Molecular Phylogenetics and Evolution 138 (2019) 193–204

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Multigene phylogeny, phylogeography and population structure of *Podarcis cretensis* species group in south Balkans

Loukia Spilani[a,b,1], Katia Bougiouri[a,b,1], Aglaia Antoniou[c,1], Nikolaos Psonis[a,b,d], Dimitris Poursanidis[e], Petros Lymberakis[a], Nikos Poulakakis[a,b,*]

[a] Natural History Museum of Crete, School of Sciences and Engineering, University of Crete, Knosos Avenue, Irakleio, GR71409, Greece
[b] Department of Biology, School of Sciences and Engineering, University of Crete, Vassilika Vouton, Irakleio, GR70013, Greece
[c] Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Gournes Pediados, Irakleio, P.O. Box 2214, GR71003, Greece
[d] Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology – Hellas, N. Plastira 100, Vassilika Vouton, Irakleio, GR70013, Greece
[e] Institute of Applied and Computational Mathematics, Foundation for Research and Technology - Hellas, N. Plastira 100, Vassilika Vouton, Irakleio, GR70013, Greece

ARTICLE INFO

Keywords:
Phylogeny
Phylogeography
Population structure
Species delimitation
Species distribution modeling
Wall-lizards

ABSTRACT

The evolutionary history of taxa with limited overseas dispersal abilities is considered to be majorly influenced by vicariant events constituting them as model organisms for the interpretation of evolutionary processes. An excellent candidate are the wall lizards of the genus *Podarcis* exhibiting an impressive level of genetic and morphological diversification and harboring several cases of recently discovered cryptic diversity. In this study, we investigated the effect of palaeogeographic events on the wall lizards' biodiversity patterns in the Aegean (Greece) as well as the evolutionary processes that acted both in space and time. To accomplish that we studied a group of three endemic *Podarcis* species (*i.e.*, *P. cretensis*, *P. levendis*, and *P. peloponnesiacus*) both at the intra and interspecific levels employing mitochondrial and nuclear DNA sequence data as well as microsatellites. Furthermore, presence information coupled with bioclimatic data (*i.e.,* species distribution modeling, and niche similarity analyses) shed light on the necessary ecological factors for the species' occurrence. These approaches revealed yet another case of cryptic diversity for this group of lizards, with the existence of two slightly overlapping lineages within *P. peloponnesiacus* and highly structured populations within *P. cretensis*. Species diversification occurred during the Pliocene with *P. peloponnesiacus* divergence into the two lineages dating back to 1.86 Mya. Furthermore, temperature and precipitation related environmental parameters were the most important ones regarding the current distribution of the studied species. Based on the results, we propose a more detailed phylogeographic scenario where both the paleogeography of the area and several environmental parameters have shaped the genetic diversity and the current distribution pattern of this species group.

1. Introduction

Lying at the crossroads of three continents (Europe, Asia and Africa), the Aegean archipelago has served as a biogeographic meeting point for species of varying origins (Lymberakis and Poulakakis, 2010). It has attracted the interest of many evolutionary biologists due to its characterization as a biodiversity hotspot, its complex geological and climatic history since the late Tertiary and the often exceptionally high percentage of endemism (Bittkau and Comes, 2005; Lymberakis and Poulakakis, 2010; Poulakakis et al., 2015). Insular systems are among the major "manufacturers" of biodiversity, especially when they present high levels of spatial, temporal and habitat heterogeneity (Drake et al., 2002). This is due to a combination of factors affecting insular species and communities, such as increased isolation, relatively small population sizes and increased susceptibility to habitat alteration.

One particularly interesting and diverse group of lacertid lizards in the Aegean is the genus *Podarcis* Wagler, 1830, commonly known as wall lizards. This genus is one of the most widespread reptile groups in the Mediterranean and comprises 23 currently recognized species (Uetz et al., 2018), with several cases of cryptic diversity that have led to a significant increase of its species number (seven new species since 2000) (Harris et al., 2002; Kaliontzopoulou et al., 2008, 2011; Lima et al., 2009; Lymberakis et al., 2008; Pinho et al., 2006, 2007, 2008; Psonis et al., 2017a; Salvi et al., 2017).

Among the four distinct groups in which the genus has been divided (*i.e.,* Western island group, southwestern group, Italian group and

* Corresponding author at: Department of Biology, School of Sciences and Engineering, University of Crete, Vassilika Vouton, Irakleio, GR70013, Greece.
E-mail address: poulakakis@nhmc.uoc.gr (N. Poulakakis).
[1] Equal contribution.

https://doi.org/10.1016/j.ympev.2019.05.026
Received 23 April 2018; Received in revised form 21 May 2019; Accepted 22 May 2019
Available online 23 May 2019

Balkan Peninsula group) (Harris and Arnold, 1999), the *Podarcis* species of the Balkan peninsula are further divided into two subgroups, the subgroup of *P. tauricus* and the subgroup of *P. erhardii* (Poulakakis et al., 2005b; Psonis et al., 2017b). Previous studies on the *P. erhardii* subgroup (Poulakakis et al., 2003, 2005b), indicated a paraphyletic relationship of *P. erhardii* (Bedriaga, 1882) with respect to *P. peloponnesiacus* (Bibron & Bory, 1833), leading to the description of two new species (Lymberakis et al., 2008) - *P. cretensis* (Wettstein, 1952) and *P. levendis* Lymberakis, Poulakakis, Kaliontzopoulou, Valakos, & Mylonas, 2008 - which were formerly considered as lineages of *P. erhardii*. From a phylogeographic point of view, it has been hypothesized that the ancestor of the *P. erhardii* species group colonized the Balkan area during the Miocene and subsequently diverged into the present-day species during the Messinian salinity crisis (Krijgsman et al., 1999) at the end of Miocene (Poulakakis et al., 2005a, 2005b) due to a series of vicariant events. It was suggested that the ancestor of the three focal species of the present study *i.e. P. cretensis, P. peloponesiacus* and *P. levendis* (hereinafter called "*P. cretensis* species group") was once distributed in the area of Peloponnisos, the island group of Pori and Crete, when these regions were united into one landmass. A series of vicariant events, namely the separation of Crete and the island group of Pori from Peloponnisos around 5–5.5 Mya, led to the diversification of the three distinct lineages which correspond to the taxa recognized today as *P. cretensis, P. levendis* and *P. peloponnesiacus*. *P. cretensis* species group form a monophyletic group which has not been thoroughly studied. All three species are endemic to the southern Aegean region: (i) *P. cretensis* is distributed in the western part of Crete and its eastern islets while it is absent from the central and eastern part of mainland Crete, (ii) *P. peloponnesiacus* is distributed in Peloponnisos, and (iii) *P. levendis* is found only on the two islets of Pori and Poreti (hereafter referred to as the island group of Pori), located between the islands of Kythira and Antikythira (Fig. 1).

The paleogeographic evolution of the southern Aegean region during the Tertiary is characterized by intense geotectonic, climatic and eustatic events that isolated land masses and created barriers for species with poor overseas dispersal abilities (also see Poulakakis et al. 2015). The permanent isolation of Crete from Peloponnisos at 5–5.5 Mya, after the refilling of the Mediterranean Sea at the end of Messinian Salinity Crisis (MSC) (Krijgsman et al., 1999), has been supported by studies based on the faunal evolution and the paleogeography of the island from the Miocene until the Plio-Pleistocene (Dermitzakis, 1990a, b). Specifically, during the Miocene, mammalian carnivores were present on Crete *i.e.,* the island had a typical mainland/balanced fauna [as defined in Van der Geer et al. (2011)], whereas in the Plio-Pleistocene its fauna shifted to a distinct type *i.e.*, typical insular/unbalanced fauna [as defined in (Van der Geer et al., 2011)], which differed significantly from the nearby mainland faunal assemblages (Dermitzakis, 1990b). This shift in faunal composition highly indicates a barrier between the two land masses. During the Pliocene, Crete was mostly submerged in the eastern part and consisted of small paleo-islands in the west. This continued until the early Pleistocene when Crete began to uprise, taking its final form (Dermitzakis, 1990b). In Quaternary, all present islands were in 'approximately' the same position as today and Crete remained isolated. Although in glacial maxima, the sea level was 200 m lower than today (Beerli et al., 1996), Crete remained isolated from Cyclades and Peloponnisos, since the Cretan sea is much deeper (Schule, 1993). Therefore, the isolation of Crete from the mainland at 5–5.5 Mya and the fact that reptiles have limited sea dispersal abilities, support this geological event as a reliable calibration point. Additionally, the transition between cold temperate and dry tropical climate as we know today only began to appear during the late Pliocene, about 3.2 Mya, as part of a global cooling trend (Blondel et al., 2010), and about 2.8 Mya today's prevailing climate became established throughout the region. Since then, the contrast between the alternating hot, dry and cold wet seasons has intensified steadily up to the present day.

The aim of this study is to further elucidate the evolutionary history of an exemplary group of species in southern Balkans (*P. cretensis* species group), focusing on how the complex geoclimatic events occurring

