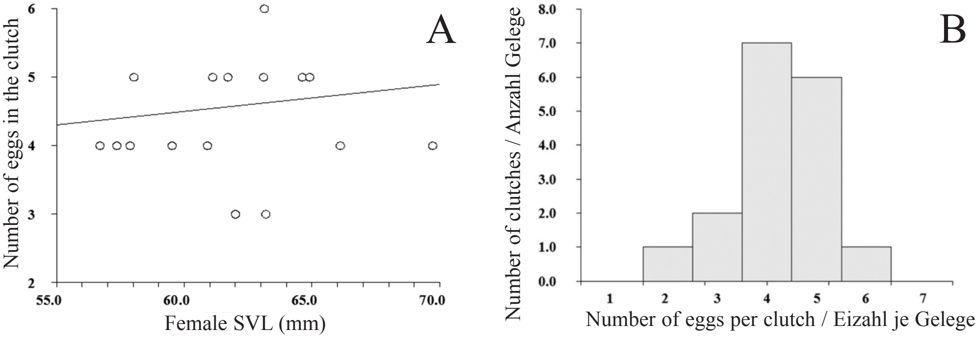

Fig.4: *Iberolacerta martinezricai* (Arribas, 1996). A – Relationship between female snout-vent-length (SVL) and the size (egg number) of her clutch. B – Frequency distribution of clutch size.

Abb. 4: *Iberolacerta martinezricai* (Arribas, 1996). A – Beziehung zwischen Kopf-Rumpf-Länge (SVL) des Weibchens und der Eizahl seines Geleges. B – Häufigkeitsverteilung der Gelegegrößen.

61.78 ± 0.93 mm (range: 56.7 - 69.71, N = 10) (Fig. 4 A). The body mass of gravid females was 5.2 ± 0.14 g (range: 5.06 - 5.48) (N = 3). After egg-laying, body mass was reduced to 3.25 ± 0.13 g (2.63 - 4.00) (N = 10).

The number of eggs per clutch was two to six (average: 4.23 ± 0.23). From 17 clutches checked or gravid females palpated by the author, one was composed of two eggs, two of three, seven of four, six of five and one of six eggs (see Figs. 4A - 4B).

This clutch size is bigger than in *Pyrenesaura* (Arribas 2004b; Arribas & Galan 2005), but smaller than in other *Iberolacerta* s. str such as *I. monticola* (the species studied best) in which it varies from two to 11 eggs (average: 7.2 in Estrela, 6.3 in Galicia lowland localities, 6.2 in Asturias or from 5.3 to 6.2 in León) (see Arribas 2014b, 2014c, 2014d, 2014e).

Female reproductive investment *sensu* Arribas & Galan (2005) (average: 19.03 ± 0.005; range: 16.01 - 21.4; N = 11 clutches) falls within the limits of the three *Pyrenesaura* species (Arribas & Galan 2005) but was not yet calculated for other *Iberolacerta* s. str. Clutch mass relative to the female's body mass (henceforth referred to as relative clutch mass – RCM, nonsystematically influenced by tail autotomy and dehydration) was 0.49 ± 0.04 (range: 0.27 - 0.65; N = 10 clutches), which is intermediate (closer to the lower limits) within *Iberolacerta* s. str. but higher than in other lacertids such as *Podarcis muralis* (Laurenti, 1768) (0.24 - 0.47), *Podarcis lilfordi* (Günther, 1874) (0.25), *Podarcis melisellensis* (Braun, 1877) (0.25), *Dalmatolacerta oxycephala* (Schlegel, 1831) (0.23) or *Algyroides nigropunctatus* (Duméril & Bibron, 1839) (0.29) (see summary in Rúa & Galan 2003 and Arribas & Galan 2005).

Other *Iberolacerta* species, such as *I. monticola* or *I. aranica* have average RCMs of 0.54 - 0.56 and 0.55, respectively, higher than in most other lacertid lizards, with the exception of *Zootoca vivipara* (Lichtenstein, 1823) which may reach up to 0.81 (Rúa & Galan 2003; Arribas & Galan 2005). *Iberolacerta* (s. str.) *horvathi* (Méhely, 1904) has a slightly lower RCM of 0.435 ± 0.028 (range: 0.155 - 0.632) (Ljubisavljevic et al. 2012).

