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Molecular biogeography of the Mediterranean lizards *Podarcis* Wagler, 1830 and *Teira* Gray, 1838 (Reptilia, Lacertidae)

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Abstract

Aim We discuss biogeographical hypotheses for the Mediterranean lizard species *Podarcis* and *Teira* within a phylogenetic framework based on partial mitochondrial DNA sequences.

Methods We derived the most likely phylogenetic hypothesis from our data set (597 aligned positions from the 12S rDNA and phenyl tRNA) under parsimony, distance and maximum likelihood assumptions.

Results The species usually included in *Teira* do not form a strongly monophyletic clade. In contrast, the monophyly of the genus *Podarcis* is rather well supported. Seven lineages are identified in the genus; in order of appearance within the tree, these are: the Balearic *pityusensis* and *lilfordi* pair, the *sicula* complex, a Tyrrhenian *tiliguerta* and *raffonei* pair, *muralis*, the Siculo-Maltese *filfolensis* and *wagleriana* pair, the Balkan group (*erhardi*, *peloponnesiaca*, *milensis*, *melisellensis* and *taurica*), and the Ibero-Maghrebian group (*bocagei*, *atrata*, *hispanica* and *vaucheri*).

Conclusions The origin of the three European genera of lacertid assayed (*Lacerta*, *Teira* and *Podarcis*) is hypothesized to have occurred in the Oligocene. For *Podarcis*, a possible scenario of a Miocene diversification is derived from the sequence data, and the zoogeography of the lineages are discussed in relation to the palaeogeography of the Mediterranean. It is hypothesized that in the early history of the genus the main lineages separated by rapid, numerous and close events that produced a starting point very similar to a polytomy, hard to resolve by parsimony analysis of the data set.

Keywords

Molecular phylogenetics, mtDNA, wall lizards, *Podarcis*, *Teira*, *Lacerta*, Mediterranean region.

INTRODUCTION

The Mediterranean region underwent a complex palaeogeographical history that significantly affected the evolution of its faunal assemblages (Alvarez *et al.*, 1974; Hsü *et al.*, 1977; Rögl & Steininger, 1984; La Greca, 1990). The availability of phylogenies is of primary importance as a means to understand the dynamic patterns of evolution underlying the region's biogeography (Oosterbroek & Arntzen, 1992). Lacertid lizards are good models in such studies because they often underwent remarkable radiation, are distributed world-wide and are particularly well differentiated in the Mediterranean region.

Phylogenetic relationships of the genera in the family Lacertidae are still incompletely known. Arnold (e.g. 1973, 1989a, 1989b, 1993) and Mayer & Benyr (1994) proposed phylogenetic hypotheses based on morphological and biochemical or immunological data sets, respectively. In the Mediterranean region the Lacertidae are represented by some 14 genera (*Acanthodactylus* Wiegmann, 1834; *Algyroides* Bibron & Bory de Saint-Vincent, 1833; *Archaeolacerta* Mertens, 1921; *Eremias* Fitzinger, 1834; *Gallotia* Boulenger, 1916; *Lacerta* Linnaeus, 1758; *Mesalina* Gray, 1838; *Ophisops* Ménétries, 1832; *Phylochortus* Matschie, 1893; *Podarcis*, Wagler, 1830; *Psammodromus* Fitzinger, 1826; *Teira* Gray, 1838; *Timon* Tschudi, 1836; and *Zootoca* Wagler, 1830) that have been scarcely studied phylogenetically in the past. Only recently have herpetologists studied the phylogeny of lacertids with combined molecular and morphological data (Harris

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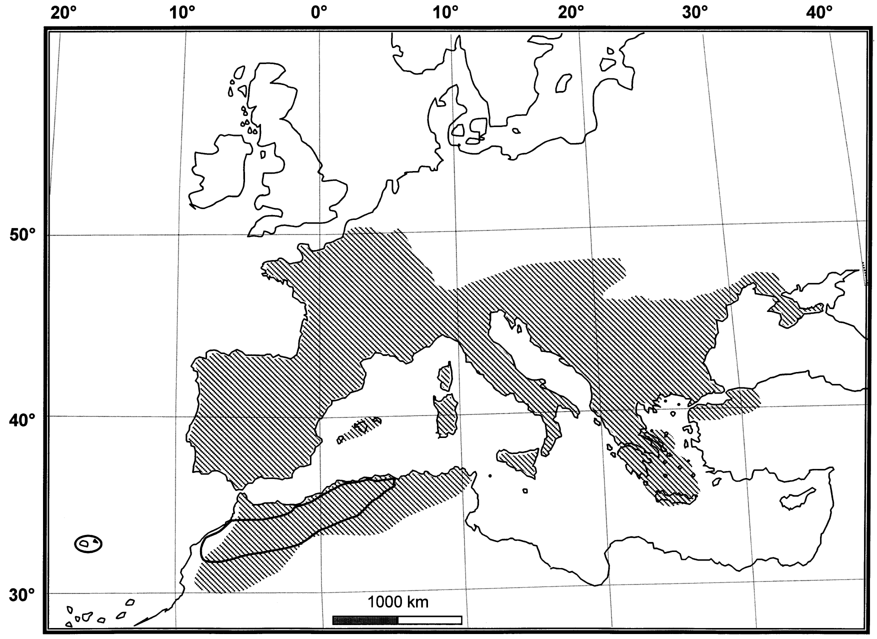

Figure 1 General distribution limits (native populations only) of the genera *Podarcis* (hatched areas) and *Teira* (areas within bold, black line).

et al., 1998), and one of the major outcome was that *Lacerta* was confirmed to be a para/polyphyletic assemblage.

So far, the taxonomy of several taxa at the specific or supraspecific level is still under debate (Mayer & Tiedemann, 1980, 1981, 1982; Tiedemann & Mayer, 1980; Böhme, 1984; Lutz & Mayer, 1985; Busack & Maxson, 1987). Some of the Mediterranean species previously referred to as *Lacerta* are presently included in the genus *Podarcis*; this genus is widespread primarily in the northern, north-eastern (except in most of Anatolia) and south-western Mediterranean, and represented also in Central Europe by a single species, *P. muralis* (Fig. 1). Several species are endemic to Mediterranean islands, and a few have been introduced by man in secondary areas. Böhme (1986) recognized 17 species and, according to Richter (1980), divided *Podarcis* into two subgenera, the nominate *P. (Podarcis)* (with 15 species) and *P. (Teira)* (with 2 species). The systematics of *Podarcis* is still controversial and *Teira* (once considered by some authors as a subgenus of *Podarcis* that included 3 species: see Table 1) has recently been elevated to generic rank by Mayer & Bischoff (1996). The most important recent deviations from Böhme's (1986) taxonomy are that *gaigeae* is presently regarded as a subspecies of *milensis* after Tiedemann (in Gasc *et al.*, 1997), while *raffonei* from the Aeolian islands (Sicily) and *atrata* from the Columbretes islands, were recently raised to specific rank by Capula (1994a) and Castilla *et al.* (1998), respectively. In the present paper, *vaucheri*, so far regarded as a subspecies of *hispanica*, from north-west Africa, is proposed as distinct species. Consequently, at present 17 species are ascribed to *Podarcis sensu stricto* and three to *Teira*; the species of both genera are listed in Table 1 with their distribution.

Within this framework, we started a project (Oliverio *et al.*, 1998a, 1998b) with the intention of clarifing the phylogeny and zoogeography of the almost strictly Mediterranean *Podarcis* and the closely related western-Mediterranean and Macaronesian *Teira* (Fig. 1). A parallel study was started on the *Podarcis* group by E. Arnold and colleagues (J. Harris, personal communication; see e.g. Castilla *et al.*, 1998; Harris & Arnold, 1999).

All *Podarcis* species are enormously variable and often remarkably plastic from a phenetic point of view, both in coloration and in scale morphology and number. There are at least two noteworthy consequences of this variability: (1) the construction of a purely morphological key to the identification of species is extremely difficult (Arnold, 1993; Bologna *et al.* in progress), and (2) the number of subspecies was greatly inflated in the past, particularly for small islands populations (e.g. Böhme, 1986). This infraspecific taxonomy requires careful taxonomic re-evaluation, and it is likely that several subspecies will need to be regarded as simple ecophenotypes (see e.g. Corti *et al.*, 1989). On the other hand, in the future certain subspecies may come to be considered as distinct species (see Discussion).

With regard to the attempts to resolve phylogenetic relationships of species, several hypotheses have been proposed, based on 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; 1994a; 1994b; 1994c; Mayer & Benyr, 1994). Unfortunately, most of these investigations included some *Podarcis* and other (often distantly related) lacertid species, but quite rarely the complete complement of *Podarcis*: the possibility of a comparative analysis is therefore greatly reduced. In some instances, biochemical analyses gave indications of the phylogenetic relationships within groups of species. Capula (1990) compared the allozyme variation of almost all *Podarcis* and one *Teira*

Table 1 Species of *Teira* and *Podarcis* with their present distribution and locality data for the samples

Taxa	Distribution	Samples
Teira Gray, 1838		
dugesii (Milne-Edwards, 1829)	Madeira Archipelago; introduced into the Azores	Tdu#1: Portugal, Madeira Island, Augua de Pena, 8.V.1988, H. in den Bosch leg. (MC).
perspicillata (Duméril & Bibron, 1839)	Morocco, Algeria; introduced in Menorca (Spain)	Tps#1: Spain, Baleares, Menorca Island, 1991 (one female born in rearing, from parents from Menorca, Ciudadela, 1987) (blood sample from a living specimen reared by Herman in den Bosch).
andreanszkyi (Werner, 1929)	Morocco	Tan#1: Morocco, High Atlas, M. Oukaimedem, 2600 m asl, 21.V.1996 A. Vigna-Taglianti leg. (MZUR R 1048).
Podarcis Wagler, 1830		
atrata (Boscá, 1916)	Spain (Columbretes Islands)	Pat#1: Spain, Columbretes Islands, Columbrete Grande Is., J. Harris leg.
bocagei (Seoane, 1884)	Western Spain, Portugal	Pbo#4: Portugal, Esposende, Costa Verde, H. in den Bosch leg. (ex NHM)
erhardii (Bedriaga, 1876)	S Balkans (from Kosovo and Albania to Crete, Bulgaria and part of Cyclades)	Per#2: Greece, Cyclades, Amorgos Island, 23.V.1989 M. Capula leg. (MC).
filfolensis (Bedriaga, 1876)	Maltese Archipelago, Linosa Is., Lampione Is. (Sicily)	Pfl#5: Italy, Sicily, Agrigento Prov., Linosa Island. 2.IV.1990, M. Bologna leg. (MZUR R830), *P. f. laurentimuelleri* (Fejérváry, 1924); Pfm#1: Malta, Gozo Island, Ramla, 21.I.1997, P. Schembri leg. (MZRT) and Pfm#2: Malta, Malta Island, Zeytun, 2.II.1997, P. Schembri leg. (MZRT), *P. f. maltensis* Mertens, 1921.
hispanica (Steindachner, 1870)	Iberian Peninsula, S France	Phi#1: Spain, Granada Prov., Sierra Nevada, Puerto de la Ragua, 9.V.1979, 1900 m, A. Vigna Taglianti & S. Bruschi leg. (MZUR R324).
lilfordi (Günther, 1874)	Baleares (Menorca, Mallorca)	Pli#1: Spain, Baleares, Cabrera Island, II.1998, collected by the National Parc personnel.
melisellensis (Braun, 1877)	Eastern Adriatic coastal area	Pme#2: Croatia, Dubrovnik, Kotor, 19.V.1986, M. Capula leg. (MC): *P. m. fiumana* (Werner, 1891).
milensis (Bedriaga, 1876)	Western Cyclades islands; including subspecies *gaigeae* (Werner, 1930) from Skyros Is., N Sporades	Pmi#1 and Pmi#2: Greece, Cyclades, Milos Island, V.1983 A. Cattaneo leg. (AC).
muralis (Laurenti, 1768)	S and central Europe, NW Anatolia	Pmn#3: Italy, Latium, Roma Prov., Castel di Leva, 50 m asl, 30.III.1996, M. Bologna leg. (MZRT), *P. m. nigriventris* Bonaparte, 1836; Pmn#6: Italy, Latium, Viterbo Prov., Monte di Canino, 250 m asl, 19.IV.1996, M. Bologna leg. (MZRT), intermediate phenotype between *P. m. nigriventris* and *P. m. brueggemanni* (Bedriaga, 1879); Pmb#7: Italy, Latium, Rieti Prov., Mt. Terminillo, Vallonina shelter, 1250 m asl, 25.V.1996, M. Bologna leg. (MZRT), 'Apennine brown phenotype', = ? *P. m. breviceps* (Boulenger, 1905); Pmn#4: Italy, Latium, Frosinone Prov., San Vittore, La Radicosa, 650 m asl, 15.IV.1996, M. Bologna leg. (MZRT), intermediate phenotype between *P. m. nigriventris* and the "Apennine brown phenotype".
peloponnesiaca (Bibron & Bory, 1833)	Greece: Peloponnese	Ppe#1: Greece, Ahaia Prov., Zahalorous, Thelmos Mt., 700 m, 23.V.1985. (MZUR R354).
pityusensis (Boscá, 1883)	Baleares (introduced in Mallorca)	Ppi#1: Baleares, Maiorca Island, 'Ses Illetes', Baie de Palma, II.1998, J. Muntaner leg.
raffonei (Mertens, 1952)	Sicily (Aeolian islands: Strombolicchio Is. and Vulcano Is.)	Pra#3: Italy, Sicily, Messina Prov., Vulcano Island, 15.IX.1986, M. Capula leg. (MC), *P. r. antoninoi* (Mertens, 1955).

