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RESEARCH ARTICLE

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Variations in age structure and growth in congeners *Lacerta viridis* and *Lacerta media*

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Abstract

Determining the age of any species allows it to be analyzed from the ontogenetic, demographic, and ecological perspectives. In the present study, we tested the hypothesis that the age structure of congener species (*Lacerta media* and *Lacerta viridis*) with the same ecological niche may vary in different areas. In this context, we applied skeletochronology method to reveal various demographic parameters, such as age structure, longevity, age at sexual maturity, growth rate, survival rate, adult life expectancy, and the relationship between age and body size in the green lizard, *L. viridis*, and the medium lizard, *L. media*. In *L. media* and *L. viridis*, the maximum lifespan was 10 and 8 years, respectively. The mean age and body size of females were significantly greater than those of males in *L. media*. However, in the examined *L. viridis* population, no appreciable variation in mean age or body size was found to exist between the sexes. It was estimated that the green lizards reach maturity at the age of 2 or 3 years. However, the *L. media* reached sexual maturity approximately 1 year later than the congener. The body size markedly increased with age in males for both studied populations. However, in females, body size positively increased with age only in *L. media*. The approach of skeletochronology that we utilized in this study to assess age structure makes it simple to gather a variety of time-dependent ecological data for such ectothermic species.

KEYWORDS

age, growth, sexual dimorphism, skeletochronology, survival rate

1 | INTRODUCTION

Understanding the life history of a species provides comprehensive data in many areas, including conservation strategies for that species. Extrinsic and intrinsic variables can influence life history features as growth rate, fertility, age at maturity, and lifespan, with natural selection acting on phenotypes (Zamudio et al., 2016). For instance, habitat quality and ecological circumstances have a direct impact on a number of demographic factors, including longevity, fertility, and age structure (Altunışık, 2018; Altunışık et al., 2022a, 2022b; Atkins et al., 2020; Ma et al., 2022). For many species, determining an animal's age is challenging. The most direct information is provided by mark-recapture analyses (Najbar et al., 2020; Roitberg & Smirina, 2006a). However they demand long-term research. But there is another method which is called skeletochronology that gives reliable results in a shorter time. In ectothermic species, the influence of climatic circumstances on metabolism may be seen in bone tissue formation, which enables the calculation of an individual's age in natural populations. The individual's physiology, life expectancy, and sexual maturity are all revealed by this alteration and histomorphological structure. Lizards are one of the vertebrate groups whose growth is unstable and in this respect, they are used as model organisms for the study of parameters related to demography and body size (Altunışık & Eksilmez, 2021; Curtin, 2006; Ma et al., 2022).

A biological feature known as sexual size dimorphism (SSD) is often seen in reptiles (Cox et al., 2007; Ramírez-Bautista et al., 2016; Rastegar-Pouyani & Fattahi, 2015). It is yet unknown what causes intraspecific SSD body size variation, despite the possibility that theories of fecundity selection and sexual selection may be able to partially explain the variance in lizard SSD (Zamudio, 1998). Apart from these major mechanisms, ecological and behavioral characteristics can also play a role in causing sexual dimorphism (Altunışık, 2017; Blanckenhorn, 2005; Fairbairn et al., 2007).

True lizards, the family Lacertidae, are represented by 362 species widespread across Eurasia and Africa (Uetz et al., 2022). As all lizards, they exhibit great ecological diversity both within and among species (Arnold, 1989; Arnold et al., 2007). Genus Lacerta include 10 species and in this study we focused on 2 of them: the green lizard, *L. viridis* (Laurenti 1768), is a bright green colored medium-sized species with a Snout-vent length (SVL) of up to 15 cm (Vacheva et al., 2022) and tail length about 40 cm (Nettmann & Rykena, 1984). The green lizard is found throughout the Balkan Peninsula, including Croatia (including some Adriatic islands), Bosnia-Herzegovina, Serbia, Montenegro, Macedonia, Albania, and Greece. It is also found in extreme North-east Italy, eastern Germany, the Czech Republic, Slovakia, Hungary, Eastern Austria, Slovenia, and Türkiye. In Türkiye, *L. viridis* is largely distributed in the area of Marmara and along the Black Sea coastal region (Budak & Göçmen, 2008). This species has a vertical distribution from sea level to 2,130 m a.s.l. in these locations. *L. viridis* is listed as Least Concern by the International Union for the Conservation of Nature (IUCN) since 2008 (Crnobrnja-Isailovic et al., 2009). The medium lizard, *L. media* (Lantz & Cyrén, 1920) can reach up to 50 cm. *L. media* is found in the Caucasus region of Northwest Iran, Armenia, extreme northeastern Azerbaijan, northeastern Georgia, southern Russia and central, southern, and eastern Anatolia (Türkiye). It also occurs in northwestern Syria, Lebanon, northwestern Jordan, and northern Israel. In Türkiye, the medium lizard is mostly found in the Central and Eastern parts of Anatolia (Budak & Göçmen, 2008). The vertical distribution of the species ranges between 600 and 2,200 m a.s.l and the species has been considered Least Concern by the IUCN since 2008 (Agasyan et al., 2009).

