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SEXUAL SIZE DIMORPHISM IN THE BALKAN SUBSPECIES OF THE SAND LIZARD *LACERTA AGILIS BOSNICA* SCHREIBER, 1912 (REPTILIA: LACERTIDAE) IN BULGARIA

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Abstract: Sexual size dimorphism (SSD) among species from the Lacertidae family is expressed mainly in body and head size, with males being larger than females, having larger heads but shorter trunks in relation to body size. Most lacertid species display male-biased SSD, but in few species, such as *Lacerta agilis*, females display larger size. The sand lizard Lacerta agilis is one of the most widespread lizard species in Eurasia and inhabits an area from central Greece and south Armenia to south Sweden in the North, and from Great Britain to the lake Baikal in the East. At least nine subspecies are currently recognized and two of them occur in Bulgaria. In the present study we analysed SSD in the poorly known subspecies Lacerta agilis bosnica, which inhabits the mountain areas on the Balkan Peninsula. We examined 9 somatometric traits, which were transformed into 10 indices, describing body proportions. All of the measurements were taken in live animals captured in mountains in Western Bulgaria (the mountains of Vitosha, Osogovo, Stara Planina, and Plana). We established statistically significant differences between the sexes in 9 of the indices. The differences could be described as follows: males have longer tails, longer legs (fore and hind legs), and larger heads (both in length and width). The similarities and differences in regards to SSD in other taxa from the genus Lacerta (for which similar research was done in Bulgaria) are further discussed.

INTRODUCTION

Many reptiles exhibit external sexual dimorphism, which among lizards is mostly expressed in body size differences between sexes (Andersson, 1994, Cox *et al.*, 2007). This type of sexual dimorphism is often called *sexual size dimorphism* (abbreviated SSD). There are two main evolution trends: (1) sexual selection for larger male size (related to male combat and guarding individual territory) (Kratochvil and Frynta, 2002) and (2) natural selection for larger female size (which gives advantage in fecundity selection) (Zamudio, 1998). SSD among species from the Lacertidae family is expressed in body length and mass, leg length and head size and shape (Ljubisavljević et al., 2008, Molina-Borja, 2003, Žagar *et al.*, 2012) with males often being larger than females, having larger heads (Kaliontzopoulou *et al.*, 2006, 2008) but shorter trunks in relation to body size (Braña, 1996, Gvozdik and Van Damme, 2003, Herrel *et al.*, 1996, Scharf and Meiri, 2013).

The Sand lizard *Lacerta agilis* Linnaeus, 1758 is a medium-sized, diurnal species. It is one of the most widespread lizards in Palearctic which include at least nine subspecies (Andres *et al.*, 2014). The species is distributed from central Greece and south Armenia to southern Sweden in the North, and from Great Britain to the lake Baikal in the East (see Ananjeva *et al.*, 2004 and Sillero *et al.*, 2014). Among the different populations and subspecies of *L. agilis*, SSD could be male-biased (Peskov *et al.*, 2013, Roitberg and Smirina, 2006a, Tuniyev and Tuniyev, 2008, Yablokov, 1976), female-biased (Amat *et al.*, 2000, Borczyk and Pasko, 2011, Olsson and Shine, 1996, Guarino et al., 2010, Gvozdik and Boukal, 1998, Tuniyev and Tuniyev, 2008) or without obvious difference between sexes. There are also differences between populations of the same subspecies (Guarino *et al.*, 2015, Roitberg and Smirina, 2006a, Roitberg, 2007, Yablokov, 1976).

The aim of our study was to analyze SSD of poorly known subspecies of the Sand lizard and to compare our results with other taxa from the genus *Lacerta* for which similar research were done in Bulgaria.

