

Original Article

Subspecies inflation hampers conservation efforts: a case study on wall lizards

Emanuele Berrilli¹, Benedetta Gambioli², Pierluigi Bombi³, Matteo Garzia¹,
Martina Muraro⁴, Claudio Pardo⁵, Marco Reale², Stéphanie Sherpa⁴,
Gentile Francesco Ficetola⁴, Leonardo Vignoli² and Daniele Salvi^{1,*}

¹Department of Health, Life & Environmental Sciences-University of L'Aquila, Via Vetoio snc, 67100 L'Aquila-Coppito, Italy

²Department of Sciences, Roma Tre University, 00146 Rome, Italy

³Institute of Research on Terrestrial Ecosystems, National Research Council, 00015 Monte libretti, Italy

⁴Department of Environmental Science and Policy, Università degli Studi di Milano, Via Celoria 10, 20133 Milan, Italy

⁵Department of Ecological and Biological Sciences, Tuscia University, 01100 Viterbo, Italy

*Corresponding author. Department of Health, Life & Environmental Sciences-University of L'Aquila, Via Vetoio snc, 67100 L'Aquila-Coppito, Italy.
E-mail: danielesalvi.bio@gmail.com

ABSTRACT

The common practice of using subspecies as conservation targets raises the question of whether efforts are focused on preserving conspicuous components of the species' phenotypic variability rather than evolutionarily significant units. To address this question, in this study we performed a comprehensive morphological and genetic assessment on all the subspecies of wall lizard described for the Aeolian Archipelago (Italy) to determine whether they represent distinct evolutionary lineages and/or discrete phenotypic partitions. Further, we applied a monophyly test to 70 subspecies belonging to seven wall lizard species occurring in Italy, based on our results and on previous phylogeographic studies. We found that none of the Aeolian subspecies represents a distinct evolutionary lineage, despite some morphological differentiation of island populations across the archipelago, suggesting a very recent origin of island populations and of the observed phenotype variation. Across seven wall lizard species, tests revealed that lizard subspecies rarely (< 9% of cases) match evolutionary units. This study demonstrates that intraspecific taxonomy of wall lizards is a poor predictor of phylogeographic partitions and evolutionary units, and therefore of limited use (if not dangerous) for defining conservation and management units. A better approach would be relying on the integration of genomic and phenotypic data to assess the evolutionary significance and conservation value of phenotypic and genetic units within species.

Keywords: *Podarcis siculus*; Mediterranean; Aeolian Islands; population genetics; phylogeography; morphology; reptiles; taxonomy; phylogeography

INTRODUCTION

Accurate biodiversity assessment and conservation management requires a solid understanding of taxonomic status and systematic relationships among species (Sites and Marshall 2003, Isaac 2004, Balakrishnan 2005, De Queiroz 2007, Petit and Excoffier 2009, Yeung *et al.* 2009). However, precisely defining the boundary between intra- and inter-specific variability, and its taxonomic significance, is not always a simple task. Multiple processes can promote the evolution of intraspecific morphological diversity in several traits, including genetic drift and founder events, and local environmental variability, which in turn can determine both phenotypic plasticity and local adaptations

(Losos 2000, Schluter 2000, O'Hara 2005, Runemark *et al.* 2010, Young and Badyaev 2010). This implies that species with wide geographic distributions, or with populations living in geographically isolated areas such as islands, may exhibit phenotypic variation across their range (Werner and Sherry 1987, Renaud and Millien 2001, Donihue *et al.* 2016, Prates *et al.* 2023). These differences result in the presence of phenotypically diverse populations, which have often stimulated their recognition as distinct subspecies.

The subspecies concept was first introduced in the mid-1800s and it has primarily been used to formalize geographical variations or units of variation within species ranges based on

morphological differences (Simpson 1961, Mayr 1963, 1982, 1999, Remsen 2010). Thus, while the concept underlying this classification has evolved and been implemented over time in an increasingly evolutionary perspective (Burgon et al. 2021, Burbrink et al. 2022, Prates et al. 2023), the majority of the currently recognized subspecies (Mayr 1946, Burt 1954, Gillham 1956, Frost 2020), have been proposed to capture differences in morphological traits observable within the species' range rather than to delimit evolutionary entities (Burbrink et al. 2000, Braby et al. 2012, Prates et al. 2023). Despite this inconsistency, the concept of subspecies remains widely used in many aspects of biodiversity research and conservation planning (Torstrom et al. 2014). As a result, legislators have frequently used described subspecies to justify the protection of specific populations or for designing protected areas (Zink 2004, Haig et al. 2006). For instance, the EU Habitat directive (Directive 92/43/EEC) identifies 17 taxa of mammals as 'priority' taxa whose conservation requires the designation of special areas of conservation, and 41% of these taxa are subspecies of more widespread species. Using subspecies as conservation targets raises the question of whether efforts have been directed at safeguarding conspicuous components of the species' phenotypic variability rather than evolutionary processes and evolutionarily significant units (ESUs, Moritz 1994, Crandall et al. 2000).

Wall lizards of the genus *Podarcis* Wagler, 1830 offer an excellent case study to assess the bias introduced by the application of the subspecies concept to conservation. *Podarcis* wall lizards are currently represented by 26 species (Uetz et al. 2023) and are the predominant reptile group of the Mediterranean Basin and its islands (Arnold and Ovenden 2002). Indeed, 11 of these species are island endemic and 21 of them have populations occurring on islands (Poulakakis et al. 2005, Sillero et al. 2014, Psonis et al. 2021, Salvi et al. 2021, Yang et al. 2021a, Bonardi et al. 2022). Insular populations exhibit a remarkable phenotypic variability (Arnold et al. 2007), which has stimulated the description of an impressive number of subspecies during the past century, often based on weak morphological characters (Böhme 1986, Henle and Klaver 1986, Poulakakis et al. 2003, Corti et al. 2010, Bellati et al. 2011, Uetz et al. 2023). Most of these subspecies are of doubtful value and are currently considered as synonyms of more widespread subspecies, whereas others have been raised to full species rank in view of their large genetic divergence (e.g. Capula 1994a, Podnar et al. 2005, Salvi et al. 2013, 2014, 2017, Senczuk et al. 2017, 2019b). This is well exemplified by subspecies of wall lizards described for islands within the Aeolian Archipelago located off the north-east coast of Sicily (southern Italy). Until the 1990s, lacertid populations found in the Aeolian Islands and islets were recognized as subspecies of either the Sicilian wall lizard, *Podarcis waglerianus* Gistel, 1868, or the Italian wall lizard, *Podarcis siculus* Rafinesque, 1810. Subsequent population genetic studies revealed that four of the described subspecies were genetically distinct from either species, and represented allopatric populations of a new distinct species, *Podarcis raffonei* (Mertens, 1952), that is facing a high extinction risk given its narrow and fragmented distribution (Capula 1994a, 2004, Gippoliti et al. 2017). On the other hand, while most of the other micro-island subspecies of *P. siculus* have been lately considered not valid (Tiedemann et al. 1994, Corti and Lo Cascio 2002, Uetz et al. 2023), some have been the target of

conservation efforts, for instance through the implementation of the Natural Regional Nature Reserve of 'Isola di Panarea e Scogli Viciniori' (D.A. 483/44 of 25-07-1997). However, the systematics of these taxa has never been assessed through genetic data, thus their taxonomic and conservation value remains uncertain. Estimating patterns of intraspecific genetic diversity allows the identification of independent evolutionary units worthy of protection, which is crucial for defining conservation priorities and planning biodiversity management actions (Zink 2004).

