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# Molecular phylogenetics of the Italian *Podarcis* lizards (Reptilia, Lacertidae)

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ABSTRACT

Phylogenetic relationships within the Italian species of the lacertid genus *Podarcis* were examined by parsimony analysis of mitochondrial DNA sequences from the genes encoding the small ribosomal RNA and the phenyl transfer RNA. *Lacerta viridis* was used as outgroup and *Teira dugesii* was also included in the ingroup. The 80 phylogenetically informative positions produced four most parsimonious trees, with the Italian *Podarcis* split into three groups: the first comprised *P. filfolensis, P. melisellensis, P. wagleriana, P. muralis,* and *P. raffonei,* the second *P. sicula* with its various subspecies. The third lineage was that of *P. tiliguerta,* whose relationships were resolved as more closely related to the first clade, when weighting transversion three times transitions. Bootstrap analyses on a subset of sequences representing all species herein studied, supported the results from the larger dataset. The present results are only partly in agreement with previous hypotheses based on morphology, immunology, and allozyme variation analyses.

KEY WORDS: Molecular systematics - 12S rDNA - Cladistics - Wall lizards - *Podarcis* - *Lacerta* - *Teira*.

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## INTRODUCTION

The reconstruction of phylogenies is of primary importance in the understanding of the dynamic patterns of evolution (i.e., the biogeography of a group, the bases of its biological diversity at any level etc.).

Phylogenetic relationships of the genera in the family Lacertidae are still incompletely clarified. Arnold (e.g., 1973, 1989a, b, 1993) proposed phylogenetic hypotheses based on morphological and biochemical datasets; contrasting arrangements, based on immunological records, were proposed by Mayer & Benyr (1994). The collective genus *Lacerta* Linné, 1758 has in the last years undergone considerable revision and been split into several genera, previously considered as subgenera by most of the specialists; however, the systematic position of a few species is still controversial and the generic value of some taxa is still debated (Mayer & Tiedemann, 1982; Böhme, 1984; Lutz & Mayer, 1985; Busack & Maxson, 1987). The Afrotropical species have been referred to distinct genera (Arnold, 1989a, b) and the genus is presently restricted to the Euro-Mediterranean region.

Several Mediterranean taxa previously ascribed to *Lacerta* are presently included in the genus *Podarcis* Wagler, 1830. This is a complex of species (Fig. 1) diffused in central Europe and in the circum-Mediterranean regions, with the exception of the xeric south-eastern areas and of Anatolia, and includes several endemic insular species. Böhme (1986) recognised 17 species and, according to Richter (1980), divided *Podarcis* into two subgenera, the nominate *P. (Podarcis)* (with 15 species) and *P. (Teira)* Gray, 1838 (with 2 species). The systematics of *Podarcis* are still controversial and *Teira*, including *dugesii* (Milne-Edwards, 1829), *perspicillata* (Duméril & Bibron, 1839) and *andreanskyi* (Werner, 1929), has recently been elevated to generic rank by Mayer & Bischoff (1996). At the species level, one of the taxa listed by Böhme (1986), namely *gaigeae* (Werner, 1930), is presently regarded (Mayer & Tiede-

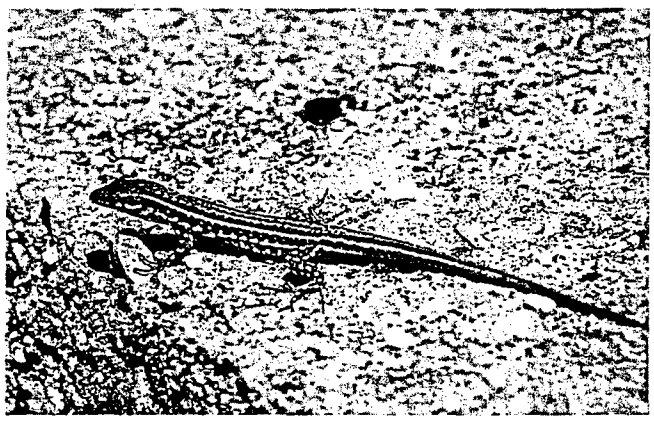

Fig. 1 - *Podarcis tiliguerta* (Gmelin, 1789). M. Gennargentu, Sardinia, Italy.

mann, 1980, 1981; Tiedemann & Mayer, 1980) as a subspecies of *milensis* (Bedriaga, 1882), while *raffonei* (Mertens, 1952) was raised to specific rank (Capula, 1994a). Consequently, at present 15 species are ascribed to *Podarcis* sensu stricto.

The following seven species are presently recognised as living in Italy: *P. filfolensis* (Bedriaga, 1876), endemic to some Sicilian Channel islands; *P. melisellensis* (Braun, 1877), a dinaric element restricted in Italy to the Julian Karst; *P. muralis* (Laurenti, 1768), largely diffused in Europe; *P. raffonei* (Mertens, 1952), endemic to a few Aeolian islands; *P. sicula* (Rafinesque-Schmaltz, 1810), widely spread in Italy and Dalmatia; *P. tiliguerta* (Gmelin, 1789), endemic to the Corso-Sardinian complex; *P. wagleriana* (Gistel, 1868), endemic to Sicily and some satellite islands. All the Italian species, as well as other *Podarcis*, are morphologically highly polymorphic, and many subspecies have been described in literature: the majority of these taxa require strong taxonomic re-evaluation (see e.g., Corti *et al.*, 1989). In fact, taxonomy at species level of *Podarcis*, as well as of other lizard genera, has mostly been based on chromatic pattern variation and/or other external and skeletal features (e.g., Böhme, 1986). More recently, karyological (e.g., Olmo *et al.*, 1986, 1987) and biochemical analyses (e.g., Gorman *et al.*, 1975; Lanza & Cei, 1977; Lanza *et al.*, 1977; Mayer, 1981; Guillaume & Lanza, 1982; Mayer & Tiedemann, 1982; Lutz & Mayer, 1984, 1985; Lutz *et al.*, 1986; Capula *et al.*, 1987, 1988; Mayer & Lutz, 1989, 1990; Capula, 1990, 1994a, b, c; Mayer & Benyr, 1994) of some species have provided useful taxonomic data. As indicated by Arnold (1993: 10), the taxonomy of this genus is still unresolved and "*Podarcis* have proved very resistant to analysis".

