

Low genetic differentiation between populations of *Podarcis sicula* (Reptilia, Lacertidae) from the Italian islands off the coast of Campania and the mainland

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The Italian wall lizard, *Podarcis sicula* (Rafinesque-Schmaltz, 1810), is a morphologically variable species, widespread in Italy (Sardinia, Sicily and most of the Tyrrhenian and Italian Ionian and Adriatic islands included), Corsica and the northern part of the east Adriatic coast (Slovenia, Croatia and many Dalmatian islands and part of Montenegro). Naturalized populations are found in Spain, Portugal, France, Turkey and USA (1). It is found on multiple small islands throughout its range, most of which contain morphologically distinctive populations. This has led to many subspecies being described. HENLE & KLAVER (2) reviewed 91 described subspecies and accepted 52, of which 47 were single island endemics. Assessment of the genetic distinctiveness of these subspecies is essential, since although the peninsular populations are generally not threatened, island populations may be vulnerable (1). Some microinsular populations may have gone extinct through environmental degradation, while a distinct subspecies, *P. s. sanctistephani* of Santo Stefano Island (Arcipelago delle Pontine), appears to have been replaced after the nominal subspecies was introduced to the island (3; 4). PODNAR et al. (5) identified six main haploclades within *P. sicula*. One of these, the “Monasterace group” is known from only one locality on the Ionian coast, indicating that extensive sampling is needed to fully determine genetic diversity. However, little diversity was found between some Adriatic island subspecies and mainland forms, corroborating doubts of the validity of several subspecies.

Our aim was to examine genetic diversity of *P. sicula* from various Campanian islands and the South-eastern Pontian Archipelago islands, some of which have been described as island endemic subspecies. This area lies on the border of the area where the “campestris-sicula” hap-

logroup was found (5), but no previous information was available regarding the majority of these island populations, only one individual from one island, Ischia, had previously been determined. We examined diversity by sequencing part of the mitochondrial DNA cytochrome b, so that our results could be compared to previously published data on *P. sicula* (5), but also so that levels of diversity could be compared to other insular *Podarcis* subspecies, such as those from the Balearics (6).

TABLE 1

Localities of samples used, their position on Fig. 1, and their respective haplotypes in Fig. 2

Code	Locality	Map Code	Haplotype
24	Ventotene	1	B
20	Ventotene	1	I
31	Santo Stefano	2	G
25	Santo Stefano	2	J
44	Ischia	3	A
45	Ischia	3	E
CA 4	Ischia	4	A
Pr 14	Procida	5	D
51	Procida	5	A
PP 3	Punta Pennata	6	A
Ni 3	Nisida	7	A
Ni 4	Nisida	7	H
Na 4	Napoli	8	B
Rv 4	Rovigliano	9	B
Vt 1	Vetara	10	A
Vt 2	Vetara	10	A
62	Castelluccia	11	F
71	Gallo Lungo	12	A
32	Capri	13	A
43	Capri	13	C
Li 9	Licosa	14	K
Li 10	Licosa	14	K
74	Camerota	15	B

