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Molecular phylogeny and biogeography of the wall-lizard *Podarcis erhardii* (Squamata: Lacertidae)

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Abstract

Erhard's wall lizard, *Podarcis erhardii* (Sauria: Lacertidae), is highly diversified in Greece and especially in the southern Aegean region. Out of the 28 recognized subspecies, 27 are found in Greece from the North Sporades island-complex in the North Aegean (grossly south of the 39th parallel) to the island of Crete in the South. The species exhibits great morphological and ecological plasticity and inhabits many different habitats from rocky islets and sandy shores to mountaintops as high as 2000 m. By examining intraspecific variability at a segment of the mitochondrial gene cytochrome *b* we have found that that extant populations of *P. erhardii* are paraphyletic. Furthermore, we have found that subspecies previously defined on the basis of morphological characteristics do not correspond to different molecular phylogenetic clades, so that their status should be reconsidered. The DNA based biogeographical and phylogenetic history of *Podarcis* in Southern Greece is congruent with available paleogeographic data of the region, which supports the view that DNA sequences may be a useful tool for the study of palaeogeography.

1. Introduction

The genus *Podarcis* (Lacertidae) comprises 17 currently recognized species in southern Europe, where they are the predominant reptile group (Harris and Arnold, 1999). The taxonomy of *Podarcis* is complex and continuously revised because it exhibits substantial intraspecific variability (Arnold and Burton, 1978). There is substantial morphological and genetic evidence that *Podarcis* is a monophyletic group (Arnold, 1973, 1989; Fu, 2000; Harris et al., 1998; Oliverio et al., 2000) and that its closest relative, as derived from morphology, is the Moroccan *Lacerta* (*Teira*) *andreanskyi* (Arnold, 1973). Within *Podarcis* relationships are poorly understood. Several karyological (Olmo et al., 1986, 1987) and biochemical (Capula, 1994, 1996, 1997; Lanza and Cei, 1977) studies exist for a few species, and produce conflicting results.

Using partial mitochondrial DNA (mtDNA) sequences, Harris and Arnold (1999) and Oliverio et al. (2000), concluded that the relationships among *Podarcis* species cannot be definitively resolved with the data sets they used. Nevertheless both these studies support the monophyly of a Balkan group of *Podarcis*, which includes *Podarcis gaigeae*, *Podarcis milensis*, *Podarcis melisellensis*, *Podarcis taurica*, "and perhaps *Podarcis wagleriana*, *Podarcis erhardii*, and *Podarcis peloponnesiaca*." However, Oliverio et al. (2000) do not agree with Harris and Arnold (1999) that of *P. wagleriana* is part of the "Balkan" clade.

Compared to other lacertids *P. erhardii* is less well studied and apart from its close relation with *P. peloponnesiaca* (Arnold, 1973), its phylogenetic relationship with other taxa is not established. *P. erhardii* exhibits notable geographic variation in morphology (Arnold and Burton, 1978; Gruber, 1971, 1987; Wettstein, 1953,

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1957), habitat preference, and behavior (Gruber, 1971; Katsadorakis, 1984; Valakos, 1986, 1987, 1990; Valakos et al., 1997). The observed morphological variation in *P. erhardii* (e.g., color pattern, shape, and size) has led to the description of 28 subspecies in Greece (Engelmann, 1993; Gruber, 1987). This high variability and remarkable plasticity makes the construction of a purely morphological key for the identification of species and subspecies extremely difficult (Arnold, 1993).

The complex geological history of the Southern Aegean (including the Peloponnisos region) during the late Tertiary has influenced the distribution of all five *Podarcis* species in the region (i.e., *P. erhardii*, *P. peloponnesiaca*, *Podarcis muralis*, *P. taurica*, and *P. milensis*), as well as *P. gaigeae* further to the north, and contributed to the diversification within *P. erhardii*. This diversity is thought to reflect the submergence and re-emergence of landmasses, due to tectonic, volcanic and eustatic events.

In the present study we examine the phylogenetic relationships of several populations of *P. enhardii* across Greece, especially South Greece using a region of the mitochondrial cytochrome *b* gene (cyt *b*). We combine this information with previously published sequences and use the results to produce a historical interpretation of the species' distribution and morphological diversification.

