

The Italian wall lizard, *Podarcis siculus campestris*, unexpected presence on Gorgona Island (Tuscan Archipelago)

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Abstract. We here report the unexpected presence of the Italian wall lizard (*Podarcis siculus campestris*) on Gorgona Island, in the Parco Nazionale Arcipelago Toscano (Tuscan Archipelago, Tyrrhenian Sea, Tuscany, Central Italy). Field observations were carried out in 2020 confirming its presence on the island, where it had never been reported before. We recorded 37 GPS points of the species in three major areas of Gorgona (with 50 lizard records) and about 180 visual counts regarding all age classes (newborns, juveniles and adults). The species was found in the urban area (site of state prison) and in two grassy and bushed areas, around and along olive tree plantations. Seven individuals were captured and their tails were used to assess the sequence variation of the mitochondrial *CYB* gene. Biometrical parameters were also evaluated for six of these individuals. We detected three distinct *CYB* haplotypes that were compared to *Podarcis siculus CYB* sequences available in public databases. They resulted identical or phylogenetically closest to those found in mainland Tuscany. One haplotype, found in three specimens, was identical to one previously detected at Orti Bottagone (WWF Oasis in Piombino), while the other two haplotypes were most similar to haplotypes reported in the Giannella peninsula and Pisa, respectively.

Keywords. Introduced species, *Podarcis siculus campestris*, Gorgona Island, Tuscan Archipelago, mtDNA *CYB* sequences.

INTRODUCTION

The Italian wall lizard *Podarcis siculus* (Rafinesque-Schmaltz 1810) is a Mediterranean species endemic to the Italian peninsula, Sardinia, Sicily, Corsica, coastal Slovenia and Croatia, and the majority of small islets of the Adriatic and Tyrrhenian Seas (Corti, 2006). It is also present, as an allochthonous taxon, in several European and non-European countries (Crnobrnja Isailovic et al., 2009; Corti et al., 2011; Silva-Rocha et al., 2012, 2014; Adamopoulou, 2015; Mizsei et al., 2016; Ribeiro and Sá-

Sousa, 2018; Clemens and Allain, 2021). According to Crnobrnja Isailovic et al. (2009) "generally it is an invasive that can displace native populations of other species in its invasive range (the southern part of its range and in the areas where it has been introduced)". Recent data on introduced populations found that the species shows a marked resilience (Burke et al., 2002), and efficient adaptation patterns (Kapsalas et al., 2016). In some cases, the species showed clear-cut ecological plasticity in adapting to a new environment, changing some anatomical and physiological traits (Herrel et al., 2008). Locally,

the species is supposed to drive the extinction of indigenous lizards (Ribeiro and Sá-Sousa, 2018). As far as we were aware, eradication projects proved successful results only in Greece (Adamopoulou and Pafilis, 2019). Thus, *P. siculus* still represents worldwide a serious threat to autochthonous species, particularly due to the facility with which it can be transferred from its native area into a new environment (Adamopoulou, 2015; Silva-Rocha et al., 2012; Mizsei et al., 2016; Clemens and Allain, 2021).

In Italy, it is widespread from the North to the South, being common in coastal and hilly areas of Northern and Central Italy, while in the South it can reach higher altitudes (Corti et al., 2011). Recent unpublished data report new introduced *Podarcis siculus* individuals in some areas in Northern Italy (province of Trento), via olive trees transfer from central Italy (K. Tabarelli de Fatis pers. comm.). The species is naturally present in Tuscany and in its insular environments, as reported by the latest regional atlas (Vanni and Nistri, 2006). Regarding the seven larger islands of the Tuscan Archipelago, it has been reported as naturally present in Capraia, Elba (with three small populations), Montecristo, Giglio and Giannutri, and it was likely introduced in Pianosa (Vanni and Nistri, 2006). However, the species was never reported from Gorgona island. Previous repeated survey sessions, whose results were published in 2006 and in 2011 (Corti, 2006; Corti et al., 2011) did not find the species on the island, thus suggesting that this unexpected presence should be a very recent introduction.

From a phylogeographic and phylogenetic point of view, several papers have evaluated the distribution and the genetic variation of this taxon (Podnar et al., 2005; Senczuk et al., 2017), also when regarding allochthonous populations (Silva-Rocha et al., 2012, 2014). Considering the above scenario regarding the dispersal ability of the species in novel places and its ecological plasticity, we have aimed at assessing the population distribution of *P. siculus campestris* on Gorgona, within the framework of a larger project granted by the “Parco Nazionale Arcipelago Toscano” on Habitats Directive species occurring in the Tuscan Archipelago, and the mitochondrial DNA (mtDNA) variation (*CYB* gene) of some individuals from the island, with the overarching goal to obtain preliminary results concerning the geographical origin of the female founders.