In this study, we combined a comprehensive morphological and genetic assessment to verify whether the subspecies of *P. siculus* described for the Aeolian Archipelago represent distinct evolutionary lineages and discrete phenotypic partitions. The significance of using wall lizard subspecies as proxies for conservation units is discussed.

MATERIAL AND METHODS

Study system and sampling

The Aeolian Islands are a volcanic archipelago in the southern Tyrrhenian Sea, near Sicily and the southern tip of the Italian peninsula (Fig. 1). The archipelago is made up of seven main islands (Alicudi, Filicudi, Lipari, Panarea, Salina, Stromboli, and Vulcano) and many islets. The seven islands are all thought to have been exposed above sea level within a timespan of a few hundred thousand years, with the oldest outcrops of the island of Lipari emerging around 270 000 years ago (Lucchi et al. 2013). The main islands were never connected to mainland Sicily or to each other during glacial low sea levels due to the deep bathymetry (between 650 and 2300 m) with the exception of the nearby islands Vulcano and Lipari (Lucchi et al. 2013). On the other hand, main islands have been connected with their small satellite islets during most of the last glacial period until part of the Holocene. Despite a relatively young age and geographic isolation, the Aeolian Archipelago hosts endemic fauna and flora, making it an interesting laboratory for biogeographic or conservation studies. The Italian wall lizard can be found on all of the Aeolian Islands, including many satellite islets (Lo Cascio and Corti 2006, Sindaco 2006). Its insular populations have been classified as four distinct subspecies, with the nomenclature and distribution in the archipelago as follows: *Podarcis siculus strombolensis* (Taddei, 1949) from Stromboli, Panarea, and the Basiluzzo Islet; *Podarcis siculus liscabiancae* (Mertens, 1952) from the Lisca Bianca Islet; *Podarcis siculus trischittai* (Mertens, 1952) from the Bottaro Islet; *Podarcis siculus siculus* from Vulcano and other islands (Fig. 1; Corti et al. 2010, Uetz et al. 2023).

We sampled 164 individuals of all currently described *P. siculus* subspecies in September 2021 (Table 1; Supporting Information, Table S1). Two additional taxa, originally described as subspecies of *P. siculus* (*Podarcis siculus raffonei* from the Strombolicchio Islet and *Podarcis siculus alverai* from the Scoglio Faraglione Islet) are not considered here because genetic studies showed they belong to a distinct species, *P. raffonei* endemic to the Aeolian Archipelago (Capula 1994a). For genetic comparison, we selected 39 specimens from Stromboli, Panarea and the three islets, and also sampled eight individuals of the nominal subspecies from the proximal mainland Sicily population of Milazzo (Fig. 1; Table 1). A small tissue sample from the tail tip of each lizard was collected and stored in pure ethanol



Figure 1. A, Geographic range of *Podarcis siculus* locating the study area (red circle). B, Close-up of the Aeolian Archipelago, with a focus on the islands of Stromboli, Panarea, and nearby islets (Bottaro, Lisca Bianca, and Basiluzzo). The sampling locations (symbols) cover the four described *P. siculus* subspecies, defined by morphological data, to be validated with genetic criteria: *P. s. siculus* (square; photo by D. Salvi); *P. s. strombolensis* (circle; photo by M. van Dijk); *P. s. liscabiancae* (triangle; photo by B. Gambioli); *P. s. trischittai* (star; photo by B. Gambioli). The aerial images are digital orthophotos with GSD 50 cm of the islands of Panarea and Vulcano taken on 11/15/2015 by **Immagini terraItaly™ 2005 - ©CGR S.p.A. -Parma**. The two orthophotos were obtained from the Geoportale Nazionale of the Italian Ministry of the Environment (<http://www.pcn.minambiente.it/>).

Table 1. Samples of *P. siculus* from the Aeolian Islands used for genetic and morphometric analyses. Sample size for genetic analyses: total number of sequences; in parentheses: number of sequences from Senczuk et al. (2017). Sample size for morphometric analyses, in parentheses: number of males ♂/number of females ♀.

Subspecies	Island	No. of individuals for genetic analysis	No. of individuals for morphometric analysis
<i>Podarcis siculus liscabiancae</i>	Lisca Bianca	9	12 (6♂/6♀)
<i>Podarcis siculus trischittai</i>	Bottaro	7	7 (5♂/2♀)
<i>Podarcis siculus strombolensis</i>	Basiluzzo	7	10 (6♂/4♀)
<i>Podarcis siculus strombolensis</i>	Panarea	8	57 (33♂/24♀)
<i>Podarcis siculus strombolensis</i>	Stromboli	14 (6)	31 (21♂/10♀)
<i>Podarcis siculus siculus</i>	Milazzo	8	-
<i>Podarcis siculus siculus</i>	Vulcano	6 (6)	47 (29♂/18♀)

for genetic analyses. For morphometric comparison, the head of each individual was photographed in dorsal view using Olympus TG-5 or TG-6 cameras in a photo light box to perform head geometric morphometrics.

DNA extraction, amplification, and sequencing

The genomic DNA of the 47 selected lizards was extracted from alcohol-preserved tissues using a standard high-salt protocol (Sambrook et al. 1989). Two mitochondrial gene fragments were amplified by polymerase chain reaction (PCR): a fragment of cytochrome b (*cytb*) and a fragment of NADH dehydrogenase subunit 4 (*nd4*). For *cytb*, we used the primers L14253mod (5'-TTTGGATCYCTRITAGGCCTCTHCC-3'; modified from Podnar et al. 2005) and H15425 (Podnar et al. 2005) with the following cycling protocol: 94 °C (3 min), [94 °C (30 s), 50 °C (30 s), 72 °C (50 s)] × 35 cycles, 72 °C (5 min). For *nd4*, we used primers and PCR protocols described in previous studies (Mendes et al. 2016). PCR products were purified and sequenced using the forward and reverse primers employed for amplification (Genewitz, UK). Forward and reverse sequence chromatograms were manually edited and assembled into consensus sequences using Geneious v.11.0.12 (Biomatters Ltd., Auckland, New Zealand). None of the chromatograms showed double peaks, and the translated amino acid sequence of both gene fragments did not have any stop codons, confirming that nuclear copies (pseudogenes) were not occurring in our mitochondrial sequence dataset. Details on sequenced specimens along with GenBank accession numbers are provided in Supporting Information, Table S2.

Phylogenetic and network analysis

Sequences were aligned with MAFFT v.7.450 using the G-INS-I progressive method algorithm, resulting in an alignment of 765 positions for *cytb* and of 862 positions for *nd4*. To investigate whether *P. siculus* specimens correspond to independent evolutionary lineages, we used a phylogenetic approach based on a *cytb* dataset of 361 sequences, including the 47 newly sequenced specimens (Table 1; Supporting Information, Table S1) and 314 sequences available in GenBank (Supporting Information, Table S2) from Podnar et al. (2005) and Senczuk et al. (2017). Phylogenetic relationships were inferred using Maximum likelihood (ML) and *Podarcis muralis* Laurenti, 1768Laurenti, 1768Laurenti, 1768 as an outgroup (Salvi et al. 2021, Yang et al. 2021a, b). ML trees were inferred in IQ-TREE

1.6.12 (Nguyen et al. 2015) using the W-IQ-TREE webserver (Trifinopoulos et al. 2016). The best substitution model was determined by the ModelFinder module, including flexible rate heterogeneity across sites (Kalyaanamoorthy et al. 2017), based on the Bayesian Information Criterion (BIC). Branch support was assessed by 1000 replicates of ultrafast bootstrapping (Minh et al. 2013, Hoang et al. 2018). Phylogenetic relationships were further investigated through a phylogenetic network approach. Haplotype network approaches are the most appropriate methods for intraspecific gene evolution, particularly when levels of divergence are low (Posada and Crandall 2001). First, haplotype networks were constructed based on alignments of *cytb* and *nd4* sequences generated for the 47 newly sampled specimens. Second, to assess the phylogenetic and haplotype diversity of Aeolian populations within the Sicilian clade of *P. siculus* (Senczuk et al. 2017), we constructed a phylogenetic network using all available *cytb* sequences of *P. siculus* from Sicily ($N = 104$) and the Aeolian Islands ($N = 57$) from this study, Podnar et al. (2005), and Senczuk et al. (2017). Haplotype networks were constructed using the median-joining network method in POPART v.1.7 (Bandelt et al. 1999, Leigh and Bryant 2015).