There were no significant correlations between female SVL and several parameters as clutch size ($r = 0.07$; $t = 0.29$, $P = 0.78$), egg length ($r = -0.20$; $t = 0.16$, $P = 0.16$), egg width ($r = -0.16$; $t = -1.08$, $P = 0.29$), egg volume ($r = -0.26$; $t = -1.8$, $P = 0.07$) or the total clutch volume ($r = -0.09$; $t = -0.28$, $P = 0.78$). The correlations between the residuals from the regression of log (clutch size) on log (SVL of mother) and of log (egg mass) on log (SVL of mother) were also not

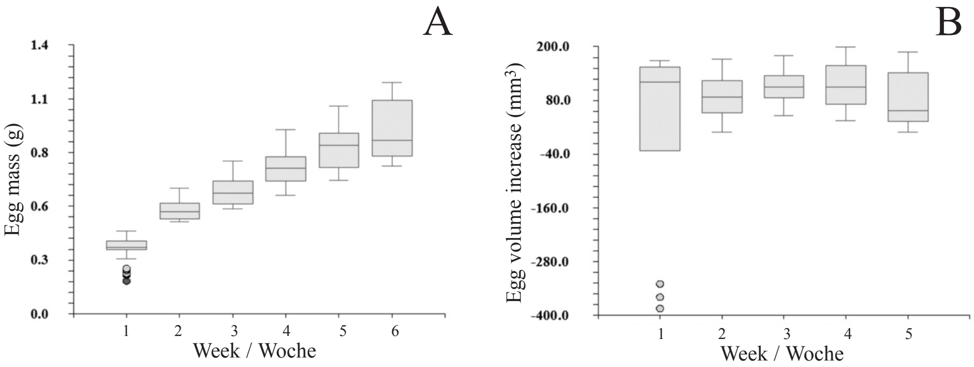

Fig. 5: *Iberolacerta martinezricai* (ARRIBAS, 1996). A – Increase of egg mass during the incubation. Changes in egg volume, not represented, are fully paralleled by that of the egg mass. Note the continuous increase of the mass from the moment of deposition ("week 1") until a date very near to eclosion ("week 6"), when eggs begin to reduce their turgidity. B – Increase of egg volume during the incubation is largely constant, but slightly raised during the first week and reduced in the week prior to eclosion. Outliers in the first week correspond to eggs that died and decayed.

Abb. 5: *Iberolacerta martinezricai* (ARRIBAS, 1996). A - Zunahme der Eimasse während der Inkubation (die Veränderung des Eivolumens, hier nicht dargestellt, entspricht jener der Eimasse). Man beachte die regelmäßige Massenzunahme vom Augenblick der Ablage ("week 1") bis kurze Zeit vor dem Schlupf ("week 6"), als die Eier begannen, ihre Turgeszenz zu verringern. B – Die Zunahme des Eivolumens während der Inkubationszeit erfolgt ziemlich gleichförmig; sie ist etwas gesteigert während der ersten Woche und reduziert in der Woche vor dem Schlupf. Die Ausreißer der ersten Woche entsprechen Eiern, die abstarben und verfaulten.

significant (r = 0.05; t = 0.36, P = 0.72). Clutch size (number of eggs) may be positively correlated with the total clutch volume but this relationship did not reach the level of significance (r = 0.58; t = 2.16, P = 0.06). However, there was a significant negative correlation between female SVL and the individual egg mass (r = -0.43; t = -3.23, P = 0.00) suggesting that bigger females lay smaller eggs, may be combined with an increase in the number of eggs per clutch, which could however, not be observed due to the small sample studied.

In a closely related species, *I. monticola*, mean egg mass was not correlated with female SVL, and negatively correlated with clutch size (r = -0.36) (RÚA & GALAN 2003), but the total clutch size and mass increased significantly along with female SVL (r = 0.53). Furthermore, RCM was not correlated with female SVL in this species, and the residuals from the regressions of log (clutch size) on log (SVL of mother) and log (egg mass) on log (SVL of mother) showed a negative correlation (r = -0.36). In conclusion, larger clutches meant smaller eggs for a determinate female size. In the three *Iberolacerta* (*Pyrenesaura*) species, clutch size was significantly positively correlated with female SVL, whereas egg length was not. Other parameters such as egg width, egg volume and total volume of the clutch, were positively correlated with female SVL in *I. aranica* only, probably due to small sample size in the other two species. Correlations of the aforementioned residuals were significant in all three species. Clutch size (number of eggs) was positively correlated with total clutch volume in the three *Pyrenesaura* species, (significant only in *I. aranica*, probably due to large sample size). In conclusion, the bigger a *Pyrenesaura* female is, the more and bigger are the eggs it lays and the bigger is the total clutch volume. This increased egg size in large clutches is different from what we find in *I. monticola* and is suggested in *I. martinezricai*.