MATERIALS AND METHODS

Study area and sampling

The study area is Gorgona Island (43.429008°N, 9.899226°E), the northernmost island of the Tuscan Archi-

pelago, about 34 km westward from the Italian coast. It is a rocky island with a very small surface (2.1 km²) and a perimeter of about 7 km (Fig. 1A). The herpetological assemblage of the island lacks amphibians, due to the absence of freshwater areas, while known reptile species are the wall lizard (*Podarcis muralis*), the Moorish gecko (*Tarentola mauritanica*), the Turkish gecko (*Hemidactylus turcicus*) and the Whip snake (*Hierophis viridiflavus*) (Vanni and Nistri, 2006), species that are still present and abundant according to recent unpublished surveys (C. Corti, pers. comm.; M.A.L. Zuffi and M. Boschetti pers. obs.). Sampling surveys were limited to *Podarcis* species and were carried out from mid to end of summer 2020, on three different occasions, on the 29th of July, 29th and 30th of September. We selected three different transects, A = 1,678 m, B = 2,073 m, and C = 2,240 m (Fig. 1B). According to the recommendation concerning the monitoring of relative small-sized lizards (Sacchi and Scali, 2016; Sindaco et al., 2016), we adopted a visual census, that shall be repeated in the following years to establish the relative abundance of the target species and other reptile species for the above-mentioned project. We counted all the lizards observed along each transect and, every 10-12 m, we marked the lizards presence with a GPSMAP® 62 series GPS. We therefore provided *i*) a distribution mediated by the GPS recording and *ii*) an overall count of observed animals along the whole transect. According to the capture feasibility, in some areas along the different transects, we also captured seven individuals of *P. siculus* by noosing. From the captured individuals (six out of seven, one escaped before measurements) we recorded multiple morphometrics and body size (body mass, snout to vent length, SVL, head length, width and height, as in Kaliontzopoulou et al., 2007; Table 1), determined sex and ontogenetic stage (male, female, juvenile) and collected the tail tip to assess the variation of the mitochondrial *CYB* gene. From its external appearance, the species shows the typical continental *P. siculus campestris* dorsal pattern, with two green parietal bands (Fig. 2). Being on this island allochthonous and likely invasive, after the measurements the captured individuals were transferred to the museum lab, maintained alive in terraria for further analyses and comparisons, waiting for the Ministry's approval for euthanasia.

Samples analysed for CYB gene variation

Seven *P. siculus* specimens collected in Gorgona were analysed for the sequence variation of the *CYB* gene at the University of Pavia. DNA was extracted from either tails or tail re-growths stored in 95% ethanol. The majority of the *CYB* gene (at least 924 bp, from np 14,357 to np 15,280) was determined for all specimens.

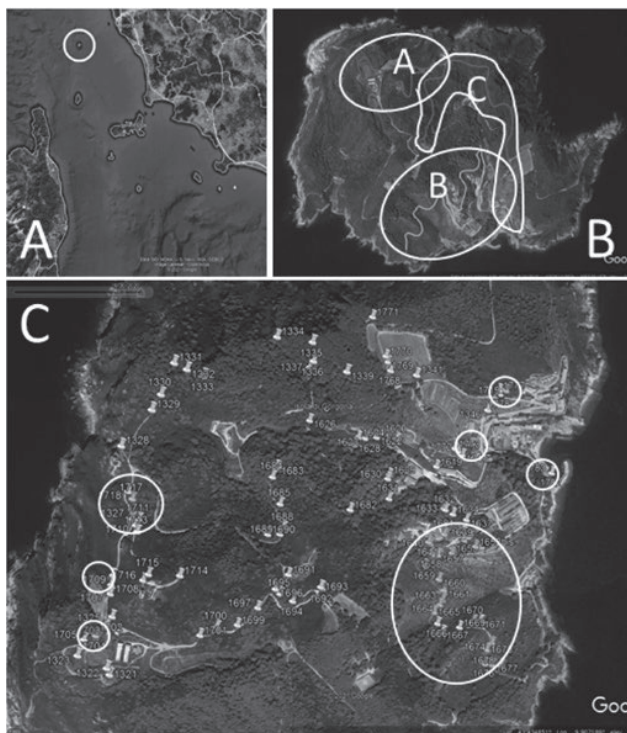


Fig. 1. A. Tuscan Archipelago islands with the Gorgona Island, marked with a white circle. B. Distribution of selected transects (A, B, C) on the Gorgona island. C. Distribution of allochthonous *Podarcis siculus* (marked with white circles) and the congeneric *P. muralis* (other unmarked waypoints). Figures 1A and B are modified from Google Earth.

At the time when our analyses were performed, there were 532 *P. siculus* *CYB* sequences in GenBank. Only 394 of these, whose *CYB* sequence covered the 764 bp between np 14,417 and 15,180 (Akopyan et al., 2017; Buglione et al., 2019; Deichsel et al., 2010; Garcia-Porta and Irisarri, 2019; Kolbe et al., 2013; Podnar et al., 2004, 2005, 2007, 2009; Senczuk et al., 2017, 2018; Taverne et al., 2020), were employed for comparisons along with the *P. siculus* reference sequence (PsRS) (NC_011609). Detailed information on the overall 402 samples (7 from this study and 394 as reference) is provided in Table S1.