Table 1 *continued*

Taxa	Distribution	Samples
sicula (Rafinesque-Schmaltz, 1810)	Italy, Tyrrhenian islands, E Adriatic coasts; introduced in several localities in Europe and U.S.A. Including subspecies *cettii* (Cara, 1872), from Sardinia	Pss#5: Italy, Campania, Salerno Prov., Sapri beach, 13.VII.1996, P. Mariottini leg. (MZRT), *P. s. sicula* (Rafinesque-Schmaltz, 1810); Psc#1: Latium, Italy, Rome Prov., Maccarese, Bocca di Leone dunes, 5.II.1995, M. Bologna leg. (MZRT) and Psc#3: Italy, Latium, Rome Prov., Roma, Prato Falcone, 50 m asl, 30.III.1996, P. Mariottini leg. (MZRT), *P. s. campestris* (De Betta, 1857); Pse#4: Italy, Sardinia, Oristano Prov., Is Aruttas dunes, 25.VI.1996, M. Bologna leg. (MZRT), *P. s. cettii* (Cara, 1872).
taurica (Pallas, 1814)	SE Europe (from Hungary to Crimea, southward to Peloponnese)	Pta#3: Greece, Kastoria Prov., Gavros, 800 m, 21.IV.1984 G.M. Carpaneto leg. (MZUR R 362), *P. t. jonica* (Lehrs, 1902).
tiliguerta (Gmelin, 1789)	Sardinia, Corsica	Pti#1: Italy, Sardinia, Nuoro Prov., Bosa, Temo valley, 300 m asl, 22.VI.1996, M. Bologna leg. (MZRT); Pti#2: Italy, Sardinia, Nuoro Prov., Macomer, Santa Maria de Sauccu, 450 m asl, 1.VII.1996, M. Bologna leg. (MZRT).
vaucheri (Boulenger, 1905)	Maghreb, from Morocco to Tunisia	Pva#1: Morocco, Tetouan Prov., 10 km W Bab-Berred, 1200 m, 10/11.V.1979, A. Vigna Taglianti and M. A. Bologna leg. (MZUR R339).
wagleriana (Gistel, 1868)	Sicily (also Egadi islands)	Pwa#1: Italy, Sicily, Palermo Prov., Godrano, 690 m asl, 31.III.1973, G. Carpaneto leg. (MZUR R-902); Pwa#2: Italy, Sicily, Trapani Prov., Egadi Islands, Marettimo Island, 4.XII.1992, M. Mei leg. (MZUR R-878).

species, analysing phylogenetically a morphological data set and deriving (contrasting) phylogenetic hypotheses from the resulting trees.

Recent studies on the lacertid genus *Gallotia* Boulenger 1916 (e.g. Thorpe *et al.*, 1993, 1994) and on the iguanid genus *Anolis* (Jackmann *et al.*, 1997; Losos *et al.*, 1997), among others, confirmed on one hand the general power of DNA sequencing as a means to reconstruct phylogenies and zoogeography, and on the other hand highlighted the limitations of these data sets when particular dynamics (such as very rapid, early speciation followed by waves of radiation) had occurred (see Jackmann *et al.*, 1999). Bearing these points in mind, the availability of molecular data nevertheless allows the creation of an independent phylogenetic framework to test biogeographical hypotheses.

The present paper includes the results of the study of all *Podarcis* and *Teira* species. These analyses aimed to reconstruct phylogenetic hypotheses on the basis of molecular characters by testing specimens from different isolated populations. In this study, as in our previous paper (Oliverio *et al.*, 1998b), we considered *Lacerta sensu stricto* as an optimal outgroup choice, and included *Teira* in the ingroup. For all species, we report the partial DNA sequences of the mitochondrial genes encoding the 12S ribosomal RNA (12S rDNA) and the phenyl transfer RNA (tRNA$^{\text{Phe}}$). Phylogenetic information recovered from the sequences was used to test previous hypotheses of relationships among species based on biochemical data, and to define zoogeographical hypotheses for the species of the related genera *Podarcis* and *Teira*.

MATERIALS AND METHODS

Errata corrige to published sequences

Eight out of 28 original sequences reported by Oliverio *et al.* (1998b) were wrongly attributed because of mislabelling of the DNA samples. The four sequences ascribed to *P. melisellensis* (Pme#1 and Pme#3, the accession numbers for tRNA$^{\text{Phe}}$/12S AJ001464/AJ001569 and AJ001465/AJ001570, respectively) were in fact based on two samples of *P. filfolensis* (Pfm#3 and Pfm#4); the four sequences ascribed to *P. raffonei* (Pr#1 and Pr#2, accession numbers AJ001472/AJ001575 and AJ001473/AJ001576, respectively) were actually based on two samples of *P. muralis* (Pmn#5 and Pmn#11). Corrections have been made to the EMBL data base. These sequences were not employed in the present work.

Specimens used

All species recognized by modern taxonomy (see Introduction) of wall lizards (Fig. 2) belonging to the genus *Podarcis* were tested, including three 'subspecies' for *P. sicula*, four 'ecophenotypes' for *P. muralis* and two 'subspecies' of *P. hispanica* (Table 1).

We have also included in this study all species referred to as *Teira*, namely *T. dugesii* (Fig. 3) from Madeira, *T. perspicillata* from Morocco and *T. andreanszkyi* from Morocco, in order

Figure 2 *Podarcis erhardi*. A male from Folegandros Is., Greece (photo by R. Sindaco).

Figure 3 *Teira dugesii* from Madera Is., Portugal (photo by M. Capula).

to test the relationships of the two genera (*Teira* and *Podarcis*) with respect to *Lacerta*, and possibly to evaluate their divergence at the molecular level. The source locations of the examined specimens are listed in Table 1. These specimens are mostly preserved in the zoological collections of 'Roma Tre' University (MZRT), in the M. Capula collection at Museo Civico di Zoologia di Roma (MC), in the Augusto Cattaneo collection in Rome (AC), in the Natural History Museum of London (NHM), 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 given denomination for subspecies refers to the taxon name currently employed for individuals originating from the relevant geographical area. The Lbi#1 specimen of *Lacerta bilineata* Daudin (1802) was from Italy, Latium, Rome Prov., Castel di Decima, 2.XI.1996, M. Bologna leg. (MZRT). According to Amann *et al.* (1997), and following Rykena (1991) and Nettmann (1995), the Italian populations once named *L. viridis* (Laurenti, 1768) should be ascribed to *bilineata*.

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 (Promega), incubated for 10 min and then extracted with standard phenol-chloroform procedure, and precipitated with ethanol. For some specimens (e.g. protected species such as *lilfordi* and *pityusensis*), the tails were cut in the field and stored in pure ethanol, while drops of blood were absorbed on stripes of sterile 3M Whatmann paper, afterwhich the specimens released. For alcohol-preserved museum specimens, tissue samples from one posterior leg and/or the tail were taken, homogenated and dehydrated. The material was then processed with Proteinase K and the standard phenol-chloroform extraction procedures described above. DNA was precipitated with isopropanol.

Purified total DNA was used as a template for the double-stranded polymerase-chain-reaction (PCR) amplification, which was performed in 50 μL 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 μM, each dNTP at 100 μM, 0.5–1 μg template DNA, and 1 unit of Taq Polymerase (Pharmacia Biotech). The PCR cycling parameters for amplification were 30 to 60 seconds at 95 °C, 60–90 seconds at 48–50 °C and 60–90 seconds at 72 °C, for 28–30 cycles.

The primers for amplifying the mitochondrial genes were designed from two regions of high sequence conservation among four vertebrates (see Oliverio *et al.*, 1998b for details). The primer sequences and the position of the 5′ end of the primer in the chicken mitochondrial DNA (mtDNA) sequence (Desjardins & Morais, 1990) are (1248) 5′-AAGCATAGCACTGAAGA-3′ for primer 1 and (1874) 5′-AGAACAGGCTCCTCTAGG-3′ for primer 2.

One-fifth of the amplified product was electrophoresed on a 2% agarose gel to visualize the corresponding DNA band. One-fiftieth of the sample was cloned using the TA Cloning® kit (Invitrogen), or the pGEM®-T easy Vector System (Promega), 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 Sequenase Version 2.0 T7 DNA polymerase (Amersham Life Science, Inc.), or by an ABI model 373 A automated DNA sequencer using a Dye Terminator Ready Reaction Kit (Perkin Elmer) according to the manufacturer's protocol.

Phylogenetic analysis

Nucleotide sequences were aligned by hand, and no ambiguous alignment positions were scored. The aligned mitochondrial sequences had a total length (including the primers) of 615–621 bp. The divergence indices (uncorrected 'p') between the sequences were calculated. To test whether multiple substitutions had a saturation effect on the analysed sites, pairwise transition and transversion proportions were plotted against the corresponding divergence indices.

The aligned lacertid sequences were then analysed by the neighbour joining (NJ: Saitou & Nei, 1987) method. Node support in the resulting tree was estimated by 1000 bootstrap replicates; the Ts/Tv ratio was then estimated along the trees.

All lacertid sequences were analysed by the maximum parsimony (MP: Farris, 1970) method with a heuristic search and node support analysed with a search on 1000 bootstrap replicates. Indels (positions including insertions/deletions, aligned by gaps) were included in a first analysis, then excluded to score the influence of the gaps on the topologies, but preference was given to results from the analyses on the gap-excluding data set. Equal weight was initially given to transitions and transversions; all analyses were then replicated by imposing a weight to transversions 2, 2.5, 3, 5 and 10 times that of transitions.

According to Harris *et al.* (1998), the group of *Lacerta sensu stricto* can be used as a direct outgroup to *Teira* and *Podarcis*. We chose *L. bilineata*, and the corresponding mtDNA sequence was also analysed from this species. In practice, in order to test the effect of outgroup choice on the tree topology, nearly all analyses involving outgroup rooting were also performed by using *Teira* as the outgroup and including *Lacerta* in the ingroup. As the results were topologically identical for *Podarcis*, we will discuss below only the results obtained when using *Lacerta* as the outgroup.

All topologies found with each search methods were finally analysed using the maximum likelihood method (Felsenstein, 1981). The Ts/Tv ratio was estimated to be 2.82, the among-site variation was estimated using a discrete approximation to a gamma-distribution with shape parameter 0.5 and four rate categories. The model used was HKY-85 (Hasegawa *et al.*, 1985), allowing for two substitution types and unequal base frequencies. All analyses were performed using the licensed package PAUP 4* (Swofford, 1999).

