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Age structure and growth in Bulgarian populations of *Ablepharus kitaibelii* (Bibron & Bory de Saint-Vincent, 1833)
(Squamata: Sauria: Scincidae)

Altersstruktur und Wachstum bei Bulgarischen Populationen von *Ablepharus kitaibelii* (Bibron & Bory de Saint-Vincent, 1833)
(Squamata: Sauria: Scincidae)

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KURZFASSUNG

Ablepharus kitaibelii (Bibron & Bory de Saint-Vincent, 1833) ist eine der kleinsten Skinkarten weltweit. Bis auf eine einzige Beobachtung an *A. kitaibelii* in Gefangenschaft, fehlen Daten über die Lebensdauer in der Gattung *Ablepharus* Lichtenstein, 1823. In der vorliegenden chronologischen Studie an Skeletten von *A. kitaibelii* betrug die maximale Anzahl an Überwinterungen in beiden Geschlechtern drei. Mit zunehmendem Alter der Individuen wurden Umgestaltungen im lamellären Aufbau der Knochen beobachtet. Nach der dritten Überwinterung traten signifikante Unterschiede in der Körperlänge zugunsten der Weibchen auf. Die Studie unterstützt die Hypothese, daß kleine Arten früh geschlechtsreif werden und eine relativ kurze Lebenserwartung aufweisen.

ABSTRACT

Ablepharus kitaibelii (Bibron & Bory de Saint-Vincent, 1833), is one of the smallest scincid species in the world. Apart from a single observation in captive *A. kitaibelii*, there is no information on the lifespan of any species of the genus *Ablepharus* Lichtenstein, 1823. In the present skeletochronological study the maximal number of hibernations was three in both sexes of *A. kitaibelii*. Remodeling of the lamellar bone structure with age was observed. The significantly longer body length of the females became most pronounced after the third hibernation. This study supports the hypotheses of small species arriving at maturity at comparativerly young age and having a rather short lifespan.

KEY WORDS

Reptilia: Squamata: Sauria: Scincidae; *Ablepharus kitaibelii*, skeletochronology, femur, lines of arrested growth, LAG, growth rates, age structure, population ecology, physiology, morphology, Bulgaria

INTRODUCTION

Skeletochronology is a commonly used method for age determination in vertebrates based on the regular deposition of bone tissue layers (usually annually or seasonally accelerated/decelerated) of varying width, paralleling cyclical processes in the animal's seasonal activity (Enlow 1969; Hemelaar & Van Gelder 1980). This technique was used in age determination of many lizards. The skeletochronological studies available on the age structure of species of the family Scincidae are commented in the Discussion. To the authors' knowledge, there is no skeletochronological study on any species of the genus *Ablepharus* Lichtenstein, 1823, which includes some of the smallest scincid species in the world.

MATERIALS AND METHODS

For age determination of *A. kitaibelii*, 55 alcohol-fixed specimens (24 males, 24 females and 7 juveniles) from different localities in Bulgaria were examined. Following Ljubisavljević et al. (2002), specimens of snout-vent-length below 32 mm were considered juveniles. The specimens came from the following 10 km x 10 km UTM squares: GM48, FM59, GL19, MG79, MG11, KG86, FM46, GL39, MF29, MH84, KH90, MG45, FP70, NJ08, MG95, NG16, GL28, NG45, LG23, NJ27, FP91, FM59, NH14, NH35, MG47, FM46, GN41, NG75, MJ75 and FN75. All specimens are stored in the collection of the National Museum of Natural History, Sofia, Bulgaria (see Appendix I). The body length of each specimen was measured from the tip of the rostrum to the posterior end of the cloacal scales. Considering the small size of the studied object, the femur bone was chosen to be most appropriate for the skeletochronological study.

The extracted femoral bone parts were washed in water for several hours, and demineralized with 5 % HNO_3 for 2-3 hours. After 24 hours of washing in running water, the specimens were dehydrated with ethanol, cleared with xylene and embedded in paraffin wax. The samples were sectioned at 15 µm. After deparaffinizing and hydration, the sections were stained with Heidenhain's iron-haematoxylin, dehydrated with ethanol and mounted in Euparal or Canada balsam. In search of a more informative staining technique, several slides were stained with Harris' haematoxylin, which resulted in pictures with higher contrast.