MATERIALS AND METHODS

In Bulgaria, the Sand lizard is represented by two subspecies: *L. agilis* chersonensis Andrzejowski, 1832 which occurs in the kettles (and occasionally in mountain areas) of West Bulgaria and sporadically in the lowlands of East Bulgaria; *L. a. bosnica* (the subject of this study) – typical for the high mountains of the country (see Stojanov et al., 2011).

The study was conducted between 2014 and 2018 in four mountains in western Bulgaria (Vitosha, Osogovo, Stara Planina, and Plana; the altitude of the visited locations is from 1100 to 1600 m). Four sites were chosen (one in each mountain) where the presence of animals were previously known, and visited

in sunny days during the active period (April - September). A total of 272 Sand lizards were captured, measured, and then released at the site of capture.

Following the adopted approach by a number of studies, in the current study individuals that have never undergone hibernation were considered as juveniles, i. e. captured in the year of their hatching. Lizards which had hibernated at least once were accepted for subadults, and if we supposed that hatching took place mainly in August, in the next year those individuals would be at age between 7 and 13 months (in the period April – September) but still won't be sexually mature. In April after the second hibernation the age of the same individuals would be around 19 months but they still might not have reached sexual maturity (probably some of them will take part in reproduction). In April after the third hibernation the age of the lizards would be 31 months (at least 3 years) and they would undoubtedly be sexually mature.

The sex of the captured lizards was determined (excluding the smallest specimens, i.e. juveniles and some of the subadults) by coloration pattern (green coloured sides of the body in males vs brown in females; a large count of black dots on the abdominal side of the body in males vs absence or only very small count in females) and size of the femoral pores (larger in males).

Standard metrics were taken (using ruler, with resolution 0,1 mm) for: body length (L.corp.) from the snout tip to the cloaca; tail length (L.cd) from the cloaca to the tail tip (only individuals with intact tails were used for the analysis); length of the front leg (P.a.) from the axillar fold to the tip of longest toe; length of the hind leg (P.p.) from the inguinal fold to the tip of the longest toe; length of the hind leg step (P.p.2) from the tip of the longest toe to the base of the first; (using digital calliper, with resolution 0,01 mm) pileus length (L.pil.); pileus width (Lat. pil.); head width (Lat.cap.) at the widest point of the jugal bones (Lat.cap). On the base of the obtained measurements, ten indices were calculated: L.corp./L. cd; P.a./L.corp.; P.p./L.corp.; P.p.2/L.corp.; L.pil./L.corp.; Lat.pil./L.corp.; Lat.pil./L.corp.; Lat.pil./L.pil.; P.a./P.p.

The results of the normality test (Kolmogorov-Smirnov) showed that the distribution in some of the measurements and indices was not normal; moreover, sample size in the sex and age groups varied significantly. Hence, comparisons were done using a nonparametric test (Mann-Whitney U Test). All of the statistical procedures were done in Statistica 10 ver. 10.0 (StatSoft. Inc. 2011).

RESULTS AND DISCUSSION

Body length and age groups

The values of L.corp. for the whole study are presented on Fig.1, arranged by months (in ascending order). Individuals captured in April (n=16) could clearly be placed in two size groups: the first from 33 to 42 mm, and the second – larger than 59 mm. Most likely the first group consisted of individuals hatched in the

previous year, that did not take part in the reproduction (subadults), and the second group was composed of adult individuals. In May all of the individuals (n=75) showed the same tendency: smaller individuals were from 38 to 46 mm, and larger – more than 57 mm. In June (n=54) and July (n=93) the transition from smaller to larger individuals was lighter and the size groups could not be easily determined. This was probably due to the fact that the individuals from the previous year had significantly grown in size towards April, and there were no hatchlings yet. The individuals found in September (n=34) could be assigned to three size groups: first from 20 to 22 mm - undoubtedly newly hatched; second from 28 to 44 mm (some of them newly hatched but others (the larger ones) could have been hatched the year before), and the third group consists of individuals larger than 56 mm (probably all of them were adults but the smaller ones might not had taken part in reproduction yet).



