

# A study in scarlet: incipient speciation, phenotypic differentiation and conservation implications of the *Podarcis* lizards of the western Pontine Islands, Italy

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During the first decades of the last century, an enigmatic extinction was documented to have occurred on the small Mediterranean island of Santo Stefano in the Pontine Archipelago. Although islands are fascinating systems for studying microevolutionary processes, they may nevertheless host unstable communities that make their populations particularly vulnerable to anthropogenic effects and, ultimately, extinction. Here, we investigated the variation of head morphology of 374 individuals of *Podarcis siculus* from mainland Italy, Sicily and the Pontine Archipelago, using geometric morphometrics. We also included in the analysis samples of the extinct population of Santo Stefano Island, aiming to shed light on the extinction and provide additional information on the historical biogeography of the Archipelago. We found a strong relationship between morphological differences and phylogeographical structure based on previously published genetic data, indicating that the western Pontine populations seem to be a case of incipient speciation. In addition, the extinct population of Santo Stefano Island clustered in all analyses with the western Pontine populations. The results not only provide information on the evolutionary history of *P. siculus*, but also lead to broader considerations of taxonomy and conservation.

ADDITIONAL KEYWORDS: extinct *Podarcis* – geometric morphometrics – insular phenotypic differentiation – Italian wall lizard – *Podarcis siculus* – species replacement.

## INTRODUCTION

Islands are fascinating natural laboratories, to which we owe the main evolutionary theories (Darwin, 1851; Wallace, 1892). To date, islands continue to attract the attention of biologists and biogeographers, contributing to bring crucial insights into evolutionary processes underlying differentiation and speciation (MacArthur & Wilson, 1967; Simberloff & Wilson, 1969; Losos & Schluter, 2000). Among the most attractive issues, islands help in explaining complex evolutionary dynamics in a simplified framework. However,

islands, so fascinating from a heuristic point of view, are unfortunately particularly vulnerable to the invasion of alien species, to habitat loss and to human-induced detrimental effects (Williamson, 1989; Biber, 2002; Sax *et al.*, 2002; Sax & Gaines, 2008; Harradine *et al.*, 2015). As an example, most of the vertebrate historical extinctions (~83%, according to the World Conservation Union) have occurred on islands. Nevertheless, a general island conservation assessment is still lacking, because the International Union for Conservation of Nature criteria are not always suitable references to evaluate insular conservation priorities (Martín, 2009). This scenario is even more complicated by the fact that insular biota may hide proper species identification.

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Indeed, on the one hand spatial segregation between island and mainland populations would de facto suggest reproductive isolation, but on the other hand it makes an empirical assessment of the biological species concept impossible (Mayr, 1995). Therefore, taxonomic ambiguities should be avoided, because they undermine conservation and management actions that refer to species lists (Gherardi *et al.*, 2002). Although a great number of studies have focused closely on many aspects of island biogeography, including colonization, extinction, gene flow and diversification, it is felt that despite great efforts, little has been translated into active conservation policies (Castilla *et al.*, 1998; Allendorf & Luikart, 2009).

*Podarcis* lizards are one of the most abundant components of the Mediterranean island endemic vertebrates. Although most lizard species are considered as 'least concern', because of no apparent threat is affecting either the mainland or large island populations, for the endemic small island populations the real extinction risk is, in general, overlooked (Corti *et al.*, 1999; Foufopoulos & Ives, 1999). Indeed, historical extinctions have been reported for a relatively large number of insular *Podarcis* populations (Mertens, 1926; Lanza & Corti, 1996; Pérez-Mellado *et al.*, 1997). For example, in 1926 Robert Mertens examined the specimens captured in 1914 by G. de Southoff (preserved in the herpetological collections of the British Museum of Natural History of London) and described an endemic *Podarcis siculus* Rafinesque-Schmaltz, 1810 subspecies (*Podarcis siculus sanctistephani*) of the small Santo Stefano Island in the eastern Pontine Archipelago. About 30 years later, Professor Benedetto Lanza was very surprised to receive a number of specimens of Santo Stefano Island corresponding to the 'ordinary' *P. siculus siculus* phenotype. During an epistolary exchange with the famous German herpetologist, B. Lanza jokingly wrote, 'On Santo Stefano Island ... we should send Sherlock Holmes to solve the mystery'. The endemic population of Santo Stefano Island probably become extinct during the first decades of the last Century and was replaced by the wider distributed phenotype, most probably coming from the nearby Ventotene Island (Mertens, 1956; Bruno, 1982; Lanza & Corti, 1996).

Trying to shed new light on this enigma and to tell the history of the colonization of the Pontine Archipelago, we focused on the morphological variation of *P. siculus* populations from mainland Italy, Sicily and the Pontine Archipelago. Owing to the strong phylogeographical structure and considerable morphological variation found within *P. siculus*, the occurrence of a species complex has been argued, with  $\geq 20$  subspecies described (Oliverio *et al.*, 1998, 2000; Harris & Sa-Sousa, 2002). However, recent studies highlighted the monophyly of this species, and a complex evolutionary history characterized by allopatric fragmentation occurred in

different Italian peninsular sub-refugia (Podnar *et al.*, 2005; Senczuk *et al.*, 2017). In addition, such genetic investigations underlined the inconsistency of almost all the subspecies described on the basis of morphological traits. The latest study, based on mitochondrial and nuclear sequences, described a new diverging lineage for the western Pontine Islands (Ponza, Zannone, Palmarola and Gavi islands), whereas populations from the eastern Pontine (Ventotene and Santo Stefano islands) were found to be genetically indistinguishable from the mainland populations (Biaggini *et al.*, 2009; Senczuk *et al.*, 2018). The genetic distances found for the western Pontine with respect to the mainland lineages are comparable to those observed in other *Podarcis* species and even twice as much as, for example, between *Podarcis waglerianus* and its sister species, the endemic Aeolian wall lizard (*Podarcis raffoneae*) (Harris *et al.*, 2005; Poulakakis *et al.*, 2005; Lymberakis & Poulakakis, 2010, Psonis *et al.*, 2017). The molecular clock was in line with the dating of the principal episodes of volcanic eruptions that led to the formation of the islands (Cadoux *et al.*, 2005), suggesting an early Pliocene colonization of the palaeo-archipelago followed by a mid-Pleistocene make-up of the current genetic variation among the islands (Senczuk *et al.*, 2018).

