

# Age determination and long bone histology in *Stellagama stellio* (Linnaeus, 1758) (Squamata: Sauria: Agamidae) populations in Turkey

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## Abstract

In this study, the age structures of the four populations of Roughtail Rock Agama, *Stellagama stellio* distributed in Turkey were determined and their long bone development was examined from a histological perspective. The ages of some 218 individuals (32 juveniles, 86 ♂♂, 100 ♀♀) captured from the Mediterranean, Transitional Mediterranean, Aegean, and Central Anatolia Regions were computed with the method of skeletochronology, and the correlation between age and the SVL (the snout-vent length) was compared. The mean SVL was greater in males than in females, and this difference was found statistically significant in all populations except for the Central Anatolian population. The mean ages for male and female individuals were calculated as  $3.44 \pm 1.26$  and  $3.18 \pm 1.18$  years in the Mediterranean population, as  $4.65 \pm 1.25$  and  $3.69 \pm 1.31$  years in the Transitional Mediterranean population, as  $4.05 \pm 1.32$  and  $3.36 \pm 1.46$  years in the Aegean population, and as  $4.20 \pm 1.87$  and  $3.60 \pm 2.01$  years in the Central Anatolian population, respectively. The oldest individuals were encountered in the Central Anatolian populations, and the maximum age detected for both sexes was 7 years. It was determined that the age at maturity was 3 in all the populations under examination and that there was not any difference in age at maturity either among the populations or between sexes. There was a significant positive correlation between age and the SVL. The impacts of environmental factors on age structures were examined, and altitude, latitude and climate were found effective on longevity. Histologically, the primary bone, which developed in the embryonic period, was preserved throughout the life of a species even in the oldest individual. Neither secondary bone development nor formation of lamellar systems was encountered. It was seen that intramembranous and endochondral ossification mechanisms played a joint role in long bone development.

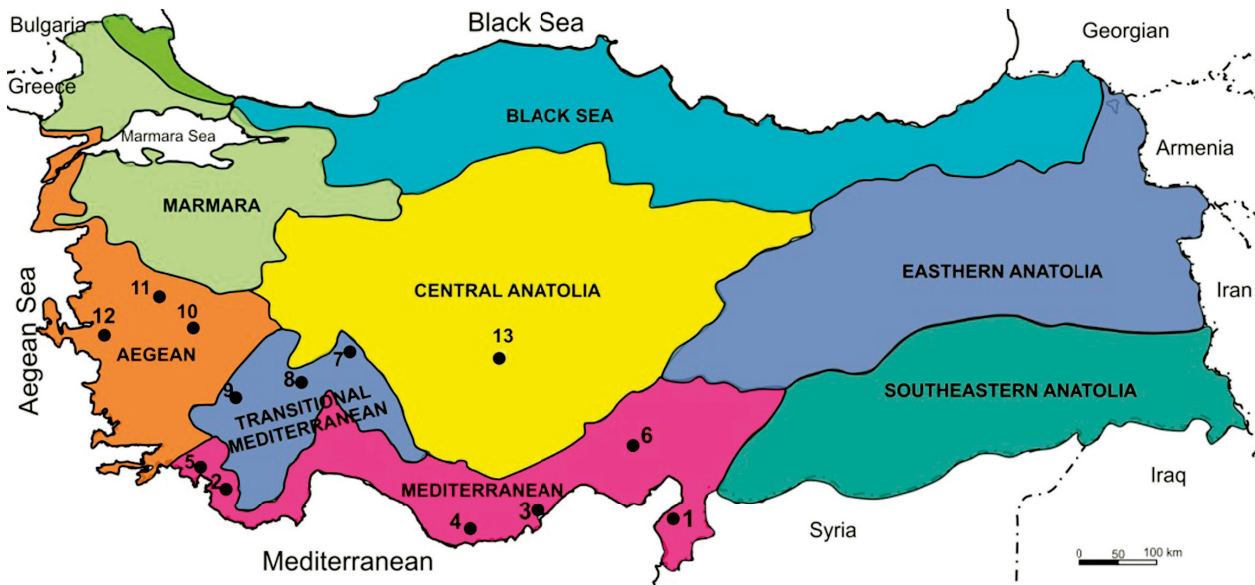
## Key words

*Stellagama stellio*, skeletochronology, age determination, growth, longevity, histology, Turkey.

## Introduction

Roughtail Rock Agama, *Stellagama stellio* is widely distributed along the Turkish islands and from Corfu to Salonika and the Cyclades (Mykonos, Delos, Paros, Antiparos, and Naxos) as well as from Anatolia, Syria, Eastern Iraq, Lebanon, Israel and Jordan to Lower Egypt and Northern Saudi Arabia (DAAN, 1967; BAŞOĞLU & BARAN, 1977; LEVITON *et al.*, 1992; SINDACO *et al.*, 2000; ALMOĞ *et al.*, 2005).

The method of skeletochronology is used as a method that allows the determination of ages of individuals on the basis of counting the lines of arrested growth which take form on the bone tissue as a result of the stopping of bone growth during hibernation or estivation in amphibians and reptiles (GIBBONS & MCCARTHY, 1983; CASTANET & GASC, 1986).



**Fig. 1.** The localities where the specimens were collected: (1. Samandag/Hatay, 2. Fethiye/Mugla, 3. Silifke/Mersin, 4. Anamur/Mersin, 5. Dalaman/Mugla, 6. Yılkale/Adana, 7. Bagkonak Village-Yalvaç /Isparta 8. Barla /Isparta, 9. Burdur/ Golhisar, 10. Pamukkale-Hierapolis Ancient City / Denizli 11. Seferihisar-Teos Ancient City/ İzmir. 12. Ahmetli-Sart Ancient City / Manisa. 13. Karapınar-Meke Tuzlası / Konya). Changed by ATALAY, 2002.

The bone tissue is used as the model tissue in many studies aiming to determine the physiological activities of ectothermic species, their growth rates, and some important parameters such as breeding and approximate longevities. Most of the studies about bone histology recorded in the literature have been on some mammalian species (CASTANET, 2006; KÖHLER & MOYA-SOLA, 2009; RICQLES *et al.*, 1991; MARGARIE, 2002) and some fossil dinosaur species (BOTHÁ & CHINSAMY, 1994; STEIN *et al.*, 2010), and the number of studies on lizards is rather small (SMIRINA & ANANJEVA, 2007; CASTANET & BAEZ, 1991; PAL *et al.*, 2009; GUARINO *et al.*, 2010). There are some age studies in other members of Agamidae. Some important biological parameters have been exhibited by researchers in *L. caucasica* (LEDENTSOV & MELKUNYAN, 1987; PANOVA & ZYKOVA, 2003), *L. stoliczkana* (SMIRINA & ANANJEVA, 2007), *Agama impalearis* (EL MOUDEN *et al.*, 1999), *Phrynocephalus melanurus* (ANANJEVA *et al.*, 2003), *Sitana ponticeriana* (PAL *et al.*, 2009).

This study aimed to make a comparative examination of the age structures of the *Stellagama stellio* populations distributed in Turkey, to determine the correlation between age and the snout-vent length, to reveal the effects of geographical factors on the distributions of ages, to make a histological examination of the age-dependent differentiation in bone development from a hatchling to the oldest individual, and to reveal the results.