To assess the congruence between morphological subspecies from the Aeolian Islands and molecular phylogenetic data we examined whether subspecies were monophyletic in gene trees and networks, i.e. whether all individual sequences of a given subspecies were more genetically similar to one another than to any other subspecies (Moritz 1994, Phillimore and Owens 2006).

To further explore the overall congruency between morphological subspecies and phylogenetic units in *Podarcis* wall lizards, we applied this monophyly test to the entire *cytb* phylogeny of *P. siculus* estimated in this study and to six additional species from Italy based on previous phylogeographic studies: *Podarcis filfolensis* (Bedriaga, 1876) (Salvi et al. 2014); *Podarcis latastei* (Bedriaga, 1879) (Senczuk et al. 2018); *Podarcis melisellensis* Braun, 1877 (Podnar et al. 2004); *Podarcis muralis* (Giovannotti et al. 2010, Bellati et al. 2011, Salvi et al. 2013); *Podarcis tiliguerta* (Gmelin, 1789) (Vasconcelos et al. 2006, Salvi et al. 2017); and *Podarcis waglerianus* Gistel, 1868 (Senczuk et al. 2019a). For subspecies represented by only one individual in the phylogeny, haplotype sharing with the other subspecies was considered as indicative of non-monophyly, but the lack of haplotype sharing was not considered as indicative of monophyly. The minimum

criteria for monophyly tests were: (1) the presence of more than one individual of a given subspecies in the phylogeny, and (2) the presence of more than one subspecies in the phylogeny.

Morphometric analysis

To establish whether the a priori classification of specimens to different subspecies was supported by head shape data, we performed a geometric morphometrics (GM) analysis of lizard heads from Stromboli, Panarea, and the three islets Basiluzzo, Bottaro, and Lisca Bianca, as well as from Vulcano as a reference outgroup. Head shape GM is a powerful tool to investigate phenotypic variation in lizards and has been successfully used in taxonomic, ecological, and evolutionary studies on *Podarcis* lizards (Capula *et al.* 2009, Kaliontzopoulou *et al.* 2010, Piras *et al.* 2011, Simbula *et al.* 2021). We used previously published digitized pictures with 28 landmarks located at intersections and borders of cephalic scales (see Muraro *et al.* 2022 for a description of landmarks). Males ($N = 100$) and females ($N = 64$) were analysed separately.

The superimposition of landmark configurations was carried out using the Procrustes Generalized Least-Squares (GLS) procedure implemented in the IMP CoordGen8 software (Sheets 2014a). Partial Warp (PW) scores, derived from the Thin-Plate Spline (TPS) of superimposed landmark configurations (Procrustes coordinates; reference form = mean of all configurations), were subjected to Principal Component Analysis (PCA) in IMP PCAGEN8 software (Sheets 2014b). Variation in head shape among island populations was assessed on the two first Principal Component (PCs), and TPS deformation grids were used to visualize changes in head shape along the PCs.

We performed multivariate analyses of variance (MANOVA) to test for between-group differences, considering: (1) four pre-defined taxonomic groups corresponding to the *P. siculus siculus* outgroup reference (Vulcano), *P. siculus strombolensis* (Panarea, Stromboli, Basiluzzo), *P. siculus trischittai* (Bottaro), and *P. siculus liscabiancae* (Lisca Bianca) and (2) six pre-defined geographical groups corresponding to islands. We then used Discriminant Function Analysis (DFA) to evaluate confidence in a priori assignments of group (1) based on cross-validation assignment rates. MANOVA and DFA of PW scores were performed in R using the packages *car* v.3.1-1 (Fox and Weisberg 2019) and *MASS* v.7.3-58.1 (Venables and Ripley 2002).

RESULTS

Phylogenetic results

The ML phylogenetic analysis of 361 *P. siculus* from its whole distribution range indicates five well-supported clades (Fig. 2): an Adriatic clade (ultrafast Bootstrap Support, uBS = 100), a Tyrrhenian clade (uBS = 94), two Calabrian clades (C1, uBS = 99; C2, uBS = 100), and a Sicilian clade (uBS = 99). Sequences of the three Aeolian subspecies of *P. siculus* were placed within the Sicilian clade with high statistical support (uBS = 99; Fig. 2). Neither the populations of the Aeolian Archipelago, nor any of the Aeolian subspecies of *P. siculus* form monophyletic groups.

Median-joining network analyses corroborate the patterns observed in the ML tree while providing increased

phylogeographic resolution (Fig. 3). The subspecies *P. s. strombolensis*, *P. s. trischittai*, and *P. s. liscabiancae* do not segregate as distinct haplogroups, neither in the *cytb* network (Fig. 3) nor in the *nd4* network (Supporting Information, Fig. S1, S2 and S3). Populations referred to these subspecies from the islands of Bottaro, Lisca Bianca, Panarea, and Vulcano share haplotypes among each other and with Sicilian populations. In particular, the most common haplotype found in the Aeolian Islands is shared among the three subspecies and the nominal subspecies from Sicily. The number of haplotypes observed, and their relationships indicate a high haplotype and nucleotide diversity within the Sicilian clade, a small proportion of which is represented within the Aeolian populations.

Results of the monophyly assessment of morphological subspecies in *Podarcis* wall lizards show that among 70 subspecies (15 from mainland, 55 from islands) belonging to seven *Podarcis* species, only six (8.6%; all from islands) were recovered as monophyletic in mitochondrial trees or networks (Table 3).

Morphometric results

Variation in head shape described by the PCA reveals a weak differentiation among islands (Fig. 4). The two first PCs accounted for 31% and 35% of total variance in males and females, respectively. PC1 differentiates Panarea and the three islets (positive values) from Stromboli and Vulcano (negative values), whereas PC2 corresponds to within-island variance. The islets of Bottaro, Lisca Bianca, and Basiluzzo each represent a subset of the morphospace occupied by Panarea (Fig. 4). TPS deformation grids showed similar deformations on PC1 and PC2 in males and females (Fig. 4), corresponding to changes in head width (PC1) and in the length of parietal scales (PC2). Higher PCs explained < 9% of total variance. Head shape was significantly different among islands (MANOVA; males: d.f. = 5, $F = 2.36$, $P = 2e-11$; females: d.f. = 5, $F = 1.84$, $P = 0.004$), as well as between a priori defined subspecies (MANOVA; males: d.f. = 3, $F = 2.09$, $P = 5e-6$; females: d.f. = 3, $F = 2.25$, $P = 0.004$). Based on Akaike's Information Criteria (AIC), the between-islands model better describes morphometric variation among individuals compared to the between-subspecies model, for both males ($\Delta AIC = 458$) and females ($\Delta AIC = 255$). Consistently, the DFA of partial warp scores revealed weak confidence in a priori assignments into subspecies: 68% for males and 55% for females (Table 2).