The following abbreviations have been used in the paper especially in the Results section and in the figure captions:

b.s. bootstrap support
pi-chrs: parsimony informative characters
CI: consistency index
HI: homoplasy index
CI*: consistency index excluding uninformative characters
HI*: homoplasy index excluding uninformative characters
RI: retention index
RC: rescaled Consistency index
NJ: neighbour-joining
MP: maximum parsimony.

RESULTS

Sequences of mtDNA were obtained from 32 specimens representing all recognized species of the genera *Podarcis* and *Teira*, plus the outgroup *L. bilineata*. The sequences with their EMBL Data Library accession numbers are reported in the Appendix 1. Multiple alignment with the sequence of the bird *G. gallus* Linnaeus (1758) (as published by Desjardins & Morais, 1990) resulted in 597 nucleotide positions. Of these, 144 positions contained phylogenetically informative base substitutions within the *Lacerta-Teira-Podarcis* data set (128 pi-chrs excluding gap positions).

Sequence percent divergences (uncorrected 'p' distance) are reported in Appendix 2. 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 Pmb#7 with Pmn#3 and Pmn#4). Species divergence within *Podarcis* ranged from 0.1% to 9.5%. Sequence divergence between the *Teira* species were 10.4%, 12% and 14.6%. Sequence divergence between *Lacerta* and *Teira* ranged from 13.5% to 16%; between *Lacerta* and *Podarcis* it ranged from 11.3% to 14.2%.

Although sequence divergence *per se* does not give direct indication on the specific status of a population, we emphasise here that the level of divergence scored between specimens currently attributed to the same species are in some cases of the same order of magnitude as those scored between different species. This is the case with *P. sicula cettii* from Sardinia compared to the other *P. sicula sensu lato* (uncorrected 'p' distance = 2.7–3.6%). *Podarcis 'hispanica' vaucheri* from Morocco shows a situation similar to that investigated by Castilla *et al.* (1998) for *P. atrata* (formerly *P. hispanica atrata*). Given these results, we suggest the status of both *cettii* and *vaucheri* be re-analysed by more focused studies; for this study, we treated *P. vaucheri* as a full species while conservatively maintaining

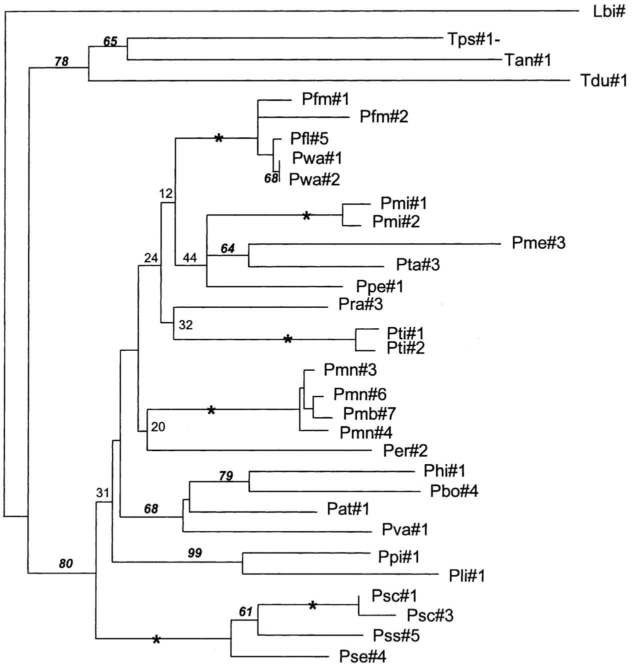

Figure 4 Neighbour-joining tree (uncorrected 'p' distance). Numbers are the bootstrap support (1000 replicates) of the relevant node. This topology has a length of 533 with equal weighing of Tv vs. Ts, of 747.5 with double weighing of Tv, and of 863 with triple weighing of Tv.

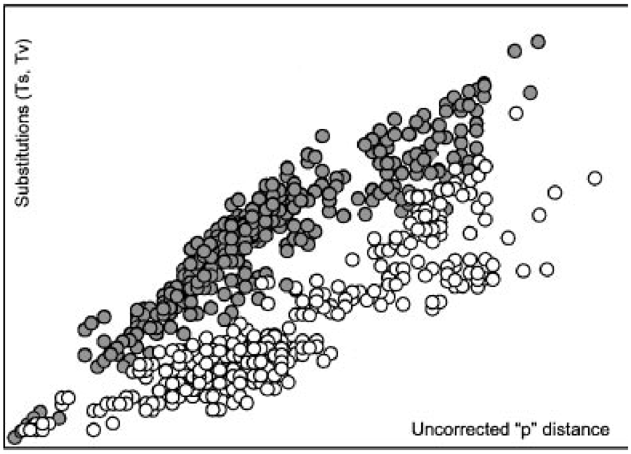

Figure 5 Pattern of nucleotide substitutions. Pairwise proportions of Transitions (●) and Transversions (○) plotted against the corresponding uncorrected ‘p’ distances.

the traditional status of *P. sicula cettii*; resulting in a total of 17 species of *Podarcis* and 3 of *Teira*, dealt with herein.

In the first phylogenetic analysis carried out by the neighbour joining method, the resulting tree (Fig. 4), rooted by *L. bilineata*, showed the *Teira* species as a monophyletic clade (78% bootstrap support), positioned as the sister group of all *Podarcis* species, with *dugesii* as the more primitive within the group. It should be noted that when using *Teira* as the outgroup and *Lacerta* in the ingroup, the topology internal to *Podarcis* did not change. There are indications that the relationships at the genus level as revealed by this study are not necessarily the true one: it is possible that an analysis on all *lacertid* genera may reveal significant differences. In any event, this does not affect the conclusion drawn in the present work. *Podarcis* also appears as a monophyletic clade (80% bootstrap support), with respect to *Teira* and *Lacerta*. Seven main lineages (mostly species pairs) are evident in the tree: an early off-shoot of the *sicula*-complex (100% b.s.), followed by the Balearic pair *pityusensis-lilfordi* (99%), then the Ibero-Maghrebian group (*hispanica*, *vaucheri*, *bocagei*, *atrata*: 68% b.s.), the *muralis* + *ehrardii* pair (20% b.s.), the *Tyrrhenian tiliguerta* + *raffonei* pair (30% b.s.), the Balkan group (*peloponnesiaca*, *milensis*, *taurica*, *melisellensis* without *erhardi*: 44% b.s., the inclusion of *erhardi* has less than 10% b.s.), and the Sicilian pair *filfolensis* + *wagleriana* (100% b.s.). Plotting transitions and transversions against the corresponding uncorrected ‘p’ distance (Fig. 5) gives indication of a moderate bias in favour of transitions, as commonly scored in mtDNA studies. The t-ratio (the averaged transition/transversion ratio over the tree length) was 2.3, and kept values between 2 and 3 along the tree, raising to higher values (up to 6) only in the final clades of very closely related sequences.

Maximum parsimony (MP) analysis of all aligned lacertid sequences by equally weighing Tv and Ts and treating the gaps as a fifth base, yielded 14 equally parsimonious trees with length 617. All trees displayed the *Podarcis* sequences as monophyletic with respect to both *Lacerta* and *Teira*. The strict consensus topology (Fig. 6) shows the phylogeny of the three *Teira* as unresolved, mostly due to the paraphyletic position of *andreanszkyi* being intermediate between *Podarcis* and the monophyletic pair *perspicillata-dugesii* (supported by 12 out of 14 trees: 86% b.s.). Several of the lineages scored in the NJ tree are confirmed, with some differences, in their relative positions: the Balearic pair *pityusensis-lilfordi* (100% b.s.) positions at the base of the *Podarcis* clade and is followed by the *sicula*-group (100% b.s.); *muralis* is the sister to nearly all the remaining species except for *erhardi* (unresolved position). This group has *raffonei* at the base, then a central Mediterranean group (*filfolensis* + *wagleriana* and *tiliguerta*) is defined (86% b.s.), and the position of the Ibero-Maghrebian group (*hispanica*, *vaucheri*, *bocagei*, *atrata*: 100% b.s.) makes paraphyletic the Balkan one (*peloponnesiaca*, *milensis*, *taurica*, *melisellensis*).

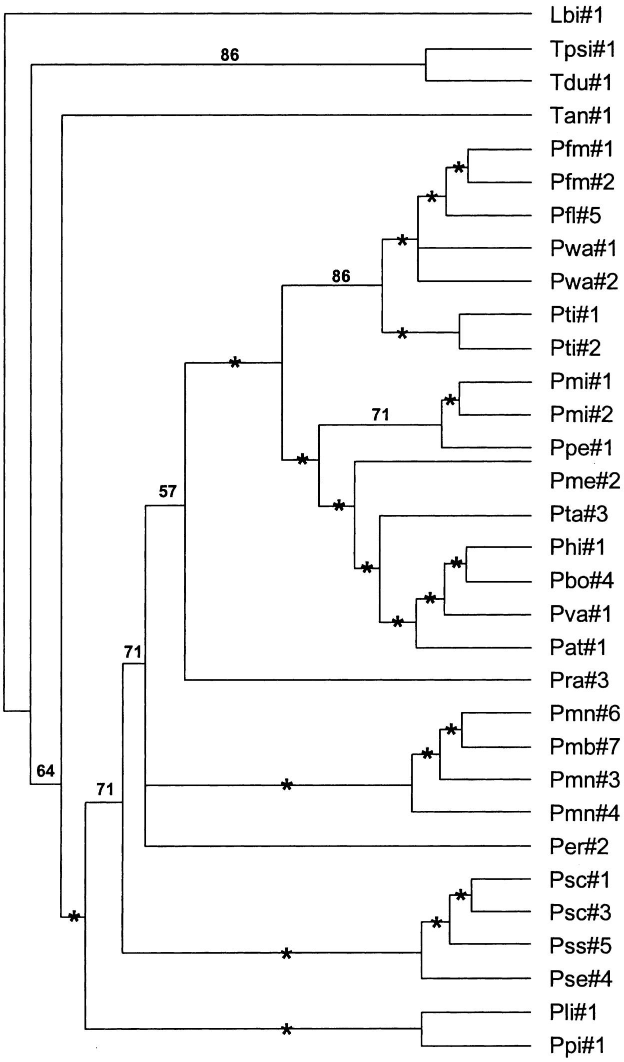

Figure 6 Majority rule consensus tree of 14 MP trees (heuristic search, including gap: asterisks indicate 100% consensus) [144 pi-chrs, length = 617, CI 0.5543, HI 0.4457, CI* 0.4489, HI* 0.5511, RI 0.6257, RC 0.3436].

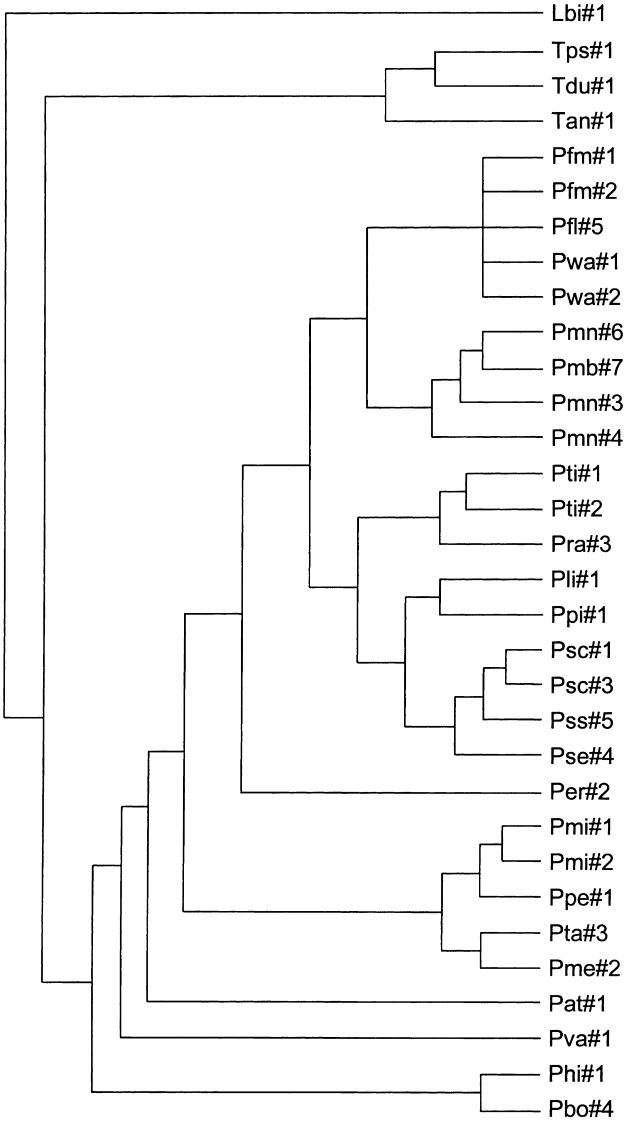

Figure 7 Consensus tree of 2 MP trees (heuristic search, excluding gap) [128 pi-chrs, length = 525, CI 0.5467, HI 0.4533, CI* 0.4360, HI* 0.5640, RI 0.6040, RC 0.3302].

