

Age estimation of *Anatololacerta anatolica* (Werner, 1902) in the vicinity of Çanakkale by skeletochronology

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Abstract: In this study, age estimation using skeletochronology was done in 43 specimens (6 ♂♂, 29 ♀♀, 8 juv.) of *Anatololacerta anatolica* living in the vicinity of Çanakkale. When the cross-sections taken from phalanges were examined, the median age for the Çanakkale population was 4 years. The maximum age was calculated as 10 years for female individuals, and the maximum snout-vent length (SVL) of female individuals was measured as 74.18 mm. The mean SVL was 57.39 (SD = 4.6) mm for males and 63.62 (SD = 8.62) mm for females. The age at sexual maturity was determined as 3 years for both sexes of this species. As a result of correlation analysis, a strong correlation was found for both males (r: 0.845) and females (r: 0.886) in terms of age and SVL. These specimens were also examined for morphological properties and pholidosis characters.

Key words: *Anatololacerta anatolica*, Lacertidae, age estimation, skeletochronology, Çanakkale, Turkey

1. Introduction

The distributional range of the species *Anatololacerta anatolica* is bordered by northwestern Anatolia, which remains above the Büyük Menderes River in the west of Turkey and extends as far as Uludağ (Bursa) in the north and Afyon in the east (Eiselt and Schmidtler, 1986). In addition, *A. anatolica aegaea*, a different subspecies of the species, is known from Samos Island (Ioannides et al., 1994). *A. anatolica* was first described as *Lacerta anatolica* from Gökçekısık (Eskişehir) (Werner, 1902). The systematic position of this species has been studied by different researchers (Méhely, 1909; Boulenger, 1920; Bird, 1936; Cyren, 1941; Bodenheimer, 1944; Mertens, 1952, 1959; Wettstein, 1967; Budak, 1976; Eiselt and Schmidtler, 1986); most recently, in the study by Arnold et al. (2007), all lacertid species distributed in the Palearctic region were considered at the genus level, and the taxon concerned was evaluated as *A. anatolica*.

Skeletochronology is the safest and most appropriate method for various age determination studies in lizards. Not only can the age of specimens be determined by skeletochronology, but antecedent information on their growth can also be acquired. Moreover, it is also possible to make demographic studies with this method (Augert, 1992). As a result of skeletochronology, how the growth

rates of the species have been affected by seasonal conditions and the approximate age of the individuals can be determined by the marks on the bone. However, there is not always a direct relationship between body size and age in animals. In other words, the animal with the largest body size may not always be the oldest. The individuals that live longer are generally those growing more slowly and gradually (Smirina, 1994).

There have been some age-determination studies on various amphibian and lizard species in Turkey to date (Erişmiş et al., 2000; Olgun et al., 2001; Erişmiş, 2005; Kutrup et al., 2005; Olgun et al., 2005; Yılmaz et al., 2005; Guarino and Erismis, 2007; Miaud et al., 2007; Çiçek, 2009; Erişmiş et al., 2009; Üzüm, 2009; Üzüm and Olgun 2009; Üstel, 2010; Üzüm et al., 2010; Gül et al., 2011; Kutrup et al., 2011; Parlak, 2011; Özdemir et al., 2012; Yakın et al., 2012; Altunışık and Özdemir, 2013; Altunışık et al., 2013; Üzüm et al., 2013). However, there have been no such studies on this species.

In this study, the age, snout-vent length (SVL), sex, and pholidosis characteristics of the population of *A. anatolica* distributed in the vicinity of Çanakkale and found in the collection of Çanakkale Onsekiz Mart University were determined and compared. Longevity of the species and the relationship between age and SVL were also revealed.

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2. Materials and methods

Specimens of *A. anatolica* were caught by hand and were then brought to the laboratory in cloth bags. Photographs of the specimens were taken while they were alive, and then the specimens were placed in ether. The specimens were fixed by injecting 96% ethanol into their body cavities and were then preserved in 70% ethanol as museum materials as described by Başoğlu and Baran (1977).

In this study, the pholidosis characteristics and body measurements of 43 specimens in total (6 ♂♂; 29 ♀♀; 8 juv.), which consisted of specimens collected from the vicinity of Çanakkale between 2010 and 2012 and preserved specimens in the collection of Çanakkale Onsekiz Mart University, were determined. Body measurements were taken using a dial caliper with an accuracy of 0.01 mm. Using the specimens, it was investigated whether or not the SVL was associated with age.