In some instances biochemical analyses gave indications of the phylogenetic relationships among species. Capula (1990) compared the allozyme variation of all *Podarcis* and one *Teira* species, analysed phylogenetically a morphological dataset and derived (contrasting) phylogenetic hypotheses from the resulting trees. Böhme & Corti (1993) listed several "groups of species" of western Mediterranean *Podarcis* and discussed the relevant zoogeographical scenarios concerning these groups.

We present here the preliminary results of a study aimed at reconstructing phylogenetic hypotheses for the genus *Podarcis* on the basis of molecular characters. Recent studies in this field on the genus *Gallotia* Boulenger, 1916 (e.g., Thorpe *et al.*, 1993, 1994) highlighted the power of mitochondrial sequences for reconstructing phylogenies within the family Lacertidae. Arnold (1993) indicated an unpublished research in progress on this topic (391 nucleotides from the mitochondrial 12S rRNA gene) concerning eleven lizard genera. The present work refers to the seven recognised Italian species of the genus *Podarcis*, with the inclusion of one representative of the (sub)genus *Teira*, namely *dugesii* (Milne-Edwards, 1829). The outgroup to *Podarcis* and *Teira* was searched for within the family Lacertidae. The systematic position of *Podarcis* emerging from the trees proposed on the basis of morphological (Arnold, 1989b), or immunological characters (Mayer & Benyr, 1994) is not completely consistent, and the sister group of *Podarcis* remains uncertain. Possible closely related taxa to *Podarcis* included *Teira*, *Algyroides* Bibron & Bory, 1833 as well as *Archeolacerta* Mertens, 1921 or different groups of the genus *Lacerta*, which in both studies is regarded as a polyphyletic taxon. For the present study, according to classical systematics (Böhme, 1986) the genus *Lacerta* s.s. was considered the optimal outgroup choice, also considering the inclusion of *Teira* in the ingroup. We report the partial DNA sequences of the mitochondrial genes encoding the 12S ribosomal RNA (12S rDNA) and the phenyl transfer RNA ($tRNA^{Phe}$). Phylogenetic information recovered from the sequences was used to test previous hypotheses of relationships among species based on biochemical data, to develop general hypotheses on the structure of the Italian *Podarcis* fauna, and to draw working hypotheses for further phylogenetic and biogeographical analysis of the entire genus *Podarcis*, currently in progress.

## MATERIALS AND METHODS

### *Specimens used*

All seven Italian species of lizards belonging to the genus *Podarcis* were tested, including three 'subspecies' for *P. sicula* and four 'ecophenotypes' for *P. muralis*, in order to test whether morphological variation was correlated with molecular divergence. All specimens were collected in Italy, with the exception of two individuals of *P. filfolensis maltensis* and two of *P. melisellensis fiumana*. We have also included in this study a specimen of *Teira dugesi* from Madeira, in order to test its relationship with the Italian *Podarcis* and to evaluate its divergence at the molecular level. The source locations (Fig. 2) of the examined specimens are listed below. These specimens are preserved in the Zoological collections of "Roma Tre" University (MZRT), in the M. Capula collection at Museo Civico di Zoologia di Roma (MC), or in the Zoological Museum of "La Sapienza" Roma University (MZUR).

Nomenclature for subspecific entities follows the current use, with no implications on their actual status: the use of a certain denomination for subspecies refers to the taxon name currently employed for individuals originating from the relevant geographic area.

*Podarcis filfolensis maltensis* Mertens, 1921: Malta, Gozo Island, Ramla, 21.I.1997, P. Schembri leg. (Pf#1) (MZRT); Malta, Malta Island, Zeytun, 2.II.1997, P. Schembri leg. (Pf#2) (MZRT).

*Podarcis melisellensis fiumana* (Werner, 1891): Croatia, Dubrovnik, Kotor, 19.V.1986, M. Capula leg. (Pme #1; Pme #3) (MC).

*Podarcis muralis nigriventris* Bonaparte, 1836: Latium (RM), Roma, Castel di Leva, 50 m asl, 30.III.1996, M. Bologna leg. (Pmn#3) (MZRT).

*Podarcis muralis* intermediate phenotype between *nigriventris* and *brueggemanni* (Bedriaga, 1879): Latium (VT), Monte di Canino, 250 m asl, 19.IV.1996, M. Bologna leg. (Pmn#6) (MZRT).

*Podarcis muralis* (Laurenti, 1802) intermediate phenotype between *nigriventris* and the "Appenninic brown phenotype": Latium (FR), San Vittore, Radicosa, 650 m asl, 15.IV.1996, M. Bologna leg. (Pmn#4) (MZRT).

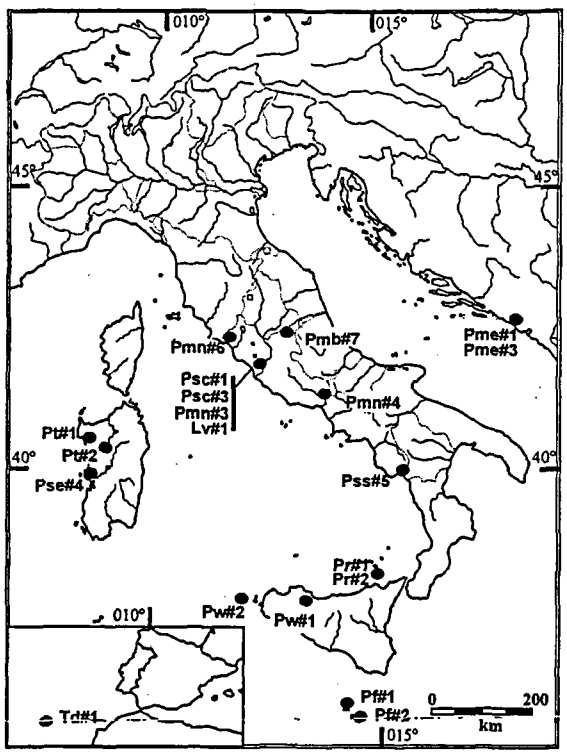


Fig. 2 - Location of the collecting sites. See Material and Methods for abbreviations. Latitude degrees are N. Longitude degrees are E.

*Podarcis muralis* "Appenninic brown phenotype" (=? ssp. *breviceps* (Boulenger, 1905)): Latium (RI), Mt. Terminillo, Vallonina shelter, 1250 m asl, 25.V.1996, M. Bologna leg. (Pmb#7) (MZRT).

*Podarcis raffonei antoninoi* (Mertens, 1955): Sicily (ME), Vulcano Island, 15.IX.1986, M. Capula leg. (Pr#1; Pr#2) (MC).

