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Comparative phylogeography of six herpetofauna species in Cyprus: late Miocene to Pleistocene colonization routes

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The colonization patterns of oceanic islands are often interpreted through transmarine dispersal. However, in islands with intense human activities and unclear geological history, this inference may be inappropriate. Cyprus is such an island, whose geotectonic evolution has not been clarified yet to the desired level for biogeographical reconstructions, leaving the questions of 'how the Cypriote biota arrived' and 'does the dispersal have the formative role in patterns of its diversification' unanswered. Here, we address these issues through a reconstruction of the evolutionary history of six herptiles (*Ablepharus budaki*, *Ophisops elegans*, *Acanthodactylus schreiberi*, *Telescopus fallax*, *Pelophylax* cf. *bedriagae*, and *Hyla savignyi*) by means of mitochondrial DNA (cytochrome *b* and 16S rRNA), applying a Bayesian phylogenetic, biogeographical, and chronophylogenetic analyses. The phylogeographical analyses show that the colonization history of those species in Cyprus started in the late Miocene and extended into the Pliocene and Pleistocene, with geodispersal, transmarine dispersal, and human-mediated dispersal having their share in shaping the diversification of Cypriote herptiles. The revealed patterns could be divided into three biogeographical categories: old colonizers that arrived in Cyprus during the late Miocene or early Pliocene either by a land bridge (geodispersal) which connected Cyprus with the mainland or by transmarine dispersal, younger colonizers that reached the island through transmarine dispersal from the Middle East, and new settlers that arrived through human-induced (voluntary or not) introductions. This work advances our knowledge of the biogeography of Cyprus and highlights the need to consider both geo- and transmarine dispersal when dealing with islands whose associations do not have a straightforward interpretation. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, **108**, 619–635.

ADDITIONAL KEYWORDS: amphibians – Bayesian approaches – east Mediterranean – geodispersal – human-mediated – reptiles – transmarine dispersal.

INTRODUCTION

In recent years, numerous phylogeographical studies have incorporated temporal information. There are two main sources of this kind of information: the fossil record and molecular clocks (Posadas, Crisci & Katinas, 2006). For a long time fossils were thought to be the basis for the introduction of a timescale in the evolutionary history of organisms. However, it appears that the use of the fossil record is prone to substantial errors (Pulquerio & Nichols, 2007). These

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errors are associated with the dating of particular fossils (i.e. radioisotope dating has intrinsic error) and the fossil record *per se*. Alternatively to the fossil record, calibration of a phylogenetic tree can be made with established geological events. Geologists can date events such as the formation of landmasses (e.g. islands) or separation of continents. But the problem here is the difficulty in assessing the dynamics of an island's formation or how well the geological date corresponds to the dates at which lineages became separated. Although large discrepancies have been found so far in dates of evolutionary events obtained using a molecular clock and fossil records, the application of recently developed methods have increased precision.

Modern methods enable users to input their assessment of uncertainty about the date of a calibration point as an upper and lower bound, or as a probability distribution (Pulquerio & Nichols, 2007). Drummond *et al*. (2006) recently developed a Bayesian method that makes no assumption about the correlation between substitution rates in the tree, allows for uncertainty in the dates attributed to calibration points, and does not impose unproven assumptions about the pattern in clock-rate variation among lineages (Drummond & Rambaut, 2007). Combining information from many species, this method enables the extent and pattern of the clock-rate variation to be roughly characterized. When this information is available, the inclusion of additional calibration points to the analysis should therefore produce more accurate clock-base dates (Pulquerio & Nichols, 2007). Thus, a method that includes a Bayesian approach on a multispecies dataset and several calibration points could shed light on the biogeographical history of the biota of an area (i.e. island) with unclear geotectonic history. Cyprus is such an island, whose geotectonic evolution has not yet been clarified to the desired level for biogeographical reconstructions.

