

Morphological and ecological divergence in two populations of European glass lizard, *Pseudopus apodus* (Squamata: Anguidae)

Olga Jovanović Glavaš¹, Paula Počanić², Vanja Lovrić², Lorena Derežanin², Zoran Tadić^{2,*}, Duje Lisičić^{2,*}

¹ Department of Biology, University of Osijek, Osijek 31000, Croatia

² Department of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb 10000, Croatia

ABSTRACT

The European glass lizard, *Pseudopus apodus* (Pallas, 1775), is a large, legless lizard with wide distribution across south-eastern Europe and eastern and central Asia. To date, morphological diversification among populations on a geographically small scale has not yet been reported in this lizard. Thus, we investigated the morphological variations and corresponding differences in habitat utilization between two populations of *P. apodus* inhabiting the same biogeographical zone within a relatively close geographic area. We hypothesized that minor differences in habitat could induce a significant level of morphological differentiation, thus indicating morphological plasticity in this species on a small geographical scale. We sampled 164 individuals (92 from the Croatian mainland and 72 from the island of Cres). Results showed that *P. apodus* indeed exhibited morphological differences between populations in the same biogeographical zone within a relatively close geographic area, with the Cres Island individuals being generally larger than the individuals from the mainland. Some ecological

characteristics were similar in both populations (e.g., soil temperature, distance to hiding place), whereas others were distinct (e.g., air temperature and humidity). In addition, vegetation cover differed between the two sites, with more vegetation present on the mainland than on the island. Furthermore, the Cres Island population showed clear sexual dimorphism, which was absent in the mainland population.

Keywords: Mediterranean; Morphology; Sauria; Sexual dimorphism

INTRODUCTION

The European glass lizard or sheltopusik, *Pseudopus apodus*, (Pallas, 1775), is a large legless lizard. With a total length of up to 120 cm (Arnold, 2002) and tail length around 150% of its body length (Obst, 1981), it is the largest lizard in Europe and the only extant species of this genus. It is diurnal, crepuscular, and often active after rain (Arnold, 2002). It lives in Asia Minor and central Asia and throughout southeastern Europe and the Balkans up to the eastern Adriatic coast (Jandzik et al., 2018). The existence of two previously recognized subspecies (Obst, 1978, 1981), i.e., nominal *P. apodus apodus* from the eastern range and *P. a. thracicus* from the western range, was recently confirmed by molecular data (Jandzik et al., 2018). However, several issues remain regarding the third, southern clade,

Open Access

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright ©2020 Editorial Office of Zoological Research, Kunming Institute of Zoology, Chinese Academy of Sciences

Received: 20 September 2019; Accepted: 27 February 2020; Online: 28 February 2020

Foundation items: This work was partly supported through grant 119-0000000-1285 of the Ministry of Science of the Republic of Croatia to Z.T.

*Corresponding authors, E-mail: duje.lisicic@biol.pmf.hr

DOI: 10.24272/zj.issn.2095-8137.2020.025

which, according to molecular analyses, also deserves a subspecies status (Jandzik et al., 2018). In addition to molecular analysis, morphological variation between the two subspecies is evident; however, in some populations, morphometric data from the original description of the subspecies (Obst, 1978, 1981) have not yet been confirmed (e.g., Jordanian population; Rifai et al., 2005). Furthermore, despite being relatively common in certain areas, there is a paucity of data on its ecology. Sexual dimorphism in this species is not very prominent, as is typical for anguids (Çiçek et al., 2014; Thomas & Hedges, 1998), although recent studies from Kukushkin & Dovgal (2018) found sexual dimorphism in a population from the Steppe Crimea.

Contemporary literature on this species indicates a level of morphological variation within the subspecies *P. a. thracicus* (Obst, 1981; Rifai et al., 2005), although only between distant populations inhabiting different biogeographical zones, and accordingly, under the influence of different environmental conditions. In this study, we investigated the morphological differences between two populations of *P. a. thracicus* in relative geographic proximity within the same biogeographical zone. One population was from the Croatian mainland and the other was from the island of Cres. We chose an island population to ensure that the level of migration between populations was minimal and that populations were isolated from one another. In addition, it is common for isolated populations to display some divergence, as recorded in other reptile species (e.g., *Testudo graeca*, Carretero et al., 2005; *Podarcis siculus*, Herrel et al., 2008; *Notechis scutatus*, Aubret, 2015).

We expect that slight differences in physical and ecological conditions between the two sites (such as composition of predatory fauna, temperature, and precipitation) may also influence species morphology, given that such conditions are known to influence the morphology of species that exhibit greater phenotypic plasticity (e.g., *Podarcis siculus*; Herrel et al., 2008). We speculate that morphological divergence will be found between the studied populations, thereby indicating morphological plasticity in *P. apodus*, even on a small geographical scale.

We anticipated that body size would differ between the insular and mainland populations, possibly due to Foster's rule (Foster, 1964). On the one hand, lizards tend to grow larger on islands (Owens et al., 2006; Pafilis et al., 2009; Palkovacs, 2003) due to high intraspecific competition or predation relaxation. On the other hand, large-bodied animals may adopt smaller body forms when in insular populations in response to limited natural resources (Lomolino, 2005). As such, the issue of body size on islands remains highly debated (Itescu et al., 2018). Habitat usage of *P. apodus* is very diverse and data obtained so far show that usage highly depends on population geography and habitat type; preferences diverge between dense vegetation in the Caucasian regions (Aleksperov, 1978), rocky slopes and shrubs in Crimea (Shcherbak, 1966), rivers and oases in middle Asia (Bogdanov, 1986), to the humid and dense

vegetation in Bulgaria (Telenchev et al., 2017). Due to the similarity of our study sites, we expected to find only small differences in habitat usage between the two populations.

MATERIALS AND METHODS

Study sites

We chose sites with similar climatic and vegetation properties, yet separated enough that morphological variability could manifest. We studied two populations located 200 km apart: a Croatian mainland population (Klis, central Dalmatia, Croatia; DMS N43°33', E16°31') and an island population (Island of Cres, Croatia; DMS N44°41', E14°24') on the eastern Adriatic Coast (Figure 1). Both sites lay within the Eumediterranean biogeographical zone, therefore sharing similar vegetation and climatic characteristics (Horvatić, 1963). An Eumediterranean climate is characterized by hot, dry summers and mild, rainy winters. Vegetation on both sites is comprised of Mediterranean macchia interspersed with pastures or small patches of agricultural land, particularly olive groves.

Cres Island is the largest Croatian island within the Eumediterranean zone of evergreen vegetation, i.e., *Quercion ilicis* alliance (Topić, 2001), with an abundant *P. apodus* population. Due to its size, Cres Island shows variability in vegetation communities similar to that observed on the mainland (Horvatić, 1963). Klis was chosen as a random site within the same zone, where the population of *P. apodus* is large enough for the study (personal observation).