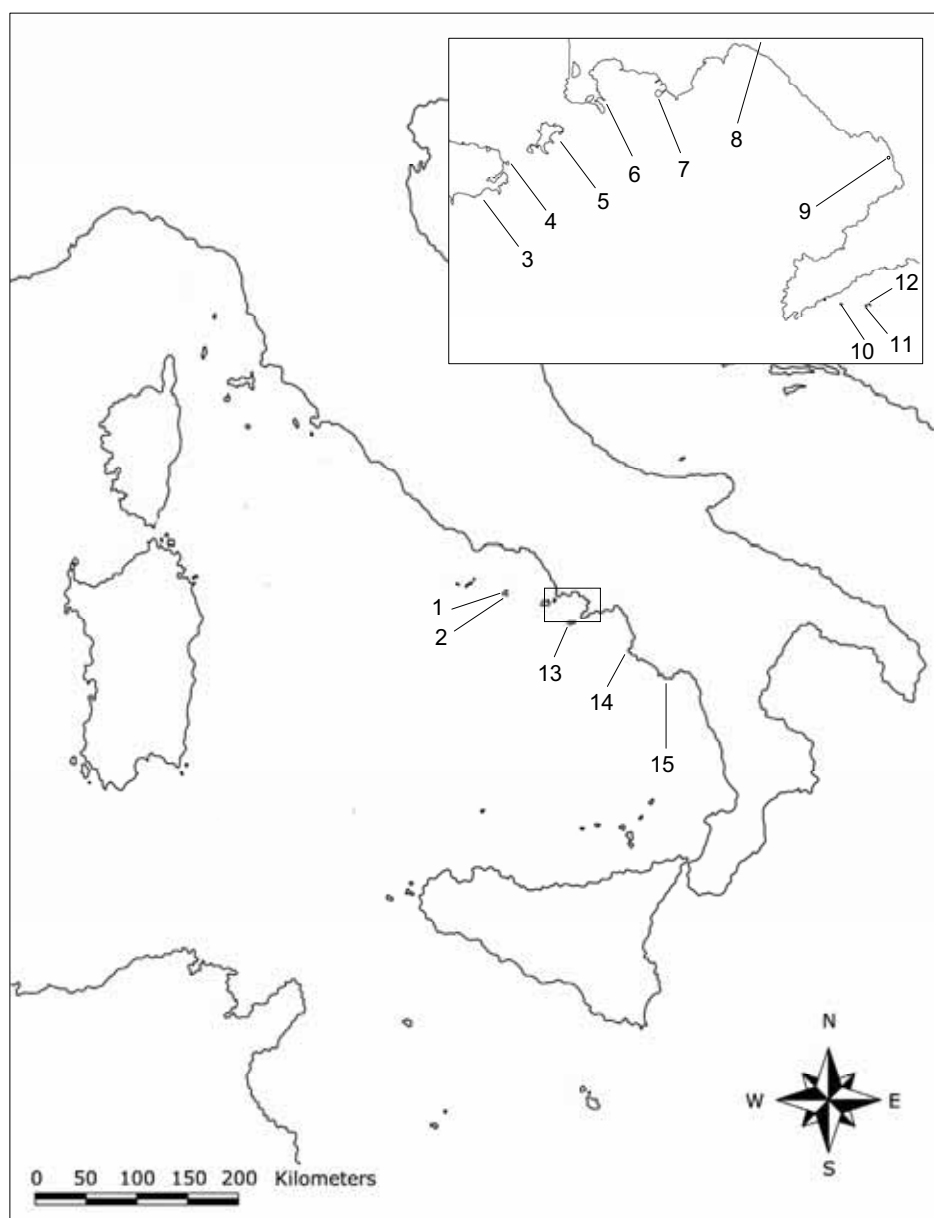


Fig. 1. – Map showing the sampling locations of *P. sicula* sequenced for this study. Codes are given in Table 1.

The number and geographic locations of the specimens used in this study are given in Table 1 and Fig. 1. Individuals were released after tail tips were collected. The tissue samples have been collected under the permits of the Ministero dell'Ambiente e della Tutela del Territorio e del Mare, DPN-1701/2006 and the Riserva Naturale Marina di Ventotene e Santo Stefano, Ente Parco Regionale dei Campi Flegrei, Ente Parco Nazionale del Cilento e Vallo di Diano.

Tissue samples were stored in 100% ethanol. Total genomic DNA was extracted from tissue samples following the SAMBROOK et al. (7) protocol. Polymerase Chain Reaction primers used in both amplification and sequencing were GluDG and Cytb2 from PALUMBI et al. (8) and KOCHER et al. (9) respectively. Amplification conditions were the same as described by HARRIS et al. (10). Amplified fragments were sequenced on a 310 Applied Biosystem DNA Sequencing Apparatus. Mitochondrial DNA

sequences were aligned by eye. Within species, when variation is low, networks are a more appropriate way of assessing genetic variation than using the more common phylogenetic tree building algorithms (11). Since initial inspection of the sequences indicated that variation was low, all the haplotypes were joined in a most parsimonious network (Fig. 2).

