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.


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 (Martin, 2009). This scenario is even more complicated by the fact that insular biota may hide proper species identification.
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 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 phyleogeographical structure and considerable morphological variation found within *P. siculus*, the occurrence of a species complex has been argued, with ≥ 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, Psomis 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 phyleogeographical 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 latestei (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 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 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 phylogenetic relationships in previous studies (Podnar et al., 2005; Senczuk et al., 2017, 2018).

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

<table>
<thead>
<tr>
<th>No.</th>
<th>Locality</th>
<th>Date</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ponza</td>
<td>1878</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>Gavi</td>
<td>1954</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>Palmarola</td>
<td>1987</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Zannone</td>
<td>1878</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>Santo Stefano</td>
<td>1878</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>Ventotene</td>
<td>1878</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>Ostia Antica</td>
<td>1953</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>Scauri</td>
<td>1953</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>Napoli</td>
<td>1953</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>10</td>
<td>Roccarainola</td>
<td>1968</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>11</td>
<td>Palermo</td>
<td>1975</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>12</td>
<td>Siracusa</td>
<td>1951</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>167</td>
<td>207</td>
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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’, ‘Tyrrenian’ 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. sanctistephanii* 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>
<thead>
<tr>
<th>Table 2. Procrustes ANOVA for the effect of sexual dimorphism and phylogeographical units on both shape and size</th>
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<tr>
<td></td>
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<tr>
<td>Size</td>
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<tr>
<td>Sexual dimorphism</td>
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<tr>
<td>Phylogeographical units</td>
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<td>Residuals</td>
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<tr>
<td>Total</td>
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<tr>
<td>Shape</td>
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<tr>
<td>Sexual dimorphism</td>
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<td>Phylogeographical units</td>
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<tr>
<td>Residuals</td>
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<tr>
<td>Total</td>
</tr>
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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.

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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).

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

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

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>WP</th>
<th>EP</th>
<th>S</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>14.4207***</td>
<td>2.154*</td>
<td>8.368***</td>
<td>5.947***</td>
<td></td>
</tr>
<tr>
<td>WP</td>
<td>0.031</td>
<td>–</td>
<td>19.267***</td>
<td>16.501***</td>
<td>15.464***</td>
</tr>
<tr>
<td>EP</td>
<td>0.011</td>
<td>0.029</td>
<td>–</td>
<td>10.767***</td>
<td>8.156***</td>
</tr>
<tr>
<td>S</td>
<td>0.029</td>
<td>0.040</td>
<td>0.029</td>
<td>–</td>
<td>12.360***</td>
</tr>
<tr>
<td>T</td>
<td>0.019</td>
<td>0.028</td>
<td>0.0191</td>
<td>0.0372</td>
<td>–</td>
</tr>
</tbody>
</table>

The effect size ($\times$) 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.

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 ×1.5.
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; Verwaijen 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 km²), 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.

ACKNOWLEDGEMENTS

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