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Phylogenetic relationships and biogeography of *Podarcis* species from the Balkan Peninsula, by bayesian and maximum likelihood analyses of mitochondrial DNA sequences

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Abstract

Wall lizards of the genus *Podarcis* (Sauria, Lacertidae) comprise 17 currently recognized species in southern Europe, where they are the predominant nonavian reptile group. The taxonomy of *Podarcis* is complex and unstable. Based on DNA sequence data, the species of *Podarcis* falls into four main groups that have substantial geographic coherence (Western island group, southwestern group, Italian group, and Balkan Peninsula group). The Balkan Peninsula species are divided into two subgroups: the subgroup of *P. taurica* (*P. taurica, P. milensis*, *P. gaigeae*, and perhaps *P. melisellensis*), and the subgroup of *P. erhardii* (*P. erhardii* and *P. peloponnesiaca*). In the present study, the question of phylogenetic relationships among the species of *Podarcis* encountered in the Balkan Peninsula was addressed using partial mtDNA sequences for cytochrome *b* (cyt *b*) and 16S rRNA (16S). The data support the monophyly of *Podarcis* and suggest that there are three phylogenetic clades: the clade A (*P. taurica*, *P. gaigeae, P. milensis*, and *P. melisellensis*); the clade B (*P. erhardii* and *P. peloponnesiaca*), and the clade C (*P. muralis* and *P. sicula*). By examining intraspecific relationships it was found that extant populations of *P. erhardii* are paraphyletic. Furthermore, subspecies previously defined on the basis of morphological characteristics do not correspond to different molecular phylogenetic clades, suggesting that their status should be reconsidered. The distinct geographic distribution of the major clades of the phylogenetic tree and its topology suggest a spatial and temporal sequence of phylogenetic separations that coincide with some major paleogeographic separations during the geological history of the Aegean Sea. The results stress the need for a reconsideration of the evolutionary history of Balkan *Podarcis* species and help overcome difficulties that classical taxonomy has encountered at both the species and subspecies level.

Keywords: *Podarcis*; Molecular phylogeny; mtDNA markers; Balkan Peninsula

1. Introduction

The reconstruction of phylogenies is of primary importance in the understating of the dynamic patterns of evolution, that is, the biogeography of a group and the bases of its biological diversity at any level. Although the phylogeny of the genus *Podarcis* has been the subject of much discussion (Carranza et al., 2004; Fu, 2000; Harris, 1999; Harris and Arnold, 1999; Harris and Sa-Sousa, 2002; Harris et al., 1998, 2002; Oliverio et al., 2000; Poulakakis et al., 2003), the relationships among the species are still unclear. Because morphology is so uniform, it provides few characters for phylogenetic analysis, and these tend to be conflicting (Arnold, 1973, 1989). Various karyological, immunological, and protein electrophoretic studies have been made, but these usually involve only a minority of species, and results from different species combinations

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are equivocal (Capula, 1994, 1996, 1997; Chondropoulos et al., 2000; Lanza and Cei, 1977; Mayer and Tiedemann, 1980, 1981; Olmo et al., 1986, 1987; Tiedemann and Mayer, 1980).

The group of wall lizards of the genus *Podarcis* (Sauria, Lacertidae) in southern Europe currently consists of 17 or 18 recognized species, depending on whether *P. carbonelli* is a distinct species (Sá-Sousa and Harris, 2002). The wall lizards are the predominant reptile group in this region, but their taxonomy is complex and continuously revised (Arnold, 2002). Recently, several works on *Podarcis* have revealed cases of hidden diversity and paraphyly, indicating the necessity of a taxonomic revision within the genus (Castilla et al., 1998a,b; Carranza et al., 2004; Harris and Sá-Sousa, 2001, 2002; Poulakakis et al., 2003; Sá-Sousa et al., 2000).

Harris and Arnold (1999) propose that the species of *Podarcis* fall into four main groups with substantial geographic coherence: (i) the Western island group (*P. filfolensis, P. pityusensis, P. tiliguerta,* and *P. lilfordi*), (ii) the southwestern group (*P. atrata, P. bocagei, P. hispanica*, and perhaps *P. carbonelli*), (iii) the Italian group (*P. muralis, P. raffonei,* and *P. sicula*), and (iv) the Balkan Peninsula group (*P. taurica, P. gaigeae, P. milensis, P. melisellensis*, and perhaps *P. peloponnesiaca, P. erhardii,* and *P. wagleriana*), although Oliverio et al. (2000) do not agree with Harris and Arnold (1999) that *P. wagleriana* is part of this clade and suggest that *P. wagleriana* and *P. filfolensis* consist of another small group (Sicily-Maltese). However, in the most comprehensive analysis using a maximum likelihood (ML) tree, which was included in the study of Harris and Arnold (1999), *P. peloponnesiaca* lies outside the Balkan group, making it paraphyletic. The same was observed by Carranza et al. (2004), where again *P. peloponnesiaca* seems to be outside the Balkan group. Only the results of Oliverio et al. (2000) support the monophyly of Balkan group, but also according to Podnar et al. (2005) some of the Oliverio et al. (2000) conclusions were questionable. The Balkan group is considered to be the less studied group within *Podarcis*, since not only the phylogenetic relationships of this group, but its monophyletic status is questionable. The only substantiated data concern the close phylogenetic relationship of *P. erhardii* to *P. peloponnesiaca*, which probably form a species complex, since *P. erhardii* seems to be paraphyletic with respect to *P. peloponnesiaca* (Poulakakis et al., 2003).

On the basis of preliminary results of a recent molecular study (Poulakakis et al., 2003), the Balkan group was divided into two subgroups, one containing *P. taurica, P. milensis,* and *P. gaigeae* and the other subgroup containing *P. erhardii* and *P. peloponnesiaca*, whose members are highly diversified and present great morphological and ecological plasticity, inhabiting many different ecotypes.

In the present study, *Podarcis* were collected from several localities of the Balkan Peninsula, and the DNA sequences were obtained from the cytochrome *b* (cyt *b*) and 16S rRNA (16S) genes to infer the phylogenetic relationships of these species. These were combined with previously published sequences to (i) examine the validity of the current taxonomy, (ii) produce a historical interpretation of the species' distribution, and (iii) evaluate alternative models of the biogeographic history of *Podarcis* in the Balkan area.

2. Materials and methods

2.1. DNA extraction, amplification, and sequencing

The number and the geographic locations of the specimens used in this study are given in Appendix A and Fig. 1. For all samples (132), voucher specimens were deposited in the Natural History Museum of Crete, Greece. Total genomic DNA was extracted from small pieces of the tail or tongue using standard methods (Sambrook et al., 1989). Two target genes were selected for molecular phylogenetic analysis: (i) a partial sequence (~400 bp) of the mitochondrial protein encoding cyt *b* and (ii) a partial sequence (~500 bp) of the non-protein coding mitochondrial 16S, using PCR conditions and primers reported elsewhere (Poulakakis et al., 2004). Sequencing was done on an ABI377 automated sequencer. For 43 individuals (40 specimens of *P. erhardii*, one specimen of *P. peloponnesiaca*, and two specimens of *P. muralis*), the cyt *b* gene region had already been published (AF486191–233) (Poulakakis et al., 2003) and therefore only the 16S from the same individuals was sequenced for this study. In addition, 11 sequences (both cyt *b* and 16S) of *P. melisellensis* (Podnar et al., 2005), one sequence of *P. taurica* (Harris et al., 1998), two sequences of *P. muralis* (Harris et al., 1998; Podnar et al., 2005), one sequence of *P. peloponnesiaca* (Beyerlein and Mayer, 1999) and two sequences of *P. sicula* (Podnar et al., 2005) were retrieved from GenBank and included in the phylogenetic analysis.

Individuals from three closely related lacertid species were used as out-group taxa: *Lacerta andreasnkyi* (cyt *b*: AF206537 and 16S: AF206603 Fu, 2000), *Gallotia stehlini* (cyt *b*: AF439949 Maca-Meyer et al., 2003; and 16S: AF149936 Beyerlein and Mayer, 1999), and *G. galloti* (cyt *b*: AF439946 and 16S: AF019651 Harris et al., 1999).