2. Materials and methods

All individuals included in this study are listed in Table 1. Locations of sampled populations are shown in Fig. 1. Single alcohol-preserved wall-lizards were used as source material. Genomic DNA was extracted from soft tissues using the standard proteinase K protocol (Hillis et al., 1996). Double-stranded PCR was used to amplify a 451 bp of the mitochondrial DNA cyt *b* gene using universal primers (L14724 and H15175, Palumbi, 1996). The PCR cycle program was comprised of an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 1 min at 94 °C, 1 min at 47 °C, and 1 min at 72 °C. The cycling was ended with 70 s sequence extension at 72 °C. All specimens were sequenced directly from the PCR products using the primers mentioned above on a Perkin–Elmer automated sequencer. Sequences for both strands were determined. GenBank accession numbers for the sequences obtained are: AF486191–AF486234. Sequences were aligned using the ClustalX program package (Thompson et al., 1997). The MEGA computer package (v.2, Kumar et al., 2001) was used to determine the number and type of nucleotide substitutions in pairwise comparisons of sequences, to measure the degree of divergence between sequences and to identify the unique sequences for phylogenetic analysis.

2.1. Phylogenetic analysis

Maximum parsimony analysis was performed with PAUP 4.0b10 (Swofford, 2002), with heuristic searches using stepwise addition and performing tree-bisection-reconnection (TBR) branch swapping (Swofford et al., 1996). Bootstraping with 1000 pseudo-replicates and heuristic search were used to examine the robustness of clades in resulting trees (Felsenstein, 1985). Branch support was also assessed from decay indices calculated for all internal branches of the tree (Bremer, 1994). Decay indices were calculated using AutoDecay (ver. 5.0) (Eriksson, 2001) for Windows.

For maximum likelihood (ML) analysis (Felsenstein, 1981), the best-fit model of DNA substitution and the parameter estimates used for tree construction were chosen by performing hierarchical likelihood ratio tests (Hulsenbeck and Crandall, 1997) in Modeltest 3.06 (Posada and Crandall, 1998). Likelihood ratio tests indicated that the Tamura and Nei (1993) model with general time reversible option was the most appropriate for subsequent ML analyses. Heuristic ML searches were performed with 10 replicates of random sequence addition and TBR branch swapping. ML bootstraps employed only 100 iterations.

2.2. Testing alternative hypotheses

All trees favored under the parsimony or likelihood criterion were used for topology testing (PAUP 4.0b10). Topologies were compared with the nonparametric multiple comparison approach of Goldman et al. (2000) and Shimodaira and Hasegawa (1999). The Likelihood Shimodaira–Hasegawa test (LSH) was used with full parameter optimizations and 1000 bootstrap replications. The Parsimony Shimodaira and Hasegawa (1999) tests (PSH) were implemented as described by Shimodaira and Hasegawa (1999), but by substituting tree lengths with negative log-likelihoods. We used two-tailed Wilcoxon signed-ranks tests (Templeton, 1983) to test whether the cladograms predicted by alternative phylogenetic hypotheses were significantly different from the most parsimonious tree obtained in our analyses. The following hypotheses were tested: (1) all conventional subspecies of *P. erhardii* are monophyletic, (2) *P. erhardii* is a monophyletic clade, and (3) the group *P. muralis*, *P. erhardii*, and *P. peloponnesiaca* is not monophyletic. Moreover, the ML trees with the highest log-likelihood ($\ln L$) score were compared to these three alternative tree hypotheses, with the Shimodaira and Hasegawa (SH) test.

2.3. Divergence time and molecular clock testing

To estimate divergence times, the net nucleotide divergence (Da) between geographic groups was calculated

Table 1
List of the specimens of *Podarcis* examined, with taxon name, museum numbers, geographic origins, population map codes (see Fig. 1), number of samples, and accession numbers

