

Article

Seasonal Phenotypic Variation in the Aeolian Wall Lizard, *Podarcis raffonei*, of the Capo Grosso (Vulcano) Population

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Abstract: In recent decades, studies on animal coloration have expanded significantly, from understanding color production and perception mechanisms to exploring the selective pressures driving these patterns. Conservation biology has begun leveraging these studies to develop effective strategies, gauge public support for conservation projects, select flagship species, and determine reproductive pairs and optimal rearing and release conditions for captive breeding. Critically endangered Aeolian wall lizards (*Podarcis raffonei*) were housed at the Fondazione Bioparco di Roma for a pilot captive breeding project following alarming hypotheses of rising numbers of “green” hybrids (*P. raffonei* × *P. siculus*) in one of the four surviving populations. Through a quantitative analysis of visible dorsal color in captive and natural populations, we aim to determine whether *P. raffonei* is characterized by seasonal variation in its dorsal coloration and if color is influenced by sex and ontogenesis. Our findings show that *P. raffonei*’s dorsal pigmentation varies between seasons. In spring, the size and age of the animals does not seem to affect any color component, while the sexes always differ in their hue, with males having consistently more yellow-green dorsa. Between-year analyses, including measurements from the wild population, indicate that yearly variation is not limited to the captive population but also occurs in nature. We caution against identifying potential hybrids based solely on phenotypic traits.



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Keywords: coloration; seasonal variation; captive breeding; *Podarcis*; reptiles

1. Introduction

The last decades have seen exponential growth in studies of animal color, from the mechanisms of color production and perception to the selective pressures that drive the expression of these patterns [1]. The phenotypic diversity that color analysis provides us with, due to the quantification of what color means to various species’ visual systems, has made coloration a highly interdisciplinary field. Among these fields, conservation biology is beginning to find guides to the implementation of effective strategies in both basic and applied color studies. Questioning how species coloration can influence popularity and public support for conservation projects can help predict the success of such projects or lead to the selection of effective flagship species [2,3]. The coloration of ornamental traits can serve as an early indicator of exposure to several pollutants [4], or as an individual quality indicator guiding the selection of reproductive couples in captive breeding programs [5]. Understanding ontogenetic changes in coloration can in turn determine rearing and release conditions of captive-bred individuals to maximize their survival in nature [6]. In addition to breeding individuals to ensure their long-term survival, captive breeding also provides optimal and standardized conditions for analyzing the color over time of species often on the verge of extinction, for which it is not always possible to conduct studies under natural conditions. Among the critically endangered species kept in captivity, the Aeolian wall lizard, a small lacertid hosted for a conservation project at the Fondazione Bioparco di Roma, allowed us to study its color variation through time.

This lizard, endemic to the Aeolian archipelago, survives today on three tiny islets and a narrow promontory of the archipelago, while on the remaining islands and islets, only the Italian wall lizard, *P. siculus*, is found, which has been introduced by man to most parts of the archipelago [7]. On the island of Vulcano, the last of the main islands of the archipelago where *P. raffonei* is still present, the species was much more widespread in the 1990's when first described by Capula [8] and has since experienced a strong decline. All the while *P. siculus* has spread across the island [9]. The Italian wall lizard is an incredibly competitive and highly adaptable lacertid [10–12], which colonized numerous islands of the Mediterranean Sea, affecting the native biota [13–15]. The partially disjointed isthmus of the Capo Grosso promontory seems to be the last standing barrier to the encounter of the two species. *P. siculus* represents the biggest threat to the survival of the *P. raffonei* Capo Grosso population, not only through competition [16,17], but also through hybridization [18]. As documented by Capula [19], discrimination between the two sympatric species on Vulcano Island on a morphological level is difficult, and hybrids appear morphologically intermediate between the parent species. The wall lizards' genus has recently proved to be a great model for color investigation thanks to the incredible phenotypic variability that characterizes it. Dorsal color variation along habitat, climatic and altitudinal conditions, has been found in *P. hispanicus* and *P. liopelis*, and the latter, together with *P. siculus*, has been shown to experience color variation throughout seasons, with a greener dorsal pigmentation during the spring months [20–22]. Ficetola et al. [23] reported that after nearly two years from the first observation in September 2015, a growing portion of *P. raffonei* lizards on Capo Grosso were characterized by a green dorsal pigmentation in May 2017. These “greenish” individuals were considered an intermediate phenotype between *P. raffonei* and *P. siculus*. However, *P. raffonei* held at the Bioparco di Roma, which portrayed a typical “raffonei” brown coloration in both May and October 2017, turned greener in March 2021 [23].

We hypothesize that *P. raffonei* from the Capo Grosso promontory, similar to *P. siculus* [21], also changes color throughout the seasons, and the green pattern documented during spring may not be a sign of hybridization but a natural phenological variation. Through a quantitative analysis of visible dorsal color in captive and natural populations, we aim to measure the extent of this variation through seasons, years, and ontogenesis, and eventually determine whether coloration alone can be used as a characteristic for the identification of hybrid *P. raffonei*. To do this, we analyzed the color pattern of the lizards hosted at the Bioparco since 2017 with a comparison to lizards in nature from the same population. We expect that (i) lizard dorsal coloration varies across the seasons (i.e., according to reproductive cycle or cryptism), years (i.e., according to stochastic or climate condition), and age (i.e., ontogenetic variation), and (ii) lizards from the population in nature (springtime) mirror the same variation observed in captivity.

2. Materials and Methods

Initially described by Mertens (1955) as a subspecies of the Sicilian wall lizard, *Lacerta wagleriana antoninoi*, *P. raffonei* was recognized as its own species in 1994 by Capula following allozymic analyses. *Podarcis raffonei* subspecies are morphologically distinct and the Vulcano population is characterized by a smaller size with a mean SVL of 54.8–68.4 mm, and with a brownish dorsal coloration with a more or less pronounced black reticulation and whole, well-defined dorso-lateral light streaks. Some individuals show an orange-red underside or black spots on the throat or both (Figure 1) [18].