DISCUSSION

A countless number of animal subspecies have been described for insular populations given the striking phenotypic variation expressed on islands (Mayr 1963). In many cases, genetic data have further corroborated the validity of island subspecies, sometimes showing they represent true species requiring special conservation attention (Mayr 1982, Braby *et al.* 2012). A global meta-analysis on birds found that a greater proportion of island-dwelling morphological subspecies coincide with evolutionary units defined by molecular data, compared to continental subspecies (Phillimore and Owens 2006). This is possibly due to the fact that, beside phenotypic divergence, island populations genetically diverge fast due to physical limitations to gene flow

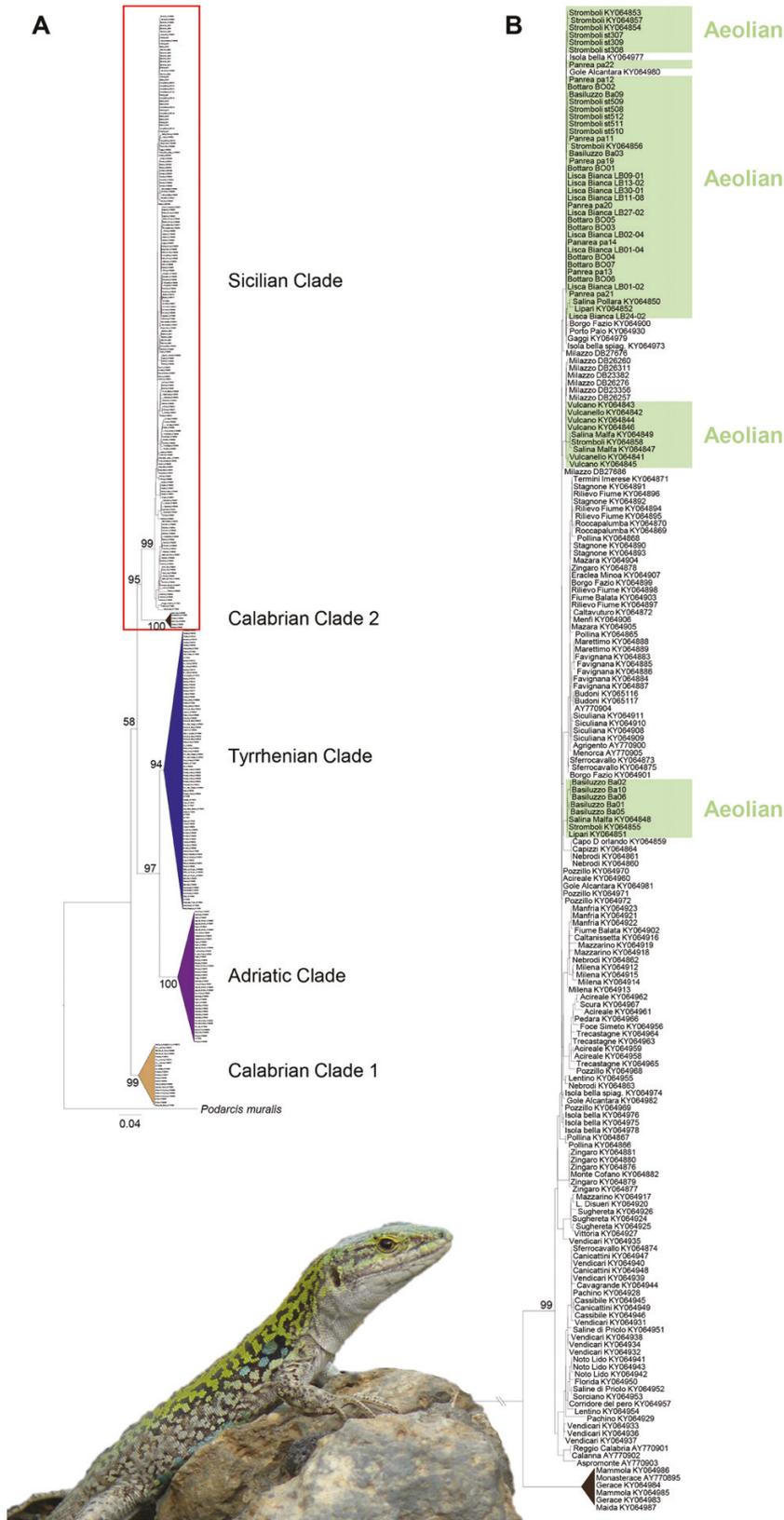


Figure 2. A, Maximum Likelihood phylogenetic tree of *Podarcis siculus* based on the *cytb* dataset. Bootstrap values (ultrafast bootstrap support) are reported in correspondence of main nodes. Except for the Sicilian clade, the other four main clades are collapsed and depicted with different colours. B, Phylogenetic relationships among specimens of the Sicilian clade. For each sequence, the locality and GenBank accession number is reported. Sequences of *P. siculus* from the Aeolian Archipelago are highlighted in green. Bottom left image: adult male of *P. siculus* from Vulcano island (photo by D. Salvi).