2.2. Alignment and genetic divergence

The alignment of the concatenated cyt *b* and 16S sequences was performed with Clustal X (Thompson et al., 1997) and corrected by eye. Additionally, the 16S was aligned based on its secondary structure, to facilitate proper alignments. Alignment gaps were inserted to resolve length differences between sequences, and

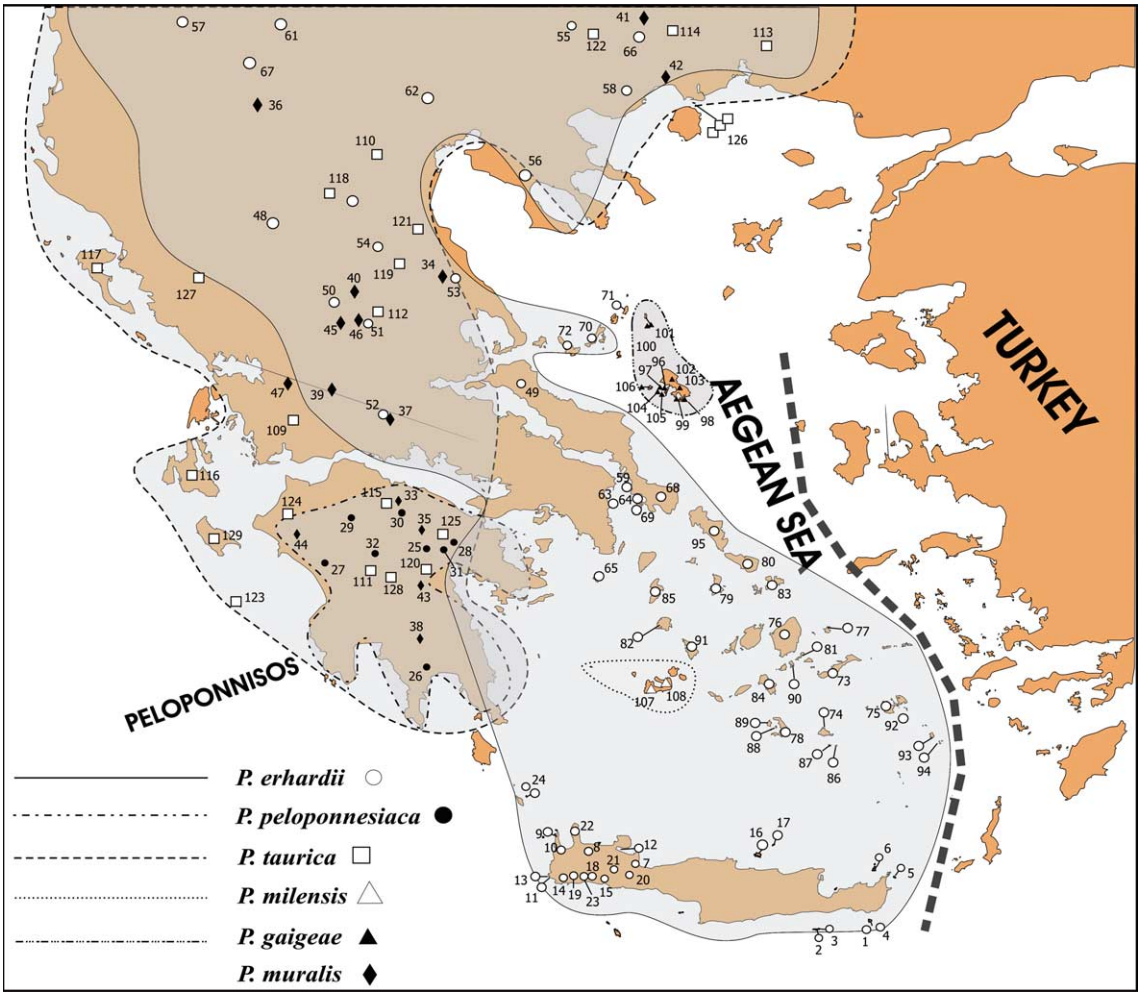

Fig. 1. Map showing the sampling localities of the 132 specimens used for the DNA analysis, which included seventy-three specimens of *P. erhardii*, eight specimens of *P. peloponnesiaca*, 23 specimens of *P. taurica*, 11 specimens of *P. gaigeae*, two specimens of *P. milensis*, 15 specimens of *P. muralis*. The dashed thick line presents the Mid Aegean trench.

positions that could not be unambiguously aligned were excluded. Cytochrome *b* sequences were translated into amino acids prior to analysis and did not show any stop codons, suggesting that all were functional. Sequence divergences were estimated using the MEGA computer package (v. 2; Kumar et al., 2001) using the Tamura–Nei model of evolution (Tamura and Nei, 1993). The alignment used is available on request from the authors. GenBank accession numbers for the sequences obtained are: AY896054–143 and AY768706–741 for cyt *b* and AY986147–242 and AY768742–777 for 16S.

2.3. Phylogenetic analyses

Phylogenetic inference analyses were conducted using ML, and Bayesian inference (BI). Nucleotides were used as discrete, unordered characters. For 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 (Huelsenbeck and Crandall, 1997) in Modeltest (v. 3.06; Posada and Crandall, 1998). Likelihood-ratio tests indicated that the general time reversible (GTR) (Rodriguez et al., 1990) model + I + G showed a significantly better fit than the other less complicated models (model parameters: Pinv = 0.4059, $a = 0.6451$; base frequencies A = 0.32, C = 0.27, G = 0.13, T = 0.28; rate matrix A/G = 3.87, C/T = 9.11). For each data set (cyt *b* and 16S), the model of evolution that showed a significantly better fit than the other were GTR + I + G (model parameters for cyt *b*: Pinv = 0.5160, a = 1.2540; base frequencies A = 0.30, C = 0.32, G = 0.10, T = 0.28; rate matrix A/G = 8.82, C/T = 10.882, and for 16S: Pinv = 0.4195, a = 0.5276; base frequencies A = 0.36, C = 0.23, G = 0.16, T = 0.25; rate matrix A/G = 4.057, C/T = 7.794). Heuristic ML searches were performed with 10 replicates of random sequence addition and TBR branch swapping using PAUP* (v.4.0b10; Swofford, 2002). Since a ML tree search with such a complex model would be NScomputationally excessive, we used a smaller data set, that included 70 sequences, and the confidence of the nodes was assessed by 100

bootstrap replicates (Felsenstein, 1985). The selection of the specimens was based on the clades of the ML tree and the genetic distances among the specimens (e.g., we used only nine of 23 specimens from the island of Crete).

Bayesian inference analysis was performed with the software MrBayes (v. 3.0B; Huelsenbeck and Ronquist, 2001) using the GTR + I + G model of substitution. The analysis was run with four chains for 10^6 generations, and the current tree was saved to file every 100 generations. This generated an output of 10^4 trees. The $-\ln L$ stabilized after approximately 10^4 generations, and the first 10^3 trees (10% "burn-in" in Bayesian terms, chain had not become stationary) were discarded as a conservative measure to avoid the possibility of including random, suboptimal trees. The percentage of samples recovering any particular clade in a BI analysis represents the clade's posterior probability (Huelsenbeck and Ronquist, 2001). We used one of the methods of Leaché and Reeder, 2002) to assure that our analyses were not trapped on local optima. In particular, the posterior probabilities for individual clades obtained from separate analyses (four runs) were compared for congruence (Huelsenbeck and Imennov, 2002), given the possibility that the analyses could appear to converge on the same ln-likelihood value while actually supporting incongruent phylogenetic trees.

