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# Hidden relationships and genetic diversity: Molecular phylogeny and phylogeography of the Levantine lizards of the genus *Phoenicolacerta* (Squamata: Lacertidae) ☆

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ABSTRACT

The Levant region witnessed dramatic tectonic events and climatic fluctuations that changed the historical landscape of the area and consequently influenced the cladogenesis and distribution of the local biota. In this study we use information from two mitochondrial and two nuclear genes and species delimitation methods in order to obtain the first robust time-calibrated molecular phylogeny of the Levantine rock lizards of the genus *Phoenicolacerta*. We sampled from across its distributional range with the aim to clarify its systematics, biogeography and evolution. Our results suggest that the genus includes two well-supported clades, one comprising solely the montane species *Phoenicolacerta kulzeri*, and the other including the three remaining species, the relatively widespread, *P. laevis*, the Syrian-Turkish *P. cyanisparsa* and the Cypriot endemic *P. troodica*. We found that both *P. laevis* and *P. cyanisparsa* are not monophyletic, as the Turkish populations of *P. laevis* branch within *P. cyanisparsa*. We found high levels of undescribed diversity within *P. laevis* which necessitate a thorough revision. We suggest that *Phoenicolacerta* started radiating during the mid-late Miocene, and that both vicariance and dispersal events shaped the diversification and distribution of the genus concomitantly with the formation of major geological structures and climatic fluctuations in the Levant. These results highlight the region as an important center of speciation, contributing to the species diversity of the eastern Mediterranean.


## 1. Introduction

The lizard family Lacertidae includes over 40 genera, and is divided into the subfamilies Gallotiinae and Lacertinae. The latter includes two main tribes, the Lacertini (distributed mainly in Eurasia) and Eremiadini (distributed mainly in Asia and Africa) (Arnold, 1973, 1989; Arnold et al., 2007; see reference therein). Arnold et al. (2007) revised the Lacertini tribe, using both morphological and molecular data. However, the systematics of the tribe remains complex and phylogenetic relationships among and within many genera are unresolved (Arnold et al., 2007; Pavlicev and Mayer, 2009; Kapli et al., 2011).

The Levantine rock lizards of the genus *Phoenicolacerta* Arnold, Arribas and Carranza, 2007 are such an example. *Phoenicolacerta* has a Levantine distribution, ranging from southwest Jordan, through central and northern Israel, western Syria and Lebanon to southern Turkey and Cyprus (Fig. 1; Arnold et al., 2007; Sindaco and Jeremčenko, 2008). These Levantine lizards, named after the land of the Phoenicians in the eastern Mediterranean, inhabit a variety of habitats in the Mediterranean and Irano-Turanian ecoregions from ~60 m below sea level to rocky cliffs and mountains over 2200 m. The genus is thought to include four species (Arnold et al., 2007; Fig. 1). *Phoenicolacerta laevis* (Gray, 1838) is the most widespread species of the genus, ranging from the Mediterranean areas of Israel northwards to southern Turkey (Sindaco and Jeremčenko, 2008) with, possibly introduced, populations along the southern coast of Anatolia (see Kariş and Göçmen, 2014). *Phoenicolacerta cyanisparsa* (Schmidtler and Bischoff, 1999) is endemic to a narrow range in northwestern Syria and adjacent southeastern Turkey. *Phoenicolacerta kulzeri* (Müller and Wettstein, 1932) has a disjunct distribution with isolated populations at high elevations in mountains from southern

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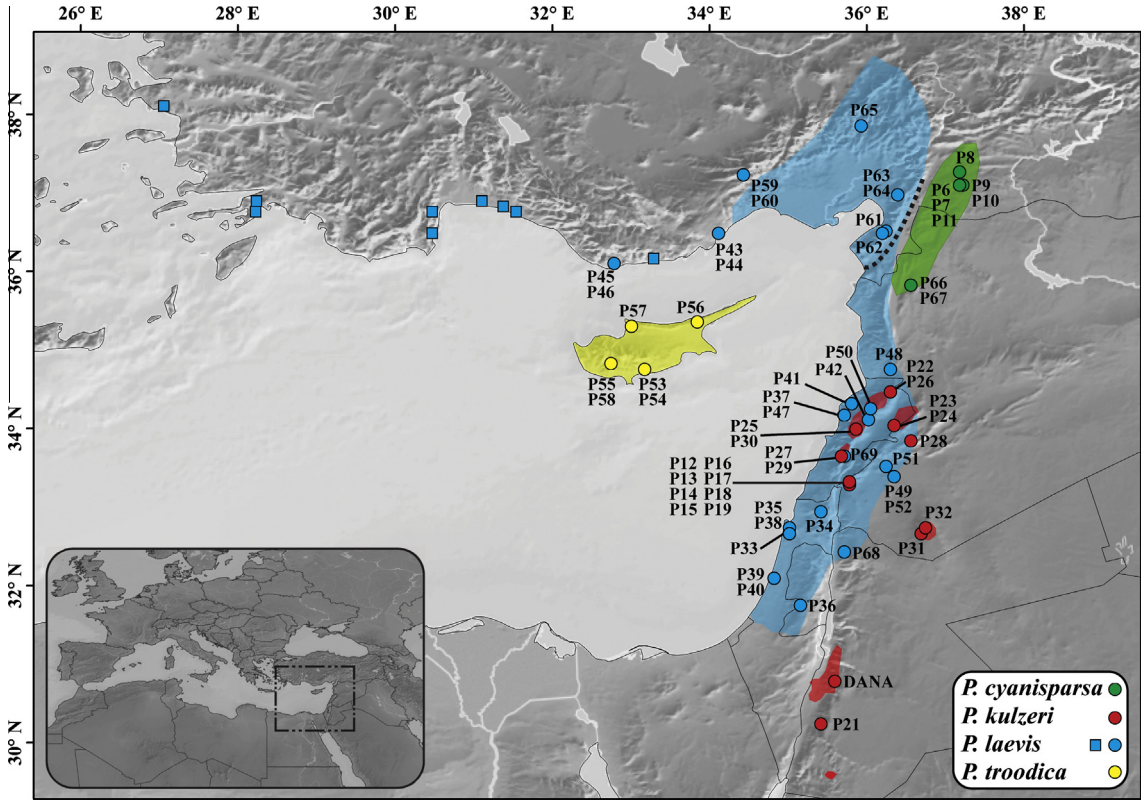


**Fig. 1.** Sampling localities of the specimens used in this study, with the global distribution range of each species (circles; data modified from IUCN, http://www.iucnredlist.org/). Recent localities of *P. laevis* in southern and West Turkey (rectangles) are added (Kariş and Göçmen, 2014; see reference therein). The black dashed line marks the Amanos Mountains and the Amik Basin in southern Turkey. Locality codes correlate to specimens in Table S1 and colours in Figs. 2–4 and S2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Jordan to Lebanon and south-western Syria (Disi et al., 2001; in den Bosch, 2002; Sindaco and Jeremčenko, 2008). It comprises three subspecies: *P. k. kulzeri* (Müller and Wettstein, 1932) from Israel, Lebanon and Syria, *P. k. petraea* (Bischoff and Müller, 1999) from southern Jordan, and *P. k. khazaliensis* Modrý et al., 2013 from Wadi Ramm in southern Jordan. The last species in the genus, *P. troodica* (Werner, 1936), is endemic to Cyprus (Baier et al., 2009).

The taxonomic history of *Phoenicolacerta* has seen many contradictions, disagreements and debates, as the classification of species and their taxonomic status often changed (Bischoff and Schmidtler, 1999; in den Bosch, 2002; see reference therein). Until the late 1990s all currently recognized species were treated as subspecies of *P. laevis* or as part of a *laevis-kulzeri* complex. Although Arnold et al. (2007) sampled only *P. laevis* and *P. kulzeri,* they accepted the differentiation between *P. kulzeri*, *P. laevis* and *P. cyanisparsa*, and elevated *P. laevis troodica* to specific level. These classifications are accepted to date (Uetz, 2015).