*Podarcis sicula sicula* (Rafinesque-Schmaltz, 1810): Campania (SA), Sapri beach, 13.VII.1996, P. Mariottini leg. (Pss#5) (MZRT).

*Podarcis sicula campestris* (De Betta, 1857): Latium (RM), Maccarese, Bocca di Leone dunes, 5.II.1995, M. Bologna leg. (Psc#1) (MZRT); Latium (RM), Roma, Prato Falcone, 50 m asl, 30.III.1996, P. Mariottini leg. (Psc#3) (MZRT).

*Podarcis sicula cettii* (Cara, 1872): Sardinia (OR), Is Aruttas dunes, 25.VI.1996, M. Bologna leg. (Pse#4) (MZRT).

*Podarcis tiliguerta* (Gmelin, 1789): Sardinia (NU), Bosa, Temo valley, 300 m asl, 22.VI.1996, M. Bologna leg. (Pt#1) (MZRT); Sardinia (NU), Macomer, Santa Maria de Sauccu, 450 m asl, 1.VII.1996, M. Bologna leg. (Pt#2) (MZRT).

*Podarcis wagleriana* (Gistel, 1868): Sicily (PA), Godrano, 690 m asl, 31.III.1973, G. Carpaneto leg. (Pw#1) (MZUR R-902); Sicily (TP), Marettimo Island, 4.XII.1992, M. Mei leg. (Pw#2) (MZUR R-878).

*Teira dugesii* (Milne-Edwards, 1829): Portugal, Madeira Is., Augua de Pena, 8.V.1988, H. in den Bosch leg. (Td#1) (MC).

*Lacerta viridis* (Laurenti, 1768): Latium, Roma, Castel di Decima, 2.XI.1996, M. Bologna leg. (Lv#1) (MZRT).

### *DNA isolation, amplification and sequencing*

Total DNA was extracted following standard methods (Hillis *et al.*, 1990) with slight modification: 100-200 µl of blood was taken directly from the heart with a 1 ml syringe containing 100-200 µl of 0.1 × SSC (150 mM NaCl, 15 mM Na Citrate, pH 7.2) to avoid coagulation. The solution was brought to 1-2 ml final volume with PK buffer (10 mM EDTA, 100 mM Tris-HCl pH 7.5, 300 mM NaCl, 2% SDS), containing 1-2 mg Proteinase K, incubated for 10 minutes and then extracted with standard phenol-chloroform procedure, and precipitated with ethanol. Alcohol preserved museum specimens were processed in a slightly different way. Tissue samples from one posterior leg and/or the tail were cut and homogenated. The material was washed several times in distilled water to eliminate any trace of ethanol. Then the homogenate was processed with Proteinase K and the standard phenol-chloroform extraction procedures described above, and DNA precipitated with isopropanol.

Purified total DNA was used as template for the double-stranded polymerase-chain-reaction (PCR) amplification, which was performed in 50 ml volume of a solution containing 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0:01% gelatin (Difco), each primer at 0.5 mM, each dNTP at 100 mM, 0.5-1 mg template DNA, and 1 unit of Taq Polymerase (Pharmacia Biotech). The PCR cycling parameters for amplification were 30 sec to 1 min at 95° C, 1 to 1.5 min at 48° C and 1 to 1.5 min at 72° C, for 28-30 cycles. The primers used were designed by alignment of the $tRNA^{Phe}$ and 12S rDNA sequences of four vertebrates, *Homo sapiens* (cf. Anderson *et al.*, 1981), *Gallus gallus* (cf. Desjardins & Morais, 1990), *Xenopus laevis* (cf. Roe *et al.*, 1985) and *Cyprinus carpio* (cf. Chang *et al.*, 1994). Primers were chosen from two regions of high sequence conservation among these taxa; the nucleotide differences observed were resolved in favour of the putative *consensus* sequence. The primer sequences and the position of the 5' end of the primer in the chicken mitochondrial DNA (mtDNA) sequence (Desjardins & Morais, 1990) are, for primer 1: (1,248) 5'-AAGCATAGCACTGAAGA-3', for primer 2: (1,874) 5'-AGAACAGGCTCCTCTAGG-3'. These oligonucleotides amplify portions of the $tRNA^{Phe}$ gene [52 base pair (bp)] and 12S rDNA (563-569 bp), for a total length of 615-621 bp. One-fifth of the amplified product was electrophoresed on a 2% agarose gel to visualise the corresponding DNA band; only in few cases did we observe a non-specific DNA band with a higher molecular weight (not shown). One-fiftieth of the sample was cloned using the TA Cloning$^R$ kit (Invitrogen), then a plasmid DNA minipreparation screening of the recombinant clones was carried out using standard procedure (Maniatis *et al.*, 1982). Plasmid DNA from positive clones was sequenced with the T7 Sequencing kit (Pharmacia Biotech), according to the manufacturer's protocol.

### *Phylogenetic analysis*

Nucleotide sequences were aligned by hand, and no ambiguous alignment positions were scored. Aligned sequences were analysed by the maximum parsimony (MP: Farris, 1970) and neighbor joining (NJ: Saitou & Nei, 1987) methods.

MP analyses were performed using the package PAUP 4* (test release: Swofford, 1998) partly replicated with the PHYLIP 3.5 package with identical results. The branch and bound procedure was used to search for the shortest tree(s) under the maximum parsimony assumptions. A subset of nine sequences representing all specific taxa herein investigated was employed in bootstrap analyses to test on 1000 replicates the robustness of the phylogenetic hypotheses derived from the larger dataset. The neighbor joining analysis was performed on the Jukes & Cantor divergence matrix. Equal weight was initially given both to transitions and transversions; all analyses were then replicated by imposing a triple weight to transversions. Indels (positions including insertions/deletions, aligned by gaps) were alternatively included and excluded in the analyses, to score the influence of gaps on the results.

*Lacerta viridis* was chosen as a direct outgroup, and the corresponding mtDNA sequence was analysed also from this species (according to Rykena (1991) and Nettmann (1995) the Italian populations should be ascribed to *Lacerta bilineata* Daudin, 1802).

## RESULTS

Sequences of mtDNA were obtained from 20 specimens representing the seven recognised species of the

genus *Podarcis* in Italy, *Teira dugesii* and the outgroup *L. viridis*. The EMBL Data Library accession no. of the $tRNA^{Phe}$ sequences are AJ001567- AJ001585 and AJ004885, those for the 12S rDNA sequences are AJ001415, AJ001463-AJ001480 and AJ004884. Multiple alignment with the sequence of the bird *G. gallus* Linné, 1758, as published by Desjardins & Morais (1990) resulted in 613 nucleotide positions (Table I). Of these, 80 positions contained phylogenetically informative base substitutions. Transition/Transversion ratios were greater than 2.00 in more than 85% of the pairwise comparisons within *Podarcis*.

