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RESEARCH ARTICLE

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First record of the Italian wall lizard *Podarcis siculus* on the island of Crete, Greece

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ABSTRACT – The Italian wall lizard *Podarcis siculus* is a species distributed in Italy and the east Adriatic coast. It is also a well-known invasive species introduced into four continents and many countries outside its native range. In 2014, an established population of this species was recorded in Athens, Greece, which was identified as *Podarcis siculus campestris* by molecular analysis. Here, we report for the first time the presence of another subspecies, *Podarcis siculus siculus*, in Greece and specifically on the island of Crete. Tissue samples from six adult and subadult lizards were analysed to reconstruct a mitochondrial phylogeny using a segment of cytochrome b and all published sequences of native and introduced populations of *P. siculus*. We identified the source-population of the Cretan *P. siculus* to be located in Sicily, while the Athenian population originated from the Adriatic coast. This indicates two independent introduction events in a period of < 10 years. The Cretan population of *P. siculus* seems to be well established and may pose a future threat to the endemic Cretan wall lizard *Podarcis cretensis*.

INTRODUCTION

Invasive species constitute one of the major current threats to global biodiversity. They can harm native species in several ways, such as through predation (Cowie, 2001; Montes et al., 2022), hybridisation (Rhymer & Simberloff, 1996; Pinho et al., 2009), by the spread of alien parasites and pathogens (Chalkowski et al., 2018) and by competition for more suitable microhabitats and access to more abundant and higher-quality food (Petren & Case, 1996; Ranat et al., 2023). As a result, in many cases, native species are displaced, sometimes to the point of complete extinction (Towns et al., 2006; Lo Cascio & Sciberras, 2020). There are numerous cases of invasive species globally, that involve species from almost every taxon, such as mammals, fishes and reptiles (Blackburn et al., 2019). In the case of reptiles, the most probable reason for translocations is involuntary human-mediated transport, e.g. via the trade of plants, building materials etc. but also the pet trade and the accidental or even voluntary release of non-native pet-reptiles (Farashi & Alizadeh-Noughani, 2021).

The natural range of the Italian wall lizard *Podarcis siculus* includes most of Italy, excluding the northern mountainous region and the eastern Adriatic coasts. It is a medium-sized species, with a snout-vent length (SVL) of approximately 9 cm, characterised by a wide variety of colour patterns. Regarding its taxonomy, fifty-two subspecies were previously described (Henle & Klaver, 1986), but these have been refuted by more comprehensive studies, primarily based on mitochondrial DNA evidence. Podnar et al. (2005) described six mitochondrial clades, which were grouped into two main clades with three subclades each. Senczuk et al. (2017), using mitochondrial DNA analyses and a larger, more representative sample, identified seven mitochondrial clades, also divided into two main clades. The first main clade includes the regions of Sicily and Calabria (southern Italy) and corresponds to the subspecies *Podarcis siculus siculus*, while the second clade encompasses central and northern Italy as well as the eastern Adriatic coasts and represents the subspecies *Podarcis siculus campestris* (Podnar et al., 2005; Speybroeck et al., 2016; Senczuk et al., 2017).

Podarcis siculus is a well-known invasive species. Numerous cases have been documented of the species' introduction and even establishment and spread to various countries around the world, including Portugal (González de la Vega et al., 2001), Spain (Mertens & Wermuth, 1960), mainland France (Orsini, 1984) and Corsica (Sindaco et al., 2006), Great Britain (Hodgkins et al., 2012), Switzerland (Schulte & Gebhart, 2011), Greece (Adamopoulou, 2015), Turkey (Başoğlu & Baran, 1977), Cyprus and Montenegro (Speybroeck et al., 2016), Azerbaijan (Iskenderov et al., 2021), Russia (Tuniyev et al., 2020), Tunisia and Libya (Arnold & Ovenden, 1978), the United States (Deichsel et al., 2010; Kolbe et al., 2013) and Canada (Hanke & Deichsel, 2020).

On a herpetological tour that took place in October 2023 across a stream at Kladisos beach, near Chania, Crete, Greece (35° 30'42" N, 24° 00'08" E; Fig. 1), AZ encountered a robust population of *Podarcis* lizards. Adults and juveniles were mainly encountered basking on a wall beside the stream, in a habitat consisting of reeds and several species of non-native palms. This stream lies at the edge of a small semi-natural area of about fifteen hectares with sand dunes, surrounded by an urban landscape. Several (> 100) lizards were detected, and the inspection of their morphological characteristics suggested that they were probably Sicilian wall lizards. The aim of the current study was to identify these lizards at the species-level and to clarify their geographical origin using molecular techniques.

