Genetic diversity within Corsican and Sardinian specimens of the Tyrrhenian Wall Lizard, *Podarcis tiliguerta*, estimated using mtDNA sequences

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Podarcis tiliguerta*, a Wall Lizard endemic to Corsica and Sardinia, has recently been shown to harbour highly genetically distinct mitochondrial lineages, that may indicate it is in fact a species complex. Here we combine 12S rRNA mtDNA sequences from previous studies with 17 newly generated sequences to better understand genetic diversity within this group. In particular we include samples from the Cerbicale islands, which were quite distinct in an earlier assessment of protein electrophoretic variation. Results confirm that distinct lineages exist on Corsica and Sardinia. The Cerbicale islands appear as part of the Corsican group. A third lineage exists, although at present it is known only from a single specimen from Sardinia. Further morphological and molecular data is needed to revise the taxonomy of this apparent species complex.*

Keywords: *Podarcis tiliguerta*, evolution, systematics, 12S rRNA, Corsica and Sardinia.

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Introduction

Although wall lizards of the genus *Podarcis* are a dominant part of the herpetofauna of much of Mediterranean Europe, exact delimitations of species is often difficult. Recent applications of molecular methods to some forms indicate that they might be species complexes. For example, *P. hispanica* contains several highly genetically distinct lineages all of which may deserve species status (Harris *et al*. 2002, Pinho *et al*. 2006). *Padarcis erhardii* is also probably a species complex (Poulakakis *et al*. 2003), while *P. sicula*, *P. melisellensis* and *P. taurica* exhibit considerable intraspecific variation (Podnar *et al*. 2004, 2005; Poulakakis *et al*. 2005). On the other hand, the many insular subspecies described for species such as *P. lilfordi*, *P. pityusensis* and *P. melisellensis* often showed little or no genetic differentiation (Terrassa *et al*. 2004, Podnar *et al*. 2004). Thus there is a need for further phylogeographic analyses of remaining *Podarcis* species, especially the insular ones.

Podarcis tiliguerta is endemic to Corsica, Sardinia and many neighbouring small islands, with at least ten recognized subspecies (Arnold & Ovenden 2002). Using allozyme electrophoresis, Capula (1996) reported that genetic variation was distributed into three geographically coherent population groups: Corsica, the small islands off the south-eastern coast of Corsica (Cerbicale and Lavezzi) and Sardinia and Meli Island. A preliminary analysis of mtDNA sequences (Harris *et al*. 2005) indicated high levels of diversification between individuals from Corsica and Sardinia, at a level more typically observed between species. Another preliminary study, including four new 12S rRNA mtDNA sequences of *P. tiliguerta* (Podnar & Mayer 2005) obtained a similar result, with one distinct lineage on Corsica and two on Sardinia. However, few subspecies were included in either study, and in particular samples from the Cerbicale islands were lacking.

The aim of this work was to complement the earlier phylogeographic analysis of *P. tiliguerta* by extending the sampling area in order to cover more of its distribution range, including islets off Northeast Sardinian – Molara, Molarotto and Tavolara – and Corsica – Finocchiarola island, and especially Toro Grande islet, one of the Cerbicale islands. Further, the data from the previous studies (Harris *et al*. 2005, Podnar & Mayer 2005) is combined for the first time to gain insight into the distribution of the mtDNA lineages.

Materials and Methods

Podarcis tiliguerta specimens were collected in the field from Corsica and Sardinia (Fig. 1) and released after a small tail clip, tissue samples being taken and stored in