Femur samples from the left hind limbs and samples of the fourth phalanx of the left hind limbs were taken from the specimens, from which various body measurements had been obtained for skeletochronological analysis. The skin and muscles of the bone samples were cleared, and then 5% nitric acid (HNO₃) was applied for 3–5 h for the phalanges and 7–12 h for the femora, according to the size of the bones. In this way, the procedure of decalcification of the bone tissue was performed.

To carry out the method of skeletochronology, a phalanx was selected from each wild-collected *A. anatolica* specimen. The use of a phalanx enables the collected specimens to be released into nature again after they have been measured and counted as required. Furthermore, it was observed that more successful results were obtained with the phalanx during the procedure of decalcification than with the femur.

Following the procedure of decalcification, the specimens were washed in running water overnight in order to remove the acid from the tissues. For the procedure of dehydration, the tissues were passed through an increasing alcohol series. Later on, the tissues were left in xylene for 2 h in total. Finally, the tissues were placed into xylene–paraffin medium in order to allow them to acclimatize to paraffin. Xylene was removed by taking the tissues from the xylene–paraffin to a pure paraffin bath;

the tissues were then embedded in paraffin (McManus and Mowry, 1964).

Sections of 10 µm in thickness were obtained from the paraffin blocks of the tissues by using a Leica 2125 RT microtome. Attention was paid to the fact that the tissues passed through the diaphyseal zone at the sections. For counting the age rings, staining was performed with Ehrlich's hematoxylin. Examinations were made under an Olympus CX21 light microscope, and the age rings in the preparations were photographed using the Olympus Analysis LS program with an Olympus BX51 light microscope.

SPSS 15.0 was used for statistical evaluations. Linear regression and Spearman's correlation analyses were used for the relationship between age and SVL. All analyses were made and evaluated at a 95% confidence interval.

3. Results

As a result of the morphological measurements, the mean SVL was determined to be 63.62 ± 8.62 mm in the female specimens, ranging from 41.1 to 74.18 mm. In the males, however, the mean SVL was 57.39 ± 4.66 mm, ranging from 52.57 to 65.23 mm. In the juvenile specimens, this value varied between 26.06 and 36.24 mm, while the mean was found to be 29.86 ± 0.92 mm (Table 1).

When the phalanx sections of the specimens were examined, the ages of juveniles were determined as being from 0 to 2 years old (3 of them were 0 years to 1 year old, 2 were 1 year old, and 3 were 2 years old) (Figure 1). Adult individuals were rarely encountered in the fieldwork performed in the summer, and a hatchling mark was seen on the ventral side of the juvenile specimens; thus, it is thought that the hatching time for the species might be June–August.

The maximum age for females was detected as 10 years, which was seen for 1 specimen with a SVL of 74.18 mm. The minimum age was found to be 3 years. When the other distribution of ages in the females was considered, it was seen that 4 and 6 years constituted the majority. In the females, the median age was found to be 5 years. For males, the maximum age was determined as 5 years in 1 specimen with a SVL of 65.23 mm, and the minimum age in the males was found to be 3 years. The median age

Table 1. Descriptive statistics of SVL of *A. anatolica* (N: number of specimens Min: minimum, Max: maximum, SE: standard error, SD: standard deviation).

Sex	N	Min (mm)	Max (mm)	Mean (mm)	SE	SD
Juv.	8	26.06	36.24	29.86	1.26	0.92
♂♂	6	52.57	65.23	57.39	1.90	4.66
♀♀	29	41.10	74.18	63.62	1.60	8.62