The distribution of the vitellus (yolk) among the eggs follows different strategies (BAUWENS & DÍAZ-URIARTE 1997; BAUWENS 1999): Lizard species which lay large

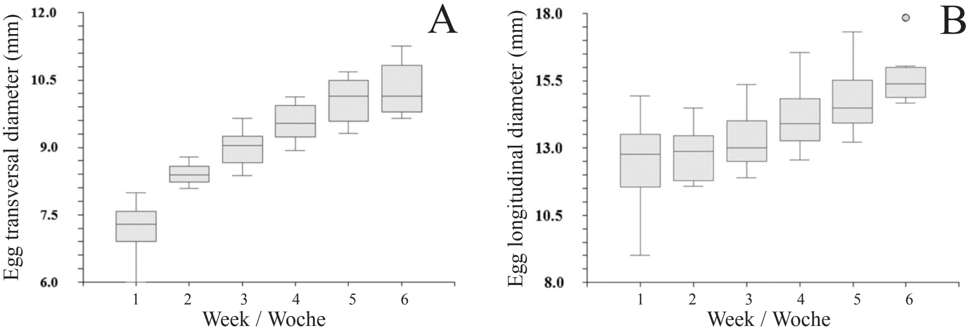

Fig. 6: *Iberolacerta martinezricai* (ARRIBAS, 1996). A – Variation of the egg transversal diameter (width) during incubation. "Week 1" corresponds to fresh-laid eggs, "Week 2" to the end of the first incubation week, and so on. Note the steep increase in width during the first third of the incubation period and see text for discussion of this phenomenon. B – Variation of the egg longitudinal diameter (length) during incubation.

Abb. 6: *Iberolacerta martinezricai* (ARRIBAS, 1996). A – Veränderung des Ei-Querdurchmessers im Verlauf der Inkubation. "Week 1" entspricht dem Querdurchmesser frisch gelegter Eier, "Week 2" jenem am Ende der ersten Inkubationswoche, usw. Man beachte den steilen Anstieg des Wertes im ersten Drittel der Inkubationszeit, der im Text diskutiert wird. B – Veränderung des Ei-Längsdurchmessers im Verlauf der Inkubation.

numbers of relatively small-sized eggs (e.g., *Lacerta* s. str.) invest in the increase of the number of eggs, not their size. Those which lay small numbers of large-sized eggs (e.g., *Podarcis*) invest in the increase of the egg's size, not its number. *Iberolacerta monticola* (and probably also *I. martinezricai*) represents an intermediate situation between these two extremes, increasing both the number and the size of the eggs (RÚA & GALÁN 2003; GALAN 2009). In those lacertids with medium egg size, the reproductive investment changes throughout their ontogeny along with the female's growth. A young *Iberolacerta* female behaves like *Podarcis* in laying few eggs because of her small size and limited quantity of vitellus available for distribution, whereas old and thus large females lay many more and bigger eggs due to the increased quantity of vitellus available for distribution among eggs. The quantity of vitellus available varies over the life time of the individual and between first and second clutches of the year (if a second exists).

The clutch size of *Iberolacerta martinezricai* is noticeably small (Figs. 4A-4B), clearly smaller than in the related species *I. monticola*, *I. galani* ARRIBAS, CARRANZA & ODIERNA, 2006, and *I. cyreni*, which all however a are slightly larger in size (see Fig. 2D). *Iberolacerta martinezricai* females reach SVL values of 68.86 mm (average 59.77 mm), whereas *I. cyreni* as much as 81.74 mm (average 65.51 mm), *I. galani* 84.42 mm (average 64.19 mm) and *I. monticola* 79.81 mm (average 61.02 mm). The similar-sized *I. horvathi* (SVL maximum 70.5 mm, average 60 mm) has a comparable clutch size (2 to 5 eggs, mode 3) (ARRIBAS 2014b, 2014c, 2014d, 2014e; DE LUCA 1989, 1992; LJUBISAVLJEVIĆ et al. 2012). This is an aspect to keep in mind considering the conservation status of *I. martinezricai*: its low reproductive potential adds another constraint to the restrictions imposed to the species by climate and habitat.