The sections were prepared from the diaphysis, where the lines of arrested growth (LAGs) were most distinct. The slides were observed under a compound microscope and the LAGs were counted independently by each author (CASTANET et al. 1977; HEMELAAR & VAN GELDER 1980). An Amplival (Carl Zeiss Jena) microscope equipped with apochromatic objectives and digital camera Canon EOS 70D were used for photography. The method of age determination and the terminology for the structures observed follow CASTANET & SMIRINA (1990) and ENLOW (1969).

Descriptive data of the body length by age group (determined by the number of LAGs) were calculated separately for males, females and juveniles. Significance of size differences between age groups was tested with ANOVA. Growth models were estimated according to Von Bertalanffy's equation (Chapman's method) used in other studies on scincids (WAPSTRA et al. 2001; GUARINO 2010). The presumed age of each specimen was calculated from the LAG number (corresponding to the number of hibernations) plus the days between the median hatching date and capture date. Since the specimens from northern and southern Bulgaria hatch at different periods (VERGILOV unpubl.), the specimens were assigned to northern and southern populations with the Stara Planina Mountains as a physical border between them. The median hatching date was estimated as 18 July for the southern and 18 August for the northern populations. Specimens with unknown date of capture and juveniles were excluded from the analyses. Seventeen females and 18 males entered the analysis. SigmaPlot ver. 12.0 (Systat Software, Inc., San Jose California USA, < www.systatsoftware.com >) and Past ver. 3.18 (HAMMER et al. 2001) were used for the calculations and representation.

RESULTS

The body length was 35 mm in the smallest studied male specimen and 45 mm in the largest; the smallest and the largest females were 35 mm and 53 mm long, respectively (Table 1). Derived from the number of LAGs on sections from the femur bones (Fig. 1A-D), the largest specimens of both sexes had gone through three hibernations, i.e., their age was four years at most. Females between 42 and 53 mm body length showed three LAGs, males from 35 to 43 mm two and those between 43.2 and 46 mm three. No difference in maximum age between sexes was observed. In cases of endosteal resorption, the hatching line can be resorbed during bone growth and remodeling (CASTANET & SMIRINA 1990). Apparently, there was never complete resorption of the endosteal bone tissue in the examined specimens since in all the hatching line was at least partially visible.

As the bone grows, processes of remodeling (resorption and deposition of bone tissue) occur, which cause modifica-

Table 1: Descriptive statistics of body length (mm) by age group in 24 males, 24 females and 7 juveniles of *Ablepharus kitaibelii* (BIBRON & BORY DE SAINT-VINCENT, 1833), from Bulgaria. SE – Standard error of the mean, SD – Standard deviation.

Tab. 1: Deskriptive Statistiken der Körperlänge (mm) in Abhängigkeit von der Altersklasse bei 24 Männchen, 24 Weibchen und 7 Jungtieren von *Ablepharus kitaibelii* (BIBRON & BORY DE SAINT-VINCENT, 1833) aus Bulgarien. SE – Standardfehler des Mittelwertes, SD – Standardabweichung.

	Hibernations Überwinterungen	N	Minimum	Maximum	Mean Mittel	SE	SD
Juveniles	0	2	20.0	24.0	22.0	2.00	2.83
Jungtiere	1	5	27.0	31.3	29.4	0.73	1.64
Males	1	3	35.0	37.0	36.2	0.60	1.04
Männchen	2	13	35.6	43.0	40.4	0.56	2.01
	3	8	43.2	46.0	44.9	0.34	0.97
Females	1	1	35.0	35.0	35.0	—	—
Weibchen	2	5	39.0	47.5	41.8	1.51	3.39
	3	18	42.0	53.0	47.5	0.71	3.01

tions in the bone structure (ENLOW 1969). Prominent changes were observed after the third hibernation, when most specimens demonstrated “drifting” of the lamellae (Fig. 1F). In nine specimens the LAG pattern looked blurred in most sections (Fig. 1E), yet in some sections countable LAGs were recognized. Absence of recognizable LAGs due to extensive remodeling was registered in one specimen only (Fig. 1E).