DNA extraction

Genomic DNA was extracted via the ReliaPrep™ (Promega Madison, WI, USA) gDNA Tissue kit, using the standard protocol for mouse tail. Roughly, 0.5-1 cm of the tail was cut into smaller parts using a scalpel and homogenised in a 2 ml test tube. We added to the samples 100 µl of Tail Lysis Buffer (TLA) and 20 µl of Proteinase K (20 mg/ml), vortexed and incubated at 56 °C

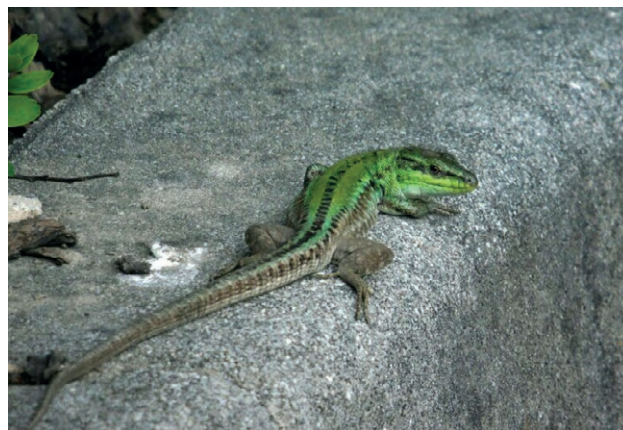


Fig. 2. Adult male of *Podarcis siculus*, showing the typical “campes-tris” pattern (picture taken on Gorgona).

overnight. Then we added 300 µl of Cell Lysis Buffer (CLD) and 20 µl RNase A, vortexed and incubated (56 °C) until clear. DNA was then purified using a standard phenol/chloroform method. Purified genomic DNAs were eluted into Promega elution buffer.

CYB sequencing and data analysis

The seven samples were Sanger sequenced. PCRs were carried out in 50 µl reactions with a standard reaction mix containing 1X Buffer (1.5 mM MgCl₂), 0.2 mM of each dNTP, 2 U of GoTaq G2 Polymerase (Promega), 0.3 µM of each primer (CytF and H15425 by Senczuk et al., 2017) and ~100 ng of DNA template, using the following PCR protocol: 95 °C (2 min); 10 cycles at 95 °C (30 s), 52 °C (30 s), 72 °C (2 min); 25 cycles at 95 °C (30 s), 50 °C (30 s), 72 °C (2 min) and a final extension at 72 °C (10 min). PCR products were visualised on a 1% agarose gel and amplicons were sequenced with standard dideoxy sequencing with primers CytF and H15425 using Dye terminator chemistry (Applied Biosystems) and following the manufacturer’s protocol. Sequences were output in For and Rev files in .ab1 format, cleaned by hand to remove ambiguous tails, aligned to PsRS (NC_011609) and exported to the standard FASTA format.

Phylogenetic analyses and age estimates of mtDNA haplogroups

A maximum likelihood (ML) tree was built with the software MEGAX using the GTR model (8γ distributed categories) with 1,000 bootstraps (extensive SPR method). It encompassed 402 *CYB* sequences (our

seven sequences, 394 from GenBank plus the reference sequence) and was rooted with the corresponding *CYB* sequence from *P. muralis* (NC_011607) using Geneious 8.1.5 (Biomatters; Kearse et al., 2012). Bayesian estimations were performed using Beast 2.6.0 (Bouckaert et al., 2019) under the HKY substitution model (gamma-distributed rates plus invariant sites) with a relaxed clock (log normal). The clock value of 1×10^{-8} base substitution per nucleotide per year (2% divergence rate Myr^{-1}), was entered as prior. The chain length was established at 50,000,000 iterations, with samples drawn every 1,000 Markov chain Monte Carlo (MCMC) steps after a discarded burn-in of 5,000,000 steps.

RESULTS

Transects

We visually counted 180 *Podarcis siculus* and, among them, we recorded 37 GPS points corresponding to 50 individuals (10 adult males, 30 adult females and 10 juveniles). We counted more than 400 *P. muralis* and, among them, we recorded 92 GPS points corresponding to 74 individuals (20 adult males, 11 adult females and 43 juveniles) (Fig. 1C). We captured seven Italian wall lizards in three different areas of the island (all variables recorded in Table 1), from which a small piece of the tail tip was obtained and preserved in 95% EtOH. Almost all the observed *P. siculus* were distributed along the transects characterized by open and sunny areas (Fig. 1B), while only three individuals were found in the urban context of the island (transect C). Specifically, only some individuals were found along transect A, three only in transect C and almost all the other lizards in transect B. This latter transect is characterized by an abundant olive tree plantation, whose establishment is relatively recent (from 1999 to 2015). On the contrary, *P. muralis* was common and widespread on the island (see Fig. 1C), being relatively

scarce only along transect B, especially where the habitat is much open, sunny and cultivated.

Podarcis siculus *CYB* sequences

We sequenced 924 bp of the *CYB* gene from the seven tails collected from Gorgona Island. We detected three haplotypes ($Hd = 0.714 \pm 0.127$) and a total of seven variable sites (Table 2). On average 3.43 ± 1.08 nucleotide differences were found between any two sequences and the average nucleotide diversity (π) was 0.373% ($\pm 0.066\%$). When considering all available *CYB* sequences ($n = 402$), we detected 229 haplotypes ($Hd = 0.994 \pm 0.001$) with 217 variable sites. On average 35.48 ± 1.14 nucleotide differences were found between any two sequences and π was 5.59% ($\pm 0.06\%$).