Several studies have attempted to resolve the relationships within and among *Phoenicolacerta* species using behavioral, ecological, morphological, serological, karyological and anatomical approaches (e.g., Bischoff and Franzen, 1993; Budak and Göçmen, 1995; Bischoff and Schmidtler, 1999; Tosunoğlu et al., 1999, 2001; in den Bosch and Zandee, 2001). Molecular studies, however, were few. The three genetic studies of the genus, focused mainly on *P. kulzeri* and *P. laevis*. Beyerlein and Mayer (1999) sequenced the mitochondrial 12S and 16S rRNA genes of four lizards in order to resolve the relationships of *P. kulzeri* and *P. laevis* with other species of the paraphyletic genus "*Lacerta*". They concluded that *P. laevis* and *P. kulzeri* are clearly close, but separate species, with unexpectedly large intraspecific variability. in den Bosch et al. (2003), used karyological and mitochondrial data (cytochrome *b*) and found low levels of intraspecific diversity within *P. kulzeri*, which was clearly separated from, but closely related to, *P. laevis*. Pavlicev and Mayer (2006) used the mitochondrial cytochrome *b* and the nuclear c-*mos* (including a pseudogene) genes to infer the phylogenetic relationships of *Phoenicolacerta* and found that *P. laevis* formed two distinct groups (northern and southern) and *P. cyanisparsa* clustered with northern *P. laevis*, making this species paraphyletic. Their phylogeny, however, failed to provide sufficient resolution for the placement of *P. kulzeri*.

The Levant region, where *Phoenicolacerta* is distributed, contains the intersections of several major geological structures. Since the Miocene, this region has been characterized by the relative tectonic movements of the Arabian, Anatolian and African plates (McKenzie, 1978; Pichon and Angelier, 1979; Dewey et al., 1986; Westaway, 1994; Over et al., 2004; Inwood et al., 2009). These movements resulted in the Levant being an intermittent land-bridge between Eurasia and Africa (Over et al., 2004; Inwood et al., 2009). Since the late Miocene, the intensive geological events in the Levant formed the Dead Sea Fault and the East Anatolian Fault, among other structures. These geological elements shaped the current known landscape such as the Taurus, Amanos and Zagros Mountains, the Amik basin and the Jordan rift valley. These geological events have affected the distribution of many vertebrates, including many reptilian taxa in Anatolia and the southern Levant (e.g., Bilgin, 2011; Kornilios et al., 2012; Ahmadzadeh et al., 2013b; Kapli et al., 2013). In addition to these tectonic events, climatic changes during the late Miocene and the Pliocene resulted in aridification and the establishment of the Mediterranean climate. Dry zones expanded and forests were replaced by woodlands and grasslands in mid-latitude regions (Hsü et al., 1977; Fauquette et al., 1999; Cavazza and Wezel,

2003). Temporal climatic oscillations between arid and wet conditions, greatly changed the regions habitat composition and subsequently the evolutionary and biogeographical history of its representative biota (Prentice and Jolly, 2000; Douady et al., 2003; Schuster et al., 2006).

Species classification is traditionally based on morphological differences, either qualitative or quantitative, though these studies at times cannot identify or differentiate between evolutionary lineages. Great similarities and incomplete appearance of distinct and differentiating characters may cause confusion between closely related taxa or cryptic species (Sáez and Lozano, 2005; Beheregaray and Caccone, 2007; Bickford et al., 2007; Kaliontzopoulou et al., 2012). Incorrect species ascription of specimens/populations may result from intraspecific variability or morphological similarities due to phenotypic plasticity or due to environmental adaptation as convergence resulted from local conditions and pressures (Nevo, 2001; Sears and Angilletta, 2003; Weitere et al., 2004; Kaliontzopoulou et al., 2010; Edwards et al., 2012; Tamar et al., 2014). Several recently published studies of Levantine reptiles have used molecular tools in order to elucidate and understand their inter and intraspecific relationships and phylogeography. These studies have revealed high levels of genetic differentiation and cryptic diversity that do not accord with the current taxonomy (e.g., Kapli et al., 2013; Ahmadzadeh et al., 2013b; Tamar et al., 2014; Bellati et al., 2015). The use of molecular data thus may help evaluate the relationships between closely related species, or reveal the presence of distinct taxa that are at times morphologically indistinguishable.

Although there are four recognized species within *Phoenicolacerta*, their intra and interspecific relationships are largely unresolved. In order to clarify the systematics and to determine the role of diversification processes in the evolutionary history of this genus, we inferred the phylogenetic relationships using multi-locus genetic data including gene and species trees and coalescent-based methods for species delimitation. We provide insight on the systematics, phylogeography and evolution of multiple populations from all four species within the genus.

## 2. Material and methods

### 2.1. Taxon sampling

We included in the phylogenetic analyses 64 samples of all four currently recognized species of *Phoenicolacerta*, from across the genus range (Fig. 1). One sequence of *P. kulzeri* from Dana, Jordan was retrieved from GenBank. As the relationships within the Lacertini tribe are unclear, in order to ensure the monophyly of *Phoenicolacerta*, we retrieved and analyzed from GenBank the 12S and *cytb* sequences from Arnold et al. (2007) coupled with the same gene fragments sequences from this study (see Fig. S1 for the tree and methods). Based on published evidence and our analysis we used *Podarcis filfolensis, P. muralis, P. sicula* and *Zootoca vivipara* (sequences retrieved from GenBank), as the close outgroups for the phylogenetic analysis (Harris et al., 1998; Pavlicev and Mayer, 2009; Kapli et al., 2011; Pyron et al., 2013). Two members of the Erimiadini tribe, *Acanthodactylus blanfordi* and *A. cantoris*, were used as distant outgroups to root the tree (Fu, 2000; Kapli et al., 2011; Pyron et al., 2013). Sample codes, vouchers, localities and GenBank accession numbers are given in Table S1. Localities are shown in Fig. 1.

### 2.2. DNA extraction, amplification, and sequence analysis

Genomic DNA was isolated from ethanol-preserved tissue samples using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. In order to identify the evolutionary units within the genus and evaluate the relationships among them, both mitochondrial and nuclear gene fragments were selected. All individuals were sequenced for both strands for two mitochondrial gene fragments, the ribosomal 12S rRNA (12S) and Cytochrome *b* (*cytb*), and two nuclear gene fragments, melano-cortin 1 receptor (MC1R) and acetylcholinergic receptor M4 (ACM4). Primers, PCR conditions and source references are listed in Tamar et al. (2014).

Chromatographs were checked manually, assembled and edited using Geneious v.7.1.5 (Biomatter Ltd.). For the nuclear genes MC1R and ACM4, heterozygous individuals were identified and coded according to the IUPAC ambiguity codes. Coding gene fragments (*cytb*, MC1R and ACM4) were trimmed so that all started by the first codon position and translated into amino acids and no stop codons were observed, suggesting that the sequences were all functional. DNA sequences were aligned for each gene independently using the online application of MAFFT v.7 (Katoh and Standley, 2013) with default parameters (Auto strategy, Gap opening penalty: 1.53, Offset value: 0.0). For the 12S ribosomal fragment we applied the Q-INS-i strategy, in which information on the secondary structure of the RNA is considered. In order to remove regions without specific conservation, and poorly aligned positions of 12S, we used G-blocks (Castresana, 2000) with low stringency options (Talavera and Castresana, 2007). Inter and intra-specific uncorrected *p*-distances with pairwise deletion of the mitochondrial fragments, and the number of variable (*V*) and parsimony informative (Pi) sites were calculated in MEGA v.5.2 (Tamura et al., 2011).

### 2.3. Phylogenetic and network analyses and hypothesis testing

Phylogenetic analyses were performed for the complete dataset simultaneously using partitions by gene and specified by PartitionFinder v.1.1.0 (Lanfear et al., 2012). Analyses using PartitionFinder were performed with the following parameters, including the model estimation for each partition: linked branch length; all models; BIC model selection; greedy schemes search; data blocks of the complete 12S and by codons for the other protein coding genes (*cytb*, MC1R and ACM4). JModelTest v.2.1.5 (Guindon and Gascuel, 2003; Darriba et al., 2012) was used to select the model of sequence evolution under the Akaike Information Criterion (AIC; Akaike, 1974) for each gene partition independently. See Table S2 for a summary of DNA partitions and relevant models.

Phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian (BI) methods. Maximum likelihood analyses were performed with RAxML v.7.4.2 (Stamatakis, 2006) using RAxMLGUI v.1.3 (Silvestro and Michalak, 2012), with a GTR + G model of evolution and parameters estimated independently for each partition. All ML analyses were performed with 100 random addition replicates and reliability of the tree was assessed by 1000 bootstrap iterations (Felsenstein, 1985). We used a likelihood-ratio test implemented in MEGA v.5.2 (Tamura et al., 2011) to test if the different partitions were evolving in a clock-like fashion. This information was used to choose between the strict-clock and the relaxed uncorrelated lognormal clock priors implemented in BEAST (Monaghan et al., 2009). Bayesian analyses were performed with BEAST v.1.8.0 (Drummond et al., 2012) with the same dataset used in the ML analysis but without outgroups. Three individual runs were performed for $5 \times 10^7$ generations with a sampling frequency of $10^4$. Models are specified in Table S2 and priors applied are as follows (otherwise by default): Coalescence: constant size process of speciation; random starting tree; substitution rate fixed to 1; strict clock; base substitution Uniform (0, 100); alpha Uniform (0, 10); clock rate Uniform (0,

1). Parameter values both for clock and substitution models were unlinked across partitions. The xml file was manually modified to "Ambiguities = true" for the nuclear partitions to account for variability in the heterozygote positions, instead of treating them as missing data.

For all analyses implemented in BEAST, each run was analyzed in Tracer v.1.5 (Rambaut and Drummond, 2003) to confirm effective sample sizes (ESS) were sufficient for all parameters (posterior ESS values >300). LogCombiner and TreeAnnotator (both available in BEAST package) were used to infer the ultrametric tree after discarding 10% of the samples from each run and the production of the chronogram. We treated alignment gaps as missing data, and the nuclear gene sequences were not phased. Nodes were considered strongly supported if they received ML bootstrap values $\geqslant$70% and posterior probability (pp) support values $\geqslant$0.95 (Wilcox et al., 2002; Huelsenbeck and Rannala, 2004).

Haplotype networks were constructed for the nuclear genes MC1R and ACM4 (using only full length sequences). To resolve the multiple heterozygous single nucleotide polymorphisms (detected in the presence of two peaks of approximately equal height at a single nucleotide site), SEQPHASE (Flot, 2010) was used to convert the input files, and the software PHASE v.2.1.1 to resolve phased haplotypes (Stephens et al., 2001; Stephens and Scheet, 2005). Default settings of PHASE were used, except for phase probabilities, which were set as $\geqslant$0.7. All polymorphic sites with a probability of <0.7 were coded in both alleles with the appropriate IUPAC ambiguity code. The phased nuclear sequences were used to generate median-joining (MJ) networks using NETWORKS v.4.6.1.1 (Bandelt et al., 1999).

In order to assess alternative topologies of the populations of *P. laevis* and *P. kulzeri*, topological constraints that could be statistically tested were constructed. We enforced alternative topologies and compared them to the unconstrained best ML tree with the Approximately-Unbiased (AU; Shimodaira, 2002) and Shimodaira–Hasegawa (SH; Shimodaira and Hasegawa, 1999) tests. Per-site log likelihoods were estimated using RAxMLGUI v.1.3 (Silvestro and Michalak, 2012) and *p*-values were calculated using CONSEL (Shimodaira and Hasegawa, 2001).

### 2.4. Species delimitation approaches

To evaluate the relationships and species boundaries within *Phoenicolacerta*, we used different species delimitation approaches including a Bayesian coalescence approach (species tree; Edwards, 2009) and two delimitation methods, using both single and multi-locus analyses. We first used the independent generalized mixed Yule coalescent (GMYC) method (Pons et al., 2006) for estimating species boundaries. As this method relies on single locus data, we used a Bayesian haplotype mitochondrial phylogenetic tree reconstructed with BEAST v.1.8.0 (Drummond et al., 2012). The analysis, priors and parameters applied were as above and the models are specified in Table S2. We preformed the GMYC function implemented in R (R Core Team, 2013) in the R "splits" package (Species Limits by Threshold Statistics; Ezard et al., 2009; package available at http://r-forge.r-project.org/projects/splits). We used a single threshold value, which has already been applied successfully to different groups of organisms (Pons et al., 2006; Fontaneto et al., 2007; Monaghan et al., 2009).

A multi-locus coalescence-based Bayesian species-tree for *Phoenicolacerta* was estimated using *BEAST (Heled and Drummond, 2010). We used the results obtained from the GMYC analyses to define the groups of individuals to be used as "species" (only linages with full dataset were included, excluding outgroups; nuclear genes phased). Three individual runs were performed for $2 \times 10^8$ generations with a sampling frequency of $2 \times 10^4$. Models are specified in Table S2 and priors applied are as follows (otherwise by default): Relaxed Uncorrelated Lognormal Clock (12S, *cytb*), strict clock (MC1R, ACM4); Molecular clock model (estimate); Yule process of speciation; random starting tree; base substitution Uniform (0, 100). Parameter values for both clock and substitution models were unlinked across partitions and the trees for the mtDNA partitions were linked.

To explore the variability within the *kulzeri* and the *laevis* clades, multi-locus coalescent species delimitation analyses were conducted using Bayesian Phylogenetics and Phylogeography (BPP v.2.2; Rannala and Yang, 2003; Yang and Rannala, 2010). We used the species-tree recovered from *BEAST as our guide tree using our phased nuclear data only (MC1R and ACM4 gene fragments). We ran the rjMCMC analyses for $5 \times 10^5$ generations (sampling intervals of five) with a burn-in of $5 \times 10^4$. Both algorithms 0 and 1 implemented in BPP were used, assigning each species delimitation model equal prior probability. As prior distributions on the ancestral population size ($\theta$) and root age ($\tau$) can affect the posterior probabilities for models (Yang and Rannala, 2010), we tested different combinations (Leaché and Fujita, 2010). We tested: (1) a relatively large ancestral population with deep divergences ($\theta = G[1, 10]$; $\tau = G[1, 10]$); (2) a relatively small ancestral population with shallow divergences ($\theta = G[2, 2000]$; $\tau = G[2, 2000]$); (3) a relatively small ancestral population with deep divergences ($\theta = G[2, 2000]$ and $\tau = G[1, 10]$). All analyses were run twice to confirm consistency between runs. We considered speciation probability values $\geqslant$0.95 as strong support for a speciation event.

### 2.5. Estimation of divergence times

Unfortunately, no fossils of *Phoenicolacerta* are currently known, precluding the use of internal calibration points and preventing a direct estimation of the time in our phylogeny. Therefore, we used several geological external calibration points of two lacertid genera, *Gallotia* and *Podarcis*, as was previously used in different lacertid phylogenies (e.g., Poulakakis et al., 2005; Kaliontzopoulou et al., 2011; Carranza and Arnold, 2012; Kapli et al., 2013). Calibrations based on the ages of the Canary Islands and the splits between the different species of the Canary Islands endemic genus *Gallotia* were based on Cox et al. (2010) and Carranza and Arnold (2012), with different priors using the islands' ages as representing times of earliest possible colonisations, thus used as the maximal node age constraints. The calibration points used in this study were as follows: (a) the split between *Gallotia* and *Psammodromus algirus* (age of the oldest islands Fuerteventura and Lanzarote; Normal distribution, mean 18, stdev 2); (b) the split between *G. galloti* and *G. caesaris* (age of La Gomera Island; Normal distribution, mean 6, stdev 3); (c) the split between *G. galloti palmae* and the ancestor of *G. g. galloti* and *G. g. eisentrauti* (age of La Palma Island; Normal distribution, mean 1, stdev 0.5); (d) the splits between *G. gomerana* and *G. simonyi machadoi* and between *G. caesaris caesaris* and *G. c. gomerae* (age of El Hierro Island; Normal distribution, mean 0.8, stdev 0.2). Other calibration points involved the separation between *Podarcis pityusensis* and *Podarcis lilfordi* (endemic to the Balearic Islands; Brown et al., 2008), and between *Podarcis cretensis* and *Podarcis peloponnesiaca* (isolation of Crete from the Peloponnesus; Poulakakis et al., 2005; *cytb* only) both coinciding with the end of the Messinian Salinity Crisis (Normal distribution, mean 5.32, stdev 0.005).