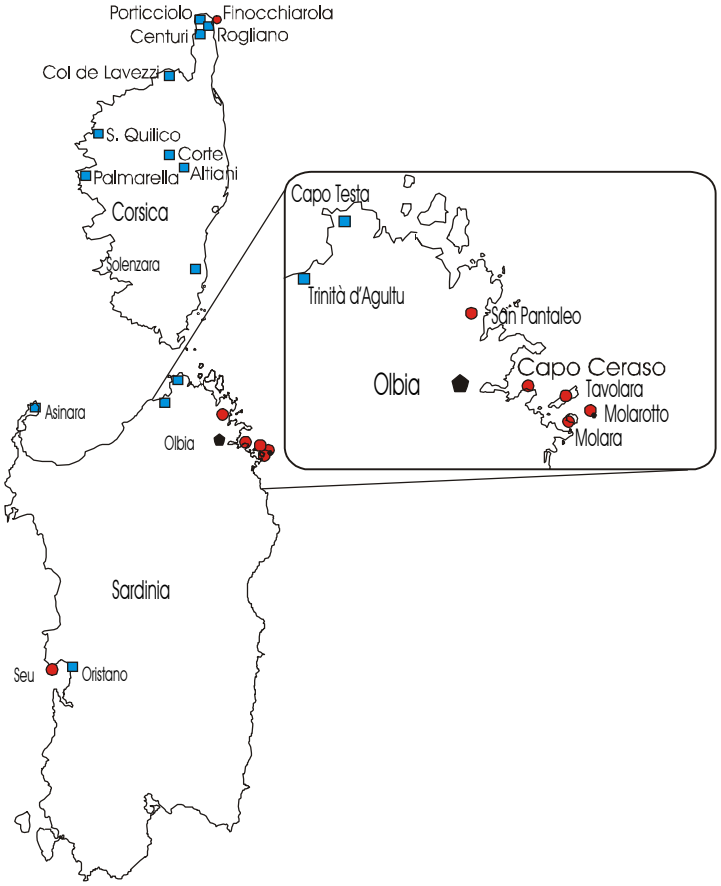

Fig. 1. Map showing sampling localities of *Podarcis tiliguerta* sequenced. Circles refer to the new localities sampled in this study and squares to the previous ones sampled by Harris *et al.* (2005).

ethanol. Genomic DNA was extracted following a standard high-salt protocol. Part of the 12S gene was amplified by PCR using 12Sa and 12Sb (Kocher *et al.* 1989) and conditions described by Harris (2001). This region was chosen to be amplified in order to include previous published sequences from Harris *et al.* (2005). The amplified products were sequenced on an automated sequencer (ABI 310 by Amersham Biosciences) and the 17 new sequences – 10 from Sardinia and seven from Corsica – were checked by eye and aligned against others previously published using BioEdit (Hall 1999) – 12 from Corsica and six from Sardinia, analysed by Harris *et al.* (2005) and four samples from Podnar & Mayer (2005). Details of the new samples and sequences used in this study are presented in the Table 1. The new sequences were deposited on GenBank, accession numbers EF 165017 to EF 165024.

Table 1. Location and sample codes of the new samples used in this study.

Location	Code
Sardinia, Molara island	SM1
Sardinia, Molara island	SM2
Sardinia, Tavolara island	ST
Sardinia, Capo Ceraso	Scc1
Sardinia, Capo Ceraso	Scc2
Sardinia, Capo Ceraso	Scc3
Sardinia, San Pantaleo	Ssp
Sardinia, Molarotto island	SMo1
Sardinia, Molarotto island	SMo2
Sardinia, Seu	Ss
Corsica, Finocchiarola island	CF1
Corsica Finocchiarola island	CF2
Corsica, Finocchiarola island	CF3
Corsica, Toro Grande islet	CTg1
Corsica, Toro Grande islet	CTg2
Corsica, Toro Grande islet	CTg3
Corsica, Toro Grande islet	CTg4

Analytical methods

To assess the relationships of the lineages of *P. tiliguerta* to other *Podarcis* and to each other, 37 12S rRNA sequences from a wide variety of *Podarics* were aligned, including representatives of the three known lineages within *P. tiliguerta*. Both Maximum Parsimony and Neighbour Joining were used to estimate relationships, and support for nodes was estimated using the Bootstrap technique. To analyse the low level of variation within each group of *P. tiliguerta* for which multiple samples were available – one consisting of the specimens from the Corsican archipelago and another of the Sardinian archipelago – the haplotypes of these groups were joined in two median-joining networks (Bandelt *et al.* 1999). In total 34 sequences of 343 base pairs length – 16 from Corsica and 18 from Sardinia – were studied.

Results

The level of divergence found within 12S sequences of *Podarcis tiliguerta* from Corsica and Sardinia was high as previously reported (Harris *et al.* 2005), with

a difference of 17-23 mutations in only 343 bp. Our estimate of phylogeny has few well supported nodes, because only this short fragment of 12S rRNA can be combined from the different studies (Fig. 2). The only relationship between species that is supported is that between the well-established sister *taxa*, *P. wagleriana* and *P. raffonei*. However, three distinct lineages within *P. tiliguerta* can be identified, two in Sardinia and one in Corsica. For one of these only a single individual from Central-Eastern Sardinia has been reported. For the other two, in order to determine variation within each lineage two different networks were constructed, one of Corsican specimens and the other of Sardinia ones (Fig. 3). A total of 11 haplotypes were found. About the same number of haplotypes were revealed within 12S rRNA sequences from Corsica and Sardinian samples, with one new haplotype in six and in five total haplotypes, respectively. Most individuals from Northeast Sardinia, including some individuals from small islands like Tavolara and Molara, shared the same haplotype for this gene. The individuals from Molarotto island presented three mutational steps from the main island haplotype and the individual