Although the age structure of many lizard species has been revealed by skeletochronology, studies on the demographic structure of the genus *Lacerta* are limited (Candan, 2021; Guarino et al., 2010; Kalaycı et al., 2018; Odabaş et al., 2019; Roitberg & Smirina, 2006b). Furthermore, neither of these investigations addressed the *L. media* nor the *L. viridis* species. Considering the information stated before, here we tested for the first time the hypothesis that the age structure of congener species (*L. media* and *L. viridis*) with the same ecological niche may vary in different geographies. In this context, we analyzed how the age structure and some life-history traits (e.g., growth and survival rate (*Sr*), longevity, body size, and sexual dimorphism) vary intra- and inter-specifically.

2 | MATERIAL AND METHODS

2.1 | Studied species and sampling

L. media and *L. viridis* specimens (Totally 57 specimens: 18 males, 13 females and 4 juveniles for *L. media*; 11 males, 10 females and 1 juvenile for *L. viridis*) were obtained from Zoology Museum of Adıyaman University in Türkiye. *L. media* specimens were collected from south and east of Türkiye, while *L. viridis* specimens were collected northwest of Türkiye (Figure 1). The map (Figure 1) was generated in ArcMap 10.3 (http://www.esri.com/software/arcgis/arcgis-for-desktop). World Geodetic System of 1984 (WGS84) datum was used as the coordinate system.

Sampling was performed with the permission of the local ethics committee (Dollvet-Hadyek, decision number: 2014/02) for animal experiments. Secondary sexual traits were employed to identify the sex of the specimens (Altunışık et al., 2022a). The juvenile and mature distinction was made according to pattern-coloration characteristics. *L. viridis* have 2–4 and *L. media* have 5 longitudinal lines on the dorsum, and these lines disappear in adults and a homogeneous green color prevails (Budak & Göçmen, 2008). According to the authors, *L. viridis* laid between 6 and 22 eggs, typically 8 (Rykena et al., 1996). The medium lizard, *L. media* inhabits woodlands with stony and rocky parts with an understory of scrub or grass. Every year, the female lays 2 clutches of 9–18 eggs (Budak & Göçmen, 2008).

A digital caliper (Mitutoyo 500-181-300, Japan) was used to measure the SVL of the specimens. Then, in accordance with Smirina's (1994) instructions, the fourth toe of the right hind limb was snipped and then stored in an ethanol solution with a 70% alcohol content.

2.2 | Age determination

Skeletochronology is a method that is frequently used to study the age composition of many ectothermic species. It