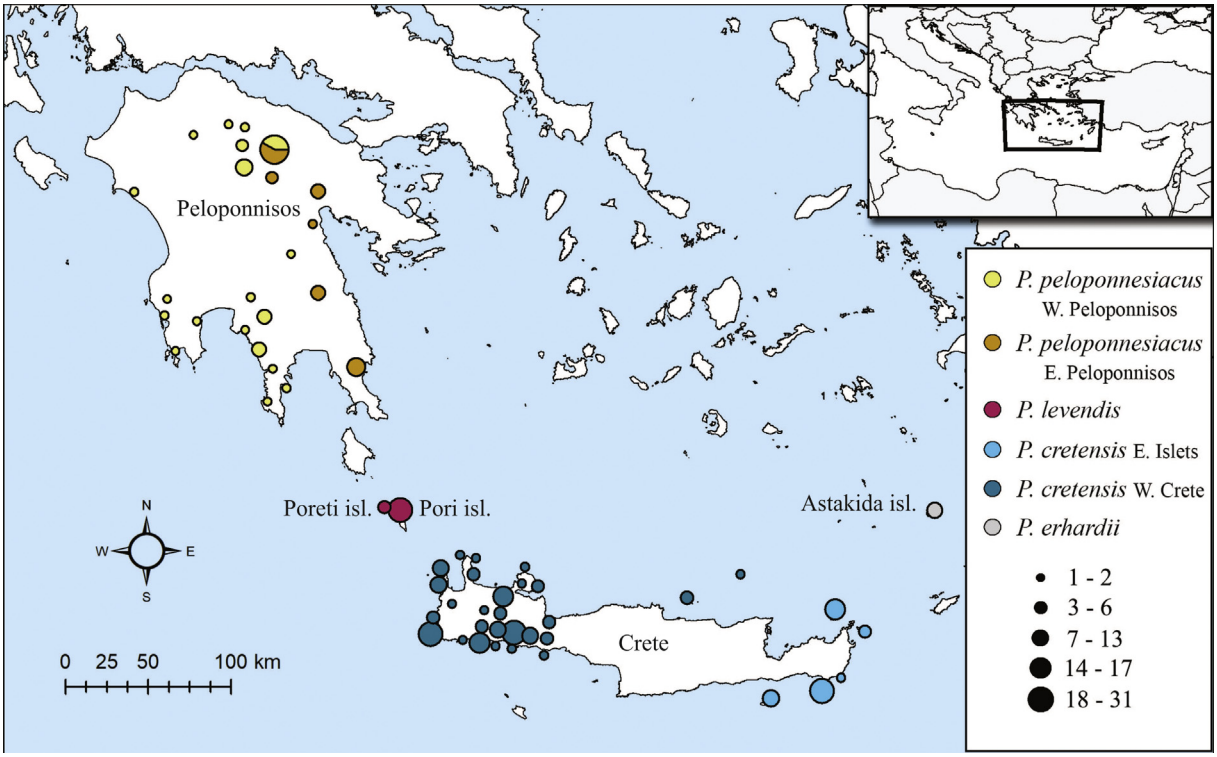

Fig. 1. Map showing the localities of the samples used in this study. Different species/lineages are represented with differently colored points while the varying point sizes are proportional to the number of analyzed samples from each sampling locality. For the sake of clarity, specimens that were in a 1 km radius were grouped together and represented by a single point.

in the area of southern Balkans have shaped the biodiversity patterns observed today. A multilateral approach was followed covering diversity from the intra to the interspecific level employing multiple nuclear (nuDNA; nuclear DNA sequences and microsatellites) and mitochondrial DNA (mtDNA) markers. Specifically, we performed several phylogenetic reconstructions, divergence time estimation, species delimitation and biogeographic analyses (*i.e.,* reconstruction of the ancestral geographic distribution) as well as population genetic analyses, which were combined with species distribution modeling and niche similarity approaches to assess the effect of ecological factors on the lineages' current distribution, while niche similarity analyses also provided a measure of niche overlap indicative of similar or different ecological demands.

2. Materials and methods

2.1. Specimens & DNA extraction

A total of 283 specimens from the collections of the Natural History Museum of Crete University of Crete (NHMC; Greece) were included in the present study (Table S1). Of those specimens, 281 represent the focal species while the remaining 2 belong to *P. erhardii*, which served as an outgroup due to its sister taxa relationship with the *P. cretensis* species group (Lymberakis et al., 2008). Total genomic DNA was extracted from 240 specimens using a standard ammonium acetate protocol (Bruford et al., 1998) or DNeasy Blood & Tissue Extraction kit (Qiagen®, Hilden, Germany), while the remaining 43 extractions were obtained from previous studies (Lymberakis et al., 2008; Poulakakis et al., 2003, 2005b).

All samples except the two outgroup samples (*i.e.,* 281 samples) were genotyped for 17 microsatellite loci and used in population genetic analyses while a representative in terms of geography and diversity number of samples (*i.e.,* subsets of the above mentioned 281 samples) was used in mtDNA (179 samples, outgroups included) and nuDNA (42 samples, outgroups included) analyses, as indicated in the relevant sections that follow.

2.2. Phylogenetic reconstruction, divergence time estimation, species delimitation & biogeographic analysis

2.2.1. Datasets, amplification & sequencing

For the phylogenetic reconstruction, the chronophylogenetic analysis and the species delimitation, several different datasets were created. Initially, a dataset of 179 specimens (including the two outgroup specimens) was assembled for which two mitochondrial (mtDNA) gene fragments [the large subunit of ribosomal RNA (16S rRNA) and the cytochrome *b* (cyt *b*) genes] were amplified using PCR. A second dataset was created by choosing 5–8 representatives from each of the major evolutionary lineages, that were identified by applying the Poisson Tree Processes (PTP) model (Zhang et al., 2013) on the phylogenetic tree produced from the initial mtDNA dataset, along with the two outgroup specimens (see Section 2.2.5). This subset of 42 specimens was selected for the amplification of six additional nuclear loci (nuDNA) [the melanocortin receptor 1 (MC1R) gene, two anonymous nuDNA markers (Pod15b and Pod55), the natural killer tumor recognition (NKTR) gene, the ubinuclein 1 (UBN1) gene and the recombination-activating gene 1 (RAG1)]. Primers used to amplify these loci, as well as PCR conditions are given in Table S2.

Double stranded sequencing of the PCR product was performed using the Big-Dye Terminator v.3.1 Cycle Sequencing kit ® on an ABI3730 automated sequencer following the manufacturer's protocol and using the same primers as in PCR. Sequences were edited using CodonCode Aligner v.3.7.1 (CodonCode Corporation ®) and the homology to the targeted loci was evaluated with BLAST search in the NCBI genetic database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

All newly determined sequences have been deposited in GenBank. Details regarding the sample codes, taxon names, voucher numbers, sampling locality, reference of the study in which they were previously used (if any) and GenBank accession numbers are presented in Table S1.

2.2.2. Alignment, genetic distances, statistical inference of allelic sequence and model selection

The alignment of the sequences was performed separately for each locus using the algorithm ClustalW as implemented in MEGA v.6 (Tamura et al., 2013). Alignment gaps were inserted to resolve length differences between non-coding sequences (16S rRNA, Pod15b, Pod55). Cytochrome *b*, MC1R, RAG1, NKTR and UBN1 sequences were translated into proteins prior to further analysis in order to ensure the absence of stop codons. Sequence divergence (uncorrected *p*-distances) was estimated using MEGA.

In the case of the nuclear loci, the algorithm of PHASE v.2.1.1 (Stephens et al., 2001) as it is implemented in DnaSP v.5.10.01 (Librado and Rozas, 2009) was used prior to alignment in order to statistically infer the allelic sequences. The program was run using the allele with the highest probability while haplotypes were considered resolved whenever the confidence of the phase call was at least 90%.

The nucleotide substitution model selection tests were carried out separately for the two types of genomes used in this study (mtDNA vs nuDNA). The mtDNA alignment was partitioned into 4 blocks, including 3 blocks for the 1st, 2nd and 3rd codon positions for the protein-coding locus cyt *b* and 1 block for 16S rRNA, while the nuDNA alignment was partitioned into 14 blocks, including 12 blocks for the 1st, 2nd and 3rd codon positions for each of the four protein-coding genes (MC1R, RAG1, NKTR and UBN1) and 2 blocks for each one of the remaining loci (Pod15b and Pod55). These initial partition schemes were loaded in PartitionFinder2 (PF) v.2.1 (Guindon et al., 2010; Lanfear et al., 2012, 2016) to find the best-fit partitioning scheme and evolutionary models for each downstream analysis according to the models that can be implemented in each software (RAxML, MrBayes). The four blocks of the mtDNA alignment and the fourteen blocks of the nuDNA alignment were considered to have linked branch lengths and the model selection was based on the Bayesian Information Criterion (BIC), which is substantially more accurate in finding the true model than AIC/AICc (Darriba et al., 2012), ignoring the evolutionary models that contain both gamma distribution and invariable sites (Yang, 2006). The greedy algorithm was selected to search for the best-fit solutions.

It should be noted that in the chronophylogenetic and the species delimitation analysis, as well as in the coalescent species tree inference, the datasets were not partitioned by coding position due to software parameterization limitations in downstream analyses.

2.2.3. Phylogenetic tree estimation on mtDNA, nuDNA and concatenated loci

Phylogenetic estimation was performed using three different datasets: (1) the initial mtDNA dataset containing the 179 specimens, (2) the nuDNA dataset comprised of the 42 selected specimens and (3) the concatenated dataset containing both mtDNA and nuDNA sequences of the 42 selected specimens. Phylogenetic reconstruction was conducted using two different methods; Maximum Likelihood (ML) and Bayesian Inference (BI). The parameters (described below) used for analysing all three datasets, were the same.

Maximum likelihood analyses were performed using RAxML v.8.1.21 as implemented in raxmlGUI v.1.5 (Silvestro and Michalak, 2011). The tree with the best likelihood for each dataset was selected among the 50 ML trees generated on distinct starting trees. Statistical confidence was assessed based on 1000 thorough bootstraps. High bootstrap values (close to 100), indicate uniform support by almost all characters being analyzed of a particular clade/branch.

Bayesian inference analyses were performed in MrBayes v.3.2.6 (Ronquist et al., 2012) conducting four runs and using eight sampling chains for each run. Each chain ran for 2×10^7 generations sampling

every 10^3 generations. Several MCMC diagnostics were used to check for convergence and stationarity [the plot of the generation versus the log probability of the data (the log likelihood values), the average standard deviation of split frequencies, the average Potential Scale Reduction Factor (PSRF) and the minimum value of minimum Estimated Sample Sizes (ESS)]. The first 35% (7×10^6) trees were discarded as burn-in, as defined after the inspection of the mcmc traces in Tracer v.1.6 (Rambaut et al., 2014), as a measure to sample from the stationary distribution and avoid the possibility of including random, sub-optimal trees. A 50% majority rule consensus tree was then produced from the posterior distribution of trees and the posterior probabilities were calculated as the percentage of samples recovering any particular clade. Posterior probabilities ≥ 0.95 indicate statistically significant support (Huelsenbeck and Ronquist, 2001).

2.2.4. Species tree and divergence time estimation

Species tree and divergence time estimations were carried out using the dataset containing nuDNA and both mtDNA and nuDNA sequences of the 42 selected specimens using StarBEAST2 package as implemented in BEAST2 v.2.4.7 (Bouckaert et al., 2014). The input files (xml format) were created using BEAUti v.2.4.7, also implemented in BEAST2. The nucleotide substitution models were specified a priori according to the results of the PF analysis. As for other priors, the Birth Death model was selected for speciation, the Constant Population model for population model and the Uncorrelated Lognormal or strict clock model for describing the molecular clock. We compared the marginal likelihood of the models in Tracer based on the Akaike's information criterion through Markov chain Monte Carlo simulation (AICM) (S.E. estimated from 100 bootstrap replicates) (Baele et al., 2012). Under the AICM, an increase in the number of parameters penalizes more complex models, and models with lower AICM values are preferred over models with higher values (Leache et al., 2014). Regarding the divergence time estimation, the isolation of Crete (*P. cretensis*) from Peloponnisos (*P. peloponnesiacus*) at 5–5.5 Mya was used as a calibration point (Dermitzakis, 1990b), [N(5.3, 0.1)] (for details regarding the choice of the calibration point, see section introduction). The MCMC analysis was run for 5×10^8 generations, saving the result every 5×10^3 generations. The first 25% of the saved trees were discarded after the inspection of the log files with Tracer v.1.6 (Rambaut et al., 2014). The Maximum Clade Credibility (MCC) tree that best represented the posterior distribution was identified using TreeAnnotator v.2.4.7 (also included in BEAST2), which subsequently annotated this selected topology with the mean ages of all the nodes.