For the estimation of divergence times using the concatenated dataset in BEAST v.1.8.0 (Drummond et al., 2012), one representative of each independent GMYC lineage was used from the ultrametric tree (gene partitions; the nuclear genes unphased; see Table S1). Three individual runs were performed for $5 \times 10^7$ generations with a sampling frequency of $5 \times 10^3$. Models are specified in Table S2 and priors applied are as follows (otherwise by default): Relaxed Uncorrelated Lognormal Clock (12S, *cytb*), strict clock

(MC1R, ACM4); Molecular clock model (estimate); Yule process of speciation; random starting tree; yule.birthRate (0, 1000); alpha Uniform (0, 10); ucld.mean of 12S and *cytb* Uniform (0, 100); clock rate of MC1R and ACM4 Uniform (1, 100). Parameter values for both clock and substitution models were unlinked across partitions.

## 3. Results

Our dataset included 64 *Phoenicolacerta* specimens: eight samples of *P. cyanisparsa*, 21 of *P. kulzeri*, 29 of *P. laevis* and six of *P. troodica*. The dataset included mitochondrial gene fragments of 12S (~388 bp; $V = 53$; Pi = 48) and *cytb* (405 bp; $V = 139$; Pi = 131), and nuclear gene fragments of MC1R (663 bp; $V = 19$; Pi = 12) and ACM4 (429 bp; $V = 7$; Pi = 6) totaling to ~1885 bp. Genetic distances (*p*-distance) between the different populations of *Phoenicolacerta* are presented in Table 1.

### 3.1. Phylogenetic trees and genetic diversity within Phoenicolacerta

The results of the phylogenetic analyses of the complete concatenated and mitochondrial datasets using ML, and without outgroups using BI (for both partition approaches, PartitionFinder and independent genes; see Section 2 and Table S2), produced very similar topologies and differed mostly at less supported nodes in the intraspecific level (Figs. 2 and S2). The genus *Phoenicolacerta* is divided into two well-supported clades, defined here as the *kulzeri* and the *laevis* clades.

The *kulzeri* clade includes only *Phoenicolacerta kulzeri* specimens. This clade is distinct from the other species in the mitochondrial, concatenated, and species trees and in the nuclear haplotype networks (Figs. 2–4 and S2). This species is genetically highly divergent from the remaining species of the genus (12S: 4.8–6.6%; *cytb*: 13.8–15.1%; Table 1). Both subspecies, *P. kulzeri petraea* from southern Jordan (Petra and Dana) and the nominate *P. k. kulzeri* from Israel, Lebanon and Syria, are not monophyletic. One *P. kulzeri petraea* from Dana, Jordan, branches with samples of *P. k. kulzeri* from North Lebanon and a *P. kulzeri petraea* specimen from Petra, Jordan, is nested within the other *P. k. kulzeri* samples (Fig. 2). The specimens from Mt. Hermon group with specimens from Jebel Barouk and Jebel Druze, and those from Jebel Sannin with those from the Anti-Lebanon Mts. (Fig. 2).

The *laevis* clade includes the three remaining species of the genus, *P. laevis*, *P. cyanisparsa* and *P. troodica*. The latter species is well-supported as a distinct, monophyletic lineage, whereas both *P. laevis* and *P. cyanisparsa* are not (Figs. 2 and S2). This clade has split into two well-supported subclades (i.e., L1 and L2). Subclade L1 is comprised solely of the southern populations of *P. laevis* from Syria, Lebanon, Jordan and Israel (i.e., *P. laevis* S). Subclade L2 is comprised of the Cypriot *P. troodica*, the Syrian-Turkish *P. cyanisparsa* and the northern lineage of *P. laevis* from southern Turkey (i.e., *P. laevis* N). The phylogenetic relationships within the southern lineage of *P. laevis* (subclade L1) in the concatenated tree are poorly supported by the ML analysis for both partition approaches (ML: 52/55%; Fig. 2). However, in the mitochondrial tree the nodes are supported by the gene partition approach, but not by PartitionFinder (Fig. S2). Within subclade L1, high genetic distances between specimens were detected (12S: 1.79%; *cytb*: 6.25%). Several geographical groupings are apparent within this southern subclade: specimens from central Israel and the Carmel Mountain group together, as do specimens from northern Israel, southern Lebanon and south-west Syria. Specimens from northern Lebanon form a third group (Figs. 2 and S2). Relationships among these population groups, however, are weakly supported. Subclade L2 is comprised of *P. troodica*, northern *P. laevis* and *P. cyanisparsa*. The latter is paraphyletic with respect to northern *P. laevis*. The northern lineage of *P. laevis* (Turkey; Figs. 2 and S2) is genetically distant from the southern *P. laevis* lineage from southern Syria, Lebanon and Israel (i.e., subclade L1; 12S: 3.8%; *cytb*: 11.9%).

### 3.2. Estimation of divergence times

In the estimation of divergence times, high effective sample sizes were observed for all parameters in all BEAST analyses and assessment of convergence statistics in Tracer indicated that all analyses had converged. The time calibrated analysis (Fig. S3) indicates that diversification between the *kulzeri* and *laevis* clades occurred in the mid-late Miocene, around 9.9 Mya (95% HPD: 6.8–13 Mya; Fig. 2). The *kulzeri* clade seems to have experienced a rather recent radiation, during the late Pliocene or early Pleistocene, approximately 2.3 Mya (95% HPD: 1.4–3.2 Mya). The separation of the *laevis* clade into the two subclades occurred during the late Miocene or early Pliocene approximately 7.1 Mya (95% HPD: 5.1–9.2 Mya). Subclade L1 (the southern lineage of *P. laevis*) started radiating around 5.2 Mya (95% HPD: 3.3–7.1 Mya). Subclade L2 started diverging approximately at the same time, around 5.4 Mya (95% HPD: 3.8–7.1 Mya) with *P. troodica* from Cyprus separating from the other subclade members. Diversification within *P. troodica* started during the late Pliocene/early Pleistocene at 2.8 Mya (95% HPD: 1.7–4 Mya). The lineage of the Syrian-Turkish species, *P. cyanisparsa* split 3.7 Mya (95% HPD: 2.5–5.1 Mya), followed by the later radiation of the southern Turkish lineage of *P. laevis* from the samples from the type locality of *P. cyanisparsa* (Al-Barah in northern Syria) around 2.9 Mya (95% HPD: 1.9–4.1 Mya).

### 3.3. Species delimitation within Phoenicolacerta

The mitochondrial tree clearly reflects the division of the concatenated tree including the non-monophyly of both *P. laevis* and *P. cyanisparsa*, and the distinction between the southern and northern lineages of *P. laevis*. The level of genetic variability within *Phoenicolacerta* is very high and this is reflected in the results of the GMYC analysis that recognized 20 different lineages with the single threshold approach ($\log L_{null} = 239.1636$, $\log L_{GMYC} = 242.7481$; LR = 7.168; $p = 0.03$; Fig. 3A). The analysis with the same dataset partitioned based on PartitionFinder yielded the same topology, with 21 ML independent lineages (sample P51, *P. laevis* from Damascus, Syria, as a distinct "species"; $\log L_{null} = 217.4245$, $\log L_{GMYC} = 220.9484$; LR = 7.047; $p = 0.03$). The GMYC results mainly differ from the concatenated dataset (Fig. 2) in the less supported nodes. The result of the likelihood ratio test was significant for both partition approaches, indicating that the null model (i.e., single population) could be rejected.