Cyprus is an oceanic island that originated from the seabed (Hadjikyriakou & Hadjisterkotis, 2002). The creation of Cyprus began between 85 and 92 Mya with the genesis of the Troodos massif. By the late Miocene, the Troodos massif was a low-lying island whereas the Kyrenia range, which had been deeply submerged, began to rise. A severe compression and drastic uplift occurred in Cyprus during the Pleistocene. The Troodos masif, Kyrenia lineament, and Mesaoria basin were uplifted together, and for the first time Cyprus acted as a single structural unit (Hadjisterkotis, Masala & Reese, 2000, and references therein). Until 6 Mya, the Mediterranean Sea was connected to the Atlantic via shallow seaways across southern Spain and northern Morocco (Krijgsman *et al*., 1999). The uplift of Spain and Morocco gradually closed these seaways and the Mediterranean basin became isolated from the Atlantic Ocean, marking the start of the Messinian Salinity Crisis (MSC) (5.96–5.33 Mya). A series of large lakes were present in the southern part of the Troodos and Pendadactylos that comprised Cyprus at that time (Hadjisterkotis *et al*., 2000). Only for this short period of the upper Miocene (Messinian) might islands of volcanic origin, such as Cyprus, have been connected to the mainland.

Cyprus is biogeographically characterized as one of the most isolated Mediterranean islands (Moores *et al*., 1984). Several researchers (Hadjisterkotis *et al*., 2000, and references therein) noted that the island was never joined by a land bridge to the neighbouring mainland. Other researchers (Hadjisterkotis *et al*., 2000, and references therein; Plötner *et al*., 2010) suggested that Cyprus was connected to the nearby mainland (Syria or southern Anatolia), and this connection probably persisted long enough to allow the penetration of biota (Zohary, 1973). On the other hand, the occurrence of a comparatively large number of endemics on the island points to the long span of time during which the island has been separated from the mainland (Hadjisterkotis *et al*., 2000). Thus, we still need to answer whether Cyprus was sometime in the past connected to the mainland (Turkey and/or Syria) and how did animal species colonize it.

The herpetofauna (*sensu lato*) of Cyprus comprises 29 species, including 11 species of snakes, 11 lizards, three sea turtles, a freshwater terrapin, and three anuran amphibians (Sindaco & Jeremčenko, 2008). Here, we use a comparative phylogeographical approach in conjuction with molecular dating and ancestral area reconstruction to compare the evolutionary histories of six herptile species which have largely overlapping ranges in Anatolia and Cyprus (Fig. 1). We aimed to understand if each species has its own idiosyncratic colonization history and geographical pattern of genetic lineages or if a general pattern could be outlined. These species are *Ablepharus budaki* (Budak's snake-eyed skink), *Ophisops elegans* (snake-eyed lizard), *Acanthodactylus schreiberi* (Schreiber's fringe-fingered lizard), *Telescopus fallax* (European cat-snake), *Pelophylax* cf. *bedriagae* (Levantine frog), and *Hyla savignyi* (tree frog). In the past, several approaches have been used to evaluate the colonization history of Cypriote biota (Poulakakis *et al*., 2005a; Lymberakis *et al*., 2007; Akin *et al*., 2010; Plötner *et al*., 2010). We propose here an alternative strategy in which several very well-established divergent events previously used as calibration points (Veith, Kosuch & Vences, 2003; Poulakakis *et al*., 2005b; Brown *et al*., 2008; Carranza *et al*., 2008) are pooled in a simultaneous phylogeographical analysis using different schemes and methods of divergence time estimation.

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MATERIAL AND METHODS

Figure 1 shows the geographical distribution of the studied taxa (data from IUCN) and the origin of the specimens used in this study (for more details see Appendix 1). For species for which sequences were not available in GenBank (see *Acanthodactylus*, *Ablepharus*, and *Ophisops*), total genomic DNA was extracted from small tissue pieces (tail or liver) using standard methods (Sambrook, Fritsch & Maniatis, 1989). Partial segments of two mtDNA genes (cyt *b* and 16S rRNA) were selected for the phylogenetic analysis. Primers and conditions used in PCR amplifications and in the cycle sequencing reactions are shown in the supporting information (Table S1). PCR products were purified with the NucleoSpin PCR purification Kit (Macherey-Nagel). Single-stranded sequencing of the purified PCR products was performed using a Big-Dye Terminator Cycle sequencing Kit (v.3.1) on an ABI 377 automated sequencer. Both strands of the amplified PCR products were sequenced for all specimens. The corresponding sequences from four other taxa (*Podarcis*, *Chalcides*, *Eumeces*, and *Rana*) were included in the analyses for outgroup comparisons.