Species	Origin				Accession Nos. in GenBank
	Museum No.	Locality	Code	Samples	
P. e. werneriana	NHMC 80.3.51.260	Crete Isl. (Xrysi islet)	1	1	AF486212
P. e. werneriana	NHMC 80.3.51.327	Crete Isl. (Koufonisi islet)*	2	1	AF486213
P. e. werneriana	NHMC 80.3.51.155	Crete Isl. (Traxilos islet)*	3	1	AF486207
P. e. werneriana	NHMC 80.3.51.277	Crete Isl. (Marmara islet)*	4	1	AF486208
P. e. werneriana	NHMC 80.3.51.157	Crete Isl. (Stroggylo islet)*	5	1	AF486210
P. e. werneriana	NHMC 80.3.51.309	Crete Isl. (Makroulo islet)*	6	1	AF486209
P. e. naxensis	NHMC 80.3.51.198	Crete Isl. (Elasa islet)	7	1	AF486214
P. e. rechingeri	NHMC 80.3.51.241	Crete Isl. (Dragonada islet)	8	1	AF486211
P. e. rechingeri	NHMC 80.3.51.235	Crete Isl. (Paximada islet)	9	1	AF486215
P. e. schiebeli	NHMC 80.3.51.237	Crete Isl. (Dia islet)	10	1	AF486206
P. e. cretensis	NHMC 80.3.51.1	Crete Isl. (Argiroupoli)	11	1	AF486216
P. e. cf. leukaorii	NHMC 80.3.51.291	Crete Isl. (Kallikratis)	12	1	AF486205
P. e. cf. cretensis	NHMC 80.3.51.528	Crete Isl. (Souda islet)	13	1	AF486194
P. e. cretensis	NHMC 80.3.51.43	Crete Isl. (Akrotiri)	14	1	AF486200
P. e. cretensis	NHMC 80.3.51.311	Crete Isl. (Therissou)	15	1	AF486201
P. e. cretensis	NHMC 80.3.51.319	Crete Isl. (Rodopos)	16	1	AF486198
P. e. cretensis	NHMC 80.3.51.318	Crete Isl. (Menies)	17	1	AF486197
P. e. cretensis	NHMC 80.3.51.509	Crete Isl. (Agia Eirini)	18	1	AF486191
P. e. cretensis	NHMC 80.3.51.501	Crete Isl. (Ballos)	19	1	AF486196
P. e. cretensis	NHMC 80.3.51.5	Crete Isl. (Kambos)	20	1	AF486199
P. e. elephonisii	NHMC 80.3.51.516	Crete Isl. (Lafonisi beach)	21	1	AF486193
P. e. elaphonisii	NHMC 80.3.51.519	Crete Isl. (Lafonisi islet)	22	1	AF486192
P. e. punctigularis	NHMC 80.3.51.534	Crete Isl. (Artemis islet)	23	1	AF486195
P. e. cf. leukaorii	NHMC 80.3.51.545	Crete Isl. (Lissos)	24	1	AF486219
P. e. cf. leukaorii	NHMC 80.3.51.13	Crete Isl. (Sougia)	25	1	AF486218
P. e. cf. leukaorii	NHMC 80.3.51.284	Crete Isl. (Tripiti)	26	1	AF486220
P. e. leukaorii	NHMC 80.3.51.177	Crete Isl. (Samaria Cemetery)	27	1	AF486204
P. e. leukaorii	NHMC 80.3.51.176	Crete Isl. (Samaria Castle)	28	1	AF486202
P. e. leukaorii	NHMC 80.3.51.3	Crete Isl. (Samaria Village)	29	1	AF486217
P. e. leukaorii	NHMC 80.3.51.310	Crete Isl. (Anopoli)	30	1	AF486203
P. erhardii	NHMC 80.3.51.279	Pori islet (near Antikythira)	31	1	AF486221
P. erhardii	NHMC 80.3.51.288	Pori islet (near Antikythira)	32	1	AF486222
P. peloponnesiaca	NHMC 80.3.54.9	Peloponnisos (Stymfalia)	33	1	AF486231
P. muralis	NHMC 80.3.53.21	Peloponnisos (Kalavrita)	34	1	AF486233
P. e. naxensis	NHMC 80.3.51.227	Cyclades (Santorini isl.)	35	1	AF486226
P. e. naxensis	NHMC 80.3.51.314	Cyclades (Santorini isl.)	36	1	AF486225
P. e. amorgenensis	NHMC 80.3.51.240	Cyclades (Anafi isl.)	37	1	AF486224
P. e. syrinae	NHMC 80.3.51.312	Cyclades (Astypalaia isl.)	38	1	AF486223
P. e. amorgenensis	NHMC 80.3.51.329	Cyclades (Amorgos isl.)	39	1	AF486229
P. e. naxensis	NHMC 80.3.51.315	Cyclades (Donousa islet)	40	1	AF486228
P. e. naxensis	NHMC 80.3.51.313	Cyclades (Naxos isl.)	41	1	AF486227
P. e. ruthveni	NHMC 80.3.51.328	Sporades (Skopelos isl.)	42	1	AF486230
P. muralis	NHMC 80.3.53.45	Thessalia (Kisavos mt)	43	1	AF486232
P. sicula	NHMC 80.3.55.1	Sicily (Italy)	44	1	AF486234
P. atrata (5 specimens)		Castilla et al. (1998)			AJ004910–AJ004911, AJ004978–AJ004980
P. bocagei (5)		Harris and Sá-Sousa (2001, 2002)			AF372087–AF372089, AF469424 and AF469426
P. carbonelli (3)		Harris and Sá-Sousa (2001, 2002)			AF372079–AF372081
P. hispanica (6)		Harris and Sá-Sousa (2001, 2002)			AF372086, AF469434, AF469436, AF469438, AF469440, and AF469442
P. lilfordi (6)		Terrasa et al. Unpublished data			AY046281–AY046286
P. tiliguerta (1)		Harris and Arnold (1999)			AF133457
P. filfolensis (1)		Harris and Arnold (1999)			AF133443
P. pityusensis (5)		Terrasa et al. Unpublished data			AY046293–AY046297
P. taurica (1)		Harris et al. (1998)			AF080280
P. milensis (1)		Harris and Arnold (1999)			AF133450