Phylogeny of *Podarcis siculus* *CYB* sequences

An initial phylogenetic survey by Senczuk et al., (2017) encompassing 277 mtDNA *CYB* sequences revealed three major haplogroups present throughout the species' distribution range. They were named A for Adriatic, T for Tyrrhenian and S for Sicily. The addition of our seven samples from Gorgona together with 118 additional sequences from GenBank (Podnar et al., 2004, 2005, 2007; Mayer et al., 2010; Kolbe et al., 2013; Akopyan et al., 2017; Senczuk et al., 2018; Buglione et al., 2019; Garcia-Porta and Irisarri, 2019; Taverne et al., 2020) provided a more in-depth resolution of the species phylogeny (Fig. 3). All samples fall within haplogroups, A, T and S whose founding nodes were dated, through Bayesian estimates, to $2,602 \pm 426$, $1,925 \pm 3,259$ and $4,709 \pm 620$ thousand years ago (kya), respectively. Haplogroups A and T are sister clades whose ancestral AT node is dated at $3,909 \pm 534$ kya. The *P. siculus* ancestral mitogenome (PsAM) was estimated at $6,150 \pm 735$ kya.

Table 1. Biometry of *Podarcis siculus* samples from Gorgona. bmass = body mass (g); svl = snout to vent length; h_l = head length; h_w = head width; h_h = head height (all length in mm).

| Sample ID | sex | age | site | Transect | bmass | svl | h_l | h_w | h_h |
|-----------|--------|----------|---------------|----------|-------|------|------|------|-----|
| GORG01 | male | adult | Torre Vecchia | A | 7.1 | 65.0 | 17.5 | 9.9 | 7.9 |
| GORG02 | male | juvenile | Capanne | B | 4.0 | 58.0 | 14.5 | 8.4 | 6.3 |
| GORG03 | male | adult | village | C | 8.1 | 71.0 | 17.6 | 10.0 | 8.2 |
| GORG04 | male | adult | Capanne | B | 8.2 | 71.0 | 17.5 | 10.1 | 8.2 |
| GORG05 | female | adult | Capanne | B | 3.1 | 55.5 | 12.7 | 7.5 | 5.4 |
| GORG06 | female | adult | village | C | 4.5 | 55.5 | 13.3 | 7.4 | 5.8 |
| GORG07 | female | adult | Torre Vecchia | A | --- | --- | --- | --- | --- |

Table 2. Nucleotide substitutions identified in the three *P. siculus* *CYB* haplotypes from Gorgona.

| Haplotype | Sample ^a | Mutations relative to the reference sequence (NC011609) | | | | | | | GenBank accession number |
|-----------|---------------------|---|-----------|-----------|-----------|-----------|-----------|------------------------|--------------------------|
| | | np 14,436 | np 14,607 | np 14,985 | np 15,027 | np 15,042 | np 15,063 | np 15,255 ^b | |
| Reference | (NC011609) | T | C | A | T | C | A | T | |
| 1 | GORG03 | C | . | G | . | . | C | . | OM925988 |
| 1 | GORG05 | | | | | | | | OM925989 |
| 1 | GORG06 | | | | | | | | OM925990 |
| 2 | GORG02 | . | . | G | C | . | T | . | OM925991 |
| 3 | GORG01 | C | T | . | . | T | T | C | OM925992 |
| 3 | GORG04 | | | | | | | | OM925993 |
| 3 | GORG07 | | | | | | | | OM925994 |

^a 924 bp (from np 14,357 to np 15,280) of the *CYB* gene were sequenced for all samples.

^b This nucleotide position was not included in phylogenetic analyses because outside of the sequence range available for most of the *CYB* sequences from GenBank.

Haplotype A (n = 112), representative of individuals with Adriatic origins, was indeed mainly sampled around the Adriatic basin (Croatia and Italy), but also in Umbria, Lazio, Campania and Calabria. It harbours the lowest intra-clade nucleotide diversity (1.565 ± 0.125 %) (Table 3) and is composed of three major sub-haplotypes: A1, A2 and A3. Haplotype A1 (n = 9) encompasses only Croatian individuals and is the youngest (359 ± 133 kya). Haplotype A2 is the most represented (n = 101) and the oldest (909 ± 164 kya). It encompasses samples from the Italian Adriatic coast, but also from Calabria, Campania, Lazio and Lombardia. Haplotype A3 (n = 11) (807 ± 219 kya) includes mainly individuals from Calabria, but also one each from Campania and Emilia-Romagna.