In the light of the above findings, we aim to assess whether the observed genetic divergence is accompanied by morphological differences in head shape and size using a geometric morphometric (GMM) approach. The extinct *P. s. sanctistephani* population of Santo Stefano Island was also included in the analysis, both to shed new light on the population previously living on the island and to provide helpful information on the biogeographical history of the Archipelago. First, we tested a phylogeographical hypothesis considering that the populations sampled belonged to five separate phylogeographical units. Indeed, according to the literature (Biaggini *et al.*, 2009; Senczuk *et al.*, 2018), the western Pontine Islands (Ponza, Zannone and Palmarola) belong to an ancient lineage distinct from the lineage inhabiting the eastern Pontine Islands (Ventotene and Santo Stefano), which is strictly related to one of the continental lineages. Moreover, Sicilian and continental populations belong to three distinct lineages (Tyrrhenian, Adriatic and Sicilian). Successively, we explored the extent of morphological variation of the head in the Pontine Archipelago and the degree of phenetic relatedness among populations (sampled at different times) in order to clarify the phenotypic relationship of the extinct Santo Stefano lizards with the other insular populations. The results obtained provide useful information to understand the colonization and extinction processes that took place on the Archipelago. Our findings are also discussed in the light of their implications in defining conservation priorities.

## MATERIAL AND METHODS

## SPECIES, STUDY AREA AND SAMPLING

The Italian wall lizard, *Podarcis siculus* is distributed in Italy, the Eastern Adriatic coast, Sicily, Sardinia and most of the Tyrrhenian, Ionian and Adriatic islands. Introduced populations are found in North Africa, the Iberian Peninsula, France, Turkey and USA (Corti *et al.*, 2010; Sillero *et al.*, 2014). The Pontine Archipelago consists of two groups of islands separated by 40 km of open sea: the western group (32 km distant from the Tyrrhenian coast) includes Ponza, Palmarola, Zannone and Gavi islands, while the eastern (50 km distant from the Tyrrhenian coast) includes Ventotene and Santo Stefano islands. All are volcanic, originating from different eruptive episodes (Cadoux *et al.*, 2005).

Several subspecies have been described for the Archipelago based on external morphology (size and colour pattern): *Podarcis siculus latastei* (Bedriaga, 1879) for Ponza Island, *Podarcis siculus patrizii* (Lanza, 1952) for Zannone Island, *Podarcis siculus lanzai* (Mertens, 1952) for Gavi Islet and *Podarcis siculus palmarolae* (Mertens, 1967) for Palmarola Island.

Three hundred and seventy-four specimens (207 males and 167 females) from the collections of the Museo di Storia Naturale dell'Università di Firenze (Sezione di Zoologia 'La Specola'; hereafter MZUF) were used in this study (Table 1). Populations from both the western (Ponza, Zannone, Gavi and Palmarola) and the eastern (Ventotene and Santo Stefano) islands were analysed. For the islands of Ponza, Zannone, Palmarola, Ventotene and Santo Stefano, we used specimens sampled at different times (1878, 1954, 1966 and 1987), including the extinct population of Santo Stefano sampled in 1878 by E. H. Giglioli. The original sampling consisted of 15 specimens, of which five were adult males, five adult females and five juveniles, although two (one male and one female) were sent in exchange by B. Lanza to the Senckenberg Museum (Frankfurt, Germany) in 1954. In addition, four mainland populations from the Tyrrhenian coast and two populations from Sicily were used for comparison (Table 1; Fig. 1). These populations were selected taking into account both the geography and the phylogenetic relationship assessed in previous studies (Podnar *et al.*, 2005; Senczuk *et al.*, 2017, 2018).

## GEOMETRIC MORPHOMETRICS

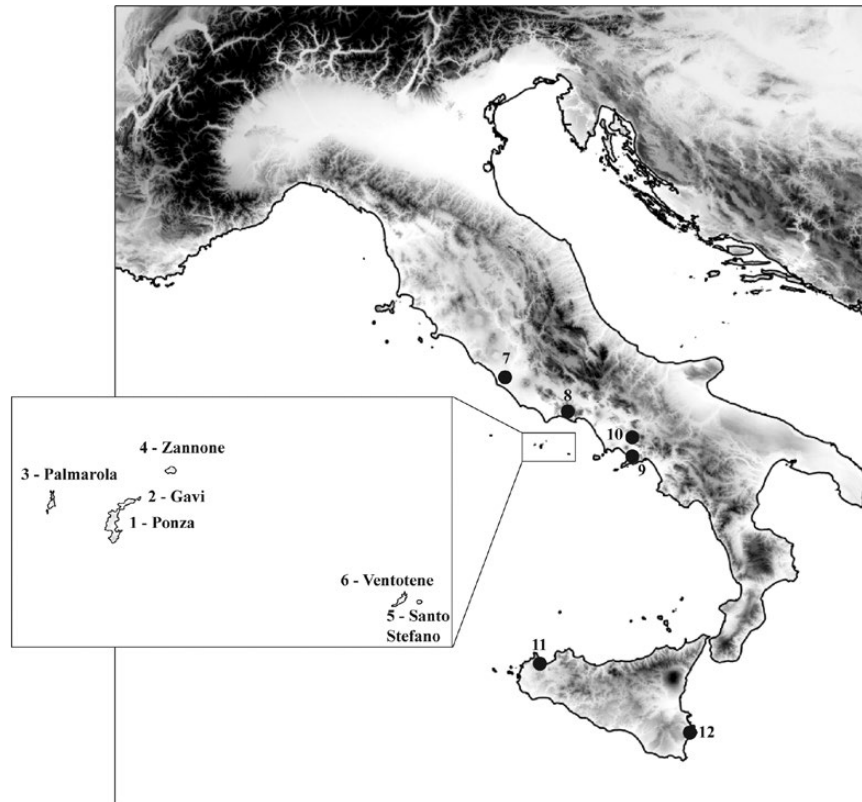
A digital camera (Nikon D7000) was used to shoot high-resolution images of the dorsal part of the head, and a ruler (positioned next to the specimens) was used as a scale reference. On the dorsal surface of the head, 32 landmarks were digitized using the software tpsDig2 (Rohlf, 2005). The positions of the landmarks and the corresponding cephalic configuration are