Table 1 (*continued*)

Species	Origin				Accession Nos. in GenBank
	Museum No.	Locality	Code	Samples	
P. gaigeae (1)		Harris and Arnold (1999)			AF133445
P. peloponnesiaca (1)		Harris and Arnold (1999)			AF133452
P. muralis (1)		Surget-Groba et al. (2001)			AF248007
P. sicula (2)		Fu (2000); Harris and Arnold (1999)			AF206531 and AF133455**
Lacerta andreanskyi (1)		Fu (2000)			AF206537
Gallotia stehlini (1)		Gonzalez et al. (1996)			Z480036
Gallotia atlantica (1)		Gonzalez et al. (1996)			Z480035

Below horizontal line: previously published sequences used in the analysis with the reference and accession number. Localities with asterisks belong to Koufonisia island group (see Fig. 1). Specimen with double asterisks (*P. sicula* of Harris and Arnold, 1999) belongs to *P. muralis* (Harris pers. comm.).

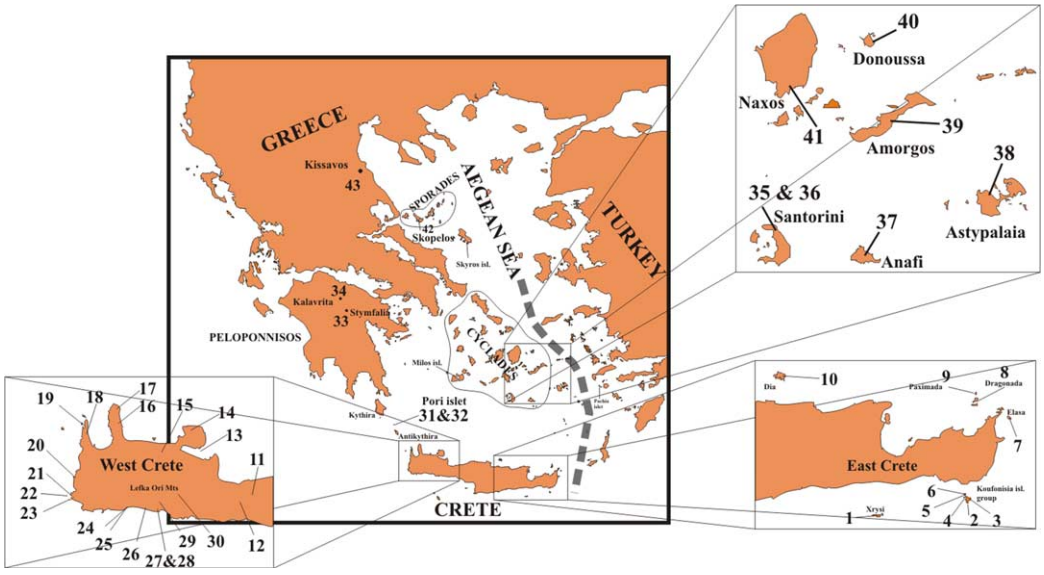

Fig. 1. The sampling localities of the 43 specimens used in this study (a 44th specimen originated from Sicily, Italy and is not shown). Thirty specimens came from the island of Crete, two from Pori islet, seven from Cyclades islands, one from Sporades islands, and three from continental Greece. The dashed thick line presents the Mid-Aegean trench.

from Tamura and Nei (1993) pairwise distances using MEGA (2.01). This metric, corresponds to the between-group variation corrected for the within-group variation in haplotypes and can be used to calculate the splitting time of groups (Nei, 1987). A molecular clock likelihood ratio test (LRT), $2\Delta = \log L_{\text{nonclock}} - \log L_{\text{clock}}$, which is distributed as χ^2 with $n-2$ degrees of freedom, where n is the number of sequences, was performed to determine whether there was a statistical difference in evolutionary rates among clades (Muse and Weir, 1992).