Fig. 1 Body length (L corp.) given in mm for all measured L. a. bosnica by months.

The definition of the age classes could be clearer if skeletochronology was applied in parallel with the measurement. This was not possible in the present study due to number of reasons. Hence, a consideration should be kept in mind that the borders between all described size classes herein were relative but sufficient for the present work. The body size for all age classes vary highly among different populations in accordance with subspecific and geographical variations (see Roitberg, 2007 and the review in Borczyk and Paśko, 2011); Gvozdik, 2000 assigned lizards in 3 age groups – hatchlings (1st year) from 24,1 to 34,5; overwintering (2nd year) from 26,5 to 66,4 and adults after 3th year –

54,8 to 83,9. Other authors defined juveniles in different size range – from 35 to 55 (Angel, 1947); 30-35 (Yabolkov, 1976); 26-31 (Barus and Oliva 1992). For Bulgaria Stojanov et al., 2011 recorded for hatchlings L.corp. at 27.2 mm and total length at 59.1 mm, and Tzankov *et al.* (2014) recorded total length for *L. a. bosnica* up to 58 mm.

In respect of what was noted above, all individuals with a value of L.corp. larger than 55 mm could be accepted as adults, respectively: subadults - those from 33 to 55 mm (in some, but not all, sex could be determined according to coloration), and as juveniles – smaller than 33 mm.

In the material collected for this study, the largest value for L.corp. in males was 87 mm, and in females - 86 mm. Nevertheless, the Mann-Whitney test showed that in adults, there was a statistically significant difference (p < 0.001) between sexes in body length, and the main tendency was that females were larger than males; in subadults the difference between sexes was not significant in respect to this measurement (see Tables 1 and 2.).

The maximum known size of *L.a.bosnica* for Bulgaria is 83 mm for males (n=65), and 88 mm for females (n=54) and for *L.a.chersonensis* is 92 mm in males (n=63) and 100 mm in females (n=60) (Stojanov *et al.*, 2011 and Tzankov et al., 2014). The larger size of females *Lacerta agilis bosnica* is confirmed by Andjelkovic *et al.* (2013) for the population of Bistra Mts., Macedonia.

In most of the populations from the Western clade, the females displayed a larger size (Borczyk and Pasko, 2011, Guarino et al., 2010, Gvozdik and Boukal, 1998 but see Guarino et al., 2015), especially in the populations from North Europe or high altitudes (Amat *et al.*, 2000, Olsson and Shine, 1996). In the Eastern (Caucasian) clade the males with larger size were predominant (Kidov *et al.*, 2014, Roitberg and Smirina, 2006a, Tuniyev and Tuniyev, 2008) but populations without significant difference between sexes were also present (Majlth *et al.*, 1997, Tuniyev and Tuniyev, 2008, Yablokov, 1976).

Body proportions

It is clear that if the difference between sexes exists in body length (as it was commented above), then similar difference could also be observed in direct comparison of the separate body parts. A much better understanding of SSD can be obtained by analysing the proportions of individual parts of the body using indices. The descriptive statistics of the used indices are presented in Table 1, and the results of Mann-Whitney test – in Table 2.

In adults, there was a statistically significant difference between sexes for nine indices. Seven of them had the highest level of significance (p < 0.001): L.corp./L.cd., P.a./L.corp., P.p./L.corp., P.p.2/L.corp., L.pil./L.corp., Lat.pil./L. corp., and Lat.cap./L.corp. In summary, males appear to have longer tails and legs, larger hind legs step, larger and wider pileus and wider head, relative to body size.

In subadults, a significant difference between sexes was found in six of the indices, and three had high level of significance: P.p./L.corp., P.p.2/L.corp., and L.pil./L.corp. In all of the six indices the main tendency was the same as in the adults. If we consider only the three indices with highest level of significance, it could be presumed that the first display of SSD in the development of young lizards is expressed in the elongation of the legs (especially the hind leg) and pileus.