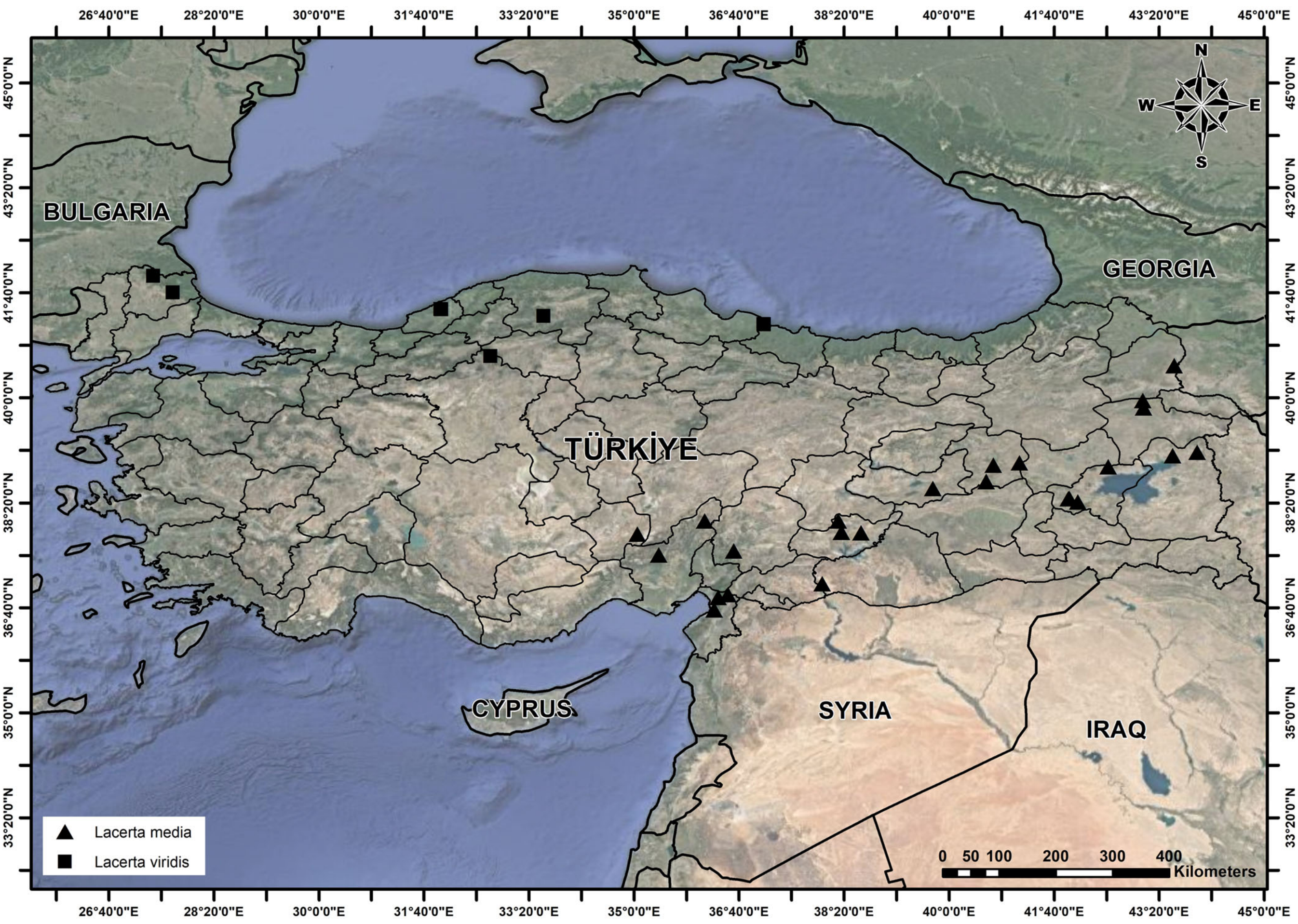

FIGURE 1 Distribution map of the *Lacerta viridis* and *Lacerta media*

is based on the regarding of traces known as "growth markers" or "Lines of Arrested Growth" (LAG), formed on different bone tissues (e.g., phalanges, metatarsals, tibias, fibulae, humeri, and femurs) due to the metabolic slowdown of bone formation during hibernation or estivation phenomones (Gibbons & McCarty, 1983; Smirina, 1994).

Altunışık and Eksilmez's (2018) modified techniques were used to conduct the skeletochronological study (Smirina, 1994). The preserved second phalanx underwent a 1-day soak in distilled water before being decalcified for roughly 2 hr in a solution of 5% HNO_3 (nitric acid). Cross-sections with a thickness of 18 μm provided by a Shandon Cryostat microtome were stained with Ehrlich's hematoxylin dye between 10 and 15 min. Sections having relatively narrow bone marrow cavity were then selected and put in a glycerine solution. At 10× and 20× magnifications, a light microscope equipped with a digital photo camera (Pixera) was utilized to examine and photograph the chosen preparations (Figure 2). The authors separately counted and confirmed the LAGs after reviewing all of the images (Altunışık et al., 2022b).

2.3 | Statistical analyses

SPSS 21.00 was used to determine statistical differences of the experimental data. Normality of the data was performed by a Shapiro–Wilk test. Student *t*-test is one of the parametric tests, which was utilized to compare sexes or species. The relationships between SVL and age were tested with Pearson's correlation coefficient.

The sexual dimorphism index (*SDI*) developed by Lovich and Gibbons (1992) was used to estimate the sexual size differences.

$$SDI = \left(\frac{\text{size of larger sex}}{\text{size of smaller sex}}\right) - 1,$$

The *Sr*s were computed using the Robson and Chapman (1961) methodology.