## Materials and methods

### Study area

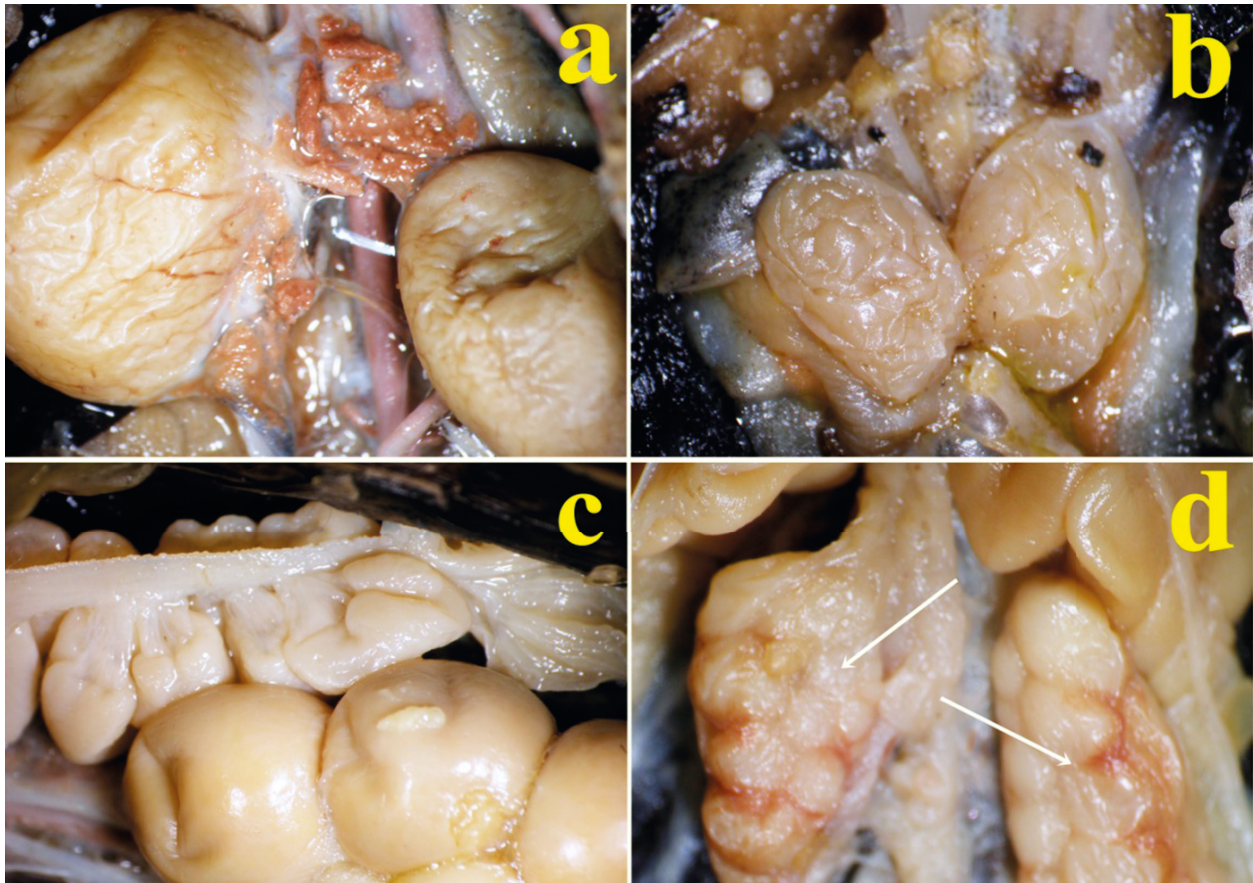
In this study, a total of 218 (86 ♂♂, 100 ♀♀, 32 juveniles) specimens were captured from the Mediterranean, Transitional Mediterranean, Aegean, and Central Anatolia Regions.

Three different types of climate prevail in the regions where the specimens were captured. The Mediterranean and Aegean Regions are influenced by the Mediterranean climate conditions, the Transitional Mediterranean Region by the semi-terrestrial climate conditions and the Central Anatolia Region by the terrestrial climate conditions (ATALAY, 2002).

The localities where the specimens used in our study had been captured (Figure 1) and some geographical data about the localities are provided in Table 1. The study procedure was approved by Decision No. 2011-027 by the Local Ethics Board for Animal Experiments, Faculty of Pharmacy, Ege University, and the studies were conducted after the permissions required for the field had been obtained from the Directorate General for Nature Conservation and National Parks, Ministry of Environment and Forestry, Republic of Turkey.

### Sex

In specimens greater than 70 mm, sex determination was made by examining secondary sexual characters. Post-anal and ventral glandular scales found in male, whereas



**Fig. 2.** Testis and ovary structures of *S. stellio*. **a.** mature testis in adults; **b.** immature testis in juveniles; **c.** mature ovary in adults; **d.** immature ovary in juveniles.

female did not have any scales at these regions. In smaller individuals, sex organs were examined under stereo microscope and those who didn't complete their development were called juveniles (Figure 2).

### Histology

The femur bone was fixed in 96% ethanol and decalcified by 5% nitric acid for 2–8 hours according to the size of bone. 15–20  $\mu\text{m}$  of transverse sections were prepared using a freezing microtome. For each femur, we selected at least three cross sections at the mid-diaphyseal level, with the smallest marrow cavity. All sections were stained with Ehrlich's Hematoxylin for 10 minutes. The diameters of medullar cavity (MC), resorption line (RI), each visible LAG, and periosteal outer margin were measured in each specimen in order to estimate the endosteal resorption rate.

For histological processes, the femur bone was fixed 96% ethanol. Transverse and longitudinal cross sections with thicknesses of 5–7  $\mu\text{m}$  were obtained from the femur, stained with Goldner's trichrome, Heidenhain's trichrome, Picro-sirius red, Alcian-Periodic acid-Schiff and Hematoxylin-Eosin stains, and examined under a light microscope. All sections were photographed with an Olympus LC20 Soft Imaging System.

### Statistical analysis

All statistical analyses were done with SPSS 17.0 and MS Office Excel. The Student t-test was applied to the parametric data, while the Mann-Whitney U test was applied to the nonparametric data. A regression analysis was applied to the data in order to compare the correlation between age and the SVL. To determine the effects of the climatic characteristics of the distributional areas of the populations, their values of altitude and their locations in latitudes on the parameters of age and the SVL, the data were statistically evaluated with the ANOVA (one-way analysis of variance). In all the tests applied, the P significance degree (confidence interval) was evaluated as 95%.

### Results

#### Body size

The summary statistics about the snout-vent lengths obtained from the juvenile, male and female individuals are presented in Table 2. In males, the highest mean SVL was recorded as  $106.76 \pm 22.88$  mm in the Aegean population,

**Table 1.** The localities where the *S. stellio* specimens were collected, dates of collection, and the number of specimens obtained.

Region	Locality	Coordinates	Altitude (m)	Capture Dates	Number of specimen
Mediterranean	Samandağ/Hatay	36,064646° N 35,948684° E	1	19–22.07.2010	16 ♂♂ 19 ♀♀ 7 juveniles
	Fethiye/Mugla	36,618561° N 29,116751° E	42	08.04.2012	1 ♀ 1 ♂
	Silifke/Mersin	36,368676° N 33,936578° E	52	02.08.2012	2 ♂♂ 2 ♀♀ 4 juveniles
	Mamure Castle /Anamur /Mersin	36,080444° N 32,895653° E	2	04.08.2012	4 ♂♂ 2 ♀♀ 6 juveniles
	Dalaman/Mugla	36,762569° N 28,797568° E	14	06.08.2012	1 ♂ 6 ♀♀
	Yılkale/Adana	37,017630° N 35,755522° E	65	29/2007	1 ♂ 3 ♀♀
Transitional Mediterranean	Bağkonak village, Yalvaç/Isparta	38,286538° N 31,182445° E	1345	2001–2004	7 ♂♂ 16 ♀♀ 1 juvenile
	Barla/Isparta	38,017146° N 30,783908° E	1194	07–08.04.2012	4 ♂♂ 3 ♀♀ 5 juveniles
	Göhlhisar/ Burdur	37,121744° N 29,607617° E	1020	07–08.04.2012 26.06.2012	2 ♂♂, 2 ♀♀ 13 ♀♀, 2 juveniles
Aegean	Hierapolis Ancient City Pamukkale/Denizli	37,909669° N 29,120193° E	363	23.04.2011	2 ♂♂ 1 ♀ 3 juveniles
				31.03.2012	7 ♂♂ 6 ♀♀ 3 juveniles
	Teos Ancient City, Seferihisar/Izmir	38,175425° N 26,786099° E	6	26.04.2012	10 ♂♂ 7 ♀♀
	Sart Ancient City, Ahmetli/Manisa	38,493994° K 28,027607° D	115	03.05.2012	12 ♂♂ 10 ♀♀
Central Anatolia	Meke Lake, Karapınar/Konya	37,690586° N 33,641506° E	1005	13–17.06.2011	10 ♂♂ 10 ♀♀