Maximum parsimony (MP) analysis of the aligned sequences ($tRNA^{Phe}$ + 12S rDNA) by equally weighting Tv and Ts yielded four equally parsimonious trees with length 218. All trees displayed the *Podarcis* sequences as monophyletic with respect to both *Lacerta* and *Teira*. The consensus topology (Fig. 3a) presents two main clades within the *Podarcis* plus a third one including the Sardo-Corsican *P. tiliguerta* samples. The first included *P. sicula*, and the second all the remaining species. In the second clade the individuals are split into two monophyletic groups. One group - the *muralis*-group - included the widely distributed *P. muralis* together with the insular *P. raffonei*. The other group - the *wagleriana*-group - included the Sicilian *P. wagleriana*, the Maltese *P. filfolensis* and the Dalmatian *P. melisellensis*, whose relationships are not resolved by this dataset. Weighting Transversions three times the Transitions resulted in a single MP tree with length 340 (Fig. 3b) with the same general topology as the consensus, but with the sequences of *P. tiliguerta* clearly representing an early off-shoot of the second clade, before the splitting of the *muralis*-group and the *wagleriana*-group. The inclusion of indels in the analyses did not significantly changed the results, with the exception of the position of *P. tiliguerta* that was constantly the sister group to *P. sicula*.

Analysis (MP) on a subset of nine sequences representing all specific taxa herein investigated confirmed the same pattern of relationships. Equal weighting of Tv *vs* Ts produced six equally parsimonious trees with length 187 differing only in the relationships within the *wagleriana*-group and in the position of *tiliguerta* (Fig. 4). Bootstrap values (1000 replicates) strongly support the resolution of three nodes, while the monophyly of *tiliguerta* and *sicula* is very poorly supported. Weighting Transversions three times the Transitions produced nearly identical results.

Topologies nearly identical to that of the MP consensus were displayed by the trees obtained by neighbor-joining analysis (Fig. 5) based on either Jukes & Cantor, or %-divergence distance matrices. The main difference was the position of the *tiliguerta* specimens, that was constantly positioned as the sister group of the second clade (*muralis*- and *wagleriana*-groups), representing an early off-shoot of this lineage.

Sequence percent divergences (Kukes & Cantor distance) are reported in Table II. Within species sequence divergence ranged from 0.0% to 3.6%. The highest values were scored between the *sicula* specimens (0.5%-3.6%); within the other species the values ranged from 0.0% to 0.8% (the latter comparing Pma#7 with Pmn#3 and Pmn#4). Between species (Lv#1 and Td#1 excluded) divergence ranged from 0.1% to 8.3%. Sequence divergence between Td#1 and the *Podarcis* specimens ranged from 13.1% to 15.1%. Sequence divergence between the outgroup (Lv#1) and the *Podarcis* sequences ranged from 11.5% to 13.3%. The highest value was that observed between Lv#1 and Td#1, diverging by 16.4% of the sequence.

## DISCUSSION

In the present study, the monophyly of *Podarcis* seems strongly supported by the cladistic analysis of the Italian species of the genus. The position of *Teira* at the root of the *Podarcis+Teira* clade and the level of divergence from the *Podarcis* sequences would give preliminary support to its distiction as a separate lineage. Anyway the low Ts/Tv ratios (Ts/Tv < 2) in nearly all pairwise comparisons involving Td#1 notwithstanding the high level of divergence would witness for a long time of separation of the lineages (*Lacerta, Teira* and *Podarcis*); a more precise definition of their position is beyond the scope of the present paper and will be analysed by including at least another *Teira* species in the study currently in progress. We replicated all analyses by imposing Td#1 as the outgroup, and the results with regard to *Podarcis* were not affected.

The hypotheses published on the phylogenetic relationships within the genus *Podarcis* are contrasting. As regards the Italian species, little information on phylogenetic relationships are available: (1) Arnold (1973), following Klemmer (1957), morphologically recognised three groups of species: the first one including *muralis, tiliguerta, filfolensis, wagleriana* and the Greek species *milensis* (Bedriaga, 1882); the second including the Iberian and Madeiran species; the third one including *sicula, melisellensis* and two eastern Mediterranean species; (2) Lanza & Cei (1977), on the basis of immunological data, considered *wagleriana* and *filfolensis* strictly related. Lanza *et al.* (1977) referred *tiliguerta* as a subspecies to *muralis*, but afterwards (Guillaume & Lanza, 1982) the two species were newly recognised, but as being closer to each other than to *sicula* and *hispanica*; (3) Lutz & Mayer (1985) included six *Podarcis* species in a study on albumin of several Lacertid genera: *sicula* and *muralis* proved closely allied, *melisellensis* was also isolated but slightly closer to three eastern Mediterranean species; a distinct group included *tiliguerta*, slightly differentiated, and *wagleriana* and *filfolensis*; (4) Capula (1990) compared all *Podarcis* (*raffonei* was reported as "Aeolian" *wagleriana*) and one *Teira* species using allozyme data, reanalysed cladisti-

TABLE I - *Mitochondrial DNA portion of tRNA$^{Phe}$ gene and of 12S rRNA gene (corresponding to sites 1266-1297 and 1298-1856, respectively, in* Gallus *mtDNA; Desjardins & Morais, 1990) of seven taxa of* Podarcis *(see Material and Methods), of* Teira dugesii (Td), *of* Lacerta viridis (Lv) *and of* Gallus gallus (Gg). *Dots (.) represent identical nucleotides with the first sequence. The gaps (-) in the sequences are introduced to improve the alignment.*