Podarcis raffonei individuals (n = 46) were collected in May 2017, at the Capo Grosso area in the island of Vulcano (Sicily; 38°25'6.98" N, 14°56'32.80" E) for an ex situ conservation project headed by the Department of Sciences, Roma Tre University and the Fondazione Bioparco di Roma. Potentially hybrid individuals, portraying a greenish dorsal coloration, were excluded, and only the lizards with a brown *P. raffonei* phenotype were selected. After capture, the individuals were transported to the facilities of the Reptile House at the Bioparco in refrigerated containers. At the Bioparco, tissue samples were taken for the genetic characterization of the collected lizards, and later confirmed the purity of

the individuals included in this study [24]. Capture, captive keeping, and authorization to perform studies were given by the Ministry of the Environment and the Protection of the Territory and the Sea (note 0008937; 2 May 2017). Sex was determined by the presence/absence of active femoral pores (present in males) and by the width and shape of the head (larger in males) [25]. All lizards were adults, and their body size (snout–vent length, SVL, to the nearest mm) was measured using a ruler. The individuals were housed in outdoor enclosures ($13.0 \times 2.0 \times 0.9$ h m), with sand as substrate and enriched with shelters, basking sites and spontaneous vegetation to recreate suitable conditions. The density was set at five individuals/m². Along with the invertebrates naturally present inside the enclosures, the lizards were fed house crickets (*Acheta domestica*), mealworms (*Tenebrio molitor*), and various fruits ad libitum.



Figure 1. *Podarcis raffonei* from Capo Grosso, in July 2020 (Photo by B. Gambioli).

To quantify lizard coloration, photographs of each individual were taken under standard conditions following Stevens et al. [26]. The dorsal pattern of the lizards was photographed using a Panasonic Lumix DMC-LX7 digital camera by placing the individuals on a white surface with a metric reference inside a white photographic box (Softbox Zectix; $30 \times 30 \times 30$ cm) illuminated with LED lights (LED: 21 Pcs., SMD 2850; color temperature: 5500 K; LED brightness: $350 \text{ lm} \times 2 = 700 \text{ lm}$; LED strip dimensions: $19.8 \times 1.8 \times 0.13$ cm). A picture of a color standard (Spydercheckr™-24 colors) was taken at the beginning of each session and under the same standard conditions to allow color calibration. The pictures were taken in RAW format. For 37 lizards, three photographic sessions were carried out in October 2017, February 2018, and June 2018. Twelve remaining lizards were photographed again in March 2021 and three more sessions were carried out in March, July, and October 2022 on the eight surviving individuals. In May 2021 and April 2022, ten lizards, five males and five females each year, were captured, measured, photographed, and immediately released on the Capo Grosso promontory. The photographs of wild lizards were taken following the same procedure described for the captive conditions. To account for the effect of temperature on the hue and luminance of reptile coloration [27,28], the pictures of the

captive lizards were taken in the internal structure of the Reptile House, where a constant temperature of 20–22 °C is maintained. To limit the effect of temperature on the wild lizards, these were kept inside individual cloth bags in the shade before the photographic session began. The color correction was carried out in Adobe Lightroom CC software and through the software associated with the color standard (SpyderCheckr 1.6 [29]) by creating a color correction profile applied to all the images of the session. Final photographs were exported in 16-bit TIFF format. The color measurements were then extracted in Image J software 1.54j [30] (<https://imagej.nih.gov/ij/>). The dorsal area was first selected using the polygon selection tool, and the black spots were excluded using the color threshold tool. The hue, saturation, and brightness of the selected area (on average 121,000 pixels) were measured through the HSB stack on Image J. The HSB or HSV (Value) color space, which is produced from the RGB system through a conversion algorithm [31], is considered a more intuitive color system where the single components are actual perceptual color features, with linear or proportional metrics [32]. In the HSV system that can be visualized as an inverted cone, brightness (V or B) is the vertical dimension where 0, the bottom, is black and 1 is white; saturation (S) is the horizontal distance from the axis, where the center of the cone (0 S) is completely gray while the edge (1 S) is completely pigmented or the “pure” color; hue (H) is the angle of the cone that, from 0° red, passes through yellow, green, cyan, blue, magenta and back to red at 360° [32] (pp. 202–207).

Evaluation of seasonal color variation in *P. raffonei*, was performed through different sets of repeated-measures analyses of variances (r-mANOVAs) and General Linear Models (GLMs) for each color component: hue, saturation, and brightness. The first r-mANOVAs tested 37 individuals’ color shifts between the 2017–2018 seasons. The effect of sex and its interaction with season (categorical predictors) was also evaluated. The second set assessed if color expression followed the same pattern in 2017–2018 and 2022 for the eight surviving lizards. To determine whether variation between years is defined by the age/ontogeny of the individuals, a further r-mANOVA was applied on each color variable collected in Spring 2017, 2021, and 2022 on the eight surviving individuals. Finally, through GLMs, we investigated the effect of captivity (categorical predictor: status = captive or wild), sex (categorical predictor), size (SVL continuous predictor) and year (categorical predictor: 2021–2022) on spring color expression. All statistical analyses were run on Statistica v.12 [33].

3. Results

3.1. Hue

As for the seasonal variations, there is a significant main effect of time on hue values (season: $F_{2,70} = 68.02$, $p < 0.001$) with autumn 2017 values being significantly higher than those in spring and summer 2018 (post hoc Tukey HSD test). While sex alone does not affect this component of color expression, hue does change differently between the sexes across time (season*sex: $F_{2,70} = 10.78$, $p < 0.001$) (Table A1). Males and females only appear to be significantly different in spring (post hoc Tukey HSD test, $p < 0.005$) with males showing higher hue values (Figure 2).

The inter-seasonal comparison between the first period (2017/2018) and 2022 has highlighted significant differences across years, seasons, and their interaction (year: $F_{1,18} = 255.47$, $p < 0.001$; season: $F_{2,18} = 15.39$, $p < 0.001$; year*season: $F_{2,18} = 14.12$, $p < 0.001$, respectively). In 2022, the hue values have significantly decreased, and while, in 2017, there is a clear difference between the seasons, in 2022, the hue remains practically unaltered from spring to autumn (Table A1).