The species-tree analysis (Fig. 3B) included 123 sequences for the 13 GMYC entities that had a full set of genes (for seven GMYC "species" the dataset did not include all genes). The topology and clusters revealed in these analyses correspond to the structure from the phylogeny of the ML and BI methods for the two clades, the *kulzeri* clade, the two *laevis* subclades, and the non-monophyly of both *P. cyanisparsa* and *P. laevis*. The analysis revealed high variability within the *kulzeri* and *laevis* clades.

The Bayesian species delimitation results (BPP; nuclear data only) for the *P. kulzeri* populations and the *laevis* clade yielded posterior probabilities all greater than 0.95 across the different prior distributions for $\theta$ and $\tau$ (Fig. 3C and D, respectively). This analysis for *P. kulzeri* supported a three species model (Fig. 3C): (1) Petra, Jordan; (2) North Lebanon Mts. – Central and northern Lebanon including the Anti-Lebanon populations (Jebel Sannin, Jebel Charbine and Ma'alula); (3) Southern Mts. – Southern Lebanon

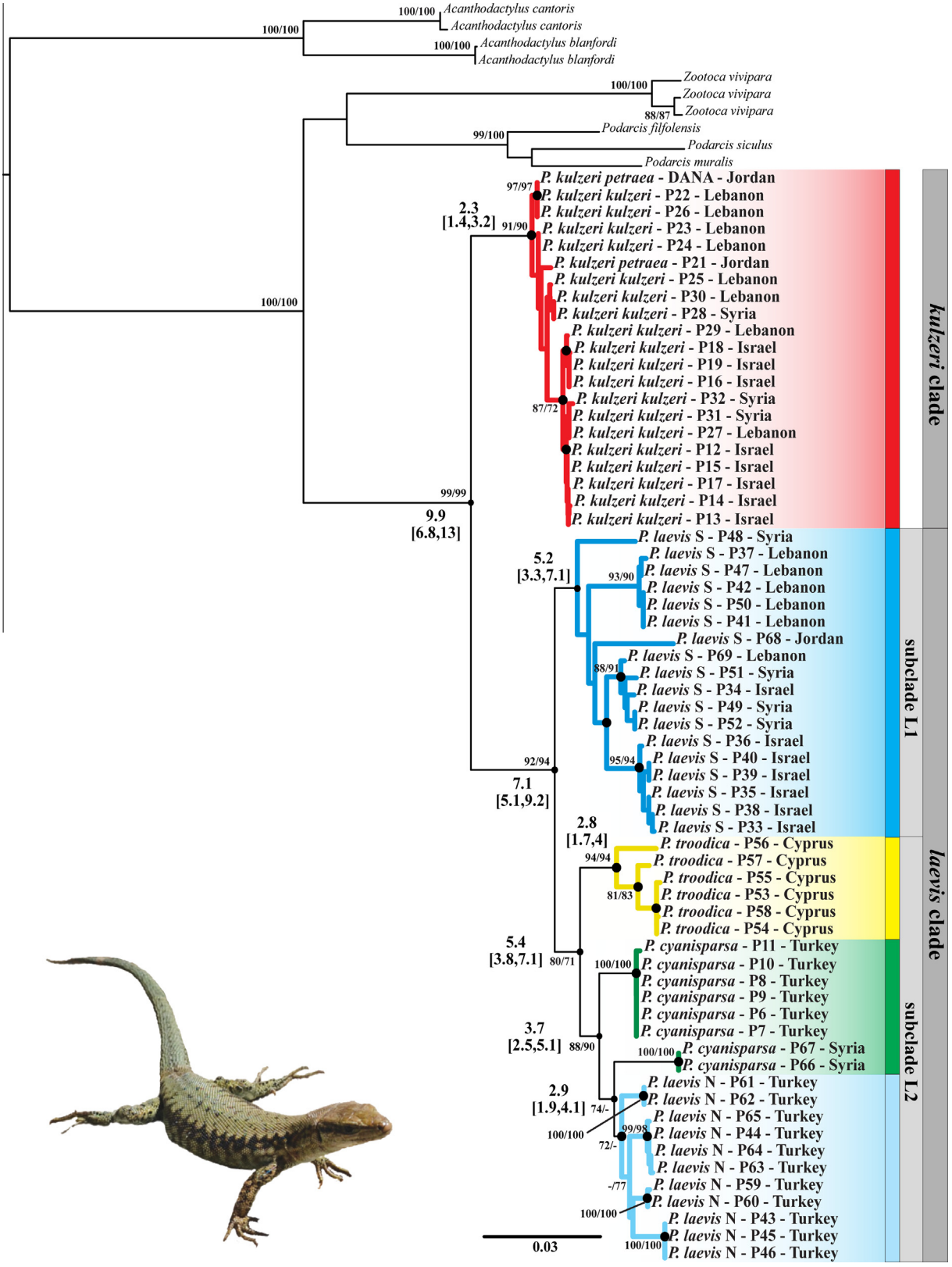


**Fig. 2.** Maximum likelihood tree of *Phoenicolacerta* inferred using 12S, *cytb* mtDNA and MC1R and ACM4 nuclear gene fragments. Posterior probability in the Bayesian analysis is indicated by black dots on the nodes (values ⩾0.95 shown, for both gene partitions and partitions by PartitionFinder [PF]), and the ML bootstrap support values are indicated near the nodes (values ⩾70% shown; ML, ML-PF). Age estimates with BEAST are indicated near the relevant nodes and include the mean and, between brackets, the HPD 95% confidence interval (millions of years ago). Sample codes correlate to specimens in Table S1 and colours in Figs. 1–4 and S2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