**Table 1.** Population number, locality and collection dates for both males and females

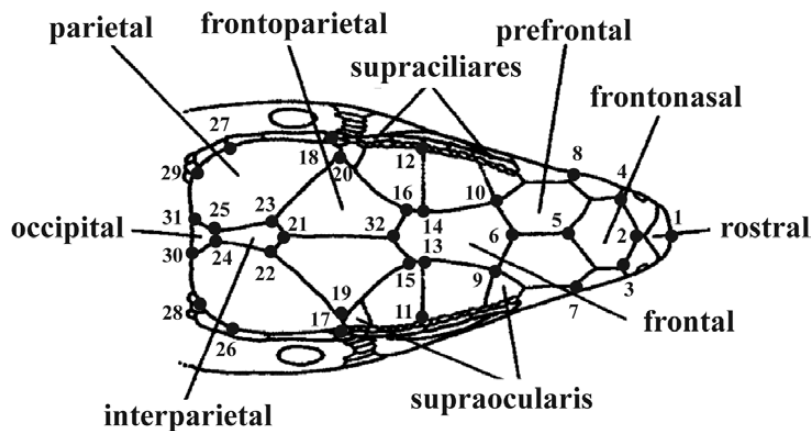
No.	Locality	Date	Females	Males
1	Ponza	1878	8	14
		1954	13	13
		1987	6	6
2	Gavi	1987	3	10
		1954	10	12
3	Palmarola	1987	5	4
		1878	6	6
		1954	17	18
4	Zannone	1966	5	6
		1878	5	5
		1954	14	13
5	Santo Stefano	1966	0	8
		1878	6	8
		1954	8	9
6	Ventotene	1966	6	4
		1990	5	9
		1956	6	9
7	Ostia Antica	1956	6	9
8	Scauri	1953	13	17
9	Napoli	1953	8	14
10	Roccarainola	1968	7	9
11	Palermo	1975	5	8
12	Siracusa	1951	11	5
	Total		167	207

shown in Figure 2. The R package geomorph v. 3.0.5 (Adams *et al.*, 2017) was used to analyse shape and size among configurations. First, a common generalized Procrustes analysis (GPA) was applied in order to align configurations and control for differences in size and orientation (Rohlf & Slice, 1990; Bookstein, 1997; Goodall, 1991).

A Procrustes ANOVA with randomized residual permutation procedure (RRPP; 1000 permutations), computed using the function *procD.lm*, was run on shape variables obtained from aligned configurations and centroid size (CS; the square root of the sum of squared distances of a set of landmarks from their centroid) to test for the contribution to the overall phenotypic variability of sex and main phylogeographical units. According to the literature, which recognized different phylogenetic clusters for mainland Italy and the islands, our samples were grouped in five phylogeographical units. In the Pontine Archipelago, two distinct lineages can be distinguished: one occurring in the western Pontine Islands (Ponza, Zannone, Palmarola and Gavi) and the other in the eastern Pontine Islands (Ventotene and Santo Stefano). Within the continental samples, on the basis of geography and genetics we can distinguish between two groups: the 'Tyrrhenian' (Ostia Antica and Scauri) and the 'Adriatic' (Naples and Roccarainola). The



**Figure 1.** Map of the sampled localities numbered as in Table 1.



**Figure 2.** Landmarks of the dorsal surface of the head with names of the scales.

latter is genetically close to the eastern Pontine group. Finally, the ‘Sicilian’ population (Palermo and Siracusa) belongs to a phylogeographical unit well differentiated from the continental and the Pontine populations (Podnar *et al.*, 2005; Senczuk *et al.*, 2017, 2018). A pairwise Procrustes ANOVA (RRPP; 1000 permutations) was then computed to assess the degree of shape divergence among phylogeographical units after having controlled for sexual dimorphism

using the function *advanced.procD.lm*. Given that sexual dimorphism represents an important source of morphological variability in *Podarcis* lizards, all the subsequent analyses were carried out for males and females separately (Kaliontzopoulou *et al.*, 2007).

To assess both the interpopulation phenotypic variation and the intrapopulation variation at different times, we first performed a cluster analysis, based on the Ward method, using the Procrustes distances

among the average configurations of populations. Successively, to explore the extent of phenotypic variation in the morphospace fully, a principal component analysis (PCA) of the Procrustes coordinates was performed. Shape changes in the whole dataset were represented by deformation grids associated with PCA axes.

Finally, in order to visualize the shape differentiation between the two Pontine lineages, deformation grids between the average head configurations of the two lineages were also computed.

## RESULTS

The Procrustes ANOVA showed that both sexual dimorphism and phylogeography contribute to the overall shape and size variation (Table 2). The phylogeographical units showed a major contribution in explaining shape variability, whereas sexual dimorphism represented the main source of size variability.

In the Procrustes ANOVA pairwise test, a significant difference was found between the western Pontine phylogeographical unit compared with all the others (Table 3). The Sicilian populations still appeared to be fairly different with respect to the 'Adriatic', 'Tyrrhenian' and eastern Pontine phylogeographical units. The divergence observed between the eastern Pontine and the Adriatic phylogeographical unit was particularly low.

The cluster analysis clearly showed that the populations of the western Pontine Islands were grouped into a distinct cluster, including the 'extinct' *P. s. sanctistefanii* (collected in 1878). The populations from the eastern Pontine (Ventotene and Santo Stefano) clustered together with the mainland populations, whereas the Sicilian populations showed a slight, but evident, phenotypic divergence (Fig. 3). The morphospace described by the first two PCA axes showed a remarkable

separation of the western Pontine populations from those of the eastern Pontine, the mainland and Sicily (Fig. 4). The first two axes explained, respectively, 22.4 and 11.9% of the variance in females and 20.2 and 10.9% of the variance in males. The morphological differences observed for the western Pontine were more evident in females than in males. Deformation grids suggested that the main morphological changes were attributable to an expansion of the most proximal region of the head, as shown by landmarks 17–27, suggesting a shift of the parietal, interparietal, frontoparietal and occipital scales. Another relevant point is that the extinct population of *P. s. sanctistefanii* sampled in 1878 overlapped with the group of the western Pontine (Ponza, Palmarola, Zannone and Gavi). In contrast, the lizards sampled on Santo Stefano in 1954 and 1966, as expected, overlapped the morphospace of the eastern Pontine population of Ventotene. In addition, both the current Santo Stefano and Ventotene populations were not distinguishable from the mainland and Sicilian populations.

Moreover, when comparing the shape of the two Pontine clusters, the deformation vectors suggested that most of the shape differences between the two phenotypic forms of the Archipelago (western Pontine populations plus the extinct *P. s. sanctistefanii* vs. the eastern Pontine populations) are located again, in the posterior head region (Fig. 5).