**Table 2.** Summary statistics about snout-vent lengths (SVL) and ages concerning each sex of *S. stellio* populations (N = Number of specimens)

	Sex	N	SVL (mm)		Age (year)	
			Mean ± SD	Range	Mean ± SD	Range
Mediterranean	Juveniles	17	39,2 ± 4,87	31,3–48,29	0,43 ± 0,23	0–1
	Males	25	93,13 ± 14,24	66,25–117,24	3,44 ± 1,26	2–5
	Females	33	85,74 ± 12,45	60,9–115,71	3,18 ± 1,18	2–6
Transitional Mediterranean	Juveniles	9	50,93 ± 10,94	39,19–64,85	1,33 ± 0,50	1–2
	Males	20	105,81 ± 11,71	71,66–122,99	4,65 ± 1,25	2–6
	Females	33	93,68 ± 15,7	53,42–123,59	3,69 ± 1,31	2–6
Aegean	Juveniles	5	50,41 ± 5,52	43,75–59,00	0,78 ± 0,54	0–1
	Males	31	106,76 ± 22,88	53,33–129,06	4,09 ± 1,32	2–6
	Females	25	90,19 ± 23,03	54,00–128,87	3,36 ± 1,46	2–6
Central Anatolia	Juveniles	—	—	—	—	—
	Males	10	98,98 ± 19,26	60,53–114,16	4,2 ± 1,87	2–7
	Females	10	86,73 ± 16,02	64,00–107,18	3,60 ± 2,01	2–7

while the lowest SVL was recorded as 93.13 ± 14.24 mm in the Mediterranean population. In females, the highest mean was determined to be 93.68 ± 15.70 mm in the

Transitional Mediterranean population, whereas the lowest mean was detected to be 85.74 ± 12.45 mm in the Mediterranean population.

In all populations, the mean SVL value was found greater in adult males than in adult females. While the difference in the SVLs between the sexes was detected to be statistically significant in the Mediterranean (t-test:  $t = 2.055$ ,  $df = 56$ ,  $P \leq 0.04$ ), Transitional Mediterranean ( $t = 2.852$ ,  $df = 51$ ,  $P \leq 0.01$ ), and Aegean populations (t-test:  $t = 2.440$ ,  $df = 54$ ,  $P \leq 0.01$ ), no significant relationship could be detected in the Central Anatolian population (t-test:  $t = 1.352$ ,  $df = 18$ ,  $P \leq 0.19$ ). Among the populations, the SVL values for males varied, whereas the difference among the females was found insignificant (one-way ANOVA, Post-hoc-Tukey; males:  $F_{4,93} = 2.165$ ,  $P \leq 0.04$ ; females:  $F_{4,108} = 1.560$ ,  $P \leq 0.19$ ).

### Age structure

The summary statistics about the data on ages determined in juveniles, males and females are provided in Table 2. For both sexes, the highest mean ages were recorded in the Transitional Mediterranean population ( $\sigma\sigma$ :  $4.65 \pm 1.25$  years;  $\varphi\varphi$ :  $3.69 \pm 1.31$  years), while the lowest mean ages were recorded in the Mediterranean population ( $\sigma\sigma$ :  $3.44 \pm 1.26$  years;  $\varphi\varphi$ :  $3.18 \pm 1.18$  years). The oldest male and female individuals were encountered in the Central Anatolian population. In this population, the maximum longevity is 7 years for both sexes. Lines of arrested growth for different age groups are seen at the transverse cross sections of the femur in Figure 3.

In all populations, the mean age of adult males is greater than that of adult females. However, no significant relationship could be detected between the distributions of ages of males and females in the intra-population comparisons except for the Transitional Mediterranean population (The Mann-Whitney U test = 192.50,  $P \leq 0.01$ ).

It was established that the first line of arrested growth on the periosteal bone was lost in only 2 of the individuals under examination and that the 1st and the 2nd lines of arrested growth were partially lost. No endosteal bone formation was observed in any of the juveniles, while the endosteal bone was seen to have formed in most of the individuals older than 3 years of age. The presence of lines of arrested growth on the endosteal bone was encountered in some individuals. No lines of arrested growth on the endosteal bone were encountered in any of the females except for a single 6-year-old female individual determined in the Mediterranean population (Figure 3c). In all other populations, these lines were encountered only in males (Figure 3).

The lines of arrested growth observed on bones were in the form of a single line in the Mediterranean and Aegean populations, while double lines (Figure 4) were encountered in 26% of the adult individuals in the Transitional Mediterranean population and in 28% of the individuals in the Central Anatolian population. This demonstrates that the individuals distributed in the Mediterranean and Aegean Regions experience a single period of arrested growth throughout the year, while some individuals in the other populations experience two periods

**Table 3.** Results of the regression analysis showing the correlation between age and the SVL in *S. stellio* populations ( $N$  = Number of specimens,  $r$  = Regression Coefficient;  $df$  = degree of Freedom,  $F$  = F table value,  $P$  = significance level  $\leq 0.05$ ).

Population	Sex	N	r	df	F	P $\leq$
Mediterranean	$\sigma\sigma$	25	0,80	23	41,092	0,01
	$\varphi\varphi$	33	0,71	31	33,348	0,01
Transitional Mediterranean	$\sigma\sigma$	20	0,62	18	11,668	0,01
	$\varphi\varphi$	33	0,55	31	13,800	0,01
Aegean	$\sigma\sigma$	31	0,68	29	24,718	0,01
	$\varphi\varphi$	25	0,91	23	107,306	0,01
Central Anatolia	$\sigma\sigma$	10	0,73	8	9,450	0,02
	$\varphi\varphi$	10	0,90	8	35,929	0,01

of arrested growth throughout the year. When the results are evaluated considering the data about altitudes, it is possible to state that lines one corresponding to the winter period and one to the summer period form in the bone tissues of the individuals inhabiting higher altitudes and distributed in further northern latitudes.

### Age at maturity

The diameters of the lines of arrested growth observed at the transverse cross sections of the femur were measured in  $\mu\text{m}$ , and their mean values were computed. It was seen that for both sexes, the first 3 years were the periods when growth was the quickest and that the distance between the lines of arrested growth rather decreased as of the 3<sup>rd</sup> year. The age at maturity was calculated as the age of 3 for both sexes. No difference in the age at maturity was observed among the populations.