Haplotype T (n = 97) is representative of individuals with Tyrrhenian origins (Toscana, Umbria and Lazio), but also from Emilia-Romagna. It is composed of two major sub-haplotypes, which were renamed from the original study (Senczuk et al., 2017) to T1 and T2 given their major split, which was not considered previously. Haplotype T1 (n = 45), dated at 990 ± 224 kya, is mainly Toscana-specific with a couple of individuals collected in Umbria. It is further sub-divided into haplotypes T1a and T1b (Ta and Tb, respectively, in Senczuk et al., 2017) though T1a only encompasses one individual. Haplotype T1b (535 ± 131 kya) was found to include all seven sequences from this study (Fig. 4). Samples GORG 03, 05 and 06 from Gorgona Island share the same haplotype (n. 1 in Table 2) with JX186543 (Kolbe et al., 2013) from Orti Bottagone (WWF Oasis in Piombino). GORG 02 harbours a novel haplotype (n. 2), though similar to those detected in samples from the Giannella peninsula (KY065091-KY065095) (Senczuk et al., 2017). Finally, GORG 01, 04 and 07 harbour the same novel haplotype

(n. 3), which appears to be closest related to JX186545 from Pisa (Kolbe et al., 2013) according to the haplotype network (Fig. 5). Thus, in all cases the closest *CYB* sequences were found in mainland Toscana.

Haplotype T2 (n = 52; 791 ± 184 kya) mainly encompasses individuals from Central Italy. It is composed of two major sub-haplotypes T2c and T2d (Tc and Td, respectively, in Senczuk et al., 2017). Haplotype T2c (444 ± 110 kya) is found in Emilia-Romagna, Toscana, Umbria and Lazio, while haplotype T2d is younger (246 ± 73 kya) and appears to be Lazio-specific. Haplotype S (n = 193), the most represented and with the largest intra-haplotype nucleotide diversity (3.185 ± 0.245 %) (Table 4), is representative of individuals with a Sicilian origin. It is composed of three main sub-haplotypes: S1, S2 and S3. Haplotype S1 ($2,170 \pm 403$ kya) is mainly found in Calabria, while haplotype S2 (404 ± 146 kya) appears to be Calabria-specific. Finally, haplotype S3, which is the most divergent ($1,423 \pm 230$ kya), is almost completely endemic to Sicily and subdivided into 13 sub-haplotypes (S3a-S3m).

DISCUSSION

Our survey on Gorgona Island reports for the first time the occurrence of the Italian wall lizard, which has probably been accidentally introduced to the island during the last few years. The species is now markedly widespread, despite never being found prior to our survey (Corti, 2006; Corti et al., 2011). The last survey on lizards was carried out at the beginning of 2000 (C. Corti, pers. comm.), and no evidence of *Podarcis siculus* occurrence was reported. Therefore, the time gap between the last and the current survey is well-defined. Nevertheless, we cannot