## DISCUSSION

Islands are exciting contexts in which to study many evolutionary processes, including colonization and extinction dynamics, adaptive radiation and speciation. Our findings, based on the GMM approach, allowed us to obtain new insights on the evolutionary history of *P. siculus* of the Pontine Archipelago, suggesting a

**Table 2.** Procrustes ANOVA for the effect of sexual dimorphism and phylogeographical units on both shape and size

	d.f.	SS	MS	$R^2$	$F$	$z$	Pr(> $F$ )
Size							
Sexual dimorphism	1	28.801	28.801	0.624	766.563	3.565	0.001
Phylogeographical units	4	3.895	0.974	0.084	25.915	5.46	0.001
Residuals	358	13.451	0.038				
Total	363	46.147					
Shape							
Sexual dimorphism	1	0.0806	0.0807	0.116	56.731	9.999	0.001
Phylogeographical units	4	0.1050	0.0262	0.151	18.462	14.453	0.001
Residuals	358	0.5091	0.0014				
Total	363	0.695					

For each analysis, we report the degrees of freedom (d.f.), the sum of squares (SS), the mean squares (MS), the  $R^2$ ,  $F$  values and  $z$  scores. In all cases, a significant effect of both factors was found.

tangled scenario dominated by ancient colonization, local extinctions and population replacement. Our results also attempt to provide useful information for the identification of appropriate conservation measures.

#### MORPHOLOGICAL DIFFERENTIATION OF THE WESTERN PONTINE ISLANDS

Multivariate analysis and GMM have been particularly useful in identifying both local adaptations and operational taxonomic units in many taxa, including lizards (Sneath & Sokal, 1973; Bruschi *et al.*, 2006; Kaliontzopoulou *et al.*, 2007; Kaliontzopoulou, 2011).

**Table 3.** Pairwise Procrustes ANOVA performed to test the degree of shape differentiation among the five phylogeographical lineages

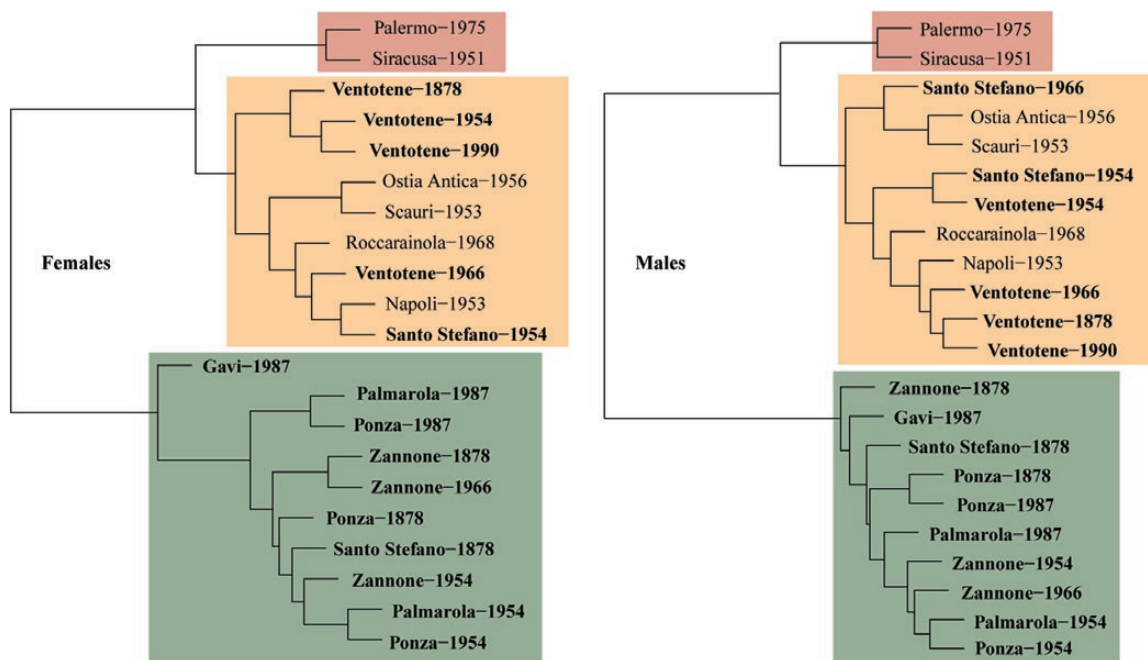
	A	WP	EP	S	T
A	–	14.4207***	2.154*	8.368***	5.947***
WP	0.031	–	19.267***	16.501***	15.464***
EP	0.011	0.029	–	10.767***	8.156***
S	0.029	0.040	0.029	–	12.360***
T	0.019	0.028	0.0191	0.0372	–

The effect size ( $\zeta$ ) with significance level (\* $< 0.05$ , \*\*\* $< 0.001$ ) above the diagonal and mean Procrustes distances between groups below the diagonal are reported. A, Adriatic; EP, eastern Pontine; S, Sicilian; T, Tyrrhenian; WP, western Pontine.