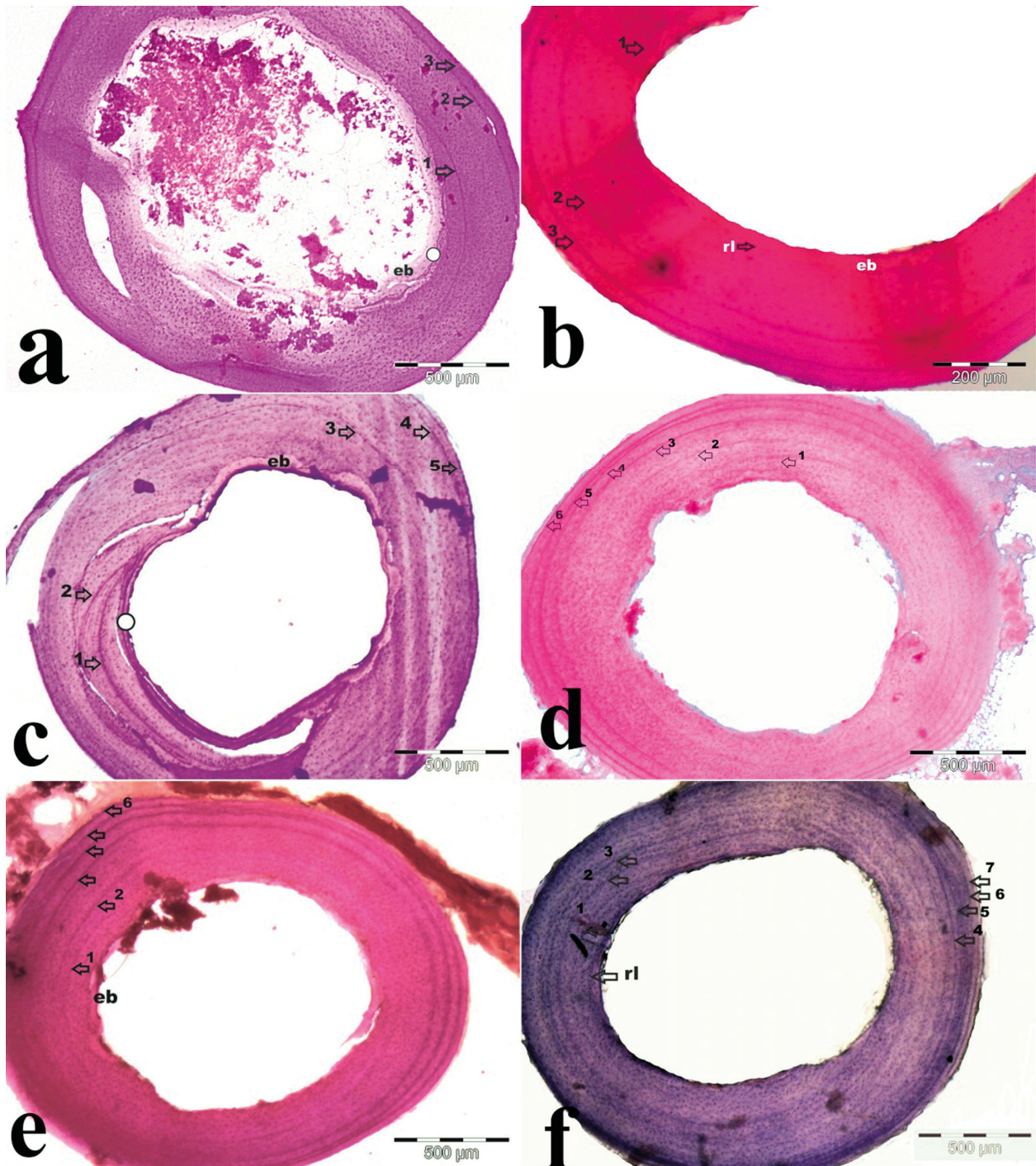
### The correlation between age and the SVL

The presence of a strong positive correlation between age and the SVL for both sexes in all populations was determined with the regression analysis, and the results are provided in Table 3.

### Effects of geographical factors on the parameters of age and snout-vent length

To determine the effects of the climatic characteristics of the distributional areas of the populations, their values of altitude and their locations in latitudes on the parameters of age and the SVL, the data were statistically evaluated with the one-way analysis of variance.

When the data about age and the SVL obtained from the individuals with these 3 different types of climate were compared, it was seen that there was a significant relationship between age and climate, but the climate



**Fig. 3.** Femur cross sections of *S. stellio* from Turkey. **a.** A 3-year-old male (SVL: 106.04 mm); **b.** A 3-year-old male (SVL: 86.12 mm); **c.** A 5-year-old female (SVL: 119.93 mm); **d.** A 6-year-old male (SVL: 122.41mm); **e.** A 6-year-old female (SVL: 115.61 mm); **f.** A 7-year-old male (SVL: 111.76 mm). eb: endosteal bone, rl: resorption line. Arrows point to lines of arrested growth and resorption lines. White rings indicate the resting lines, formed on the endosteal bone. Stain: Ehrlich's Hematoxylin.

conditions were not effective on the SVL (ANOVA, the one-way analysis of variance: Age-climate:  $F_{2,216} = 18.103$ ,  $P < 0.01$ ).

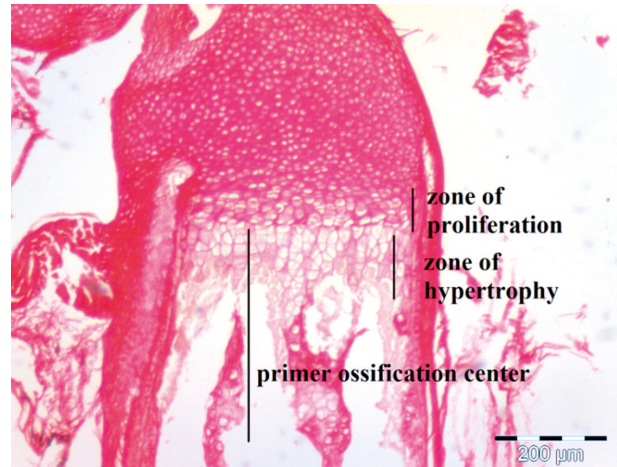
When an examination was made in terms of the values of altitudes of the distributional areas of the populations, it was seen that the relationship between altitude and age was significant, but the relationship between altitude and the SVL was insignificant (ANOVA, the one-

way analysis of variance: Altitude-age:  $F_{2,216} = 4.998$ ,  $P \leq 0.008$ ; altitude-SVL:  $F_{2,216} = 1.543$ ,  $P \leq 0.216$ ).

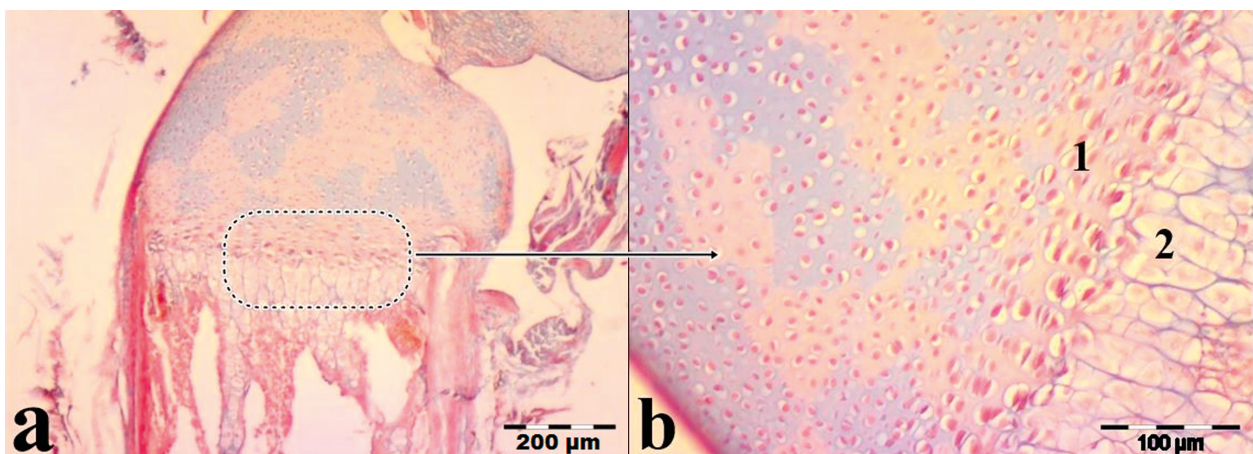
On the other hand, when they were compared depending on latitude, it was statistically determined that latitude was effective on both parameters (ANOVA, the one-way analysis of variance: Latitude-age relationship:  $F_{2,216} = 5.927$ ,  $P \leq 0.003$ ; Latitude-SVL relationship:  $F_{2,216} = 7.543$ ,  $P \leq 0.001$ ).



**Fig. 4.** Femur cross section of the femur through in a 6-year-old male individual (SVL: 114.83 mm); (eb: endosteal bone, dl: double line). Arrows point to lines of arrested growth, formed on periosteal bone. White ring indicate the line of arrested growth, formed on the endosteal bone. Stain: Ehrlich's Hematoxylin.



**Fig. 5.** Longitudinal section of the femur in the young individual that had not experienced any hibernation (SVL: 35.21 mm). Stain: Picro-sirius red.



**Fig. 6. a.** An overall view of the long bone in the young individual that had not experienced any hibernation. **b.** The zone of proliferation (1) and the zone of hypertrophy (2) in the distal part of the epiphysis (SVL: 46.2 mm). Stain: Heidenhain's Trichrome.