2.2.5. Identification of major evolutionary lineages and species delimitation

The evaluation of the putative species' boundaries was performed using two different Bayesian approaches: Bayesian Phylogenetics and Phylogeography - BPP v.3.3 (Yang, 2015) and Species Tree And Classification Estimation, Yarely - STACEY v.1.2.2 (Jones, 2016) implemented in BEAST2. For both analyses, a dataset containing only the six nuDNA markers was used, since nuclear and mitochondrial loci have very different characteristics, including different mutation rates and effective population sizes, while the latter by being inherited as a single unit, thus have a single evolutionary history. Species were determined in both cases, based on the model with the number of species that had the highest posterior probability.

In BPP analyses the fourteen clades and subclades of *P. cretensis* group, revealed in the phylogenetic analyses, were considered as potential distinct species. Joint species delimitation and species-tree estimation *i.e.*, unguided species delimitation (analysis A11) was performed, using informative priors for the ancestral population size (θ) and root age (τ_0) that are roughly equal to the average differentiation observed between the major evolutionary lineages. Both priors were assigned a gamma G(α, β) distribution with $\alpha_\theta = 50$, $\beta_\theta = 2000$ and $\alpha_{\tau 0} = 34$ and $\beta_{\tau 0} = 1000$ respectively. The nearest neighbour interchange (NNI) and subtree pruning and regrafting (SPR) algorithms were used to change the species tree topology, while rjMCMC was used to split one species into two or to join two populations into one species (Yang and Rannala, 2014). The rjMCMC analyses (algorithm 1) were performed for 1.25×10^6 generations (sampling interval of five) with a burn-in period of 2×10^4 samples and each species delimitation model was assigned equal prior probability. Each analysis was run twice, initiated with different starting seeds, to confirm consistency between runs. The topology of the starting tree for both runs was that of BI analysis on the combined dataset.

In STACEY, the input files (xml format) were created using BEAUti. The nucleotide substitution models were specified according to the results of the PF analysis. Regarding other priors, the Phylodynamics: Birth Death model was selected for speciation and the Uncorrelated Lognormal or strict clock model for describing the molecular clock, the "collapse height" parameter was set at 0.001 and each specimen was considered as potential distinct species. We compared the marginal likelihood of the models in Tracer based on the Akaike's information criterion through Markov chain Monte Carlo simulation (AICM) (S.E. estimated from 100 bootstrap replicates) (Baele et al., 2012). Under the AICM, an increase in the number of parameters penalizes more complex models, and models with lower AICM values are preferred over models with higher values (Leache et al., 2014). The MCMC analysis was run for 5×10^8 generations, saving the result every 10^6 generations and the first 10% were discarded as a burn-in phase after the inspection of the obtained log files with Tracer. This was also conducted in order to verify that the convergence of the analysis had been achieved and that satisfactory effective sample sizes had been obtained. The analysis and the display of the results of the species delimitation and its statistical support were made by SpeciesDelimitationAnalyser (Jones et al., 2015).

2.2.6. Reconstruction of the ancestral geographical distribution of P. Cretensis species group and closely related species (P. erhardii)

The broad scale geographic range evolution of the *P. cretensis* species group and closely related species was studied using a combination of phylogenetic and distributional information. The ancestral area reconstruction method of choice was the LAGRANGE (Ree and Smith, 2008), known also as Dispersal-Extinction-Cladogenesis analysis (DEC) as implemented in RASP v.3.2 (Yu et al., 2015). The phylogenetic tree used was the one produced from the BI analysis of the combined dataset (see Section 3.1). We considered species/populations to be distributed within 5 broad areas: (i) the island group of Pori where *P. levendis* is distributed (area A), (ii) Peloponnisos, corresponding to the distribution of *P. peloponnesiacus* (area B), (iii) western part of mainland Crete (west of the city of Rethymno) where the mainland populations of *P. cretensis* are distributed (area C), (iv) satellite islets of eastern Crete including all the corresponding *P. cretensis* specimens (area D) and finally (v) Cyclades corresponding to *P. erhardii* (area E, the two specimens of *P. erhradii* from Astakida isl. acting as a proxy). Additionally, based on the major paleogeographic events of the area, different dispersal constraints were defined for two time slices corresponding to the pre- and post-isolation time of Crete, where in each time slice connectivity between the designated areas is considered constant. Based on the divergence time estimations (see Section 3.1) in conjunction with the paleogeography of the area (see Section 1) the two time slices were defined as follows: 0–5.07 Mya and 5.07–5.23 Mya.

2.3. Population genetics

2.3.1. Microsatellite amplification & genotyping

Seventeen microsatellite primer pairs previously isolated from lacertid lizards were tested on a total of 281 individuals of the *P. cretensis* species group, including 202 specimens of *P. cretensis*, 52 of *P. peloponnesiacus* and 27 of *P. levendis*. Information concerning PCR primers and conditions are listed in Table S3.

Single PCR products were mixed with an internal size standard (GeneScan 500 LIZ, Applied Biosystems) and the amplified allele sizes

were visualized on an automated sequencer, type ABI3730 (Applied Biosystems). Genotyping was conducted with the program STRand v.2.4.109 (Toonen and Hughes, 2001, http://www.vgl.ucdavis.edu/STRand) and the microsatellite allele binning was conducted using the program FlexiBin v.2 (Amos et al., 2007).

2.3.2. Statistical analyses

Deviations from Hardy Weinberg Equilibrium (HWE) were tested using Genepop Web (Raymond and Rousset, 1995; Rousset, 2008) and measures of genetic diversity in the form of F_{ST} and heterozygosity were calculated using the GENETIX v.4.05 software (Belkhir et al., 2001).

Patterns of population structure were investigated using a model based Bayesian clustering approach implemented in STRUCTURE v.2.3 (Pritchard et al., 2000). The program assigns individuals on the basis of their genotypes and provides the proportion of each specimen's genome that originated from each inferred cluster (Q: membership coefficient). The MCMC parameters included 10^6 replicates, with a burn-in length of 3.5×10^5 and the use of the admixture model along with correlated allele frequencies. Ten replicates were performed for each value of K that ranged from 1 to 15. The number of clusters of individuals was detected employing the ΔK Evanno method (Evanno et al., 2005) as implemented in STRUCTURE HARVESTER (Earl and von Holdt, 2012). Following that, a second level of hierarchical structuring was carried out for each of the inferred clusters of the first level of analysis, using only individuals which were assigned to each cluster with high membership coefficient values ($Q \geq 90\%$). Cases of label switching and multimodality problems were dealt with using the software CLUMPP (Jakobsson and Rosenberg, 2007), which assists in aligning the output from multiple cluster analyses of the same dataset.

In an additional attempt to investigate the relationships among groups that does not require the assumption of HWE to be met, a non-model based assignment was also employed, that of Discriminant Analysis of Principal Components (DAPC) (Jombart et al., 2010). This method was employed using the R package Adegenet v.1.3.1 (Jombart and Ahmed, 2011), in which the number of retained Principal Components (PC) was selected after performing cross-validation as suggested by the developers. Group priors were not specified and were instead estimated by the K-means clustering of PC to identify groups of individuals. Similarly to STRUCTURE, we run K-means clustering with different numbers of clusters, each of which gives rise to a statistical model and an associated likelihood. We used BIC to assess the best supported model and therefore the number and nature of clusters.

2.4. Species distribution modeling and niche similarity analyses

2.4.1. Dataset assembly

Presence only datasets for the following species and identified lineages (see Sections 3.1 and 3.2) were prepared: (1) *P. levendis*, (2) *P. peloponnesiacus*, (3) *P. peloponnesiacus* W. Peloponnisos, (4) *P. peloponnesiacus* E. Peloponnisos, (5) *P. cretensis*, (6) *P. cretensis* from the westernmost part of its distribution (W. Crete) and (7) *P. cretensis* from Crete's eastern satellite islets (E. Islets). All the presence points used in the analyses were extracted from the NHMC collections database and ranged from 34 in *P. levendis* to 166 in *P. peloponneisacus*. The exact count of points for each species/lineage is given in Table S4.

2.4.2. Species distribution modeling

The combination of algorithms, biophysical variables and species distribution data establish complex mathematical relationships. Species Distribution Modeling can be used to estimate the potential distribution of certain species, the probability of occurrence under the studied conditions and the combination of them with other analyses in order to provide answers to certain questions (Soberon and Nakamura, 2009) such as the presence or absence of a taxon from a certain locality which is otherwise unexplained.

To calculate the models we used the Maximum Entropy method implemented in the software MaxEnt v.3.4.1 (Phillips et al., 2018). MaxEnt is a general-purpose machine learning method (Phillips et al., 2006) that uses presence-only occurrence data and is consistently competitive with the highest performing methods (Wisz et al., 2008). It was selected among others as it has been shown to outperform other established methods, among both presence only and absence-presence models and techniques, especially in the cases of small sample size (Elith et al., 2006; Hernandez et al., 2006; Peterson et al., 2011; Phillips et al., 2006; Phillips and Dudik, 2008).

Environmental data, at 1*1 km spatial resolution, from the CHELSA database were used (Bobrowski and Schickhoff, 2017; Karger et al., 2017). The bioclimatic variables annual mean temperature (BIO1), mean diurnal range (BIO2), isothermality (BIO3), temperature seasonality (BIO4), minimum temperature of coldest month (BIO6), temperature annual range (BIO7), precipitation of driest month (BIO14) and precipitation of the coldest quarter (BIO19) were used for all species and lineages. The subset of variables used was selected by: (a) choosing those that are more ecologically relevant based on expert's opinion, (b) the percent contribution, the permutation importance and the jackknife results in initial runs and (c) removing those variables that were highly correlated under the Pearson's r rank and retaining those with values ≤ 0.8. A list of all the available bioclimatic variables out of which the selection was made is given in Table S5.