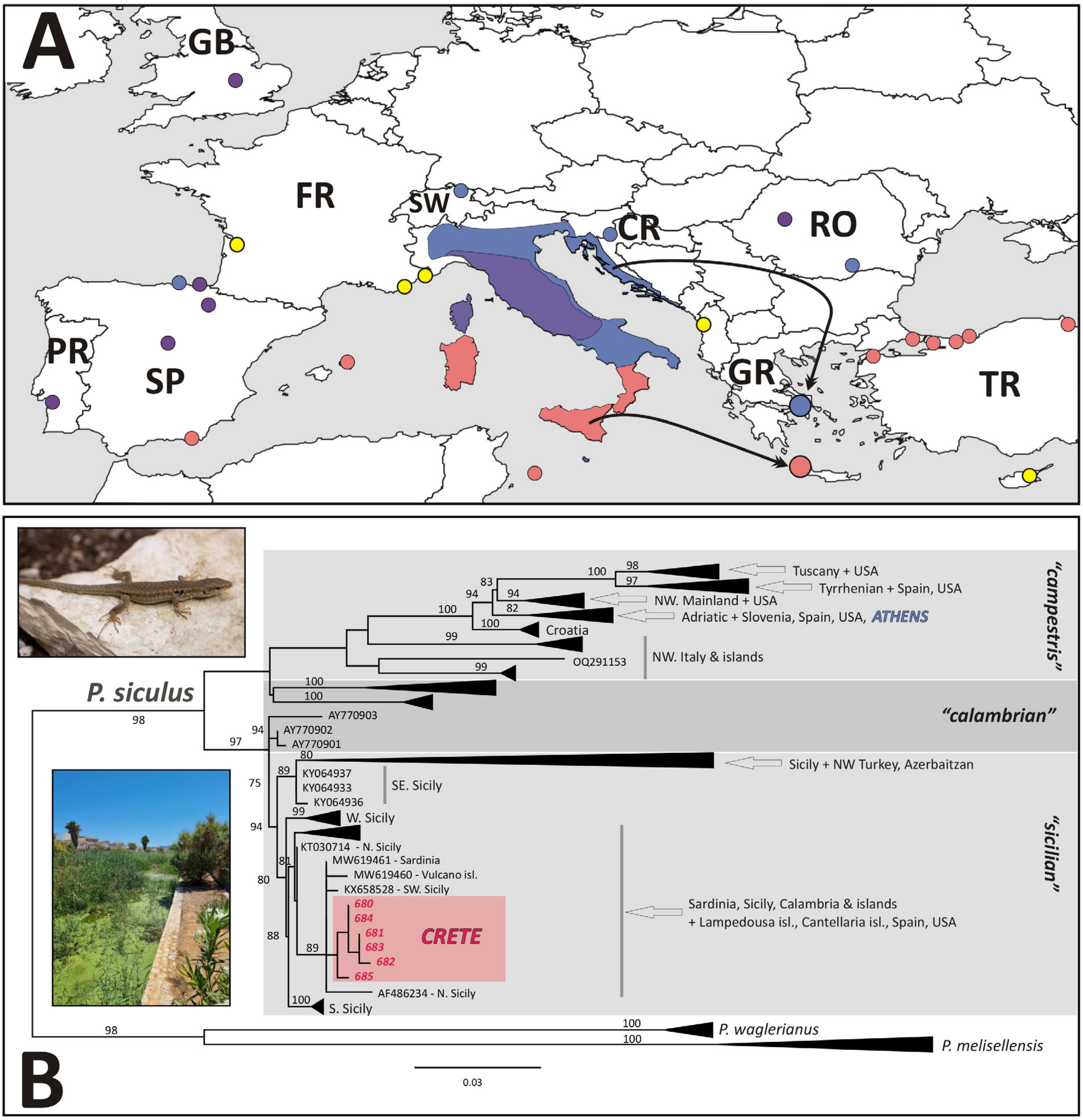

Figure 1. **A.** Map of the native geographical distribution of *Podarcis siculus* (coloured area) and the respective distribution of the main three mitochondrial lineages identified by Senćzuk et al. (2017) (pink, blue and purple). Introduced populations around the Mediterranean are shown with coloured circles, according to their origin (country names given with a two-letter code (after Mizsei et al., 2016: Speybroeck et al,, 2016; Koç et al., 2018; Oskyrko et al., 2022a). Yellow circles denote introduced populations of unknown origin. Big circles show the location of the two alien populations of Greece (Athens, blue; Crete, pink) and arrows show the source-location. **B.** The maximum-likelihood (ML) mitochondrial gene tree. The major phylogenetic clades are collapsed, except in the case where the Cretan specimens are placed. Nodal support is given in the values besides each remaining node as posterior probabilities and are shown only when > 50. The main three mitochondrial lineages are shaded and the geographical location of the individuals included in each lineage is given. The inserted photographs depict the habitat of the introduced Cretan population (Kladisos stream) and one of its individuals.

MATERIALS & METHODS

We visited the stream at Kladisos beach to collect tissue samples from the focal population of *Podarcis* lizards. Six individuals, two adults and four juveniles, were captured by hand and the autotomised tips of their tails were collected by gently pulling on the end of the tail until a small segment broke off along a fracture plane. Tail-tips preserved in 95% alcohol were transferred to the Evolutionary Biology Lab, Department of Biology, University of Patras, Greece, and deposited in its herpetological collection (voucher numbers 680–685). These tissue samples were processed through

DNA analysis to confirm the initial identification at the species level and to assess the phylogenetic position and geographical origin of the Cretan population.

Total DNA was extracted and the commonly used mitochondrial marker cytochrome b (cytb) was PCR-amplified, using the primers GluDG-L (5’ TGA CTT GAA RAA CCA YCG TTG 3’) and CB3H (5’ GGC AAA TAG GAA RTA TCA TTC 3’) from Palumbi (1991) and the sequencing protocol of Oskyrko et al. (2022b). PCR-products were sequenced in both forward and reverse directions and final sequences were approximately 500 bps long. The six sequences from the Cretan population were individually compared against the many thousands of sequences belonging to numerous representative species of