$$Sr = \frac{T}{(R + T - 1)}.$$

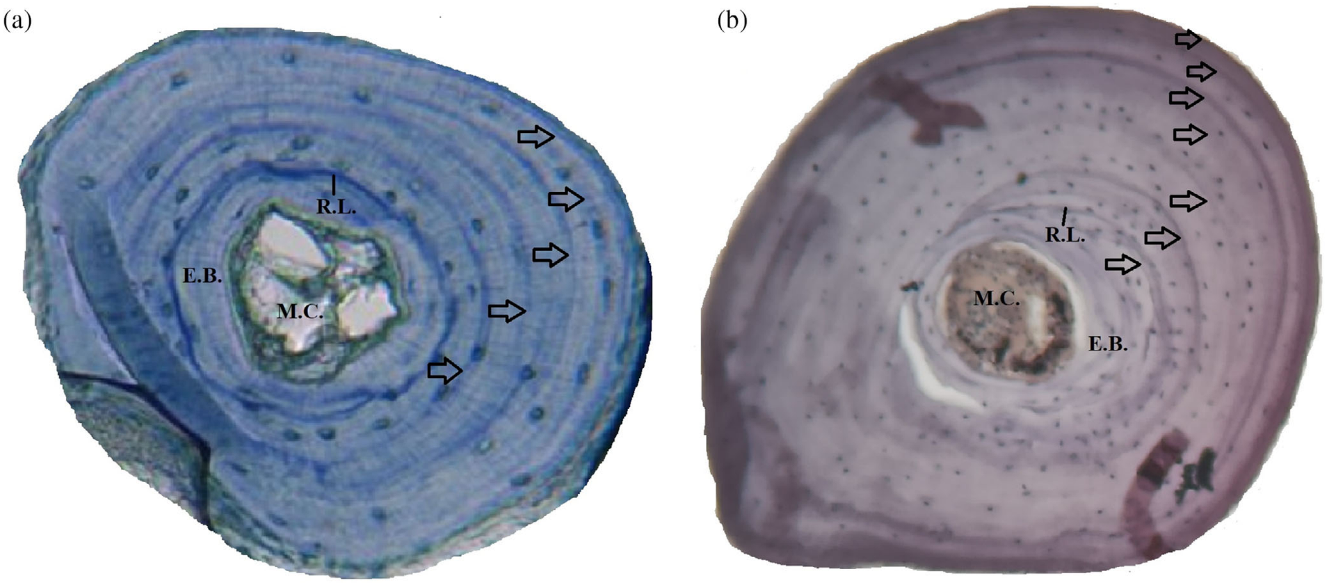

FIGURE 2 Cross-sections (16 μm thick) at the diaphysis level of the phalange bone of *Lacerta viridis* (a) and *Lacerta media* (b) specimen at the age of 5 and 7. E.B., endosteal bone; M.C., marrov cavity; R.I., resorption line.

The computation uses an assumed constant *Sr* ($T = n_1 + 2n_2 + 3n_3 + 4n_4...$, $R = \Sigma n_x$, and n_x = number of individuals in the age group *x*). Limited annual survival rate is represented by *Sr* in the calculation.

The age at which an animal reaches sexual maturity was considered to be the minimum age among the breeding individuals (Altunışık et al., 2022b). Using Seber's (1973) method, the adult life expectancy (*ESP*), which represents the anticipated longevity of animals reaching sexual maturity, was calculated.

$$ESP = 0.5 + \frac{1}{1 - Sr}.$$

The von Bertalanffy growth model was employed to conclude growth trends, as in other investigations (Eroğlu et al., 2018; Guarino et al., 2010; Roitberg & Smirina, 2006b). von Bertalanffy growth has the following generalized formula:

$$SVL_t = SVL_{\max}\left(1 - e^{-k[t - t_0]}\right)$$

In this formula "SVL_t" refers to size at age *t*, "$SVL_{\max}$" indicates the asymptotic highest *SVL*, *e* is the Euler's number (2.718...), "*k*," the growth coefficient, define the curve's shape, and hatching age (t_0) is the age at metamorphosis. Hatching *SVL* was 35 mm for *L. viridis* (Elbing, 2001). Since hatching size of *L. media* is not available for the studied specimens, we used size at hatching (SVL_{t0} = 29.2 mm) as indicated by In Den Bosch (1998).

Using the formula $r = k\ (SVL_{\max} - SVL_t)$ and MS Excel application, we computed the growth rates, $SVL_{\max}$ and *k*. To examine growth rate variations within and between populations, we employed the *t*-test.

3 | RESULTS

The results of body size and age parameters are summarized in Table 1. Darkly stained hematoxylinophilic narrow lines (LAGs) were visible in all cross-sections of the phalangeal bones (Figure 2). Since LAGs form every winter season, each LAG refers to a year. Endosteal resorption, which results in partial erosion of the periosteal bone on the margin of the medullar cavity, was seen in cross-sections of 24% of the specimens.