MaxEnt was run with 10.000 background points, beta multiplier of 2 in order to reduce model overfitting (Radosavljevic and Anderson, 2014), autofeatures and by selecting at random 70% of the presence records as training data and 30% as test data for each species. Model output was set at cloclog format. Bootstrapping was selected as replicated run type. The final model for each species was the mean of 10 different replicative models with the exception of *P. levendis* (2 replicates due to the low number of presence points). Occurrence thinner was applied in order to have one presence point per pixel (1 km radius) (Table S4). The importance and effects of each bioclimatic variable were evaluated exploring percent contribution, permutation importance and jackknife tests generated by MaxEnt. Model results were tested with Receiver Operating Characteristics (ROC) plots, on which the curves present true-positive rate against false-positive rate (Phillips et al., 2004), while the Area Under the Curve (AUC) was used as a measure of the overall fit of the model. It is calculated by plotting model sensitivity (fraction of true presences against total presences) against 1-specificity (fraction of true absences against total absences) for all available probability thresholds (Manel et al., 2001). The term sensitivity refers to presences that are predicted as presences, while specificity refers to the absences that are predicted as absences. AUC values > 0.7 correspond to good model performance (Araujo and Guisan, 2006).

2.4.3. Niche similarity

Niche similarity was calculated using the metrics and approach proposed by Warren et al. (2008). Both Schoener's D and Hellinger I indices, ranging from 0 to 1, were calculated among the studied taxa using the ENMtools (Warren et al., 2011). Values with ranges from 0 to 0.2 correspond to "no or limited" niche overlap, 0.2–0.4 correspond to "low" overlap, 0.4–0.6 correspond to "moderate" overlap, 0.6–0.8 correspond to "high" overlap and 0.8–1.0 correspond to "very high" overlap (Rodder and Engler, 2011).

3. Results

3.1. Phylogenetic reconstruction, divergence time estimation, species delimitation & biogeographic analysis

Regarding the mtDNA dataset, a total of 918 base pairs (bp) were obtained (cyt *b* 412 bp and 16S rRNA 506). Out of the 412 sites comprising the cyt *b* sequences, 113 were variable when examining the complete dataset, 92 when the outgroup was excluded. For the 16S

Table 1
Genetic p-distances of the cyt b/16S rRNA (below diagonal–left) and MC1R/Pod15b/Pod55/RAG1/NKTR/UBN1 (above diagonal–right) sequences among the major clades.

	Clade	1	2	3	4	5	6	7
1.	*P. peloponnesiacus* W. Peloponnisos		1.0/0.5/0.9/0.9/0.9/0.8	1.0/1.6/0.8/0.3/0.5/0.9	1.0/1.8/0.9/0.4/0.4/1.1	1.1/1.6/0.7/0.3/0.4/0.9	0.9/1.0/1.0/0.7/0.5/0.7	0.8/1.1/1.2/0.4/1.2/1.9
2.	*P. peloponnesiacus* E. Peloponnisos	5.8/3.4		0.5/1.5/0.3/0.7/0.9/0.9	0.5/1.7/0.4/0.7/0.8/0.9	0.5/1.6/0.3/0.6/0.8/0.8	0.4/1.0/0.5/0.8/0.9/0.7	0.9/1.0/0.8/0.7/1.6/1.9
3.	*P. cretensis* W. Crete 1	7.4/3.9	7.8/4.6		0.2/0.7/0.2/0.1/0.1/0.2	0.2/0.6/0.0/0.1/0.1/0.2	0.4/1.3/0.3/0.5/0.3/0.7	0.8/1.5/0.5/0.1/1.0/1.5
4.	*P. cretensis* W. Crete 2	6.8/4.4	7.5/5.2	5.8/2.9		0.2/0.7/0.2/0.1/0.0/0.2	0.4/1.5/0.4/0.5/0.2/0.9	0.8/1.6/0.6/0.2/0.8/1.6
5.	*P. cretensis* E. Islets	6.9/3.6	7.3/4.1	4.6/2.3	5.7/3.1		0.5/1.4/0.3/0.4/0.2/0.7	0.9/1.5/0.5/0.1/0.9/1.4
6.	*P. levendis*	7.0/2.8	7.3/3.6	7.8/5.0	9.9/4.7	9.0/4.4		1.7/0.8/0.8/0.8/0.5/1.0
7.	*P. erhardii*	13/4.3	12.7/6.0	12.4/5.3	13.1/4.5	14.6/5.1	12.7/4.4	

rRNA sequences, the corresponding numbers were 50 and 45 sites, respectively. Accordingly, a total of 3734 bp were obtained from the nuDNA markers (MC1R 662 bp, Pod15b 528 bp, Pod55 411 bp, RAG1 983 bp, NKTR 590 and UBN1 560 bp). In MC1R, 19 sites were variable when examining the complete dataset and 17 when excluding the outgroup. The corresponding numbers for Pod15b were 20 and 18 sites, for Podd55 10 and 8 sites, for RAG1 18 and 17 sites, for NKTR 16 and 11 sites and for UBN1 22 and 15 sites.

Uncorrected pairwise genetic distances (p-distances) for the mtDNA loci ranged from 0 to 14.9% for cyt *b* and from 0 to 7.2% for 16S rRNA. For the nuDNA loci sequence divergence varied from 0 to 1.2% for MC1R, from 0 to 2.4% for Pod15b, from 0 to 1.2% for Pod55, from 0 to 1.1% for RAG1, from 0 to 1.7% for NKTR and from 0 to 2% for UBN1. The mean genetic distances among the major lineages, as revealed by the phylogenetic analyses, for each locus are given in Table 1, whereas the pairwise genetic distances are given in Tables S6, S7 & S8.

The best - fit partitioning scheme for each downstream analysis, as well as the selected nucleotide substitution models are presented in Table S9.

Maximum Likelihood and Bayesian Inference analyses produced fairly similar topologies for all three examined datasets (Figs. 2, S1 & S2). However, different levels of resolution were reached (*i.e.*, trees with distinct branch support values within the major lineages) between the different analyses and/or datasets. BI analysis of the mtDNA dataset was the one with the highest resolution. The monophyly of both *P. levendis* and *P. cretensis* is well supported by all analyses and datasets, while the monophyly of *P. peloponnesiacus* is only supported by the analyses performed with the combined dataset. Within *P. cretensis*, three additional, well supported, clades were recovered when analysing either the mtDNA or the combined dataset. Two of the clades contain individuals originating from western Crete (W. Crete 1, W. Crete 2) while the remaining clade corresponds to individuals from the eastern satellite islets of Crete (E. Islets) (Fig. 2). However, when analysing the nuDNA dataset, none of the three clades was statistically supported. Furthermore, two distinct subclades were recovered within *P. peloponnesiacus*, which correspond to the western and the eastern regions of Peloponnisos (W. & E. Peloponnisos) and whose monophyly is very well supported in all three datasets. However, the relationships between the three focal species remain unclear in all cases.

In the chronophylogenetic analyses on the combined dataset, the model comparison based on AICM favoured the relaxed molecular clock (AICM = 29,005.557) model versus the strict clock model (AICM = 32,309.156). According to the results of the chronophylogenetic analysis (Fig. 2), the divergence of the *P. levendis* and *P. peloponnesiacus* lineages started approximately 2.47 Mya in the late Pliocene/early Pleistocene. Intraspecific diversification was estimated around 1.86 Mya for *P. peloponnesiacus*, 210 Kya for *P. cretensis* and 40 Kya for *P. levendis*. It is worth mentioning that similar results were obtained when only nuDNA dataset was used (the divergence of *P. levendis* and *P. peloponnesiacus* at 2.27 Mya, and the intraspecific diversification of *P. peloponnesiacus* at 1.86 Mya). However, in this dataset, the AICM favoured the strict clock model (AICM = 30,265.213) versus the relaxed one (AICM = 36,595.557).

The species tree resulting from StarBEAST2 (Fig. 2) based on the combined dataset is topologically similar with the Bayesian tree obtained from the same dataset. Specifically, the monophyly of the focal species group is very well supported [posterior probability (pp) = 1.00] and the same applies for *P. cretensis* and *P. levendis* individually (pp = 1.00 in both cases). On the contrary, the monophyly of *P. peloponnesiacus* is not strongly supported (pp = 0.93) although the two aforementioned subclades of *P. peloponnesiacus* were also recovered (*P. peloponnesiacus* W.: pp = 1.00, *P. peloponnesiacus* E.: pp = 1.00). Regarding the intraspecific relationships of *P. cretensis*, only the subclade of E. Islets (pp = 0.97) was statistically supported. The corresponding species tree based on the nuDNA dataset is also similar with the one from the combined dataset. The only difference is that none of the

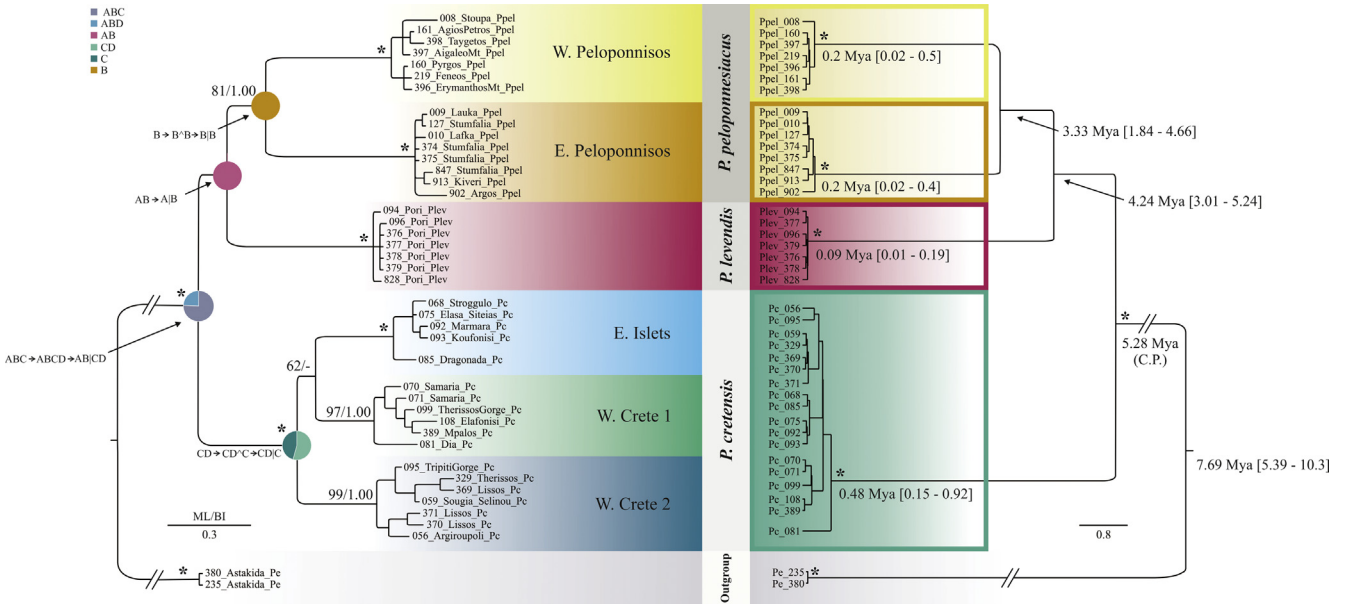

Fig. 2. Phylogeny, species delimitation and temporal and geographic aspects of the *P. cretensis* species group diversification. Left: Bayesian Inference tree based on the concatenated (mtDNA & nuDNA) dataset. The posterior probabilities (> 0.95) and bootstrap support (> 50%) of both the phylogenetic methods used are given on top of the branches. No values or dashes means low statistical support and asterisks indicate absolute support by both methods (ML/BI). Pie diagrams at nodes indicate probability of various ancestral area combinations from the LAGRANGE analysis and the most probable event route is given underneath each one of these nodes. The caret symbol (^) indicates an event of migration while the vertical bar (|) indicates a vicariant event. The scale bar represents the estimated number of nucleotide substitutions per site. Right: The resulted calibrated starBEAST2 species tree of the chronophylogenetic analysis based on the combined dataset. The calibration point (C.P.) as well as the calculated node ages of the major nodes and their respective credible intervals [95% HPD] are given underneath each of these nodes. The lined frames represent distinct entities based on both the BPP and STACEY analyses. Asterisks indicate a p.p. value equal to 1. The scale bar represents the estimated number of nucleotide substitutions per site.

subclades of the *P. cretensis* were statistically supported.