Comparing the hue values in spring of 2017, 2021, and 2022, there is a clear effect of sex and year (sex: $F_{1,6} = 54.62$, $p < 0.001$; year: $F_{2,12} = 20.78$, $p < 0.001$) (Table A1). Hue is always lower in females, and it’s highest in spring 2021. Finally, hue in spring (march–may) does not differ between captive and wild individuals, nor is it affected by the body size (SVL) or year (2021–2022) (Figure 3). Sexes show differences, with males having significantly higher values than females ($F_{1,32} = 13.74$, $p < 0.001$) (Table A1).

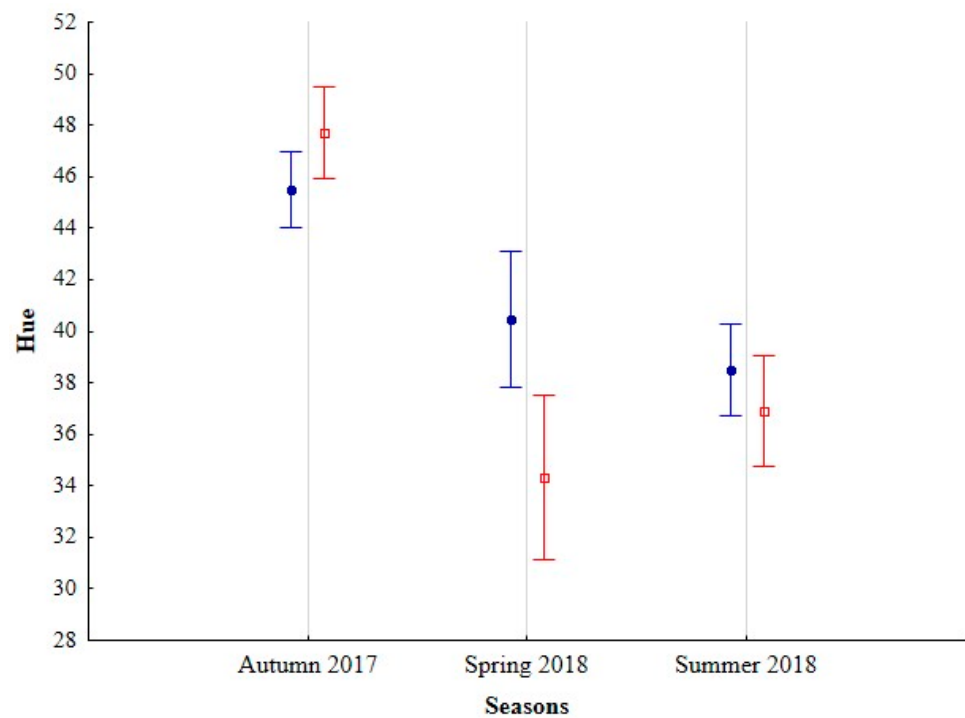


Figure 2. Mean hue values for the 37 lizards measured between autumn 2017 and summer 2018. Males (full circles) and females (empty squares) are significantly different only in spring 2018. Vertical bars represent 95% confidence intervals.

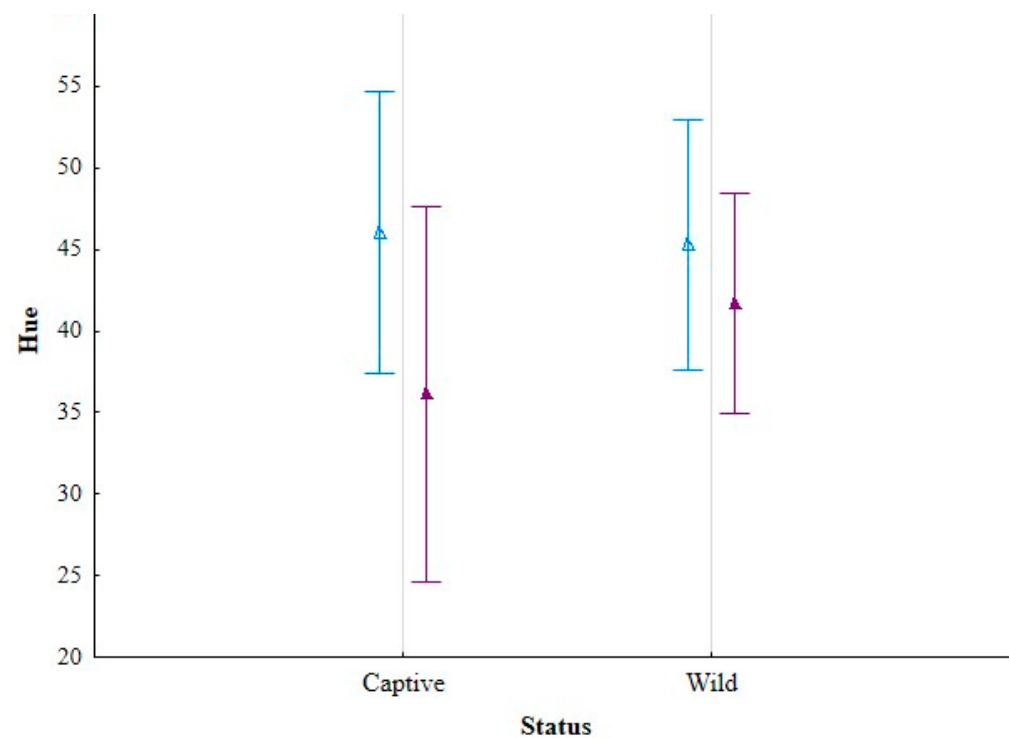


Figure 3. Mean hue for captive and wild *P. raffonei* in spring 2021 (empty triangles) and spring 2022 (full triangles); the vertical bars represent 95% confidence intervals. Neither status nor the years are significantly different in their hue values.