Egg characteristics.- Measurements of fresh, recently laid eggs are shown in Table 1. There is no data from the embryonic developmental stages as no eggs were opened for this purpose.

Transformation of the egg during incubation.- For metric traits (length, width, mass, volume, as well as weekly increase in these parameters) of eggs during the incubation see Table 1 and Figs. 5-6. Eggs increase their volume and, thus mass constantly during the incubation

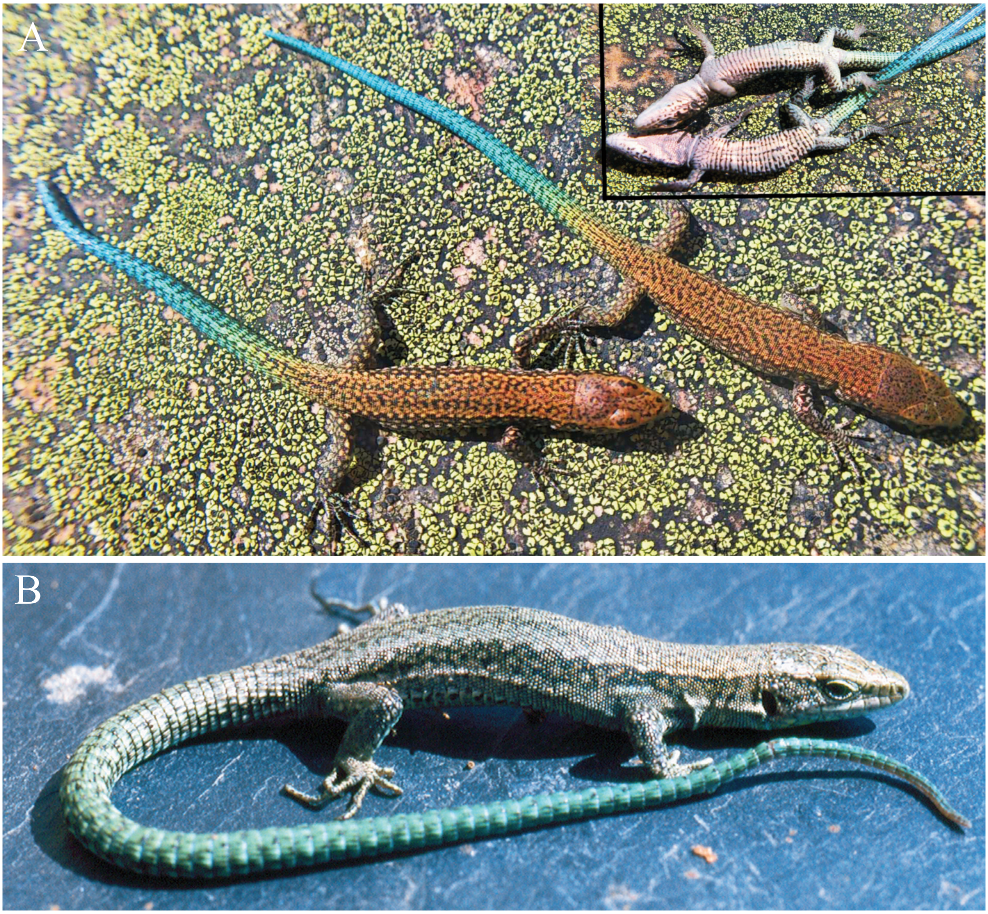

Fig. 7: *Iberolacerta martinezricai* (ARRIBAS, 1996). A – Recently hatched specimens (one of them lacking the tail tip) (inset: ventral view of the same specimens). B – A yearling. Note that the bright blue color of the tail fades and disappears during the second calendar year of life.

Abb 7: *Iberolacerta martinezricai* (ARRIBAS, 1996). A – Kürzlich geschlüpfte Individuen, eines ohne Schwanzspitze (Insert: Bauchansicht derselben Tiere). B – Einjähriges Jungtier. Man beachte, daß die leuchtend blaue Schwanzfarbe im zweiten Kalender-Lebensjahr verblaßt und schließlich verschwindet.

(Figs. 5A-5B). In the week preceding eclosion, this increase is very small as eggs reduce their turgidity about 24 hours before hatching.