In *L. viridis*, the age ranged from 2 to 8 years in males (mean: 5.81 ± 0.57) and 3–8 years in females (mean: 5.70 ± 1.70). Regarding the average age in this group, there is no statistical difference between male and female specimens (*t*-test, $t = -0.150$, $df = 19$, $p = .882$). The sixth age class is the most prevalent in the population with 28.57% ($n = 6$; Figure 3). Age upon attaining sexual maturity was found to be 2–3 years for both breeding individuals. *ESP*, which represents the anticipated longevity of animals reaching sexual maturity, was calculated as 6.80 years for male individuals and 6.27 years for female individuals. *Sr* was calculated as 0.82 and 0.84 for females and males, respectively (Table 2). A survival of 0.84 means that 84% of the population survives from one year to the

TABLE 1 Body size (Snout-vent length [SVL]), longevity, and median age in some representative lacertid populations and references

		Mean or range *SVL* (mm)		Mean or range age (years)		Longevity		
Species	**Location**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Reference(s)**
Lacerta agilis	Daghestan, Russia	35–105 (male + female)		2–8		6–7	5–6	Roitberg and Smirina (2006a)
Lacerta agilis	North-western Italy	69.3	79.3	2–4	3	4	3	Guarino et al. (2010)
Lacerta agilis	North-eastern Türkiye	74.11	71.05	4.56	4.74	6	8	Candan (2021)
Lacerta strigata	Daghestan, Russia	35–100		1–5	1–5	5		Roitberg and Smirina (2006a)
Lacerta schreiberi	Portugal	–	–	1–7	1–8	7	8	Luís et al. (2004)
Lacerta trilineata	North-western Türkiye	105	100	4–6	3–7	6	7	Kalaycı et al. (2018)
Lacerta trilineata	Western Türkiye	110	109	9.07	8.42	13	10	Odabaş et al. (2019)
Lacerta viridis	North-western Türkiye	78.04	85.81	5.82	5.70	8	8	This study
Lacerta media	Eastern Türkiye	75.33	99.46	5.33	7.23	9	10	