3.2. Saturation

Saturation of the lizards' dorsa varies across seasons ($F_{2,70} = 326.00, p < 0.001$), while neither sex nor its interaction with time has an effect (Table A2). The values were signifi-

cantly different between all seasons (post hoc Tukey HSD test) with the highest levels of saturation reached in summer 2018 and the lowest in autumn 2017.

The individual comparison between the first period (2017/2018) and 2022 reveals an effect of the year, the season, and their interaction (year: $F_{1,18} = 17.39$, $p < 0.001$; season: $F_{2,18} = 19.14$, $p < 0.001$; year*season: $F_{2,18} = 46.69$, $p < 0.001$) (Table A2). While mean saturation is overall higher in 2022, the main difference between the years is in autumn, when measurements from 2022 show higher saturation levels (post hoc Tukey HSD test, $p < 0.01$): saturation significantly grows from spring to summer of both years, and it grows still in autumn 2022, with its lowest value in autumn 2017 (Figure 4).

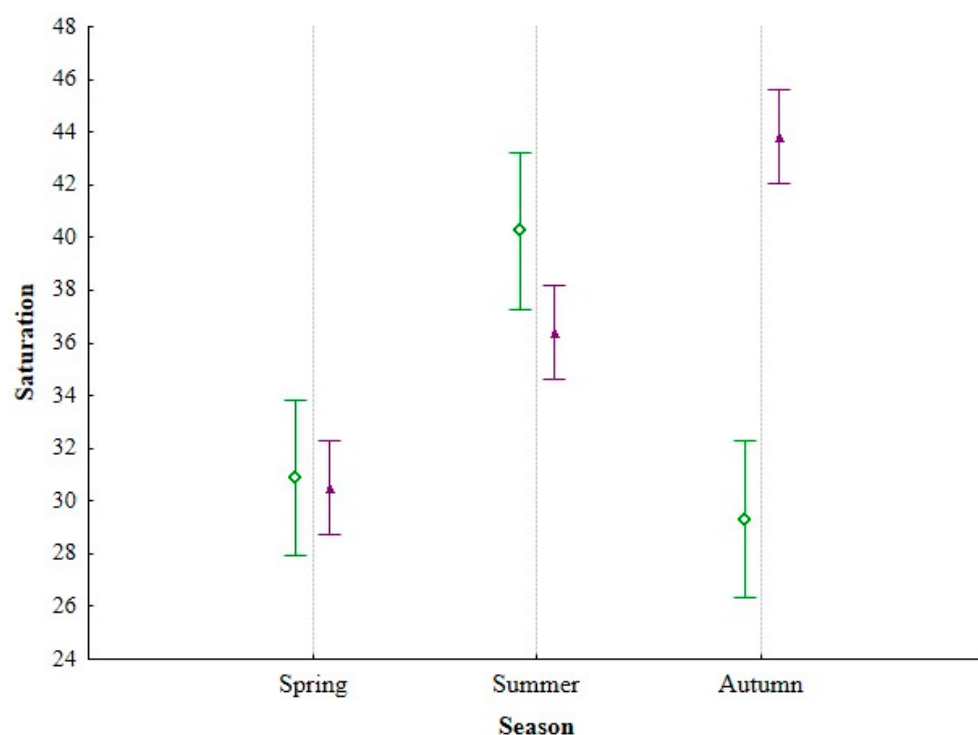


Figure 4. Mean saturation across the seasons of 2017/18 (empty rhombuses) and 2022 (full triangles); the vertical bars represent 95% confidence intervals.

Saturation with age showed differences among years, with a peak in 2021 ($F_{2,12} = 212.85$, $p < 0.001$) with no difference between sexes. The analysis with captive and wild lizards shows an overall effect of time (year: $F_{1,32} = 40.45$, $p < 0.001$), but no differences between the two groups of lizards and no effect of sex or body size (Table A2).

3.3. Brightness

In 2017–2018, the brightness significantly varies across seasons ($F_{2,70} = 40.47$, $p < 0.001$; post hoc Tukey HSD test: for all tests $p < 0.001$). The sex does not affect it, while the interaction season*sex is significant ($F_{2,70} = 22.56$, $p < 0.001$) (Table A3). Autumn 2017 shows the lowest values, and only in summer is there a significant difference between the brightness of the males (highest) and that of the females.

The comparison between the individuals' brightness from the first period (2017/18) and 2022 does not show an effect of year. Brightness slightly varies among seasons, and this variation shows a different pattern in the two years (season: $F_{2,18} = 5.11$, $p = 0.02$; year*season: $F_{2,18} = 83.45$, $p < 0.001$) (Table A3): in 2017, significantly lighter dorsa in spring turned dark in summer, while the opposite occurred in 2022, with darker lizards brightening up in summer; autumn of both years showed intermediate levels of brightness. (Figure 5).

In spring 2021, the lizards had even darker dorsal colorations than in spring 2022 and spring 2017 (r-mANOVA; year: $F_{2,12} = 20.14$, $p < 0.001$) (Table A3).

Brightness differs between years even in wild conditions ($F_{1,32} = 41.58, p < 0.001$) but, in this case, the wild lizards from spring 2021 had significantly darker dorsa than the lizards in all other conditions (post hoc Tukey HSD; $p < 0.001$). Sex, size, and status do not affect this variable (Table A3).