Our results suggest that males have longer tails and legs, larger hind legs step, larger and wider pileus and wider head, related to the body size. Males have bigger home range (Olsson, 1986) and longer tails and legs might give advantage in guarding and defending the territory and seeking for females. This also gives an advantage in general locomotor performance (Kaliontzopoulou et al., 2012, Van Damme *et al.*, 2008). The larger body and head size in males is related to the male-male competition for females and defending territory. Males also display a larger head size which is associated with the differences in the bite force. Multiple studies has indicated that the males have a stronger bite force (Herrel et al., 1996, 2001, Lappin et al., 2006, McBrayer and Anderson, 2007). On one hand, the differences in the bite force and head dimension could be driven by the necessity of holding females during copulation, which in evolutionary aspect is related to the increasing trunk size in the females (Braña, 1996, Schwarzkopf, 2005). On the other hand, these differences give opportunity to choose harder and bigger prey to reduce trophic competition (but see Gvozdik and Boukal, 1998) allowing the avoidance of intraspecific competition (Brecko et al., 2008, Lopez-Darias et al., 2014, Hierlihy et al., 2013, Vincent and Herrel, 2007). The females in many lacertid species display smaller body size and shorter and slender heads but larger trunk (Kratochvil et al., 2003). Larger trunk in females is related to the fecundity selection, increasing the space available for eggs (Olsson et al., 2002).

Table 1. Descriptive statistic of biometric characters and indices for measured L. a. bosnica presented by age and sex groups (for the abbreviations, see "Material and methods").