2.4. Testing alternative hypotheses

Where appropriate, topological constraints were generated with MrBayes (v. 3.0B; Huelsenbeck and Ronquist, 2001) and compared with our optimal topologies using the Shimodaira–Hasegawa (SH) test that was used statistically to compare alternative phylogenetic hypotheses (Goldman et al., 2000; Shimodaira and Hasegawa, 1999) implemented in PAUP* (v. 4.0b10), and employing RELL bootstrap with 1000 replicates. The following hypotheses were tested: (i) *P. erhardii* is monophyletic, and (ii) all conventional subspecies of *P. erhardii, P. taurica,* and *P. gaigeae* are monophyletic.

2.5. Calibration of molecular clock and estimation of divergence times

Divergence times were estimated using the nonparametric rate smoothing (NPRS) method (Sanderson, 1997) with Powell algorithm, implemented in the program r8s (v. 1.7 for Apple OS X; Sanderson, 2003), which relaxes the assumption of a molecular clock. As calibration point, we used a dated paleogeographical event, the isolation of the island of Crete and the island of Pori (a small islet that lies half-way between Crete and Peloponnisos) from neighboring mainland. This event happened at 5.2 MYA (Beerli et al., 1996), after the end of the Messinian salinity crisis. Mean divergence times and confidence intervals were obtained for each node based on the procedure described in r8s manual (http://ginger.ucdavis.edu/r8s). Mean divergence times and confidence limits were obtained from ML tree.

3. Results

Of the 927 sites examined, there were 158 variable cyt *b* sites, of which 146 were parsimony informative (174 and 146, respectively, when the out-groups were included in the analysis), and 95 variable 16S sites, 86 of which were parsimony informative (160 and 114, respectively, including out-groups). For cyt *b*, in-group sequence divergence (Tamura and Nei model) ranged from 0 to 21.5%, while for 16S sequence divergence ranged between 0 and 8.91% (Table 1).

For the phylogenetic analyses, a data set of 152 combined sequences, including out-groups, were used. ML analysis under the GTR + I + G model resulted in two topologies with $\ln L = -7682.2844$ (Fig. 2), differing in a single node, that is, the relationship of the specimen of *P. taurica* from Kerkyra Island with the other specimens of the same species from continental Greece (Agrinio, Olympos, Farsala and Larisa).

For the BI analysis, identical topologies were recovered for each of the runs with the full data set, although posterior probabilities for some of the nodes differed slightly between each of the Bayesian runs. Fig. 2 shows the $4*9*10^4$ trees remaining after burn-in combined as a 50% majority-rule consensus tree.

In all analyses, three very well-supported clades of *Podarcis* were identified (Fig. 2), corresponding to different species and/or to separate geographic regions throughout the Balkan Peninsula. Clade A (78%, 0.88) consists of *P. taurica* (94%, 1.00) from the entire area studied (subclades A1, A2, and A3), *P. milensis* (95%, 1.00) from the Milos island group (subclade A4), *P. gaigeae* (94%, 1.00) from the island group of Skyros (subclade A5), and *P. melisellensis* (93%, 1.00) from Croatia (subclade A6). Clade B (75%, 0.90) consists of two distinct groups of haplotypes. All haplotypes of *P. erhardii* from Crete (subclade B5), *P. erhardii* from the island of Pori (subclade B4), and *P. pelopponnesiaca* from Peloponnisos (subclade B3), which is the southernmost part of the Greek mainland, form one distinct lineage with high bootstrap support (88%) and posterior probability (0.99). The other group (72%, 0.85) includes specimens of *P. erhardii* from the island group of Cyclades (central Aegean) (subclade B2) and the continental Greece (subclade B1) [continental area, Northern Sporades (North Aegean), and the islets of Saronikos/Evoikos gulf]. In other words, *P. erhardii* is not a monophyletic species and this was avouched by the results of the SH test, based on which the hypothesis that *P. erhardii* as a whole is a monophyletic species is rejected ($p < 0.0001$). Clade C (78%, 0.85) consists of

Table 1
Nucleotide divergences (Tamura-Nei model) of cyt *b* (below diagonal) and 16S (above diagonal) sequences among the major mtDNA clades/lineages of *Podarcis* included in the study

Groups	1	2	3	4	5	6	7	8	9	10	11	12	13
1. *P.e* – Crete (B5)	—	2.58	4.91	5.78	2.9	6.23	7.51	6.11	6.04	6.45	6.11	6.15	7.36
2. *P.e* – Pori (B4)	9.01	—	4.03	4.69	2.5	5.98	7.5	5.61	6.07	6.64	5.42	5.88	6.25
3. *P.e* – Cyclades (B2)	15.43	13.1	—	3.43	4.4	4.41	4.75	4.22	3.94	4.34	4.03	5.09	5.52
4. *P.e* – Greece (B1)	14.57	14.11	11.21	—	5.15	5.26	6.86	4.73	6.45	6.05	6.45	6.11	6.65
5. *P.p* (B3)	8.5	8.4	13.62	13.62	—	5.42	6.81	5.4	5.8	6.28	5.97	6.03	6.64
6. *P.t* – Greece (A2)	15.11	17.3	17.66	15.31	16.81	—	2.5	2.9	4.52	4.7	5.08	5.48	6.73
7. *P.t*– Ionian (A1)	16.23	17.15	16.51	15.88	16.72	7.03	—	3.4	5.1	4.98	5.33	6.61	7.76
8. *P.t* – (A3)	15.34	14.96	18.32	17.07	17.51	9.93	10.97	—	4.38	4.39	4.93	5.79	6.51
9. *P.g* (A5)	16.99	18.29	19.77	17.4	18.19	12.18	13.74	12.85	—	3.1	3.9	5.81	5.92
10. *P. mi* (A4)	16.55	16.25	17.25	15.86	16.97	11.99	12.85	12.69	11.38	—	4.1	5.95	6.58
11. *P. me* (A6)	17.77	16.96	19.2	15.42	18.76	13.02	14.97	13.83	12.36	12.16	—	4.54	5.21
12. *P. mu* (C2)	14.93	15.22	16.77	15.46	16.74	15.41	16.45	16.79	18.88	18.12	16.88	—	4.89
13. *P.s* (C1)	18.76	18.37	18.03	15.38	19.02	16.1	17.78	17.74	17.93	17.75	14.59	15.97	—

P.e, *P.erhardii*; P.p, *P. peloponnesiaca*; P.t, *P. taurica*; P.g, *P. gaigeae*; P.mi, *P. milensis*; P.me, *P. melisellensis*; P.mu, *P. muralis*; P.s, *P. sicula.*

P. muralis from Greece and the rest of Europe (subclade C2) (99%, 1.00), which is placed as the sister group to *P. sicula* (subclade C1) (93%, 1.00).

The NPRS method applied to 100 bootstrapped phylograms produced divergence times and confidence intervals (Table 2). The mean evolutionary rate calculated for the ML phylogram was 0.0064 per site per million years. According to the calibration reference point, which was the separation of Crete and Pori islands from Peloponnisos at 5.2 MYA, the diversification of *Podarcis* in the Balkan Peninsula occurred at 10.6 MYA during the Late Miocene, and the speciation within this group assemblage started approximately 8.6 MYA. These dates are very close to the ones reported by Carranza et al. (2004) for *Podarcis* species and the corresponding ages evaluated using the genetic distance method (Poulakakis et al., 2003).

4. Discussion

The results of the present study revealed a well-resolved phylogeny at the species level and identified a number of haplotype clades that, based on the observed levels of sequence divergence (Table 1), represent long-separated lineages and diverse evolutionary histories within *Podarcis*. The analyses of molecular data do not agree with the species and, especially, subspecies taxonomy of *Podarcis* in Balkan Peninsula. To understand further the evolutionary relationships between these seven species, the geographical origin (site and region) and the subspecies status of the observed haplotypes are given in Fig. 2. All phylogenetic analysis indicated that the genus is monophyletic (bootstrap value 100% and posterior probability 1.0 for ML and BI analyses, respectively), which is in agreement with previous molecular studies (Carranza et al., 2004; Harris and Arnold, 1999; Oliverio et al., 2000; Poulakakis et al., 2003).