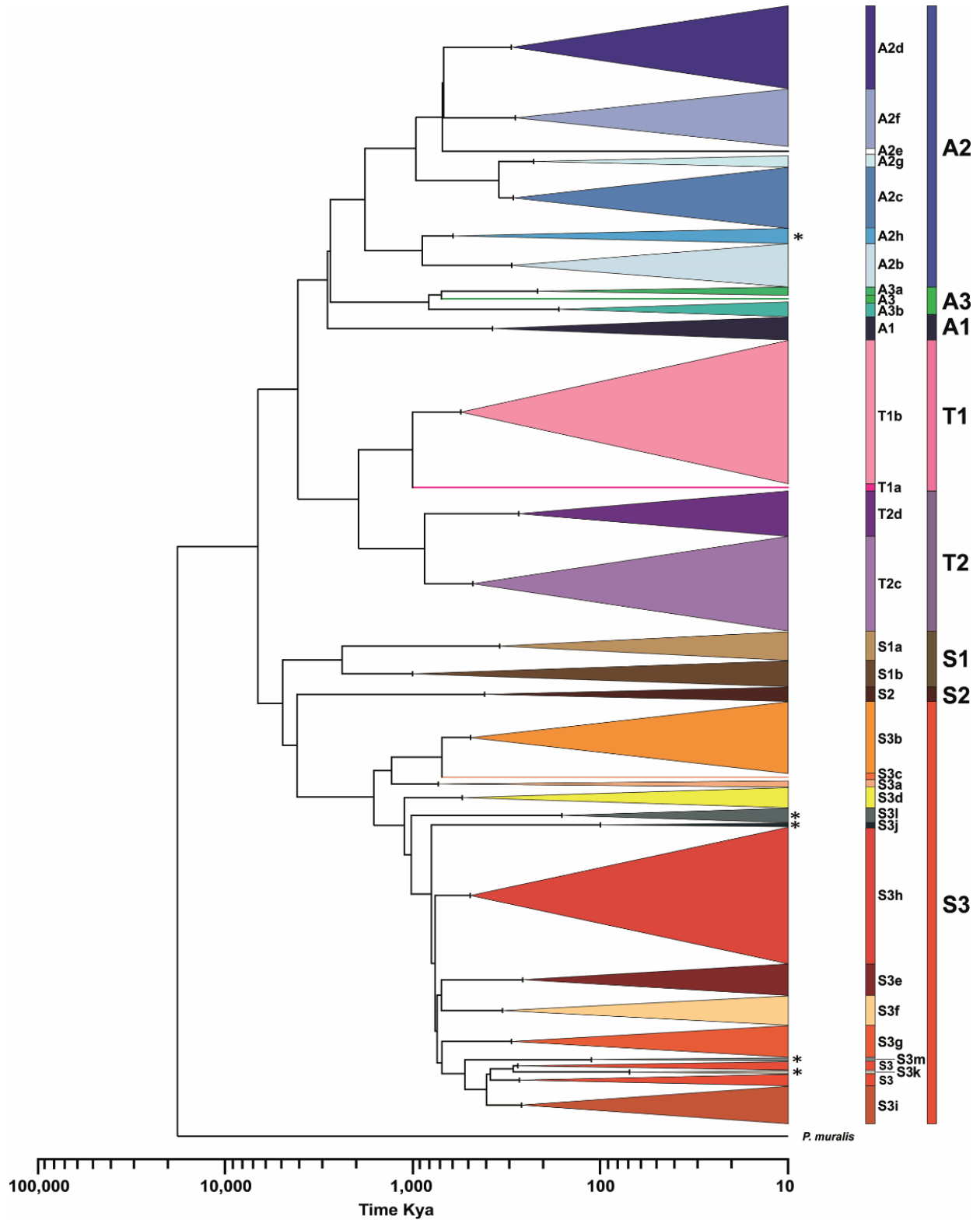


Fig. 3. Bayesian inference phylogeny of *Podarcis siculus* CYB sequences. This tree was obtained via the Bayesian method. It encompasses 402 partial CYB sequences (764 bp, nps 14417-15180) and was rooted using *Podarcis muralis* (NC_011607). The time scale is in thousands of years ago (kya). Coloured bars indicate haplogroup/sub-haplogroup affiliation, following colour scheme and nomenclature from Senczuk et al., (2017). New sub-haplogroups are indicated by an asterisk. A2a was removed due missing regions within the sequence.

Table 3. Nucleotide diversity (%) within and between *P. siculus* *CYB* sequences belonging to different haplogroups and from different geographic areas. Intra-group nucleotide diversities (π) are on the diagonal.

| | Haplogroup A n = 112 | Haplogroup T n = 97 | Haplogroup S n = 193 |
|--------------|-------------------------|------------------------|-------------------------|
| Haplogroup A | 1.565 ± 0.125 | 5.586 ± 0.148 | 7.283 ± 0.157 |
| Haplogroup T | --- | 2.154 ± 0.037 | 8.093 ± 0.147 |
| Haplogroup S | --- | --- | 3.185 ± 0.245 |

state precisely when this species reached Gorgona Island. It is worth underscoring that olive trees and grapevines have been transplanted on the island in the last two decades for agricultural purposes, according to Regional and EU projects. Thus, passive transport with plants is a possible scenario, as recently reported in the UK (Clemens and Allain, 2021). Passive transportation of animals, and especially reptiles, has been documented worldwide (Burke et al., 2002; Silva-Rocha et al., 2014; Adamopoulou, 2015; Mizsei et al., 2016; D’Amico et al., 2018; Ribeiro and Sá-Sousa, 2018; Clemens and Allain, 2021). In addition, on Gorgona Island, cattle, horses and sheep have increased in number and much more fodder is imported from the mainland, via boats from Piombino harbour.

Most reptile invaders have a survivorship rate usually at about 10% of the total (Ferreira et al., 2012), supporting the idea that new colonizers frequently survive the introduction and may be more competitive than resident species (Mangiacotti et al., 2013; Kapsalas et al., 2016; Ribeiro and Sá-Sousa, 2018; Damas-Moreira et al., 2020). In particular, the conclusion of Detwiler and Criscione (2014) “invasive metapopulation has rapidly reached the establishment stage as indicated by relatively constant effective sizes and migration rates among introduced subpopulations”, appears to fit very well with the high number of adult and juvenile *P. siculus* that we observed on Gorgona Island.

Colonization times and population structure of introduced species are often underestimated and genetic data of insular populations may provide correct information on the original distribution of analysed species (Silva-Rocha et al., 2019). Importantly, some research underlined the different invasion origins (Toscana, Sardegna, Calabria, Sicilia, Silva-Rocha et al., 2012), and possible times of introduction, ranging from the Middle Age for the Balearic Islands to the first half of the XX century for the Almeria and Cantabrian populations, or even more recently (Silva-Rocha et al., 2012). Our data indicate the arrival of the lizards on Gorgona Island from the area occurring between Pisa and Orbetello, particularly because of the overlap (or close relationship) of the three haplotypes observed on the island with those pre-