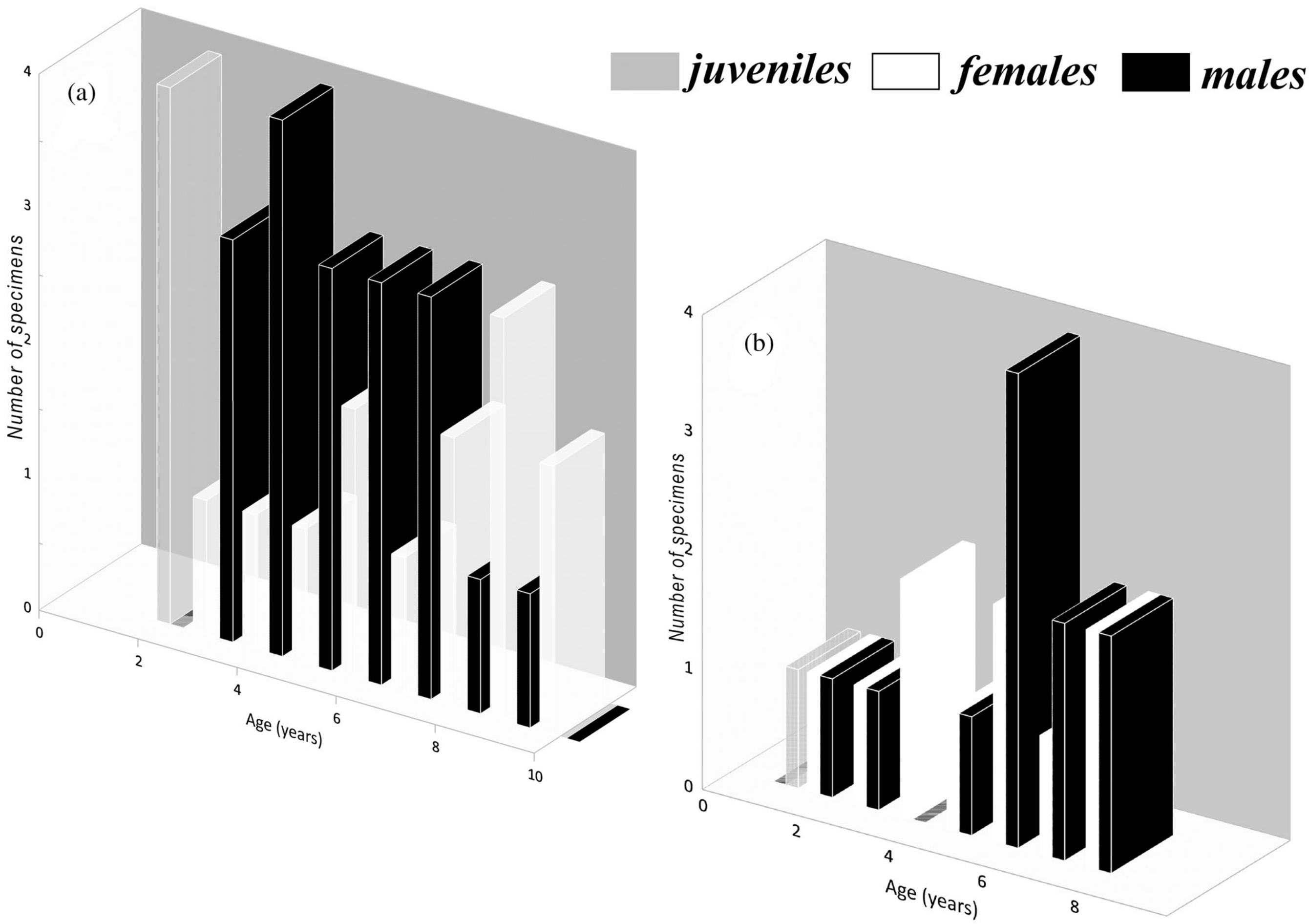

FIGURE 3 Age distribution graphic of *Lacerta media* (a) and *Lacerta viridis* (b)

next on average. Growth rates of the males (mean: 6.78 ± 5.28 mm per year) and females (mean: 5.05 ± 4.81 mm per year) were similar in this population (*t*-test, $t = 1.581$, $df = 11$, $p = .142$). Although females were slightly larger than males, there was not meaningful disparity between sexes (*t*-test, $t = 1.129$, $df = 19$, $p = .273$), and *SDI* also supported this, which was computed as 0.10.

TABLE 2 Descriptive statistics of growth rate (mm per year), growth coefficient (*k*), adult life expectancy and annual survival rate in the studied populations of *Lacerta media* and *Lacerta viridis* adults from Türkiye

Species	Altitude (m)	Sex	*N*	Growth rate ± *SE*	*k*	SVL_{max}	*ESP*	*Sr*	*SDI*
Lacerta media	552	Males	18	8.91 ± 3.45	0.13	142.42	5.03	0.77	0.32
		Females	13	9.66 ± 3.87	0.11	170.22	7.16	0.85	
Lacerta viridis	387	Males	11	6.78 ± 5.28	0.22	95.99	6.80	0.84	0.10
		Females	10	5.05 ± 4.81	0.67	86.30	6.27	0.82	

Abbreviations: ESP, adult life expectancy; N, number of specimens; SDI, sexual dimorphism index; Sr, survival rate.

In *L. media*, the age ranged from 3 to 9 years in males (mean: 5.33 ± 1.78) and from 3 to 10 years in females (mean: 7.23 ± 2.27) (Table 1). The mean age of males was noticeably lower than females (*t*-test, $t = 2.603$, $df = 29$, $p < .05$). The groups with the highest frequency in the age distribution of this population are 4 and 6 years-old individuals (Figure 3). Age at reaching maturity was estimated as 3 and 4 years for both males and females. On the other hand, *ESP* was calculated as 5.03 years for male individuals and 7.16 years for female individuals. *Sr* which expresses the *Sr* of individuals in the population from one year to the next was computed as 0.77 years for male individuals and 0.85 years for female individuals. Although growth rates of the females (mean: 9.66 ± 3.87 mm per year) were greater than males (mean: 8.91 ± 3.45 mm per year), this was not statistically significant (*t*-test, $t = -0.479$, $df = 13$, $p = .640$). Females were significantly larger than males (*t*-test, $t = 2.616$, $df = 29$, $p < .05$), and *SDI* was computed as 0.32.

When comparing two congeneric species, neither male (m) and female (f) nor both (mf) green lizards combined were significantly distinct from medium lizard with respect to the average age (m: $t = 0.695$, $df = 27$, $p = .493$; f: $t = -1.774$, $df = 21$, $p = .091$; mf: $t = -0.180$, $df = 55$, $p = .858$) and *SVL* (m: $t = 0.322$, $df = 27$, $p = .75$; f: $t = -1.470$, $df = 21$, $p = .56$; mf: $t = 0.001$, $df = 55$, $p = 1.00$).

The body size markedly increased with age in males for both the green and medium lizard populations (*L. media:* $r = .802$, $p < .001$; *L. viridis*: $r = .827$, $p < .01$). However, in females, body size positively increased with age in only *L. media* ($r = .918$, $p < .001$). Regardless of sexes, the *SVL* also increased with age in both species (*L. media*: $r = .920$, $p < .001$; *L. viridis*: $r = .780$, $p < .001$). The growth parameters predicted by von Bertalanffy equation, showed a fit that accurately depicted the link between *SVL* and age (Figure 4). The mean annual growth rate of *L. media* (8.58 mm) was the same as that of *L. viridis* (9.01 mm) ($t = -0.360$, $df = 26$, $p = .722$).