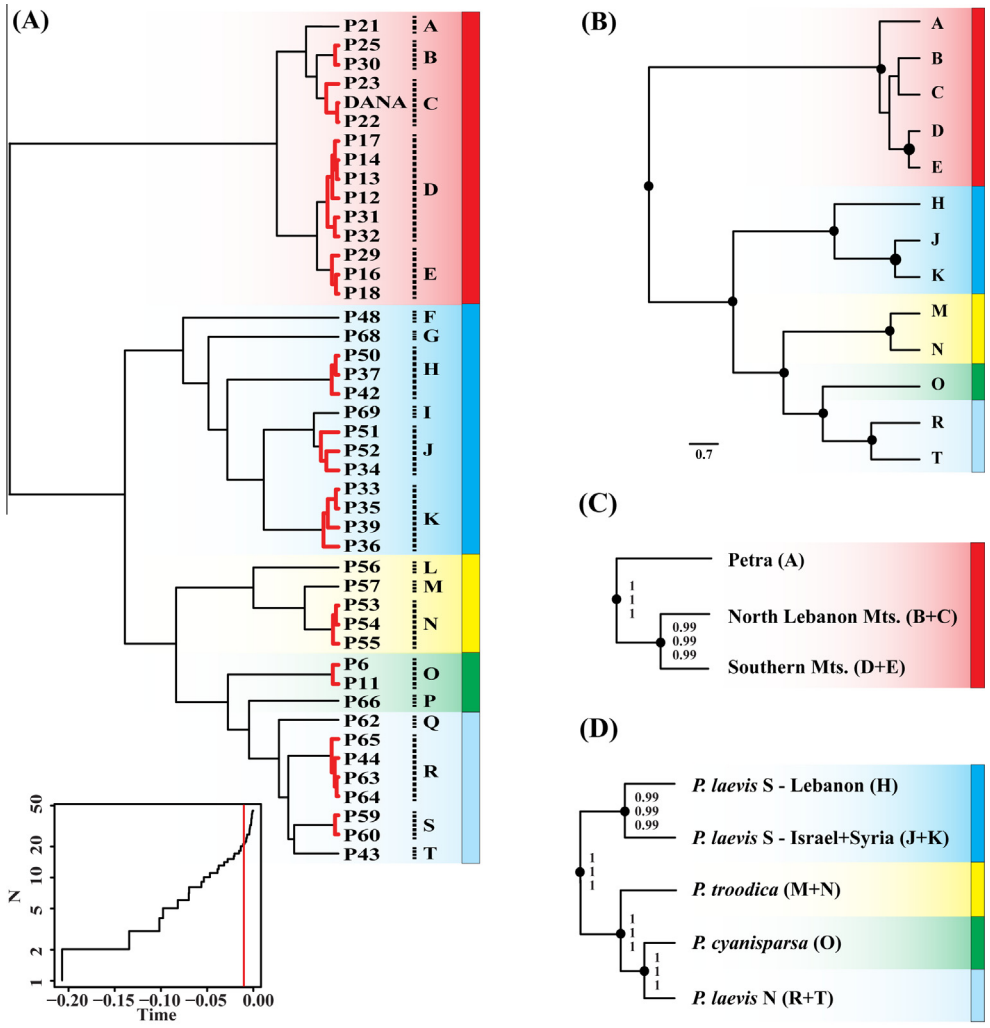


**Fig. 3.** *Phoenicolacerta* trees inferred from the species delimitations and species-trees analyses. Sample codes correlate to specimens in Table S1 and colours in Figs. 1–4 and S2. (A) Ultrametric tree obtained in BEAST of the species delimitation analysis according to the GMYC single-threshold model. Putative species are represented by letters. Lineage-through-time plot based on the ultrametric tree show in vertical red line the sharp increase in branching rate. (B) *BEAST species tree with posterior probabilities indicated by black dots on the nodes (values $\geqslant$0.95 shown). (C) Results of the species delimitation analyses inferred by BPP (nuclear genes only) for *P. kulzeri*. The posterior estimates for $\theta$ and $\tau$ are provided near the nodes. (D) Results of the species delimitation analyses inferred by BPP (nuclear genes only) for the *laevis* clade. The posterior estimates for $\theta$ and $\tau$ are provided near the nodes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and Syria, and northern Israel (Jebel Barouk, Jebel Druze and Mt. Hermon). The BPP results for the *laevis* clade supported a five species model (Fig. 3D): (1) *P. laevis* S – Lebanon (Byblos, Bcharre); (2) *P. laevis* S – Israel and Syria; (3) *P. troodica*; (4) *P. cyanisparsa*; (5) *P. laevis* N.

### 3.4. Nuclear haplotype networks

Forty-four haplotypes were identified among the 64 specimens using 793 bp of the combined 12S and *cytb* datasets (Table S1). The networks constructed for the phased haplotypes of the full length nuclear markers MC1R (35 unique haplotypes) and ACM4 (26 haplotypes) are presented in Fig. 4. The nuclear networks show similar patterns for the two gene fragments and mostly agree with the phylogenetic tree. Nuclear network analyses reveal no allele sharing in the MC1R gene fragment for all four species, as well as showing private alleles for *P. kulzeri petraea* from Petra (sample P21). No alleles are shared between the northern and southern lineages of *P. laevis,* which are clearly distinct from each other. The ACM4 network shows private alleles for *P. kulzeri* and the southern lineage of *P. laevis*, whereas *P. kulzeri petraea* and *P. k. kulzeri* share alleles. *Phoenicolacerta cyanisparsa* from its type locality in northern Syria shares alleles with *P. troodica* and with the northern lineage of *P. laevis* (from the eastern edge of the distribution range). The northern and southern lineages of *P. laevis* are clearly distinct from each other as no alleles are shared in the ACM4 gene fragment as well, in agreement with the MC1R haplotype network.

### 3.5. Constrained topology tests

In order to better understand the relationships within the *laevis* clade we performed a topology test where we forced the following monophyletic groupings: (1) of *P. laevis* (the southern and northern lineages unite together; AU: $p = 0.043$; SH: $p = 0.046$); (2) of *P. cyanisparsa* (AU: $p = 0.164$; SH: $p = 0.174$). The results of these tests reject the hypothesis of monophyly of *P. laevis*, but not of *P. cyanisparsa*.

In addition we enforced constrained topologies to test the monophyly of the subspecies of *P. kulzeri*, the Jordanian *P. k. petraea* and the nominate *P. k. kulzeri*: (1) separate monophyly of *P. k. kulzeri* only (AU: $p = 0.003$; SH: $p = 0.014$); (2) separate monophyly of *P. k. petraea* only (AU: $p = 0.002$; SH: $p = 0.012$); (3) *P. k.*

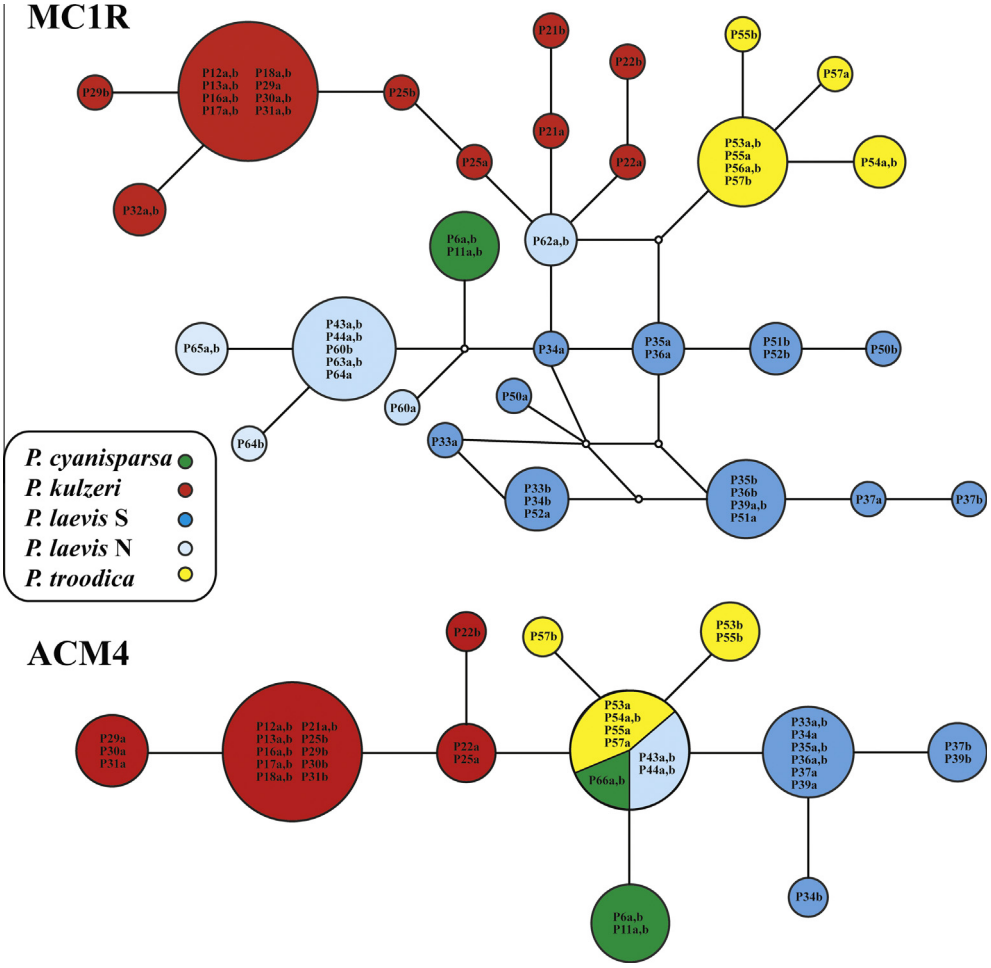


**Fig. 4.** Haplotype networks of the MC1R and ACM4 nuclear gene fragments, with colours corresponding to Figs. 1–3 and S2. Codes correlate to the two alleles (i.e., a and b) of specimens in Table S1. Circle sizes are proportional to the number of alleles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 1**
Pairwise uncorrected genetic divergence (*p*-distance) between the *Phoenicolacerta* taxa, derived from the mitochondrial genes 12S (below the diagonal) and *cytb* (above the diagonal), and within the taxa (12S/*cytb*).

| | *P. cyanisparsa* | *P. kulzeri kulzeri* | *P. kulzeri petraea* | *P. laevis S* | *P. laevis N* | *P. troodica* |
|---|---|---|---|---|---|---|
| *P. cyanisparsa* | 0/3.51 | 14.8 | 14.4 | 11.9 | 7.8 | 9.7 |
| *P. kulzeri kulzeri* | 6.6 | 0.94/2.68 | 3.9 | 13.9 | 15.1 | 14.2 |
| *P. kulzeri petraea* | 6.5 | 1.4 | 1.55/2.61 | 13.8 | 15.4 | 14.9 |
| *P. laevis S* | 4.1 | 4.9 | 4.8 | 1.79/6.25 | 11.9 | 12.4 |
| *P. laevis N* | 2.1 | 6.1 | 6.1 | 3.8 | 1.16/3.83 | 10.3 |
| *P. troodica* | 4 | 5.9 | 5.4 | 4.3 | 4 | 1/3.53 |

*kulzeri* and *P. k. petraea* reciprocally monophyletic (AU: $p = 0.001$; SH: $p = 0.019$). These tests reject the monophyly of both *P. k. kulzeri* and *P. k. petraea*.