Within the clade A, the four focal species (*P. taurica*, *P. gaigeae, P. milensis*, and *P. melisellensis*) form monophyletic groups with very high bootstrap support and posterior probabilities (Fig. 2). Within this major clade, the first that diverge from the others is *P. taurica*, while *P. gaigeae*, *P. milensis,* and *P. melisellensis* form a monophyletic group, in which *P. gaigeae* and *P. milensis* are more closely related to each other than to *P. melisellensis.* This observation agrees with previously published results (Harris and Arnold, 1999; Mayer and Tiedemann, 1980, 1981; Oliverio et al., 2000; Tiedemann and Mayer, 1980). Within the lineage of *P. taurica*, there are three groups of haplotypes: A1 corresponds to populations confined in the Ionian Islands, A2 includes populations from continental Greece and Peloponnisos, and A3 represents the haplotypes from Thasopoula Island, northwest Greece, and the eastern Balkan Peninsula.

Within the clade B, *P. erhardii* is paraphyletic with respect to *P. peloponnesiaca*, and given that the SH test provided further support of this assertion, the above results confirmed the taxonomic arrangement proposed earlier (Poulakakis et al., 2003). Harris and Sá-Sousa, 2001 and Harris, 2002) have also reported an analogous case of paraphyly in *P. hispanica* and suggested a revision of the existing taxonomy of this species. The average Tamura–Nei degree of genetic differentiation (Tamura and Nei, 1993) between *P. erhardii* from Crete and *P. erhardii* from the Cyclades Islands and continental Greece is ~15%, whereas the mean pairwise distance between *P. erhardii* from the islands of Crete and Pori and *P. peloponnesiaca* from Peloponnisos is ~8.5%. Thus, *P. erhardii* populations from the island of Crete or Pori are more closely related to the populations of *P. peloponnesiaca* than they are to conspecific populations from the Cyclades Islands or continental Greece.

This high level of genetic differentiation observed in the mtDNA data between the *P. erhardii* populations from the Crete and Pori islands and the rest of *P. erhardii*

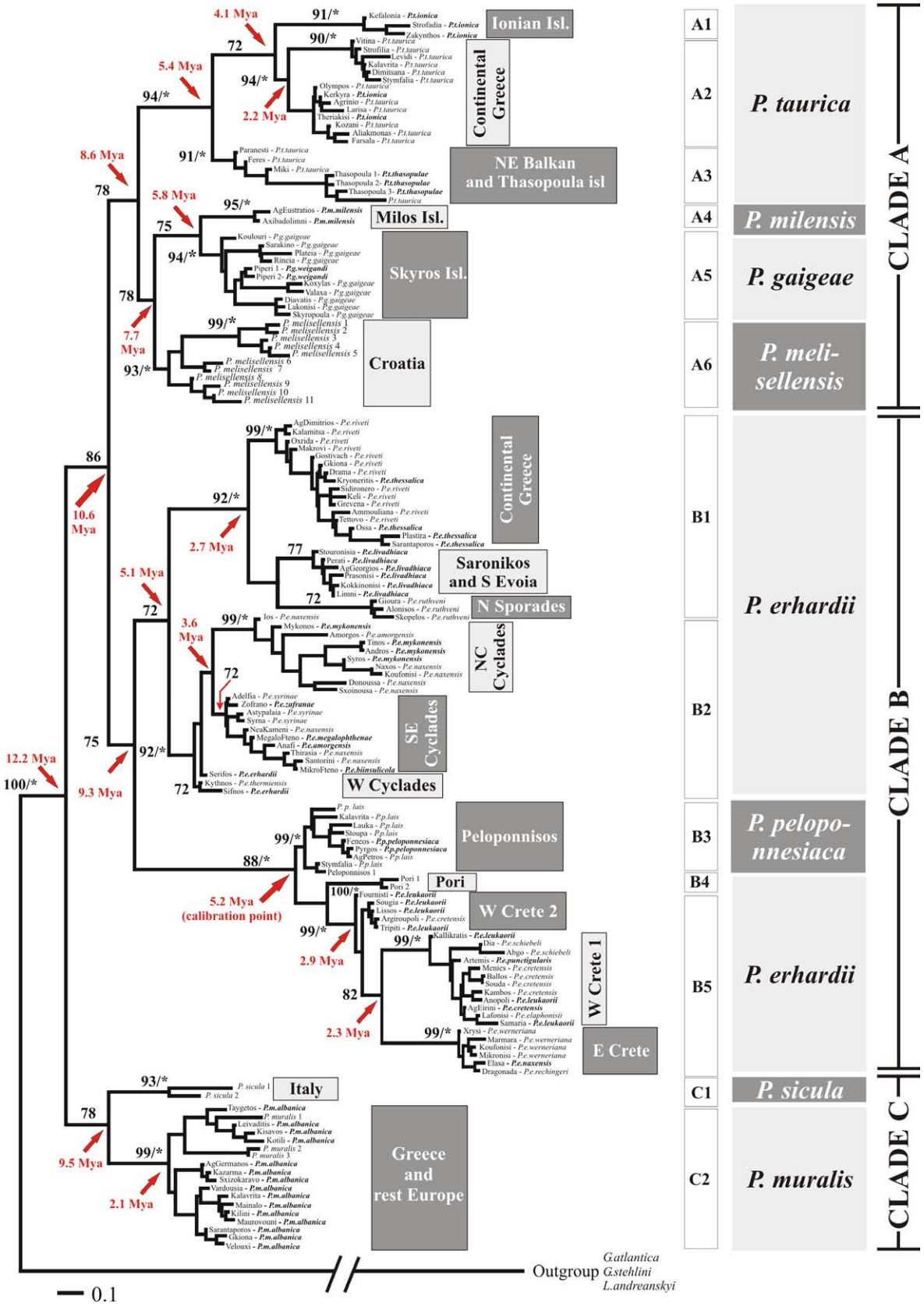

Fig. 2. Phylogenetic relationships among the seven *Podarcis* species encountered in the Balkan Peninsula. Individuals from *L. andreanskyi*, *G. stehlini* and *G. galloti,* three closely related species, were used as outgroup taxa. Phylogenetic analyses of maximum likelihood (ML) and Baynsian inference (BI) produced trees with the same topology with regard to the major lineages. Only the BI tree is presented. Numbers above branches are bootstrap values on ML greater than 50% based on 100 replicates. Asterisks indicate posterior probabilities values of BI above 95%.

Table 2
Estimated ages in MYA and the correspondent 95% confidence limits in parenthesis for selected nodes, obtained using the NPRS method (see also Fig. 2)

Separation event	Age
Clade A and B vs. Clade C	12.2 (10.3–14.1)
Clade A vs. Clade B	10.6 (8.9–12.3)
Subclades A1, A2, A3 vs. subclades A4, A5, A6	8.6 (7.2–10.0)
Subclades A6 vs. subclades A4 and A5	7.7 (5.9–9.5)
Subclade A4 vs. subclade A5	5.8 (4.9–7.7)
Subclades A1, A2 vs. subclade A3	5.4 (3.9–6.9)
Subclade A1 vs. subclade A2	4.1 (2.6–5.6)
Subclades B1, B2 vs. subclades B3, B4, B5	9.3 (7.5–11.1)
Subclade B1 vs. subclade B2	5.1 (3.4–6.8)

samples from the Cyclades Islands and continental Greece (Table 1) and its phylogenetic position (Fig. 2) raises questions regarding the inclusion of the Crete and Pori populations within *P. erhardii*, the type locality of which is Sifnos Island in the Cyclades island group. For the cyt *b* data, these divergences are higher than the average genetic distance between congeneric reptile species (13.6%; Harris, 2002). The easiest solution to avoid the paraphyly of *P. erhardii* could be the consideration of *P. peloponnesiaca* as a synonym of *P. erhardii*. However, since the scientific name of *P. peloponnesiaca* is older than that of *P. erhardii* and based on morphology, *P. peloponnesiaca* (subclade B3) is a well-defined species found in sympatry with *P. e. livadiaca* in Peloponnisos (Mayer, 1986) and accepted today as such, then *Podarcis* from Crete and Pori (subclades B4, B5) would also need to be raised to specific rank to avoid the paraphyly of *P. erhardii*. It is characteristic that several biometric characters, such as snout-vent length, a combination of pholidotic characters, the number of femoral pores, and temporal scales (Lymberakis et al. submitted) revealed significant differences between animals of the same sex among different groups (e.g., Crete, Peloponnisos, Pori, and Cyclades). However, although the differentiation of populations from Crete and Pori Islands is clear, we suggest the use of PhyloCode to avoid taxonomic confusion (Pennisi, 2001) at least until further research mainly on morphology becomes available. Thus, for the time being, we refer to wall lizards of Crete and Pori islands as *P. erhardii**.