In a single specimen, sections from different parts of the diaphysis showed signs of a large fracture. The femur appeared elliptical (not circular) in cross section with irregular endosteal and peripheral surfaces. Despite the fracture, two LAGs and remnants of the hatching line were counted.

Sex-specific differences in body length, although not prominent, appeared after the first hibernation. The growth curves of the males and females are presented in Fig. 2.

The ANOVA analysis of dispersion showed that the body length differed significantly between the male (F = 35.6, P < 0.0001) and female (F = 26.3, P < 0.0001) age groups.

Comparison of the body length between male and female specimens at the age of two hibernations showed non-significant (Student’s t-test, t = 1.15, P = 0.268), and at the age of three hibernations significant (t = 2.4, P = 0.024) differences. Thus, differences in body length between males and females were most pronounced after the third hibernation. After the third hibernation the males reduced their growth rates, whereas the females continued growing. Considering the short lifespan of the species, this probably enables females to develop more (up to five – FUHN 1970) eggs in their abdomina.

DISCUSSION

The present skeletochronological study is the first on the age and growth of free ranging specimens of the genus *Ablepharus*. Its results are in line with studies on other scincid species (ROHR 1997; WAPSTRA et al. 2001; KUMBAR 2008; GUARINO 2010), supporting the expected correlation between the body length and the age of the specimens and contradict other studies on scincid species where no strong or expressed correlation between body length and number of LAGs was found (see MCCOY et al. 2010; ANDREONE & GUARINO 2003). Similar to other scincid species (DUBEY et al. 2013), both sexes of *A. kitaibelii* have the same maximum longevity, despite differences in maximum snout-vent-length. Both female and male *A. kitaibelii* continue to grow up to four years. The accelerated growth of the female body (Fig. 2) is most probably related to demands to increase reproductive success in terms of high egg numbers. GUARINO