The exclusion of the indels (gap positions treated as 'missing') yielded two trees of length 525 (vs. the 533 step of the NJ topology) whose consensus is polytomic only with regard to the *filfolensis-wagleriana* sequences. In this analysis, the *Teira* sequences are monophyletic with *andreanszkyi* as the most primitive of the three (Fig. 7). The Ibero-Maghrebian group is placed paraphyletically at the base of the *Podarcis* clade, while most lineages of the NJ topology are again supported. The Balkan group is followed by *erhardi*, then the two clades split: one includes the *filfolensis* + *wagleriana* pair and *muralis*, the other the Tyrrhenian *tiliguerta* + *raffonei* pair as the sister to a clade including *sicula* and the Balearic pair *pityusensis* + *lilfordi*.

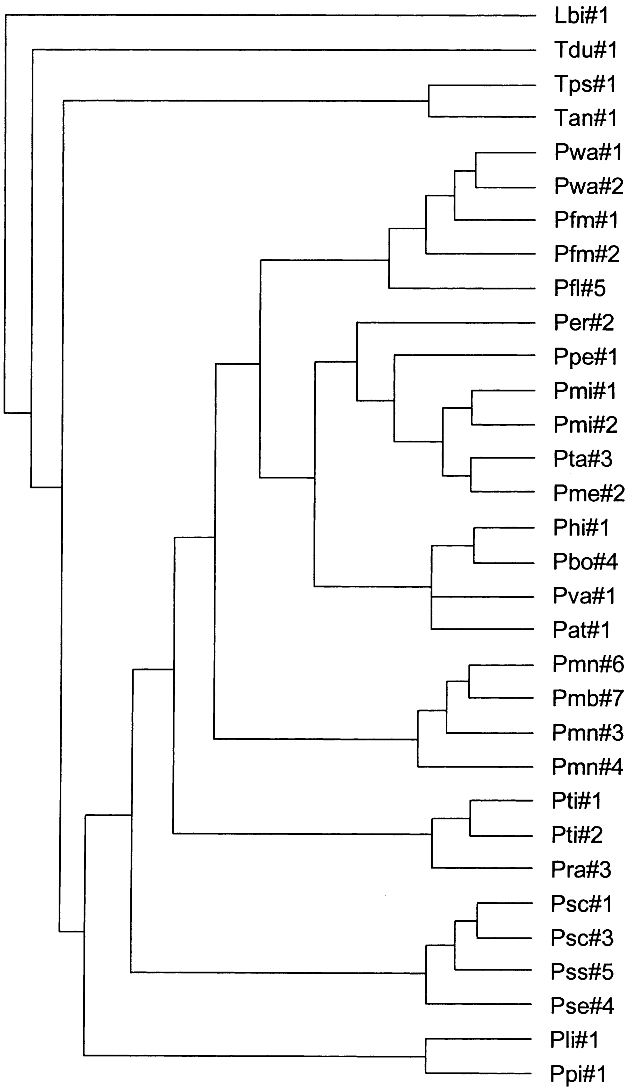

Figure 8 Consensus tree of 2 MP trees (heuristic search, excluding gap) with triple weighing of Tv [131 pi-chrs, mpt = 849, CI 0.5925, HI 0.4075, CI* 0.4897, HI* 0.5103, RI 0.6160, RC 0.3649].

Weighing Transversions 2, 2.3, 3 and 10 times the Transitions, yielded at each analysis two trees that strengthened a topology (Fig. 8), where the monophyly of *Teira* was unsupported (due to the exclusion of *dugesii*). *Podarcis* was always a monophyletic clade with most of the lineages previously recognized; its status is confirmed here. The Balearic pair *pityusensis-lilfordi* is positioned at the base of the clade, followed by the *sicula-complex* then the Tyrrhenian *tiliguerta* + *raffonei* pair, and a group of lines that appear to have originated from a *muralis* stock: *muralis*, the Tyrrhenian *filfolensis* + *wagleriana* group of sequences and the Balkan group (this time also including *erhardi*) as the sister of the Ibero-Maghrebian clade. This topology is constantly much shorter than the NJ one under the

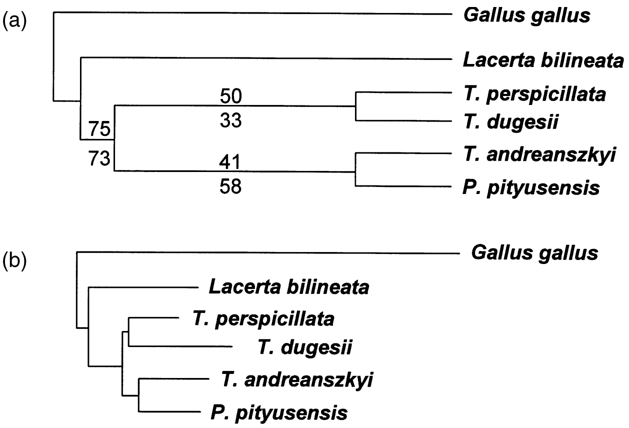

Figure 9 (a) Majority rule consensus tree of a bootstrap analysis (1000 replicates) with branch-and-bound. Figures above lines are bootstrap support including gaps [84 pi-chrs, length = 447, 1 tree, CI 0.8635, HI 0.1365, CI* 0.6592, HI* 0.3408, RI 0.3370, RC 0.2910]; figures below lines are bootstrap support excluding gaps [69 pi chrs, length = 396, 2 trees not shown, CI 0.8662, HI 0.1338, CI* 0.6319, HI* 0.3426, RI 0.3026, RC 0.2621]. (b) Neighbour-joining tree (uncorrected 'p' distance).

same conditions (Tv/Ts weighing). All 19 trees found with either method (inclusion vs. exclusion of gaps, equal vs. differential weighing of Transitions and Transversions, and NJ vs. MP searching algorithm) were tested with a maximum likelihood analysis. The MP trees with differential Tv/Ts weighing resulted the two trees with much the best scores (log likelihood –3428.7 and –3429.3 vs. log likelihood < –3440 for all other trees): the tree with the best score (–3428.7) was that with *P. atrata* as the most primitive in the Ibero-Maghrebian clade. The consensus of these two trees is the phylogenetic hypothesis, which we discuss in the next section.

To define the relationships among the *Teira* species, we analysed with the branch-and-bound method the three sequences, plus one *Podarcis* (*P. pityusensis*) and *Lacerta*, using the sequence of *Gallus gallus* as the outgroup (Fig. 9a, b). *Podarcis pityusensis* was chosen due to the primitive position it assumed in most of the preceding analyses within the *Podarcis* clade. Inclusion of indels yielded one tree of length 447: *Teira* was not monophyletic (only 13.7% bootstrap support by 1000 replicates), and *andreanskyi* was positioned repeatedly with *pityusensis* (41% of the bootstrap trees); *Teira* and *Podarcis* were regarded as a monophyletic unit (although with only 75% bootstrap support) with respect to *Lacerta*. In the neighbour-joining tree, *Teira* and *Podarcis* again formed a monophyletic group (75% bootstrap support) but *Teira* was paraphyletic. A branch-and-bound search excluding the indels yielded two equally parsimonious trees of length 396, both with *andreanszkyi* as the sister to the other lacertid sequences and *pityusensis* linked to *Lacerta bilineata*. Remarkably, a 1000 replicates bootstrap analysis was unable to support any bifurcation in the group (no clade with more than 50% bootstrap support). Weighing transversions 2, 2.3, 3 and 10 times the transitions resulted in less resolved topologies.

DISCUSSION

According to our results, especially given the observed levels of divergence, the analysed species belong to at least three groups. Barbadillo *et al.* (1997) give 18–22 Ma (Lower Miocene) as a probable dating of the generic differentiation of some genera, including *Lacerta* and *Podarcis*, mainly according to the immunological data of Lutz *et al.* (1986) and Mayer & Lutz (1990). With our data, such an estimate would mean a rate of 0.6–0.7% sequence divergence per Myr. Böhme & Corti (1993) reconstructed a hypothesis of correlation of palaeogeography with phylogeny of lacertids, with the split of *Podarcis* and *Teira* from *Lacerta sensu stricto* located at *c.* > 30 Ma; that would mean a rate of about 0.5% sequence divergence per Myr. Substitution rates ranging from 0.5–1% per Myr are common among vertebrates (Mindell & Honeycutt, 1990; Hillis & Dixon, 1991; see also Caccone *et al.*, 1997). With an evolutionary rate of about 0.5% sequence divergence per Myr for our data set, we estimate the divergence of the three lizard lineages examined herein to have occurred during the Oligocene (32–22 Ma). This is possibly referable to the age following the formation of the western European 'Tyrrhenis' macroplate (La Greca, 1990). This is compatible with the fossil record, within which is seen the Eocene precursors of the Recent lacertids (lizards 'similar' to *Lacerta*, such as *Eolacerta* and *Plesiolacerta*) in Europe (Augé, 1993).

In our study, the *Teira* species do not appear as a strictly monophyletic clade. In the gap-including analyses (Fig. 6), *T. andreanszkyi* was positioned as the sister group of *Podarcis* and not within a *Teira* clade; a finding similar to that of Harris & Arnold (1999). In the gap-excluding heuristic analyses (Fig. 7), *T. andreanszkyi* was positioned as the more ancient off-shoot of *Teira*, although this finding is not supported well in the bootstrap analyses; while in the NJ tree (Fig. 4), *perspicillata* was found to be more closely related to *andreanszkyi* than to *dugesii*, all in a *Teira* clade. Differential weighing of Ts/Tv in the parsimony analyses produced topologies where *Teira* is not monophyletic. The specific analyses of the *Teira* sequences (plus *P. pityusensis*, *L. bilineata* and with *G. gallus* as the outgroup) (Fig. 9a, b) did not produce any evidence of monophyly for the genus, under either of the conditions. Thus, monophyly of *Teira* is not supported by our present data, although the species analysed here certainly do not belong in *Podarcis*.