As for the species delimitation results, in both approaches, the model with the highest posterior probability indicated that there are 4 species present within the *P. cretensis* species group, 5 when considering the outgroup. Specifically, *P. levendis* and *P. cretensis* are considered to be single species, whereas *P. peloponnesiacus* seems to contain two species, which is consistent with the two subclades recovered from all previous phylogenetic analyses. In the BPP analysis, the 5-species scheme had the highest posterior probability equal to 0.92 with the second best being the one with 6 species and posterior probability equal to 0.06. All five species were strongly supported with posterior probabilities ranging from 0.95 to 1.00. In STACEY, the model comparison based on AICM simulation ranked the strict model (AICM = 19,893.76) over the Relaxed one (AICM = 20,236.945). The scheme with the highest posterior probability (p.p. = 0.90) was again the one with 4 species (5 when considering the outgroup).

Finally, the biogeographic reconstruction for the 4 major nodes is presented in Fig. 2**.** The ancestor of the ingroup was reconstructed to have been located in Pori, the entire area of Peloponnisos and western Crete. It is worth mentioning that similar results were obtained when only the nuDNA dataset was used (results not shown).

3.2. Microsatellite genotyping, population structure & demographic analysis

Thirteen out of the seventeen microsatellite loci amplified were deemed suitable for downstream analyses in the present study, while the remaining four either could not be successfully genotyped (Pod3, Pb47 and Pb50) or were monomorphic (Pod1A). Specimens for which less than 10 loci were genotyped, were not included in the final dataset. Therefore, a total of 233 out of 281 specimens were used for the analyses.

At the first hierarchical level of STRUCTURE analysis, runs across the selected K were variable even when longer runs and longer burn-in periods were used, with ln Pr(X|K) appearing to be bimodal, indicating that the MCMC scheme is finding two clustering solutions with equally high posterior probability *i.e.*, equally likely solutions. This can be the result of difficulties in the search for the possible membership coefficients space or of true biological factors (Jakobsson and Rosenberg, 2007). The two equally probable modes that were derived from the highest level of hierarchical analysis in STRUCTURE (*i.e.,* Mode1 & Mode2), are depicted in Figs. 3 & S3, respectively. At this level K = 4 was the most probable number of clusters. Specifically, Mode1 consisted of the clusters: (1) *P. cretensis* from eastern islets of Crete (E. Islets), (2) *P. cretensis* from western Crete (W. Crete), (3) *P. peloponnesiacus* and (4) *P. levendis,* while Mode2 supported the existence of the following clusters: (1) *P. cretensis* E. Islets, (2) *P. cretensis* W. Crete, (3) *P. peloponnesiacus* W. Peloponnisos and (4) *P. peloponnesiacus* S. Peloponnisos coupled with *P. levendis*. From the analyzed individuals, 82% were assigned to their respective clusters that reflected their geographic origin, with probability equal or higher than 90%, while only 6.5% of the individuals had probabilities lower than 70% for Mode1, while for Mode2 the corresponding numbers were 75.5% and 9.9% respectively.

A second level of hierarchical structuring was carried out for each of the inferred clusters of the first level of analysis, using only individuals which were assigned to each cluster with high membership coefficient values (Q ≥ 90%). In order to have 'pure' clusters to proceed with the hierarchical clustering approach, a threshold of 0.9 was selected in an attempt to acquire less admixed individuals within clusters or/and individuals with more accurate assignment. At the second level of hierarchical structuring, both modes presented further population subdivision and supported the discrimination of the *P. peloponnesiacus* populations into two geographically distinct clusters. Indications for additional population structure within the inferred clusters of *P. cretensis* E. Islets and *P. cretensis* W. Crete were also supported (with K = 3–5 and K = 2–4 respectively depending on the mode selected), though not corresponding to geographically distinct groups, while genuine multimodality issues also occurred. Within *P. levendis*, there were indications (*i.e.*, when analysing one of the two modes) for two

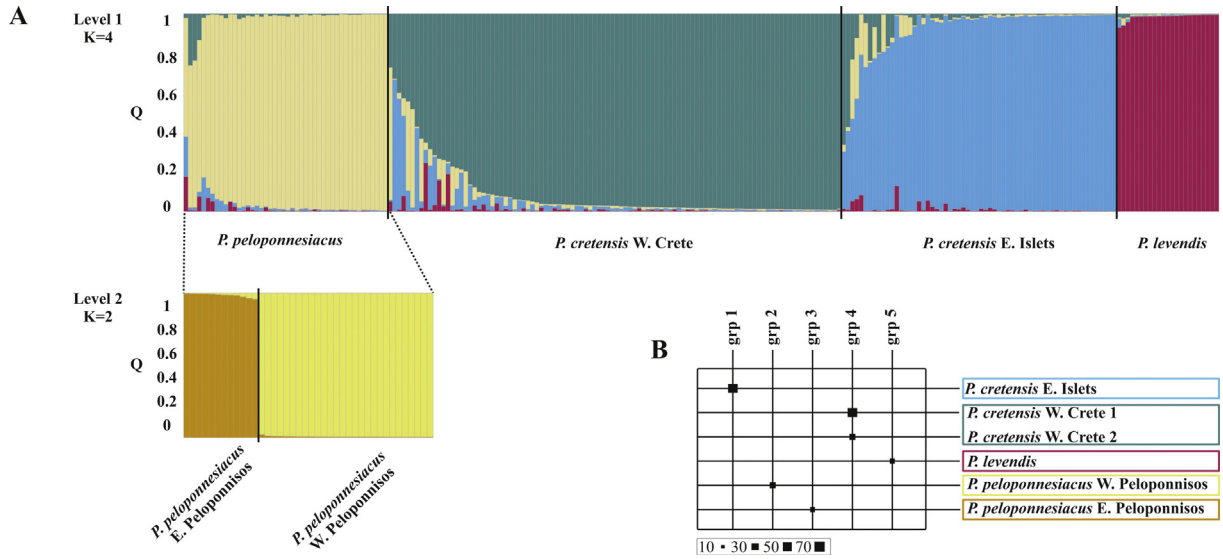

Fig. 3. A: The estimated population structure of the *P. cretensis* species group after two steps of the hierarchical STRUCTURE analysis using microsatellites for all the data. Every individual is represented by a thin vertical line that consist of K number of colors. The Q value corresponds to the percentage of estimated assignment of the individual to each one of the K clusters. The dashed lines show the process of this hierarchical approach, in which subgroups of data are analyzed subsequently (Mode 1). B: Contingency table produced by the DAPC analysis. Rows correspond to groups formed based on the lineages present in the mtDNA tree, while columns correspond to inferred groups. The size of the squares is proportional to the number of individuals.

clusters, one containing the individuals from the islet of Pori while the other the individuals from the islet of Poreti. However, since only two specimens from Poreti were used in the analysis, this further substructuring was considered ambiguous.

Following the BIC proposed in Adegenet, the optimal number of clusters for describing the analyzed microsatellite data was K = 5 (Fig. S4). All individuals were clearly assigned to the five following groups: (1) *P. cretensis* W. Crete, (2) *P. cretensis* E. Islets, (3) *P. peloponnesiacus* W. Peloponnisos, (4) *P. peloponnesiacus* E. Peloponnisos and (5) *P. levendis* that coincide with the results of STRUCTURE analysis. The correspondence of population structure analyses to phylogenetic analyses was high with the only difference that of the two W. Crete phylogenetic clades that were not retrieved in any of the population structure analyses. DAPC analysis results are presented in Fig. S5.

All F_{ST} values assessed by the estimator θ, were statistically significant (at $\alpha = 0.01$) and ranged between 0.038 (*P. cretensis* E. Islets - *P.cretensis* W. Crete) and 0.225 (*P. levendis* – *P. peloponnesiacus* W. Peloponnisos) (Table S10). All groups deviated from HWE for the majority of the loci due to heterozygote deficit. High mean heterozygosity per group was observed, ranging from $H_{obs} = 0.5597$ for *P. peloponnesiacus* E. Peloponnisos to $H_{obs} = 0.7180$ for *P. cretensis* W. Crete (Table S11).

3.3. Species distribution modeling and niche similarity

Almost all model outputs showed a potential distribution of the studied group close to the actual one (Figs. 4 & S6). However, the models corresponding to the individual lineages of *P. peloponnesiacus* and *P. cretensis* performed differently. In the first case, there was an overlap between the two lineages while in the latter, the model was consistent with the actual distribution of the lineages of the species with almost no overlap between the two lineages (*i.e.*, West Crete and East Crete lineages).

Test AUC values for almost all models were above 0.9, indicating high accuracy. Only for *P. peloponnesiacus* lineages (W. Peloponnisos and E. Peloponnisos), the test AUC values were 0.81 and 0.88 respectively (Fig. S7). Jackknife results are presented in Fig. S8. The environmental parameters with the highest impact differed between the analyzed species and/or lineages. At the species level, isothermality, temperature seasonality and mean diurnal range were the most significant for *P. peloponnesiacus* and *P. cretensis* while mean diurnal range, mean annual temperature and precipitation of driest month were the most important for *P. levendis*. For each lineage the most prominent parameters were identified as follows: isothermality, mean diurnal range and temperature seasonality for *P. peloponnesiacus* E. Peloponnisos, and W. Peloponnisos, temperature annual range, minimum temperature of the coldest month and isothermality for *P. cretensis* E. Islets lineage and temperature seasonality, precipitation of driest month and isothermality for *P. cretensis* W. Crete (see Table S12 for percent contribution and permutation importance for all bioclimatic variables). It is worth mentioning that for *P. levendis,* due to the low number of records and despite the high statistical accuracy obtained in this study, the results are indicative and further data are needed in order to reach solid conclusions.