The complete dataset was divided into two subsets. The first included only the amphibians (*P. bedriagae* and *H. savignyi*) and the second comprised the four reptile taxa (*A. budaki*, *O. elegans*, *A. schreiberi*, *T. fallax*). The alignment of the sequences was performed separately for each gene with MAFFT v.6 (Katoh *et al*., 2002).

The best-fit models of DNA substitution was chosen according to the Akaike Information Criterion (Akaike, 1974) (see Posada & Buckley, 2004) as implemented in jModeltest (Posada, 2008). However, we tested only for Gamma (G) model, not for Gamma (G) plus Invariable models (I) (G + I model) following the statement that this model (G + I) is somewhat pathological as the gamma distribution with $\alpha \leq 1$ already allows for sites with very low rates. As a result, adding a proportion of invariable sites creates a strong correlation between I and α, making it impossible to estimate both parameters reliably (for more details and other drawbacks of this model see Yang, 2006).

Phylogenetic analyses

Bayesian phylogenetic analyses (Bayesian inference, BI) on each one of the two subsets were conducted in MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). The analysis was run four times with eight chains each run for 10^7 generations, sampling from the chain every 100 generations. Convergence was measured by values of the average standard deviation of split frequencies (an average standard deviation of split frequencies of less than 0.01 was considered convergence of the two simultaneous runs). This generated an output of 10^5 trees. To confirm that the chains had achieved stationarity, we evaluated 'burn-in' by plotting log-likelihood scores and tree lengths against generation number using Tracer v.1.5.0 (Rambaut & Drummond, 2008). The $-ln$L stabilized after approximately 10^6 generations and the first 10^4 trees were discarded as a conservative measure to avoid the possibility of including random, sub-optimal trees. A majority rule consensus tree was then calculated from the posterior distribution of trees, and the posterior probabilities were calculated as the percentage of samples recovering any particular clade, where probabilities $\geq 95\%$ indicate significant support.

Maximum-likelihood (ML) analyses were performed with RAxML v.7.2.8 (Stamatakis, 2006) with 100 random addition replicates in RAxML Black box (Stamatakis, Hoover & Rougemont, 2008). A GTR+GAMMA model was used and parameters were estimated independently for each gene partition. Reliability of the ML tree was assessed by bootstrap analysis (Felsenstein, 1985) including 100 replications.

Sequence divergences were estimated in MEGA v.5.05 (Tamura *et al*., 2011), using the Tamura & Nei (TrN) (Tamura & Nei, 1993) model of evolution among the Cypriote and all the other lineages of the six studied species.

A neighbour-joining analysis with TrN model was also performed in MEGA only on the dataset of *Acanthodactylus* including all the available *A. schreiberi* samples (i.e. the Israeli samples of *A. schreiberi* for which only the 16S rRNA gene was available). This was done to evaluate the taxonomic status of *A. schreiberi* from Israel.

Chronophylogenetic analyses and relaxed dating

Chronophylogenetic analyses were conducted under the Bayesian framework implemented in BEAST v.1.6.1 (Drummond & Rambaut, 2007) using a fully partitioned dataset. This strategy permits the simultaneous estimation of divergence times, tree topology, and rates of molecular evolution. A normal prior distribution for all calibration points was used. As for the priors, the Tree Prior category was set to Yule Process and the uncorrelated lognormal model was used to describe the relaxed clock. Model parameters were unlinked across partitions. The analysis was run for 10^8 generations with a 1000-step thinning. Results were analysed in Tracer to assess convergence and effective sample sizes (ESSs) for all parameters. The $-ln$L was stabilized prior to 10^8, and the first 10% of the 100 000 sampled generations was discarded as