The presence of *T. dugesii* at Madera is probably related to an ancient event, rather than to more recent dispersal. This species, in fact, although related to the Maghrebian stock, shows a remarkable isolation level from the other two species. *Teira*'s absence on the Canary Islands is thus remarkable, and could be explained by the outcompeting *Gallotia*. The diversification in this group can be dated to the Oligocene, when the region corresponding to the Alboran microplate (which gave rise to the Atlanto-Cabylian region, presently inhabited by *T. perspicillata* and *T. andreanszkyi*) could be occupied by their ancestors. The two Maghrebian species are differentiated ecologically (Schleich *et al.*, 1996). *T. perspicillata* needs a relatively high air humidity and prefers to live close to water; *T. andreanszkyi* is a typical high-mountain species, and is endemic to the High Atlas mountains where it lives above

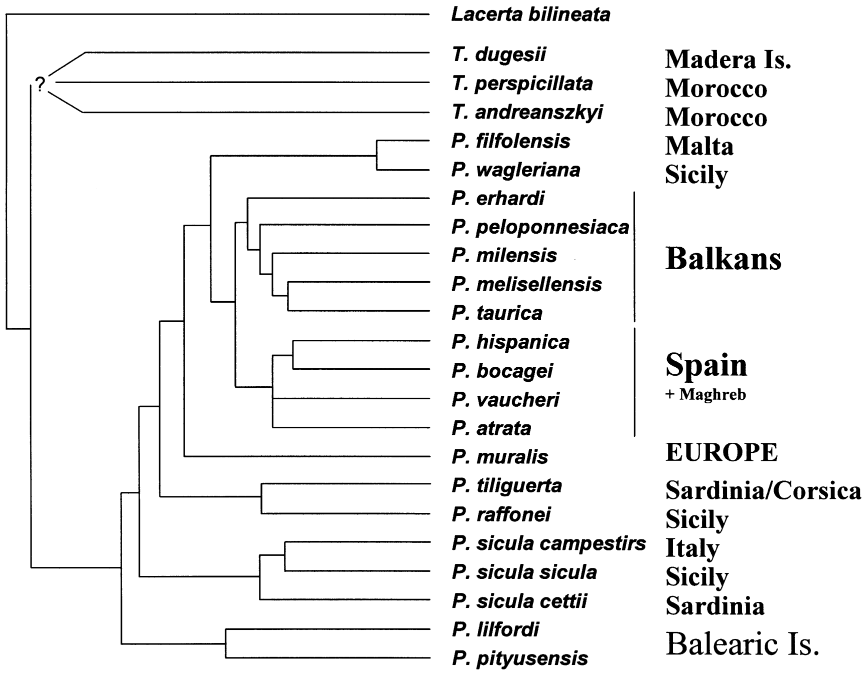

Figure 10 Our preferred phylogenetic hypotheses for the species studied here, along with their main distribution.

2000 m a.s.l. The latter is similar (convergent?) to *Zootoca vivipara*, a European species typical of mountain or cold-plane habitats. The presence of *T. perspicillata* on the Balearic Islands is almost certainly due to its introduction by man (Alcover & Mayol, 1981).

All *Podarcis* species form a monophyletic group. The genus is widely distributed in southern Europe, with a single species also found in central Europe (*P. muralis*), while being represented in north-west Africa only by *P. vaucheri* (related to the Ibero-Maghrebian *P. hispanica*-group). This would confirm the European origin of the group.

The different analyses performed on the same data set (inclusion vs. exclusion of gaps, equal vs. differential weighing of transitions and transversions, and NJ vs. MP searching algorithm; analysed using maximum likelihood evaluation of the trees) highlighted the existence of seven main lineages, although relationships among them were not fully resolved: the Balearic *pityusensis-lilfordi* pair, the *sicula*-complex, the Tyrrhenian *tiliguerta* + *raffonei* pair, *muralis*, the Siculo-Maltese *filfolensis* + *wagleriana* pair, the Balkan group (*peloponnesiaca*, *milensis*, *taurica*, *melisellensis*, *erhardi*) and the Ibero-Maghrebian group (*hispanica*, *vaucheri*, *bocagei*, *atrata*).

The few recently published phylogenetic hypotheses for the genus *Podarcis* based on allozymes and mtDNA sequences (Capula, 1990, and Harris & Arnold, 1999, respectively) are not completely comparable with our trees because of the partial differences in the number of taxa examined. Some of the groups defined by our analyses are evident in either or both of these works. Major differences exist for the relative relationships among such groups. In addition, regarding the relationship among the above lineages, in our analysis it was hard to recover unequivocal phylogenetic signals from the mitochondrial sequences available (and also from some nuclear genes, unpublished data). Jackman *et al.* (1999) concluded after an extensive study on mitochondrial data from *Anolis* sand lizards that difficulties in defining phylogenetic relationships can be related to the effects of early and rapid diversification. The difficulties in recovering robust topologies deep in the tree of *Podarcis* suggest that in this case rapid diversification early in the evolutionary history of the genus produced short, but relatively ancient, branches that hamper the recovery of phylogenetic signals from them. In fact, the levels of divergence scored between such lines indicate (applying the rate of 0.5% b.s. per Myr) that the diversification among the lineages, and even within some such groups of species, was concentrated during the Miocene (from 16 to 10 Ma). Regrettably, the available fossil record does not help in any way at this level.

Of the topologies recovered, we are inclined to give more credit to that of Fig. 10 (derived from Fig. 8) because it is the more geographically plausible, while also being largely in agreement with the few and scattered phylogenetic hypotheses so far published (e.g. Capula, 1990; Oliverio *et al.*, 1998b; Harris & Arnold, 1999).

The majority of the species are western Mediterranean (only five Balkan species) and the most primitive lineages (in any of the analyses) also have the same distribution. This supports both an origin of the genus in this region and the possibility that the bulk of the diversity could have originated from vicariance events mainly related to the western microplates Miocene fragmentation (Alvarez *et al.*, 1974). The absence in Anatolia of *Podaris* (except for a little range extension of *muralis*) is in full agreement with this hypothesis.

At the base of the *Podarcis* clade, the first off-shoot is that of the Balearic pair *pityusensis-lilfordi.* Their level of divergence is relatively high (approaching a fully acquired specific status) and is in contrast with the low level of genetic distance scored by several authors (e.g. Capula, 1990; Pérez-Mellado, 1998, and references therein). Their absence on peninsular Spain can probably be explained by extinction due to competition with other species: Plio-Pleistocene fossils from Balearic Islands referable to the *lilfordi* and *pityusensis* lineages are reported by Kotsakis (1981).

The next lineage in the tree is the *sicula* clade. This is a very remarkable complex usually regarded as a polytypic species. Our data (see also Oliverio *et al*., 1998b for further details) confirm the heterogeneity of the complex. The levels of divergence among the tested 'subspecies' are high and seem to be evidence of a long lasting isolation (especially for the Sardinian *P. s. cettii*). It is remarkable that the most primitive *sicula* is the Sardinian *cettii*, and that the sequence passes through the Sicilian (and southern Italy) nominate subspecies, with the *s. campestris* subspecies (Central and Northern Italy, and Dalmatia) as the more derived. This is congruent with the position of the clade in the tree and with an origin from a western Mediterranean stock. In all our analyses, *P. sicula* does not show any relation to *P. muralis*, with which it has been repeatedly correlated (e.g. Capula, 1990; Harris & Arnold, 1999). In Corsica, two subspecies are presently found: *s. cettii* around Bonifacio in the southern part of the island, and *s. campestris* in the northern and central parts, particularly along the coastal sites and valleys (Delaugerre & Cheylan, 1992). Both could be interpreted as having very recent dispersal, probably by man (as is also supported by allozyme data: Capula, 1994c). Dispersal by humans have affected repeatedly *Podarcis sicula* on a world-wide scale (populations are presently known from, for example, the Balearic Islands, Turkey and USA; the latter under study, Oliverio *et al*., in press). The present distribution of *s. campestris* in northern Italy is scattered and fluctuations in its range are determined by annual thermal conditions. This supports the hypothesis that this form spread repeatedly in central and northern regions, and from here to Corsica and along the Mediterranean coasts of Dalmatia, after Pleistocene glacial events probably confined it to certain warmer, south Italian regions. The record of fossils referred to as 'cf. *sicula*' in Poland (Mlynarski, 1964) should be re-checked.

Another pair with a characteristic Sardo-Sicilian split in their range is *P. tiliguerta* (Sardinia and Corsica) and *P. raffonei* (Aeolian islands). The absence in northern Africa of strict correlates to the pair is evidence of its ancient origin. Our data suggest a Miocene date (*c.* 13 Ma) of the separation of the two species, well before the Messinian crisis. While *P. tiliguerta* is present in both Corsica and Sardinia, *P. raffonei* is presently found only on active volcanic islands of relatively Recent origin (Quaternary?), thus indicating a probable relict distribution of an ancient stock. This species is presently in competition with invading populations of *P. sicula* in some of the Aeolian Islands (Capula, 1994a). According to allozymes divergence (Capula, 1996), the Corsican and Sardinian populations of *P. tiliguerta* are greatly differentiated, denoting a very high isolation; this finding is in agreement with present hypothesis. Affinities between *tiliguerta* and *raffonei* are in disagreement with those determined by allozyme analysis which consider *raffonei* as strictly related to *wagleriana* (Capula, 1994a).

All remnant species seem to be derived from a southern European stock, presently represented by the widely distributed *P. muralis*. It is the most mesic species of the genus, and has even been able to colonize Central Europe, while in the southernmost part of its present range it lives at higher altitudes in mountain habitat (e.g. southern Apennine and southern Greece) and is absent from the Mediterranean islands, except some Tuscan islands and Samothrace (Greece). The presence of the *muralis*-lineage in Central Europe is recorded since at least the Plio-Pleistocene of Austria (Rauscher, 1992). In contrast to the high morphological variation all over its range, the Italian specimens we have assayed show remarkable molecular homogeneity, indicating a possible high degree of phenotypic plasticity.

The Siculo-Maltese *filfolensis* + *wagleriana* pair, whose closeness was already hypothesized by Lanza & Cei (1977) based on immunological data, represents the first offshoot within the *muralis*-derived group. The low level of sequence divergence is remarkable and indicates a very recent separation of the two species. This is in agreement with the fact that the Maltese archipelago has been repeatedly connected to Sicily during the Quaternary marine regressions. *Podarcis filfolensis* is also present on the volcanic Linosa Islands (an old record from Lampione islet (see Böhme, 1986) has been recently reconfirmed (M. Capula, pers. comm.)).

The Balkan group includes five species, with either wide or restricted range. The origin of this group from the southern European stock probably occurred during the middle Miocene, after Europe's connection to the Balkans allowed (cf. Rögl & Steininger, 1984) *Podarcis* to colonize the former from the latter; this hypothesis is also supported by the levels of sequence divergence observed in our work. In the Balkan group, *P. erhardi* is the most primitive species according to the trees, and it is also the most similar morphologically to *muralis*. Of note is the fact that it is the most widely distributed of the group, ranging from the southern Balkans to many of the Aegean islands and Crete (it has also been found as a Pleistocene fossil: Kotsakis, 1977). It is replaced by *milensis* only in some Cyclades (Milos group) and Northern Sporades (Skyros group, ssp. *gaigae*, previously considered as a distinct species). The next species include one subinsular endemic to Peloponnese (*peloponnesiaca*), and an insular Aegean endemic (*milensis*); the latter is fragmented into two separate subranges (Skyros in Northern Sporades, and Milos and other Cyclades islands), probably due to the relatively recent arrival of the more euriecious and competitive *erhardi* from the mainland. Another pair of species (*melisellensis* and *taurica*) includes two evident vicariants (Gasc *et al*., 1997), found in the north-eastern Adriatic coastal area and in the southern and eastern Balkans. Both are restricted to mediterranean habitats, while *taurica* also extends its range into steppe–submediterranean habitats along the Danube valley.

The last lineage to emerge from the phylogenetic analysis, as the sister group of the Balkan lineage, is the Ibero-Maghrebian clade. It includes *vaucheri*, *atrata*, *bocagei* and *hispanica*. Relationships within the clade are not fully resolved. Castilla *et al.* (1998) obtained a closer relationship between *atrata* and *bocagei*, perhaps as a result of the splitting of an ancient stock by the range extension of *hispanica*. The hypotheses of *bocagei* and *atrata* as more closely related would require 3 steps more (536) in our analysis. *Podarcis atrata*, which is endemic to the Columbretes Islands and which has a very restricted distribution, is likely to be an insular relict. *Podarcis vaucheri* is here considered worthy of specific recognition but, as in the case of *P. sicula cettii*, the precise definition of its status would require a more focused study. According to our data, its divergence from the Iberian stock can probably be traced back to the Miocene, when the Rif block and the Kabilian plate separated from the Baetic plate. Busack (1986) cautiously estimated a younger Pliocene separation (3.4 Ma) between the Moroccan and the Spanish population previously referred to as the single species *hispanica*.