Pairwise comparison of niche similarity between the studied taxa indicated “no or limited” to “low” niche overlap, in all cases except between the two lineages of *P. peloponnesiacus* (Table S13) which showed high niche overlap with one another for both D & I*s*.

4. Discussion

Elucidating the evolutionary history of a group of species over a large temporal scale requires a robust phylogenetic tree and solid divergence time estimations. These are necessary in order to infer the group’s phylogeographic history, as well as applying genetic and demographic analyses at the interspecific level in order to explain its current distribution and population structure. Especially in an area such as the Aegean with its complex paleogeographic history, it is important to seek information from several different approaches.

4.1. Phylogenetic relationships and taxonomic re-evaluation of P. Cretensis species group

When examining the mtDNA and the nuDNA markers separately (Figs. S1 & S2), it becomes apparent that although both phylogenetic trees have identical topology, their support values are different. MtDNA tree displays significant support for the within *P. cretensis* lineages, whereas the support of those lineages is not statistically significant for

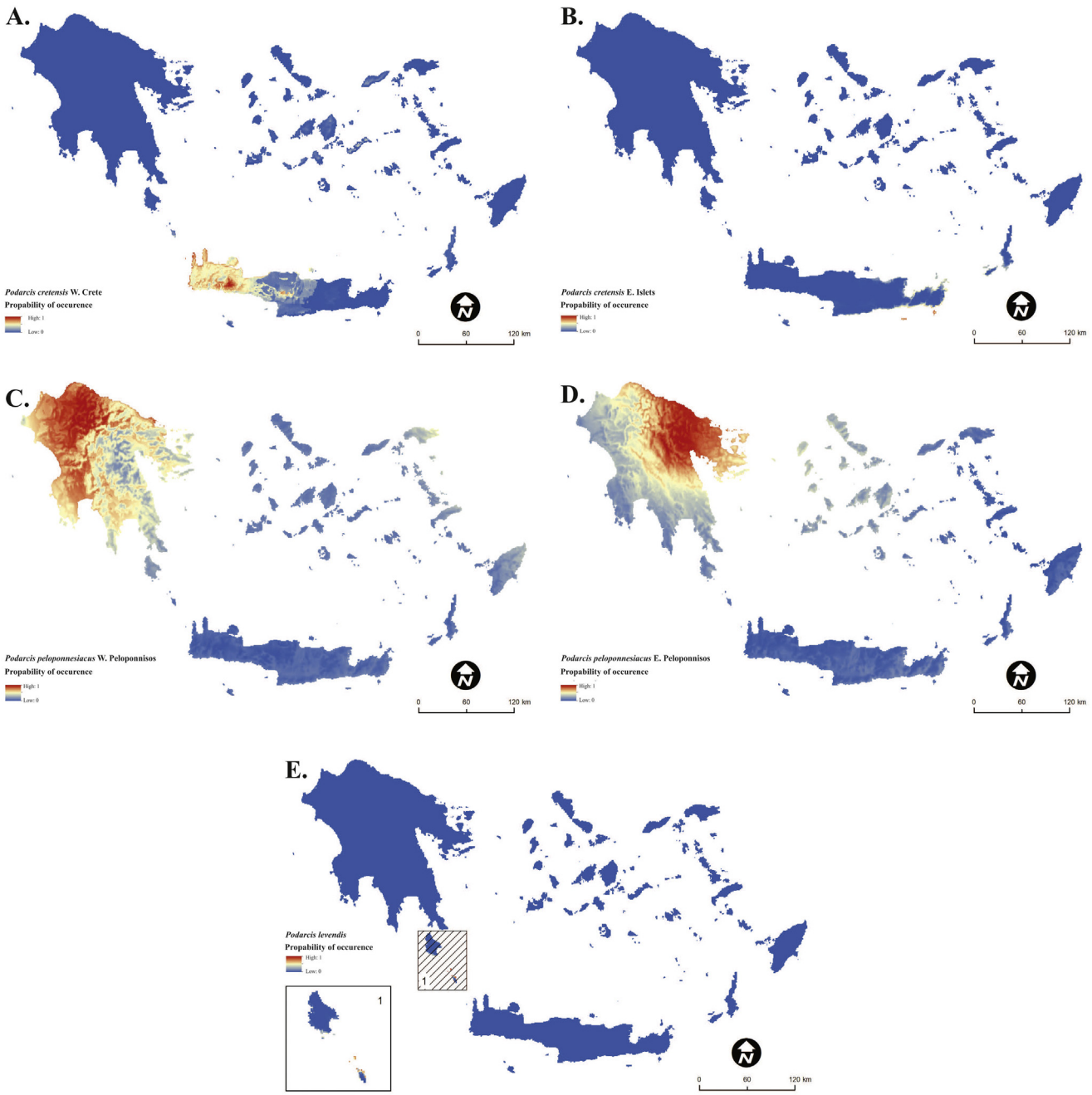

Fig. 4. Potential distribution of the studied species/lineages. A: *P. cretensis*, B: *P. cretensis* E. Islets, C: *P. cretensis* W. Crete, D: *P. peloponnesiacus*, E: *P. peloponnesiacus* W. Peloponnisos, F: *P. peloponnesiacus* E. Peloponnisos and G: *P. levendis*. The projections correspond to current conditions.

the nuDNA tree. Although this topology persists in the tree produced by the phylogenetic analyses on the concatenated dataset (mtDNA and nuDNA), the species tree based on the combined dataset, supported a topology similar to the topology produced by the nuDNA dataset probably indicating that the former is mostly driven by mtDNA information.

The most parsimonious hypothesis regarding the existence of the three mitochondrial lineages within the island of Crete involves the existence of multiple mitochondrial haplotypes present in the area of Crete that could be attributed to the faster substitution rate of mtDNA in respect to the nuclear genes sequenced in this study. This, in combination to the smaller effective population size of the mtDNA provides a fertile ground for random genetic drift and incomplete lineage sorting to act upon. A similar case of mitonuclear pattern in which multiple mitochondrial lineages correspond to a single nuclear lineage have also be found in the Iberian *P. hispanicus* complex (Kaliontzopoulou et al., 2011 and references therein). Furthermore, when nuclear markers with higher mutation rates are employed (*i.e.*, microsatellite loci), the discrimination of two lineages within *P. cretensis*, one distributed in the western part of the island and one to the eastern islets, is evident.

In concordance with the species tree estimation, species delimitation analyses clearly support the existence of four species in the *P. cretensis* species group. Two of these species coincide with the currently recognized *P. cretensis* and *P. levendis*, whereas the other two species correspond to two lineages of *P. peloponnesiacus*. Although there seems to be minimal ecological differentiation between the two *P. peloponnesiacus* lineages, their genetic distances are similar to species-level distances observed within the genus *Podarcis* (Table 1, Psonis et al.,

2017a), as well as in other members of the family Lacertidae [e.g. *Lacerta* (Sagonas et al., 2014) and *Mesalina* (Kapli et al., 2008)]. However, this lack of ecological differentiation should be taken with a pinch grain of salt, due to the lack of presence points that could be assigned with certainty to one of the two lineages. It is also worth noting, that although these lineages are, in most part, geographically isolated, there is at least one "contact zone" close to lake Stymfalia in northeast Peloponnisos (Fig. 1). The existence of this zone, reinforces the idea that there are probably two distinct species since there is no evidence of gene flow between these two lineages, as indicated by the microsatellite data, even though they also occur in sympatry.

Regarding *P. cretensis*, one could argue that even though the species delimitation analyses do not support its split into two species (East and West), they could be considered as separate conservation units based on both mtDNA and microsatellite data analyses. This is further supported based on their distinct allopatric distribution, the reduced dispersal abilities of the lizards, and the lack of gene flow between these two groups of populations, that differ significantly, as it is indicated by the microsatellite data. Furthermore, there is no indication (excluding the human aided dispersal) that this could change in the foreseeable future, therefore it is expected that these two populations would continue to diverge further apart. Diversification in the populations' ecological preferences seems to have already begun as supported by the niche similarity analysis results which indicated that the eastern and western populations show no or limited niche overlap.

4.2. Population structure of the P. Cretensis species group

Observed heterozygosity within population clusters was lower than expected for the majority of microsatellite loci, indicating genetic structuring. This notion was further supported from the results of the STRUCTURE and DAPC analysis, and the inferred population structure within *P. cretensis* and *P. peloponnesiacus*.

Overall, both methods of population structure analysis obtained the same 5 clusters within the *P. cretensis* species group, while clearly revealing further population division within *P. cretensis* and *P. peloponnesiacus*. The species of *P. cretensis* is discriminated into two clusters, each geographically corresponding to either western or eastern Crete. Lower hierarchical levels of analyses indicated further population subdivision within each Cretan cluster, though, based on our present data, those do not correspond to geographically distinct groups. Within the eastern cluster, the probable number of population subdivisions may relate to the different islets or islet groups occupied by the species, while the implied sub-structuring within the western populations could be attributed to the complex geomorphology and geological history of the island.

In contrast with the unclear genetic structuring within the two *P. cretensis* clusters based on microsatellite loci, the individuals of *P. peloponnesiacus* were unequivocally assigned to two geographically distinct clusters; W. and E. Peloponnisos. Remarkably, the aforementioned results coincide with the two phylogenetic clades of *P. peloponnesiacus*, strongly supporting the discrimination of the *P. peloponnesiacus* populations into two divergent groups, rendering reevaluation of the species current taxonomic status indispensable.

In the case of the *P. levendis* cluster, the isolated islets of Pori and Poreti may have created sub-structuring within the species and therefore could be responsible for the estimated heterozygote deficiency. However, since only two specimens from Poreti were used in the analysis, this proposition cannot be supported and other alternatives should be taken into consideration.

4.3. Phylogeographic patterns

In general, it seems that the biogeographical history of the *P. cretensis* species group in the region is mainly the result of recurring vicariance events, such as the vicariant isolation of the three major regions (Crete, Peloponnisos, Pori) leading to the three major clades of the phylogenetic tree (*P. cretensis*, *P. pelonnesiacus* and *P. levendis*).