		Adult males			Adult females	
	z	Mean (± SD) Median (25 ÷ 75%) Min ÷	÷ Max	N Mean (± SD)	Median (25 ÷ 75%)	Min ÷ Max
L.corp.		53 64.98 (± 5.77) 64.50 (61.00 ÷ 69.50) 56.00) ÷ 87.00	100 70.12 (± 7.82)	70.00 (63.75 + 75.75)	$55.50 \div 86.00$
L.corp./L.cd.		23 0.62 (± 0.05) 0.61 (0.58 ÷ 0.64) 0.54	÷ 0.75	53 0.69 (± 0.05)	0.68 (0.66 ÷ 0.72)	$0.60 \div 0.80$
P.a./L.corp.		53 0.33 (± 0.02) 0.33 (0.31 ÷ 0.34) 0.28	÷ 0.38	100 0.30 (± 0.02)	0.30 (0.28 + 0.31)	0.26 ± 0.34
P.p./L.corp.		52 0.45 (± 0.03) 0.45 (0.43 ÷ 0.47) 0.38	÷ 0.52	100 0.41 (± 0.03)	0.41 (0.38 ÷ 0.43)	$0.34 \div 0.48$
P.p.2/L.corp.		49 0.21 (± 0.02) 0.21 (0.19 ÷ 0.23) 0.15 ·	÷ 0.24	98 0.19 (± 0.03)	0.19 (0.17 ÷ 0.21)	$0.14 \div 0.25$
L.pil./L.corp.		52 0.23 (± 0.01) 0.23 (0.22 ÷ 0.24) 0.21	÷ 0.25	98 0.20 (± 0.01)	0.20 (0.19 ÷ 0.21)	0.18 + 0.22
Lat.pil./L.corp.		50 0.12 (± 0.01) 0.12 (0.11 ÷ 0.12) 0.10	÷ 0.14	99 0.10 (± 0.01)	0.10 (0.09 ÷ 0.10)	0.08 + 0.12
Lat.cap./L.corp		53 0.15 (± 0.01) 0.15 (0.15 ÷ 0.16) 0.13	÷ 0.18	99 0.13 (± 0.01)	0.13 (0.12 ÷ 0.14)	$0.11 \div 0.15$
Lat.pil./L.pil.		50 0.51 (± 0.03) 0.51 (0.49 ÷ 0.53) 0.44	÷ 0.59	99 0.49 (± 0.03)	0.49 (0.48 + 0.51)	$0.41 \div 0.56$
Lat.cap./L.pil.		53 0.66 (± 0.03) 0.66 (0.65 ÷ 0.68) 0.58 ·	÷ 0.74	98 0.65 (± 0.03)	0.65 (0.63 + 0.67)	$0.57 \div 0.74$
P.a./P.p.		52 0.73 (± 0.04) 0.72 (0.70 ÷ 0.75) 0.63	÷ 0.81	99 0.74 (± 0.04)	0.73 (0.71 ÷ 0.76)	$0.63 \div 0.84$
		Subadult males		S	ubadult females	
	z	Mean (± SD) Median Min ÷	÷ Max	N Mean (± SD)	Median	Min ÷ Max
L.corp.		29 50.86 (± 3.24) 52.00 (48.00 ÷ 53.00) 43.50) ÷ 55.00	21 50.07 (± 4.45)	$52.00(46.00 \div 54.00)$	$43.00 \div 55.00$
L.corp./L.cd.		15 0.60 (± 0.03) 0.60 (0.57 ÷ 0.62) 0.55	÷ 0.65	16 0.64 (± 0.06)	$0.64 (0.60 \div 0.68)$	$0.57 \div 0.76$
P.a./L.corp.		27 0.34 (± 0.02) 0.34 (0.32 ÷ 0.36) 0.29	÷ 0.38	21 0.32 (± 0.02)	0.32 (0.31 + 0.33)	$0.29 \div 0.36$
P.p./L.corp.		29 0.47 (± 0.04) 0.48 (0.44 ÷ 0.50) 0.38	÷ 0.53	21 0.44 (± 0.03)	0.44 (0.42 ÷ 0.47)	$0.40 \div 0.50$
P.p.2/L.corp.		28 0.24 (± 0.02) 0.23 (0.22 ÷ 0.25) 0.19	÷ 0.29	21 0.21 (± 0.02)	0.21 (0.19 + 0.22)	0.18 + 0.24
L.pil./L.corp.		27 0.23 (± 0.01) 0.23 (0.23 ÷ 0.24) 0.22	÷ 0.25	21 0.22 (± 0.01)	0.22 (0.21 + 0.23)	$0.20 \div 0.25$
Lat.pil./L.corp.		28 0.12 (± 0.01) 0.12 (0.11 ÷ 0.12) 0.09	÷ 0.13	21 0.11 (± 0.01)	0.11 (0.11 + 0.12)	$0.10 \div 0.13$
Lat.cap./L.corp	-	29 0.15 (± 0.01) 0.15 (0.14 ÷ 0.16) 0.12	÷ 0.17	21 0.14 (± 0.01)	$0.15(0.14 \div 0.15)$	0.12 + 0.16
Lat.pil./L.pil.		28 0.50 (± 0.03) 0.49 (0.48 ÷ 0.52) 0.44	÷ 0.55	21 0.51 (± 0.03)	0.51 (0.48 + 0.53)	$0.46 \div 0.55$
Lat.cap./L.pil.		28 0.65 (± 0.03) 0.65 (0.63 ÷ 0.67) 0.61 ·	÷ 0.71	21 0.65 (± 0.04)	0.64 (0.62 + 0.68)	$0.59 \div 0.74$
P.a./P.p.		28 0.71 (± 0.04) 0.71 (0.69 ÷ 0.73) 0.59	÷ 0.79	20 0.72 (± 0.03)	$0.72 (0.70 \div 0.74)$	$0.67 \div 0.77$