The mainland study site (Klis) is located a few kilometers from the coast on the south-facing slopes at an elevation of 115–235 m a.s.l. The site is divided into two neighboring locations of roughly 200 m×300 m. The island of Cres is 4.2 km from the mainland, measuring 66 km×12 km (405.8 km²). The Cres study site is situated in the southern part of the island on the southern slopes at an elevation of 30–110 m a.s.l. It consists of three neighboring locations, two of similar size (100 m×200 m) and a third larger one (200 m×300 m).

Although extremely similar in vegetation and climate, the two sites still express differences in faunal composition, especially that of predatory species. In Klis, predators like wolves (*Canis lupus*), foxes (*Vulpes vulpes*), stone marten (*Martes foina*), least weasel (*Mustela nivalis*), and badgers (*Meles meles*) are common, but are mostly absent from Cres, except for the stone marten and least weasel (Sušić & Radek, 2007). Although both martens and weasels typically feed on small mammals (Nowak, 1991), stone martens have been recorded preying on *P. apodus* (Georgiev & Raichev, 2009), though this is considered uncommon. In addition, dissimilarities in mammalian fauna may influence other components of the food chain, thus generating differences between locations. On the other hand, a species known to prey upon *P. apodus*, the short-toed eagle (*Circus gallicus*; e.g., Bakaloudis et al., 1998), is present at both sites (Tutiš et al., 2013) and thus should not create different predation pressure between sites.

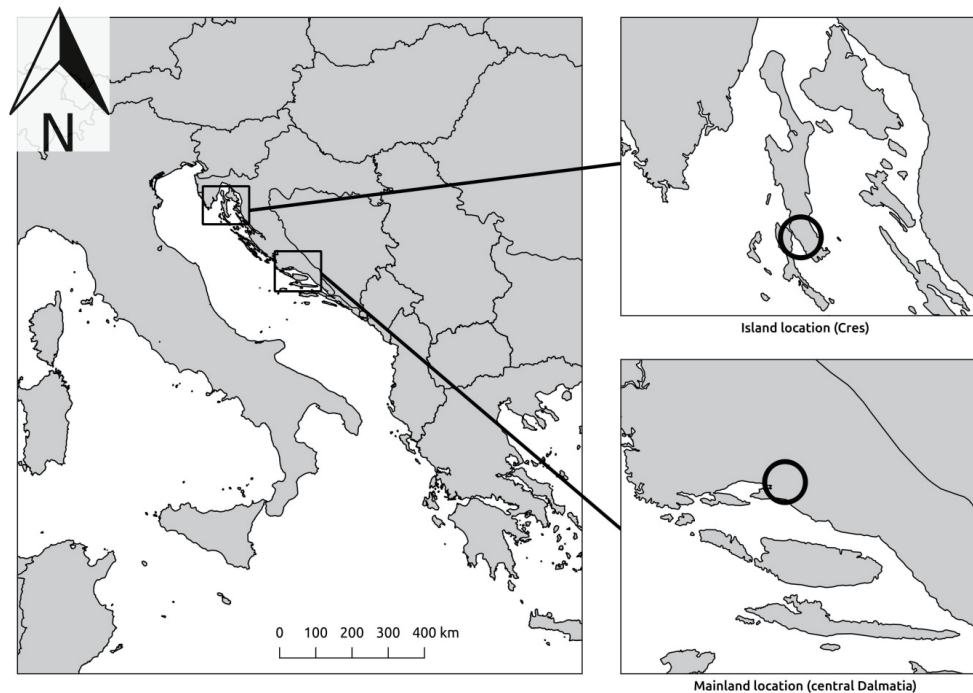


Figure 1 Geographical location of mainland (Klis, Croatia) and island (Cres) populations included in study
 Enlarged locations on right are not drawn to scale.

Data collection

A pilot study carried out in 2011 at both sites showed that *P. apodus* is most abundant during spring. Consequently, this study was performed from mid-April to the end of June over a period of two years at both sites (2011, 2014), as well as in 2012 at the mainland (Klis) site. Each site (mainland and island) was checked for the presence of animals over five consecutive days. Surveys were conducted all day, from sunrise to late dusk. For each animal located, the following morphological parameters were recorded using a hand-held meter with a precision of 1 mm, rope, pair of calipers with a precision of 0.01 mm, and 600 g Pesola spring scale with a precision of 5 g: total body length (TL), snout-vent length (SVL), body width near head (BWH—thinnest part of body after head), body width at mid-body (BWM—widest part at mid-body region), body width near cloaca (BWC—body width at cloaca), tail length (TLL), body mass (BM), head length (HL), head width (HW), head height (HH), mouth length (ML), mouth width (MW), interorbital distance (IOD), and internarial distance (IND) (see Figure 2A for head dimension measurements). Based on these data, we calculated the HL/HW ratio, which is often used to differ between subspecies (Obst, 1978). All captured animals were marked to avoid duplicating the results. If the same animal was re-captured, only the environmental data were recorded. In some specimens, the tail was partially missing, and this condition was also recorded. All applicable international, national, and/or institutional guidelines for the care and use of animals were strictly followed. All animal sample collection protocols

complied with the current laws of the Republic of Croatia.

We recorded several physical characteristics of the environment at the site of capture: i.e., air temperature, soil temperature, air humidity, air movement, and air pressure, recorded using a Kestrel 4000 Pocket Weather Tracker (precision of 0.1 °C, wind speed 3% of reading, relative humidity (RH) 3%, and barometric pressure 1.5 hPa) and a K-type Thermocouple (Appa Technology Corp., Taiwan, China, precision of 0.1 °C). In addition, we measured six microhabitat characteristics at each site of individual animal capture: (a) microhabitat type (seven subtypes: (1) open meadow with grass less than 10 cm in height—usually pasture or mowed meadow; (2) open meadow with grass more than 10 cm in height—usually untended meadow; (3) ground, surrounded by bushes; (4) bush; (5) under object—e.g., log, branch, stone; (6) open stonewall—typical stonewall that allows animal to pass through its crevices from one side to the other; and (7) closed stonewall—stonewall bordered on one side with earth or rocks, animal can enter only from one side and hide deep inside crevices); (b) substrate type (five subtypes: (1) dry vegetation; (2) earth with grass; (3) earth with rocks; (4) earth; and (5) stone); (c) nearest hiding place (five subtypes: (1) pile of rocks, under stones or in stonewall; (2) stones covered with bushes; (3) under log, branch, or manmade debris; (4) under dense bush; and (5) dense grass or herbaceous plants); (d) approximate distance of animal to hiding place (measured in cm, from the central point of animal's body); (e) vegetation cover (measured as approximative percentage of predefined subtype in a radius of 5 m around each capture site: (1) total