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BIOSKETCHES

Marco Oliverio obtained his PhD in Evolutionary Biology at 'La Sapienza' University of Rome with a thesis in evolutionary ecology. He is presently Research Scientist at the Department of Animal and Human Biology and works mainly on systematics, phylogeny and evolutionary ecology. This work began as part of his postdoctoral fellowship at the laboratories of Marco Bologna and Paolo Mariottini.

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Paolo Mariottini is Associate Professor of Molecular Biology at 'Roma Tre' University of Rome (Department of Biology). He works on the structure and evolution of mitochondrial genomes. He is also involved with the application of molecular data to phylogenetic reconstruction.

Appendix 1 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 species of *Podarcis*, *Teira* and of *Lacerta bilineata*, aligned with that of *Gallus gallus* (*Gga*). The gaps (-) in the sequences are introduced to improve the alignment. EMBL accession numbers are reported at the end of each sequence.

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                       tRNAPhe           ><      12S rDNA  →
              10        20        30        40        50        60        70        80        90       100
               .         .         .         .         .         .         .         .         .         .
Lbi#1 TGCTGAGATGAGGAACAGGAAAA-CCTCCACAGACAATAGTCTTGGTCCTAGGCTTACCGTTGTTTTTTTTCAAAATTCAAGTTTCAAGTTTCAACGCA-
Tps#1 TGCCGAGATGAGGAACAGAAAAA-CCTCCACAGACAATAGCTTTGGTCCTGGGCTTACCGTTATTTTTTATCAAGATTATACATGCAAGCTTCAACAAT-
Tdu#1 TGCTGAGATGAGGAACAGAAAAA-CCTCCACAGACAACAGTTTTGGTCCTGGGCTTACCGTTCTTTTTTTTCAAGATTATACATGCAAGCCTCAACAAA-
Tan#1 TGCCGAGATGAGGAACAGAAAAA-CCTCCACAAACAATAGCTTTGGTCCTGGGCTTACCGTTATTTTTTATCAAGATTATACATGCAAGCCTCAACAAAT
Pfm#1 TGCCGAGATGAGGAACAAAAAAA-CCTCCACAGACAATAGCTTTGGTCCTGGGCTTACCGTTATTTTTTATCAAGATTATACATGCAAGCCTCAACA-AC
Pfm#2 TGCCGAGACGAGGAACAAAAAAA-CCTCCACAGACAATAGCTTTGGTCCTGGGCTTACCGTTATTTTTTATCAAGATTATACATGCAAGCCTCAACA-AC
Pfl#5 TGCCGAGACGAGGAAACAAAAAAACCTCCACAGACAATAGCTTTGGTCCTGGGCTTACCGTTATTTTTTATCAAGATTATACATGCAAGCCTCAACA-AC
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Gga   TGCCAAGATG-GTACCTACTATA-CCTGTGGGCAAAAGA-CTTAG-TCCTAACCTTTCTATTGGTTTTTGCTAGACATATACATGCAAGTATCCGCA-TC

             110       120       130       140       150       160       170       180       190       200
               .         .         .         .         .         .         .         .         .         .
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Appendix 1 *continued*

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             210       220       230       240       250       260       270       280       290       300
               .         .         .         .         .         .         .         .         .         .
Lbi#1 ACGGCT--ATCAGCAGTAATAAACATTGGGCCATAGGTG-AAAACCTGACCCAGTTATGATAA-AT--AGGGCTGGTCAATTTCGTGCCAGCCACCGCGG
Tps#1 ACGGGTTCATCAGCAGTAATAAACATTGGGCTATAAGTG-AAAACTTGACCCTACTATGATAAC-TT-GGGGCTGGTCAATTTCGTGCCAGCCACCGCGG
Tdu#1 ACGGGTCAATCAGCAGTAATAAACATTGGGCTATAAGCG-AAAGCTTGACCCAACTATGACAAC-TT-AGGGCTGGTTAATTTCGTGCCAGCCACCGCGG
Tan#1 ACGGGCT-ACCAGCAGTAATAAACATTGGGCTATAAGTG-AAAACTTGACCCAACTATGATAAC-TT-AGGGCTGGTCAATTTCGTGCCAGCCACCGCGG
Pfm#1 ACGGCTC-ACCAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAAC-TT-AGGGCTGGTCAATTTCGTGCCAGCCA-CGCG-
Pfm#2 ACGGCTC-ACCAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAAC-TT-AGGGCTGGTCAATTTCGTGCCAGCCA-CGCG-
Pfl#5 ACGGCTC-ACCAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAAC-TT-AGGGCTGGTCAATTTCGTGCCAGCCACCGCGG
Pwa#1 ACGGCTC-ACCAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAAC-TT-AGGGCTGGTCAATTTCGTGCCAGCCA-CGCG-
Pwa#2 ACGGCTC-ACCAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAAC-TT-AGGGCTGGTCAATTTCGTGCCAGCCA-CGCG-
Pra#3 ACGGCTT-ACCAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAAC-TT-AGGGCTGGTTAATTTCGTGCCAGCCACCGCGG
Pmn#3 ACGGCTC-ACCAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAAC-TT-AGGGCTGGTCA-TTTCGTGCCAGCCA-CGCG-
Pmn#4 ACGGCTC-ACCAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAAC-TT-AGGGCTGGTCA-TTTCGTGCCAGCCA-CGCG-
Pmn#6 ACGGCTC-ACCAGCAGTAATAAACATTGGACCATAAGTG-AAAACTTGACCCAACTATGATAAC-TT-AGGGCTGGTCA-TTTCGTGCCAGCCA-CGCG-
Pmb#7 ACGGCTC-ACCAGCAGTAATAAACATTAGACCATAAGTG-AAAACTTGACCCAACTATGATAAC-TT-AGGGCTGGTCA-TTTCGTGCCAGCCA-CGCG-
Psc#1 ACGGCTT-ATCAGCAGTAATTAACATTGGGCTATAAGTG-AAAACTTGACCCAACTATAATA-TATT-AGGGCTGGTCA-TTTCGTGCCAGCCA-CGCG-
Psc#3 ACGGCTT-ATCAGCAGTAATTAACATTGGGCTATAAGTG-AAAACTTGACCCAACTATAATA-TATT-AGGGATGGTCA-TTTCGTGCCAGCCA-CGTG-
Pse#4 ACGGCTT-ATCAGCAGTAATAAACATTGGGCTATAAGTG-AAAACTTGACCCAACTATAATAATATTTAGGGCTGGTCAATTTCGTGCCAGCCA-CGCG-
Pss#5 ACGGCTC-ACCAGCAGTAATTAACATTGGGCTATAAGTG-AAAACTTGACCCAACTATAATAACATT-AGGGCTGGTCAATTTCGTGCCAGCCA-CGCG-
Pti#1 ACGGCTC-ACCAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAA-GTT-AGGGCTGGTCA-TTTCGTGCCAGCCA-CGCG-
Pti#2 ACGGCTC-ACCAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAA-GTT-AGGGCTGGTCAATTTCGTGCCAGCCA-CGCG-
Ppi#2 ACGGGCTTACCAGCAGTAATAAACATTGGGCTATAAGTG-AAAACTTGACCCAGCTATGATAA--TTTAGGGCTGGTCAATTTCGTGCCAGCCACCGCGG
Pli#2 ACGGGCTTACCAGCAGTAATAAACATTGGGCTATAAGTG-AAAACTTGACCCTGCTATATTGAC-TTTGGGGCTGGTTTGTTTCGTGCCAGCCACCGCGG
Per#2 ACGGGCTCACCAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAAC-TT-AGGGCTGGTCAATTTCGTGCCAGCCACCGCGG
Pmi#1 ACGGGCTTA-CAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAAC-TT-AGGGCTGGTCAATTTCGTGCCAGCCACCGCGG
Pmi#2 ACGGGCTTA-CAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAAC-TT-AGGGCTGGTCAATTTCGTGCCAGCCACCGCGG
Pme#3 ACGGGGTTACCAGCAGTAGTAAACATTGGGCTATAAGTG-AAAACTTGACCCAACTATGATAAC-TT-AGGGCTAGTCAATTTCGTGCCAGCCACCGCGG
Pta#3 ACGGGCTTACCAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAA--TTTAGGGCTGGTCAATTTCGTGCCAGCCACCGCGG
Ppe#2 ACGGGCTTACCAGCAGTAATAAACATTGGGCTATGAGTG-AAAACTTGACCCAACTATGATAAC-TT-AGGGCTGGTCAATTTCGTGCCAGCCACCGCGG
Phi#1 ACGGGTCCACCAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAA--TTTAGGGCTGGTCAATTTCGTGCCAGCCACCGCGG
Pva#3 ACGGGTTCACCAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAA--TT-AGGGCTGGTCA-TTTCGTGCCAGCCACCGCGG
Pbo#4 ACGGGCCCACCAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAA--TTTAGGGCTGGTCAATTTTGTGCCAGCCACCGCGG
Pat#1 ACGGGCTCACCAGCAGTAATAAACATTGGGCCATAAGTG-AAAACTTGACCCAACTATGATAA--TTTAGGGCTGGTCAATTTTGTGCCAGCCACCGCGG
Gga   ACGGGTACTC-AGCAGTAATTAACCTTAAGCAATAAGTGTAAAACTTGACTTAGCCATAGCAAC--CCAGGGTTGGTAAATCTTGTGCCAGCCACCGCGG