## 4. Discussion

This study provides the first robust phylogenetic reconstruction and assessment of the inter- and intra-specific relationships and diversity of the genus *Phoenicolacerta*, and an evaluation of its evolution and phylogeography. The data and analyses presented here stem from nearly complete taxon sampling, with representatives of all presently recognized species and most subspecies of the genus. We included samples from across the species' distributional ranges (Fig. 1) and applied both the traditional, single-locus, and the modern, multi-locus, coalescent-based methods of phylogenetic inference and species delimitation. Our findings mostly support current taxonomic designations (Arnold et al., 2007), but also present several systematic discrepancies.

### 4.1. Phylogenetic relationships within Phoenicolacerta

The different phylogenetic analyses, both of the mitochondrial and the concatenated datasets, with both partition approaches, present the same picture of two major clades, *kulzeri* and *laevis* (Figs. 2 and S2). The two clades are divided into four distinct lineages fully supported by the mitochondrial data and the nuclear gene MC1R, but show incomplete allele sorting in the nuclear marker ACM4 (Figs. 4 and S2). Mitochondrial divergence within each

lineage and species is relatively high, especially compared to variation between them (Table 1).

The *kulzeri* clade corresponds only to *P. kulzeri*, including two recognized subspecies: *P. k. kulzeri* and *P. k. petraea* (samples of the recently described third subspecies, *P. k. khazaliensis*, were unavailable). The high level of genetic mitochondrial differentiation of *P. kulzeri* from the other species of the genus (*p*-distance, 12S: 4.8–6.6%; *cytb*: 13.8–15.1%) and the absence of allele sharing in the nuclear gene fragments (Fig. 4), suggests restricted gene flow due to physical or ecological barriers. These results support the specific status of *P. kulzeri* in concordance with taxonomic classifications (Bischoff and Schmidtler, 1999; Arnold et al., 2007). However, the subspecific status of the South Jordanian *P. k. petraea* (type locality: Petra, Jordan), and the northern nominate *P. k. kulzeri*, are not supported. Samples of the former (from Petra and Dana, Jordan; Disi et al., 2001; in den Bosch, 2002) are distinct from each other and are nested within the samples of the latter in the concatenated tree (Fig. 2), though the sample from Petra is distinct in the mitochondrial tree (Fig. S2). The constrained topology tests statistically rejected the monophyly of each subspecies individually, and the reciprocal monophyly of them both.

The samples of *P. kulzeri* within its range represent five (Bischoff and Schmidtler, 1999) or seven populations (or taxonomic forms; in den Bosch and Bischoff, 1996; in den Bosch, 2002). The results of the species delimitations and species-tree analyses have revealed within *P. kulzeri* five entities in the GMYC and *BEAST, and three clear entities in the BPP analysis (Fig. 3). These entities correlate to geographically close populations within the species (Petra in Jordan, North Lebanon Mts., and the southern Mountains around the jointed borders of Syria, Lebanon and Israel). This geographic pattern is more pronounced in the mitochondrial analyses (Fig. S2), but is less obvious in the concatenated tree (Fig. 2). This pattern may result from loss of information due to less variation within the nuclear genes, or due to relatively recent divergence, which obscure both morphological characters and clear lineages identification. The isolation and non-overlapping geographic distributions of the populations of *P. kulzeri*, and the strong association of both nuclear and mitochondrial genetic diversity with the geographic pattern suggest a history of allopatric divergence within the species. Though given the fairly strict conditions that the Bayesian species delimitation (BPP) method assumes to designate species, as well as morphological variation (in den Bosch and Bischoff, 1996; Bischoff and Schmidtler, 1999; in den Bosch, 2002), we suggest more data is needed to assume that at least three taxa may be recognized within the current *P. kulzeri*. A broader sampling of more populations, integrative taxonomy and ecological data are essential to resolve this issue (as was published for other lacertid genera, Miralles et al., 2010; Fitze et al., 2011; Vasconcelos et al., 2012; Ahmadzadeh et al., 2013a).

The *laevis* clade includes the three remaining recognized species: *P. laevis*, *P. cyanisparsa* and *P. troodica*. *Phoenicolacerta troodica*, the Cyprus endemic, was originally described as a subspecies of *P. laevis* by Werner (1936). Arnold et al. (2007) elevated it to the species level based on its morphological distinctiveness. Our phylogenetic results, haplotype networks, and high levels of genetic divergence (12S: 4–5.9%; *cytb*: 9.7–14.9%; Table 1) suggest the Cypriot *P. troodica* is indeed closely related to *P. laevis*, but has been genetically isolated for a long period of time (Figs. 2–4). It thus merits specific status. The long segregation in Cyprus may have also generated high level of genetic variability (Table 1).

The relationships between the two non-monophyletic species, *P. cyanisparsa* and *P. laevis*, conflict with the known taxonomic classifications. *Phoenicolacerta cyanisparsa* is genetically distant from its closest relatives in both mitochondrial and nuclear data (12S: 2.1–4.1%; *cytb*: 7.8–11.9%; Fig. 4) and is morphologically distinct from the geographically close species *P. laevis* (i.e., in pholidosis and coloration; Schmidtler and Bischoff, 1999). The northern lineage of *P. laevis* from southern Turkey is closer to *P. cyanisparsa* and to *P. troodica* than to *P. laevis* from the southern Levant (Figs. 2–4 and S2; see also Pavlicev and Mayer, 2006). The two samples of *P. cyanisparsa* (paratypes), P66 and P67, do not include the entire dataset of genes (Table S1). This may explain their position in relations to both the other samples of *P. cyanisparsa* and those of the Turkish *P. laevis* (Figs. 2 and 3 and S2). The genetic differentiation between the northern and the southern lineages of *P. laevis* are clearly shown in the nuclear networks (Fig. 4). The mitochondrial distances between these lineages (3.8% for 12S and 11.9% for *cytb*) are much higher than those found between other lacertid species (e.g., 7.4–8.2% of *cytb* among *Iberolacerta aranica, I. aurelioi, I. bonnali*, and 4.1–5.8% of *cytb* between *Lacerta bilineata* and *L. viridis*; Crochet et al., 2004; Godinho et al., 2005, respectively). They are, however, close to those between the subspecies of *Timon princeps* (i.e., *T. p. princeps* and *T. p. kurdistanicus*; ca. 15% for *cytb*) for which the authors recommended elevation to full species (i.e., *Timon princeps* and *Timon kurdistanicus*; Ahmadzadeh et al., 2012).

The constrained topology test presents an additional support for the distinction between the two lineages of *P. laevis* as they clearly show that the enforced monophyly of the *P. laevis* species is rejected (while not rejecting the monophyly of *P. cyanisparsa*). The species delimitation approaches within the *laevis* clade using multi-locus data reveals the potential for recognizing at least five genetically distinct species within the three recognized species (Fig. 3D). Two potential species coincide with *P. troodica* and *P. cyanisparsa*, whereas within *P. laevis* three entities were observed, two from the southern populations (one from Lebanon, and one from Syria and Israel), and one from the northern populations in Turkey. These delimitation analyses all agree with the results of the phylogenetic gene trees, particularly with the distinctiveness of the northern populations of *P. laevis* from the southern ones (*P. laevis* N and *P. laevis* S, respectively).