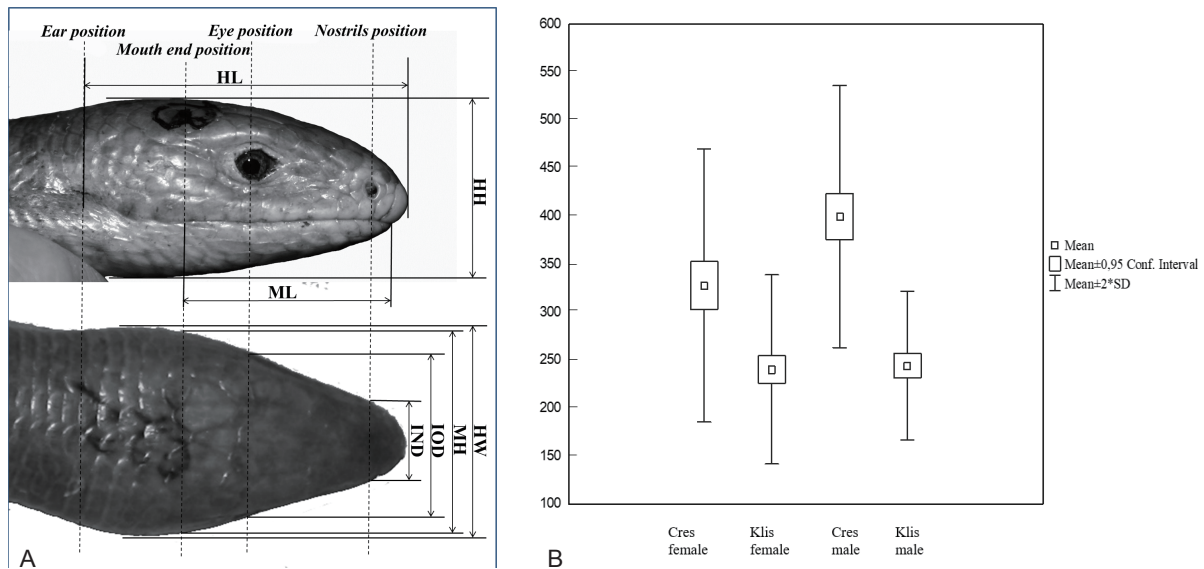


Figure 2 Head dimension measurements of *P. apodus* (A) and scaled mass index (SMI) values for Cres Island and Klis shown separately for each sex (B)

HL: Head length; HW: Head width; HH: Head height; ML: Mouth length; MW: Mouth width; IOD: Interorbital distance; IND: Internarial distance.

cover of vegetation; (2) short vegetation up to 10 cm; (3) herbaceous vegetation up to 50 cm; (4) shrubby vegetation; and (5) tree vegetation); and (f) other habitat characteristics besides vegetation (measured as approximate percentage of predefined subtype in a radius of 5 m around each capture site: (1) stone walls; (2) soil; (3) gravel/small stones; (4) concrete; and (5) rocks and stones). Hiding places were defined as any place where the animal can hide and not to be visible by a predator such as a fox or buzzard, e.g., hole in ground, crevice between rocks, under a log or boulder, or in dense vegetation. After measuring, the animals were released at the site of capture.

Scaled mass index (SMI) and residual analysis

General morphometric measurements (e.g., SVL, total length, head measurements) can be informative of the ecology of the species, and their variation can provide an indication of ecological or evolutionary processes, such as responses to predation or sexual selection pressure (Losos, 1990; Meiri, 2008). In that context, different indices are commonly used to summarize these data, including the SMI.

Body condition was estimated from mass/length data, and the SMI was calculated according to Peig & Green (2009). This index represents an improvement over previously used condition indices as it is based on the central principle of scaling, and includes many methodological, biological, and conceptual advantages (Peig & Green, 2009). Here, the calculation was carried out in three steps. First, to identify the extreme values, we plotted the recorded length (L) and mass (M) data of all individuals. Second, we calculated the scaling exponent b_{SMA} by standardized major axis regression on \ln -transformed data using online software (Bohonak & van der Linde, 2004). Third, we calculated the SMI using the formula:

$$\hat{M}_i = M_i \left[\frac{L_0}{L_i} \right]^{b_{SMA}} \quad (1)$$

where M_i and L_i are the body mass and total length of individual i , respectively, and L_0 is the arithmetic mean of L . The SMI was calculated separately for each site and sex.

Statistical analysis

All statistical analyses were carried out using Statistica software (TIBCO Software Inc, 2018). Due to their very low number (four), juveniles were excluded from the analyses. Morphometric data were analysed using multivariate analysis of covariance (MANCOVA), with site and sex as factors and SVL as the covariate. Subsequent univariate analysis of covariance (ANCOVA) was run for each variable separately. Values obtained for SMI were tested with a Kruskal-Wallis test (K-W Test). In addition, tail condition (intact vs. partial; autotomy rate) was analysed using log-linear model analysis to compare groups (sex and site). The HL/HW ratio was tested for normality and then used for t -test.

Ecological and physical habitat data (e.g., air temperature, soil temperature, air movement, relative humidity, air pressure, and distance to hiding place) and vegetation cover data were log transformed and then used for two separate factor analyses. Ecological and physical habitat data, as well as vegetation cover data, were checked for correlation. As no variables showed correlation (all values were between -0.7 and 0.7), none seemed to be redundant. Extracted factors for each dataset were analysed using MANOVA to test for site, sex, and interaction between site and sex. Data on the time of day were not included in the analyses as Mann-Whitney U -test showed no significant differences regarding when the animals were caught.

RESULTS

During the three years of study, we caught 164 animals in total, including 92 from Klis (49 females, 40 males, and three juveniles) and 72 from Cres Island (34 females, 37 males, and one juvenile). This species exhibited marked differences in coloration between juveniles and adults: juveniles were gray with black patches and spots, and had coarse scales, whereas adults were uniformly brown with smooth and shiny scales. As we did not catch animals intermediate in color/pattern, differentiation between juveniles and adults was considered straightforward.