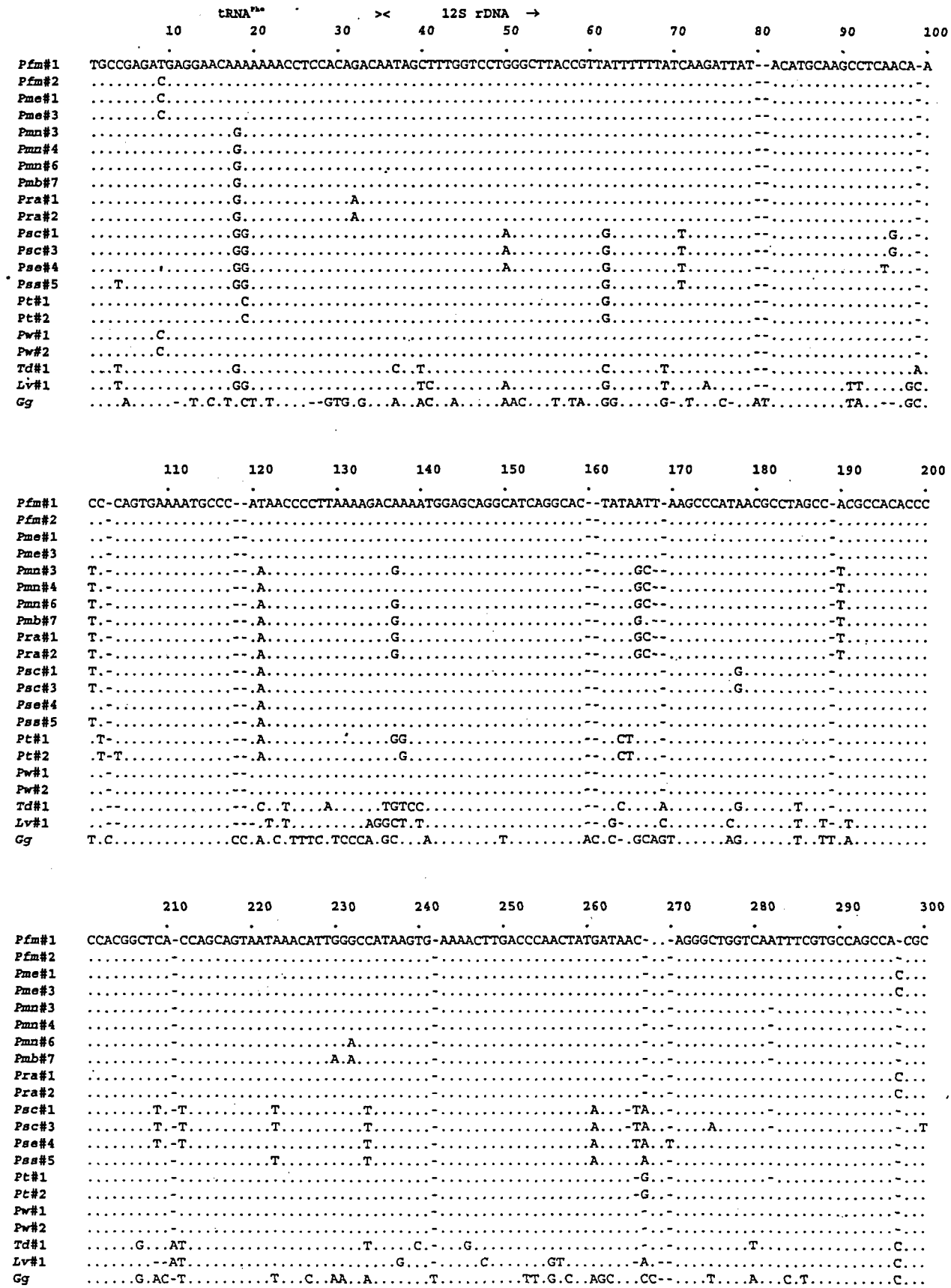


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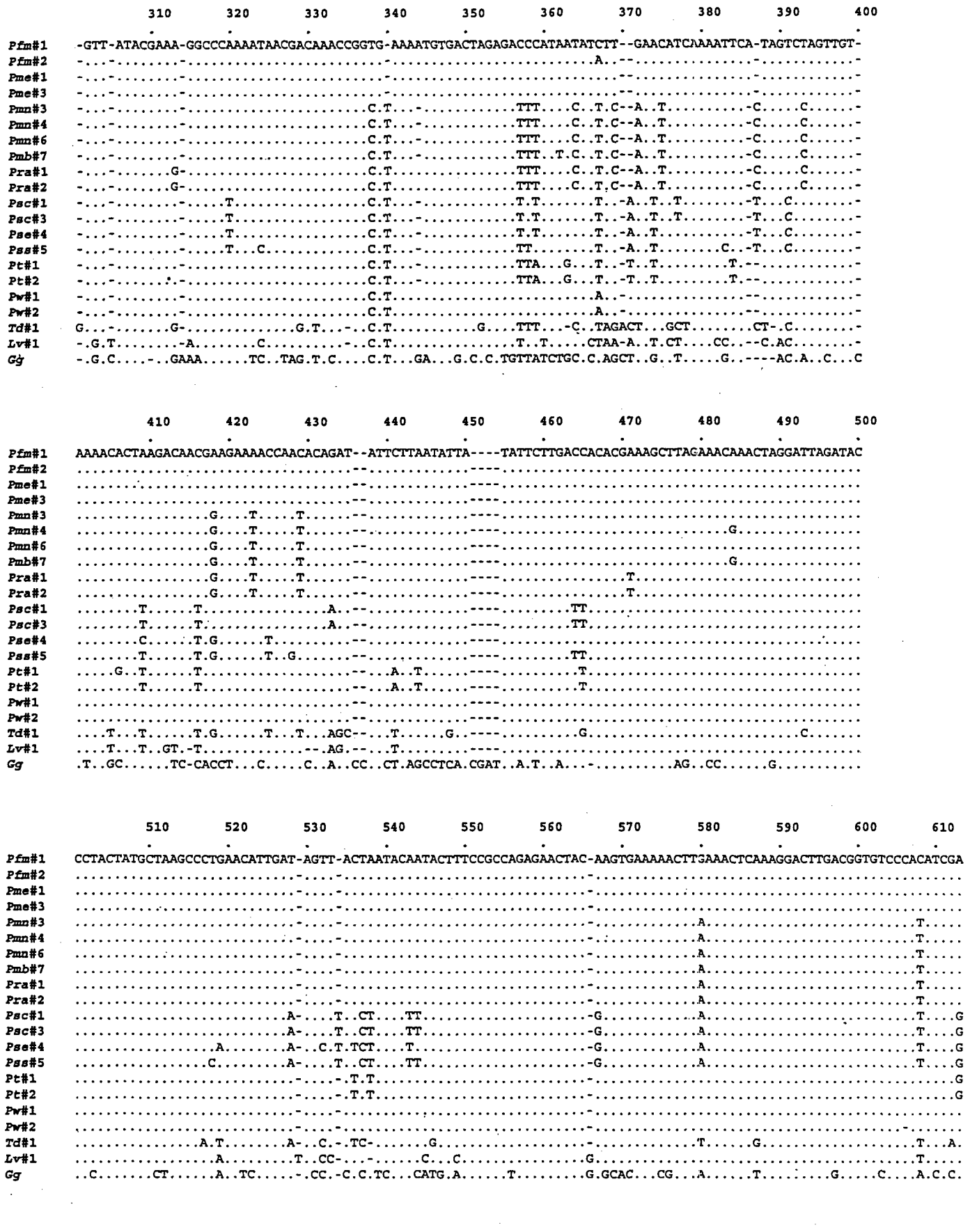
TABLE I - *(continued)*