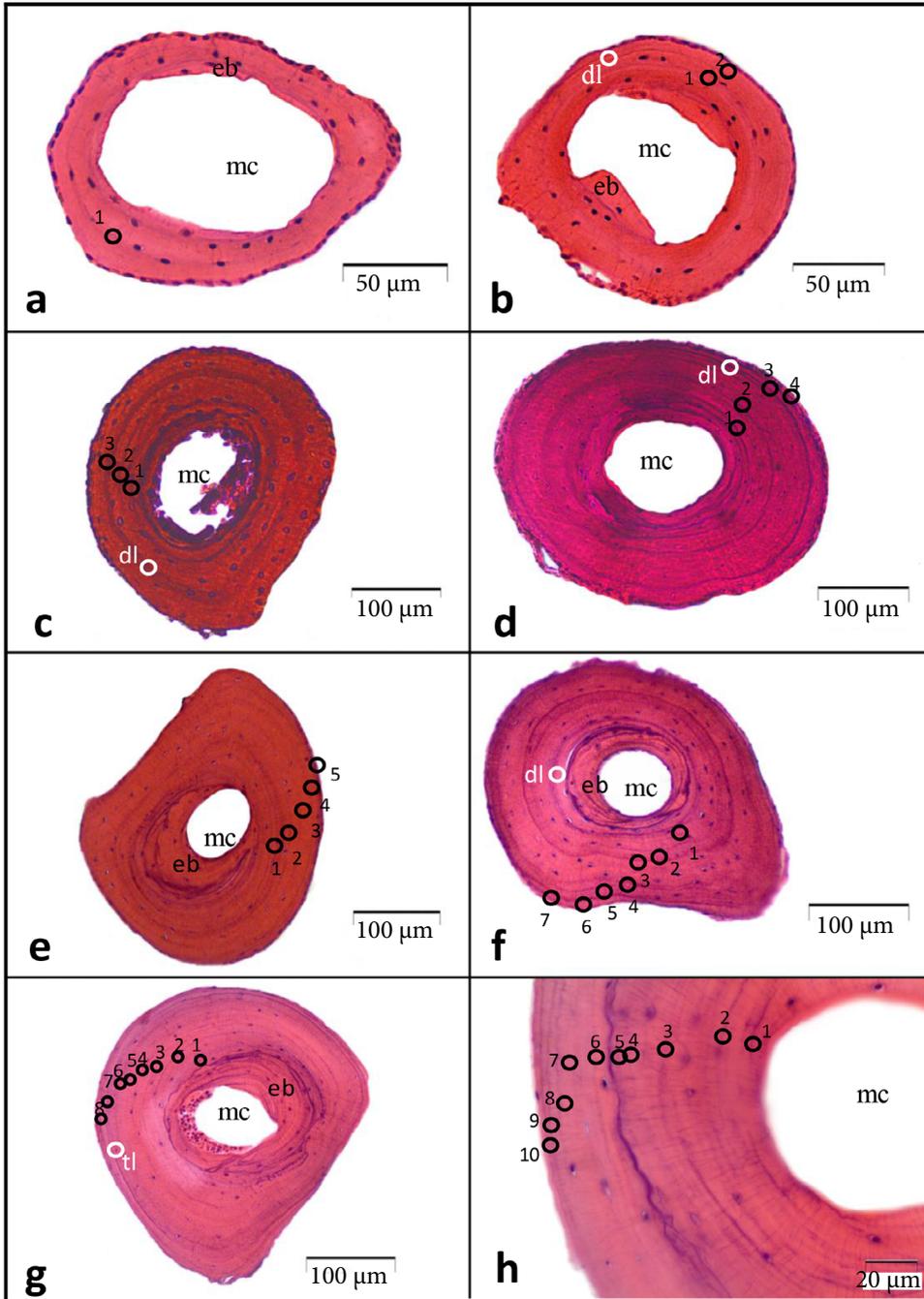


Figure 1. Cross-sections through phalanges of male, female, and juvenile *A. anatolica*. **a)** 1 year old; **b)** 2 years old; **c)** 3 years old (♂); **d)** 4 years old (♀); **e)** 5 years old (♂); **f)** 7 years old (♀); **g)** 8 years old (♂); **h)** 10 years old (♀) (mc: medullar cavity, eb: endosteal bone, dl: double LAG, tl: triple LAG, o: LAG).

was calculated as 3 years in the males. Similar results were obtained from the sections of the femora. Some phalanx sections of adult specimens are provided in Figure 1.

The descriptive statistical values for the sex-based distribution of ages and the SVLs of *A. anatolica* specimens

are given in Table 2. A graph showing the age-SVL relationship of the female and male specimens is presented in Figure 2.

When the relationship between age and SVL in the female specimens was examined by means of regression

Table 2. Biometric values of SVL in all age classes of *A. anatolica* (N: number of specimens, Min: minimum, Max: maximum, SE: standard error, SD: standard deviation).