Morphology

Individuals from Cres were generally larger than individuals from Klis, which was the case for all morphometric traits (Table 1). Morphometric data analysis showed an influence of site (MANCOVA; $F=9.20$, $P<0.05$) and sex (MANCOVA; $F=23.12$, $P<0.05$) on almost all examined variables. The only variables not influenced by sex were TLL (ANCOVA; $F=0.11$, $P=0.74$) and BWM (ANCOVA; $F=2.39$, $P=0.12$) and not influenced by site were TLL (ANCOVA; $F=2.05$, $P=0.16$) and IND (ANCOVA; $F=2.08$, $P=0.15$). Similarly, significant interactions between site and sex (MANCOVA; $F=2.18$, $P<0.05$) were detected, indicating that males and females differed in morphology depending on the site. Variables not

affected by this interaction were HH (ANCOVA; $F=3.59$, $P=0.06$), ML (ANCOVA; $F=2.88$, $P=0.09$), IOD (ANCOVA; $F=0.10$, $P=0.76$), BWM (ANCOVA; $F=1.64$, $P=0.20$), BWC (ANCOVA; $F=3.23$, $P=0.08$), and TLL (ANCOVA; $F=0.68$, $P=0.41$). All morphometric variables were influenced by SVL as a covariate (MANCOVA; $F=25.71$, $P<0.05$), except for TLL (ANCOVA; $F=0.89$, $P=0.35$). We excluded TL from this analysis as it was strongly correlated to TLL and SVL as a covariate. Log-linear model analysis of tail condition against site and sex showed that the interaction between site and tail condition, as well as site and sex, was not significant, but the interaction between tail condition and sex was highly significant (Table 2; $df=1$; $P=0.001$). The HL/HW ratio (Table 3) showed no differences between the sites, but was slightly lower in males than in females, though not significantly ($P>0.05$).

The SMI differed significantly between two populations, with the Cres population having higher values (K-W Test; $P<0.05$), indicating that the animals from Cres were larger than those from Klis. A within-population comparison of Cres individuals showed that males had higher SMI values (K-W Test; $P<0.05$) than females. The individuals from Klis, however, did not show such differences between the sexes (K-W Test; $P>0.05$; Table 4, Figure 2B).

Table 1 Means±SD, including range (in parenthesis), and MANCOVA results for morphometric measurements at each site

	Cres		Klis		MANCOVA site×sex	MANCOVA sex	MANCOVA site
	F (n=34)	M (n=37)	F (n=49)	M (n=40)			
TL (cm)	89.01±5.36 (79.10–98.00)	91.80±5.95 (78.50–107.40)	84.07±6.95 (55.60–95.7)	82.96±6.78 (64.50–97.60)	–	–	–
SVL (cm)	34.99±2.60 (30.50–40.50)	37.34±2.54 (31.30–41.9)	32.74±2.85 (24.00–39.90)	32.87±2.11 (28.30–36.50)	–	–	–
HL (mm)	40.45±4.40 (25.77–52.30)	48.58±5.73 (36.32–69.62)	37.44±3.67 (26.32–43.37)	41.15±3.47 (34.97–49.83)	$P<0.05$	$P<0.05$	$P<0.05$
HW (mm)	22.40±1.91 (19.65–29.82)	27.23±2.89 (19.50–31.20)	20.27±1.33 (16.39–23.03)	27.23±2.89 (18.59–27.14)	$P<0.05$	$P<0.05$	$P<0.05$
HH (mm)	21.34±1.89 (18.99–27.72)	25.76±2.87 (19.87–33.57)	20.27±1.68 (16.54–24.26)	22.65±1.81 (18.66–26.38)	n.s.	$P<0.05$	$P<0.05$
ML (mm)	20.53±3.11 (17.18–29.71)	24.93±3.51 (20.63–38.29)	18.16±1.96 (13.71–23.20)	20.17±2.05 (15.70–25.51)	n.s.	$P<0.05$	$P<0.05$
MW (mm)	18.75±1.92 (16.03–25.81)	22.37±1.98 (17.64–25.24)	17.24±1.66 (13.73–20.82)	18.67±1.72 (15.21–22.70)	$P<0.05$	$P<0.05$	$P<0.05$
IOD (mm)	15.03±2.21 (7.86–19.52)	17.00±2.40 (8.86–21.66)	13.49±1.26 (11.36–16.78)	14.58±1.31 (10.74–17.45)	n.s.	$P<0.05$	$P<0.05$
IND (mm)	6.81±0.68 (5.80–8.37)	8.03±0.88 (6.10–9.34)	6.59±0.55 (5.25–7.61)	7.02±0.61 (5.71–8.33)	$P<0.05$	$P<0.05$	n.s.
BWH (mm)	21.36±1.83 (18.47–25.43)	24.48±2.69 (18.18–29.96)	19.29±1.86 (16.12–25.11)	20.30±1.90 (16.72–26.95)	$P<0.05$	$P<0.05$	$P<0.05$
BWM (mm)	26.78±3.17 (20.96–32.38)	29.40±3.91 (22.61–36.65)	23.44±3.35 (18.24–31.44)	23.64±2.17 (18.76–30.2)	n.s.	n.s.	$P<0.05$
BWC (mm)	22.14±1.76 (19.35–28.39)	24.13±1.88 (20.20–29.48)	19.56±1.52 (16.16–23.44)	19.95±1.11 (17.40–22.86)	n.s.	$P<0.05$	$P<0.05$
TLL (cm)	52.99±4.68 (44.10–59.70)	52.11±6.58 (19.90–57.50)	49.55±9.43 (0.70–60.50)	50.00±5.96 (33.40–63.80)	n.s.	n.s.	n.s.
BM (g)	325.29±63.87 (220–490)	404.11±79.54 (220–552)	240.45±57.24 (105–400)	243.48±36.09 (150–315)	$P<0.05$	$P<0.05$	$P<0.05$

F: Female; M: Male. n: Number of individuals. TL: Total length; SVL (co-variate): Snout-vent length; HL: Head length; HW: Head width; HH: Head height; ML: Mouth length; MW: Mouth width; IOD: Interorbital distance; IND: Internarial distance; BWH: Body width near head; BWM: Body width at mid-body; BWC: Body width near cloaca; TLL: Tail length; BM: Body mass. –: Not available; n.s.: Not significant.

Table 2 Number of individuals with intact and autotomized tails at both sites and for both sexes

	Klis		Cres	
	M	F	M	F
Original	9	17	6	20
Autotomized	31	32	31	14

In both populations, females had significantly more often intact tails than males ($P < 0.05$). F: Female; M: Male.

Table 3 Means \pm SD of head length (HL) to head width (HW) ratios (HL/HW) for studied populations

Site	M	F
Klis	1.81 \pm 0.14	1.85 \pm 0.15
Cres	1.78 \pm 0.19	1.82 \pm 0.14

M: Male; F: Female. *t*-test showed no significant differences between sexes or between sites. ($P > 0.05$).

Table 4 Scaled mass index (SMI)

Locality	<i>n</i>	Sex	b_{SMA}	95 % CI	L_0	SMI
Cres	34	F	3.24	2.20, 4.2889.01		327.98
	34	M	3.89	2.85, 4.9392.78		398.69
Klis	48	F	3.59	2.84, 4.3484.66		239.38
	39	M	2.08	1.49, 2.6683.43		243.94

n: Number of individuals; F: Female; M: Male; b_{SMA} : Scaling exponent; L_0 : Arithmetic mean of *L* (individual total length).