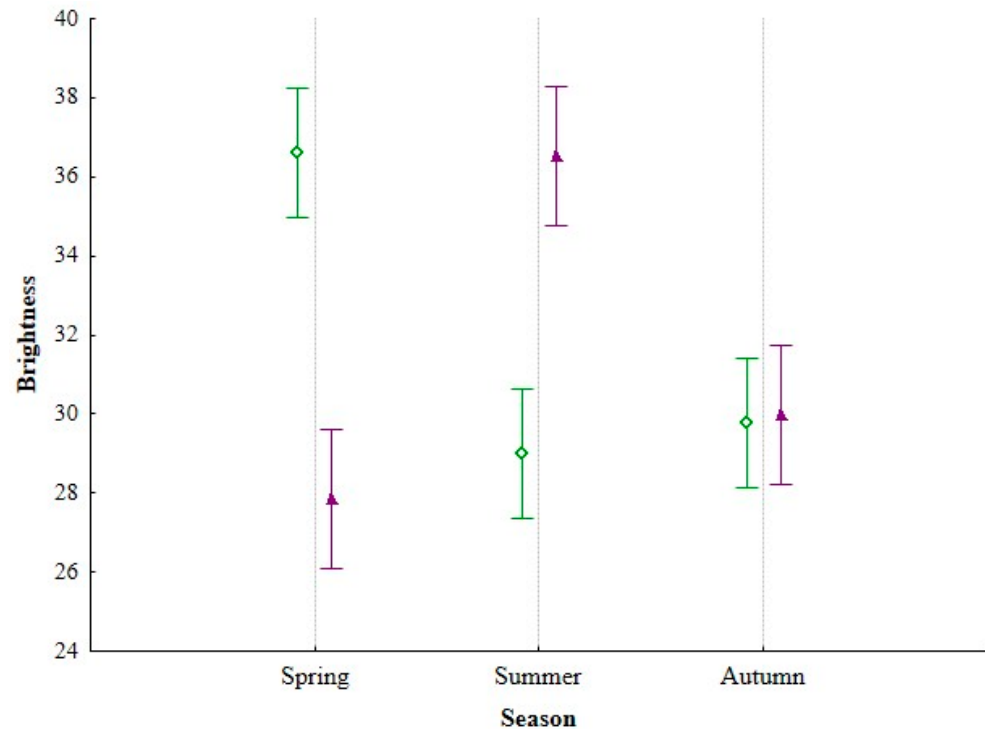


Figure 5. Mean brightness across the seasons of 2017/18 (empty rhombuses) and 2022 (full triangles); the vertical bars represent 95% confidence intervals.

4. Discussion

Our main finding demonstrates that, like several of its congeneric species, the Aeolian wall lizard varies its dorsal pigmentation throughout the seasons (Table 1). Time has a clear effect on all three color components of the lizards held in captive conditions and monitored for 12 months (2017–2018). After four years (2022), a seasonal variation still occurred, but these changes were different between the two periods. Considering the latter measurements were from individuals five years older, ontogenetic variation in color pattern could be claimed to be behind the variation process; yet, when considering only spring, for which measurement was available also in 2021, these changes did not follow a trend for either hue, saturation, or brightness, and must therefore be driven by other factors. We studied captive lizards; hence, what we observe could be influenced by the captive conditions (i.e., different climate and microclimate, food). However, the comparison between captive and wild populations in springtime did not reveal a difference in coloration variation patterning. The between-year analyses, including the measurements from the wild population from Capo Grosso, determined that the yearly variation is not limited to the captive population, but also occurs in nature (Table 1). As opposed to the other color variables, mean hue varies significantly between the sexes, with males always having significantly higher values than females in spring, consistently throughout the years, and both in captive and in situ lizards (Table 1).

Table 1. A summary of the factors and interactions (×) that significantly ($p < 0.001$) affect the hue, saturation, and brightness of the lizards' dorsa. Detailed results can be found in Appendix A.

	Hue	Saturation	Brightness
r-mANOVA 2017/18	Season Season × Sex	Season	Season Season × Sex
r-mANOVA 2017/18–2022	Year Season Year × Season	Year Season Year × Season	Season Year × Season
r-mANOVA Spring 2017–2021–2022	Year Sex	Year	Year
GLM Spring 2021–2022 Captive and Wild	Sex	Year	Year Year × Status

Analysis of the Aeolian wall lizards hosted at the Bioparco demonstrates the effect of time, across both seasons and years, on the dorsal coloration of this species. The observed strong variation of values between all three years, especially between 2021 and 2022, suggests that dorsal color in *P. raffonei* is not affected by the age of the individuals and must hence be determined by different factors. Green dorsal coloration was not previously described in this population of *P. raffonei*, while green pigmentation related to the reproductive season is known in *P. waglerianus*, *P. bocagei* [34,35], and the sympatric *P. siculus* [20,21], probably leading to the misconception of hybrid prevalence on Capo Grosso. Color variations have been correlated with seasonal changes in environmental conditions to favor crypsis [21], and with different strategies between males (communication) and females (crypsis), with the former growing greener, darker, and more saturated with age (size) and with a more pronounced seasonal pattern [20]. Perhaps due to the limited number of captive males analyzed in spring across 2021 and 2022, this effect of size on dorsal coloration was not highlighted by our analyses, although it seems to be a significant factor in some *Podarcis* lizards (*P. siculus*, *P. filfolensis*) but not others (*P. bocagei*) [36,37]. In our analyses, it appears that females and males only differ in the hue component of color, partially mirroring what was found in *P. siculus*, where there is no difference in saturation between sexes, but there is in hue and brightness [20]. As for females, the dorsal pattern variation might increase their crypsis, decreasing the probability of being detected and preyed upon [38].

This study has its limitations, mainly the small number of lizards tested in 2022 and the fact that only one male was still alive during this year. Nonetheless, the models run with the different databases, including those with more males than females (2017–2018), produce the same significant effects within each color component. Also, the findings related to the comparisons between captive and wild lizards were obtained from measurements gathered in the spring and cannot be generalized to other seasons. Breeding season color expression in lizards where color varies across months could be under sexual selection [39]. Indeed, coloration in the other seasons could be affected by other drivers or constraints. Further analyses in wild conditions are thus necessary to define the trends of color expression in *P. raffonei* across all seasons, and to identify possible drivers of color variation in other phases of the reproductive cycle of these lizards. A long-term analysis could also investigate what conditions determine color variation across the years.