	Age	N	Min (mm)	Max (mm)	Mean (mm)	SE	SD
Juvenile	0-1	3	26.06	28.49	26.89	0.79	1.38
	1	2	27.19	32.24	29.71	2.52	3.57
	2	3	30.41	36.24	32.94	1.72	2.98
♂♂	3	4	52.57	55.85	54.62	0.71	1.43
	4	1	60.65	60.65	60.65	-	-
	5	1	65.23	65.23	65.23	-	-
♀♀	3	4	49.09	58.55	54.12	2.04	4.08
	4	8	54.91	66.23	60.30	1.93	4.7
	5	4	58.45	68.2	65.59	2.3	4.7
	6	9	65.41	71.29	68.99	0.7	2.1
	7	2	72.9	73.35	73.12	0.22	0.31
	8	1	73.38	73.38	73.38	-	-
	10	1	74.18	74.18	74.18	-	-

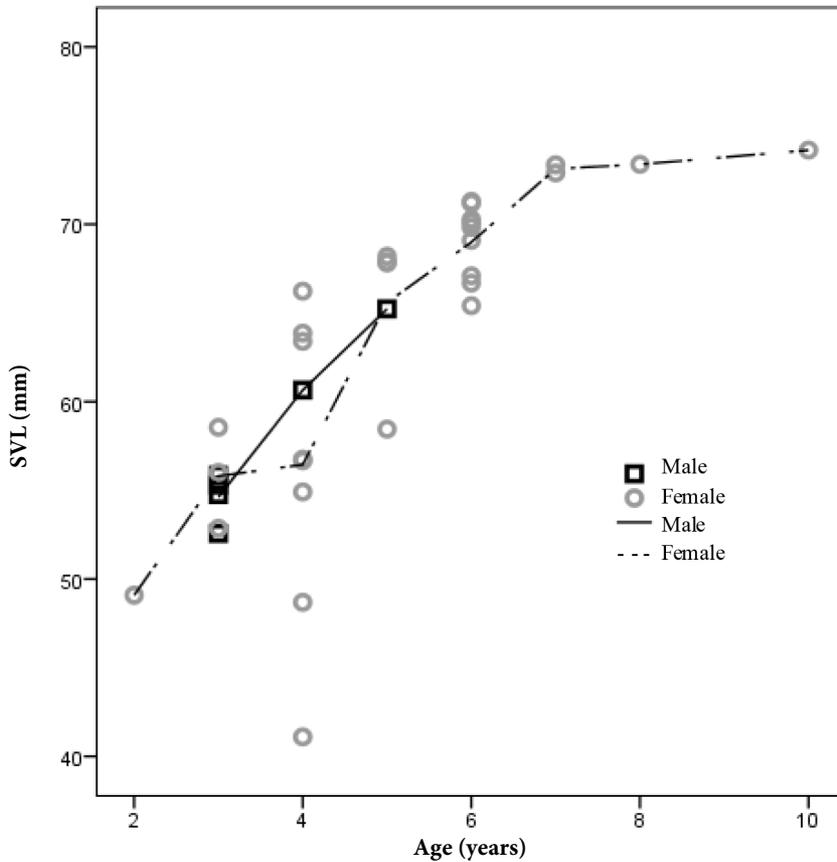


Figure 2. The relationship between age and SVL of *A. anatolica* specimens.

analysis, the unstandardized constant coefficient was calculated as 43.17, and the unstandardized coefficient corresponding to age was computed as 3.98. Accordingly, the formula that shows the relationship between age and SVL is $SVL = 43.17 + (3.98 \times \text{age})$. As a result of the linear regression analysis, it was concluded that the relationship between age and SVL in the female individuals was statistically significant ($R^2 = 0.604$, $P \leq 0.01$) (Figure 3). Spearman's correlation coefficient was calculated as $r_s = 0.886$. Accordingly, the relationship between age and SVL was determined to show a significantly positive correlation.

When the relationship between age and SVL in the male specimens was examined by means of regression analysis, the unstandardized constant coefficient was computed as 38.46, and the unstandardized coefficient corresponding to age was calculated as 5.4. Accordingly, the formula for the relation between age and SVL was $SVL = 38.46 + (5.4 \times \text{age})$. As a result of the linear regression analysis, the relationship between age and SVL in the male individuals was found to be statistically significant ($R^2 = 0.939$, $P \leq 0.01$) (Figure 4). Spearman's correlation coefficient was calculated as $r_s = 0.845$. According to this result, the relationship between age and SVL in the male individuals showed a significantly positive correlation.

