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

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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. thracius* from the western range, was recently confirmed by molecular data (Jandzik et al., 2018). However, several issues remain regarding the third, southern clade,
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. thracius (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. thracius 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 (Alekerov, 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., Quercus 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 (Circaetus gallicus; e.g., Bakaloudis et al., 1998), is present at both sites (Tutuš et al., 2013) and thus should not create different predation pressure between sites.

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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 1 Geographical location of mainland (Klis, Croatia) and island (Cres) populations included in study

Enlarged locations on right are not drawn to scale.
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 approximative 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 In-transformed data using online software (Bohonak & van der Linde, 2004). Third, we calculated the SMI using the formula:

$$Mi = M_i \left[ \frac{L_0}{L_i} \right]^{b_{SMA}}$$

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=0.10, P=0.76) and BWM (ANCOVA; F=3.29, P=0.12) and not influenced by site were TLL (ANCOVA; F=2.05, P=0.16) and IND (ANOVA; 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 (ANOVA; F=3.59, P<0.05), ML (ANOVA; F=2.88, P=0.09), IOD (ANOVA; F=0.10, P=0.76), BWM (ANOVA; F=1.64, P=0.20), BWC (ANOVA; F=3.23, P=0.08), and TLL (ANOVA; 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 (ANOVA; 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

<table>
<thead>
<tr>
<th></th>
<th>Cres</th>
<th>Klis</th>
<th>MANCOVA sex</th>
<th>MANCOVA site×sex</th>
<th>MANCOVA site</th>
<th>MANCOVA site×sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL (cm)</td>
<td>82.0±4.66</td>
<td>84.0±3.65</td>
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<tr>
<td></td>
<td>(79.10–84.20)</td>
<td>(78.50–85.50)</td>
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<tr>
<td>SVL (cm)</td>
<td>37.34±2.54</td>
<td>37.24±2.54</td>
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<tr>
<td></td>
<td>(35.30–41.10)</td>
<td>(35.00–41.00)</td>
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<tr>
<td>HL (mm)</td>
<td>22.04±1.91</td>
<td>22.02±1.91</td>
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<tr>
<td></td>
<td>(19.65–25.32)</td>
<td>(19.50–25.20)</td>
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<tr>
<td>HW (mm)</td>
<td>22.30±2.31</td>
<td>22.37±2.31</td>
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<tr>
<td></td>
<td>(19.65–25.32)</td>
<td>(19.50–25.20)</td>
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<tr>
<td>HH (mm)</td>
<td>21.34±1.89</td>
<td>21.34±1.89</td>
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<td></td>
<td>(18.99–27.72)</td>
<td>(18.97–27.72)</td>
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<tr>
<td>ML (mm)</td>
<td>18.75±1.92</td>
<td>18.75±1.92</td>
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<tr>
<td></td>
<td>(17.18–29.71)</td>
<td>(17.18–29.71)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>MW (mm)</td>
<td>18.75±1.92</td>
<td>18.75±1.92</td>
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<td></td>
<td>(17.18–29.71)</td>
<td>(17.18–29.71)</td>
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<tr>
<td>IOD (mm)</td>
<td>18.75±1.92</td>
<td>18.75±1.92</td>
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<td>(17.18–29.71)</td>
<td>(17.18–29.71)</td>
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<tr>
<td>IND (mm)</td>
<td>8.03±0.88</td>
<td>8.03±0.88</td>
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<tr>
<td></td>
<td>(5.80–8.37)</td>
<td>(5.80–8.37)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>BWH (mm)</td>
<td>25.80±2.55</td>
<td>25.80±2.55</td>
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<td>--</td>
<td>--</td>
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<td></td>
<td>(24.10–28.00)</td>
<td>(24.00–27.90)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BWM (mm)</td>
<td>22.14±1.88</td>
<td>22.14±1.88</td>
<td>--</td>
<td>--</td>
<td>--</td>
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</tr>
<tr>
<td></td>
<td>(19.35–26.89)</td>
<td>(19.35–26.89)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLL (cm)</td>
<td>325.29±63.87</td>
<td>325.29±63.87</td>
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<td>--</td>
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<tr>
<td></td>
<td>(220.490)</td>
<td>(220.490)</td>
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</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Tail condition</th>
<th>Kis</th>
<th>F</th>
<th>M</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>9</td>
<td>17</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>Autotomized</td>
<td>31</td>
<td>32</td>
<td>31</td>
<td>14</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Site</th>
<th>M</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kis</td>
<td>1.81±0.14</td>
<td>1.85±0.15</td>
</tr>
<tr>
<td>Cres</td>
<td>1.78±0.19</td>
<td>1.82±0.14</td>
</tr>
</tbody>
</table>

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

Table 4 Scaled mass index (SMI)

<table>
<thead>
<tr>
<th>Locality</th>
<th>n</th>
<th>Sex</th>
<th>b_{BMA}</th>
<th>95 % CI</th>
<th>L₀</th>
<th>SMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cres</td>
<td>34</td>
<td>F</td>
<td>3.24</td>
<td>2.20, 4.2889.01</td>
<td>327.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>M</td>
<td>3.89</td>
<td>2.85, 4.9392.78</td>
<td>396.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>F</td>
<td>3.59</td>
<td>2.84, 4.3484.66</td>
<td>239.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>M</td>
<td>2.08</td>
<td>1.49, 2.6683.43</td>
<td>243.94</td>
<td></td>
</tr>
</tbody>
</table>

n: Number of individuals; F: Female; M: Male; b_{BMA}: Scaling exponent; L₀: 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 Kis (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 η²=0.49), but not between the sexes (F=0.82, P=0.49, Partial η²=0.03) nor the interaction between sex and site (F=0.42, P=0.74, Partial η²=0.02).

Total vegetation, short vegetation up to 10 cm, herbaceous vegetation up to 50 cm, and tree vegetation were more abundant in Kis, 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 η²=0.20), but no differences between the sexes (F=1.12, P=0.33, Partial η²=0.01) nor the interaction between sex and site (F=1.55, P=0.22, Partial η²=0.02).

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Kis</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recorded air temperature (°C)</td>
<td>21±4.3</td>
<td>24±5.5</td>
</tr>
<tr>
<td>Annual air temperature (°C)</td>
<td>15.3</td>
<td>16.1</td>
</tr>
<tr>
<td>Recorded soil temperature (°C)</td>
<td>19.4±4.0</td>
<td>19.5±2.5</td>
</tr>
<tr>
<td>Air movement</td>
<td>2.1±2.2</td>
<td>2.1±1.9</td>
</tr>
<tr>
<td>Relative air humidity at capture site (%)</td>
<td>43.0±10.5</td>
<td>56.1±13.5</td>
</tr>
<tr>
<td>Annual air humidity (%)</td>
<td>70±4</td>
<td>58.1</td>
</tr>
<tr>
<td>Recorded air pressure (hPa)</td>
<td>1 010.7±4.0</td>
<td>999.9±6.3</td>
</tr>
<tr>
<td>Annual air pressure (hPa)</td>
<td>1 009.6±1</td>
<td>1 000.4±1</td>
</tr>
<tr>
<td>Distance to hiding place</td>
<td>223.8±319.4</td>
<td>226.6±318.1</td>
</tr>
</tbody>
</table>

*: 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 Kis (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 Kis 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 (Blankenhorn, 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. thracius* (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 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; Dillion 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; Verwaijen 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. thracius* 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**

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**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.

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