             310       320       330       340       350       360       370       380       390       400
               .         .         .         .         .         .         .         .         .         .
Lbi#1 TTATACGAAAAG-CCCAAAACAACGACAAAC-GGCGTAAAATGTGACTAGAGATCCTTAATACTAAA-AATACTAAAACCCAC-AACCTAGTTGT-AAAA
Tps#1 TTAAACGAAAAGCCCCAAAACAACGGTTAAC-GGCGTAAAATGTGACTAGAGGACTTCTATATTAAG-AACACTAAAAACCCACTGTCCGGTTGT-AAAA
Tdu#1 TTATACGAA-GGGCCCAAAATAACGGCTAAC-GGCGTAAAATGTGACTGGAGATTTATACATTAGAC-TAACGCTAAAATTCACTGCCTAGTTGT-AAAA
Tan#1 TTAAACGAAAGGCCCTGAATAACGGCAAAAC-GGCGTAAAATGTGACTAGATATGCTTAATTTTAAA-AATATTAAAATCTA-CAACTTAGT----AAAA
Pfm#1 TTATACGAAA-GGCCCAAAATAACGACAAACCGGTG-AAAATGTGACTAGAGACCCATAATATCTTG-AACATCAAAATTCA-TAGTCTAGTTGT-AAAA
Pfm#2 TTATACGAAA-GGCCCAAAATAACGACAAACCGGTG-AAAATGTGACTAGAGACCCATAATATATTG-AACATCAAAATTCA-TAGTCTAGTTGT-AAAA
Pfl#5 TTATACGAAA-GGCCCAAAATAACGACAAACCGGTG-AAAATGTGACTAGAGACCCATGATCTCTTG-AACATCAAAATTCA-TAGTCTAGTTGT-AAAA
Pwa#1 TTATACGAAA-GGCCCAAAATAACGACAAACCGGCGTAAAATGTGACTAGAGACCCATAATATATTG-AACATCAAAATTCA-TAGTCTAGTTGT-AAAA
Pwa#2 TTATACGAAA-GGCCCAAAATAACGACAAACCGGCGTAAAATGTGACTAGAGACCCATAATATATTG-AACATCAAAATTCA-TAGTCTAGTTGT-AAAA
Pra#3 TTATACGAAA-GGCCCAAAATAACGACAAACCGGCGTAAAATGTGACTAGAGACCAATAATATTTTT-AACATCAAAATTTA-TAGTCTAGTTGT-AAAA
Pmn#3 TTATACGAAA-GGCCCAAAATAACGACAAACCGGCGTAAA-TGTGACTAGAGATTTATAACATTTCA-AATATCAAAATTC-CTAGTCCAGTTGT-AAAA
Pmn#4 TTATACGAAA-GGCCCAAAATAACGACAAACCGGCGTAAA-TGTGACTAGAGATTTATAACATTTCA-AATATCAAAATTC-CTAGTCCAGTTGT-AAAA
Pmn#6 TTATACGAAA-GGCCCAAAATAACGACAAACCGGCGTAAA-TGTGACTAGAGATTTATAACATTTCA-AATATCAAAATTC-CTAGTCCAGTTGT-AAAA
Pmb#7 TTATACGAAA-GGCCCAAAATAACGACAAACCGGCGTAAA-TGTGACTAGAGATTTATTACATTTCA-AATATCAAAATTC-CTAGTCCAGTTGT-AAAA
Psc#1 TTATACGAAA-GGCCCTAAATAACGACAAACCGGCGTAAA-TGTGACTAGAGATCTATAATATTTTA-AATATTAAAATTCATTAGCCTAGTTGT-AAAA
Psc#3 TTATACGAAA-GGCCCTAAATAACGACAAACCGGCGTAAA-TGTGACTAGAGATCTATAATATTTTA-AATATTAAAATTCATTAGCCTAGTTGT-AAAA
Pse#4 TTATACGAAA-GGCCCTAAATAACGACAAACCGGCGTAAA-TGTGACTAGAGATCTATAATATTTTA-AATATCAAAATTCATTAGCCTAGTTGT-AAAA
Pss#5 TTATACGAAA-GGCCCTAAACAACGACAAACCGGCGTAAA-TGTGACTAGAGATTCATAATATTTTA-AATATCAAAATCCATTAGCCTAGTTGT-AAAA
Pti#1 TTATACGAAA-GGCCCAAAATAACGACAAACCGGCGTAAA-TGTGACTAGAGATTAATAGTATTTTT-AATATCAAAATTTA-TAGTCTAGTTGT-AAAA
Pti#2 TTATACGAAA-GGCCCAAAATAACGACAAACCGGCGTAAA-TGTGACTAGAGATTAATAGTATTTTT-AATATCAAAATTTA-TAGTCTAGTTGT-AAAA
Ppi#2 TTAT-CGAAA-GGCC-AAAATAACGAAAAACCGGCGTAAAATGTGACTAGAGAA-TATAATATTTTA-AATATCAAAATTCA-TAATCTAGTTGT-AAAA
Pli#2 TTAT-CGAAA-GGCC-AAAATAACGACAAACCGGCGTAAAATGTGACTAGAGATCTATAATATTTTA-AACATCAAAATTCT-TAGTCTAGTTGT-AAAA
Per#2 TTATACGAAA-GGCCCAAAATAACGACAAACCGGCGTAAAATGTGACTAGAGATCTTTAATATTTTA-AGCTTCAAAATCCA-TAGTCTAGTTGT-AAAA
Pmi#1 TTATACGAAA-GGCCCAAAATAACGACAAATCGGCGTAAAATGTGACTAGAGATCTATAATATTCTT-AACATCAAAATTTT-TAGCCTAGTTGT-AAAA
Pmi#2 TTATACGAAA-GGCCCAAAATAACGACAAATCGGCGTAAAATGTGACTAGAGATCTATAATATTCTT-AACATCAAAATTTT-TAGCCTAGTTGT-AAAA
Pme#3 TTATACGAAA-GGCC-AAAATAACGATAAACCGGGGTAAAATGTGACTAGAGATCCATAGTATTTTT-AATATTAAAATTTTT-AGTCTAGTTGT-AAAA
Pta#3 TTATACGAAA-GGCCCAAAATAACGAAAAACCGGCGTAAAATGTGACTAGAGATTCATAATATTCTT-AATATTAAAATTTTT-AGCCTAGTTGT-AAAA
Ppe#2 TTATACGAAA-GGCCCAAAATAACGACAAACCGGCGTAAAATGTGACTAGAGATCCATAATATATTG-AACATCAAAATTTT-TAGTCTAGTTGT-AAAA
Phi#1 TTATACGAAA-GGCCCAAAA-AACGACAAACCGGCGTAAAATGTGACTAGAGATTTATTATATTTTA-AATACCAAAATCCATCAACCCAGTTGT-AGAA
Pva#3 TTATACGAAA-GGCCCAAAATAACGACAAACCGGCGTAAAATGTGACTAGAGATTCATAATATTTTA-AGTATTAAAACTTATTAGCCTAGTTGT-AAAA
Pbo#4 TTATACGAAA-GGCCACTAATAACGACAAACCGGCGTAAAATGTGACTAGAGATTCATAATATTTTA-AATACTAAAACTCATCAACCTAGTTGT-AAAA
Pat#1 TTATACGAAA-GGCCCAAAATAACGACAAACCGGCGTAAAATGTGACTAGAGATTAATAATATTCTA-AATATTAAAATTCATCAGCCTAGTTGT-AAAA
Gga   TCATACAAGAAA-CCCAAATCAATAGCTACCCGGCGTAAAGAGTGGCCACATGTTATCTGCACCAGCTAAGATTAAAAT---GCAACCAAGCTGTCATAA
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Appendix I *continued*

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              410       420       430       440       450       460       470       480       490       500       510
               .         .         .         .         .         .         .         .         .         .         .
Lbi#1 TACTTAGGTA-TGAAGAAAACCAACA---AGTATTTTTAA----TATAATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Tps#1 TACTTAGACAATGGGGAAAACCAACACAAAGTGTTTTTA-----TGTTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Tdu#1 TACTTAGACAATGGAGAAAATCAATACAAAGCATTTTTAA----TAGTATATTCTTGACGACACGAAAGCTTAGAAACAAACTAGGACTAGATACCCTACTATGCTAAGC
Tan#1 TACTTAAGCTATAGAGAAAACCAATACAAA-TATTTTTAA----CCTTCAACCCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pfm#1 CACTAAGACAACGAAGAAAACCAACACAGA-TATTCTTAA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pfm#2 CACTAAGACAACGAAGAAAACCAACACAGA-TATTCTTAA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pfl#5 CACTAAGACAACGAAGAAAACCAACACAGA-TATTCTTAA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pwa#1 CACTAAGACAACGAAGAAAACCAACACAGA-TATTCTTAA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pwa#2 CACTAAGACAACGAAGAAAACCAACACAGA-TATTCTTAA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pra#3 CACTAAGACAAAGGAGAACACCAACACAGA-TATTATTAA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pmn#3 CACTAAGACAACGGAGAATACCAATACAGA-TATTCTTAA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pmn#4 CACTAAGACAACGGAGAATACCAATACAGA-TATTCTTAA----TATTATATTCTTGACCACACGAAAGCTTAGAAACGAACTAGGATTAGATACCCTACTATGCTAAGC
Pmn#6 CACTAAGACAACGGAGAATACCAATACAGA-TATTCTTAA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pmb#7 CACTAAGACAACGGAGAATACCAATACAGA-TATTCTTAA----TATTATATTCTTGACCACACGAAAGCTTAGAAACGAACTAGGATTAGATACCCTACTATGCTAAGC
Psc#1 CACTTAGACAATGAAGAAAACCAACACAAA-TATTCTTAA----TATTATATTCTTGATTACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Psc#3 CACTTAGACAATGAAGAAAACCAACACAAA-TATTCTTAA----TATTATATTCTTGATTACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pse#4 CACTCAGACAATGGAGAAAATCAACACAGA-TATTCTTAA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pss#5 CACTTAGACAATGGAGAAAATCAGCACAGA-TATTCTTAA----TATTATATTCTTGATTACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pti#1 CGCTTAGACAATGAAGAAAACCAACACAGA-TATTATTTA----TATTATATTCTTGACTACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pti#2 CACTTAGACAATGAAGAAAACCAACACAGA-TATTATTTA----TATTATATTCTTGACTACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Ppi#2 CACTCAGATAATGAAGAAAACCACCACAGA-TATTTTTA-----TATTATACTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pli#2 CACTTAGACAATGAAGAAAGCCACCACAGA-TATTTTTA-----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Per#2 CACTAGGACTATGAAGAAAACCAACACAAG-TGTTCTTAA----TGTTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pmi#1 TACTAAGACAATAAAGAAAACCAACACAGA-TATTATTAA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pmi#2 TACTAAGACAATAAAGAAAACCAACACAGA-TATTATTAA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pme#3 CACTAAGACAATAAAGAAAACCAAAACAAA-TATTCTTAA----TACCATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pta#3 CACTAAGATAATAAAGAAGACCAACACAAA-TATTCTTGA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Ppe#2 CACTAAGACAATAAAGAAAACCAACACAGA-TATTCTTAA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Phi#1 CACTAAGGTAATGGAGAAAACCAACACAGG-TGTTCTTTA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pva#3 CACTAAGGTAACGAAGAAAACCAATACAGA-TGCTCTTAA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pbo#4 CACTAAGGTAATGGAGAAAACCAATACAGG-TATTCTTTA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Pat#1 CACTAAGGTAACGGAGAAAACCAACACAGA-TATTCTTAA----TATTATATTCTTGACCACACGAAAGCTTAGAAACAAACTAGGATTAGATACCCTACTATGCTAAGC
Gga   GCCTAAGATC-CACCTAAACCCAACCCAAATCCATCTTAGCCTCAACGATTAATTTTAACCCACGAAAGCTAGGACCCAAACTGGGATTAGATACCC-ACTATGCCTAGC