3. Results

The analysis of sequences from 44 individuals from 43 localities (Fig. 1) revealed 28 unique haplotypes of *Podarcis*. The lengths of these sequences ranged from 390 to 415 bp. No deletions or insertions were detected. The level of similarity in number and distribution of transitions and transversions in the cyt *b* fragment, the absence of stop codons, and the presence of strand bias against guanine on the light strand (G = 13.6, A = 27.0, T = 32.2, and C = 27.2) were comparable to the average of lacertids (Harris and Sá-Sousa, 2002), suggesting that the fragment is of mitochondrial origin rather than nuclear copy.

For phylogenetic analysis, a data set of 70 cyt *b* sequences were used (Table 1). Of these 28 are unique haplotypes from this study, 39 come from other *Podarcis spp*. and three are outgroup haplotypes (for references see Table 1). Thus, sequences representing 18 species in total (15 *Podarcis*, 1 *Lacerta*, and 2 *Gallotia* species) were used. A total of 419 base pairs were aligned, of which 169 sites (40.3%) were variable among

the *Podarcis* species (44.6% including outgroups) and 133 (31.7%) were parsimony informative (33.6% including outgroups). Tree length distribution, determined from random sampling of 10^6 unweighted trees, was significantly skewed to the left ($g_1 = -0.471$), suggesting a strong phylogenetic signal in the data ($p < 0.01$; Hillis and Huelsenbeck, 1992).

The heuristic parsimony analysis produced 288 equally parsimonious trees of 787 steps (CI = 0.342, RI = 0.765). Fig. 2A shows the 50% majority rule tree. Two ML trees were identified, differing in a single node ($-\ln L = 3903.911$; final parameters estimates: base frequencies A = 0.27, C = 0.31, G = 0.13, T = 0.29, $\alpha = 1.1413$, $P_{inv} = 0.4349$, and A/G = 9.95, C/T = 19.62). The ML tree shown in Fig. 2B, differ from the MP trees at several ingroup nodes. However, according to the LSH test, none of the MP trees is significantly worse than the ML trees ($p = 0.341$) under the ML criterion. The same result was produced under the MP criterion by the PSH test ($p = 0.354$).

The two focal species of this study, *P. erhardii* and *P. peloponnesiaca*, form a monophyletic group. To understand further the evolutionary relationship between these two species we have added in Fig. 2 the geographical origin (site and region) of the observed haplotypes. We also provide information about the assignment of the examined specimens into species and subspecies according to presently accepted classification (Gruber, 1987). It can be seen the all haplotypes of *P. erhardii* from Crete, of *P. erhardii* from Pori (a small island that lies half-way between Crete and Peloponnisos) and of *P. pelopponnesiaca* from Peloponnisos (the southern-most part of the Greek mainland) form one distinct clade (bootstrap support, BS, of 89 and 87% for MP and ML analyses, respectively). Specimens of *P. erhardii* from the island groups of Northern Sporades (North Aegean), Cyclades (Central Aegean), and Dodecanissa (South-Eastern Aegean) form another distinct clade (BS 86 and 82% for MP and ML). The first clade is further divided into three subclades, which reflect the three geographic regions (Crete, Pori, and Peloponnisos), while the more extensively sampled subclade of Crete is further subdivided into three clusters, again in accordance with the geographical site of samples. Thus,