4. Discussion

A difference in widths was observed among the 5 lines of arrested growth (LAGs) that were seen when the 4-year-old specimens were examined. The space between the fourth and fifth lines was narrower than the others. It has been stated that sexual maturation may be seen at an evident rate of decrease from the annual periosteal layers (Klevezal and Kleinberg, 1967; Klevezal, 1988). However, it is rather complicated to determine the maturation time of bone sections in rock lizards, as mature individuals keep growing (Arakelyan and Danielyan, 2000). In this case, when the annual width of the layers of the sections of the *A. anatolica* specimens under examination is considered, it might be concluded that the specimens became mature at the age of 3 years.

The endosteal resorption rate may vary among individuals. Therefore, the annual layers that develop in the early years may be either wholly or partially destroyed. Nevertheless, the endosteal resorption rate is rather low in rock lizards, as in the other members of *Lacerta* (Arakelyan and Danielyan, 2000). Thus, endosteal resorption was disregarded in this study, too.

The determination of longevity in rock lizards is facilitated by counting the LAGs. They display considerable differences in terms of the maximum and minimum age intervals in the lacertid species. Pilorge and Castanet

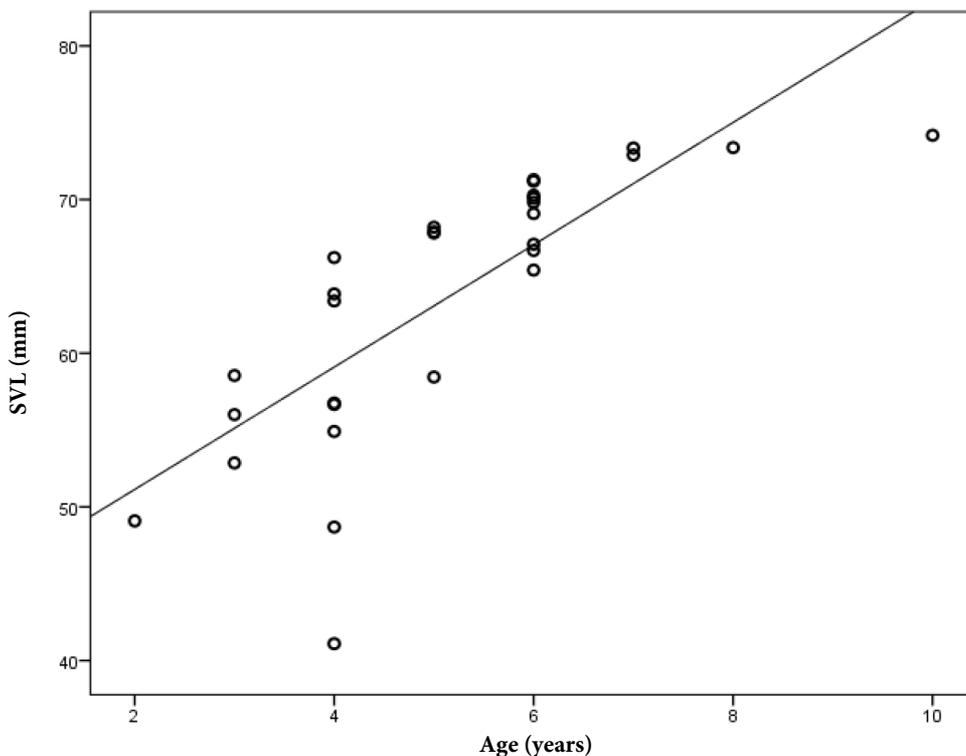


Figure 3. Age (years)–SVL relationship of females.