	Adults			Subadults		
	U	Z	Ρ	U	Z	р
L.corp.	1590,5	4,06	< 0.001	289,5	0,29	n.s.
L.corp./L.cd.	147	5,22	< 0.001	58	-2,43	< 0.05
P.a./L.corp.	893	-6,74	< 0.001	160	2,56	< 0.05
P.p./L.corp.	818,5	-6,92	< 0.001	152,5	2,98	< 0.01
P.p.2/L.corp.	1551,5	-3,49	< 0.001	119,5	3,52	< 0.001
L.pil./L.corp.	97,5	-9,68	< 0.001	124	3,3	< 0.001
Lat.pil./L.corp.	240	-8,98	< 0.001	235	1,18	n.s.
Lat.cap./L.corp	257	-9,15	< 0.001	201	2,02	< 0.05
Lat.pil./L.pil.	1739	-2,96	< 0.01	222	-1,44	n.s.
Lat.cap./L.pil.	1922	-2,63	< 0.01	267	0,54	n.s.
P.a./P.p.	2199	1,47	n.s.	241	-0,81	n.s.

Table 2. Mann-Whitney U test results (sex as a grouping variable); for the bbreviations,see "Material and methods".

The only published research on SSD in lizards from Bulgaria (Grozdanov and Tzankov, 2014) is related to Lacerta agilis chersonensis and Lacerta viridis (Laurenti, 1768). The used morphometric indices in this study are similar to the ones in our study, which allowed a comparison of SSD between these three taxa (Table 3). It appears that SSD is more pronounced in L. a. bosnica (9 of the indices), than the other two taxa (in 7 indices), with the consideration that the sampling size in our study was larger. In six of the indices a significant difference between sexes occurred in all of the three taxa, and according to the correlation between the fore and hind limbs, SSD was not observed in any of them. The differences between the taxa are expressed as follows: the sexual dimorphism in L. a. bosnica is expressed in the correlation between the pileus width and length, while it is absent in the other two taxa; sexual dimorphism is not observed in the correlation of the hind limbs and the body length in L. a. chersonensis, and the correlation of body length and tail length in L. viridis, while L. a. bosnica expressed SSD in both of those indices. It is not clear if those differences in SSD have a taxonomical significance or are related to subjective reasons (for example, different or insufficient in size samplings; the use of different statistical tests or other).

	L. a. bosnica	L. a. chersonensis	L. viridis
L.corp./L.cd.	< 0.001	< 0.01	n.s.
P.a./L.corp.	< 0.001	< 0.05	< 0.01
P.p./L.corp.	< 0.001	n.s.	< 0.01
P.p.2/L.corp.	< 0.001	< 0.05	< 0.01
L.pil./L.corp.	< 0.001	< 0.001	< 0.001
Lat.pil./L.corp.	< 0.001	< 0.001	< 0.001
Lat.cap./L.corp.	< 0.001	< 0.001	< 0.001
Lat.pil./L.pil.	< 0.01	n.s.	n.s.
Lat.cap./L.pil.	< 0.01	< 0.01	< 0.001
P.a./P.p.	n.s.	n.s.	n.s.

Table 3. Statistical significance (p level) of the differences in indices between sexes of *L. agilis bosnica* (this study), *L. agilis chersonensis* and L. viridis (after Grozdanov and Tzankov, 2014).

CONCLUSION

The Balkan subspecies of the Sand lizard *Lacerta agilis bosnica* shows a clear sexual size dimorphism in favor of females which was also confirmed in other studies from the Balkan Peninsula for this subspecies. The larger body size in females could be restricted to the fecundity selection in respect of its wider distributional range and the preferred higher altitude which would be connected with the fact that it is the smallest subspecies of the sand lizard and the only egglaying Lacerta species occupying high altitudes on the Balkan Peninsula.

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The authors declare that there is no conflict of interest.

AUTHORSHIP STATEMENT Conceived and designed the analysis: S.P, E.V., N.T. Collected the data: S.P, E.V., N.T. Contributed data or analysis tools: S.P, E.V., N.T. Performed the analysis: S.P, E.V., N.T. Wrote the paper: S.P, E.V.

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