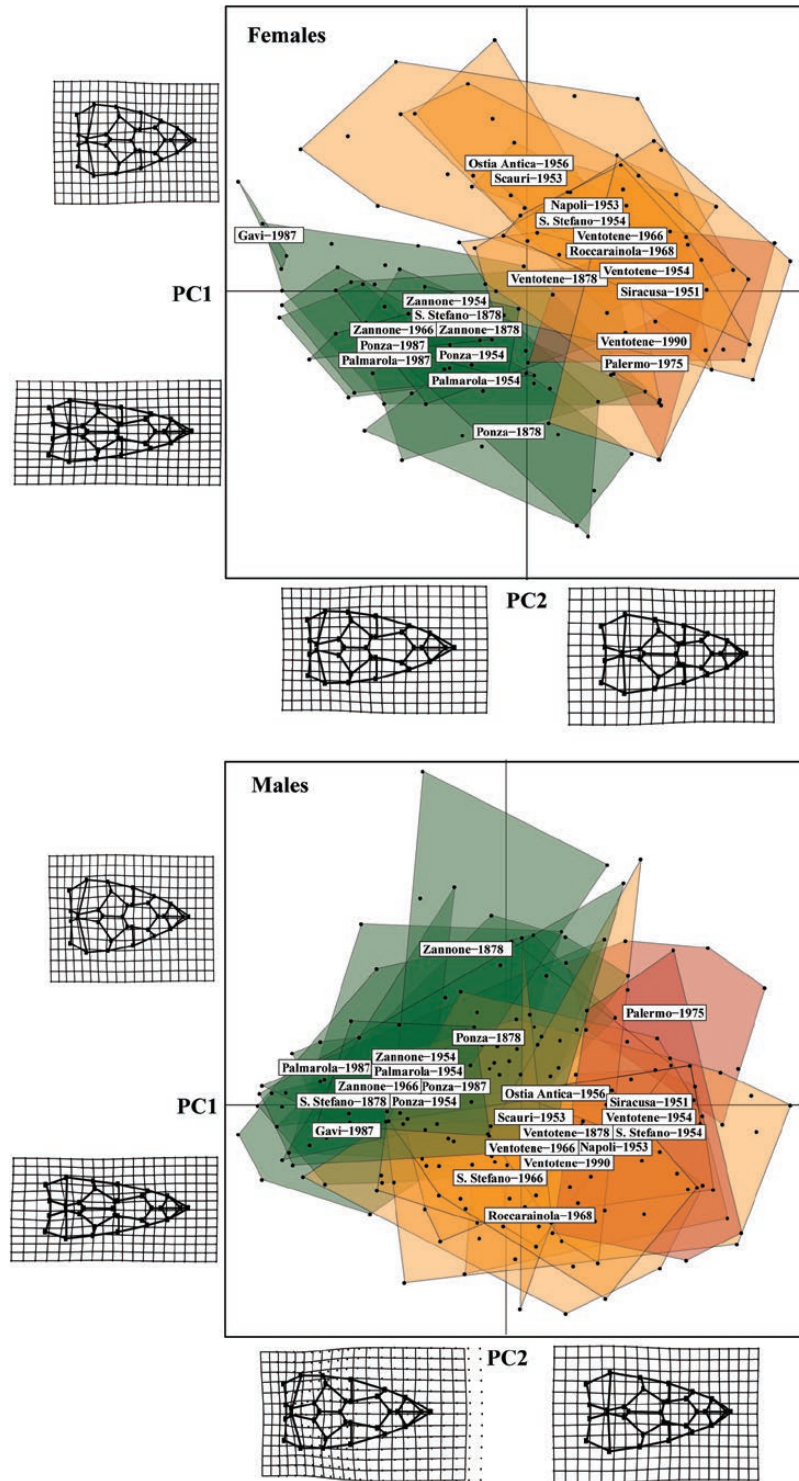
Our results, based on variation in head shape, showed that the populations of the western Pontine Islands are significantly different from those of the eastern Pontine Islands and the mainland. Results from PCA showed two principal groups, with a certain level of discrimination for both sexes albeit more pronounced in females (Fig. 3). However, the Ward cluster indicated that morphological differentiation between the mainland and Sicilian populations does not reach the morphological divergence observed between the western Pontine Islands and all the other *P. siculus* populations.

Although based on external morphology and colour patterns, at least four subspecies were described for the Archipelago, but our analysis did not detect any significant intraspecific difference in head morphology.

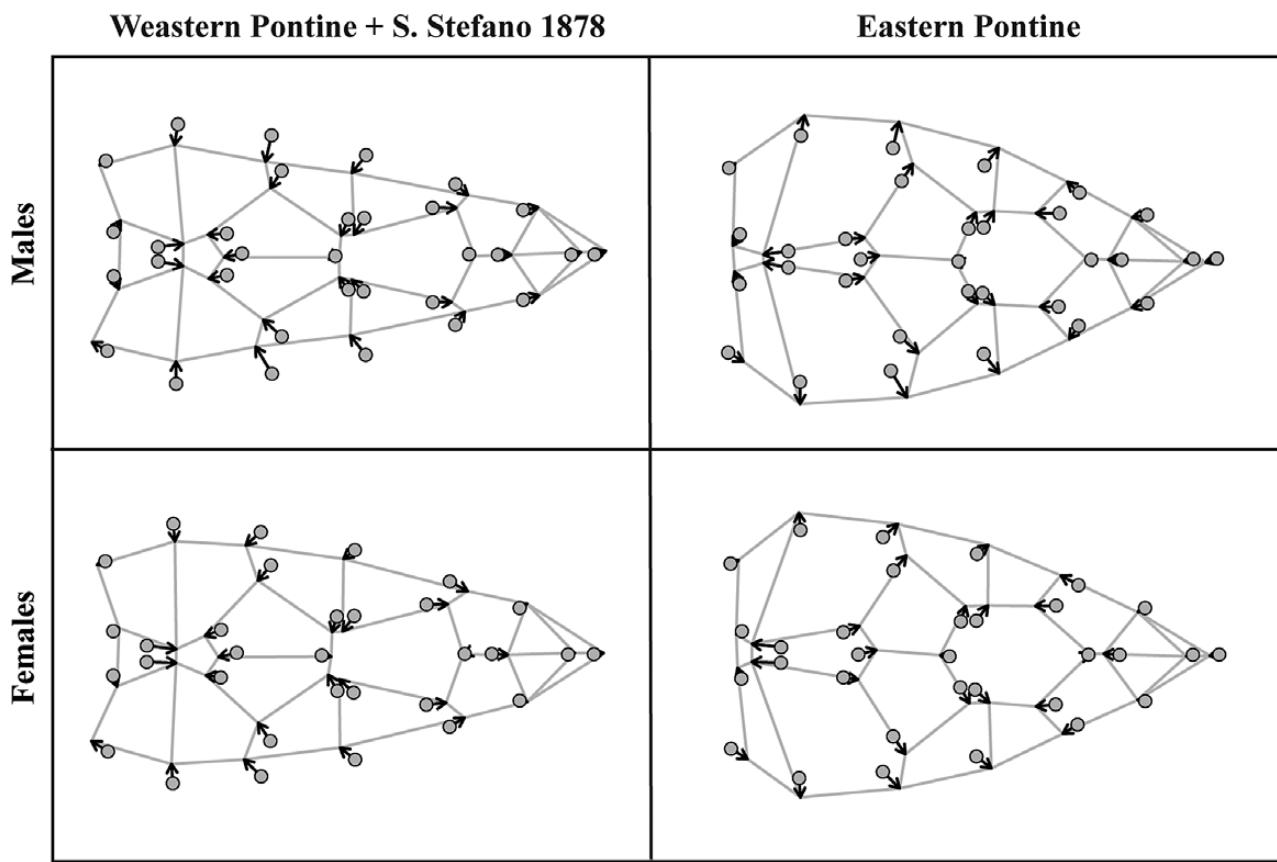
The overall head shape of the western Pontine populations shows wider cephalic scales in both sexes, which is particularly evident in the posterior part of the head, including the parietal, interparietal, frontoparietal and occipital scales. The shape of the posterior head region in lizards has been demonstrated to be a powerful predictor for bite force performance in sexual selection, including male–male competition and copulation with females (Herrel *et al.*, 2001; Husak *et al.*, 2006; Kaliontzopoulou *et al.*, 2007). Indeed, a wider parietal region could provide more space for the insertion of the adductor muscles, resulting in an improved bite performance. The differences found in the posterior region of the head, although generally evident in males, when



**Figure 3.** Cluster based on Procrustes distances separated according to sex. The cluster shows that the western Pontine lizards (green) are phenotypically well differentiated from the lizards sampled in other localities. The Sicilian populations (red) also differ from the Eastern Pontine, Adriatic and Tyrrhenian lineages. Populations from the Pontine Archipelago are in bold.