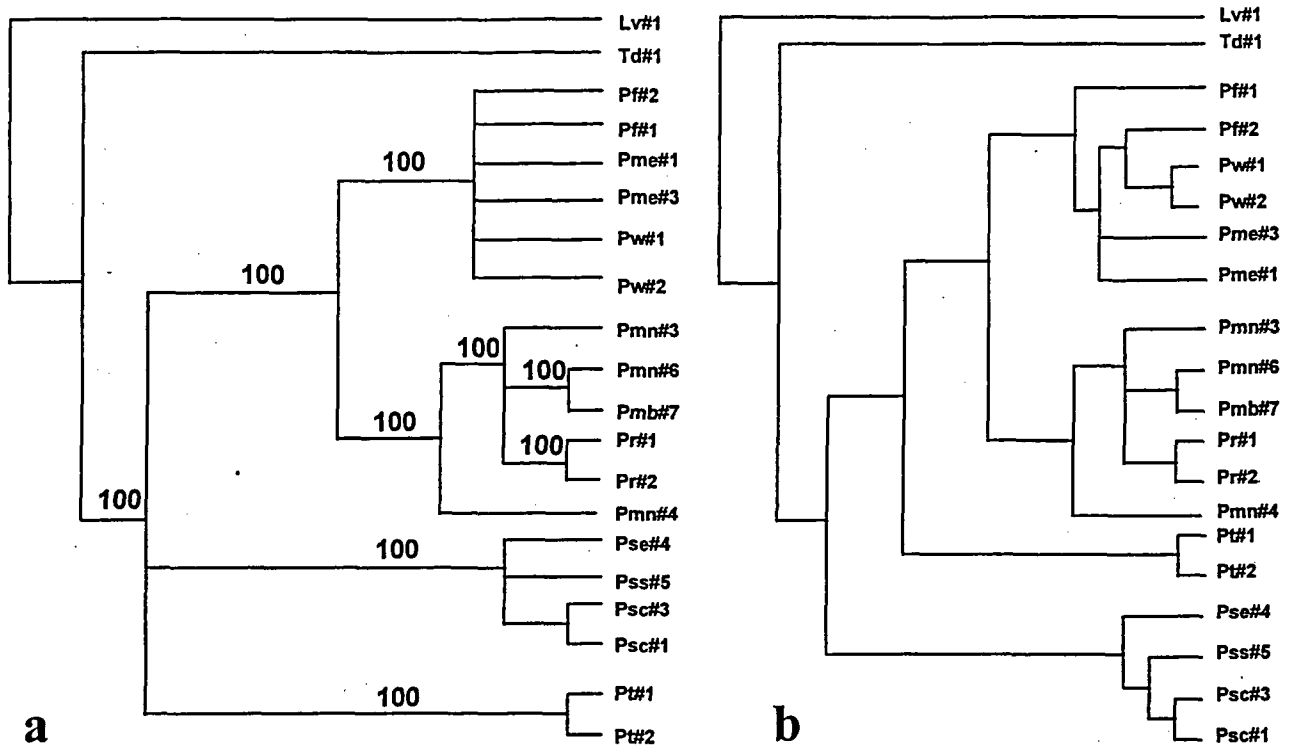



Fig. 3 - Maximum Parsimony analysis of 20 individual sequences (tRNA$^{Phe}$ + 12S rDNAs) of *Podarcis*, *Teira* and *Lacerta*: a - majority rule consensus four equally parsimonious trees (length 218, CI = 0.789, HI = 0.211, CI* = 0.683, HI* = 0.317) by equally weighting Tv *vs* Ts; b - MP tree (length 340) by weighting Tv three times Ts.

cally a morphological dataset and proposed possible phylogenetic schemes. In the genetic analysis, *filfolensis* was evidently isolated and apparently closer to the western Mediterranean *hispanica* (Steindachner, 1870) and *bocagei* (Seoane, 1884); *sicula, muralis*, and *tiliguerta* were strictly related and closer to an eastern Mediterranean group of species which also included *melisellensis*; *wagleriana* and the "Aeolian" *wagleriana* (= *raffonei*) formed an isolated cluster more related to the western Mediterranean species. In the same work Capula (1990) pointed out the specific ranking of *filfolensis, sicula* and *wagleriana*, the possible monotypy of *sicula*, usually considered polytypic at least in the Italian peninsula, and the reduced genetic variability of *filfolensis*. The high genetic variability of *wagleriana* was afterwards clarified (Capula, 1994a) raising *raffonei* to specific level, and suggesting a closer relationship of *P. raffonei* to *P. wagleriana* than to *P. sicula*, on the basis of allozyme data.

The present results are only partially in agreement with the above hypotheses. Not considering the oscillating position of *P. tiliguerta*, the Italian species herein analysed, with *L. viridis* as outgroup, were split into two clades: the first comprised the *wagleriana*-group (*P. wagleriana, P. filfolensis* and *P. melisellensis*) and the *muralis*-group (P. *muralis* and *P. raffonei*); the second *P. sicula* (with its various subspecies).

The *wagleriana*-group and the *muralis*-group seem sister clades. As for the *wagleriana*-group, the close relationship of *melisellensis* (often correlated to *P. sicula*) with *wagleriana* and *filfolensis* is remarkable. In the *muralis*-group, the strongly unsupported monophyly of *muralis* with respect to *raffonei*, traditionally ascribed to the *wagleriana* complex is even more surprising. In both cases, the negligible levels of divergence of the sequences will necessary lead to a re-analysis of the taxonomy of the entities involved, possibly by means of population genetics studies; it will have also to be tested whether one or more of these 'species' are of hybrid origin, a phenomenon that could affect a phylogenetic analysis based on mitochondrial DNA. *Podarcis sicula* proves isolated in quite all trees. Its true relationships (either with the western or with the eastern Mediterranean complexes) as well as that of the isolated *tiliguerta* will probably be delineated in detail when the remaining species of the genus are analysed.