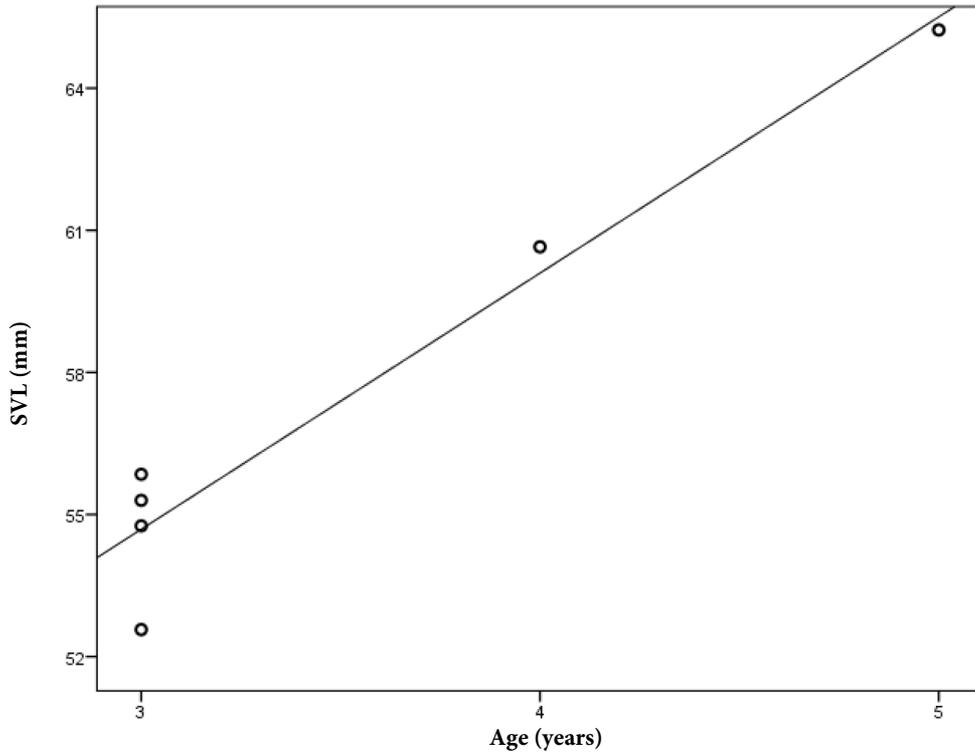


Figure 4. Age (years)–SVL relationship of males.

(1981) determined that longevity did not exceed 4 years in *Lacerta vivipara* specimens. In the species *L. agilis* and *L. strigata*, the maximum ages were found to be 6 to 7 years (Roitberg and Smirina, 1995). In another study, however, the maximum age was found to be 7 years in the species *L. armeniaca*; 6 years in the species *L. unisexualis*, *L. dahli*, and *L. raddei*; and 5 years in the species *L. nairensis* (Arakelyan and Danielyan, 2000). On the other hand, Guarino et al. (2010) determined the age values as 2–3 in females but 3–4 in males in the species *L. agilis*. In another lacertid study, Yakın et al. (2012) computed the maximum age as 8 in the species *Parvilacerta parva*.

However, it has been reported that differences in femur and phalanx sections were observed in some species (Castanet and Smirina, 1990). Thus, along with the phalanges, femora were obtained from some specimens, and the femur and phalanx sections were comparatively examined. As a result of the examination, it was seen that no difference in the numbers of the femur and phalanx sections was found in the available specimens.

The appearance of double LAGs, encountered in most of the sections, might indicate another growth arrest occurring apart from hibernation in this species. In other words, the presence of a secondary period of arrested growth may be mentioned, depending on the fact that

adult individuals of this species were rarely encountered in the fieldwork carried out in the summer months. It was reported that the formation of double lines might be either due to a secondary period of arrested growth, like estivation, or a result of the occurrence of sudden climatic changes (Castanet et al., 1993; Castanet, 1994). No such comment could be made since the climatic data from the localities of the *A. anatolica* specimens distributed in and around Çanakkale were not recorded. Another feature that appears much more rarely as compared with double LAGs is the occurrence of triple LAGs. They again make it possible to speak of the presence of both a second period of arrested growth and an external factor, like weather conditions, which would negatively affect the adequate food uptake or development of the living thing in the medium where it is found.

By making a more comprehensive ecological study of this species, it could be determined—as a result of comparing the marks on bones—in which periods exactly the species stopped its growth. Moreover, the formation of more than 2 LAGs demonstrates that various environmental effects also have a great impact on the growth of these animals, along with hibernation and estivation. It is thought that the formation of such double or triple LAGs results from food scarcity and the negative

weather conditions of that year. We are of the opinion that, when evaluating sections of the animals in order to produce a definite conclusion in such cases, it is essential to evaluate the necessary local meteorological data of those years and interpret them accordingly.

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