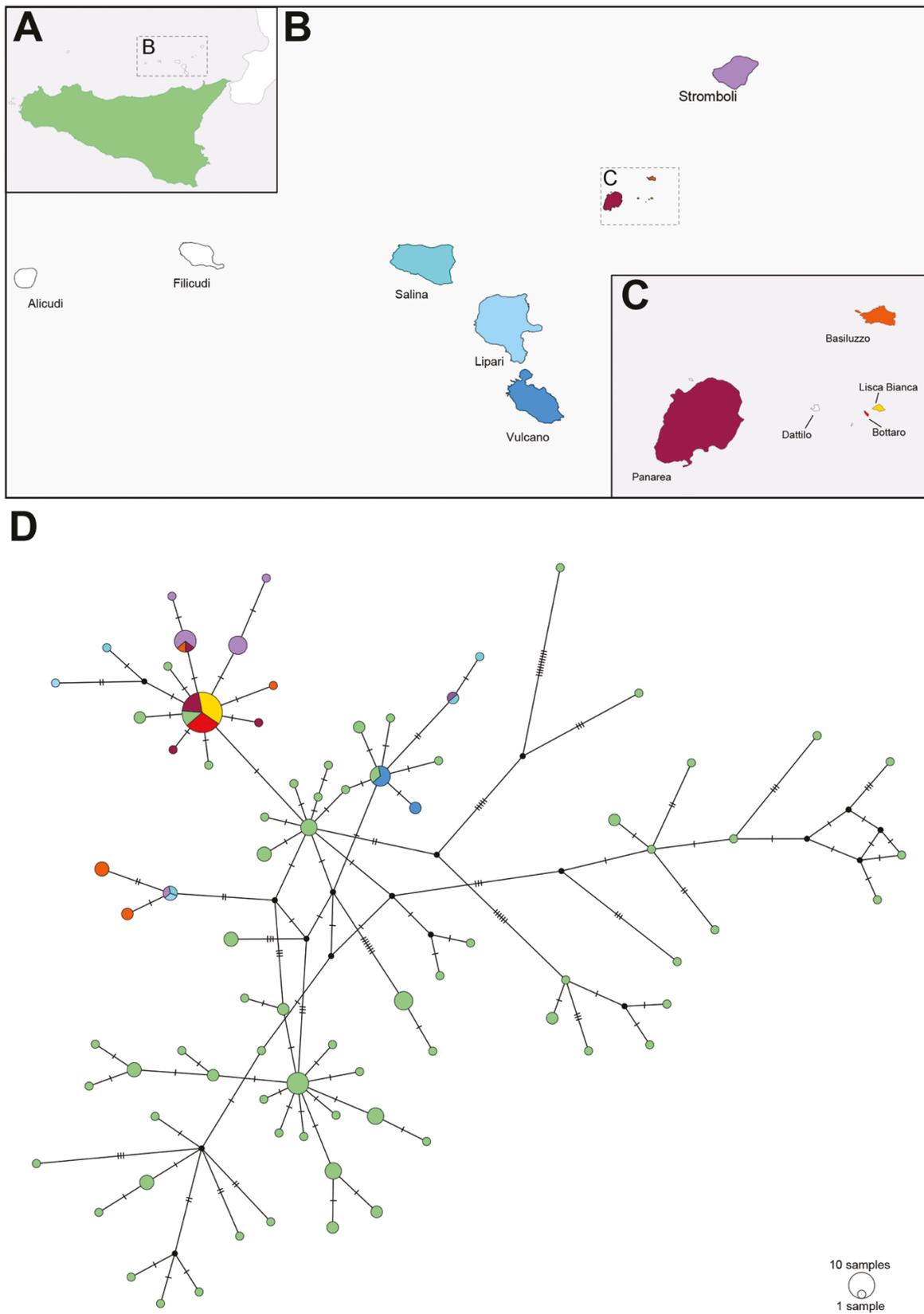


Figure 3. Phylogenetic network and geographic distribution of *cytb* haplotypes of *Podarcis siculus* from Sicily and the Aeolian Islands. a, Study area including Sicily and the Aeolian Islands, (b) with a focus on the Aeolian Islands, and (c) Panarea and the satellite islets. (d) Haplotype median-joining network showing the phylogenetic relationships among *cytb* haplotypes; haplotypes are represented by circle charts with slices coloured according to their geographic origin, and with (chart and slice) size proportional to their frequency.

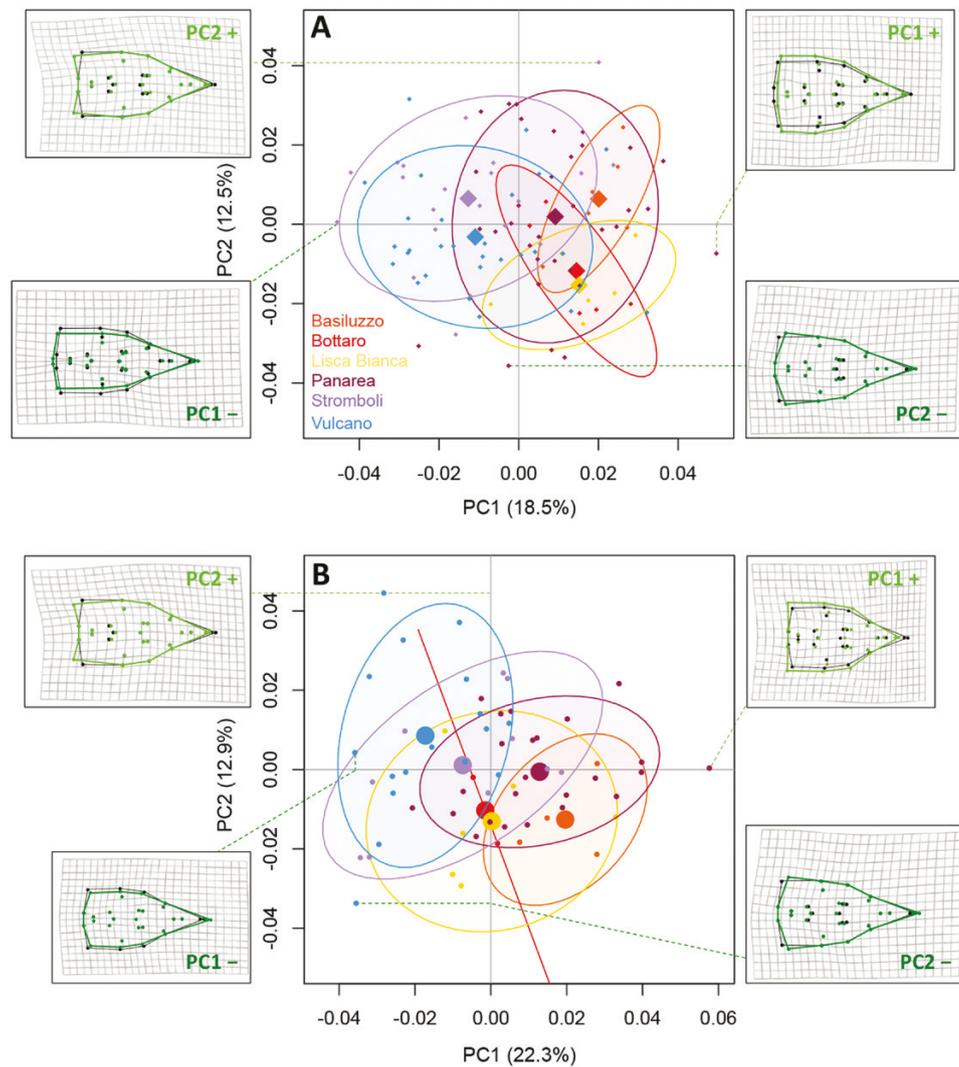


Figure 4. Results of PCA of warp scores (relative warp analysis). Head shape variation among islands on PC1 vs. PC2 in (a) males (diamonds) and (b) females (circles). Small symbols: individual scores; large symbols: island centroids. Thin-plate spline deformation grids showing shape variation as maximum : along PC1 in positive (right, light green) and maximum negative (left, dark green) directions deformations; along PC2 (PC1=0) and PC1 (PC2=0) in positive (top, light green) and negative (bottom, dark green) directions deformations; reference form (mean of all configurations) in black.

caused by geographical isolation and the fixation of neutral alleles due to founder events (Mayr 1963). The high level of genetic distinctiveness of island subspecies found in highly vagile species like birds led to hypothesize that a similar (or even stronger) pattern would apply for animals with considerably lower over-sea dispersal capacity such as lizards. Results from this study do not corroborate this expectation, indicating that none of the Aeolian Island subspecies of the Italian wall lizard is phylogenetically distinct.

The genetic variation of the Aeolian subspecies is shared with other Aeolian or Sicilian populations and, despite using fast-evolving mitochondrial markers (Palumbi et al. 2001), none of the island subspecies or populations emerged as a distinct phylogenetic lineage (Figs 2, 3). Patterns of haplotype sharing and non-monophyly are affected by effective population size and sample size. In this respect, it is remarkable that these patterns are evident even if lizard population sizes are large on both islands and mainland Sicily, and our sample size for island

populations was limited ($N < 10$). The lack of phylogenetic differentiation among Aeolian Island populations indicates that insufficient time has elapsed for coalescence to take place. This would corroborate the hypothesis that the presence of *P. siculus* in many Aeolian Islands and islets is due to recent colonization through human translocation (Capula 1994a, Lo Cascio and Corti 2006, Ficetola et al. 2021, Bonardi et al. 2022, Sherpa et al. 2023). Indeed, Panarea and the satellite islets (Lisca Bianca, Dattilo, Bottaro, and Basiluzzo) have been visited by humans since the Upper Neolithic (Brea and Cavalier 1968, Martinelli 2021), and ruins of human settlements from the Bronze Age in Panarea (Punta Milazzo dated between 1500 and 1200 B.C.; Caracuta et al. 2012) and from the Hellenistic and Roman Ages on the Basiluzzo Islet (a Roman villa and a submerged wharf dated between 50 B.C. and 50 A.D.; Anzidei et al. 2014) are visible today. As demonstrated by the submerged archaeological remains in Basiluzzo, these islets experienced relevant subsidence over the past 2000 years (Anzidei et al. 2014) and were formerly

Table 2. Assignment test of collected *P. siculus* to the four described Aeolian subspecies based on head shape geometric morphometrics, using Discriminant Function Analysis of partial warp scores. In bold: support for a priori subspecies assignments