Ecological variables

Mean recorded values of physical habitat data showed slight differences between the two sites, with Cres Island having lower average recorded temperature and humidity but higher air pressure than Klis (Table 5). Factor analysis of ecological and physical habitat data yielded three factors that explained 71.52% of the variability. Air and soil temperature showed significant factor loadings for factor 1 (0.81 and 0.84), relative humidity and air pressure for factor 2 (−0.74 and 0.77), and distance to hiding place for factor 3 (−0.77). MANOVA of the extracted factors showed differences between the two sites ($F = 31.40$, $P < 0.05$, Partial $\eta^2 = 0.49$), but not between the sexes ($F = 0.82$, $P = 0.49$, Partial $\eta^2 = 0.03$) nor the interaction between sex and site ($F = 0.42$, $P = 0.74$, Partial $\eta^2 = 0.02$).

Total vegetation, short vegetation up to 10 cm, herbaceous vegetation up to 50 cm, and tree vegetation were more abundant in Klis, whereas shrubby vegetation was more prominent on Cres. Factor analysis of the variables describing vegetation cover yielded two factors that explained 67.97% of the variability. Total and herbaceous vegetation showed significant factor loadings for factor 1 (0.91 and 0.78) and short vegetation up to 10 cm showed significant factor loadings for factor 2 (0.80). MANOVA of extracted factors showed differences between the two sites ($F = 18.98$, $P < 0.05$, Partial $\eta^2 = 0.20$), but no differences between the sexes ($F = 1.12$, $P = 0.33$, Partial $\eta^2 = 0.01$) nor the interaction between sex and site ($F = 1.55$, $P = 0.22$, Partial $\eta^2 = 0.02$).

Table 5 Measured parameters of physical and ecological habitat data with significant factor loadings from a factor analysis expressed as means \pm SD

Variable	Cres	Klis
Recorded air temperature (°C)	21.6 \pm 4.3	24.3 \pm 5.5
Annual air temperature (°C)	15.3*	16.1*
Recorded soil temperature (°C)	19.4 \pm 4.0	19.5 \pm 2.5
Air movement	2.1 \pm 2.2	2.1 \pm 1.9
Relative air humidity at capture site (%)	43.0 \pm 10.5	56.1 \pm 13.5
Annual air humidity (%)	70.4*	58.1*
Recorded air pressure (hPa)	1 010.7 \pm 4.0	999.9 \pm 6.3
Annual air pressure (hPa)	1 009.6*	1 000.4*
Distance to hiding place	223.8 \pm 319.4	226.6 \pm 318.1

*: Taken from Meteorological and Hydrological Service of Croatia (Zaninović et al., 2008); standard deviation (SD) data not available.

DISCUSSION

In our study, we compared the morphology and ecology of two populations of European glass lizard, one insular (Cres) and one from the mainland (Klis). The ecological and physical habitat data showed that the studied locations differed in the amount of vegetation, with less vegetation being present in the Cres site. Vegetation cover is an important variable that influences *P. apodus* distribution within the habitat, with individual distribution differing between different vegetation types (Telenchev et al., 2017). However, to truly differentiate to what extent our data represent true habitat or transitional habitat, we still lacked detailed information on species vagility and habitat use. Currently available data on vagility of *P. apodus*, based on a single research, show that recaptured individuals were found the furthest 5 m away from the site of their previous capture, suggesting very low mobility in this species (Telenchev et al., 2017).

In addition, two of the measured physical parameters, i.e., air temperature and relative humidity, were higher on Klis (Table 5). Interestingly, data from the Meteorological and Hydrological Service of Croatia for both areas showed only slight differences in annual temperature (less than 1 °C between locations) but larger differences in annual relative humidity. The annual humidity data correspond well to our mainland location data, but the island location showed almost two times higher values compared to our data. This finding indicates that humidity may be a parameter that makes a difference between the island and mainland locations. Yet, some parameters were strikingly similar at both sites, e.g., soil temperature (which corresponds to the temperature experienced by the animal when it is hiding underground) did not differ between sites. Humidity can directly affect vegetative growth, but in our case less vegetation was present at the island site. Another important difference between the sites was the macro- and meso-faunal composition. Cres lacks many of the predators present in the Klis area (e.g., medium-sized predators like foxes or badgers). The lack of larger predators could be connected to vegetation cover, i.e., wild

(deer) and domestic (sheep) grazers are present all over Cres, thus limiting vegetative growth (Dumančić, 1992; Sušić & Radek, 2007). The absence or removal of top predators can lead to simplified or degraded habitats, which impact lower trophic levels (Estes et al., 2001; Ripple & Beschta, 2004; Soulé et al., 2003), and might be the case on Cres. However, according to our results, the detected vegetation and humidity differences between the two selected habitats did not directly influence the presence of either sex or how they use the habitat (for ecological parameters see section Materials and methods–Data collection). In addition, during preliminary study, we observed no animals during mid-summer at either site, consistent with the fact that they are most active at temperatures between 22 °C and 31 °C (Telenchev et al., 2015) and estivate under higher air temperatures (Abe, 1995; Buhlmann, 1995; Storey, 2001). Overall, the studied populations did not differ significantly in the general ecological parameters recorded.

The most influenced phenotypic character, dependent on habitat and important from an evolutionary perspective, is body size (Werner & Gilliam, 1984). It is also the most important trait in niche determination (Blanckenhorn, 2000; Janzen, 1993; Wikelski, 2005). In general, both of our studied populations could be regarded as morphologically small, as all animals measured were smaller than those reported in the original description of *P. a. thracicus* (where numerous individuals were recorded with a SVL greater than 400; Obst, 1978). In addition, the HL/HW ratios in our populations were slightly lower than the average from the original description (1.82), although they fell within the range (1.72–2.12, Obst, 1978; Table 3).

Comparative analysis of morphological data showed strong discrimination between the two study populations. Animals from Cres were larger than those from Klis. The higher SMI values for the Cres animals also suggest better physical fitness (Cooper et al., 2009). The smaller body size observed in Klis may indicate stronger predation pressure, as many predators are absent from Cres Island (e.g., foxes and badgers, Sušić & Radek, 2007). This predation risk may influence not only survival, but also the size of the animals. In addition, predation pressure can influence population age structure, i.e., a higher number of older individuals being captured by predators can decrease the overall size of individuals as size in reptiles is commonly dependent on age (Tomašević Kolarov et al., 2010).

Another possible explanation for the larger body size could be the abundance of potential prey items. *Pseudopus apodus* is a generalist, although arthropod and mollusc prey are dominant, with some vertebrates (Rifai et al., 2005). Rifai et al. (2005) showed dominance of different prey items at different localities (e.g., orthopterans in Jordan, coleopterans in Caucasus, molluscs in Crimea). On the other hand, food abundance is often higher on large islands, which may influence not only body size, but also species density (Pafilis et al., 2009).