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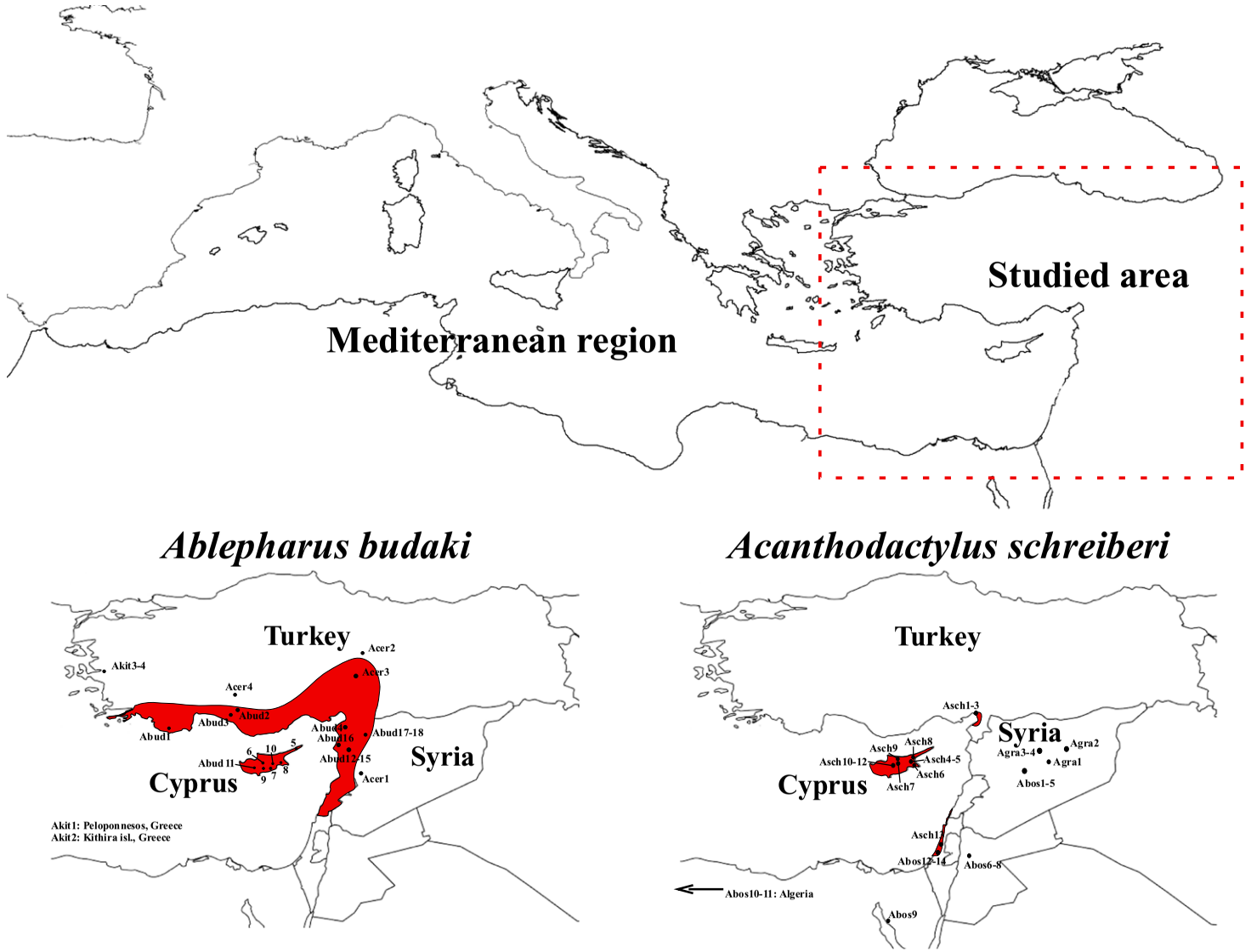


Figure 1. Map showing the geographical distribution (shaded areas) of the six studied species in the Middle East based on IUCN (http://www.iucnredlist.org/apps/redlist/search) (Abud for *A. budaki*, Asch for *A. schreiberi*, Oele for *O. elegans*, Pbed for *P. bedriagae*, Tf for *T. fallax*, and Hsav for *H. savignyi*). All the specimens used in this study (see Appendix 1) are located on the map. It is worth noting here that (1) some species (*O. elegans*, *T. fallax*, *H. savignyi*) have a larger distribution (outside of the range of this map) than those presented here and (2) in some species the current distribution range (data from IUCN) need re-evaluation, as several samples of the target species are located outside of the shaded area (i.e. *P. bedriagae*).