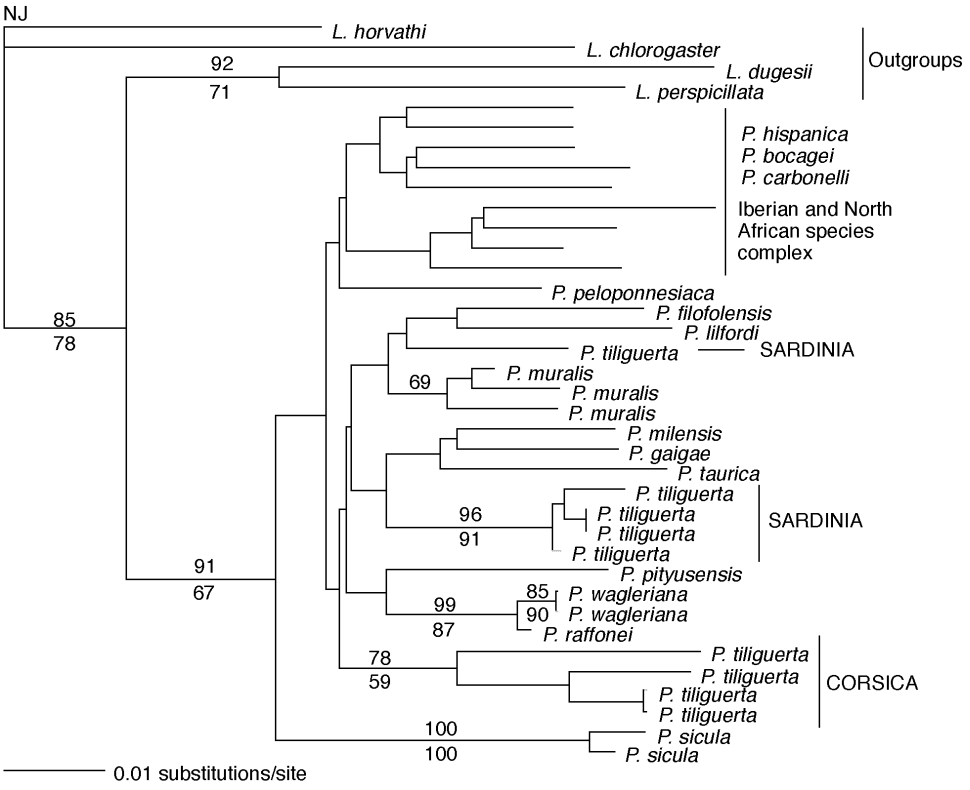

Fig. 2. Estimate of relationships derived from an uncorrected NJ analysis. Bootstrap support (500 replicates) from NJ and MP are indicated above and below nodes respectively.