In total, 23 new specimens were included for a total of 390 base pairs. Five closely related individuals from GenBank, with three distinct haplotypes were also included, and assigned the same codes as in the original publications (12; 5). Alignment was facile as this is a protein coding gene, and no insertions or deletions were needed. New haplotypes have been submitted to GenBank with accession numbers EU916814 to EU916824. In total 11 new haplotypes were recovered that differed by at most four nucleotide substitutions across this region.

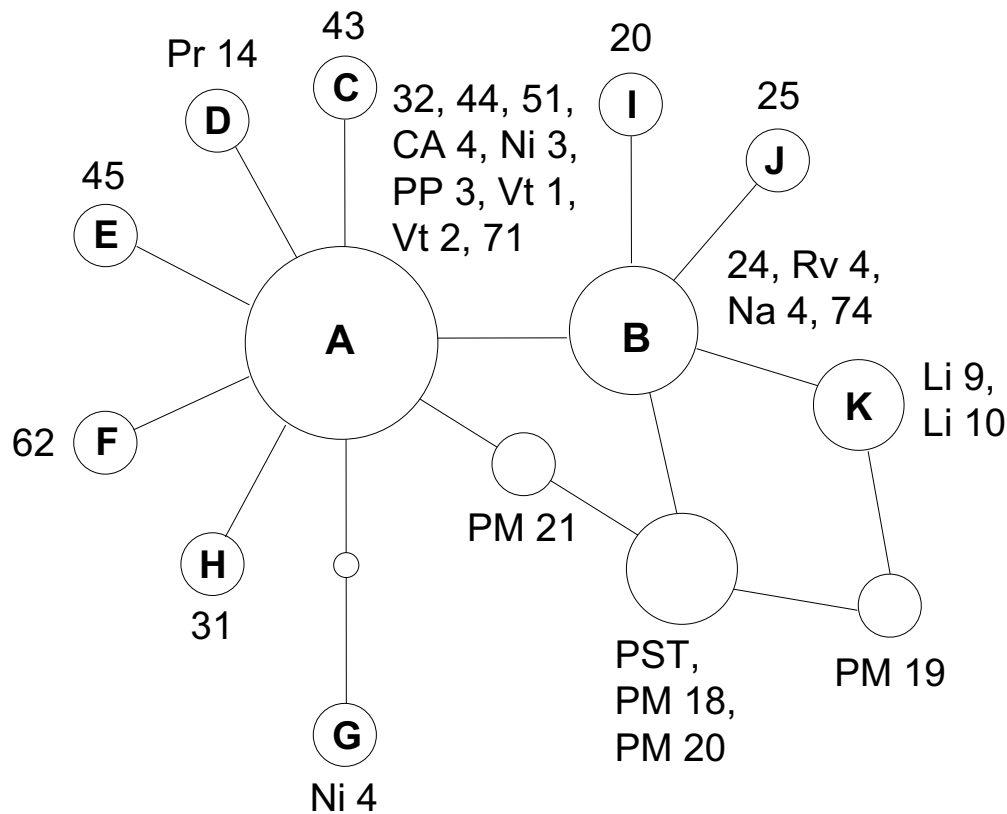


Fig. 2. – Most parsimonious network of variation of the 11 new haplotypes (A-K) plus three closely related haplotypes from GenBank (PM21, PM19 and individuals PST, PM18 and PM20). Size of circles indicates relative number of individuals with that haplotype. The small circle between haplotypes A and G indicates the sole presumed missing haplotype. Individual codes are given in Table 1.