5. Conclusions

In 2020, the Capo Grosso population was described as extinct due to the observation of only *P. siculus* or hybrid individuals detected on the promontory during a 2019 survey [40]. However, in addition to the fact that the characteristics used to define hybrid individuals are not at all clear, this statement conclusively does not correspond to the truth, given that *P. raffonei* was continuously observed on the promontory from 2015 to 2024 by our research

group and that very recent genomic analyses [24] confirm that almost all the lizards present on the Capo Grosso promontory were genetically pure. In 2017 [23], the frequency of individuals with a “hybrid phenotype”, intermediate between that of the Aeolian wall lizard and that of the Italian wall lizard, was said to be increasing sharply. This study reported that, from 2015 to 2017, the percentage of potentially hybrid lizards would have increased from 3 to 53 percent, thus threatening the genetic purity of the Capo Grosso population, the last relict population of Vulcano Island. This intermediate phenotype would be characterized by a particularly green dorsal coloration. During samplings in May 2021–2024 (unpublished data) on the Capo Grosso promontory, the same frequency of green individuals (nearly 50%) was found, confirming what was described by Ficetola et al. [23]. However, the study carried out on lizards maintained in captivity at the Bioparco in Rome cautions against inferring the presence and possible expansion of Capo Grosso hybrids on a phenotypic basis alone. The lizards studied all exhibited the typical phenotype of *P. raffonei* at the time of capture in May and October 2017, and only later did the green dorsal coloration emerge (March 2021). Genomic analysis of Aeolian wall lizards from Capo Grosso has shown that hybridization rates are extremely low (3%) on the promontory; this analysis included, among others, the individuals that were then translocated to the Bioparco for captive breeding and analyzed in this study, and none were hybrid [24]. Thus, this frequency of hybrids in the population is such that we can ascribe the greenish color variation of most, if not all, specimens to seasonal variation.

Regardless of the drivers of this varying color expression, we must be guarded in identifying potential hybrids on a phenotypic basis alone.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d16080485/s1>.

Author Contributions: Conceptualization, L.V.; methodology, B.G.; software, L.V. and B.G.; validation, L.V. and B.G.; formal analysis, L.V. and B.G.; investigation, L.V. and B.G.; resources, L.V. and B.G.; data curation, L.V. and B.G.; writing—original draft preparation, B.G. and L.V.; writing—review and editing, L.V., B.G. and D.M.; visualization, L.V., B.G. and D.M.; supervision, L.V.; project administration, L.V. and D.M.; funding acquisition, L.V. and B.G. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in the study are included in the Supplementary Materials, further inquiries can be directed to the corresponding author.

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Appendix A

Table A1. Repeated-measures ANOVAs and GLMs for analyses on hue.

Repeated-Measures ANOVAs					
2017/18					
Source	Sum of Squares	df	Mean sq.	F	Sig.
Intercept	176,069.1	1	176,069.1	4751.502	<0.001
Sex	89.6	1	89.6	2.418	0.129
Error	1296.9	35	37.1		
Month	1949.6	2	974.8	68.012	<0.001
Month × Sex	309.3	2	154.6	10.789	<0.001
Error	1003.3	70	14.3		
2017/18–2022					
Source	Sum of Squares	df	Mean sq.	F	Sig.
Intercept	43,571.68	1	43,571.68	3964.010	<0.001
Season	338.35	2	169.18	15.391	<0.001
Error	197.85	18	11.99		
Year	2519.98	1	2519.98	255.474	<0.001
Year × Season	278.57	2	139.28	14.120	<0.001
Error	177.55	18	9.86		
Spring 2017-2021-2022					
Source	Sum of Squares	df	Mean sq.	F	Sig.
Intercept	20,331.86	1	20,331.86	921.495	<0.001
Sex	1205.30	1	1205.30	54.627	<0.001
Error	132.38	6	22.06		
Year	481.71	2	240.86	20.783	<0.001
Year × Sex	90.33	2	45.17	3.897	0.050
Error	139.07	12	11.59		
GLMs					
Source	Sum of Squares	df	Mean sq.	F	Sig.
Intercept	0.11	1	0.11	0.001	0.973
Sex	1257.07	1	1257.07	13.739	<0.001
Status	14.33	1	14.33	0.157	0.695
Year	351.52	1	351.52	3.842	0.059
Size	120.95	1	120.95	1.322	0.259
Error	2927.84	32	91.50		

Table A2. Repeated-measures ANOVAs and GLMs for analyses on saturation.

Repeated-Measures ANOVAs					
2017/18					
Source	Sum of Squares	df	Mean sq.	F	Sig.
Intercept	131,187.4	1	131,187.4	11,863.48	<0.001
Sex	15.0	1	15.0	1.36	0.252
Error	387.0	35	11.1		
Month	7645.0	2	3822.5	326.00	<0.001
Month × Sex	45.1	2	22.6	1.92	0.154
Error	820.8	70	11.7		

Table A2. *Cont.*

Repeated-Measures ANOVAs					
2017/18–2022					
Source	Sum of Squares	df	Mean sq.	F	Sig.
Intercept	52012.90	1	52012.90	4439.017	<0.001
Season	448.54	2	224.27	19.140	<0.001
Error	210.91	18	11.72		
Year	124.30	1	124.30	17.386	<0.001
Year × Season	667.59	2	333.79	46.686	<0.001
Error	128.70	18	7.15		
Spring 2017–2022					
Source	Sum of Squares	df	Mean sq.	F	Sig.
Intercept	14,422.90	1	131,187.4	1483.638	<0.001
Sex	14.42	1	14.42	1.484	0.269
Error	58.33	6	9.72		
Year	1682.51	2	841.26	212.850	<0.001
Year × Sex	59.17	2	29.59	7.486	<0.001
Error	47.43	12	3.95		
GLMs					
Source	Sum of Squares	df	Mean sq.	F	Sig.
Intercept	379.47	1	379.47	7.785	0.009
Sex	3.24	1	3.24	0.067	0.798
Status	0.77	1	0.77	0.016	0.901
Year	1971.83	1	1971.83	40.453	<0.001
Size	57.73	1	57.73	1.184	0.285

Table A3. Repeated-measures ANOVAs and GLMs for analyses on brightness.