the genus *Podarcis* that are deposited in GenBank. Specifically, we used the BLAST search-algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for highly similar sequences (Mega BLAST) and found maximum similarity to *P. siculus*. One representative from several lineages of the peri-Mediterranean *Podarcis*, i.e. Spanish, Italian and Balkan wall-lizards (see Yang et al., 2021), was retrieved from GenBank and a preliminary, backbone phylogeny (not shown) was built including our own samples from Crete. The Cretan samples were placed in the *P. siculus* clade, verifying the original macroscopic identification at the species level.

In order to detect the source of the introduced Cretan population, we downloaded all available cytb sequences of *P. siculus* published in GenBank (> 600), excluding those of a dubious or unknown origin, a length of < 400 bps or a high percentage of missing data. The remaining sequences were aligned with ClustalX v.2.0.12 (Larkin et al., 2007). Our final dataset included the majority of published sequences from across the native range of *P. siculus*, all the localities where introduction of this species has been reported, and the newly founded population of Crete. Sequences of two closely related wall-lizard species, *Podarcis waglerianus* and *Podarcis melisellensis* served as outgroup, adding to a total of 786 sequences (Supplementary Table S1). A Maximum Likelyhood (ML) tree was reconstructed under the 3-codon partitioned scheme implemented in IQ-TREE v.1.6.7 (Nguyen et al., 2015). The best substitution model was determined with the modelfinder option (Chernomor et al., 2016; Kalyaanamoorthy et al., 2017) and nodal support of the tree was tested via 10,000 replicates of the SH-like approximate likelihood ratio test (SH-aLRT; Guindon et al., 2010). The analysis was repeated twice to ensure convergence.

RESULTS

Our six cytb sequences revealed four very similar haplotypes, differing among each other only in one or two single-site polymorphisms. None of these haplotypes was identical to any of the other analysed sequences either of native or introduced Sicilian wall lizards, but was almost identical to one haplotype from Alghero, north-west Sardinia and another from the Vulcano Isle (north-east Sicily) (Salvi et al., 2021). It should be noted that the lack of exact similarity among the Cretan haplotypes and other previously published sequences of *P. siculus* may not reflect true genetic divergence but could be due to differences in the methodological approach applied in each case (primer-pair, PCR-conditions, PCR-product length etc.).