**Figure 4.** Principal component analysis showing deformation grids separated according to sex. The first two axes account, respectively, for 22.4 and 11.9% of the total variance for females (above) and for 20.2 and 10.9% of the total variance for males (below). Colours depict the three principal groups as identified by the cluster analyses. Deformation grids represent shape modification along principal component 1 (PC1) and principal component 2 (PC2) magnified  $\times 1.5$ .



**Figure 5.** Deformation vectors (magnified  $\times 3$ ) showing shape differences for males (upper row) and females (lower row) between the eastern Pontine (right) and the western Pontine (left) lineages.

comparing the western Pontine populations and continental ones, are particularly evident also in females, with a fourfold difference compared with that observed for sexual dimorphism. This result might suggest that other ecological pressures are acting on lizards living on the islands rather than a strong discrepancy in bite force related to sexual selection. For example, it is known that some insular *Podarcis* species, when arthropods became scarce, feed on plant matter that requires stronger adductor muscles to be consumed (Pérez-Mellado & Corti, 1993; Herrel *et al.*, 2001; Verwajen *et al.*, 2002). Nonetheless, the significant differences, by far exceeding those observed for sexual dimorphism, do not exclude the dominant role of other forces, either stochastic or selective, related to the long evolutionary history of the western Pontine lizards. However, given that we focused only on the external head morphology, such considerations may remain speculative, and further studies should be addressed to functional anatomy.

Finally, the differences in sexual dimorphism, a dominant component in the overall morphological variations observed in *Podarcis* lizards, are in agreement with previous studies indicating that they are mainly linked to size, with males larger than females having a

more robust head (Olsson *et al.*, 2002; Kaliontzopoulou *et al.*, 2007; Piras *et al.*, 2011). However, as a general pattern, a size reduction was also observed for all the studied insular populations.

#### SHEDDING LIGHT ON THE MYSTERY

In 1878, when Sir Arthur Conan Doyle wrote about Sherlock Holmes seeking for a test to detect human haemoglobin, the Italian zoologist E. H. Giglioli was collecting lizards, cruising the Mediterranean islands with the *Regia Goletta Olga*. Among others, 15 specimens were collected on Santo Stefano Island (Pontine Archipelago) and deposited in the MZUF. In 1926, R. Mertens described the subspecies *P. s. sanctistephani* based on specimens captured in 1914 by G. de Southoff. Over the following 30 years, the endemic island population became extinct and was replaced by the ordinary mainland phenotype, as attested by B. Lanza in 1954. This small circular island (0.27 km<sup>2</sup>), the easternmost of the Archipelago, is located 1.4 km far from Ventotene and characterized by a rocky coast and rich, low vegetation cover (Lanza & Corti, 1996).



Interestingly, the extinct population of Santo Stefano Island clustered in all analyses with the populations of the western Pontine Islands, whereas the two populations sampled in 1954 and 1966 clustered with the mainland and Ventotene populations. In addition, all the chronological series of the western Pontine Islands are part of the same group, suggesting that head morphology did not change over the past 150 years, as observed for other insular lizard populations (Losos *et al.*, 1997; Herrel *et al.*, 2008; Stuart *et al.*, 2014; de Amorim *et al.*, 2017). In contrast, a rapid phenotypic differentiation of the colour pattern of another reptile species of the Pontine Archipelago was discussed by Avella *et al.* (2017).

The GMM analysis indicated a strong overlapping of the morphological head shape of the extinct population and the current populations inhabiting the western Pontine Islands, suggesting that a similar ancient lineage was also present on the eastern Pontine Islands. In addition, considering the geographical setting of the eastern Pontine Islands, we could argue with reasonable confidence that an earlier extinction also occurred on Ventotene Island. Concerning this possible extinction, we can only speculate on the relevance of the ancient Roman port, which might have facilitated the introduction of the 'ordinary' phenotype from mainland Italy to Ventotene Island.

On the basis of molecular analyses, seemingly no traces of introgression were observed in the current population of Santo Stefano Island (Biaggini *et al.*, 2009), and our morphometric analysis also suggests that hybridization probably did not occur. Indeed, if the native population were to have mixed with the allochthonous one, we would have expected at least an intermediate shape in the individuals collected in 1953. The possible absence of hybridization between the two forms could be attributable to several non-exclusive factors. First, it could be hypothesized that the extinction of *P. s. sanctistephani* happened before the arrival of the current population, for instance because of habitat loss, pathogens or the introduction of predators. However, it should be emphasized that habitat loss and anthropogenic pressures have not increased significantly over the last century in Santo Stefano Island, because it was a penal colony lasting less than 200 years. The second hypothesis takes into consideration the possibility that the population of Santo Stefano became extinct after the arrival of *P. siculus* from the mainland or, more plausibly, from Ventotene Island. If this is the case, we cannot rule out the possibility that any behavioural or ecological modification that occurred during the allopatric divergence, such as differences in competitive abilities, mating success or other incipient reproductive isolation mechanisms, might have contributed to the extinction of the endemic population.

#### CONCLUDING REMARKS

One of the most challenging tasks in evolutionary biology is to assess whether a taxon diverging in allopatry has acquired enough differences to deserve its own specific status. Indeed, although the recognition of new taxa can be trivial for island endemic taxa that show deep divergences and evident differences in morphology and behaviour, as for oceanic islands, the identification of incipient species can be problematic when considering island populations that have diverged recently. The identification of new species in this context requires integrated approaches to provide evidence from multiple disciplines, such as genetics, morphology and ecology (Carretero, 2008). In the present study, we exploited the potential of GMM to disclose biogeographical dynamics of *P. siculus* populations in the Pontine Archipelago. We found a strong correlation between the reconstruction of evolutionary history based on GMM and the one obtained using genetic data (Senczuk *et al.*, 2018). The morphological differentiation that we found suggests that the western Pontine populations seem to be a case of incipient speciation, therefore in need of a taxonomic revision in order to define and implement proper conservation measures. Indeed, populations such as those inhabiting islands are more subject to both natural and anthropic pressures and are therefore particularly prone to extinction (Olson, 1989; Williamson, 1989). In view of this, the species replacement reported here suggests that further introduction from the mainland could be fatal and should be prevented.