Repeated-Measures ANOVAs					
2017/18					
Source	Sum of Squares	df	Mean sq.	F	Sig.
Intercept	111,221.6	1	111,221.6	6964.543	<0.001
Sex	0.3	1	0.3	0.018	0.893
Error	558.9	35	16.0		
Month	489.7	2	244.8	40.466	<0.001
Month × Sex	273.1	2	136.5	22.568	<0.001
Error	423.5	70	6.1		
2017/18–2022					
Source	Sum of Squares	df	Mean sq.	F	Sig.
Intercept	41,981.66	1	41,981.66	6556.818	<0.001
Season	65.46	2	32.73	5.112	0.017
Error	115.25	18	6.40		
Year	1.16	1	1.16	0.416	0.527
Year × Season	466.46	2	233.23	83.452	<0.001
Error	50.31	18	2.79		
Spring 2017–2022					
Source	Sum of Squares	df	Mean sq.	F	Sig.
Intercept	8994.485	1	8994.48	916.091	<0.001
Sex	2.583	1	2.58	0.263	0.626
Error	58.910	6	9.82		
Year	241.144	2	120.57	20.136	<0.001
Year × Sex	2.569	2	1.28	0.215	0.810
Error	71.856	12	5.99		

Table A3. Cont.

Repeated-Measures ANOVAs					
GLMs					
Source	Sum of Squares	df	Mean sq.	F	Sig.
Intercept	129.11	1	129.11	6.486	0.016
Sex	13.26	1	13.26	0.666	0.421
Status	75.40	1	75.40	3.787	0.060
Year	827.87	1	827.87	41.587	<0.001
Year × Status	410.65	1	410.65	20.629	<0.001
Size	28.52	1	28.52	1.433	0.240

References

1. Cuthill, I.C.W.; Allen, L.; Arbuckle, K.; Caspers, B.; Chaplin, G.; Hauber, M.E.; Hill, G.E.; Jablonski, N.G.; Jiggins, C.D.; Kelber, A.; et al. The biology of color. *Science* **2017**, *357*, eaan0221. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Stokes, D.L. Things we like: Human preferences among similar organisms and implications for conservation. *Hum. Ecol.* **2007**, *35*, 361–369. [\[CrossRef\]](#)
3. Prokop, P.; Fančovičová, J. Does colour matter? The influence of animal warning coloration on human emotions and willingness to protect them. *Anim. Conserv.* **2013**, *16*, 458–466. [\[CrossRef\]](#)
4. Lifshitz, N.; St. Clair, C. Coloured ornamental traits could be effective and non-invasive indicators of pollution exposure for wildlife. *Conserv. Physiol.* **2016**, *4*, cow028. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Qiu, X.; Whiting, M.J.; Du, W.; Wu, Z.; Luo, S.; Yue, B.; Fu, J.; Qi, Y. Colour Variation in the Crocodile Lizard (*Shinisaurus crocodilurus*) and its Relationship to Individual Quality. *Biology* **2022**, *11*, 1314. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Mynott, S.; Daniels, C.; Widdicombe, S.; Stevens, M. Using camouflage for conservation: Colour change in juvenile European lobster. *bioRxiv* **2018**. [\[CrossRef\]](#)
7. Sherpa, S.; Salvi, D.; Silva-Rocha, I.; Capblancq, T.; Paris, J.R.; Carretero, M.A.; Ficetola, G.F. Reconstructing the complex colonisation histories of lizards across Mediterranean archipelagos. *J. Biogeogr.* **2024**, *51*, 157–172. [\[CrossRef\]](#)
8. Capula, M. Genetic variation and differentiation in the lacertid lizard, *Podarcis wagleriana* (Reptilia: Lacertidae). *Biol. J. Linn. Soc.* **1994**, *52*, 177–196. [\[CrossRef\]](#)
9. D'Amico, M.; Bastianelli, G.; Faraone, F.P.; Lo Valvo, M. The spreading of the invasive Italian wall lizard on Vulcano, the last island inhabited by the critically endangered Aeolia wall lizard. *Herp. Con. Bio.* **2018**, *13*, 146–157.
10. Damas-Moreira, I.; Riley, J.L.; Harris, D.J.; Whiting, M.J. Can behaviour explain invasion success? A comparison between sympatric invasive and native lizards. *Anim. Behav.* **2019**, *151*, 195–202. [\[CrossRef\]](#)
11. Downes, S.; Bauwens, D. An experimental demonstration of direct behavioural interference in two Mediterranean lacertid lizard species. *Anim. Behav.* **2002**, *63*, 1037–1046. [\[CrossRef\]](#)
12. Herrel, A.; Huyghe, K.; Vanhooydonck, B.; Backeljau, T.; Breugelmans, K.; Grbac, I.; Van Damme, R.; Irschick, D.J. Rapid large-scale evolutionary divergence in morphology and performance associated with exploitation of a different dietary resource. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 4792–4795. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Rocha, I. *Podarcis sicula*. In *Invasive Species Compendium*; CAB International: Wallingford, UK, 2016. [\[CrossRef\]](#)
14. Nevo, E.; Gorman, G.C.; Soulé, M.; Yang, E.J.; Clover, R.; Jovanovic, V. Competitive exclusion between Insular *Lacerta* species (Sauria, Lacertidae). *Oecologia* **1972**, *10*, 183–190. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Nikolic, B.; Josic, P.; Buric, D.; Tkalec, M.; Lisicic, D.; Blazevic, S.A.; Hranilovic, D. Coexisting lacertid lizard species *Podarcis siculus* and *Podarcis melisellensis* differ in dopamine brain concentrations. *J. Comp. Physiol.* **2019**, *205*, 451–456. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Capula, M. Competitive exclusion between *Podarcis* lizards from Tyrrhenian islands: Inference from comparative specie distribution. In Proceedings of the IV Ordinary General Meeting of the Societas Europea Herpetologica, Budapest, Hungary, 19–23 August 1991; pp. 89–93.
17. Caruso, Y.; Macale, D.; Luiselli, L.; Vignoli, L. Thermoregulation comparisons between a threatened native and an invasive lizard species. *Herpetol. J.* **2021**, *31*, 70–76. [\[CrossRef\]](#)
18. Capula, M.; Lo Cascio, P. *Podarcis raffonei* (Mertens, 1952). In *Fauna d'Italia, Reptilia, Edizioni Calderini de*; Corti, C., Capula, M., Luiselli, L., Razzetti, E., Sindaco, R., Eds.; Il Sole 24 ORE: Bologna, Italy, 2011; pp. 401–407.
19. Capula, M. Natural hybridization in *Podarcis sicula* and *P. wagleriana* (Reptilia: Lacertidae). *Biochem. Syst. Ecol.* **1993**, *21*, 373–380. [\[CrossRef\]](#)
20. Storniolo, F.; Zuffi, M.A.L.; Coladonato, A.J.; Di Vozzo, L.; Giglio, G.; Gini, A.E.; Leonetti, F.L.; Luccini, S.; Mangiacotti, M.; Scali, S.; et al. Patterns of variations in dorsal colouration of the Italian wall lizard *Podarcis siculus*. *Biol. Open* **2021**, *10*, bio058793. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Pellitteri-Rosa, D.; Gazzola, A.; Todisco, S.; Mastropasqua, F.; Liuzzi, C. Lizard colour plasticity tracks background seasonal changes. *Biol. Open* **2020**, *9*, bio052415. [\[CrossRef\]](#) [\[PubMed\]](#)