In the first week after deposition, the eggs' gain in volume and mass is not so much due to an increase in length (Fig. 6 A) but in width (Fig. 6B). The egg is laid with a reasonable length, but the width is unproportionally small as determined by the opening size of the pelvis and the cloaca. It is the increase in width from which the egg gains volume rapidly after laying. To a lesser extent this gain in width is continued during the second week of incubation, being the increase of longitude and width more synchronized during the third and fourth incubation weeks. Finally, egg length increases abruptly while the growth in width decelerates in the last week before hatching.

Incubation period.- The incubation period lasts from 33 to 42 days (average 37.75 ± 0.86 days; N = 12 eggs) under the conditions as outlined in Arribas (2004b) and Arribas & Galan (2005).

In other *Iberolacerta* s. str. species the incubation period is of similar length, i.e., about 46 days in *I. cyreni* (Barbadillo 1985) (but 32-33 days in two Bejar clutches – Arribas unpublished), 45-54 days in *I. monticola* from the Galician coast at low altitude (Galán 1991) and 37-41 days (Bischoff 1984) or 40-47 days (Ljubisavljević et al. 2012) in *I. horvathi*. In the subgenus *Pyrenesaura*, eggs are more embryonated at the moment of deposition and the incubation period is somewhat shorter, corresponding to the more extreme high mountain climate at the lizards' habitat (on average 30, 34 and 36 days for *I. aranica*, *I. bonnali* and *I. aurelioi*, respectively) (Arribas & Galan 2005).

Eclosion.- In the lab, eclosion occurred between 3:00 to 22:00 h (GTM) (average 9.40 ± 1,71 h; N = 12). The duration of the eclosion, from the egg's first crack until the total disentanglement of the hatchling, lasted from 4.10 to 12.0 hours (average 6.9 ± 1.1 hours; N = 6) (Figs. 3C-3D).

Hatchling morphology and behavior.- Hatchling SVL ranged from 24.9 to 27.6 mm (average 26.11 ± 0.26 mm; N = 11) and body mass from 0.37 to 0.48 g (average 0.41 ± 0.008 g; N = 11). Newborns had 27-29 transversal series of ventral scales (average 28.27 ± 0.23; median = 28; mode = 29; N = 11), the umbilical scar being positioned beginning at ventral scale rows 19-21 (average 20.09 ± 0.21; median and mode = 20) and ending at rows 21-24 (average 23.09 ± 0.28; median and mode = 23; N = 11).

There were no significant correlations between hatchling and female SVL (r = -0.25; t = -0.81, P = 0.44), or hatchling body mass and female SVL (r = -0.39; t = -1.35, P = 0.21).

Dorsum and pileus of the hatchlings are brown, finely spotted with black, in some more pigmented specimens almost finely reticulated. Costal (= temporal) bands with black reticulation, leaving room for brown centered ocelli, and a row of whitish ocelli in their lower part. Legs also reticulated, with bright centered ocelli (Fig. 7A). The belly is white, with irregular spots originating from the anterior edge of the ventral scales and decreasing in their extent from the external towards the innermost rows. Submaxillary plates irregular but conspicuously spotted (Fig. 7 A, inset). The color of the tail base passes gradually from the brown of the dorsum, across greenish brown, to a bright turquoise blue that covers the main part of the tail, also stippled with very small black marks (Fig. 7A). The underside of the tail is also turquoise blue, stippled with black except the two central scale rows which are almost immaculate (Fig. 7A, inset).

Egg-teeth persisted from 8 to 36 hours after hatching (average 23.6 ± 2.57 hours; N = 10).

From 12 individuals hatched under lab conditions six were females and six males.

Iberolacerta martinezricai hatchlings were able to autotomize their tail from birth. The hatchlings (1 CY) observed in nature had intact tails, thus the frequency of tail loss in hatchlings must be low. The bright color of the juveniles' tail disappeared during their 2 CY (Fig. 7B) and seemed to be an important component of the antipredator strategy of hatchlings (e.g., Castilla et al. 1999; Hawlena et al. 2006)

Hatchlings of the Batuecan Lizard were able to attract the attention of potential predators to their tail by a striking whirling movement of its distal part, in a fashion that adults are unable to do with their stiffer tails, which are moved in cases of danger or excitement, however only to a comparatively limited extent.(Arribas 2014a).

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