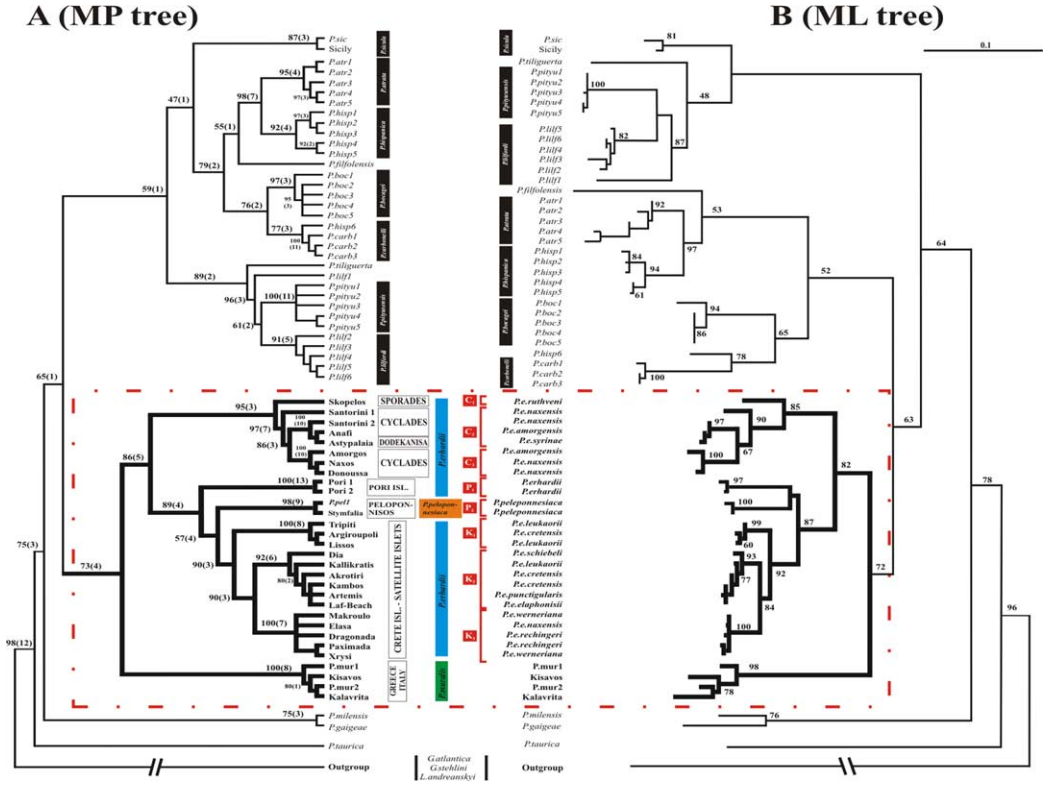

Fig. 2. Phylogenetic relationships among 70 unique cyt *b* haplotypes. (A) The 50% majority rule cladogram from the MP analysis. Branch numbers represent bootstrap support for 1000 iterations and Bremer support values (in parenthesis). (B) One of the two ML trees produced from the TrN + I + G model of evolution ($-\ln = 3903.91$). The ML tree not shown differed in the position of the short branches within *P. pityusensis*. Numbers indicate bootstrap values based on 100 replicates. Bold lines show the species that are the main focus of this study. The name at the tips of branch is the site from which the specimen was obtained (part A) or the subspecies to which the specimen belonged (part B). Boxes in part A give the wider geographical regions of the samples sites. C, P, or K in part B indicate specific clades. Black boxes provide species names. *Lacerta*, *Gallotia stehlini*, and *G. atlantica* were used as outgroups.

cluster K1 (Fig. 2) includes specimens from the Lefka Ori mountains in the South-Western part of the island (BS 100 and 99% for MP and ML), K2 includes specimens from Western Crete and the satellite islet of Dia in Central Crete (BS 92 and 93% for MP and ML), and K3 includes specimen from satellite islets of Eastern Crete (BS 100% for both MR and ML). Finally, the Cyclades clade is also divided in two subgroups of haplotypes, which correspond to two geographically distinct cluster of islands, one in the north (subclade C3 with BS = 100%) and the other in the southeast C2 (BS = 100 and 97%).

It is clear from the above analysis that the phylogenetic affiliations produced from the molecular data do not agree with the species and subspecies groupings predicted from the morphological classification of our sampled populations. The results from the Wilcoxon signed-rank and the Shimodaira–Hasegawa tests provide further support of this assertion. The Wilcoxon test rejects the hypothesis that the various subspecies of *P. erhardii* (Fig. 2) are monophyletic ($p < 0.0001$) and also the hypothesis that *P. erhardii* as a whole is a monophyletic species ($p = 0.039$), while it does not reject the hypothesis that *P. muralis*, *P. peloponnesiaca*, and *P. erhardii* form a monophyletic group ($p = 1$). The corresponding probabilities from the Shimodaira–Hasegawa tests are $p = 0.000,\ 0.026$, and 0.379, respectively.