With regard to morphological subspecies of *P. taurica, P. gaigeae*, and *P. erhardii*, it is clear that they do not represent monophyletic units and should be reevaluated in light of new evidence. Enforcing the monophyly of the subspecies, the SH test always revealed that the constraint tree was statistically worse than the best tree. More alarmingly, none of the traditionally recognized subspecies within *Podarcis* species in the Balkan Peninsula formed reciprocally monophyletic evolutionary lineages, with the exception of *P. e. livadiaca* from the Saronikos Gulf and *P. e. ruthveni* from the Northern Sporades, thus seriously question the validity of the currently designated subspecies units (Fig. 2).

4.1. Phylogeography

The paleogeographic evolution of the Hellenic region during the Tertiary is described by six paleogeographic sketches (Fig. 3),that indicate the relative positions of various paleogeographic domains during some critical periods (from 17 MYA to 0.02 MYA). Geomorphological changes in the Mediterranean region were driven by the collision of the African and Arabian plates with the Eurasian plate (Steininger and Rögl, 1984). The late Seravallian to early Tortonian (12–8 MYA) tectonic movements probably initiated the modern history of the Aegean region and the surrounding areas. These movements caused the breakup of a southern Aegean landmass. During the Messinian salinity crisis, the Mediterranean islands became mountains in a steppe or desert, so that overland migration between islands and mainland was possible. However, Crete was isolated from the Cyclades Islands and Peloponnisos by deep canyons during the Messinian salinity crisis (Schüle, 1993). Some 5.3 MYA (Krijgsman et al., 1999; Duggen et al., 2003), the Strait of Gibraltar reopened and the basin was refilled from the Atlantic Ocean in about 100 years (Blondel and Aronson, 1999). The island of Crete became permanently isolated before 5.2 MYA (Beerli et al., 1996). In the Pleistocene, all of today's islands were in the same position as present and remained completely isolated.

The genetic distances and the phylogenetic tree suggest that the evolutionary history of *P. taurica* in the Balkan Peninsula does not consist of a simple invasion occurring during the past (Fig. 4). The distribution of clade C (*P. taurica, P. gaigeae, P. milensis,* and *P. melisellensis*) mainly in the Balkan Peninsula and its absence from the rest of Europe suggest that the ancestral species of this group originated somewhere in the Balkan Peninsula and expanded to this area. This probably happened after the formation of the mid-Aegean trench (Figs. 1 and 3), which was fully completed about 10.6 MYA (Poulakakis et al., 2003). 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 of Pachia near the island of Nisyros (Valakos et al., 1999), which could be a case of recent colonization from the Cyclades. This information fits well with the divergence time estimated in this study for the beginning of the diversification of Balkan *Podarcis* species, excluding that of *P. muralis* (10.6 MYA), which do not belong to Balkan group. A historical fact, probably the arrival 9 MYA ago of the ancestral form of *P. erhardii* (Poulakakis et al., 2003) from the northwest, following the eastward path of the Dinaric Alps and the

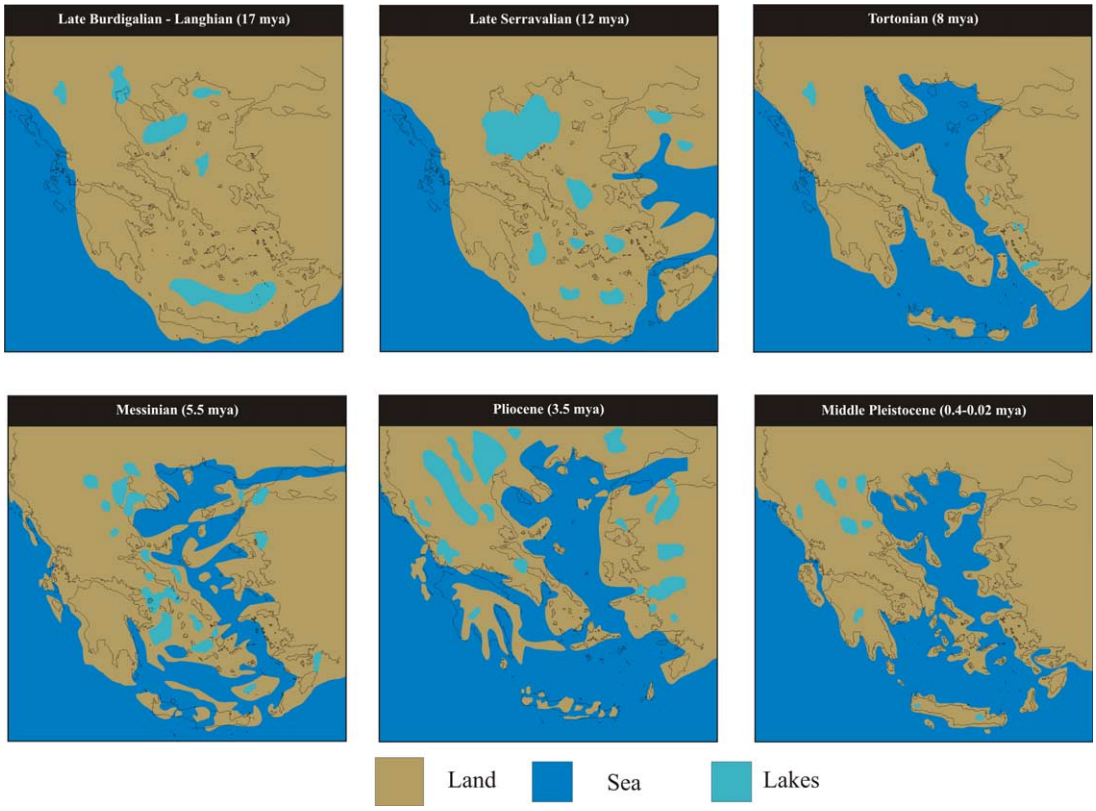

Fig. 3. Greece from the Miocene era to present (redrawn after Creutzburg, 1963; Dermitzakis and Papanikolaou, 1981; Dermitzakis, 1990). The maps were drawn based on present geography. The actual positions of landmasses have been constantly changing from the upper Serravalian, due to the fan-like, southwards, expansion of the southern Aegean region. The Aegean Sea region was part of a united landmass (known as Agäis) during the Upper and Middle Miocene (23-12 MYA). At the end of the middle Miocene (12 MYA), the forming of the Mid-Aegean trench (east of Crete and west of Kasos-Karpathos) (see also Fig. 1) began and was fully completed during the early late Miocene (10.6 MYA). In the Messinian (latest Miocene, 5.96 MYA) the entire Mediterranean basin dried up, as a result of the closing of the Strait of Gibraltar (Krijgsman et al., 1999; Duggen et al., 2003).