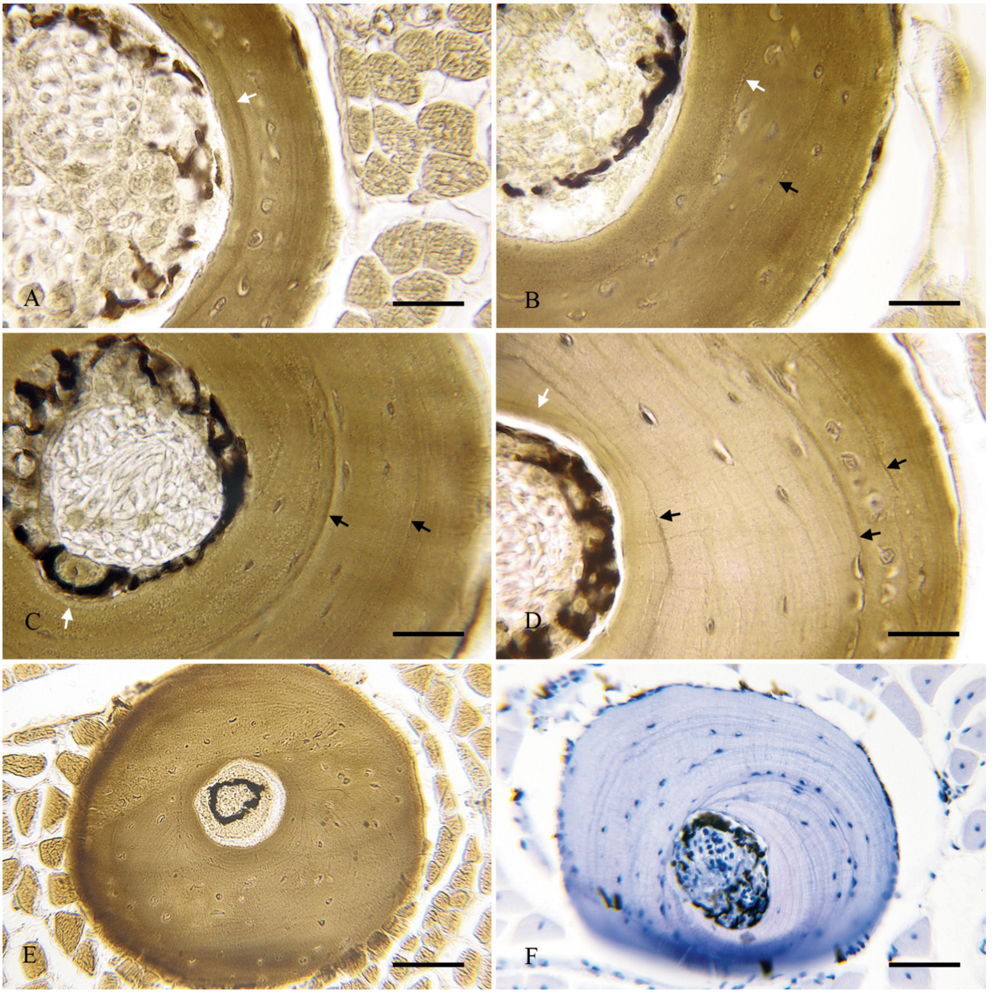

Fig. 1: Sections of femoral diaphyses from specimens of *Ablepharus kitaibelii* (BIBRON & BORY DE SAINT-VINCENT, 1833) of different age. A – no hibernation; B – one hibernation; C – two hibernations; D – three hibernations; E – undefined number of hibernations, no LAGs are recognizable due to extensive bone remodeling; F – "drifting" of the lamellae, the hatching line is still detectable in the right half of the section. The white arrow denotes the hatching line, black arrows denote LAGs. Scale bars represent 20 µm in A-D and 50 µm in E-F. Sections A-D and E were stained with Heidenhain's iron-haematoxylin, section F was with Harris' haematoxylin.

Abb. 1: Histologische Schnitte von Oberschenkeldiaphysen unterschiedlich alter *Ablepharus kitaibelii* (BIBRON & BORY DE SAINT-VINCENT, 1833). A – keine Überwinterung; B – eine Überwinterung; C – zwei Überwinterungen; D – drei Überwinterungen; E – Anzahl an Überwinterungen unbestimmt; aufgrund starker Umgestaltung des Knochengewebes sind keine Wachstumslamellen erkennbar; F – Verschiebung der Lamellen, die „hatching line" (Schlupflinie) ist in der rechten Hälfte des Querschnittes noch erkennbar. Der weiße Pfeil bezeichnet die „hatching line" (Schlupflinie); schwarze Pfeile Wachstumslamellen. Der Maßstab entspricht 20µm bei A-D, 50µm bei E-F. Die Schnitte A-D und E wurden mit Eisenhämatoxylin nach Heidenhain, F wurde mit Hämatoxylin nach Harris gefärbt.

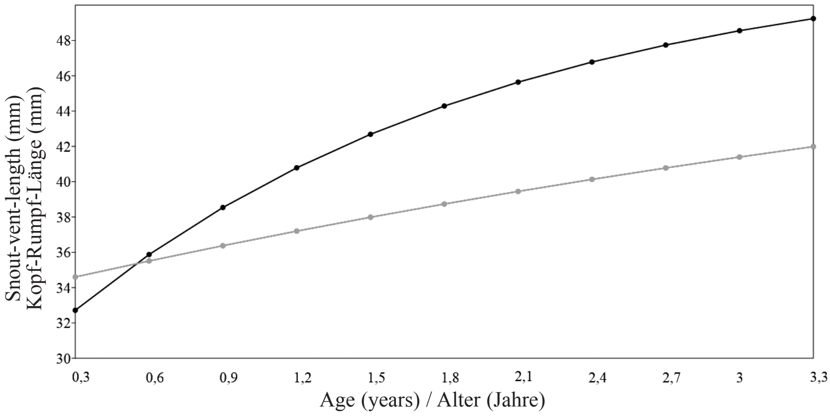

Fig. 2: Von Bertalanffy growth curves of females (black) and males (grey) of *Ablepharus kitaibelii* (BIBRON & BORY DE SAINT-VINCENT, 1833) from Bulgaria. Note that the increase in length is more rapid in females.