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#### REFERENCES

- Adams C, Collyer ML, Kaliontzopoulou A, Sherratt E. 2017. *Geomorph: software for geometric morphometric analyses. R package version 3.0.5*. Available at: <https://cran.r-project.org/package=geomorph>
- Allendorf FW, Luikart G. 2009. *Conservation and the genetics of populations*. New York City: John Wiley & Sons.
- de Amorim ME, Schoener TW, Santoro GRCC, Lins ACR, Piovia-Scott J, Brandão RA. 2017. Lizards on newly created islands independently and rapidly adapt in morphology and diet. *Proceedings of the National Academy of Sciences of the United States of America* **114**: 8812–8816.

- Avella I, Castiglia R, Senczuk G. 2017.** Who are you? The genetic identity of some insular populations of *Hierophis viridiflavus* sl from the Tyrrhenian Sea. *Acta Herpetologica* **12**: 209–214.
- Biaggini M, Nulchis V, Carretero MA, Cipolla RM, Corti C, Nappi A, Harris DJ. 2009.** Low genetic differentiation between populations of *Podarcis sicula* (Reptilia, Lacertidae) from the Italian islands off the coast of Campania and the mainland. *The Belgian Journal of Zoology* **139**: 169–172.
- Biber E. 2002.** Patterns of endemic extinctions among island bird species. *Ecography* **25**: 661–676.
- Bookstein FL. 1997.** *Morphometric tools for landmark data: geometry and biology*. Cambridge: Cambridge University Press.
- Bruno S. 1982.** Catalogo sistematico, zoogeografico e geonomico dei Lacertidae di Corsica, Italia e isole Maltesi. *Natura Bresciana* **19**: 39–95.
- Bruschi S, Corti C, Carretero MA, Harris DJ, Lanza B, Leviton AE. 2006.** Comments on the status of the Sardinian-Corsican Lacertid lizard *Podarcis tiliguerta*. *Proceedings-California Academy of Sciences* **57**: 225.
- Cadoux A, Pinti DL, Aznar C, Chiesa S, Gillot PY. 2005.** New chronological and geochemical constraints on the genesis and geological evolution of Ponza and Palmarola volcanic islands (Tyrrhenian Sea, Italy). *Lithos* **81**: 121–151.
- Carretero MA. 2008.** An integrated assessment of a group with complex systematics: the Iberomaghrebian lizard genus *Podarcis* (Squamata, Lacertidae). *Integrative Zoology* **3**: 247–266.
- Castilla AM, Fernández Pedrosa V, Backeljau T, González A, Latorre A, Moya A. 1998.** Conservation genetics of insular *Podarcis* lizards using partial cytochrome *b* sequences. *Molecular Ecology* **7**: 1407–1411.
- Corti C, Biaggini M, Capula M. 2010.** *Podarcis siculus* (Rafinesque-Schmaltz, 1810). In: Corti C, Capula M, Luiselli L, Razzetti E, Sindaco R, eds. *Fauna d'Italia. Reptilia*. Bologna: Edizioni Calderini de Il Sole 24 Ore Editoria Specializzata. ISBN 9788850653621.
- Corti C, Böhme W, Delfino M, Masseti M. 1999.** Man and Lacertids on the Mediterranean islands: Conservation perspectives. *Natura Croatica* **8**: 287–300.
- Darwin C. 1851.** *Geological observations on coral reefs, volcanic islands, and on South America: being the geology of the voyage of the Beagle, under the command of Captain Fitzroy, RN, during the years 1832 to 1836*. London: Smith, Elder.
- Foufopoulos J, Ives AR. 1999.** Reptile extinctions on land-bridge islands: life-history attributes and vulnerability to extinction. *The American Naturalist* **153**: 1–25.
- Gherardi F, Corti C, Gualtieri M. 2002.** Biodiversity conservation and habitat management. In: Gherardi F, Corti C, Gualtieri M, eds. *Encyclopedia of life support systems*. Oxford: Eolss Publishers/UNESCO, 325–362.
- Goodall C. 1991.** Procrustes methods in the statistical analysis of shape. *Journal of the Royal Statistical Society, Series B (Methodological)* **53**: 285–339.
- Harradine E, How RA, Schmitt LH, Spencer PBS. 2015.** Island size and remoteness have major conservation significance for how spatial diversity is partitioned in skinks. *Biodiversity and Conservation* **24**: 2011–2029.
- Harris DJ, Pinho C, Carretero MA, Corti C, Böhme W. 2005.** Determination of genetic diversity within the insular lizard *Podarcis tiliguerta* using mtDNA sequence data, with a reassessment of the phylogeny of *Podarcis*. *Amphibia-Reptilia* **26**: 401–407.
- Harris DJ, Sá-Sousa P. 2002.** Molecular phylogenetics of Iberian wall lizards (*Podarcis*): is *Podarcis hispanica* a species complex? *Molecular Phylogenetics and Evolution* **23**: 75–81.
- Herrel A, Damme RV, Vanhooydonck B, Vree FD. 2001.** The implications of bite performance for diet in two species of lacertid lizards. *Canadian Journal of Zoology* **79**: 662–670.
- Herrel A, Huyghe K, Vanhooydonck B, Backeljau T, Breugelmanns K, Grbac I, 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**: 4792–4795.
- Husak JF, Lappin AK, Fox SF, Lemos-Espinal JA. 2006.** Bite-force performance predicts dominance in male venerable collared lizards (*Crotaphytus antiquus*). *Copeia* **2006**: 301–306.
- Illera JC, Spurgin LG, Rodriguez-Exposito E, Nogales M, Rando JC. 2016.** What are we learning about speciation and extinction from the Canary Islands? *Ardeola* **63**: 15–33.
- Kaliontzopoulou A. 2011.** Geometric morphometrics in herpetology: modern tools for enhancing the study of morphological variation in amphibians and reptiles. *Basic and Applied Herpetology* **25**: 5–32.
- Kaliontzopoulou A, Carretero MA, Llorente GA. 2007.** Head shape allometry and proximate causes of head sexual dimorphism in *Podarcis* lizards: joining linear and geometric morphometrics. *Biological Journal of the Linnean Society* **93**: 111–124.
- Lanza B, Corti C. 1996.** Evolution of the knowledge on the Italian herpetofauna during the 20th century. *Bollettino del Museo Civico di Storia Naturale di Verona* **20**: 373–436.
- Losos JB, Schluter D. 2000.** Analysis of an evolutionary species-area relationship. *Nature* **408**: 847–850.
- Losos JB, Warheit K, Schoener TW. 1997.** Adaptive differentiation following experimental island colonization in *Anolis* lizards. *Nature* **387**: 70–73.
- Lymberakis P, Poulakakis N. 2010.** Three continents claiming an archipelago: the evolution of Aegean's herpetofaunal diversity. *Diversity* **2**: 233–255.
- MacArthur RH, Wilson EO. 1967.** *The theory of island biogeography*. Princeton: Princeton University Press.
- Martín JL. 2009.** Are the IUCN standard home-range thresholds for species a good indicator to prioritise conservation urgency in small islands? A case study in the Canary Islands (Spain). *Journal for Nature Conservation* **17**: 87–98.
- Mayr E. 1995.** Species, classification, and evolution. In: Arai R, Kato M, Doi Y, eds. *Biodiversity and evolution*. Tokyo: National Science Museum Foundation, 3–122.
- Mertens R. 1926.** Zwei neue Inselrassen der Gattung *Lacerta*. *Zoologischer Anzeiger* **68**: 319–322.
- Mertens R. 1934.** Die Inselreptilien, ihre Ausbreitung, Variation und Artbildung. *Zoologica Stuttgart*, **84**: 1–108.
- Mertens R. 1956.** Das Rätsel der Eidechsen von Santo Stefano. *Zoologische Jahrbücher, Abteilung Systematik* **92**: 91–102.