              520       530       540       550       560       570       580       590       600        EMBL Acc. numbers
               .         .         .         .         .         .         .         .         .
Lbi#1 CCTAAACATTGATTAGCC-ACT-ATACAACACTCTCCGCCAGAGAACTACGAAGTGAAAAACTTGAAACTCAAAGGACTTGACGGTGTCCCATATCAA AJ001585-AJ001480
Tps#1 CCTAAACATTGAT-AGCT-ATC-ATACAACGCTTTCCGCCAGAGAACTAC-AAGTGAAATACTTGAAACTCAAAGGACTTGACGGTGTCCCATATCGA AJ250154
Tdu#1 CATTAACATTGAA-AGCT-ATC-ATACAATGCTTTCCGCCAGAGAACTAC-AAGTGAAAAACTTTAAACTCGAAGGACTTGACGGTGTCCCATATCGA AJ004885-AJ004884
Tan#1 CCTAAACATTGATAAGCT-AAC-ATACTATGCTTTCCGCCAGAGAACTAC-AAGTGAAAAACTTGAAACTCAAAGGACTTGGCGGTGTCCCATATCGG AJ250155
Pfm#1 CCTGAACATTGAT-AGTT-ACTAATACAATACTTTCCGCCAGAGAACTAC-AAGTGAAAAACTTGAAACTCAAAGGACTTGACGGTGTCCCACATCGA AJ001567-AJ001415
Pfm#2 CCTGAACATTGAT-AGTT-ACTAATACAATACTTTCCGCCAGAGAACTAC-AAGTGAAAAACTTGAAACTCAAAGGACTTGACGGTGTCCCACATCGA AJ001568-AJ001463
Pfl#5 CCTGAACATTGAT-AGTT-ACTAATACAATACTTCGCGCCAGAGAACTAC-AAGTGAAAAACTTGAAACTCAAAGGACTTGACGGTGTCCCACATCGA AJ250156
Pwa#1 CCTGAACATTGAT-AGTT-ACTAATACAATACTTTCCGCCAGAGAACTAC-AAGTGAAAAACTTGAAACTCAAAGGACTTGACGGTGTCCCACATCGA AJ001583-AJ001466
Pwa#2 CCTGAACATTGAT-AGTT-ACTAATACAATACTTTCCGCCAGAGAACTAC-AAGTGAAAAACTTGAAACTCAAAGGACTTGACGGTGTCC-ACATCGA AJ001584-AJ001467
Pra#3 CCTGAACATTGAT-AGTT-ATTTATACAATACTTCCCGCCAGAGAACTAC-AAGTGAAAAACTTGAAACTCAAAGGACTTGACGGTGTCCCATATCGG AJ250157
Pmn#3 CCTGAACATTGAT-AGTT-ACTAATACAATACTTTCCGCCAGAGAACTAC-AAGTGAAAAACTTAAAACTCAAAGGACTTGACGGTGTCCCATATCGA AJ001571-AJ001468
Pmn#4 CCTGAACATTGAT-AGTT-ACTAATACAATACTTTCCGCCAGAGAACTAC-AAGTGAAAAACTTAAAACTCAAAGGACTTGACGGTGTCCCATATCGA AJ001572-AJ001469
Pmn#6 CCTGAACATTGAT-AGTT-ACTAATACAATACTTTCCGCCAGAGAACTAC-AAGTGAAAAACTTAAAACTCAAAGGACTTGACGGTGTCCCATATCGA AJ001573-AJ001470
Pmb#7 CCTGAACATTGAT-AGTT-ACTAATACAATACTTTCCGCCAGAGAACTAC-AAGTGAAAAACTTAAAACTCAAAGGACTTGACGGTGTCCCATATCGA AJ001574-AJ001471
Psc#1 CCTGAACATTGAA-AGTTTACCTATACTTTACTTTCCGCCAGAGAACTAC-GAGTGAAAAACTTAAAACTCAAAGGACTTGACGGTGTCCCATATCGG AJ001577-AJ001474
Psc#3 CCTGAACATTGAA-AGTTTACCTATACTTTACTTTCCGCCAGAGAACTAC-GAGTGAAAAACTTAAAACTCAAAGGACTTGACGGTGTCCCATATCGG AJ001578-AJ001475
Pse#4 CCTAAACATTGAA-AGCTTATCTATACTATACTTTCCGCCAGAGAACTAC-GAGTGAAAAACTTAAAACTCAAAGGACTTGACGGTGTCCCATATCGG AJ001579-AJ001476
Pss#5 CCCGAACATTGAA-AGTTTACCTATACTTTACTTTCCGCCAGAGAACTAC-GAGTGAAAAACTTAAAACTCAAAGGACTTGACGGTGTCCCATATCGG AJ001580-AJ001477
Pti#1 CCTGAACATTGAT-AGTT-ATTTATACAATACTTTCCGCCAGAGAACTAC-AAGTGAAAAACTTGAAACTCAAAGGACTTGACGGTGTCCCACATCGG AJ001581-AJ001478
Pti#2 CCTGAACATTGAT-AGTT-ATTTATACAATACTTTCCGCCAGAGAACTAC-AAGTGAAAAACTTGAAACTCAAAGGACTTGACGGTGTCCCACATCGG AJ001572-AJ001479
Ppi#2 CCTGAACATCGAT-AGTT-ACCTATACAGTACTTTCCGCCAGAGAACTAC-AAGTGAAAAACTTGAAACTCAAAGGACTTGACGGTGTCCCATATCGG AJ250158
Pli#2 CCTGAACATTGAT-AGTT-ACTTACACAATACTTCCCGCCAGAGAACTAC-AAGTGAAAAGCTTGAAACTCAAAGGACTTGACGGTGTCCCATATCGG AJ250159
Per#2 CCTGAACATTGAT-AGT--TTTAATACAACGCTTCCCGCCAGAGAACTAC-AAGTGAAAAACTTGAAACTCAAAGGACTTGACGGTGTCCCATATCGG AJ250160
Pmi#1 CCTGAACATTGAA-AGTT-A-TAATACAAGACTTCCCGCCAGAGAACTAC-AAGTGAAAAACTTGAAATTCAAAGGACTTGACGGTGTCCCATATCGG AJ250161
Pmi#2 CCTGAACATTGAA-AGTT-A-TAATACAAGACTTC-CGCCAGAGAACTAC-AAGTGAAAAA-TTGAAACTCAAAGGACTTGACGGTGTCCCATATCGG AJ250162
Pme#3 CCTGAACATTGAA-AGTT-ACAAATACAATACTCTC-GCCAGAGAACTAC-AAGTGAAAAACTTGAAACTCAAAGGACTTGACGGTGTCCCATATCGA AJ250163
Pta#3 CCTGAACATTGAA-AGTT-ATAAGTACAATACTTCCCGCCAGAGAACTAC-AAGTGAAAAACTTAAAACTCAAAGGACTTGACGGTGTCCCATATCGA AJ250164
Ppe#2 CCTGAACATTGAA-AGTT-ACTAATACAATACTTTCCGCCAGAGAACTAC-AAGTGAAAAACTTGAAACTCAAAGGACTTGACGGTGTCCCACATCGA AJ250165
Phi#1 CCTGAACATTGAT-TGCT-ATTAATACAATACTTTCCGCCAGAGAACTAC-AAGTGAAAAACTTGAAGCTCAAAGGACTTGACGGTGTCCCATATCGA AJ250166
Pva#3 CCTGAACATTGAT-AGCT--CCAATACAATACTTCCCGCCAGAGAACTAC-AAGTGAAAAACTTGAAACTCAAAGGACTTGACGGTGTCCCATATCGG AJ250167
Pbo#4 CCTGAACATTGAT-AGCT-ACTAATACATTACTTCCCGCCAGAGAACTAC-AAGTGAAAAACTTGAAACTCAAAGGACTTGACGGTGTCCCATATCGA AJ250168
Pat#1 CCTGAACATTGAT-AGTT-ACTCATACAAGACTTCCCGCCAGAGAACTAC-AAGTGAAAAACTTGAAACTCAAAGGACTTGACGGTGTCCCATATCGA AJ250169
Gga   CCTAAATCTAGAT-ACCT-CCCATCACACATGTATCCGCCTGAGAACTACGAGCACAAACGCTTAAAACTCTAAGGACTTGGCGGTGCCCCAAACCCA X52392
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Appendix 2 Pairwise sequence divergence indices. Uncorrected ‘p’ distance.

Tv	Lbi#1	Tps#1	Tdu#1	Tan#1	Pfm#1	Pfm#2	Pfl#5	Pwa#1	Pwa#2	Pra#1	Pmn#3	Pmn#4	Pmn#6	Pmb#7	Psc#1	Psc#3	Pse#4	Pss#5	Pti#1	Pti#2	Ppi#2	Pli#2	Per#2	Pme#3	Pta#3	Ppe#2	Pmi#1	Pmi#2	Phi#1	Pva#1	Pbo#4	Pat#1
Lbi#1																																
Tps#1	0.135																															
Tdu#1	0.160	0.120																														
Tan#1	0.151	0.104	0.146																													
Pfm#1	0.113	0.102	0.123	0.122																												
Pfm#2	0.115	0.104	0.123	0.123	0.003																											
Pfl#5	0.125	0.112	0.133	0.132	0.012	0.012																										
Pwa#1	0.113	0.102	0.121	0.121	0.005	0.002	0.014																									
Pwa#2	0.113	0.102	0.121	0.122	0.005	0.002	0.014	0.000																								
Pra#1	0.127	0.105	0.126	0.116	0.038	0.036	0.043	0.034	0.034																							
Pmn#3	0.122	0.094	0.116	0.106	0.038	0.040	0.050	0.038	0.038	0.050																						
Pmn#4	0.124	0.097	0.120	0.108	0.038	0.040	0.050	0.038	0.038	0.050	0.003																					
Pmn#6	0.124	0.095	0.118	0.108	0.040	0.042	0.052	0.040	0.040	0.052	0.002	0.005																				
Pmb#7	0.127	0.099	0.122	0.115	0.043	0.045	0.054	0.043	0.043	0.055	0.009	0.009	0.007																			
Psc#1	0.124	0.115	0.128	0.121	0.068	0.070	0.080	0.068	0.068	0.076	0.069	0.069	0.071	0.075																		
Psc#3	0.129	0.120	0.134	0.126	0.073	0.075	0.085	0.073	0.073	0.082	0.075	0.075	0.076	0.080	0.005																	
Pse#4	0.118	0.109	0.119	0.115	0.062	0.064	0.075	0.062	0.062	0.064	0.064	0.064	0.066	0.069	0.027	0.033																
Pss#5	0.125	0.123	0.126	0.118	0.066	0.068	0.078	0.066	0.066	0.076	0.064	0.064	0.066	0.069	0.029	0.034	0.036															
Pti#1	0.125	0.117	0.118	0.126	0.045	0.047	0.057	0.045	0.045	0.050	0.056	0.059	0.057	0.061	0.069	0.074	0.066	0.067														
Pti#2	0.125	0.118	0.120	0.126	0.043	0.045	0.055	0.043	0.043	0.048	0.057	0.057	0.059	0.062	0.067	0.073	0.064	0.066	0.005													
Ppi#2	0.129	0.113	0.134	0.110	0.061	0.060	0.068	0.058	0.058	0.068	0.068	0.072	0.070	0.073	0.073	0.078	0.071	0.077	0.068	0.069												
Pli#2	0.142	0.114	0.137	0.131	0.070	0.068	0.073	0.066	0.066	0.066	0.073	0.077	0.075	0.079	0.080	0.085	0.081	0.085	0.080	0.083	0.047											
Per#2	0.127	0.088	0.137	0.101	0.055	0.057	0.064	0.055	0.055	0.057	0.055	0.055	0.057	0.064	0.078	0.084	0.076	0.083	0.073	0.071	0.078	0.078										
Pme#3	0.141	0.121	0.137	0.124	0.063	0.061	0.069	0.061	0.061	0.076	0.080	0.080	0.082	0.085	0.085	0.090	0.088	0.095	0.080	0.078	0.085	0.095	0.088									
Pta#3	0.128	0.111	0.124	0.106	0.054	0.056	0.062	0.054	0.054	0.060	0.061	0.061	0.063	0.066	0.071	0.076	0.069	0.078	0.062	0.061	0.069	0.083	0.067	0.045								
Ppe#2	0.125	0.109	0.123	0.113	0.031	0.031	0.043	0.029	0.029	0.050	0.056	0.056	0.057	0.061	0.073	0.078	0.068	0.074	0.057	0.055	0.068	0.071	0.059	0.041	0.057							
Pmi#1	0.120	0.109	0.123	0.114	0.050	0.052	0.059	0.050	0.050	0.052	0.063	0.063	0.064	0.071	0.075	0.080	0.068	0.081	0.061	0.059	0.073	0.073	0.059	0.067	0.055	0.047						
Pmi#2	0.119	0.108	0.123	0.113	0.049	0.047	0.052	0.045	0.045	0.047	0.061	0.061	0.063	0.070	0.073	0.078	0.066	0.080	0.059	0.057	0.070	0.069	0.057	0.005	0.062	0.043	0.040					
Phi#1	0.120	0.101	0.132	0.119	0.066	0.065	0.073	0.063	0.063	0.073	0.063	0.063	0.064	0.066	0.091	0.096	0.081	0.085	0.071	0.070	0.078	0.099	0.071	0.083	0.097	0.070	0.075	0.078				
Pva#1	0.122	0.120	0.138	0.113	0.061	0.059	0.062	0.057	0.057	0.068	0.064	0.064	0.066	0.071	0.078	0.083	0.076	0.081	0.075	0.073	0.064	0.083	0.068	0.073	0.081	0.059	0.069	0.068	0.060			
Pbo#4	0.114	0.125	0.143	0.115	0.066	0.068	0.075	0.066	0.066	0.078	0.066	0.066	0.068	0.073	0.085	0.090	0.083	0.085	0.081	0.080	0.083	0.100	0.078	0.081	0.093	0.067	0.074	0.080	0.048	0.057		
Pat#1	0.114	0.107	0.127	0.106	0.049	0.050	0.057	0.049	0.049	0.059	0.052	0.052	0.054	0.057	0.073	0.078	0.071	0.074	0.066	0.064	0.062	0.074	0.062	0.055	0.078	0.041	0.060	0.055	0.053	0.045	0.044	
Tv	Lbi#1	Tps#1	Tdu#1	Tan#1	Pfm#1	Pfm#2	Pfl#5	Pwa#1	Pwa#2	Pra#1	Pmn#3	Pmn#4	Pmn#6	Pmb#7	Psc#1	Psc#3	Pse#4	Pss#5	Pti#1	Pti#2	Ppi#2	Pli#2	Per#2	Pme#3	Pta#3	Ppe#2	Pmi#1	Pmi#2	Phi#1	Pva#1	Pbo#4	Pat#1