The use of molecular divergence as time indicator relies on the molecular-clock or rate constancy hypothesis (Zuckerkandl & Pauling, 1965). The existence of rate heterogeneities among different vertebrate groups has been confirmed by several studies (e.g., Meyer & Wilson, 1990; Mindell & Honeycutt, 1990; Hillis & Dixon, 1991; Allard *et al.*, 1992; Martin *et al.*, 1992; Cantatore *et al.*, 1994; Martin 1995a, b; Caccone *et al.*, 1997), and was tentatively explained claiming different metabolic rates and/or body temperature as evolutionary constraints. Caccone *et al.* (1997) address the concept that though the molecular clock is somewhat troublesome

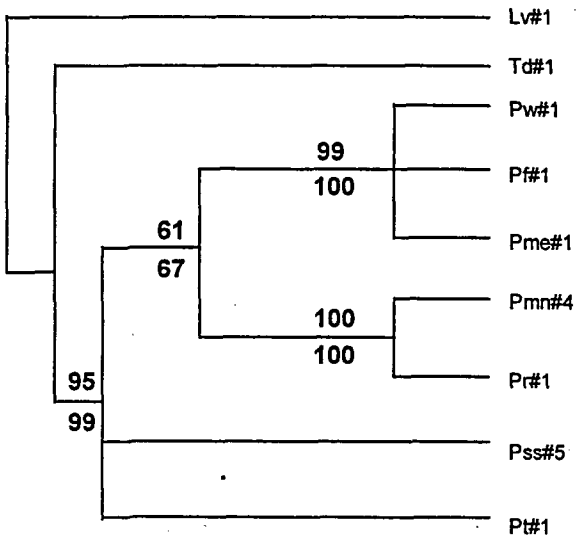



Fig. 4 - Maximum Parsimony analysis of nine individual sequences ($tRNA^{Phe}$ + 12S rDNAs) representing all species assayed in the present study. Majority rule consenus of six equally parsimonious trees (Tv *vs* Ts equal weighting: length 187, CI = 0.860, HI = 0.140, CI* = 0.720, HI* = 0.280; Tv *vs* Ts 3+1 weighting: length 303, CI = 0.884, HI = 0.115, CI* = 0.760, HI* = 0.239). Numbers at the nodes are bootstrap values (1000 replicates, with the same Majority rule consensus topology) supporting the nodes: figures above the nodes are after equal weighting of Tv *vs* Ts, figures below the nodes are after 3+1 weighting of Tv *vs* Ts.

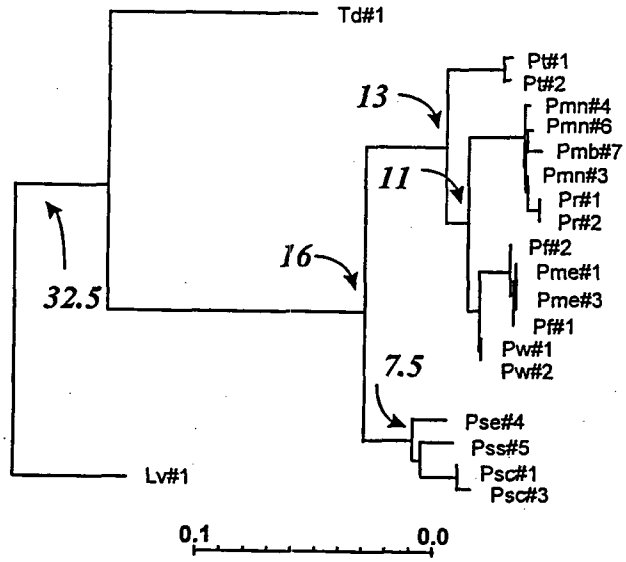



Fig. 5 - Neighbor-joining tree (Jukes & Cantor distance) of all *Podarcis, Teira* and *Lacerta* sequences herein analysed. The figures at the nodes are estimates of divergence time using an evolutionary rate (0.38% per MY) derived by Caccone *et al.* (1997) for *Euproctus* newts.

when comparing distantly related taxa, its use among closely related taxa is generally acceptable. Mitochondrial ribosomal genes are regarded as more rate-homogeneous within vertebrates than e.g., protein-coding mitochondrial ones. As a consequence, given the availability of absolute time (i.e., geologically based divergence dating) against which the clock for a group can be calibrated, the observed percent-divergence matrix can be used to estimate the time of cladogenetic events. In the present study we lacked geologically supported divergence dates and the phylogenetic hypotheses we present still concern only a part of the species. As a very preliminary attempt to estimate times of lineage splitting from molecular sequence divergence data, we can use a known rate pertaining to cold-blooded vertebrates. If we roughly apply the rate of 0.38% sequence-divergence per MY for mitochondrial ribosomal genes, recently derived for European newts by Caccone *et al.* (1997), the splitting of the *Podarcis, Teira,* and *Lacerta* (here represented by *L. viridis*) lineages would date to ca. 35 MY BP (see Fig. 4). *P. sicula* would have diverged ca 17-15 MY BP, while the Sardo-Corsican *P. tiliguerta* would have been separated from the rest of the Italian species for ca 13 MY. The Sardinian 'subspecies' *P. sicula cettii* would have been separated from the nominal *P. s. sicula* for ca 7 MY, and the peninsular *P. sicula campestris* would have diverged ca 6 MY BP from the south Italian stock. The *wagleriana*-group and the *muralis*-group would have diverged ca 11 MY BP. The levels of divergence withing the *wagleriana*-group and the *muralis*-group would indicate that their diversification occurred during the Pleistocene. These datings are slightly in agreement with previous estimates, based on biochemical data, that the diversification of the genus *Podarcis* started in Miocene and continued during Pliocene and Pleistocene (Gorman *et al.*, 1975; Lanza & Cei, 1977; Guillaume & Lanza, 1982; Mayer & Tiedemann, 1982; Lutz & Mayer, 1984, 1985; Lutz *et al.*, 1986). Anyway, those estimates were based on phylogenetic relationships different from our scheme, and direct and detailed comparisons make little sense. Furthermore, when molecular data cover a large time span (as in our case) differential rates should be calibrated along the tree(s).