On Cres, the lack of predators and possibly higher food

abundance, may have influenced the size of the studied individuals, which could be a plastic trait. However, this must be confirmed experimentally, as some traits show more plasticity (e.g., hindlimb length in *Anolis sagrei*, Losos et al., 2000), whereas others are more conservative (e.g., dry mass growth rate in *Sceloporus graciosus*, Sears & Angilletta, 2003).

Apart from the size differences between populations, we also detected intersexual size differences within the Cres population, with males being significantly larger than females. In both populations, males had larger heads than females, but this difference was more prominent in Cres. Larger heads imply bigger muscles and greater bite force (Sagonas et al., 2014; Santana et al., 2010), which may evolve for a number of reasons (Christiansen & Wroe, 2007; Dewar et al., 2015; Dollion et al., 2017; Jones & Ruff, 2011; Vincent & Herrel, 2007). A greater bite force can allow an animal to extend its food niche, enabling it to feed on larger or harder prey items (Herrel et al., 2001; Verwajen et al., 2002; Wyckmans et al., 2007) or to eat different types of food, such as plants (Herrel et al., 2004a, 2004b, 2008; Sagonas et al., 2014). The larger head (accompanied with larger body size) in *P. apodus* is crucial for mating success, as male to male combat has been observed in this species (Jablonski, 2018) and individuals that can inflict stronger bites are more likely to win such contests. Therefore, larger individuals may be more successful in mating, which could, through intrasexual selection, result in the increased size of males in the population. Intersexual selection may also play a role, with females preferring larger individuals as a sign of better general fitness. This pronounced sexual dimorphism in morphology was observed in all morphometric variables in the Cres population, which was characterized by larger, more robust males compared to females.

However, there was an exception, with TLL being slightly longer in females than in males. This was the only morphometric variable that was not influenced by sex, location, or by the interaction between site and sex. This finding is surprising, as all other parameters indicated that the animals from Cres were generally larger. Log-model analysis confirmed this difference and clearly showed that males suffer more often from autotomy than females (see Table 2). Tail autotomy is directly connected to predator evasion strategies in lizards (Higham et al., 2013). This suggests that the observed TLL values may be dependent on increased predation and that males are more active and exposed to predation than females. Intrasexual selection (e.g., male-male fights) is unlikely to have a significant influence on the occurrence of autotomy, as observations on male to male combat have shown no bites to the tail region (Jablonski, 2018). This is an interesting finding as previous study suggests that *P. apodus* lizards rarely autotomize their tails (Obst, 1981).

The general differences between populations observed in this paper could also be a consequence of the island effect (e.g., Foster's rule). On islands that lack predators, lizards

tend to increase their body size in order to reduce intraspecific competition (Case, 1978; Meiri, 2008). In addition, selection on islands with reduced predation risk may favor larger males, resulting in larger body size in future generations (Case, 1978). This may partially explain the phenomenon we observed, as *P. apodus* may be very aggressive towards conspecifics during the breeding period (Lisičić pers. observ.; Jablonski, 2018). However, detailed studies on territoriality and sexual selection in this species are lacking.

The evolutionary background of the detected intrapopulation differences is speculative. Even though *P. apodus* is common on many islands along the eastern Adriatic coast, information on its dispersal history is limited. Island populations of *P. apodus* are probably remnants from the last Ice Age when most islands in this region were connected to the mainland, some 10 000–15 000 years ago, as was the case with the herpetofauna on other Mediterranean islands (Corti et al., 1999). Previous experiments on the reciprocal introduction of the Italian wall lizard (*Podarcis siculus*) on two Adriatic islets have shown that even for genetically identical populations, 35 years of separation can result in profound morphological, behavioural, and physiological differences (Herrel et al., 2008). These differences confirm the plasticity of this species in terms of dietary shift, which can be observed in skull dimensions, dentition, gut morphology, and digestive performance (Herrel et al., 2008; Vervust et al., 2010).

Detailed genetic data on our studied populations are not available, so we can only speculate to what extent these differences result from environmental and/or genetic variability. The only genetic data on these populations refer to the subspecies (Jandzik et al., 2018), which show very low genetic diversity within the whole range of the *P. a. thracicus* subspecies, both at the nuclear and mitochondrial level. It has also been suggested that *P. apodus* has lower genetic plasticity than the genus *Anguis*, possibly explaining its much narrower distribution (Jandzik et al., 2018).

Genetic data that can account for the evolutionary history in some lizard species, e.g., genetic data of *Podarcis erhardii* in Greece suggests that spatial fragmentation contributes more to genetic variation than geographic proximity (Hurston et al., 2009), should be taken with caution as *P. apodus* obviously differs in this aspect from other European lizards. Certainly, detailed genetical analyses would contribute to a better understanding of the underlying causes of the observed plasticity.

Regardless of the causal mechanism, morphological differences between the two studied populations were evident. Here, we showed that *P. apodus* exhibited morphological differences between two geographically close populations inhabiting the same biogeographical zone. Specifically, not only did we observe an increase in body size but also clear sexual dimorphism in the island population compared with the mainland population. This indicates that phenotypic plasticity occurs, even on a small geographical scale, in *P. apodus*. However, more studies on different populations of *P. apodus* are needed to confirm the level of phenotypic plasticity on a

wider scale and to put it in a broader ecological context.

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Fieldwork in both localities in Croatia was partly supported through grant 119-0000000-1285 of the Ministry of Science of the Republic of Croatia. Permission for the field survey was issued by Department of Nature Protection, Ministry of Culture of the Republic of Croatia (permit No.: UP/I-612-07/10-33/762, 532-08-01-01/3-10-02 issued 29 June 2010), and by the Ministry of Environmental and Nature Protection of the Republic of Croatia (permit No.: UP/I-612-07/14/48/77, 517-07-1-1-1-14-4 issued 04 June 2014).

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

D.L. and Z.T. designed the study and revised the manuscript. O.J.G. performed the analyses and wrote the manuscript. D.L., P.P., V.L., and L.D. conducted the fieldwork. All authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

We thank Herrel A. for reading the manuscript and for suggestions to improve it. We also want to thank P.L. and S.L. for unlimited logistical support during field work.