Our results show that the island populations are genetically very similar to each other and to the mainland populations, representing the “*campestris-sicula*” haplogroup of PODNAR et al. (5). The two commonest haplotypes, A and B are found on several islands and on the continent, while the remaining rare haplotypes are generally island specific. This is despite morphological differences, such that some island forms have been assigned subspecific status, such as *P. s. ventonensis* from Ventotene, *P. s. klemmeri* from Licosia and *P. s. gallensis* from Gallo Lungo and Castelluccia. Our results suggest that these differences are more likely due to founder effects, drift phenomena and rapid adaptations to local island condition rather than longer term evolutionary isolation. All of these islands, except for Ventotene and Santo Stefano, lie in relatively shallow water (less than 100m), and so would have been connected to the continent during sea level fluctuations associated with the last glacial cycles.

Despite the low levels of differentiation, overall diversity was not particularly low, with 11 haplotypes determined from 23 individuals. This implies that during the last glacial period populations of *P. sicula* were large enough in this area to maintain considerable diversity, something that is not surprising given that populations of *Podarcis* in the southern Iberian Peninsula and North Africa were also relatively large (13). Moreover, since the Olocene the Campanian islands vertebrate fauna seem to be related mainly to human intervention rather than to natural colonization (14). During the XX Century, for

example, on Gallo Lungo Island, some continental lizard individuals have been introduced to control insect population (G. Casa, pers. comm., 16-05-2007). The “star-like” network configuration is also consistent with a population expansion, presumably following the last glacial maxima. At this time the islands were colonized, and drift effects led to the current morphological differences. CAPULA & CECCARELLI (15) also reported low diversity between Pontine Archipelago populations of *P. sicula* and mainland populations based on allozyme electrophoretic data. A similar situation occurred in the colonization of the Berlengas islands off the West coast of Portugal by individuals of *Podarcis carbonelli* (16) that are morphologically distinct from their coastal relatives (17). Similar low-differentiation of microinsular populations is also seen in *P. tiliguerta* on islands off Sardinia (18) and in *P. lilfordi* in the Balearics (6). Our results thus further support the view of PODNAR et al. (5) that most of the island endemic subspecies of *P. sicula*, and perhaps of many other species of *Podarcis*, are not genetically distinct entities. However, this is not always the case, the endemic *Podarcis* from the Aeolian Islands once thought to be a subspecies of *Podarcis wagleriana* is now considered a distinct and critically endangered species, *Podarcis raffonei*, after genetic and ecological investigations (19). Considerable cryptic variation within *Podarcis hispanica* has also been reported (20, 21). Thus other island forms of *Podarcis* still need to be evaluated in case other unidentified genetically-distinct lineages exist.