### 4.2. Biogeography and diversification of Phoenicolacerta

The estimated divergence times support the initial differentiation of the lineages in the genus *Phoenicolacerta* during the mid-late Miocene, around 9.9 Mya (95% HPD: 6.8–13 Mya; Fig. 2). The geological instability resulted from the tectonic movements of the Arabian, Anatolian and African plates in the eastern Mediterranean during this period (Over et al., 2004; Inwood et al., 2009) may have triggered the divergence of the genus and the formation of the *kulzeri* and *laevis* clades.

*Phoenicolacerta kulzeri* started radiating during the late Pliocene or early Pleistocene at approximately 2.3 Mya (95% HPD: 1.4–3.2 Mya). Due to the location, the separation and close relationships of its geographically adjacent populations, the radiation of *P. kulzeri* may has been caused by climatic fluctuations during the postglacial aridification in the eastern Mediterranean as suggested for other taxa (Veith et al., 2003; Kornilios et al., 2012; Ahmadzadeh et al., 2013b). Cooling periods may have led *P. kulzeri* to lower elevations, where merging of populations was possible (evident in the allele sharing in the nuclear networks; Fig. 4). During warmer periods the connections between the mountain ranges were lost and the glacial relict distributions on different mountains established their disjunct populations. We suggest that members of this species could have become isolated from each other as a result of habitat fragmentation, which drove to allopatric distribution of its populations.

The *laevis* clade started radiating during the late Miocene to early Pliocene around 7.1 Mya (95% HPD: 5.1–9.2 Mya), splitting into two distinct subclades (Fig. 2). This period corresponds to the tectonic events and dry climate in the eastern Mediterranean

at the time (Hsü et al., 1977; Over et al., 2004; Inwood et al., 2009). It may be that the geological events caused the fragmentation of the ancestral populations of this clade and that late-Pliocene aridification and Quaternary climatic oscillations resulted in speciation. This pattern of cladogenesis was also suggested for other taxa in the region (Veith et al., 2003; Kornilios et al., 2012; Ahmadzadeh et al., 2013b; Kapli et al., 2013).

*Phoenicolacerta troodica* is endemic to Cyprus, where it is found across the island from sea level to 1500 m (Baier et al., 2009). Our phylogeny shows that this species diverged approximately 5.4 Mya (95% HPD: 3.8–7.1 Mya) during the late Miocene and early Pliocene. This date coincide well with the period proposed for the Messinian salinity crisis at the end of the Miocene and early Pliocene (Krijgsman et al., 1999), when several lizard species are thought to have arrived to Cyprus (e.g., *Acanthodactylus schreiberi*, *Ablepharus budaki*; Tamar et al., 2014; Poulakakis et al., 2013; respectively). That said, transmarine dispersal has also been suggested for some Cypriot reptiles (Poulakakis et al., 2013). Whether Cyprus was connected to the mainland during the Messinian crisis is debated, as are suggestions of a land connection at later periods (Steininger and Rögl, 1984; Jolivet et al., 2006; Bache et al., 2012). Such connections could have provided opportunities for terrestrial organisms with poor overseas dispersal ability, such as lizards, to colonize the island. Several studies argue that post-Messinian sea level changes are unlikely to have formed connections between Cyprus and the mainland (Steininger and Rögl, 1984; Jolivet et al., 2006). Thus our dating of the split of *P. troodica* at ca. 5.4 Mya leads us to suggest that the Messinian crisis may have provided the possible route to colonize Cyprus – potentially from southern Turkey and north-west Syria where its living closest relatives, *P. cyanisparsa* and northern *P. laevis* reside.

*Phoenicolacerta cyanisparsa* was described as a form within the *laevis-kulzeri* complex, inhabiting rocky and drier habitats in the inner Levant (Schmidtler and Bischoff, 1999). The divergence between *P. cyanisparsa* and the northern lineage of *P. laevis* occurred during the late Pliocene or mid Pleistocene, around 3.7–2.9 Mya (95% HPD: 1.9–5.1 Mya; Fig. 2). This split overlaps with the time of the uplift of the Amanos Mountains and the formation of the Amik Basin in southern Turkey in the same area (Over et al., 2002, 2004; see reference therein). The emergence of these geological structures was suggested to drive the cladogenesis of other lacertid taxa in this region (*Lacerta media*, Ahmadzadeh et al., 2013b; *Apathya cappadocica wolteri*, Kapli et al., 2013). We suggest the same scenario for *Phoenicolacerta* – It may be that specimens of the ancestral population of this *P. cyanisparsa*-northern *P. laevis* lineage, were separated between modern Syria and Turkey due to the formation of these geological features, acting as biogeographic barriers. The establishment of the Irano-Turanian landscape in the eastern front in northern Syria and southern Turkey may have driven the morphological divergence of *P. cyanisparsa*. Coastal southern Turkey and the southern Levant probably remained similar to each other in climate and vegetation (today: Mediterranean maquis) and thus the *Phoenicolacerta* populations inhabiting these regions (southern and northern *P. laevis*) either retained the ancestral morphology or co-evolved to follow similar adaptive regimes, and therefore remained morphologically alike, while diverging genetically.

### 4.3. Systematic and taxonomic implications

The intraspecific diversity within *P. kulzeri, P. laevis* and *P. cyanisparsa* conflicts with the currently known taxonomy. Our results support the distinctiveness of *Phoenicolacerta kulzeri*, but question its contemporary accepted intraspecific classification. Our species delimitation methods using multi-locus data reveal the potential for recognizing at least three genetically distinct entities within *P. kulzeri* (Fig. 3). These lineages could have become isolated from each other as a result of habitat fragmentation, which drove allopatric divergence. In addition, preliminary laboratory crossing experiments have shown severe hybridization problems between these populations (in den Bosch and Zandee, 2001), and differences in chromosomal morphology (in den Bosch et al., 2003). Thus, we suggest that the taxonomy of *Phoenicolacerta kulzeri* needs a re-evaluation, considering both morphological (e.g., Bischoff and Müller, 1999; in den Bosch, 2002; Modrý et al., 2013) and genetic variation, as well as additional ecological data.

The non-monophyly of *P. laevis* and *P. cyanisparsa* necessitates taxonomic attention. Species designations are hindered by the fact that the type locality of *P. laevis* is unknown (Gray, 1838 did not provide a type locality for the holotype, BMNH 1946.9.3.2). We suggest that, though genetically distinct, the morphological similarities between the northern and southern lineages of *P. laevis* can be explained by vicariance, dispersal and ecological adaptation processes or combinations thereof. Our results show that the *laevis* clade is comprised of four distinct units (i.e., southern *P. laevis, P. troodica*, *P. cyanisparsa* and northern *P. laevis*; Figs. 2–4 and S2). This is partially supported by morphology (i.e., the differentiation of *P. laevis*, *P. cyanisparsa* and *P. troodica*). Nevertheless, a more definitive elucidation of *P. laevis* taxonomy necessitates thorough examinations. These include close examinations of the type and populations from the entire distribution range of *P. laevis* (from both the southern and northern lineages) and determination of the proper identity of the *P. laevis* holotype.

## 5. Conclusions

Since the Miocene, the Levant has dramatically transformed from a tropical domain into a southern province of the Palearctic region due to intensive geological events with a wide range of climatic shifts (Hsü et al., 1977; Tchernov, 1992). These abiotic phenomena are suggested to have affected the ranges of species and the cladogenesis of *Phoenicolacerta*, as well as of other taxa (e.g., Ahmadzadeh et al., 2013b; Kapli et al., 2013). Based on thorough sampling of the four species of the genus *Phoenicolacerta* and using both mitochondrial and nuclear data, we found both some agreement and some disagreement with the currently accepted taxonomy and species status. Though morphologically the populations of *P. laevis* are similar, distinct evolutionary lineages and high genetic differentiation were revealed within this taxon. Therefore, the *laevis* clade requires further taxonomic revision as to the true nature of *P. laevis.*

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2015.05.002.

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