- Oliverio M, Bologna MA, Mariottini P. 2000.** Molecular biogeography of the Mediterranean lizards *Podarcis* Wagler, 1830 and *Teira* Gray, 1838 (Reptilia, Lacertidae). *Journal of Biogeography* **27**: 1403–1420.
- Oliverio M, Bologna MA, Monciotti A, Annesi F, Mariottini P. 1998.** Molecular phylogenetics of the Italian *Podarcis* lizards (Reptilia, Lacertidae). *Italian Journal of Zoology* **65**: 315–324.
- Olson SL. 1989.** Extinction on islands: man as a catastrophe. In: Western D, Pearl M, eds. *Conservation for the twenty-first century*. New York: Oxford University Press, 50–53.
- Olsson M, Shine R, Wapstra E, Uivari B, Madsen T. 2002.** Sexual dimorphism in lizard body shape: the roles of sexual selection and fecundity selection. *Evolution* **56**: 1538–1542.
- Pérez-Mellado V, Corti C. 1993.** Dietary adaptations and herbivory in lacertid lizards of the genus *Podarcis* from western Mediterranean islands (Reptilia: Sauria). *Bonner zoologische Beiträge* **44**: 193–220.
- Pérez-Mellado V, Corti C, Lo Cascio P. 1997.** Tail autotomy and extinction in Mediterranean lizards. A preliminary study of continental and insular populations. *Journal of Zoology* **243**: 533–541.
- Piras P, Salvi D, Ferrara G, Maiorino L, Delfino M, Pedde L, Kotsakis T. 2011.** The role of post-natal ontogeny in the evolution of phenotypic diversity in *Podarcis* lizards. *Journal of Evolutionary Biology* **24**: 2705–2720.
- Podnar M, Mayer W, Tvrtković N. 2005.** Phylogeography of the Italian wall lizard, *Podarcis sicula*, as revealed by mitochondrial DNA sequences. *Molecular Ecology* **14**: 575–588.
- Poulakakis N, Lymberakis P, Valakos E, Pafilis P, Zouros E, Mylonas M. 2005.** Phylogeography of Balkan wall lizard (*Podarcis taurica*) and its relatives inferred from mitochondrial DNA sequences. *Molecular Ecology* **14**: 2433–2443.
- Psonis N, Antoniou A, Kukushkin O, Jablonski D, Petrov B, Crnobrnja-Isailović J, Sotiropoulos K, Gherghel I, Lymberakis P, Poulakakis N. 2017.** Hidden diversity in the *Podarcis tauricus* (Sauria, Lacertidae) species subgroup in the light of multilocus phylogeny and species delimitation. *Molecular Phylogenetics and Evolution* **106**: 6–17.
- Rohlf FJ. 2005.** *tpsDig, digitize landmarks and outlines, version 2.05*. Stony Brook: Department of Ecology and Evolution, State University of New York.
- Rohlf FJ, Slice D. 1990.** Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Biology* **39**: 40–59.
- Sax DF, Gaines SD. 2008.** Species invasions and extinction: the future of native biodiversity on islands. *Proceedings of the National Academy of Sciences of the United States of America* **105**(Supplement 1): 11490–11497.
- Sax DF, Gaines SD, Brown JH. 2002.** Species invasions exceed extinctions on islands worldwide: a comparative study of plants and birds. *The American Naturalist* **160**: 766–783.
- Senczuk G, Colangelo P, De Simone E, Aloise G, Castiglia R. 2017.** A combination of long term fragmentation and glacial persistence drove the evolutionary history of the Italian wall lizard *Podarcis siculus*. *BMC Evolutionary Biology* **17**: 6.
- Senczuk G, Havenstein K, Milana V, Ripa C, De Simone E, Tiedemann R, Castiglia R. 2018.** Spotlight on islands: on the origin and diversification of a new lineage of the Italian wall lizard *Podarcis siculus* in the western Pontine Islands. *bioRxiv* doi: <https://doi.org/10.1101/293985>.
- Sillero N, Campos J, Bonardi A, Corti C, Creemers R, Crochet PA, Crnobrnja Isailović J, Denoël M, Ficetola FG, Gonçalves J, Kuzmin S, Lymberakis P, De Pous P, Rodríguez A, Sindaco R, Speybroeck J, Toxopeus B, Vieites DR, Vences M. 2014.** Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. *Amphibia-Reptilia* **35**: 1–31.
- Simberloff DS, Wilson EO. 1969.** Experimental zoogeography of islands: the colonization of empty islands. *Ecology* **50**: 278–296.
- Sneath PH, Sokal RR. 1973.** *Numerical taxonomy. The principles and practice of numerical classification*. San Francisco: Freeman, 573.
- Stuart YE, Campbell TS, Hohenlohe PA, Reynolds RG, Revell LJ, Losos JB. 2014.** Rapid evolution of a native species following invasion by a congener. *Science* **346**: 463–466.
- 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**: 842–850.
- Wallace AR. 1892.** *Island Life; or the phenomena and causes of insular faunas and floras: including a revision and attempted solution of the problem of geological climates*. New York City: Macmillan and company.
- Williamson M. 1989.** Natural extinction on islands. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **325**: 457–466; discussion 466.