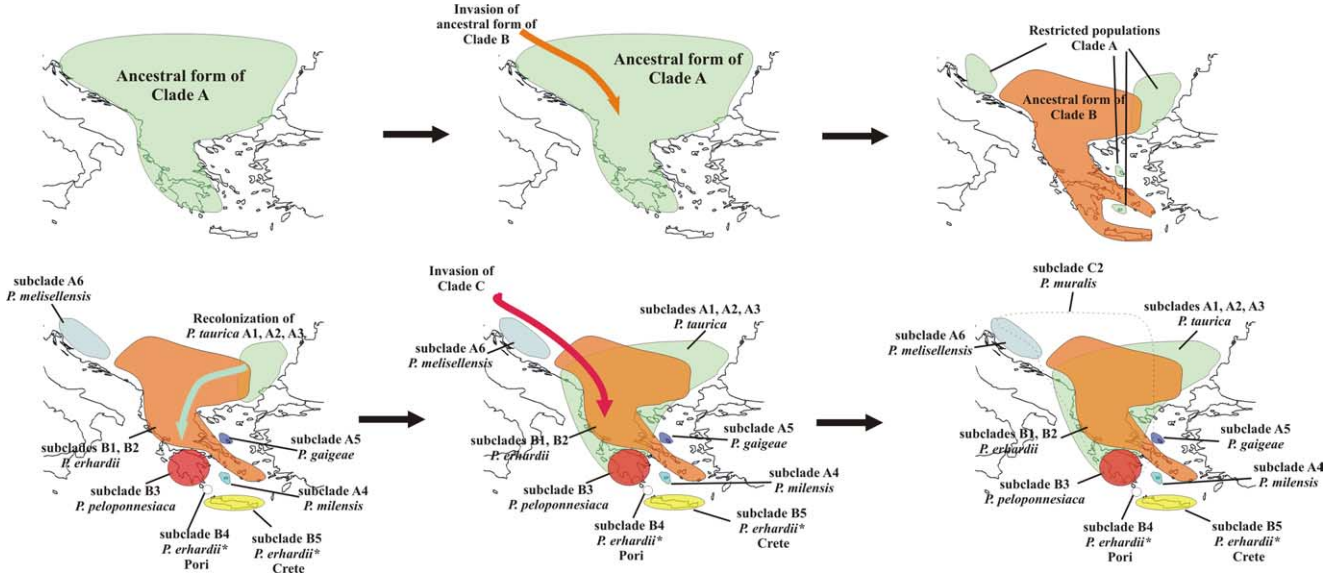

Fig. 4. The hypothetical biogeographic scenario of *Podarcis* species in the Balkan Peninsula.

Hellenides, led to the restriction of the distribution of the ancestral form of *P. taurica* subgroup in few small populations.

One of the above-mentioned population restrictions occurred, perhaps, near the Dalmatian coast and produced the species recognized today as *P. melisellensis*.

Two other populations, the first in the southeast and the second in the central-east part of Greece (corresponding to the area of Milos and Skyros island groups), produced the taxa recognized today as *P. milensis* and *P. gaigeae*, respectively. Concerning the fourth species of this clade (*P. taurica*), the topology of phylogenetic tree and the corresponding genetic distances of the subclades of this species (A1, A2, and A3) suggest the remaining one population in northeast Greece or the northeast part of Balkan Peninsula (clade A3), which, when the situation from the arrival of the ancestral form of *P. erhardii* was stabilized, recolonized the area of Greece, producing subclades A2 (continental Greece) and A1, the Ionian islands (except Kerkyra) that based on the paleogeographic maps was a paleogeographic unit (Dermitzakis, 1990). From this evidence, and given that the morphological and molecular data suggest that all *P. taurica* specimens consist of a clade, it appears that colonization of Greece came from the hypothetical ancestral population of *P. taurica* of northeast Greece or the Balkan Peninsula before 5.4 MY, following the dispersal route mentioned above, in which the latter population gave the haplotypes group of the Ionian islands (A2) before 4.1 MY.

The distribution of *P. erhardii*, which is rare in the Adriatic and Ionian coasts, suggests that the ancestral species descended to Greece from the northwest, following the eastward path the Dinaric Alps and the Hellenides (Fig. 4). This probably happened before ~9 MYA, as mentioned above. During this time, southern Greece was made of two large peninsulas (Fig. 3), 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). This geological information fits well with the molecular phylogeny of *Podarcis* (Fig. 2), assuming that the first group evolved in the area that produced the present complex of *P. erhardii* of continental Greece and Cyclades Islands and subsequently differentiated into subclades B1 (continental Greece, Northern Sporades, Saronikos/Evoikos islets) and B2 (Cyclades island group). The second group evolved in the area of Peloponnisos and Crete Island, when these regions were united into one landmass. After the splitting of the island of Crete from Peloponnisos before 5.2 MYA, this lineage produced the taxon recognized today as *P. peloponnesiaca* in Peloponnisos, whereas in Crete and its satellite islands, and Pori Island produced the taxon recognized as *P. erhardii** (present study). It should be noted that the ages 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 data (Table 2).

Harris and Arnold, 1999) reported that *P. muralis* is distributed in southern, western, and central Europe, but it is morphologically very uniform over most of this area except Italy, where it exhibits more diversity. Genetic variability estimated using allozyme electrophoresis also suggested that genetic diversity in populations in Italy is much higher than in Spain or Austria (Capula, 1997). This observations and the fact that the apparent close relatives *P. muralis* and *P. sicula* have their primer range in Italy suggest that *P. muralis* arose in Italy and then spread to the other areas quite recently. The genetic distance between the specimens of *P. muralis* from the Balkan area and the rest of Europe (Austria, Italy, Spain, and France) is low (3.8% for cyt *b* and 1.3% for 16S). The estimated divergence time for this species is 2.2 MYA (Fig. 2). This is in agreement with the opinion of Harris and Arnold, 1999), and we believed that the last species that reached Balkan area was *P. muralis* from Italy, following the eastward path of the Dinaric Alps and the Hellenides (Fig. 4).

As a whole, the examination of mtDNA lineages in the lizards of the genus *Podarcis* may contribute substantially to the refining of its taxonomic status. Phylogenetic information can now be added to the knowledge of their morphology and distribution, producing a more accurate taxonomy for this group. The present results also confirm that the molecular information in conjunction with geological data can be used to resolve questions about the paleogeography of a region or the phylogeography of a species.