4 | DISCUSSION

In this study, two species (*L. viridis* and *L. media*) of the genus *Lacerta* in Türkiye were investigated for the first time using skeletochronology to determine the age, growth and *Sr*, *ESP*, and *SDI*. The two species' comparative data related to the parameters mentioned above were compared with other species belonging to the Lacertidae family (Table 1). Environment (such as resources as food and space; temperature: Roff, 1992 Stearns, 1992) and genetics (Ballinger, 1979; Dunham & Miles, 1985) are the two key factors that affect the variety in life-history evolution. Within the same genus or species, there are significant differences in the life histories of lizards' populations, sexes, and different ecological environments.

It was estimated that individuals of the *L. media* live up to 10 years, whereas both sexes of *L. viridis* live for 8 years. Contrarily, males and females of the congener *Lacerta agilis* have lifespan of 7 and 8 years, respectively (Candan, 2021; Guarino et al., 2010; Roitberg & Smirina, 2006b). Similarly, the minimum longevity for Iberian Emerald Lizard, *Lacerta schreiberi* has been reported to be 7 and 8 years by Luís et al. (2004) for males and females, respectively. *Lacerta trilineata* stands out as the species with the longest lifespan (13 years for males and 10 years for females) among the members of the Lacertidae family analyzed by the skeletochronological method (Kalaycı et al., 2018; Odabaş et al., 2019). On the other hand, the species with the shortest lifespan among the studied Lacertidae species is *Lacerta strigata* with 5 years (Roitberg & Smirina, 2006a; Table 1). It is possible that the lifespan of these lacertid species living in different geographical regions was affected by local conditions and food abundancy in addition to genetic structure.

Males and females of the green lizard attained maturity at the age of 2 or 3 years. However, the medium lizard reached sexual maturity approximately 1 year later than the congener. The females' age is no longer than the males in *L. viridis*. On the other hand, female specimens of *L. media* have higher mean age compared with those

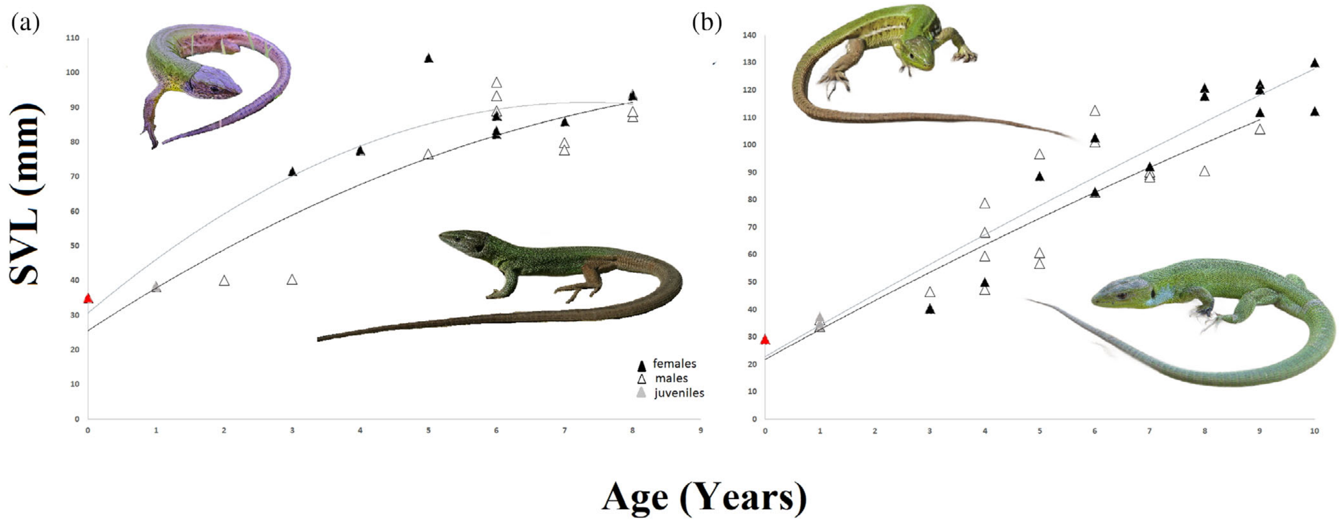

FIGURE 4 Relationship between age and body size (Snout-vent length) of *Lacerta viridis* (a) and *Lacerta media* (b)

male specimens. Findings of *L. viridis* show similarity to the *L. agilis* that reached maturity at 2 years, while females attained at the third year of their lifetime (Candan, 2021; Guarino et al., 2010), like in Daghestan specimens of same species (Roitberg & Smirina, 2006a). Similarly, Schreiber's green lizards have been reported to reach maturity at the age of 3 years (Luís et al., 2004). In contrast, *Psammodromus algirus*, which is a member of Lacertidae, matured in the second year of their life (Bouragaoui & Nouira, 2019). This result shows that the age of sexual maturity may change for different species or climatic conditions.