## Long bone development

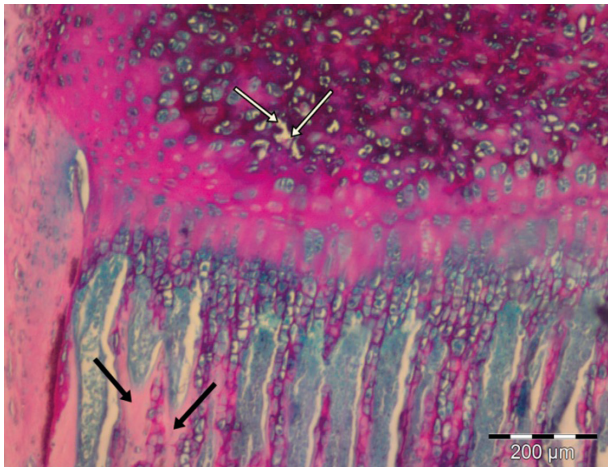
At the transverse and longitudinal cross sections examined, no difference in the ossification mechanisms was detected among the populations. Development was addressed jointly for all populations, and the results were revealed.

In the juvenile individuals that have not experienced any hibernation yet, the first cartilage differentiation is seen in the region between the growth plate and the metaphysis. The hyaline cartilaginous cells embedded in the circular lacuna in the epiphyseal zone formed cell clusters by starting to converge with the decrease in the contact among the cells towards the growth plate. Referred to as the zone of proliferation, this zone consists of the areas where cells rapidly proliferate. Following this, some growth in volume occurs in the cells towards the metaphyseal zone that remains between the epiphyseal and diaphyseal zones and occupies a narrow area. The

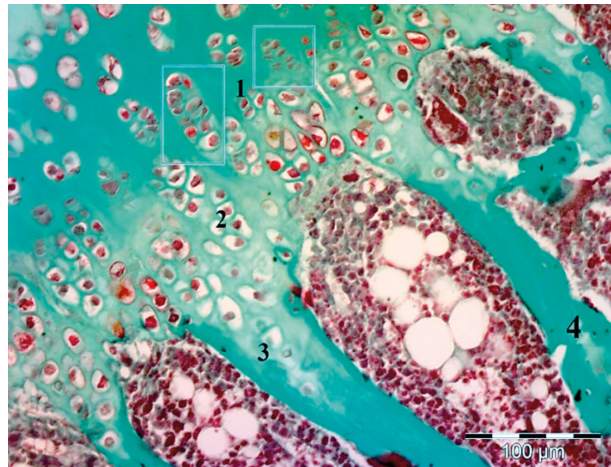
chondrocytes occupying these areas constitute the hypertrophic zone (Figures 5, 6). In this process, the epiphyseal zones completely appear to be a hyaline cartilage. Bone differentiation has not started in these zones yet.

After the 1<sup>st</sup> hibernation, degeneration was seen in the chondrocytes constituting the hypertrophic areas, and zones of mineralized cartilage called osteoids began to form in these areas (Figure 7). Some of the chondrocytes in these areas were destroyed and disappeared, while some of them were mineralized and entered the ossification process.

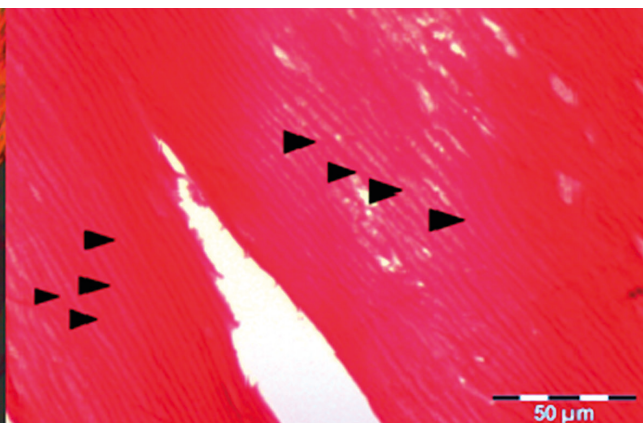
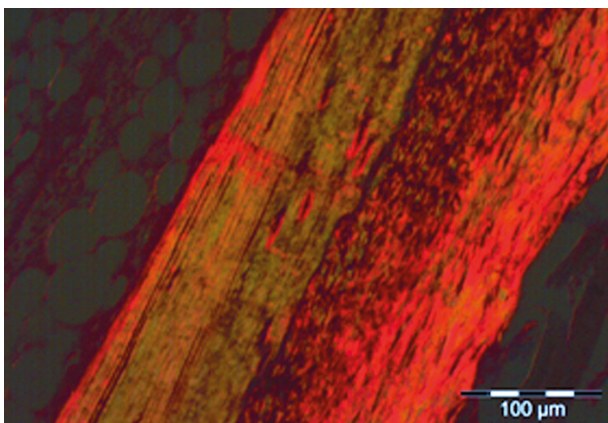
Occurring in the diaphyseal zones in the process of bone development and continuing to take form throughout the life of a species, the primary ossification event is also accompanied by the epiphyseal zones as of the first hibernation, and bone formation takes place in these zones as well through endochondral ossification. Degeneration of the hyaline cartilaginous cells located at the center of this zone is seen, and bone marrow cavities



**Fig. 7.** Frontal cross section of the femur through the epiphyseal and diaphyseal zones in a 2-year-old male individual (SVL: 83.91 mm). Black arrows: The newly shaped parts of bone; white arrows indicated to forming bone marrow cavity. Stain: Alcian blue-PAS.



**Fig. 8.** Cartilage differentiation in the endochondral ossification process at the frontal cross section through the metaphyseal and diaphyseal parts of the femur in a 4-year-old female (SVL: 106.89 mm). Zone of proliferation (1) zone of hypertrophy (2) zone of mineralized cartilage (3) ossification zone (4). Stain: Goldner's Trichrome.



**Fig. 9.** Collagen fibrils on the primary bone (SVL: 84.32 mm). Stain: Picro-sirius red (under polarized light).

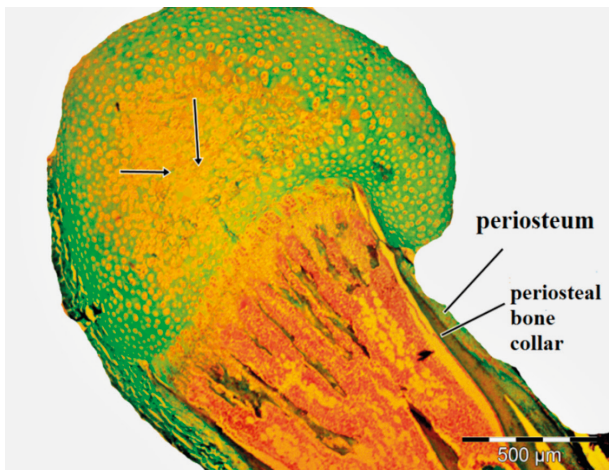
form in these areas (Figure 7). The overwhelming majority of the areas which have emptied out upon the degeneration of the chondrocytes fill with the marrow and fat cells, while trabeculae of bone take form in the mineralized areas upon the synthesis of collagen fibrils by the osteoblasts.

With the 2<sup>nd</sup> hibernation, 5 different zones where the cartilage is gradually replaced by bone as of the epiphysis are clearly distinguished. In addition to the zones of resting, proliferation and hypertrophy, there occurs an ossification zone where the mineralized cartilage and then these cells disappear and are replaced by the bone tissue. Great cavities form in these areas that have remained from the cartilage. First of all with the synthesis of collagen fibrils the organic components of bone into some of these areas by osteoblasts, zones of mineralized bone called osteoid are formed. Later on, the ossification process is completed with the accumulation of the inorganic components of bone in these areas (Figure 8). All these synchronous events have an effect on the longitudinal growth of the diaphysis. The trabeculae of bone take

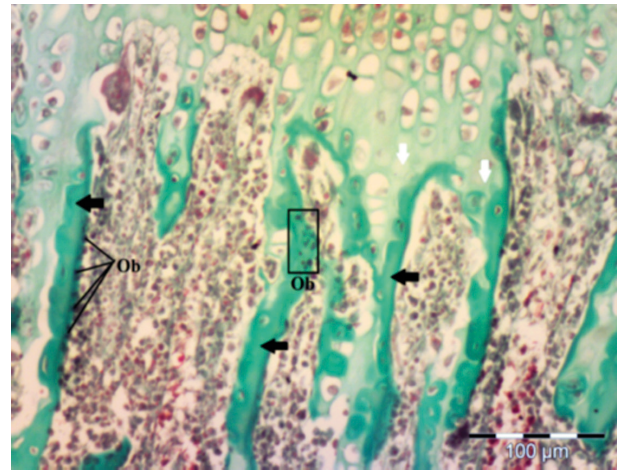
form as a result of the differentiation of the chondrocytes located in the epiphyseal plate. Since bone development is very slow but very regular, the collagen fibrils display a parallel sequence from one end to the other end of the bone (Figure 9). At the cross sections, it was seen that the zones where ossification had been completed were stained in a darker color than the zones where ossification had not been completed yet.