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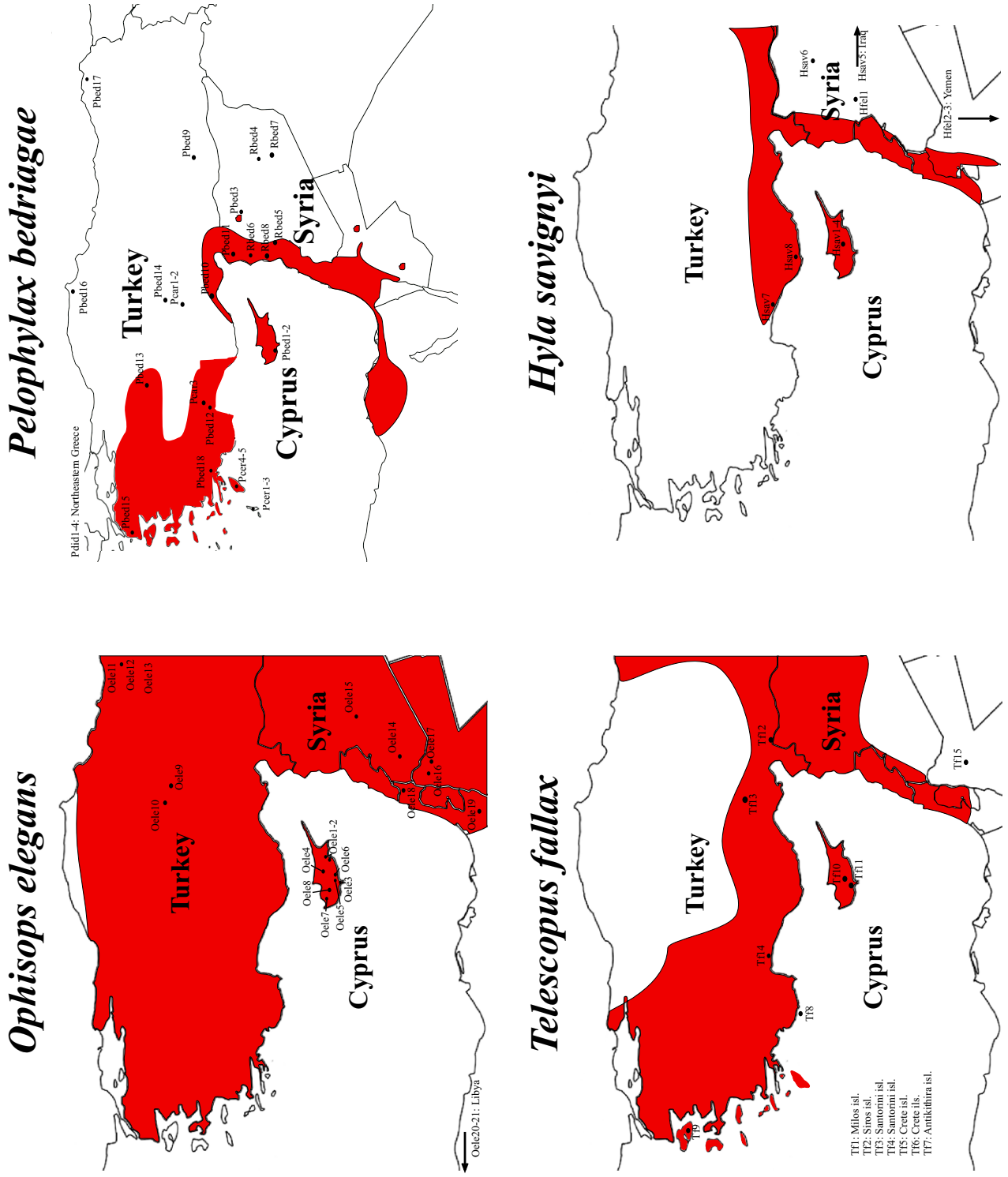


Figure 1. *Continued*

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Table 1. Calibration points used for the dating of Cypriote herptile divergences

	Split	Age (Mya)	Reference
1	*C. viridanus – C. sphenopsiformis*	7.2	Carranza *et al*., 2008
2	*P. cretensis – P. peloponnesiaca*	5.3	Poulakakis *et al*., 2005b
3	*P. bocagei – P. hispanica*	5.17-6.05	Kaliontzopoulou *et al*., 2011
4	*Rana – Pelophylax*	9.32	Veith *et al*., 2003
5	*R. a. arvalis – R. a. wolterstorffi*	1.03	Veith *et al*., 2003

recommended by Tracer. The final tree with divergence estimates and their 95% highest posterior densities (HPDs) was computed in TreeAnnotator v.1.6.1.