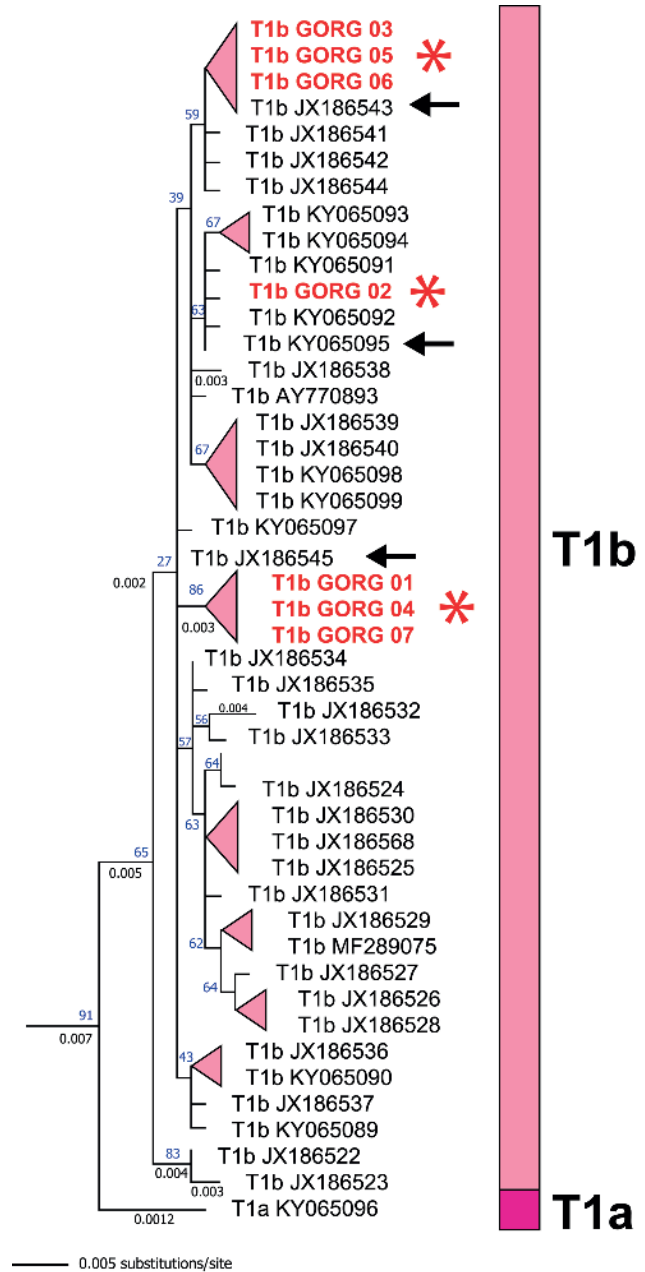


Fig. 4. Maximum likelihood phylogeny of haplogroup T1 sequences. This tree is a subset of the one in Figure S1, encompassing only haplogroup T1 sequences. Numbers at nodes indicate the bootstrap values. Asterisks indicate samples from Gorgona Island and arrows indicate their closest related relative.

viously reported in a wide area of the coast of Toscana. To explain the detection of three distinct *CYB* haplotypes, at least three unrelated female founders from the mainland have to be postulated, individuals that most likely reached Gorgona Island through distinct introduction events. Different and not related introduction events,