The longitudinal growth in the diaphysis progresses towards the ends of the epiphysis upon the formation of the periosteal ring or the periosteal surfaces. The transverse growth of the diaphysis is provided by the periosteal collar in the middle of the diaphysis, and it is seen that the ossification mechanism takes place through the intramembranous type of ossification (Figure 10). The bone matrix is continually being synthesized by the osteoblasts, and in this way, the periosteal surfaces widen. The osteoblasts which synthesize the bone matrix are sequenced in a single row in the ossified zones. These cells were generally encountered around the newly formed bone (the osteoid) (Figure 11).

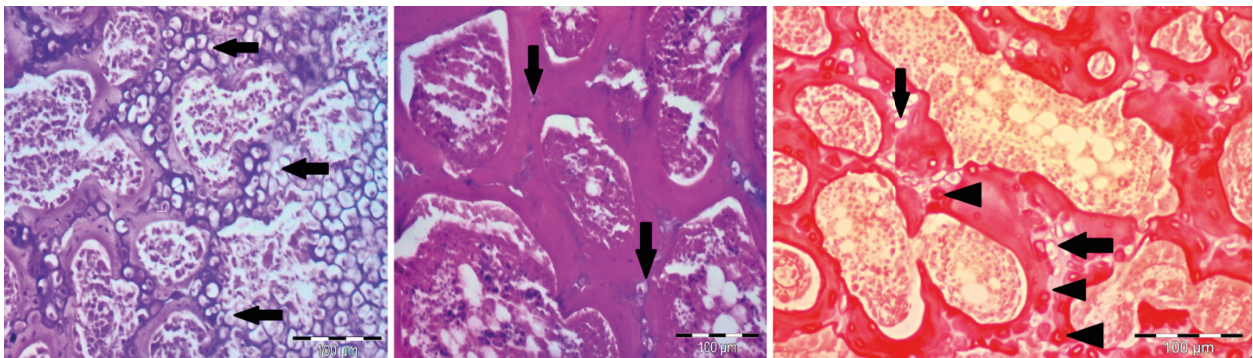




**Fig. 10.** The longitudinal cross section of the femur in a 2-year-old female individual (SVL: 86.73 mm). Black arrows: bone marrow cavities. Stain: Goldner's Trichrome (Scale: 500  $\mu$ m).



**Fig. 11.** Bone matrix which was newly synthesized by osteoblasts cells (SVL: 96.44 mm). Ob: Surface osteoblasts; black arrows: The newly formed bone; white arrows: The zone of mineralized cartilage. Stain: Goldner's Trichrome.

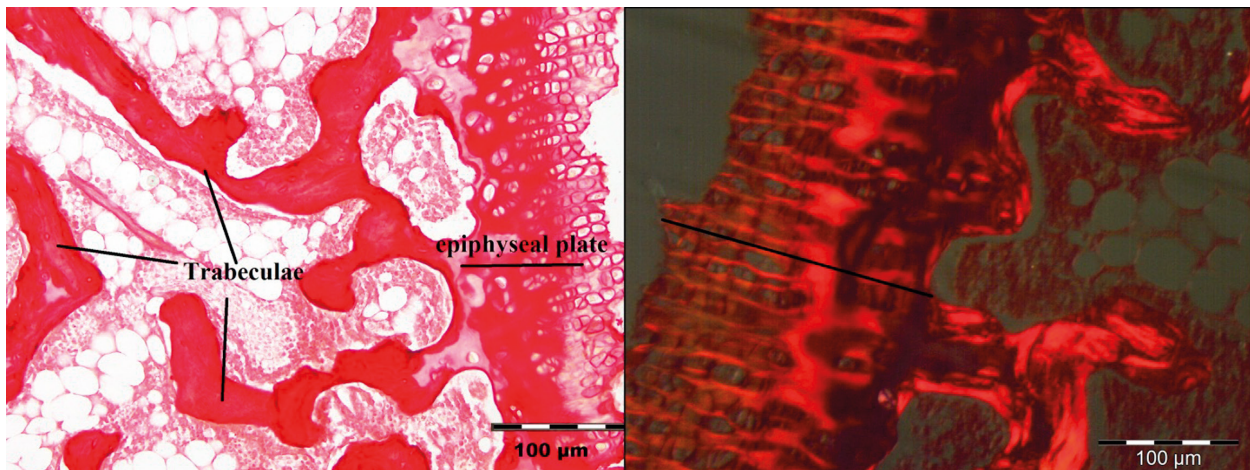


**Fig. 12.** Transverse cross section of the epiphysis in different age groups. **a.** A 2-year-old female (SVL: 60.53 mm). **b.** A 3-year-old female (SVL: 87.45 mm). Stain: Ehrlich's H&E. **c.** A 3-year-old male (SVL: 105.61 mm). Stain: Picro-sirius red. Blue arrows: osteoblasts; black arrows: degenerated chondrocytes.

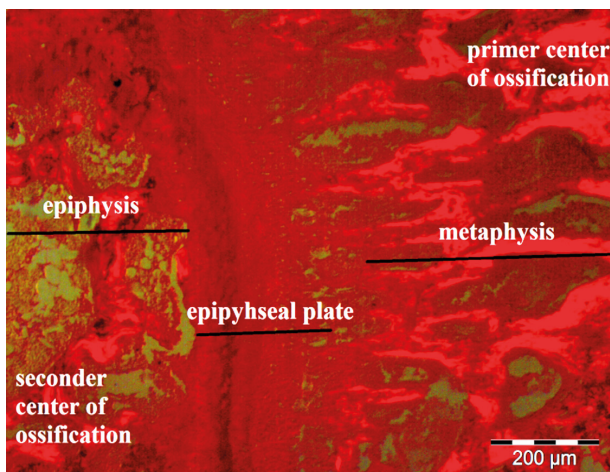
In all the specimens under examination, it was seen that the ossification which had started in the diaphyseal zone progressed towards the ends of the epiphysis as of 3 years of age and that the trabeculae of bone began to take form. As also seen in Figure 12a, on the one hand the chondrocytes undergo hypertrophy and degenerate, but on the other, the bone matrix is synthesized in the remaining areas by the surface osteoblasts. In this way, the trabeculae of bone keep forming. The trabeculae of bone take form as a result of the differentiation of the chondrocytes in the epiphyseal plate. In Figure 12b and 12c, it is seen that ossification is at a more advanced level. The number of hypertrophic chondrocytes further decreased, and the trabeculae of bone further thickened (Figure 13). It was observed that even in the oldest individuals under examination, ossification on these surfaces was not completed and that the chondrocytes continually differentiated and changed into a bone tissue (Figure 14). It is thought that construction of the trabeculae of bone with a cancellous bone structure is a process which continues throughout the life of a species.

Bone development slows down upon reaching sexual maturity. It was seen that even in the oldest individuals under examination, bone kept developing, although slightly. The fact that bone growth continues throughout the life of a species but decreases gradually is also followed from the growth rings occurring on the bone. The regular inclusion of a line of arrested growth every year indicates that bone development is permanent, but the gradual decrease in the distance between the lines and its movement towards the outermost margin of the bone indicate that growth has slowed down.