22. Ortega, J.; Marti'N, J.; Crochet, P.-A.; López, P.; Clobert, J. Seasonal and interpopulational phenotypic variation in morphology and sexual signals of *Podarcis liolepis* lizards. *PLoS ONE* **2019**, *14*, e0211686. [CrossRef]
23. Ficetola, G.F.; Silva-Rocha, I.; Carretero, M.A.; Vignoli, L.; Sacchi, R.; Melotto, A.; Scali, S.; Salvi, D. Status of the largest extant population of the critically endangered Aeolian lizard *Podarcis raffonei* (Capo Grosso, Vulcano island). *PLoS ONE* **2021**, *16*, e0253631. [CrossRef]
24. Paris, J.R.; Ficetola, G.F.; Ferrer Obiol, J.; Silva-Rocha, I.; Carretero, M.A.; Salvi, D. Does hybridisation with an invasive species threaten Europe's most endangered reptile? Genomic assessment of Aeolian lizards on Vulcano island. *bioRxiv* **2024**. [CrossRef]
25. Corti, C.; Lo Cascio, P. I lacertidi italiani. In *Mediterraneo, Guide Naturalistiche*; L'Epos: Palermo, Italy, 1999; Volume 10.
26. Stevens, M.; Párraga, C.A.; Cuthill, I.C.; Partridge, J.C.; Troscianko, T.S. Using digital photography to study animal coloration. *Biol. J. Linn. Soc.* **2007**, *90*, 211–237. [CrossRef]
27. Smith, K.R.; Cadena, V.; Endler, J.A.; Kearney, M.R.; Porter, W.P.; Stuart-Fox, D. Color change for thermoregulation versus camouflage in free ranging lizards. *Am. Nat.* **2016**, *188*, 668–678. [CrossRef]
28. Guo, K.; Zhong, J.; Zhu, L.; Xie, F.; Du, Y.; Ji, X. The thermal dependence and molecular basis of physiological color change in *Takydromus septentrionalis* (Lacertidae). *Biol. Open* **2021**, *10*, bio058503. [CrossRef]
29. Datacolor. SpyderCHECKR Camera Color Correction for Photo and Video. Version 1.6. 2022. Available online: <https://www.datacolor.com/spyder/> (accessed on 16 July 2024).
30. Rasband, W.S. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, 1997–2018. Available online: <https://imagej.net/ij/> (accessed on 16 July 2024).
31. Chernov, V.; Alander, J.; Bochko, V. Integer-based accurate conversion between RGB and HSV color spaces. *Comput. Electr. Eng.* **2015**, *46*, 328–337. [CrossRef]
32. Burger, W.; Burge, M.J. *Principles of Digital Image Processing—Fundamental Techniques*; Springer: London, UK, 2009; pp. 202–207.
33. StatSoft, Inc. STATISTICA (Data Analysis Software System), Version 12. 2014. Available online: www.statsoft.com (accessed on 16 July 2024).
34. Faraone, F.P.; Lo Valvo, M. Seasonal variation in colour of the Sicilian wall lizard *Podarcis wagleriana*. In Proceedings of the Riassunti del 6° Congresso Nazionale della Societas Herpetologica Italica (SHI), Rome, Italy, 27 September–1 October 2006; p. 25.
35. Galán, P. Cambios estacionales de coloración y comportamiento agonístico, de cortejo y de apareamiento en el lacértido *Podarcis bocagei*. *Rev. Esp. Herp.* **1995**, *9*, 57–75.
36. Sacchi, R.; Scali, S.; Mangiacotti, M.; Ruffo, D. Colour variation of the Maltese wall lizards (*Podarcis filfolensis*) at population and individual levels in the Linosa island. *Rend. Lincei Sci. Fis. Nat.* **2021**, *32*, 565–575. [CrossRef]
37. Galán, P. Ontogenetic and sexual variation in the coloration of the lacertid lizards *Iberolacerta monticola* and *Podarcis bocagei*. Do the females prefer the greener males? *Anim. Biol.* **2008**, *58*, 173–198. [CrossRef]
38. Merilaita, S.; Stevens, M. Crypsis through Background Matching. In *Animal Camouflage: Mechanisms and Function*; Stevens, M., Merilaita, S., Eds.; Cambridge University Press: Cambridge, UK, 2017; pp. 17–33.
39. Olsson, M.; Stuart-Fox, D.; Ballen, C. Genetics and evolution of colour patterns in reptiles. *Semin. Cell Dev. Biol.* **2013**, *24*, 529–541.
40. Lo Cascio, P.; Sciberras, A. "Cold-blooded" travellers around Sicily and Recent herpetofauna of circum-Sicilian and Maltese Islands. In *Life on Islands. 1 Biodiversity in Sicily and Surrounding Islands*; La Mantia, T., Badalamenti, E., Carapezza, A., Lo Cascio, P., Troia, A., Eds.; Edizioni Danaus: Palermo, Italy, 2020; pp. 368–369.

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