A priori assignment	Statistical assignment			
	<i>P. siculus siculus</i>	<i>P. siculus strombolensis</i>	<i>P. siculus trischittai</i>	<i>P. siculus liscabiancae</i>
Males				
<i>P. siculus siculus</i> (Vulcano)	0.793	0.138	0.000	0.069
<i>P. siculus strombolensis</i>	0.200	0.667	0.050	0.083
Basiluzzo	0.000	0.667	0.167	0.167
Panarea	0.182	0.727	0.030	0.061
Stromboli	0.286	0.571	0.048	0.095
<i>P. siculus trischittai</i> (Bottaro)	0.000	0.400	0.600	0.000
<i>P. siculus liscabiancae</i> (Lisca Bianca)	0.000	0.667	0.000	0.333
Females				
<i>P. siculus siculus</i> (Vulcano)	0.611	0.222	0.000	0.167
<i>P. siculus strombolensis</i>	0.184	0.579	0.000	0.237
Basiluzzo	0.250	0.750	0.000	0.000
Panarea	0.125	0.583	0.000	0.292
Stromboli	0.200	0.600	0.000	0.200
<i>P. siculus trischittai</i> (Bottaro)	0.000	0.000	0.500	0.500
<i>P. siculus liscabiancae</i> (Lisca Bianca)	0.167	0.333	0.333	0.167

larger and perhaps partially connected one each other (Cavaliere 1994). More recently, during the past few centuries, Basiluzzo and Lisca Bianca have been used for agriculture, goat and sheep grazing, and sulphur mining (Lo Cascio and Corti 2006 and reference therein). Such an intense and prolonged human presence on these islands provides a likely scenario for the recent introduction of lizards from Panarea to its satellite islets.

Morphometric analyses of head shape, on the other hand, revealed a significant but weak morphological differentiation of the four Aeolian subspecies, although no island subspecies was diagnosable from all others. More evident is the morphological differentiation between island populations across the whole archipelago, as previously observed by Muraro *et al.* (2022). The island-specific morphological differences, despite a lack of genetic differentiation, indicate that local phenotype variation occurred in a short amount of time. Morphological changes in insular habitats are known to occur rapidly (Madsen and Shine 1993, Millien 2006, Eloy de Amorim *et al.* 2017), such as in the case of *Podarcis* lizards (Herrel *et al.* 2008, Vervust *et al.* 2010, Taverne *et al.* 2021). Some of these phenotypic changes can emerge over the course of just a few generations due to neutral processes linked to island colonization (genetic drift) or to rapid adaptation and phenotypic plasticity in response to diverse habitats or resource types (Herrel *et al.* 2008, Runemark *et al.* 2010, Vervust *et al.* 2010, Young and Badyaev 2010, Levis *et al.* 2018, Sherpa *et al.* 2023).

The lack of genetic distinctiveness reported for Aeolian subspecies of *P. siculus* is not restricted to this archipelago or to this species. Previous range-wide phylogeographic studies of *P. siculus* (Podnar *et al.* 2005, Senczuk *et al.* 2017) included 26 of the 52 subspecies recognized by Henle *et al.* (1986) and none of them was recovered as phylogenetically distinct in mitochondrial or nuclear gene trees (Table 3). Likewise, comprehensive phylogeographic surveys on six other *Podarcis* species occurring

in Italy revealed that subspecies rarely match evolutionary units (Podnar *et al.* 2004, 2005, Giovannotti *et al.* 2010, Bellati *et al.* 2011, Salvi *et al.* 2013, 2014, 2017, Senczuk *et al.* 2017, 2018, 2019a).

The lack of congruence between traditional subspecies boundaries and phylogenetic units in wall lizards is clearly due to their extensive phenotypic variability in colour pattern, biometry, and pholidosis (Kaliontzopoulou *et al.* 2010, 2012, Piras *et al.* 2011), especially in insular environments, combined with the tendency of taxonomists in the past century to describe subspecies based on ambiguous and weak morphological characters (Braby *et al.* 2012, Prates *et al.* 2023). About two hundred subspecies are currently listed for *Podarcis*, most of them described between 1930 and 1970 to designate island populations (Uetz and Stylianou 2018, Uetz *et al.* 2023). Such subspecies inflation has impacts on systematics, with a complex and confusing infra-specific taxonomy, but most importantly hampers conservation applications. Uncertainties concerning the criteria used to identify subspecies are exacerbated in conservation because legislation is implemented based on uncertain intra-specific taxonomy, with rare subsequent re-evaluations of the validity of subspecies during the selection of conservation targets (Stanford 2001, Haig *et al.* 2006, Gippoliti and Amori 2007). In this respect, the Aeolian Archipelago provides an emblematic case of the peril of assessing conservation priorities based on outdated or inflated taxonomy. On one side, the critically endangered Aeolian wall lizard *Podarcis raffonei* is underprotected because it was recognised as a distinct species two years after the adoption of the Habitats Directive (Council Directive 92/43/EEC; Gippoliti *et al.* 2017). On the other side, lizard populations of Panarea and satellite islets, assigned in the past century to distinct subspecies that are actually considered as not valid (Tiedemann *et al.* 1994, Corti and Lo Cascio 2002, Uetz *et al.* 2023, this study), have been identified as conservation targets

Table 3. Molecular assessment of phylogenetic monophyly of *Podarcis* wall lizard's subspecies. We considered species occurring in Italy for which at least two subspecies were sampled in molecular studies. The minimum criterion for monophyly testing of a subspecies is that more than one individual ($N \geq 2$) of that particular subspecies was sampled in the phylogeny (* flags subspecies with small sample size: $N < 5$). For those subspecies for which a single individual was sampled we applied a criterion of haplotype sharing with other subspecies as an indication on non-monophyly (whereas in these cases the lack of haplotype sharing is not sufficient as a proof of monophyly)