Based on the paleogeographic history of the island and the results of the present study, we hypothesize that the western populations, which had colonized Crete coming from the northwest prior to its isolation from Peloponnisos, expanded their distribution towards the eastern part of the island, when those were one united landmass (Dermitzakis, 1990b). Throughout the eustatic events of the Pleistocene, where most of the Aegean islands were continuously reconnected and isolated with each other and the mainland, Crete did not come in contact with any nearby land masses due to the deep trenches of the Cretan sea (Schule, 1993). During this period, the eastern islets separated from the main island where *P. cretensis* continued to inhabit them until today, but for unknown reasons became extinct from the eastern part of the island of Crete. This distinction into eastern and western populations is evident when examining the microsatellite data and the mtDNA, while it is absent when nuclear genes are analyzed. Regardless of the way the species disappeared from eastern mainland Crete, there is still the question of why this happened and what type of barrier is preventing recolonization of the area. One plausible hypothesis is that the eastern part of the island does not satisfy the ecological requirements of the species. This notion is supported by the SDM analysis which indicates that the eastern part of Crete is not suitable for either population. However, further examination of the species' ecology is required in order to be able to definitely conclude which bioclimatic factors are responsible for its' current distribution. It should be noted that this disjunct distribution is unique among all species of "herptiles" studied in Crete (Kyriazi et al., 2013 and references therein; Sagonas et al., 2014), even among those that share the same colonization route as *P. cretensis* [*Telescopus fallax* (Kyriazi et al., 2013) and *Pelophylax cretensis* (Lymberakis et al., 2007)].

Around the same time that Crete was permanently isolated from mainland Greece, it seems that the island group of Pori also became separated from Peloponnisos, leading to the lineage of *P. levendis*. The neighboring islands of Kythira and Antikythira, which are located at a small distance from the island group of Pori, are not inhabited by *P. levendis*. Its absence in Kythira could be attributed to the islands' submergence throughout the Pliocene (Meulenkamp, 1985), while for Antikythira no informative explanation can be given. The SDM model showed potential distribution in Antikythira isl., south Kythira isl. and in the coastal area of central Crete (Rethymno area).

Peloponnisos became isolated from continental Greece approximately 3.5 Mya and remained as an island for about 2 million years until the Pleistocene (Creutzburg, 1963; Dermitzakis, 1990a, b). The populations of *P. peloponnesiacus* diverged from the rest of the *P. cretensis* species group with the separation of Crete and the island group of Pori from Peloponnisos. Diversification within the species was estimated at 1.86 Mya leading to the two aforementioned geographically distinct clades, W. Peloponnisos and E. Peloponnisos. Similar discrimination into eastern and western lineages within the Peloponissos region have also been found in the genus *Lacerta* (Sagonas et al., 2014). Interestingly, the SDM analysis in this case, showed partial overlap between the putative distributions of the two lineages despite there being strong evidence for their isolation. However, this could be due to the aforementioned limited number of usable presence points. Regarding the co-occurrence of the two lineages around the area of lake Stymfalia and because there is no evidence of gene flow between them, we assume that this is a case of secondary conduct of previous isolated populations.

Overall, the proposed phylogeographic scenario is in agreement with a previous study by Poulakakis et al. (2005b). They suggested that the species comprising the *P. cretensis* species group evolved from an ancestral form that was distributed in the area of Peloponnisos, the island group of Pori and Crete, when these regions were united into one landmass and that a series of vicariant events, namely the separation of Crete and the island group of Pori from Peloponnisos around 5–5.5

Mya, led to the diversification of the three distinct lineages which correspond to the taxa recognized today as *P. cretensis*, *P. levendis* and *P. peloponnesiacus*. Adding to that pre-existing scenario, we also hypothesize that an ancestral form of the two *P. cretensis* lineages seen today, was initially distributed throughout Crete and its satellite islets that were still connected at this point in time. The separation of the eastern islets from mainland Crete and the extinction of the taxon from the eastern part of the island led to the populations observed today. Furthermore, the ancestral form of the two *P. peloponnesiacus* lineages observed today was probably distributed throughout Peloponnisos and was subdivided into two populations by a vicarianistic event around 1.89 Mya.

4.4. Conclusions

In conclusion, the present study highlights the fact that the lizards of the genus *Podarcis* can be considered as a model species with their evolutionary past reflecting the history of the areas in which they are distributed thus shedding light to the interplay of paleogeography, environment and selection that has shaped the genetic diversity we see today. Lizards of the genus *Podarcis* as well as other taxa with similar dispersal abilities and limitations, are highly impacted not only by the geological history of the area that they inhabit where isolation (e.g. in the case of *P. peloponnesiacus*) and insularity (e.g. in the cases of *P. cretensis* and *P. levendis*) play an important role in speciation, but also by the current environmental conditions that maintain the generated genetic distinction. Extensive sampling both in terms of samples and markers analyzed is needed when designing similar studies combined with multilateral analysis methods as to obtain the clearest possible view of the issue in question.

Acknowledgements

We wish to express our gratitude to Panayiotis Pafilis (Department of Biology, National & Kapodistrian University of Athens) for sending us several tissue samples. We also thank Manolis Nikolakakis (Natural History Museum of Crete, University of Crete) for helping in map drawing and Apostolos Trichas (Natural History Museum of Crete, University of Crete) for providing the photo that was used on the graphical abstract. This study was funded by NSFR 2007-2013 programme for development, European Social Fund, Operational Programme, Education and Lifelong Learning investing in knowledge society, Ministry of Education and Religious Affairs, Managing Authority, Co-financed by Greece and the European Union.

Appendix A. Supplementary material

The supporting information is provided in five different files containing supporting figures and tables. Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2019.05.026.

References

Amos, W., Hoffman, J.I., Frodsham, A., Zhang, L., Best, S., Hill, A.V.S., 2007. Automated binning of microsatellite alleles: problems and solutions. Mol. Ecol. Notes 7, 10–14.
Araujo, M.B., Guisan, A., 2006. Five (or so) challenges for species distribution modelling. J. Biogeogr. 33, 1677–1688.
Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M.A., Alekseyenko, A.V., 2012. Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. Mol. Biol. Evol. 29, 2157–2167.
Beerli, P., Hotz, H., Uzzell, T., 1996. Geologically dated sea barriers calibrate a protein clock for aegean water frogs. Evolution 50, 1676–1687.
Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, F., 2001. GENETIX, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions CNRS UMR 5000: Université de Montpellier II, Montpellier, France.
Bittkau, C., Comes, H.P., 2005. Evolutionary processes in a continental island system: molecular phylogeography of the Aegean *Nigella arvensis* alliance (Ranunculaceae) inferred from chloroplast DNA. Mol. Ecol. 14, 4065–4083.
Blondel, J., Aronson, J., Bodiou, J.-Y., Boeuf, G., 2010. The Mediterranean Region. Biological Diversity in Space and Time. Oxford University Press Inc., New York.
Bobrowski, M., Schickhoff, U., 2017. Why input matters: selection of climate data sets for modelling the potential distribution of a treeline species in the Himalayan region. Ecol. Model. 359, 92–102.
Bouckaert, R., Heled, J., Kuhnert, D., Vaughan, T., Wu, C.H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 10, e1003537.
Bruford, M.W., Hanotte, O., Burke, T., 1998. Multi and single locus DNA fingerprinting. In: Hoelzel, A.R. (Ed.), Molecular Genetic Analysis of Populations: A Practical Approach. IRL Press, pp. 225–269.
Creutzburg, N., 1963. Paleogeographic evolution of Crete from Miocene till our days. Cretan Ann. 15 (16), 336–342.
Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9 772 772.
Dermitzakis, D.M., 1990a. The colonisation of Aegean islands in relation with the paleogeographic evolution. Biologia Gallo - Hellenica 17, 99–130.
Dermitzakis, D.M., 1990b. Paleogeography, geodynamic processes and event stratigraphy during the late Cenozoic of the Aegean area. Atti Convegni Lincei 85, 263–288.
Drake, D.R., Mulder, C.P.H., Towns, D.R., Daugherty, C.H., 2002. The biology of insularity: an introduction. J. Biogeogr. 29, 563–569.
Earl, D.A., von Holdt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4, 359–361.
Elith, J., Graham, C.H., Anderson, R.P., Dudik, M., Ferrier, S., Guisan, A., Hijmans, R.J., Huettmann, F., Leathwick, J.R., Lehmann, A., Li, J., Lohmann, L.G., Loiselle, B.A., Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., Overton, J.M., Peterson, A.T., Phillips, S.J., Richardson, K., Scachetti-Pereira, R., Schapire, R.E., Soberon, J., Williams, S., Wisz, M.S., Zimmermann, N.E., 2006. Novel methods improve prediction of species' distributions from occurrence data. Ecography 29, 129–151.
Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620.
Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321.
Harris, D.J., Arnold, E.N., 1999. Relationships of wall lizards, *Podarcis* (Reptilia: Lacertidae) based on mitochondrial DNA sequences. Copeia 749–754.
Harris, D.J., Carranza, S., Arnold, E.N., Pinho, C., Ferrand, N., 2002. Complex biogeographical distribution of genetic variation within *Podarcis* wall lizards across the Strait of Gibraltar. J. Biogeogr. 29, 1257–1262.
Hernandez, P.A., Graham, C.H., Master, L.L., Albert, D.L., 2006. The effect of sample size and species characteristics on performance of different species distribution modeling methods. Ecography 29, 773–785.
Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
Jakobsson, M., Rosenberg, N.A., 2007. CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23, 1801–1806.
Jombart, T., Ahmed, I., 2011. adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics 27, 3070–3071.
Jombart, T., Devillard, S., Balloux, F., 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11.
Jones, G.R., 2016. Algorithmic improvements to species delimitation and phylogeny estimation under the multispecies coalescent. J. Math. Biol. 74, 447–467.
Jones, G., Aydin, Z., Oxelman, B., 2015. DISSECT: An assignment-free Bayesian discovery method for species delimitation under the multispecies coalescent. Bioinformatics 31, 991–998.
Kaliontzopoulou, A., Brito, J.C., Carretero, M.A., Larbes, S., Harris, D.J., 2008. Modelling the partially unknown distribution of wall lizards (Podarcis) in North Africa: ecological affinities, potential areas of occurrence, and methodological constraints. Canadian Journal of Zoology-Revue Canadienne De Zoologie 86, 992–1001.
Kaliontzopoulou, A., Pinho, C., Harris, D.J., Carretero, M.A., 2011. When cryptic diversity blurs the picture: a cautionary tale from Iberian and North African *Podarcis* wall lizards. Biol. J. Linn. Soc. 103, 779–800.
Kapli, P., Lymberakis, P., Poulakakis, N., Mantziou, G., Parmakelis, A., Mylonas, M., 2008. Molecular phylogeny of three *Mesalina* (Reptilia: Lacertidae) species (*M. guttulata, M. brevirostris* and *M. bahaeldini*) from North Africa and the Middle East: Another case of paraphyly? Mol. Phylogen. Evol. 49, 102–110.
Karger, D.N., Conrad, O., Bohner, J., Kawohl, T., Kreft, H., Soria-Auza, R.W., Zimmermann, N.E., Linder, H.P., Kessler, M., 2017. Data Descriptor: Climatologies at high resolution for the earth's land surface areas. Sci. Data 4.
Krijgsman, W., Hilgen, F.J., Raffi, I., Sierro, F.J., Wilson, D.S., 1999. Chronology, causes and progression of the Messinian salinity crisis. Nature 400, 652–655.
Kyriazi, P., Kornilios, P., Nagy, Z.T., Poulakakis, N., Kumlutaş, Y., Ilgaz, Ç., Avcı, A., Göçmen, B., Lymberakis, P., 2013. Comparative phylogeography reveals distinct colonization patterns of Cretan snakes. J. Biogeogr. 40, 1143–1155.
Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29, 1695–1701.
Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., Calcott, B., 2016. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. 34, 772–773.
Leache, A.D., Wagner, P., Linkem, C.W., Bohme, W., Papenfuss, T.J., Chong, R.A., Lavin, B.R., Bauer, A.M., Nielsen, S.V., Greenbaum, E., Rodel, M.O., Schmitz, A., LeBreton, M., Ineich, I., Chirio, L., Ofori-Boateng, C., Eniang, E.A., El Din, S.B., Lemmon, A.R.,