REFERENCES

- Abe AS. 1995. Estivation in South American amphibians and reptiles. *Brazilian Journal of Medical and Biological Research*, **28**(11–12): 1241–1247.
- Alekperov AM. 1978. Amphibians and reptiles of Azerbaijan. Elm, Baku.
- Arnold EN. 2002. Reptiles and Amphibians of Europe. 2nd ed. Princeton: Princeton University Press.
- Aubret F. 2015. Island colonisation and the evolutionary rates of body size in insular neonate snakes. *Heredity*, **115**(4): 349–356.
- Bakaloudis DE, Vlachos CG, Holloway GJ. 1998. Habitat use by short-toed eagles *Circaetus gallicus* and their reptilian prey during the breeding season in Dadia Forest (north-eastern Greece). *Journal of Applied Ecology*, **35**(6): 821–828.
- Blanckenhorn WU. 2000. The evolution of body size: what keeps organisms small?. *The Quarterly Review of Biology*, **75**(4): 385–407.
- Bogdanov OP. 1986. Presmikayushchiesya Srednei Azii. Tashkent, Uzbekistan, Ukituvchi. (in Russian)
- Bohonak AJ, van der Linde K. 2004(2016-03-28). RMA: software for reduced major axis regression, Java version. <http://www.kimvdlinde.com/professional/rma.html>.
- Buhlmann KA. 1995. Habitat use, terrestrial movements, and conservation of the turtle, *Deirochelys reticularia* in Virginia. *Journal of Herpetology*, **29**(2): 173–181.
- Carretero M, Znari M, Macé J, Harris DJ. 2005. Morphological divergence among populations of *Testudo graeca* from west-central Morocco. *Animal*

- Biology*, **55**(3): 259–279.
- Case TJ. 1978. A general explanation for insular body size trends in terrestrial vertebrates. *Ecology*, **59**(1): 1–18.
- Christiansen S, Wroe S. 2007. Bite forces and evolutionary adaptations to feeding ecology in carnivores. *Ecology*, **88**(2): 347–358.
- Çiçek K, Tok CV, Hayrettaş S, Ayaz D. 2014. Data on the food composition of European glass lizard, *Pseudopus apodus* (Pallas, 1775) (Squamata: Anguillidae) from Canakkale (Western Anatolia, Turkey). *Acta Zoologica Bulgarica*, **66**(3): 433–436.
- Cooper Jr W, Pérez-Mellado V, Hawlena D. 2009. Morphological traits affect escape behaviour of the Balearic lizards (*Podarcis lilfordi*). *Amphibia-Reptilia*, **30**(4): 587–592.
- Corti C, Masseti M, Delfino M, Pérez-Mellado V. 1999. Man and herpetofauna of the Mediterranean islands. *Revista Española de Herpetología*, **13**: 83–100.
- Dewar EW, Crocker CA, Bauchiero AV, Livingstone JP. 2015. Is skull shape related to aggressive displays in Pinnipeds?. *FASEB Journal*, **29**(S1): 696–698.
- Dollion AY, Measey GJ, Cornette R, Carne L, Tolley KA, da Silva JM, Boistel R, Fabre AC, Herrel A. 2017. Does diet drive the evolution of head shape and bite force in chameleons of the genus *Bradypodion*?. *Functional Ecology*, **31**(3): 671–684.
- Dumančić D. 1992. Improvement of lawn production in Mediterranean as a precondition for sheep and goat farming. *Agronomski Glasnik*, **1–88**: 105–111.
- Estes J, Crooks K, Holt RD. 2001. Predators, ecological role of. In: Levin SA. Encyclopedia of Biodiversity. San Diego: Academic Press, 857–878.
- Foster JB. 1964. Evolution of mammals on islands. *Nature*, **202**(4929): 234–235.
- Georgiev D, Raichev E. 2009. A record of Horned viper *Vipera ammodytes* (L.) in the diet of the Stone marten *Martes foina* (Erxl.) (Mammalia: Mustelidae) in Bulgaria. *ZooNotes*, **5**: 1–2.
- Herrel A, Van Damme R, Vanhooydonck B, De Vree F. 2001. The implications of bite performance for diet in two species of lacertid lizards. *Canadian Journal of Zoology*, **79**(4): 662–670.
- Herrel A, Vanhooydonck B, Van Damme R. 2004a. Omnivory in lacertid lizards: adaptive evolution or constraint?. *Journal of Evolutionary Biology*, **17**(5): 974–984.
- Herrel A, Vanhooydonck B, Joachim R, Irschick DJ. 2004b. Frugivory in polychrotid lizards: effects of body size. *Oecologia*, **140**(1): 160–168.
- Herrel A, Huyghe K, Vanhooydonck B, Backeljau T, Breugelmans K, Grbac I, Van Damme R, Irschick DJ. 2008. Rapid large-scale evolutionary divergence in morphology and performance associated with exploitation of a different dietary resource. *Proceedings of the National Academy of Sciences of the United States of America*, **105**(12): 4792–4795.
- Higham TE, Russell AP, Zani PA. 2013. Integrative biology of tail autotomy in lizards. *Physiological and Biochemical Zoology*, **86**(6): 603–610.
- Horvatić S. 1963. Biogeographical position and articulation of our coast in the light of modern phytocenological research. *Acta Botanica Croatica*, **22**: 27–81.
- Hurston H, Voith L, Bonanno J, Fougopoulos J, Pafilis P, Valakos E, Anthony N. 2009. Effects of fragmentation on genetic diversity in island populations of the Aegean wall lizard *Podarcis erhardii* (Lacertidae, Reptilia). *Molecular Phylogenetics and Evolution*, **52**(2): 395–405.
- Itescu Y, Schwarz R, Donihue CM, Slavenko A, Roussos SA, Sagonas K, Valakos ED, Fougopoulos J, Pafilis P, Meiri S. 2018. Inconsistent patterns of body size evolution in co-occurring island reptiles. *Journal of Biogeography*, **27**(5): 538–550.
- Jablonski D. 2018. Male-male combat in *Pseudopus apodus* (Reptilia: Anguillidae). *Russian Journal of Herpetology*, **25**(4): 293–298.
- Jandzik D, Jablonski D, Zinenko O, Kukushkin OV, Moravec J, Gvozdík V. 2018. Pleistocene extinctions and recent expansions in an anguid lizard of the genus *Pseudopus*. *Zoologica Scripta*, **47**(1): 21–32.
- Janzen FJ. 1993. An experimental analysis of natural selection on body size of hatchling turtles. *Ecology*, **74**(2): 332–341.
- Jones KE, Ruff CB. 2011. Male-male combat drives bite force evolution in the absence of mastication. *FASEB Journal*, **25**(1): 867.
- Kukushkin OV, Dovgal IV. 2018. Sexual dimorphism in *Pseudopus apodus* (Reptilia: Sauria: Anguillidae) from the Steppe Crimea. *Ecologica Montenegrina*, **19**: 1–21.
- Lomolino MV. 2005. Body size evolution in insular vertebrates: generality of the island rule. *Journal of Biogeography*, **32**(10): 1683–1699.
- Losos JB. 1990. Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecological Monographs*, **60**(3): 369–388.
- Losos JB, Creer DA, Glossip D, Goellner R, Hampton A, Roberts G, Haskell N, Taylor P, Ettling J. 2000. Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*. *Evolution*, **54**(1): 301–305.
- Meiri S. 2008. Evolution and ecology of lizard body sizes. *Global Ecology and Biogeography*, **17**(6): 724–734.
- Nowak RM. 1991. Walker's Mammals of the World. 5th ed. Baltimore: The Johns Hopkins University Press.
- Obst FJ. 1978. On geographical variability of Scheltopusik, *Ophisaurus apodus* (Pallas). *Zoologische Abhandlungen Staatliches Museum für Tierkunde in Dresden*, **35**: 129–140.
- Obst FJ. 1981. *Ophisaurus apodus* (Pallas, 1775)-Scheltopusik. In: Handbuch der Reptilien und Amphibien Europas, vol. 1. Echsen (Sauria). Akademische Verlags gesellschaft, Wiesbaden, 259–274. (in German)
- Owens IP, Scott SN, Robinson SI, Clegg SM, Kikkawa J. 2006. Large body size in island-dwelling passerines: the roles of insular specialization, niche expansion and ecological release. *Acta Zoologica Sinica*, **52**(S1): 262–266.
- Pafilis P, Meiri S, Fougopoulos J, Valakos E. 2009. Intraspecific competition and high food availability are associated with insular gigantism in a lizard. *Naturwissenschaften*, **96**(9): 1107–1113.
- Palkovacs EP. 2003. Explaining adaptive shifts in body size on islands: a life history approach. *Oikos*, **103**(1): 37–44.
- Peig J, Green AJ. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos*, **118**(12): 1883–1891.
- Rifai L, Baker MA, Al Shafei D, Disi AM, Manhasneh A, Amr Z. 2005. *Pseudopus apodus* (Pallas, 1775) from Jordan, with notes on its ecology (Squamata: Sauria: Anguillidae). *Herpetozoa*, **18**(3–4): 133–140.
- Ripple WJ, Beschta RL. 2004. Wolves and the ecology of fear: can predation risk structure ecosystems?. *BioScience*, **54**(8): 755–766.
- Sagonas K, Pafilis P, Lymberakis P, Donihue CM, Herrel A, Valakos ED. 2014. Insularity affects head morphology, bite force and diet in a Mediterranean lizard. *Biological Journal of the Linnean Society*, **112**(3):