The Hulsenbeck and Crandall (1997) likelihood ratio test did not reject the null hypothesis of a homogeneous clocklike rate for the tree produced by the *Podarcis* sequences from Greece ($\mathrm{LRT} = 61.967$, $\mathrm{df} = 47$, $\chi_{\mathrm{critical}} = 64.001$). This suggests that we can use the genetic distances between populations inhabiting different geographical regions in conjunction with the geological information about the age of the tectonic events that are responsible for the separation of these regions to estimate a global rate of evolution for the *P. erhardii* species. The splitting of the island of Crete from Peloponnisos is dated between 5.5 and 5 Mya (Meulenkamp, 1985; Schüle, 1993). Given that the corrected pairwise divergence (*Da*) between *Podarcis* of Peloponnisos and Crete is 7.97%, the evolutionary rate is 1.45–1.59% per million years. On the basis of these evolutionary rates we infer that the ancestral population of Cyclades was separated from that of Peloponnisos some 7.8–8.6 Mya and from that of Crete some 8.9–9.7 Mya. The splitting of the Cyclades population into NW from SE populations must have occurred some 3.3–3.6 Mya.

4. Discussion

The results from our molecular data disagree in several important points from the currently held views about the taxonomy of lacertid lizards of the genus *Podarcis*. All used outgroups indicate that the genus is a monophyletic group (BS 98 and 96% for MP and ML), in agreement with previous mtDNA studies (Harris and Arnold, 1999; Oliverio et al., 2000). The phylogenetic relationships among *Podarcis* species that do not occur in Greece have been discussed by Harris and Arnold (1999), Oliverio et al. (2000), Harris and Sá-Sousa (2001) and Terrasa et al. (unpub.) and will not be discussed here. Seven species of *Podarcis* are known to occur in the Balkan Peninsula: *P. muralis*, *P. erhardii*, *P. peloponnesiaca*, *P. taurica*, *P. gaigeae*, *P. milensis*, and *P. melisellensis*. According to Harris and Arnold (1999) and Oliverio et al. (2000) all these species, except *P. muralis*, form one monophyletic group, which in turn consists of two subgroups, the subgroup of *P. taurica* and the subgroup of *P. erhardii*. The first subgroup contains *P. taurica* (Northern mainland of Greece), *P. milensis* (island of Milos in Southwestern Cyclades), *P. gaigeae* (island of Skiros in Northern Aegean) and *P. melisellensis* (from Slovenian and Croatian coast to Bosnia-Hersegovina and Montenegro coastal area, as far south as Albania). The second subgroup contains *P. peloponnesiaca* (Peloponnisos, Southern mainland of Greece) and *P. erhardii* of the Aegean islands and Crete.

As shown in Fig. 2, our results recognize four of the above seven species as separate phylogenetic clades (*P. melisellensis* is not included in this study), but fail to separate between *P. erhardii* and *P. peloponnesiaca*. In addition, *P. taurica* separates first from all other species, while *P. gaigeae* and *P. milensis* form a closely related pair, an observation that agrees with previously published results (Harris and Arnold, 1999; Mayer and Tiedemann, 1980; Oliverio et al., 2000; Tiedeman and Mayer, 1980). Finally, with regard to *P. muralis* our results suggest that this species not only does not fall outside the group made up by all other Balkan species of the genus, but rather it is a sister species of *P. erhardii*, the main inhabitant of insular Greece.

Within *P. erhardii* our analysis points to two distinct clades (Fig. 2), one which includes the populations inhabiting the island complexes of Sporades (subclade C1) and Cyclades (subclades C2 and C3), and one that includes the populations that inhabit Crete, the islet of Pori and the representatives from Peloponnisos. The average Tamura–Nei (Tamura and Nei, 1993) degree of genetic differentiation between the two clades is 14.4%, whereas the mean pair-wise distance between Crete, Pori and Peloponnisos populations is 9.2%. Thus, *P. erhardii* populations from Crete or the islet of Pori are more closely related to populations of *P. peloponnesiaca* than they are to conspecific populations from Cyclades or Sporades. Using also cyt *b* data, Harris and Sá-Sousa (2001) reported a mean genetic distance of 13.6% for congeneric reptilian species, a figure that is comparable to that we report here between the two main clades of *P. erhardii*. These authors (Harris and Sá-Sousa, 2001, 2002) have also reported an analogous case of paraphyly

in *P. hispanica* and suggested a revision of the existing taxonomy of this species.