When the overall structure of the diaphysis was examined, it was seen that the primary bone which had developed in the embryonic period was preserved throughout the life of the species and that the secondary bone covered a very small area around the marrow cavity. Between the endosteal surface and the periosteal surface is the endosteal resorption line which separates these two zones from each other (Figure 15a). No endosteal surfaces form in juvenile individuals. Formation of the endosteal surfaces was only encountered in the individu-



**Fig. 13.** Formation of bone columns towards the inner parts of the diaphysis from the epiphyseal plate in a 3-year-old male (SVL: 106.04 mm). Stain: Picro-siriusred (a. light microscope, b. under a polarized microscope).



**Fig. 14.** Longitudinal section of the femur of a 7-year-old male through the epiphyseal and diaphyseal zones (SVL: 114.16 mm). Stain: Picro-sirius red (under polarized light).

als older than 2 years of age. Mineralization of bone is scarce, and bone is rich in cells. Osteocytes are flattened cells and show a regular sequence along the whole surface of the bone. Nourishment of the cells of the bone is ensured by means of the canaliculi cytoplasmic extensions of cells by the vascular channel (Figure 15b).

## Discussion

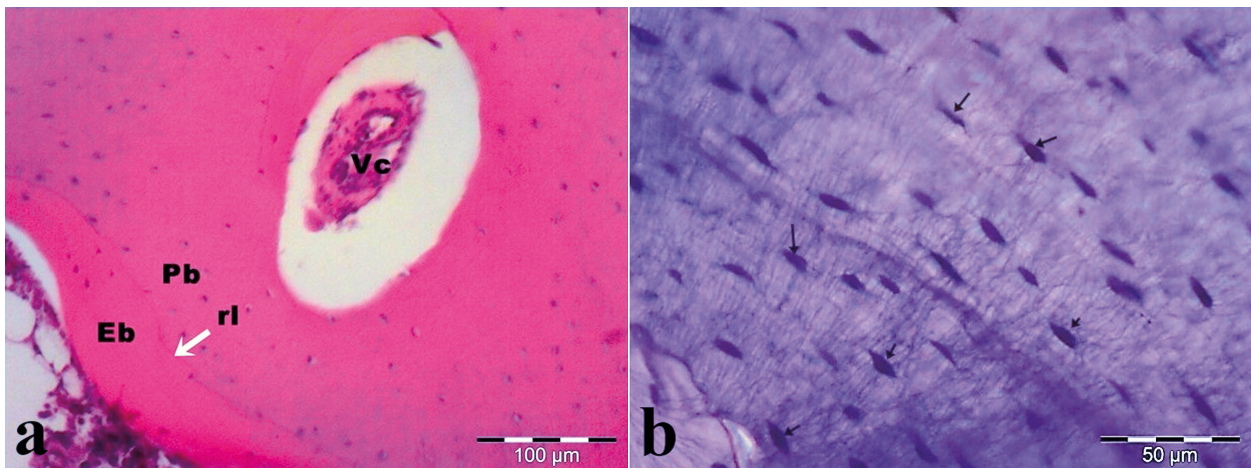
The method of skeletochronology is frequently used in demographic studies on the lizard species inhabiting temperate and tropical regions (e.g. PANOV & ZYKOVA, 2003; SMIRINA & ANANJEVA, 2007; EL MOUDEN *et al.*, 1999; LEDENTSOV & MELKUNYAN, 1987; CASTANET & BAEZ, 1991; GUARINO *et al.*, 2010; ÇIÇEK *et al.*, 2012; LUIS *et al.*, 2004). The relationships among the life history pa-

rameters of some lizard species, such as longevities, age at maturity, and growth, were revealed in the previous studies concerned.

Of squamate reptile species, lizards in particular are frequently used in the studies of age determination, for there is no secondary bone formation as the bone structure does not contain lamellar systems in these individuals and the endosteal layers hardly exist (PAL *et al.*, 2009). For all the above-mentioned reasons, the lines of arrested growth observed on the periosteal bone are preserved throughout the life of a species (SMIRINA, 1974; CASTANET *et al.*, 1988; CASTANET, 1994). This enables to make age studies on these species accurately and in a reliable fashion.

It was stated that in species *Gallotia galloti*, which was endemic to the Canary Islands, the lines of arrested growth could be counted easily in most of the individuals and that the first 2 Lags disappeared in the oldest individuals, but the endosteal resorption was generally low (CASTANET & BAEZ, 1991). LUIS *et al.* (2004) determined that the first 2 lines of arrested growth observed in the femur bone underwent resorption in species *Lacerta schreiberi*. It was established that the first line of arrested growth had been lost only in 2 of the individuals we examined in species *Stellagama stellio* and that the 1<sup>st</sup> and the 2<sup>nd</sup> lines of arrested growth had been partially lost due to the low resorption rate.

When the studies on the species of the Agamidae were examined, it was determined that the maximum longevity in *Laudakia caucasia* was 6–7 years in its populations which inhabited the foot of mountains but 10–11 years in its populations which inhabited mountains (LEDENTSOV & MELKUNYAN, 1987). On the other hand, the maximum age was detected to be 12–13 years in the long-term marking studies on this species (PANOV & ZYKOVA, 2003). However, the maximum age was provided as 5 years in species *Agama impalearis* (EL MOUDEN *et al.*, 1999). The maximum longevity was determined to be 9–10 years in species *Agama stoliczkana* (SMIRINA & ANANJEVA, 2007).



**Fig. 15. a.** Periosteal and endosteal bones; cross sections through the center of the diaphysis. Pb: periosteal bone, Eb: endosteal bone; Vc: vascular channel; white arrows: resorption line (rl). Stain: Ehrlich's H&E. **b.** Osteocytes regularly sequenced within the matrix and canalicula, cross section of the femur (SVL: 115.61 mm). Arrows: Osteocyte. Stain: Ehrlich's Hematoxylin.

The age studies on the lizard species are rather limited in number in our country. For species *Anatololacerta anatolica*, distributed in the vicinity of Çanakkale, the maximum age was recorded as 10 years for female individuals (YAKIN, 2012). In another study, those populations of species *Ophisops elegans* which were distributed around Çanakkale and Gökçeada were comparatively examined. It was determined that ages ranged from 1 year to 4 years in the Çanakkale population but from 1 year to 6 years in the Gökçeada population (PARLAK, 2011). ÇIÇEK *et al.* (2012) stated that longevities varied between 2 and 5 years for both sexes in the Mt. Ağrı (İğdir) population of species *Phrynocephalus horvathi* a species included in family Agamidae. In our study, the maximum longevity determined for both sexes in species *Stellagama stellio* was 7 years. The oldest individuals were obtained from the Central Anatolia Region. The mean age of males was determined to be greater than that of females in all populations. Apart from the Transitional Mediterranean population, no significant difference in the mean ages was seen between the sexes.

In ectothermic species, growth rates may be affected by environmental conditions such as food abundance and temperature (EL MOUDEN *et al.*, 1999). Among squamate reptiles, body size, growth rates, age at maturity and longevities quite vary even in different populations of the same species. As a rule, the individuals inhabiting higher altitudes and northern latitudes live longer than the individuals inhabiting lower altitudes and southern latitudes (WAPSTRA *et al.*, 2001; ROITBERG & SMIRINA, 2006). The effects of altitude on age were explored in age studies on different populations of the same species which were distributed in areas at different altitudes.

ROITBERG & SMIRINA (2006) determined that in species *Lacerta agilis* and *Lacerta strigiata*, the maximum longevities ranged from 5 to 6 years in the specimens they had captured on the foot of a mountain and in plain areas at lower altitudes (20–600 m), while the maximum longevities varied between 7 and 8 years in their popula-

tions which inhabited mountainous areas (960–1,900 m). In another study on species *Lacerta agilis*, the maximum longevity determined for males was 4 years, while the maximum longevity was computed as 3 years for female individuals in the population of the species distributed in North-western Alps at high altitudes (1,790–1,890 m) (GUARINO *et al.*, 2010). The researchers stressed that the maximum longevities they had detected in this area at a high altitude were very low and different from the previous studies.

When the data about the ages of the specimens of species *Lacerta schreiberi*, endemic to the Iberian Peninsula, captured from altitudes of 500, 510 and 760 m were examined by LUIZ *et al.* (2004), it was stated that geographical factors were not effective on the age structure and that the longevities were similar in each of the three populations.