Acknowledgments

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Appendix A

List of the specimens of *Podarcis* used in molecular analyses

Code	*Species*	Locality	Museum No.	Acc. No. Cyt *b*	Acc. No. 16S
1	*P. e. werneriana*	Crete isl. (Koufonisi isl.)	NHMC 80.3.51.327	Poulakakis et al., 2003	AY896147
2	*P. e. werneriana*	Crete isl. (Xrysi isl.)	NHMC 80.3.51.260	Poulakakis et al., 2003	AY896148
3	*P. e. werneriana*	Crete isl. (Mikronisi isl.)	NHMC 80.3.51.100	AY896054	AY896149
4	*P. e. werneriana*	Crete isl. (Marmara isl.)	NHMC 80.3.51.277	Poulakakis et al., 2003	AY896150
5	*P. e. naxensis*	Crete isl. (Elasa isl.)	NHMC 80.3.51.198	Poulakakis et al., 2003	AY896151
6	*P. e. rechingeri*	Crete isl. (Dragonada isl.)	NHMC 80.3.51.241	Poulakakis et al., 2003	AY896152
7	*P. e. cretensis*	Crete isl. (Argyroulopi)	NHMC 80.3.51.1	Poulakakis et al., 2003	AY896153
8	*P. e. cretensis*	Crete isl. (Kambos)	NHMC 80.3.51.5	Poulakakis et al., 2003	AY896154
9	*P. e. cretensis*	Crete isl. (Ballos)	NHMC 80.3.51.501	Poulakakis et al., 2003	AY896155
10	*P. e. cretensis*	Crete isl. (A. Eirini)	NHMC 80.3.51.504	Poulakakis et al., 2003	AY896156
11	*P. e. elaphonisii*	Crete isl. (Lafonisi isl.)	NHMC 80.3.51.537	Poulakakis et al., 2003	AY896157
12	*P. e. cretensis*	Crete isl. (Souda)	NHMC 80.3.51.542	Poulakakis et al., 2003	AY896158
13	*P. e. punctigularis*	Crete isl. (Artemis isl.)	NHMC 80.3.51.515	Poulakakis et al., 2003	AY896159
14	*P. e. leukaorii*	Crete isl. (Sougia)	NHMC 80.3.51.13	Poulakakis et al., 2003	AY896160
15	*P. e. leukaorii*	Crete isl. (Samaria)	NHMC 80.3.51.177	Poulakakis et al., 2003	AY896161
16	*P. e. schiebeli*	Crete isl. (Dia isl.)	NHMC 80.3.51.237	Poulakakis et al., 2003	AY896162
17	*P. e. schiebeli*	Crete isl. (Avgo isl.)	NHMC 80.3.51.663	AY896055	AY896163
18	*P. e. leukaorii*	Crete isl. (Tripiti)	NHMC 80.3.51.284	Poulakakis et al., 2003	AY896164
19	*P. e. leukaorii*	Crete isl. (Fournisti)	NHMC 80.3.51.567	AY896056	AY896165
20	*P. e. leukaorii*	Crete isl. (Kallikratis)	NHMC 80.3.51.291	Poulakakis et al., 2003	AY896166
21	*P. e. leukaorii*	Crete isl. (Anopoli)	NHMC 80.3.51.310	AY896057	AY896167
22	*P. e. cretensis*	Crete isl. (Menies)	NHMC 80.3.51.318	Poulakakis et al., 2003	AY896168
23	*P. e. leukaorii*	Crete isl. (Lissos)	NHMC 80.3.51.545	Poulakakis et al., 2003	AY896169
24	*P. erhardii*	Pori isl. (1 and 2)	NHMC 80.3.51.279 and 284	Poulakakis et al., 2003	AY896170–71
25	*P. p. lais*	Peloponnisos (Stymfalia)	NHMC 80.3.54.9	Poulakakis et al., 2003	AY896172
26	*P. p. peloponnesiaca*	Peloponnisos (Feneos)	NHMC 80.3.54.29	AY896116	AY896173
27	*P. p. peloponnesiaca*	Peloponnisos (Pyrgos)	NHMC 80.3.54.25	AY896117	AY896174
28	*P. p. lais*	Peloponnisos (Ag. Petros)	NHMC 80.3.54.26	AY896118	AY896175
29	*P. peloponnesiaca*	Peloponnisos 1	NHMC 80.3.54.27	AY896119	AY896176
30	*P. p. lais*	Peloponnisos (Kalavrita)	NHMC 80.3.54.2	AY896121	AY896177
31	*P. p. lais*	Peloponnisos (Lauka)	NHMC 80.3.54.19	AY896122	AY896178
32	*P. p. lais*	Peloponnisos (Stoupa)	NHMC 80.3.54.7	AY896124	AY896179
33	*P. m. albanica*	Peloponnisos (Kalavrita)	NHMC 80.3.53.21	Poulakakis et al., 2003	AY896180
34	*P. m. albanica*	Thessalia (Kisavos)	NHMC 80.3.53.45	Poulakakis et al., 2003	AY896181
35	*P. m. albanica*	Peloponnisos (Kilini)	NHMC 80.3.53.118	AY896125	AY896182
36	*P. m. albanica*	Makedonia (Ag. Germanos)	NHMC 80.3.53.4	AY896126	AY896183
37	*P. m. albanica*	Sterea Ellada (Gkiona)	NHMC 80.3.53.123	AY896128	AY896184
38	*P. m. albanica*	Peloponnisos (Taygetos)	NHMC 80.3.53.125	AY896130	AY896185
39	*P. m. albanica*	Sterea Ellada (Vardousia)	NHMC 80.3.53.131	AY896132	AY896186
40	*P. m. albanica*	Thessalia (Kazarma)	NHMC 80.3.53.79	AY896134	AY896187
41	*P. m. albanica*	Xanthi (Kotili)	NHMC 80.3.53.28	AY896135	AY896188
42	*P. m. albanica*	Xanthi (Leivaditis)	NHMC 80.3.53.115	AY896136	AY896189
43	*P. m. albanica*	Peloponnisos (Mainalo)	NHMC 80.3.53.144	AY896137	AY896190
44	*P. m. albanica*	Peloponnisos (Mavrovouni)	NHMC 80.3.53.146	AY896138	AY896191
45	*P. m. albanica*	Thessalia (Sxizokaravo)	NHMC 80.3.53.84	AY896139	AY896192
46	*P. m. albanica*	Thessalia (Sarantaporo)	NHMC 80.3.53.109	AY896143	AY896193
47	*P. m. albanica*	Sterea Ellada (Velouxi)	NHMC 80.3.53.116	AY896141	AY896194
48	*P. e. riveti*	Makedonia (Grevena)	NHMC 80.3.51.793	AY896059	AY896195
49	*P. e. thessalica*	Evoia (Kryoneritis)	NHMC 80.3.51.555	AY896060	AY896196
50	*P. e. thessalica*	Thessalia (Plastira)	NHMC 80.3.51.573	AY896061	AY896197
51	*P. e. thessalica*	Thessalia (Sarantaporos)	NHMC 80.3.51.583	AY896062	AY896198
52	*P. e. riveti*	Sterea Ellada (Gkiona)	NHMC 80.3.51.552	AY896064	AY896199
53	*P. e. thessalica*	Thessalia (Ossa)	NHMC 80.3.51.544	AY896065	AY896200
54	*P. e. riveti*	Thessalia (Kalamitsa)	NHMC 80.3.51.574	AY896067	AY896201
55	*P. e. riveti*	Makedonia (Sidironero)	NHMC 80.3.51.776	AY896087	AY896202
56	*P. e. riveti*	Makedonia (Ag. Dimitrios)	NHMC 80.3.51.761	AY896069	AY896203
57	*P. e. riveti*	Serbia (Gostivach)	NHMC 80.3.51.760	AY896070	AY896204
58	*P. e. riveti*	Makedonia (Keli)	NHMC 80.3.51.764	AY896071	AY896205
59	*P. e. livadiaca*	Evoikos (Kokinonisi isl.)	NHMC 80.3.51.770	AY896072	AY896206
60	*P. e. riveti*	Serbia (Makrovi)	NHMC 80.3.51.763	AY896073	AY896207
61	*P. e. riveti*	Skopje (Tettovo)	NHMC 80.3.51.759	AY896075	AY896208

Appendix A. (*continued*)