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REFERENCES

1. CORTI C (2006). *Lucertola campestre* / Italian wall lizard. In: SINDACO R, DORIA G, RAZZETTI E & BERNINI F (eds), *Atlante degli Anfibi e dei Rettili d'Italia / Atlas of Italian Amphibians and Reptiles*, Societas Herpetologica Italica, Edizioni Polistampa, Firenze.
2. HENLE K & KLAVER CJJ (1986). *Podarcis sicula* (Rafinesque-Schmaltz, 1810), Ruineneidechse. In: BÖHME W (ed), *Handbuch der Reptilien und Amphibien Europas*, Band 2/II Ehsen (Sauria) III (Lacertidae III: *Podarcis*), Aula-Verlag, Wiesbaden: 254-342.
3. CORTI C & LO CASCIO P (2002). The Lizards of Italy and Adjacent Areas. Chimaira Verlag, Frankfurt-am-Main.
4. LANZA B & CORTI C (1996). Evolution of the knowledge on the Italian herpetofauna during the 20th century. *Bollettino del museo civico di storia naturale di Verona*, 20, (1993):373-436.
5. PODNAR M, MAYER W & TVRTKOVIC N (2005). Phylogeography of the Italian wall lizard, *Podarcis sicula*, as revealed by mitochondrial DNA sequences. *Molecular Ecology*, 14 (2):575-588.
6. TERRASA B, PICORNELL A, CASTRO JA & RAMON MM (2004). Genetic variation within endemic *Podarcis* lizards from the Balearic Islands inferred from partial Cytochrome b sequences. *Amphibia-Reptilia*, 25 (4):407-414.
7. SAMBROOK J, FRITSH EF & MANIATIS T (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbour Press, Cold Spring Harbour, NY.
8. PALUMBI S, MARTIN A, ROMANO S, McMILLAN WO, STICE C & GRABOWSKI G (1991). *Fool's guide to PCR*. Univ. Hawaii, Honolulu, HI.
9. KOCHER TD, THOMAS WK, MEYER A, EDWARDS SV, PÄÄBO S, VILLABLANCA FX & WILSON AC (1989). Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences*, 86:6196-6200.
10. HARRIS DJ, ARNOLD EN & THOMAS RH (1998). Relationships of lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 265 (1409):1939-1948.
11. POSADA D & CRANDALL KA (2001). Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology & Evolution*, 16 (1):37-45.
12. PODNAR M, MAYER W & TVRTKOVIC N (2004). Mitochondrial phylogeography of the Dalmatian wall lizard, *Podarcis melisellensis* (Lacertidae). *Organisms Diversity & Evolution*, 4 (4):307-317.
13. PINHO C, HARRIS DJ & FERRAND N (2007). Contrasting patterns of population subdivision and historical demography in three western Mediterranean lizard species inferred from mitochondrial DNA variation. *Molecular Ecology*, 16 (6):1191-1205.
14. NAPPI A, CIPOLLA RM, GABRIELE R, MASSETI M, CORTI C & ARCIDIACONO G (2007). Anfibi, Rettili e Mammiferi delle isole del Golfo di Napoli: check-list commentata. *Studi Trent Scienze Naturali, Acta Biologica*, 83 (2007):93-97.
15. CAPULA M & CECCARELLI A (2003). Distribution of genetic variation and taxonomy of insular and mainland populations of the Italian wall lizard, *Podarcis sicula*. *Amphibia-Reptilia*, 24 (4):483-495.
16. HARRIS DJ & SA-SOUSA P (2002). Molecular phylogenetics of Iberian wall lizards (*Podarcis*): Is *Podarcis hispanica* a species complex? *Molecular Phylogenetics and Evolution*, 23 (1):75-81.
17. LUZ AC, MONASTERIO C & VICENTE L (2006). How variable can be *Podarcis carbonelli berlengensis*? A study of comparative morphology. In: CORTI C, LO CASCIO P & BIAGGINI M (eds), *Mainland and Insular Lacertid Lizards: a Mediterranean Perspective*, Firenze University Press, Firenze: 111-123.
18. HARRIS DJ, PINHO C, CARRETERO MA, CORTI C & BÖHME W (2005). Determination of genetic diversity within the insular lizard *Podarcis tiliguerta* using mtDNA sequence data, with a reassessment of the phylogeny of *Podarcis*. *Amphibia-Reptilia*, 26 (3):401-407.
19. CAPULA M (1994). Genetic-variation and differentiation in the lizard, *Podarcis wagleriana* (Reptilia, Lacertidae). *Biological Journal of the Linnean Society*, 52 (2):177-196.
20. HARRIS DJ, CARRANZA S, ARNOLD EN, PINHO C & FERRAND N (2002). Complex biogeographical distribution of genetic variation within *Podarcis* wall lizards across the Strait of Gibraltar. *Journal of Biogeography*, 29 (9):1257-1262.
21. PINHO C, FERRAND N & HARRIS DJ (2006). Reexamination of the Iberian and North African *Podarcis* (Squamata: Lacertidae) phylogeny based on increased mitochondrial DNA sequencing. *Molecular Phylogenetics and Evolution*, 38 (1):266-273.

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