Taxonomy of the species of genus *Podarcis* has traditionally been based mostly on chromatic pattern variation and/or other external and skeletal features (e.g., Böhme, 1986). In our study, all *muralis* individuals are molecularly more homogeneous than the *sicula* ones: this would give support to the (morphologically based) separation at subspecific level of the *sicula* populations, whilst for *muralis* the high morphological diversification throughout its range, would rather be correlated to a great phenotypic plasticity. Recent evidence from a work (Losos *et al.*, 1997) on the rapid morphological divergence after colonisation of new islands by the Caribbean iguanid lizards of the genus *Anolis* highlights the possibility that, especially in insular environments, lizards can be adaptively very plastic, and their morphology can thus be extremely misleading being highly correlated to environmental parameters. From this perspective, the tight phylogenetic relationships between *P. muralis* and *P. raffonei,* contrasted by their divergent morphologies, could be reinterpreted by considering colonisation of the Aeolian Islands by a *muralis*-like stock. Dating of the event (probably very recent) could

TABLE II - *Pairwise percent sequence divergence matrix for the 20 sequences of* Podarcis *and* Lacerta *herein studied (Jukes & Cantor distance).*

| | Lv#1 | Td#1 | Pf#1 | Pf#2 | Pme#1 | Pme#3 | Pw#1 | Pw#2 | Pmn#3 | Pmn#4 | Pmn#6 | Pma#7 | Pr#1 | Pr#2 | Psc#1 | Psc#3 | Pse#4 | Pss#5 | Pt#1 | Pt#2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lv#1 | 0.000 | | | | | | | | | | | | | | | | | | | |
| Td#1 | 0.164 | 0.000 | | | | | | | | | | | | | | | | | | |
| Pf#1 | 0.115 | 0.137 | 0.000 | | | | | | | | | | | | | | | | | |
| Pf#2 | 0.117 | 0.137 | 0.003 | 0.000 | | | | | | | | | | | | | | | | |
| Pme#1 | 0.117 | 0.139 | 0.001 | 0.001 | 0.000 | | | | | | | | | | | | | | | |
| Pme#3 | 0.117 | 0.139 | 0.001 | 0.001 | 0.000 | 0.000 | | | | | | | | | | | | | | |
| Pw#1 | 0.115 | 0.135 | 0.005 | 0.001 | 0.003 | 0.003 | 0.000 | | | | | | | | | | | | | |
| Pw#2 | 0.115 | 0.135 | 0.005 | 0.001 | 0.003 | 0.003 | 0.000 | 0.000 | | | | | | | | | | | | |
| Pmn#3 | 0.127 | 0.131 | 0.038 | 0.040 | 0.040 | 0.040 | 0.038 | 0.038 | 0.000 | | | | | | | | | | | |
| Pmn#4 | 0.129 | 0.135 | 0.038 | 0.040 | 0.040 | 0.040 | 0.038 | 0.038 | 0.003 | 0.000 | | | | | | | | | | |
| Pmn#6 | 0.129 | 0.133 | 0.040 | 0.042 | 0.042 | 0.042 | 0.040 | 0.040 | 0.001 | 0.005 | 0.000 | | | | | | | | | |
| Pma#7 | 0.133 | 0.137 | 0.044 | 0.046 | 0.045 | 0.045 | 0.044 | 0.044 | 0.008 | 0.008 | 0.006 | 0.000 | | | | | | | | |
| Pr#1 | 0.133 | 0.133 | 0.044 | 0.045 | 0.045 | 0.045 | 0.044 | 0.044 | 0.005 | 0.008 | 0.006 | 0.013 | 0.000 | | | | | | | |
| Pr#2 | 0.133 | 0.133 | 0.044 | 0.045 | 0.045 | 0.045 | 0.044 | 0.044 | 0.005 | 0.008 | 0.006 | 0.013 | 0.000 | 0.000 | | | | | | |
| Psc#1 | 0.124 | 0.145 | 0.069 | 0.071 | 0.071 | 0.071 | 0.069 | 0.069 | 0.072 | 0.072 | 0.073 | 0.077 | 0.077 | 0.077 | 0.000 | | | | | |
| Psc#3 | 0.130 | 0.151 | 0.075 | 0.077 | 0.077 | 0.077 | 0.075 | 0.075 | 0.077 | 0.077 | 0.079 | 0.083 | 0.082 | 0.082 | 0.005 | 0.000 | | | | |
| Pse#4 | 0.120 | 0.132 | 0.064 | 0.066 | 0.065 | 0.065 | 0.064 | 0.064 | 0.066 | 0.066 | 0.067 | 0.071 | 0.071 | 0.071 | 0.027 | 0.033 | 0.000 | | | |
| Pss#5 | 0.130 | 0.143 | 0.067 | 0.069 | 0.069 | 0.069 | 0.067 | 0.067 | 0.066 | 0.066 | 0.068 | 0.071 | 0.071 | 0.071 | 0.029 | 0.035 | 0.036 | 0.000 | | |
| Pt#1 | 0.129 | 0.131 | 0.046 | 0.047 | 0.047 | 0.047 | 0.046 | 0.046 | 0.057 | 0.060 | 0.059 | 0.062 | 0.062 | 0.062 | 0.072 | 0.077 | 0.067 | 0.069 | 0.000 | |
| Pt#2 | 0.127 | 0.131 | 0.044 | 0.046 | 0.045 | 0.045 | 0.044 | 0.044 | 0.058 | 0.058 | 0.060 | 0.064 | 0.064 | 0.064 | 0.069 | 0.075 | 0.066 | 0.068 | 0.005 | 0.000 |
| | Lv#1 | Td#1 | Pf#1 | Pf#2 | Pme#1 | Pme#3 | Pw#1 | Pw#2 | Pmn#3 | Pmn#4 | Pmn#6 | Pma#7 | Pr#1 | Pr#2 | Psc#1 | Psc#3 | Pse#4 | Pss#5 | Pt#1 | Pt#2 |

be attempted by assaying more rapidly evolving genes or by allozyme electrophoresis. The case of the *wagleriana*-group is more complicated, and several distinct biogeographic scenarios can be equally admitted. We would favour a *melisellensis-filfolensis-wagleriana* sequence, with an eastern origin of the group (a *melisellensis*-like ancestor), and subsequent colonisation of the Maltese Islands (*filfolensis*) and of Sicily (*wagleriana*). The other two hypotheses, starting with either a *wagleriana*-like or a *filfolensis*-like ancestor cannot be disregarded. None of the above interpretations consider the possibility of much more recent events of accidental transport, even by man (as in the case of *raffonei*, as well). In this respect, the high morphological divergence of transplanted insular populations of the Caribbean *Anolis* after 10-14 years from the colonisation (Losos *et al.*, 1997), would witness for this being possible.

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