Burbrink, F.T., 2014. A hybrid phylogenetic-phylogenomic approach for species tree estimation in African Agama lizards with applications to biogeography, character evolution, and diversification. Mol. Phylogen. Evol. 79, 215–230.
Librado, P., Rozas, J., 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.
Lima, A., Pinho, C., Larbes, S., Carretero, M.A., Brito, J.C., Harris, D.J., 2009. Relationships of *Podarcis* wall lizards from Algeria based on mtDNA data. Amphibia-Reptilia 30, 483–492.
Lymberakis, P., Poulakakis, N., 2010. Three continents claiming an archipelago: the evolution of Aegean's herpetofaunal diversity. Diversity 2, 233–255.
Lymberakis, P., Poulakakis, N., Kaliontzopotilou, A., Valakos, E., Mylonas, M., 2008. Two new species of *Podarcis* (Squamata; Lacertidae) from Greece. Syst. Biodivers. 6, 307–318.
Lymberakis, P., Poulakakis, N., Manthalou, G., Tsigenopoulos, C.S., Magoulas, A., Mylonas, M., 2007. Mitochondrial phylogeography of *Rana (Pelophylax)* populations in the Eastern Mediterranean region. Mol. Phylogen. Evol. 44, 115–125.
Manel, S., Williams, H.C., Ormerod, S.J., 2001. Evaluating presence-absence models in ecology: the need to account for prevalence. J. Appl. Ecol. 38, 921–931.
Meulenkamp, J.E., 1985. Aspects of the late Cenozoic evolution of the Aegean region. In: Stanley, D.J., Wezel, F.C. (Eds.), Geological Evolution of the Mediterranean Basin. Springer, New York, pp. 307–321.
Peterson, A.T., Soberón, J., Pearson, R.G., Anderson, R.P., Martínez-Meyer, E., Nakamura, M., Bastos, M., 2011. Cological Niches and Geographic Distributions. Princeton University Press.
Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecol. Modell. 190, 231–259.
Phillips, S.J., Dudik, M., 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography 31, 161–175.
Phillips, S.J., Dudík, M., Schapire, R.E., 2004. A maximum entropy approach to species distribution modeling. In: Proceedings of the 21st International Conference on Machine Learning. ACM, Banff, Canada, pp. 655–662.
Phillips, S.J., Dudík, M., Schapire., R.E., 2018 [Internet]. Maxent software for modeling species niches and distributions (Version 3.4.1). Available from: < http://biodiversityinformatics.amnh.org/open_source/maxent/ > (accessed on 2018-11-22).
Pinho, C., Ferrand, N., Harris, D.J., 2006. Reexamination of the Iberian and North African *Podarcis* (Squamata: Lacertidae) phylogeny based on increased mitochondrial DNA sequencing. Mol. Phylogen. Evol. 38, 266–273.
Pinho, C., Harris, D.J., Ferrand, N., 2007. Comparing patterns of nuclear and mitochondrial divergence in a cryptic species complex: the case of Iberian and North African wall lizards (Podarcis, Lacertidae). Biol J Linn Soc Lond 91, 121–133.
Pinho, C., Harris, D.J., Ferrand, N., 2008. Non-equilibrium estimates of gene flow inferred from nuclear genealogies suggest that Iberian and North African wall lizards (Podarcis spp.) are an assemblage of incipient species. BMC Evol. Biol. 8.
Poulakakis, N., Kapli, P., Lymberakis, P., Trichas, A., Vardinoyiannis, K., Sfenthourakis, S., Mylonas, M., 2015. A review of phylogeographic analyses of animal taxa from the Aegean and surrounding regions. J. Zool. Syst. Evol. Res. 53, 18–32.
Poulakakis, N., Lymberakis, P., Antoniou, A., Chalkia, D., Zouros, E., Mylonas, M., Valakos, E., 2003. Molecular phylogeny and biogeography of the wall-lizard *Podarcis erhardii* (Squamata: Lacertidae). Mol. Phylogen. Evol. 28, 38–46.
Poulakakis, N., Lymberakis, P., Valakos, E., Pafilis, P., Zouros, E., Mylonas, M., 2005a. Phylogeography of Balkan wall lizard (*Podarcis taurica*) and its relatives inferred from mitochondrial DNA sequences. Mol. Ecol. 14, 2433–2443.
Poulakakis, N., Lymberakis, P., Valakos, E., Zouros, E., Mylonas, M., 2005b. Phylogenetic relationships and biogeography of *Podarcis* species from the Balkan Peninsula, by Bayesian and maximum likelihood analyses of mitochondrial DNA sequences. Mol. Phylogen. Evol. 37, 845–857.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Psonis, N., Antoniou, A., Kukushkin, O., Jablonski, D., Petrov, B., Crnobrnja-Isailovic, J., Sotiropoulos, K., Gherghel, I., Lymberakis, P., Poulakakis, N., 2017a. Hidden diversity in the *Podarcis tauricus* (Sauria, Lacertidae) species subgroup in the light of multilocus phylogeny and species delimitation. Mol. Phylogen. Evol. 106, 6–17.
Psonis, N., Antoniou, A., Kukushkin, O., Jablonski, D., Petrov, B., Crnobrnja-Isailović, J., Sotiropoulos, K., Gherghel, I., Lymberakis, P., Poulakakis, N., 2017b. Hidden diversity in the *Podarcis tauricus* (Sauria, Lacertidae) species subgroup in the light of multilocus phylogeny and species delimitation. Mol. Phylogenet. Evol. 106, 6–17.
Radosavljevic, A., Anderson, R.P., 2014. Making better MAXENT models of species distributions: complexity, overfitting and evaluation. J. Biogeogr. 41, 629–643.
Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1.6. Available from: < http://beast.bio.ed.ac.uk/Tracer > .
Raymond, M., Rousset, F., 1995. Genepop (Version-1.2) - Population-genetics software for exact tests and ecumenicism. J. Hered. 86, 248–249.
Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Syst. Biol. 57, 4–14.
Rodder, D., Engler, J.O., 2011. Quantitative metrics of overlaps in Grinnellian niches: advances and possible drawbacks. Glob. Ecol. Biogeogr. 20, 915–927.
Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.
Rousset, F., 2008. GENEPOP ' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol. Ecol. Resour. 8, 103–106.
Sagonas, K., Poulakakis, N., Lymberakis, P., Parmakelis, A., Pafilis, P., Valakos, E.D., 2014. Molecular systematics and historical biogeography of the green lizards (*Lacerta*) in Greece: Insights from mitochondrial and nuclear DNA. Mol. Phylogen. Evol. 76, 144–154.
Salvi, D., Pinho, C., Harris, D.J., 2017. Digging up the roots of an insular hotspot of genetic diversity: decoupled mito-nuclear histories in the evolution of the Corsican-Sardinian endemic lizard Podarcis tiliguerta. BMC Evol. Biol. 17.
Schule, W., 1993. Mammals, vegetation and the initial human settlement of the Mediterranean islands: a paleoecological approach. J. Biogeogr. 20, 399–412.
Silvestro, D., Michalak, I., 2011. raxmlGUI: a graphical front-end for RAxML. Org. Divers. Evol. 12, 335–337.
Soberon, J., Nakamura, M., 2009. Niches and distributional areas: Concepts, methods, and assumptions. Proc. Natl. Acad. Sci. USA 106, 19644–19650.
Stephens, M., Smith, N.J., Donnelly, P., 2001. A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. 68, 978–989.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.
Toonen, R.J., Hughes, S., 2001. Increased throughput for fragment analysis on an ABI PRISM (R) automated sequencer using a membrane comb and STRand software. Biotechniques 31, 1320–1324.
Uetz, P., Freed, P., Hošek, J., 2018. The Reptile Database. Available from: < http://www.reptile-database.org/ > .
Van der Geer, A., Lyras, G., De Vos, J., Dermitzakis, M., 2011. Evolution of Island Mammals: Adaptation and Extinction of Placental Mammals on Islands. John Wiley & Sons.
Warren, D.L., Glor, R.E., Turelli, M., 2008. Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. Evolution 62, 2868–2883.
Warren, D.L., Glor, R.E., Turelli, M., 2011. Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. (vol 62, pg 2868, 2008). Evolution 65 1215-1215.
Wisz, M.S., Hijmans, R.J., Li, J., Peterson, A.T., Graham, C.H., Guisan, A., Distribut, N.P.S., 2008. Effects of sample size on the performance of species distribution models. Divers. Distrib. 14, 763–773.
Yang, Z., 2006. Computational Molecular Evolution. Oxford University Press, Oxford, New York.
Yang, Z.H., 2015. The BPP program for species tree estimation and species delimitation. Curr. Zool. 61, 854–865.
Yang, Z.H., Rannala, B., 2014. Unguided species delimitation using DNA sequence data from multiple Loci. Mol. Biol. Evol. 31, 3125–3135.
Yu, Y., Harris, A.J., Blair, C., He, X.J., 2015. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. Mol. Phylogenet. Evol. 87, 46–49.
Zhang, J., Kapli, P., Pavlidis, P., Stamatakis, A., 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29, 2869–2876.