Species	Subspecies	Mainland (M)/ Island (I)	Distribution	mtDNA marker	N	Criterion	Monophyly	Reference
<i>Podarcis filfolensis</i>	<i>filfolensis</i>	I	Malta, Filfol island (= Filfla)	<i>nd4</i>	≥ 5	Monophyly	Yes	Salvi et al. 2014
	<i>generalensis</i>	I	Malta, General's Island (= Fungus Rock)		≥ 5	Monophyly	No	Salvi et al. 2014
	<i>kieselbachi</i>	I	Malta, San Paul Island (= Selmunett)		≥ 5	Monophyly	No	Salvi et al. 2014
	<i>laurentiimuelleri</i>	I	Italy, Linosa and Lampione islands		≥ 5	Monophyly	No	Salvi et al. 2014
	<i>maltensis</i>	I	Malta, Gozo, Kemmuna (= Comino)		≥ 5	Monophyly	No	Salvi et al. 2014
<i>Podarcis latastei</i>	<i>lanzai</i>	I	Italy, Gavi Islet	<i>cytb</i>	≥ 5	Monophyly	No	Senczuck et al. 2018
	<i>latastei</i>	I	Italy, Ponza Island		≥ 5	Monophyly	No	Senczuck et al. 2018
	<i>palmarolae</i>	I	Italy, Palmarola Island		≥ 5	Monophyly	No	Senczuck et al. 2018
	<i>patrizii</i>	I	Italy, Zannone Island		≥ 5	Monophyly	Yes	Senczuck et al. 2018
<i>Podarcis melisellensis</i>	<i>aeoli</i>	I	Croatia, Mali Opuh Island	<i>cytb</i>	1	Haplotype sharing	No	Podnar et al. 2004
	<i>bokicae</i>	I	Croatia, Vrtlac Island		1	Haplotype sharing	No	Podnar et al. 2004
	<i>caprina</i>	I	Croatia, Kaprije Island		1	Haplotype sharing	No	Podnar et al. 2004
	<i>curzolensis</i>	I	Croatia, Korčula Island		1	Haplotype sharing	No	Podnar et al. 2004
	<i>digenea</i>	I	Croatia, Svetac Island		3	Monophyly	No	Podnar et al. 2004
	<i>fumana</i>	M	Croatia		≥ 5	Monophyly	No	Podnar et al. 2004
	<i>galvagnii</i>	I	Croatia, Kamik Islet		2	Monophyly	No	Podnar et al. 2004
	<i>gigantea</i>	I	Croatia, Sv. Andrija Islet		2	Monophyly	No	Podnar et al. 2004
	<i>gigas</i>	I	Croatia, Vis Island		2	Monophyly	No	Podnar et al. 2004
	<i>jidulae</i>	I	Croatia, Jidula Island		1	Haplotype sharing	No	Podnar et al. 2004
	<i>kornatica</i>	I	Croatia, Kornati Archipelago		2	Monophyly	No	Podnar et al. 2004
	<i>lissana</i>	I	Croatia, Lissa Archipelago		≥ 5	Monophyly	No	Podnar et al. 2004
	<i>melisellensis</i>	I	Croatia, Brusnik Island		3	Monophyly	No	Podnar et al. 2004
	<i>mikavicae</i>	I	Croatia, Mikavica Island		≥ 5	Monophyly	No	Podnar et al. 2004
	<i>pomoensis</i>	I	Croatia, Jabuka Islet		2	Monophyly	No	Podnar et al. 2004
<i>thetidis</i>	I	Croatia, Veliki Opuh Island		1	Haplotype sharing	No	Podnar et al. 2004	
<i>Podarcis muralis</i>	<i>albanica</i>	M	Bulgaria, Romania, Greece (etc.)	<i>cytb</i>	≥ 5	Monophyly	No	Giovannotti et al. 2010; Salvi et al. 2013

Table 3. Continued

Species	Subspecies	Mainland (M)/ Island (I)	Distribution	mtDNA marker	N	Criterion	Monophyly	Reference
	<i>baldasseronii</i>	I	Italy, Palmaiola Island		4	Monophyly	No	Bellati <i>et al.</i> 2011
	<i>beccarii</i>	I	Italy, Porto Ercole Islet		4	Monophyly	No	Bellati <i>et al.</i> 2011
	<i>breviceps</i>	M	Italy, Calabria		≥ 5	Monophyly	No	Giovannotti <i>et al.</i> 2010; Salvi <i>et al.</i> 2013
	<i>brogniardii</i>	M	NW Spain, An- dorra, France, W Germany, Switzerland		≥ 5	Monophyly	No	Salvi <i>et al.</i> 2013
	<i>colosii</i>	I	Italy, Elba Island		≥ 5	Monophyly	No	Bellati <i>et al.</i> 2011
	<i>insulanica</i>	I	Italy, Pianosa Island		≥ 5	Monophyly	No	Bellati <i>et al.</i> 2011
	<i>maculiventris</i>	M	S Switzerland, W Austria, N Italy, W Slovenia, NW Croatia, SE Ger- many		≥ 5	Monophyly	No	Giovannotti <i>et al.</i> 2010; Bellati <i>et</i> <i>al.</i> 2011; Salvi <i>et</i> <i>al.</i> 2013
	<i>maruccii</i>	I	Italy, Argentarola Islet		3	Monophyly	No	Bellati <i>et al.</i> 2011
	<i>muellerlorenzi</i>	I	Italy, La Scola Islet		3	Monophyly	No	Bellati <i>et al.</i> 2011
	<i>muralis</i>	M	Balkans, Aus- tria, Slovakia, Czech Republic, Poland, Ukraine, NE Italy (the Alps), and Turkey		≥ 5	Monophyly	No	Giovannotti <i>et al.</i> 2010; Salvi <i>et al.</i> 2013
	<i>nigriventris</i>	M	Italy (incl. Elba and neighbouring islands)		≥ 5	Monophyly	No	Giovannotti <i>et al.</i> 2010; Bellati <i>et</i> <i>al.</i> 2011; Salvi <i>et</i> <i>al.</i> 2013
	<i>vinciguerrai</i>	I	Italy, Gorgona Island		2	Monophyly	Yes*	Bellati <i>et al.</i> 2011
<i>Podarcis</i>	<i>astorgae</i>	I	Croatia, Astorga Island	<i>cytb</i>	1	Haplotype sharing	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
<i>siculus</i>	<i>bagnolensis</i>	I	Croatia, Bagnola (= Banjol) Island		1	Haplotype sharing	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>calabra</i>	M	Italy, Calabria		≥ 5	Monophyly	No	Senczuck <i>et al.</i> 2017
	<i>campana</i>	M	Italy		≥ 5	Monophyly	No	Senczuck <i>et al.</i> 2017
	<i>campestris</i>	M	Italy, Croatia, France, Slovenia		≥ 5	Monophyly	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>cattaroi</i>	M	Montenegro, Kotor		2	Monophyly	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>cazzae</i>	I	Croatia, Sušac and Pod Kopište islands		2	Monophyly	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>cettii</i>	I	Sardinia, Menorca, S Corsica		3	Monophyly	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>dupinici</i>	I	Croatia, Veli and Mali Dupinić		2	Monophyly	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017

Table 3. Continued

Species	Subspecies	Mainland (M)/ Island (I)	Distribution	mtDNA marker	N	Criterion	Monophyly	Reference
	<i>hieroglyphicus</i>	M	Turkey		≥ 5	Monophyly	No	Silva-Rocha <i>et al.</i> 2014
	<i>insularum</i>	I	Croatia, La Longa Island		2	Monophyly	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>kolombatovici</i>	I	Croatia, Velika and Mala Kluda islets		2	Monophyly	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>laganiensis</i>	M	Croatia, peninsula of Istria		1	Haplotype sharing	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>mediofasciata</i>	I	Croatia, Dužac and Mala Sestrica islets		2	Monophyly	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>nikolici</i>	I	Croatia, Gusti Školj Islet		1	Haplotype sharing	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>pelagosae</i>	I	Croatia, Velika Palagruža Islet		1	Haplotype sharing	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>pirosoensis</i>	I	Croatia, Piroso Grande Island (= Piruzi Veliki)		1	Haplotype sharing	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>pohlibensis</i>	I	Croatia, Pohlib, Planičić, Hrid Sip, Planik, Olib, and Morovnik islands		4	Monophyly	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>premudana</i>	I	Croatia, Premuda, Hripa and Masarine islands		1	Haplotype sharing	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>premudensis</i>	I	Croatia, Lutrošnjak Islet		1	Haplotype sharing	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>pretneri</i>	I	Croatia, Gustinja Cliffs (= Gustigna), Pisulj (= Pusiglio) Cliffs		1	Haplotype sharing	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>ragusae</i>	M	Croatia, Dubrovnik		1	Haplotype sharing	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>samogradi</i>	I	Croatia, Samograd, Vrtlić		2	Monophyly	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>siculus</i>	M	Italy (including Sicily and many islands), Slovenian and Croatian Adriatic coast and offshore islands		≥ 5	Monophyly	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>vesseljuchi</i>	I	Croatia, Veseljuh Island		1	Haplotype sharing	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017
	<i>zeii</i>	I	Croatia, Kal Cliffs (= Sc. la Calle)		1	Haplotype sharing	No	Podnar <i>et al.</i> 2005; Senczuck <i>et al.</i> 2017