In our study areas an increase in the mean ages was seen in the Transitional Mediterranean Region and the Central Anatolia Region as one further progressed towards the inner parts from the coastal sections. The distributional areas of the specimens collected from the Transitional Mediterranean Region were located at altitudes of 1,000–1,400 m above sea level, and the distributional areas of the specimens collected from the Central Anatolia Region were located at the altitude of 1,005 m above sea level. In the statistical examinations carried out, it was determined that there was a significant relationship between altitude and the data about ages. On the basis of all these data, it was seen that the climate conditions were effective on the distribution of ages in the populations. It is thought that the approximate longevities of individuals do not extend as terrestriality increases. In addition, it is seen that besides terrestriality, latitude is also effective on the distributions of ages and the SVL. The individuals inhabiting further northern latitudes were determined to have greater values of the snout-vent length and longer longevities than the further southern ones.

There are differences in the ages at maturity even between different populations of the same species. Especially in a study by CASTANET & BAEZ (1991), it was seen that the age at maturity was between the 3<sup>rd</sup> and the 4<sup>th</sup> years in *G. g. galloti*, a subspecies of species *G. galloti*, whereas it ranged from 2 to 3 years in other subspecies. It was stated that the age at maturity varied between 4 and 5 years for *G. stehlini*. On the other hand, it was revealed that the appearance of rings did not provide information about the age at maturity in species *G. atlantica*. It was suggested that because the lines of arrested growth were regularly distributed up to the 15<sup>th</sup>–16<sup>th</sup> years in species *G. goliath*, it would not be so sound to give an approximate value of age. The species included in genus *Laudakia* reach sexual maturity at 3 years of age after the 2<sup>nd</sup> hibernation (STUBBE *et al.*, 1981; PETERS, 1971; ANANJEVA *et al.*, 2004). The age at maturity we determined for species *Stellagama stellio* was in agreement with the literature findings. In all the populations under examination, the age at maturity was determined to age of 3.

The growth and development rates seen in the allopatric populations of the same species vary depending on the environmental factors which the distributional areas of individuals have. According to the generally accepted view, development is slower in the individuals distributed in habitats at higher altitudes depending on altitude and latitude (ANGILLETTA *et al.*, 2004). Temperature and latitude and altitude are inversely proportionally correlated. Temperature variations have significant effects on growth between species and even in different populations of the same species. The individuals distributed in colder habitats reach sexual maturity later (ASHTON, 2004; BERRIGON & CHARNOV, 1994). Despite their low growth rates, the individuals in cold habitats reach greater snout-vent lengths, for the extension of breeding means that species use their energy more to grow (ANGILLETTA *et al.*, 2004). CASTANET *et al.* (1988) stated that the growth rate correlated with temperature in species *Sphenodon punctatus* and that the individuals inhabiting the Lady Alice Islands developed more quickly than the individuals inhabiting the Stephen's Islands. It was established that in species *Sceloporus occidentalis*, the age at maturity increased with altitude and that the individuals inhabiting high areas became mature a year later than the individuals distributed in lower areas (ANGILLETTA *et al.*, 2004). In the same study, they emphasized that the populations of species *Sceloporus undulatus* which were distributed in cold areas tended to grow more slowly and that the Bergmann's rule was disputable over lizard species. It was determined that the age at maturity in the Transitional Mediterranean and Central Anatolian populations with the highest distributional ranges among our study areas was similar to that of the populations distributed in lower areas. Considering all these findings, it is seen that the age at maturity and altitude do not correlate for species *L. stellio* and that they do not affect the growth rates either.

There is a positive correlation between age and the snout-vent length in amphibians and squamate reptiles (HALLIDAY & VERRELL, 1988). In the majority of the

previous skeletochronological studies, the correlation between age and the snout-vent length was stated to be positive and strong (PARLAK, 2011; YAKIN, 2012; ÇİÇEK *et al.*, 2012). In all the examined populations of species *Stellagama stellio*, it was seen that there was a strong positive correlation between age and the SVL.

Long bone development has a slow and variable growth rate in ectothermic species, whereas it takes place at a quicker and constant rate in endothermic species (KÖHLER & MOYA-SOLA, 2009). While endotherms are characterized by the (azonal) uninterrupted fibrolamellar bone, which develops quickly around the cortex, ectotherms are characterized by a more slowly and regularly developing lamellar-zonal bone. This bone takes form periodically as a result of storage of the parallel fibrolamellar bone, and its development stops periodically (RICQLÈS *et al.*, 1991; MARGERIE, 2002). With the studies carried out, it was revealed that the fibrolamellar bone type was not unique to endotherms and that this bone structure was also seen in many dinosaur groups (CASTANET, 2006).

It was established that in species *Stellagama stellio* the bone structure was of lamellar-zonal type and that the primary bone was preserved throughout the life of a species. In the skeletochronological studies performed, it was shown that the primary bone in lizards was preserved throughout the life of the species, and our results were in agreement with the literature findings (SMIRINA & ANANJEVA, 2007; CASTANET & BAEZ, 1991; PAL *et al.*, 2009; GUARINO *et al.*, 2010).

Further, it was stated that bone development was interrupted depending on environmental factors even in mammals and some fossil dinosaur groups that displayed quicker development than lizards (KÖHLER & MOYA-SOLA, 2009; RICQLÈS *et al.*, 2003; STEIN *et al.*, 2010; BOTHA & CHINSAMY, 2004). In this respect, it is possible to state that development depends largely on the abiotic factors in the areas where species display activities.

Particularly in lizards among squamate reptiles, the bone structure does not contain lamellar systems, and there is no secondary bone formation (PAL *et al.*, 2009). It was stated that in lizards, secondary bone development was generally seen in individuals older than 2 years of age (ROZENBLUT & OGIELSKA, 2005). Secondary bone development, defined as endosteal resorption, is rather slow in species *Stellagama stellio*, and secondary bone development could not be encountered in any of the individuals younger than 3 years of age. The rate of endosteal resorption observed on the femur bone was calculated to be 6.42% in the Aegean population, 3.58% in the Transitional Mediterranean population, 3.02% in the Mediterranean population, and 2.19% in the Central Anatolian population, respectively. No significant connection could be established between the amount of the resorption rates determined and the environmental factors of the populations.

The apposition of bone leads to the change in the structures of the primary and secondary bones and to the loss of the lines of arrested growth observed on the bone

(CASTANET, 2006). The bone is not remodelled in species *Stellagama stellio*. It was seen that the development in the bone structure was only an increase in the thickness of the cortex and that there was no change in the structure of the periosteal bone. The lines of arrested growth are preserved even in the oldest individual. Data on the histological development of bone were encountered in very few studies on reptile species. It was stated that in species *Sphenodon* and *Iguana tuberculata*, no lamellar systems existed, that the primary bone was preserved and that the secondary bone covered a narrow area around the marrow cavity, whereas both bones developed in species *Crocodilus niloticus* and *Testudo graeca* (CRAWFORD, 1940). It was reported that in species *Alligator mississippiensis*, the ratio of endosteal surfaces to the cortex of bone was ten fold greater than that of the periosteal surfaces and that reductions in bone formation with age were seen (ROBERTS *et al.*, 1988). In all the specimens we examined in our study, it was seen that the primary bone was preserved even in the oldest individual and that secondary bone development covered a very narrow area in the exterior of the marrow cavity, and no fibrolamellar bone formation could be encountered on the bone. In *Stellagama stellio*, the collagen fibrils located in the matrix of the primary bone are regularly sequenced and extend parallel from one end to the other end of the bone. The findings of our study are in agreement with CRAWFORD (1940).

For what purpose energy is going to be used, it directly correlates with the breeding periods in ectothermic species, whose metabolism rates vary depending on environmental factors. While individuals use their energy completely to grow and develop before the breeding period, they use their energy to breed upon reaching sexual maturity in order to carry on their generations (SHINE & CHARNOV, 1992). Depending on the distances between the lines of arrested growth, it was determined that bone development decreased with transition to the breeding period in species *S. stellio*. It was established that bone development took place more quickly in young individuals and that there was a decrease in bone development with age in adult individuals.

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