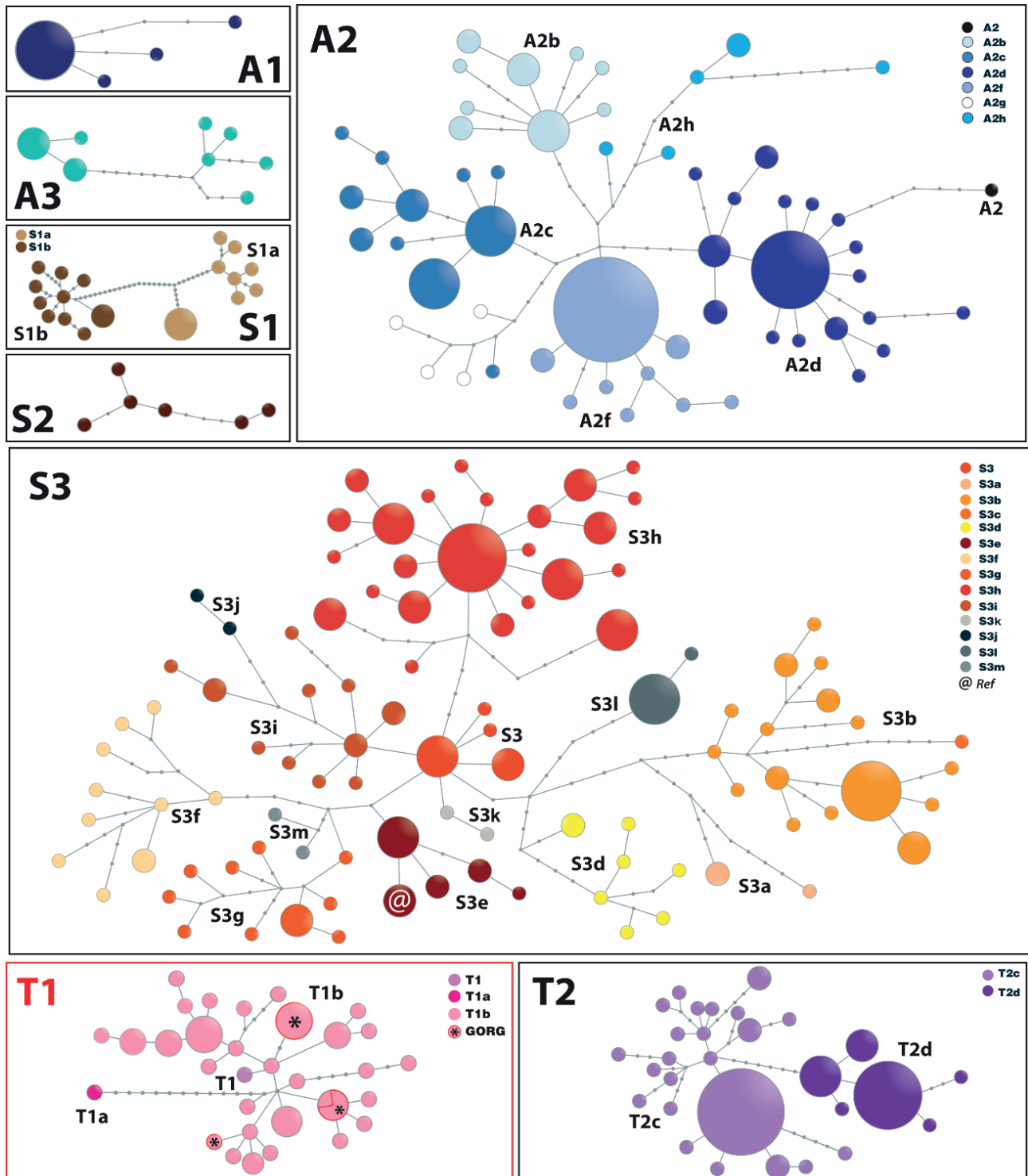


Fig. 5. Phylogeny of the 293 haplotypes found in the 402 *P. siculus* CYB sequences. Partial CYB sequences are subdivided into main haplogroups (Senczuk *et al.*, 2017). It was constructed using Fitchi (Matschiner M. (2015), <https://evoinformatics.group/fitchi.html>). Sizes of circles are proportional to the number of CYB sequences, with the smallest circle (for each panel) representing one individual. Dots on branches represent intermediate haplotypes and '@' is the reference sequence. Gorgona Island samples are highlighted with an asterisk.

Table 4. Bayesian age estimates for *P. siculus* haplogroups and sub-haplogroups. PsACYB indicates the *Podarcis siculus* Ancestral CYB sequence.

| Summary Statistic | Age – kya | St Dev – kya |
|-------------------------|-----------|--------------|
| <i>Podarcis muralis</i> | 15,670 | 2550 |
| PsACYB | 6,150 | 735 |
| AT | 3,909 | 534 |
| A | 2,602 | 426 |
| A1 | 359 | 133 |
| A2 | 909 | 164 |
| A2'3 | 1,742 | 317 |
| A2b | 268 | 85 |
| A2c | 271 | 69 |
| A2d | 283 | 70 |
| A2f | 252 | 74 |
| A2g | 207 | 65 |
| A2h | 575 | 145 |
| A3 | 807 | 219 |
| A3a | 201 | 83 |
| A3b | 148 | 52 |
| S | 4,709 | 620 |
| S1 | 2,170 | 403 |
| S1a | 331 | 112 |
| S1b | 992 | 248 |
| S2 | 404 | 146 |
| S2'3 | 3,902 | 586 |
| S3 | 1,423 | 230 |
| S3a | 672 | 202 |
| S3b | 473 | 123 |
| S3d | 528 | 158 |
| S3e | 239 | 80 |
| S3f | 312 | 88 |
| S3g | 297 | 82 |
| S3h | 483 | 102 |
| S3i | 225 | 65 |
| S3j | 100 | 59 |
| S3k | 67 | 41 |
| S3l | 142 | 49 |
| S3m | 113 | 64 |
| T | 1,925 | 325 |
| T1 | 990 | 224 |
| T1b | 535 | 131 |
| T2 | 791 | 184 |
| T2c | 444 | 110 |
| T2d | 246 | 73 |

are the unique logical explanation for having found the three distinct CYB haplotypes, similarly to what has been found in the Iberian peninsula (e.g., Silva-Rocha et al., 2012) and in some other countries, where pathways and origins have been determined (Silva-Rocha et al., 2014).

These introductions were accidental and the lizards possibly arrived with olive trees and other plants (Clemens and Allain, 2021), rather than together with the fodder for domestic animals or using man-made objects, confirming the high invasive potential of the species (Silva-Rocha et al., 2014; Clemens and Allain, 2021).

Further surveys and molecular analyses are required to understand *i*) the number of colonization events and *ii*) if other founder haplotypes are present. Finally, it could be important to monitor and study ecological and behavioural patterns of Gorgona Island population(s) with respect to those living on the continent, to assess how *P. siculus* interacts with the locally adapted *P. muralis*, and to evaluate if the eradication of this allochthonous species from Gorgona Island should be performed. Experiments on competitive interactions (i.e., chemical avoidance, territorial behaviours, food preference) and biometric analyses of head shape and body size between the two *Podarcis* species may give useful insights into the ecological plasticity of both the residential and the alien lizard.

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at <<http://www-9.unipv.it/webshi/appendix/index.html>> manuscript number 12388

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