Code	*Species*	Locality	Museum No.	Acc. No. Cyt *b*	Acc. No. 16S
62	*P. e. riveti*	Makedonia (Ammouliana)	NHMC 80.3.51.765	AY896076	AY896209
63	*P. e. livadiaca*	Evoikos (Perati isl.)	NHMC 80.3.51.768	AY896077	AY896210
64	*P. e. livadiaca*	Evoikos (Prasonisi isl.)	NHMC 80.3.51.767	AY896078	AY896211
65	*P. e. livadiaca*	Saronikos (Ag. Georgios isl.)	NHMC 80.3.51.693	AY896080	AY896212
66	*P. e. riveti*	Makedonia (Drama)	NHMC 80.3.51.686	AY896081	AY896213
67	*P. e. riveti*	Skopje (Oxrida)	NHMC 80.3.51.766	AY896083	AY896214
68	*P. e. livadiaca*	Evoia (Limni)	NHMC 80.3.51.772	AY896085	AY896215
69	*P. e. livadiaca*	Evoikos (Stouronisia isl.)	NHMC 80.3.51.775	AY896090	AY896216
70	*P. e. ruthveni*	N Sporades (Alonisos isl.)	NHMC 80.3.51.783	AY896091	AY896217
71	*P. e. ruthveni*	N Sporades (Gioura isl.)	NHMC 80.3.51.684	AY896092	AY896218
72	*P. e. ruthveni*	N Sporades (Skopelos isl.)	NHMC 80.3.51.328	Poulakakis et al., 2003	AY896219
73	*P. e. amorgensis*	Cyclades (Amorgos isl.)	NHMC 80.3.51.329	Poulakakis et al., 2003	AY896220
74	*P. e. amorgensis*	Cyclades (Anafi isl.)	NHMC 80.3.51.240	Poulakakis et al., 2003	AY896221
75	*P. e. syrinae*	Cyclades (Astypalaia isl.)	NHMC 80.3.51.312	Poulakakis et al., 2003	AY896222
76	*P. e. naxensis*	Cyclades (Naxos isl.)	NHMC 80.3.51.313	Poulakakis et al., 2003	AY896223
77	*P. e. naxensis*	Cyclades (Donousa isl.)	NHMC 80.3.51.315	Poulakakis et al., 2003	AY896224
78	*P. e. naxensis*	Cyclades (Santorini isl.)	NHMC 80.3.51.227	Poulakakis et al., 2003	AY896225
79	*P. e. mykonensis*	Cyclades (Syros isl.)	NHMC 80.3.51.390	AY896093	AY896226
80	*P. e. mykonensis*	Cyclades (Tinos isl.)	NHMC 80.3.51.717	AY896094	AY896227
81	*P. e. naxensis*	Cyclades (Koufonisi isl.)	NHMC 80.3.51.698	AY896096	AY896228
82	*P. e. erhardii*	Cyclades (Serifos isl.)	NHMC 80.3.51.531	AY896098	AY896229
83	*P. e. mykonensis*	Cyclades (Mykonos isl.)	NHMC 80.3.51.550	AY896099	AY896230
84	*P. e. naxensis*	Cyclades (Ios isl.)	NHMC 80.3.51.548	AY896100	AY896231
85	*P. e. thermiensis*	Cyclades (Kythnos isl.)	NHMC 80.3.51.546	AY896102	AY896232
86	*P. e. megalophthenae*	Cyclades (Megalo Fteno isl.)	NHMC 80.3.51.593	AY896103	AY896233
87	*P. e. biinsulicola*	Cyclades (Mikro Fteno isl.)	NHMC 80.3.51.607	AY896104	AY896234
88	*P. e. naxensi*	Cyclades (Nea Kameni isl.)	NHMC 80.3.51.633	AY896105	AY896235
89	*P. e. naxensi*	Cyclades (Thirasia isl.)	NHMC 80.3.51.644	AY896106	AY896236
90	*P. e. naxensis*	Cyclades (Sxoinousa isl.)	NHMC 80.3.51.589	AY896107	AY896237
91	*P. e. erhardii*	Cyclades (Sifnos isl.)	NHMC 80.3.51.649	AY896108	AY896238
92	*P. e. syrinae*	Cyclades (Dio Adelfia isl.)	NHMC 80.3.51.661	AY896110	AY896239
93	*P. e. syrinae*	Cyclades (Syrna isl.)	NHMC 80.3.51.688	AY896113	AY896240
94	*P. e. zafranae*	Cyclades (Zofrano isl.)	NHMC 80.3.51.689	AY896114	AY896241
95	*P. e. mykonensis*	Cyclades (Andros isl.)	NHMC 80.3.51.672	AY896115	AY896242
96	*P. g. gaigeae*	Skyros isl. (Koulouri isl.)	NHMC 80.3.56.29	Poulakakis et al., 2005	AY768766
97	*P. g. gaigeae*	Skyros isl. (Sarakino isl.)	NHMC 80.3.56.14	Poulakakis et al., 2005	AY768773
98	*P. g. gaigeae*	Skyros isl. (Plateia isl.)	NHMC 80.3.56.15	Poulakakis et al., 2005	AY768771
99	*P. g. gaigeae*	Skyros isl. (Rineia isl.)	NHMC 80.3.56.16	Poulakakis et al., 2005	AY768772
100	*P. g. weigandi*	Skyros isl. (Piperi isl.)	NHMC 80.3.56.37	Poulakakis et al., 2005	AY768769
101	*P. g. weigandi*	Skyros isl. (Piperi isl.)	NHMC 80.3.56.38	Poulakakis et al., 2005	AY768770
102	*P. g. gaigeae*	Skyros isl. (Koxylas)	NHMC 80.3.56.34	Poulakakis et al., 2005	AY768767
103	*P. g. gaigeae*	Skyros isl. (Valaxa)	NHMC 80.3.56.1	Poulakakis et al., 2005	AY768775
104	*P. g. gaigeae*	Skyros isl. (Diavatis isl.)	NHMC 80.3.56.11	Poulakakis et al., 2005	AY768765
105	*P. g. gaigeae*	Skyros isl. (Lakonisi isl.)	NHMC 80.3.56.25	Poulakakis et al., 2005	AY768768
106	*P. g. gaigeae*	Skyros isl. (Skyropoula isl.)	NHMC 80.3.56.22	Poulakakis et al., 2005	AY768774
107	*P. m. milensis*	Milos isl. (Ag. Eustratios.)	NHMC 80.3.52.3	Poulakakis et al., 2005	AY768776
108	*P. m. milensis*	Milos isl. (Axibadolimni.)	NHMC 80.3.52.2	Poulakakis et al., 2005	AY768777
109	*P. t. taurica*	Sterea Ellada (Agrinio)	NHMC 80.3.50.15	Poulakakis et al., 2005	AY768742
110	*P. t. taurica*	Makedonia (Aliakmonas)	NHMC 80.3.50.16	Poulakakis et al., 2005	AY768743
111	*P. t. taurica*	Peloponnisos (Dimitsana)	NHMC 80.3.50.17	Poulakakis et al., 2005	AY768744
112	*P. t. taurica*	Thessalia (Farsala)	NHMC 80.3.50.31	Poulakakis et al., 2005	AY768745
113	*P. t. taurica*	Xanthi (Feres)	NHMC 80.3.50.34	Poulakakis et al., 2005	Y768746
114	*P. t. taurica*	Makedonia (Miki)	NHMC 80.3.50.35	Poulakakis et al., 2005	AY768747
115	*P. t. taurica*	Peloponnisos (Kalavrita)	NHMC 80.3.50.18	Poulakakis et al., 2005	AY768748
116	*P. t. ionica*	Kefalonia isl.	NHMC 80.3.50.26	Poulakakis et al., 2005	AY768749
117	*P. t. ionica*	Kerkyra isl.	NHMC 80.3.50.22	Poulakakis et al., 2005	AY768750
118	*P. t. taurica*	Makedonia (Kozani)	NHMC 80.3.50.27	Poulakakis et al., 2005	AY768751
119	*P. t. taurica*	Thessalia (Larisa)	NHMC 80.3.50.29	Poulakakis et al., 2005	AY768752
120	*P. t. taurica*	Peloponnisos (Levidi)	NHMC 80.3.50.23	Poulakakis et al., 2005	AY768753
121	*P. t. taurica*	Thessalia (Olympos)	NHMC 80.3.50.19	Poulakakis et al., 2005	AY768754
122	*P. t. taurica*	Makedonia (Paranesti)	NHMC 80.3.50.37	Poulakakis et al., 2005	AY768755
123	*P. t. ionica*	Strofadia isl.	NHMC 80.3.50.3	Poulakakis et al., 2005	AY768756

(*continued on next page*)

Appendix A. (*continued*)

Code	*Species*	Locality	Museum No.	Acc. No. Cyt *b*	Acc. No. 16S
124	*P. t. taurica*	Peloponnisos (Strofilia)	NHMC 80.3.50.1	Poulakakis et al., 2005	AY768757
125	*P. t. taurica*	Peloponnisos (Stymfalia)	NHMC 80.3.50.24	Poulakakis et al., 2005	AY768758
126	*P. t. thasopoulae*	Thassopoula isl. (1, 2, 3)	NHMC 80.3.50.33, 36, 32	Poulakakis et al., 2005	AY768759–61
127	*P. t. ionica*	Ipeiros (Theriakisi)	NHMC 80.3.50.2	Poulakakis et al., 2005	AY768762
128	*P. t. taurica*	Peloponnisos (Vitina)	NHMC 80.3.50.21	Poulakakis et al., 2005	AY768763
129	*P. t. ionica*	Zakynthos isl.	NHMC 80.3.50.20	Poulakakis et al., 2005	AY768764

Map code, species name, samples localities, and GenBank accession numbers of sequence data in our analysis.

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