The ML analyses returned the same tree (Fig. 1B), with a topology very similar to the numerous published mitochondrial phylogenies of *P. siculus*, for example those in Podnar et al. (2005), Silva-Rocha et al. (2014), Senczuk et al. (2017) and Oskyrko et al. (2022b). As in all previous phylogenies, we also retrieved several mitochondrial clades and subclades. The two major ones correspond to the two subspecies *P. s. campestris* and *P. s. siculus* which are geographically located at the north and south, respectively, of the natural distribution of the two species and meet in Calabria (southern Italy). In general, all sequences from Crete were placed in the same clade with sequences natively distributed in southern Italy (Calambria), Sicily and adjacent islands (Fig. 1B). This clade, named “the Sicilian” clade by many authors (e.g. Silva-Rocha et al., 2014), is also the origin of many worldwide, overseas invasions of the species, such as Sardinia (Senczuk et al., 2017), Menorca (Podnar et al., 2005) and California (Kolbe et al., 2013). Interestingly, another introduced population of this species that had been recently found in Athens, Greece (Adamopoulou, 2015; Fig. 1A) is placed in another clade, namely “the Campestris-sicula”, which corresponds to one of the subclades within the *P. s. campestris* subspecies (see also Silva-Rocha et al., 2014).

DISCUSSION

The Sicilian wall lizards found in west Crete share a very similar mtDNA haplotype with the Sicilian populations, a fact that implies that they are probably a result of a recent human-induced dispersal from there. However, we cannot exclude the possibility of a secondary dispersal from other introduced populations, such as Turkish or Sardinian ones. As documented in other cases, e.g. in Spanish or North American populations which originated from several different sources, the two currently known populations of *P. siculus* introduced in Greece originate from geographically distant regions and were established after two recent but distinct human-mediated events.

The Cretan population is probably very recent, as these lizards had not been observed until now, despite the high herpetological interest focused on Crete and the numerous scientific expeditions and herpetological tours of the past years. The ongoing transportation of people and products across the Mediterranean has acted as an agent for the passive dispersal of many reptiles (Bisbal-Chinesta et al., 2020 and references therein). In the case of *P. siculus* in Crete, we consider the plant trade to be the most probable means of the lizards’ transportation, since many exotic ornamental plants (i.e. *Trachycarpus fortunei, Livistona chinensis, Phoenix canariensis* and *Washingtonia robusta*) have been recently planted in the area around the Kladisos stream. The plant trade is also the most likely explanation for the Athenian population (Silva-Rocha et al., 2014) and for other invasive populations, i.e. in Catalonia (Rivera et al., 2011), Gorgona Island (Zuffi et al., 2022), and Great Britain (Clemens & Allain, 2021). Alternatively, the lizard’s transportation could be a result of the high touristic flow, e.g. yacht tours connecting popular touristic destinations, such as Sicily and Crete or the pet trade as suggested for various populations introduced to the United States (Kolbe et al., 2013).

The introduction and establishment of the Sicilian wall-lizard may eventually pose a serious threat to native *Podarcis* species. It has been documented that the extinction of *P. melliselensis* on several Adriatic islands was probably due to competitive exclusion following the introduction of *P. siculus* (Nevo et al., 1972). Hybridisation of introduced *P. siculus* with *Podarcis tiliguerta* (Capula, 2002), *P. waglerianus* (Capula, 1994) and *Podarcis raffonei* (Capula et al., 2002) has also been observed in Sardinia, Sicily and the Aeolian islands, respectively. In other cases, introduced *P. siculus* has now spread across the entire range of islands, such as Menorca, where the species had been introduced in historical times (Alcover, 1981), or the Gorgona Island where it has been recently introduced (probably within the last 25 years; Zuffi et al., 2022). In the case of Crete, it is possible that *P. siculus* will eventually constitute a significant threat to the endemic Cretan wall lizard, *Podarcis cretensis*. Currently, both species have been found to coexist in the same habitat, although the second one seems to be less abundant. Extensive future studies focusing on the geographical distribution and population size of the introduced lizard population are needed to map and estimate the extent of its establishment and spread. Population surveys and behavioural experiments investigating the interaction between *P. siculus* and *P. cretensis* will form the basis for designing management measures to prevent further spread of the species on the island. In several cases, the eradication of the established *P. siculus* population has been proposed or applied, wherever potential threats to the native species have been identified (Hodgkins et al., 2012; Adamopoulou & Pafilis, 2019). Given the endemic status of the Cretan wall lizard, such measures might also be implemented in Crete.

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Data accessibility

Mitochondrial cytb data are deposited in the NCBI Nucleotide Database (accession numbers: PQ591857 - PQ591862).

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