Abb. 2: Von Bertalanffy Wachstumskurven für Weibchen (schwarz) und Mänchen (grau) von *Ablepharus kitaibelii* (BIBRON & BORY DE SAINT-VINCENT, 1833) aus Bulgarien. Man beachte die raschere Längenzunahme bei den Weibchen.

(2010) revealed that the females of *Calcides chalcides* (LINNAEUS, 1758) grow more rapidly than males (similar to *A. kitaibelii*), but live longer. The current study also accords with the hypothesis of small species having short lifespan and being of young age at maturity (ANDREWS 1982; SHINE & CHARNOV 1992; STEARNS 1992; BAUWENS & DIAZ-URIARTE 1997; KOLAROV et al. 2010; ÇIÇEK et al. 2012; ARAKELYAN et al. 2013). The growth in males and females, established here (Fig. 2), is similar to those in other reptile species (FLOWER 1925; STEARNS 1984; DUNHAM & MILES 1985; AVERY 1994; BAUWENS & DIAZ-URIARTE 1997; O'MEARA & ASHER 2015).

LAGs are easily recognized in many Lacertidae (CASTILLA & CASTANET 1986; ROITBERG & SMIRINA 2006; GUARINO et al. 2010; KOLAROV et al. 2010; ARAKELYAN et al. 2013; TOK et al. 2013), Agamidae (ERGÜL et al. 2014) and Liolaemidae (PIANTONI et al. 2006; GUTIÈRREZ et al. 2013; CABEZAS-CARTES et al. 2015), whereas they are not always well defined in *A. kitaibelii* and other Scincidae (KUMBAR 2008; GUARINO 2010), especially in older specimens. This could be explained with bone remodelling and specifics in bone formation in the species of Scincidae. Similar cyclical depositions in the bones were typical even for tropical lizards such as *Eutropis carinata* (SCHNEIDER, 1801) (KUMBAR 2008), *Calotes versicolor* (DAUDIN, 1802) (PATNAIK & BEHERA 1981), *Psammophilus dorsalis* (GRAY, 1831) (MAHAPATRO et al. 1989), *Hemidactylus brooki* (GRAY, 1835) (PANCHARATNA & KUMBAR 2005) and *Sitana ponticeriana* CUVIER, 1829 (RATH & PAL 2007).

The persistence of the hatching line, at least partially, is observed also in other species (GUARINO 2010). The correct interpretation of the LAG number is supported by the reported lifespan of 3.5 years in captive *A. kitaibelii* (GRUBER 1981), which roughly corresponds to the maximal LAG number of three (i. e., hibernations).

In the present study, only Bulgarian specimens, i.e., individuals from the middle of the species' distribution were examined. Considering its vast range area from southern Slovakia to Anatolia covering various climatic belts from Continental to Mediterranean, minor skeletochronological differences to the Bulgarian populations are to be expected in other parts of the species' territory.

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Appendix I

Museum numbers of the studied specimens of *Ablepharus kitaibelii* (BIBRON & BORY DE SAINT-VINCENT, 1833), from the National Museum of Natural History, Sofia, Bulgaria - NMNHS.

Inventarnummern der untersuchten Exemplare von *Ablepharus kitaibelii* (BIBRON & BORY DE SAINT-VINCENT, 1833) des Naturhistorischen Nationalmuseums, Sofia, Bulgarien - NMNHS.

NMNHS 39, 40, 42, 44-48, 54-56, 58-60, 89, 94, 96, 98, 111, 122, 131, 135, 137, 140, 148, 151-152, 155-156, 161, 166, 168-169, 184, 187-188, 190, 192, 195, 197, 199, 203-209, 211-212, 215, 223, 226, 227 and 230.

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