It is obvious that a similar and in many ways deeper revision of the current taxonomy is needed for the genus *Podarcis* in the Balkan Peninsula in general and for the species *P. erhardii* specifically. We have already referred to the inconsistencies between the molecular and morphological groupings of the various species within the genus *Podarcis*. With regard to morphological subspecies of *P. erhardii*, it is clear that they do not represent monophyletic units with regard to *P. peloponnesiaca*. This brings into question the practice of subspecies recognition and subsequent assignment of local populations into these subspecies on the basis of an exclusive or limited collection of characters, be that morphological, behavioral or molecular. An interesting example in support of this claim is the assignment of the population from the satellite islet Elasa in Eastern Crete to *P. e. naxensis* (Chondropoulos, 1986). In our analysis an individual from this island was assigned to the subclade K3 (Fig. 2), a genuine *P. erhardii* division, together with specimens from neighboring islets, whereas other *P. e. naxensis* individuals were assigned to subclades C2 and C3, which are separated from K3 by a genetic distance of 16.4%.

In addition to providing a means for evaluating the validity of morphological taxonomy, the molecular data may provide insights concerning the biogeography of the genus. The contemporary distribution of *P. muralis* and *P. erhardii*, both of which are rare in the Adriatic and Ionian coasts, suggests that the ancestral species descended to Greece from the Northwest, following the eastward path of Dinaric Alps and the Hellenides. It probably reached the Aegean after the formation of the Mid-Aegean trench (Fig. 1), which began splitting 12 million years ago (Mya) (Creutzburg, 1963) and was fully completed about 10.6 Mya (Dermitzakis, 1990). This hypothesis is based on the fact that species of the genus *Podarcis* are not found presently in any Aegean island to the east of the trench. An exception is the small islet Pachia near the island of Nisyros (Valakos et al., 1999), which could be a case of recent colonization from the Cyclades.

During the Upper Miocene Southern Greece was made of two large peninsulas, one in the southwest, which at present corresponds to the area of Peloponnisos and Crete and another in the southeast, which at present corresponds to the area of Cyclades (Dermitzakis, 1990). The split of Cyclades from Crete is placed in the Middle to Late Miocene (9–11 Mya) and that from Peloponnisos in the Late Miocene (7–9 Mya). The split of Cyclades into the Northwest and Southeast complexes of islands is placed in the Pliocene (3–4 Mya) (Dermitzakis, 1990). This geological information fits well with the molecular phylogeny of Fig. 2 if we assumed that the first clade evolved in the area that produced the present complex of Cyclades islands and subsequently differentiated into subclades C1, C2, and C3 (Fig. 2). These subclades are presently recognized as belonging to *P. erhardii*. The second clade evolved in the area of Peloponnisos and Crete, when these regions were united into one landmass. After the splitting of Crete from Peloponnisos, this clade produced the taxon we recognize today as *P. peloponnesiaca* in Peloponnisos, whereas in Crete and its satellite islands it produced the subclades K1, K2, and K3, which are recognized as belonging to *P. erhardii*. The fact that *P. erhardii* from the islet of Pori has a similar genetic distance from *P. peloponnesiaca* and from *P. erhardii* from Crete suggests that this population is a relict from the time Crete was connected to Peloponnisos. There is no evidence that Pori was affected by the submergence of the larger island of Kythira to the North during the Pliocene (Meulenkamp, 1985), which may explain the absence of *Podarcis* in Kythira. Antikythira, a larger islet to the south of Pori, also seems not to have undergone a submergence during the past 5 My, but in spite of this it carries no lizards of the genus *Podarcis*. It should noted that the ages we have sited above for the tectonic events that allegedly separated the ancestral populations of *P. erhardii* and produced the present-day subdivisions of the species are in good agreement with the ages produced from the molecular clock (see Section 3).

In conclusion, our molecular data of *Podarcis* in the Balkan Peninsula stress the need for a reconsideration of the classification of this main lizard of Greece and highlights the difficulties that classical taxonomy is faced with when attempting to produce divisions below the species level. These data also show that the molecular information can be used in conjunction with geological data to through light on the paleogeography of a region or on the phylogeography of a species.

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