The Srs of the green and the medium lizards showed different trends in males and females in this study. For example, while the Sr of males (0.84) was higher compared to females (0.82) in the green lizard, this rate was higher in females (0.85) than males (0.77) in the middle lizard (Table 2). On the other hand in the Lacertidae family, relatively low Srs of 0.51 were reported in *Podarcis tauricus* (Altunışık et al., 2016), and also in *Dinarolacerta mosorensis* which Sr was determined as 0.71 and 0.75 for males and females, respectively (Tomašević Kolarov et al., 2010).

Although sexual size differences are less known in reptiles compared to other vertebrate groups (Fitch, 1981), SSD has been documented in several reptile species (Altunışık et al., 2013; Cruz-Elizalde et al., 2020a; Roitberg & Smirina, 2006b). In the case of lizards, SSD can be observed in favor of females (Altunışık & Eksilmez, 2021; Guarino et al., 2010) and males (Cruz-Elizalde et al., 2020b; Kalaycı et al., 2018; Odabaş et al., 2019) or without obvious difference between sexes (Ramírez-Bautista et al., 2013). Different subspecies or populations within a species may occasionally differ from one another (Altunışık & Eksilmez, 2021; Roitberg & Smirina, 2006b). Differentiated evolutionary adaptations to ecological situations in males and females are just one of the many probable explanations of differential SSD patterns between and within species (John-Alder & Cox, 2007; Roitberg, 2007). Natural selection, sexual selection, and fecundity selection are the three main hypotheses used to explain sexual dimorphism (Anderson, 1994). The first, known as natural selection, exposes an inclination that aids in survival; for instance, competition for food among individuals may contribute to sexual dimorphism in a different manner (Fairbairn, 1997). But neither males nor females benefit from this theory. In this study, intersexual differences in body size for both *L. viridis* and *L. media* are female-biased. Moreover, the difference in *SVL* in *L. media* was also statistically significant. The *SDI*, which we calculated as 0.32, roughly means that the female individuals of the medium lizard have a 32% greater size than the male individuals. Additionally, in numerous reptile species (John-Alder & Cox, 2007; Tomašević Kolarov et al., 2010; Werner, 1993), SSD is caused by a disparity in growth rates that depends on food availability and temperature circumstances. The similar growth rates between sexes in *L. viridis* may explain a weakly female biased SSD in the present study.

Numerous research on reptile species found a favorable link between age and SVL (Altunışık et al., 2022b; Ma et al., 2022; Piantoni et al., 2006; Yıldırım et al., 2019). But not all species exhibit this positive link (Odabaş et al., 2019), and in some, the correlation may differ based on sex (Cabezas-Cartes et al., 2015). Both sexes in this study showed a positive association between age and body size.

5 | CONCLUSION

Our preliminary data presented on age structure, longevity, age upon attaining sexual maturity, growth and Sr, body size, and ESP of two lacertid species (*L. media* and *L. viridis*) from Türkiye have contributed to ecological knowledge of these lizard species.

It was concluded that the age structure of congener species (*L. media* and *L. viridis*) with the same ecological niche varies in different geographies. Various time-dependent ecological data can be easily gathered with the skeletochronology approach we employed to assess age in this study. Our findings serve as a guide for using skeletochronology in these relatively large lizards.

AUTHOR CONTRIBUTIONS

Abdullah Altunışık: Conceptualization (equal); data curation (equal); formal analysis (supporting); methodology (supporting); resources (equal); supervision (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Mehmet Zülfü Yıldız:** Conceptualization (equal); data curation (equal); investigation (equal); methodology (equal); resources (equal); visualization (equal); writing – review and editing (supporting). **Bahadır Akman:** Conceptualization (equal); investigation (equal); methodology (equal); resources (equal); writing – review and editing (supporting). **Naşit İğci:** Data curation (equal); investigation (equal); methodology (equal); resources (equal); writing – review and editing (supporting). **Mert Karış:** Conceptualization (equal); data curation (equal); investigation (equal); methodology (equal); writing – review and editing (supporting). **Mehtap Sömer:** Data curation (equal); formal analysis (equal); methodology (equal); writing – review and editing (supporting).

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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