469–484.

- Santana SE, Dumont ER, Davis JL. 2010. Mechanics of bite force production and its relationship to diet in bats. *Functional Ecology*, **24**(4): 776–784.
- Sears MW, Angilletta Jr MJ. 2003. Life-history variation in the sagebrush lizard: phenotypic plasticity or local adaptation?. *Ecology*, **84**(6): 1624–1634.
- Shcherbak NN. 1966. Amphibians and Reptiles of the Crimea (Herpetologia Taurica). Kiev: Naukova Dumka. (in Russian).
- Soulé ME, Estes JA, Berger J, del Rio CM. 2003. Ecological effectiveness: conservation goals for interactive species. *Conservation Biology*, **17**(5): 1238–1250.
- Storey KB. 2001. Turning down the fires of life: Metabolic regulation of hibernation and estivation. In: Storey KB. Molecular Mechanisms of Metabolic Arrest. Oxford: BIOS Scientific Publishers, 1–21.
- Sušić G, Radek V. 2007. Biodiversity through ponds of Cres Island. Istraživačko-edukacijski centar za zaštitu prirode Eko Centar Insulae, Beli. (in Croatian)
- Telenchev I, Simeonovska-Nikolova D, Natchev N, Tzankov N. 2015. A preliminary study on the habitat selection of European glass lizard (*Pseudopus apodus*) in southeast Bulgaria. In: First National Conference of Biotechnology. Sofia, 2014.
- Telenchev I, Simeonovska-Nikolova D, Tzonev R. 2017. Habitat use and activity of European glass lizard, *Pseudopus apodus* (Pallas, 1775), in southeastern Bulgaria. *Turkish Journal of Zoology*, **41**(2): 286–293.
- Thomas R, Hedges SB. 1998. New anguid lizard (*Diploglossus*) from Cuba. *Copeia*, **1998**(1): 97–103.
- TIBCO Software Inc. 2018. Statistica (data analysis software system), version 13. <http://tibco.com>.
- Tomašević Kolarov N, Ljubisavljević K, Polović L, Džukić G, Kalezić ML. 2010. The body size, age structure and growth pattern of the endemic Balkan Mosor rock lizard (*Dinarolacerta mosorensis* Kolombatović, 1886). *Acta Zoologica Academiae Scientiarum Hungaricae*, **56**(1): 55–71.
- Topić J. 2001. Vegetation (Flora) of Croatia. In: Lovstvo JJ. Croatia: Strossmayer University of Osijek, 105–124. (in Croatian)
- Tutiš V, Kralj J, Radović D, Ćiković D, Barišić S. 2013. Red Data Book of Birds of Croatia. Republic of Croatia: Ministry of Environmental and Nature Protection, State Institute for Nature Protection.
- Vervust B, Pafilis P, Valakos ED, Van Damme R. 2010. Anatomical and physiological changes associated with a recent dietary shift in the lizard *Podarcis sicula*. *Physiological and Biochemical Zoology*, **83**(4): 632–642.
- Verwaijen D, Van Damme R, Herrel A. 2002. Relationships between head size, bite force, prey handling efficiency and diet in two sympatric lacertid lizards. *Functional Ecology*, **16**(6): 842–850.
- Vincent SE, Herrel A. 2007. Functional and ecological correlates of ecologically-based dimorphisms in squamate reptiles. *Integrative and Comparative Biology*, **47**(2): 172–188.
- Werner EE, Gilliam JF. 1984. The ontogenetic niche and species interactions in size-structured populations. *Annual Review of Ecology and Systematics*, **15**: 393–425.
- Wikelski M. 2005. Evolution of body size in Galapagos marine iguanas. *Proceedings of the Royal Society B: Biological Sciences*, **272**(1576): 1985–1993.
- Wyckmans M, Van Wassenbergh S, Adriaens D, Van Damme R, Herrel A. 2007. Size-related changes in cranial morphology affect diet in the catfish *Clariallabes longicauda*. *Biological Journal of the Linnean Society*, **92**(2): 323–334.
- Zaninović K, Gajić-Čapka M, Perčec Tadić M, Vučetić M, Bajić A, Cindrić K, Cvitan L, Katušin Z, Kaučić D, Likso T, Lončar E, Lončar Ž, Mihajlović D, Pandžić K, Patarčić M, Srnc L, Vučetić V. 2008. Atlas of Croatia 1961–1990, 1971–2000. Zagreb: Meteorological and Hydrological Service of Croatia.