Table 3. Continued

Species	Subspecies	Mainland (M)/ Island (I)	Distribution	mtDNA marker	N	Criterion	Monophyly	Reference
<i>Podarcis tiliguerta</i>	<i>rodulphisimonii</i>	I	France, Finocchiarola, Isolotto di Mezzo and Isolotto di Terra islets	12S	3	Monophyly	No	Vasconcelo <i>et al.</i> 2006, Salvi <i>et al.</i> 2017
	<i>maresi</i>	I	France, Toro Grande and Toro Piccolo islets		4	Monophyly	Yes*	Vasconcelo <i>et al.</i> 2006, Salvi <i>et al.</i> 2017
	<i>ranzii</i>	I	Italy, Molarotto Islet		2	Monophyly	Yes*	Vasconcelo <i>et al.</i> 2006, Salvi <i>et al.</i> 2017
	<i>tiliguerta</i>	I	Italy, Sardinia		≥ 5	Monophyly	No	Vasconcelo <i>et al.</i> 2006, Salvi <i>et al.</i> 2017
<i>Podarcis waglerianus</i>	<i>waglerianus</i>	I	Italy, Sicily, Favignana, Levanzo islands, and La Scola islands	<i>nd4</i>	≥ 5	Monophyly	No	Senczuk <i>et al.</i> 2019a
	<i>marettimensis</i>	I	Italy, Marettimo Island		≥ 5	Monophyly	Yes	Senczuk <i>et al.</i> 2019b

in the establishment of the Nature Reserve of ‘Isola di Panarea e Scogli Viciniori’ (Establishment measures: D.A. 483/44 of 25-07-1997). Likewise, the wall lizard populations of Linosa and Lampione (Pelagian Archipelago), once assigned to the subspecies *P. filfolensis laurentiimuelleri*, have been listed as one of the conservation targets of the Natural Reserve ‘Isole di Linosa e Lampione’ (D.A. 82/44 of 18-04-2000), although genetic data do not support this subspecies and challenge the native status of these populations (Capula 1994b, Salvi *et al.* 2014). Although molecular studies help reduce the burden of subspecies inflation, conservation legislation often relies on outdated taxonomy based on morphological subspecies. It can be contended that only molecular validated subspecies are eligible as conservation targets. However, oddly enough, there are still studies that maintained the validity of subspecies that failed any molecular assessment of phylogenetic distinction and monophyly, such as in the case of three subspecies of the recently recognised *Podarcis latastei* (Senczuk *et al.* 2018; Table 3). A much safer option would therefore be to abandon the subspecies rank as proxy for units of conservation in wall lizards and rely on integrative assessment of the evolutionary significance of phenotypic and genetic units.

CONCLUDING REMARKS: TOWARDS A GENOMIC DISSECTION OF PHENOTYPIC VARIATION FOR EVOLUTIONARY AND CONSERVATION APPLICATIONS

This study demonstrates that the intraspecific taxonomy of wall lizards is a poor predictor of phylogeographic partitions and evolutionary units, and therefore of limited use for defining conservation and management units. However, the use of mitochondrial genealogies to assess subspecies limits has its own limitations,

including applying the phylogenetic concepts to subspecies validation (Patten 2015), and the nature of mitochondrial DNA variation. Such an approach implicitly assumes that subspecies should represent monophyletic and diagnosable populations (Zink 2004, Phillimore and Owens 2006). However, under a coalescent neutral model lack of monophyly is often expected for recently diverged populations such as those commonly designated as subspecies (Neigel and Avise 1986, Hudson 1990, Avise 2000, Hudson and Turelli 2003, Rosenberg 2003). Moreover, because the population tree is expected to differ from gene trees (Rosenberg 2002), we cannot rely on a single gene tree to infer historical population sundering.

A key aspect requiring clarification is whether the units we want to delimit or validate (the subspecies) as proxies of conservation units should represent historical partitions within a population tree, or local adaptation. The concept of ESU has long focused on the recognition of sets of populations that have been historically isolated and, accordingly, are likely to have a distinct potential (Moritz 1994). However, many modern subspecies concepts refer to ‘heritable geographic variation in phenotype’, and ‘phenotypic variation of adaptive significance’ (Crandall *et al.* 2000, Patten 2015) that implies that informative genes for subspecies delimitation would be non-neutral, and that in most cases a handful of genes sparsely distributed across the genome might be associated with the key phenotypic trait that varies geographically.

In this respect, an excellent example is provided by the common wall lizard *P. muralis*. In this lizard, Italian populations showing a green dorsal and black ventral coloration have been referred to the subspecies *P. m. nigriventris*. This colour pattern is correlated with a suite of sexually selected traits (syndrome) including large body and head size, and aggressive behaviours (While *et al.* 2015). Although mitochondrial genealogies have

failed to recover this subspecies as monophyletic (Giovannotti *et al.* 2010, Bellati *et al.* 2011, Salvi *et al.* 2013), genomic studies have shown that the *nigriventris* phenotype is associated with Single Nucleotide Polymorphism (SNP) variation representing only 2% of the genome (Yang *et al.* 2018). Males with the *nigriventris* phenotype show a competitive advantage in male-male competition under hot and dry climates (Heathcote *et al.* 2016, MacGregor *et al.* 2017, Ruiz Miñano *et al.* 2021, 2022). This explains why this phenotype has introgressed into a distantly related lineage along the north-western Italian coast, but did not spread within the same lineage from coastal to inland mountain populations (While *et al.* 2015, Yang *et al.* 2018, Ruiz Miñano *et al.* 2021, 2022). As a consequence, genomic clusters based on thousands of neutral SNPs do not match phenotypic partitions, whereas genomic clusters based on syndrome-associated SNPs group all individuals carrying the *nigriventris* phenotype in a monophyletic clade (Yang *et al.* 2018). These studies exemplify how the complex genomic architecture of intraspecific phenotypic variation cannot be captured by discrete intraspecific taxonomic partitions nor by historical partitions, no matter how accurately they are defined (mitochondrial genealogies vs. thousands of neutral loci across the genome).

Nevertheless, genotyping-by-sequencing data of *P. siculus* show that populations of the eastern Aeolian Islands (from Vulcano to Stromboli) form a genetic cluster with populations of eastern Sicily (Milazzo), from which they recently originated (Sherpa *et al.* 2023), thus supporting the results from this study. However, all island populations can be further differentiated in genomic and morphometric traits (this study; Muraro *et al.* 2022, Sherpa *et al.* 2023). Future genome-wide studies of the Aeolian populations of *P. siculus* can unravel the actual importance of historical, selective, and plastic processes underlying the observed phenotypic variation.

In conclusion, the burden of available intraspecific names assigned to wall lizard populations and their loose association with significant evolutionary units suggests that intraspecific names rarely help to identify conservation units. We highlight that, while some evolutionary and conservation units may well fit some subspecies definitions laid upon both the biological and the phylogenetic species concept (see for example Patten 2015), these do not need to be named to receive conservation attention. Instead, the best way for defining conservation targets is framed upon the integration of phenotypic and population genomic data. Population genomic data offer an essential view over the genetic basis of the phenotypic variation and its evolutionary and conservation significance, and allow disentangling adaptive and sexually selected phenotypic variation from historical (phylogenetic) units. The increasing availability of genomic resources (Formenti *et al.* 2022) suggests that this approach will be suitable for an increasing number of non-model species.

SUPPORTING INFORMATION

Supplementary data is available at *Biological Journal of the Linnean Society* online.

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CONFLICT OF INTEREST

None declared.

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DATA AVAILABILITY

Data supporting the findings of this study are available in the Supporting Information including GenBank accession numbers of the DNA sequences generated or analysed in this study.

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