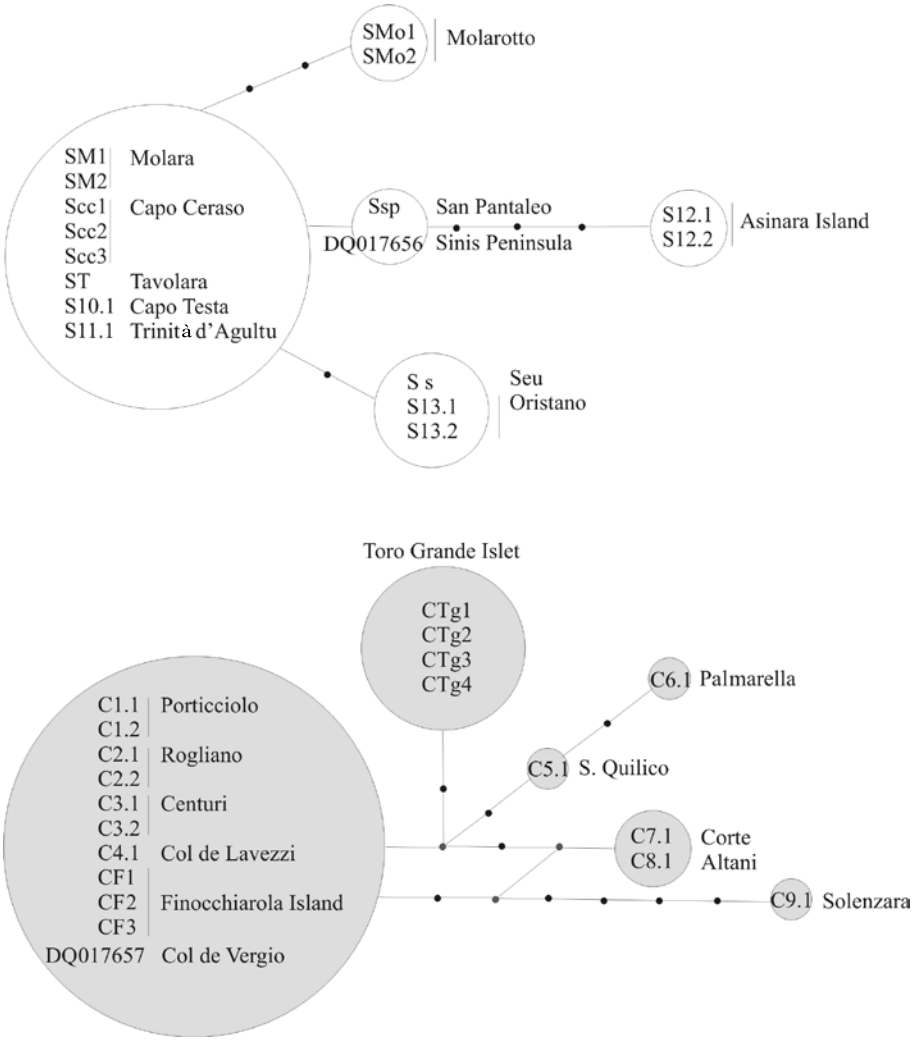

Fig. 3. Median-joining network of the 12 S rRNA sequences for *Podarcis tiliguerta.* The Corsican samples are in grey and the Sardinian ones in white. Filled circles indicate presumed missing haplotypes. Codes for new sequences are given in table 1; all others are from Harris *et al.* (2005) and Podnar & Mayer (2005).

from San Pantaleo one. Interestingly, in Corsica most genetic variation seems to occur in the southern populations. However, individuals from the Cerbicale island of Toro Grande, that are considered a distinct subspecies (*P. t. maresi*) and that formed a distinct group based on analysis of electrophoretic data (Fig. 4; Capula 1996), are not especially different from other Corsican populations.

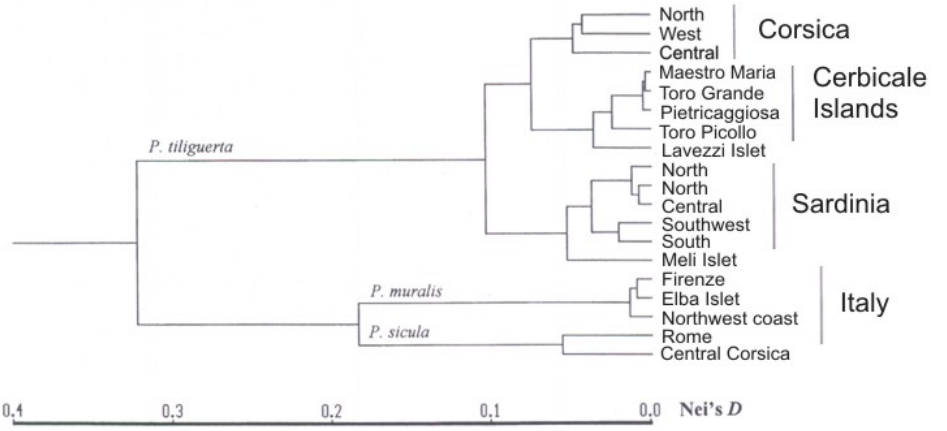

Fig. 4. Estimate of relationships derived from protein electrophoretic analysis, adapted from Capula (1996).

Discussion

These results generally support the conclusions of Harris *et al.* (2005) and Podnar & Mayer (2005). The high genetic variation (5-6%) between *Podarcis tiliguerta* sequences from Corsica and Sardinia does not conflict with the hypothesis of separate species in each archipelago. The Cerbicale islands appear to form part of the Corsican clade. A third lineage also exists in Sardinia, although at present only one sample is known so few conclusions can be drawn from this. The diversity of *P. tiliguerta* in Sardinia and in South Corsica is, however, clearly underestimated, as shown by the appearance of several new haplotypes. On the other hand, newly sequenced subspecies, such as *P. t. maresi*, do not appear to be very distinct. Thus the present taxonomy is clearly inadequate. Further sampling in broader areas, especially in the west of Sardinia and in the rest of the Southestern Corsican Cerbicale islands, is needed. This, followed by morphological and molecular studies with nuclear makers with these specimens, will help to clarify its phylogeny and also its evolutionary and geological history and eventually to a revised taxonomy. Since the geology of the Corso-Sardinia microplate is well known, the area also promises to be an excellent region for calibrating molecular clocks and for other comparative phylogeographic studies. In particular, assessment of genetic variation within the other endemic lacertids, *Archedacerta bedriagae* and *Algyroides fitzingeri*, will be especially interesting.

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