The divergence times for the phylogenetic clades of the studied species were estimated by using 'external' calibration age constraints (Table 1). In the first subset (Cypriote amphibians), the corresponding sequences of three brown frogs (*Rana graeca*, *R. arvalis arvalis*, and *R. a. wolterstorffi*) were included in the analyses (Veith *et al*., 2003), whereas for the reptile lineages (second subset), three Scincidae (*Chalcides viridanus*, *C. sphenopsiformis*, and *Eumeces algeriensis*) and 12 Lacertidae specimens (three *P. cretensis*, two *P. peloponnesiaca*, four *P. hispanica*, two *P. carbonelli*, and one *P. bocagei*) were included in the analyses (Poulakakis *et al*., 2005b; Brown *et al*., 2008; Kaliontzopoulou *et al.*, 2011; Carranza *et al*., 2008).

Biogeographical analysis

The distribution range of each species was divided into several areas, based on its current distribution pattern. These areas are: A (Turkey, Syria, Jordan, Israel, Iraq), B (Cyprus), C (East Aegean Islands), D (Greece), E (Saudi Arabia), and F (North Africa). We used recently developed Bayesian Binary Markov chain Montel Carlo (MCMC) (BBM) analysis implemented in RASP v 2.0b (Yu, Harris & He, 2010) to reconstruct the possible ancestral ranges of each species on the phylogenetic trees. In this method, the frequencies of an ancestral range at a node in ancestral reconstructions are averaged over all trees. For each studied species, a separately analysis was performed in BEAST. The input file was formatted with the BEAUti utility included in the software package. The analysis was run for 75×10^6 generations with a 1000-step thinning from which 10% were discarded as burn-in. Models and prior specifications applied were as follows (otherwise by default): *cyt b* – HKY+G, 16S rRNA – GTR+G (see Results section); Relaxed Uncorrelated Lognormal Clock (estimate); Yule process of speciation; random starting tree. The trees made by BEAST were turned into NEWICK format and used as a base in a BBM biogeography analysis. To account for uncertainties in phylogeny, we used 75 000 trees from BEAST output. The possible ancestral ranges at each node on a selected tree were obtained. The MCMC chains were run simultaneously for 5×10^6 generations. The state was sampled every 100 generations. Fixed JC +G (Jukes-Cantor + Gamma) were used for BBM analysis with null root distribution. The maximum number of areas for this analysis was kept as 2.

RESULTS

Phylogenetic and chronophylogenetic analyses were done using a partitioned dataset (HKY+G, and GTR+G models of evolution for cyt *b* and 16S rRNA, respectively, in both subsets).

Phylogenetic trees and genetic distances

The BI and ML phylogenetic analyses on each one of the two subsets using the corresponding models of evolution resulted in similar topologies (for amphibians: $ln\text{L} = -3546.14$ for BI and $ln\text{L} = -3515.11$ for ML, for reptiles: $ln\text{L} = -10\,877.74$ for BI and $ln\text{L} = -10\,860.61$ for ML) (Fig. 2). In the case of amphibians, the analysis partitioned the mtDNA diversity into three major clades that correspond to the three different taxa (the two ingroup taxa: *Pelophylax* – Fig. 2B1, and *Hyla* – Fig. 2B2, and the outgroup taxon: *Rana* – Figs 2, 3). Correspondingly, the analysis revealed six major clades in reptiles (the four ingroup taxa: *Ophisops* – Fig. 2A1, *Ablepharus* – Fig. 2A2, *Telescopus* – Figs 2, 3, *Acanthodactylus* – Fig. 2A6, and the outgroup taxa: *Chalcides* – Fig. 2A4, and *Podarcis* – Fig. 2A5).

The TrN genetic distances between the Cypriote and the other lineages varied from 0.9 to 22.0% in cyt *b* and from 0.3 to 12.5% in 16S rRNA (Table 2). The taxa with the largest genetic distances between the Cypriote and the remaining conspecific lineages are *O. elegans* (16.9% in cyt *b* between Cyprus and Turkey) and *A. budaki* (10.2% in cyt *b* between Cyprus and Syria). On the other hand, the smallest genetic distances were found between *A. schreiberi* of Cyprus and Turkey (0.9% in cyt *b*).

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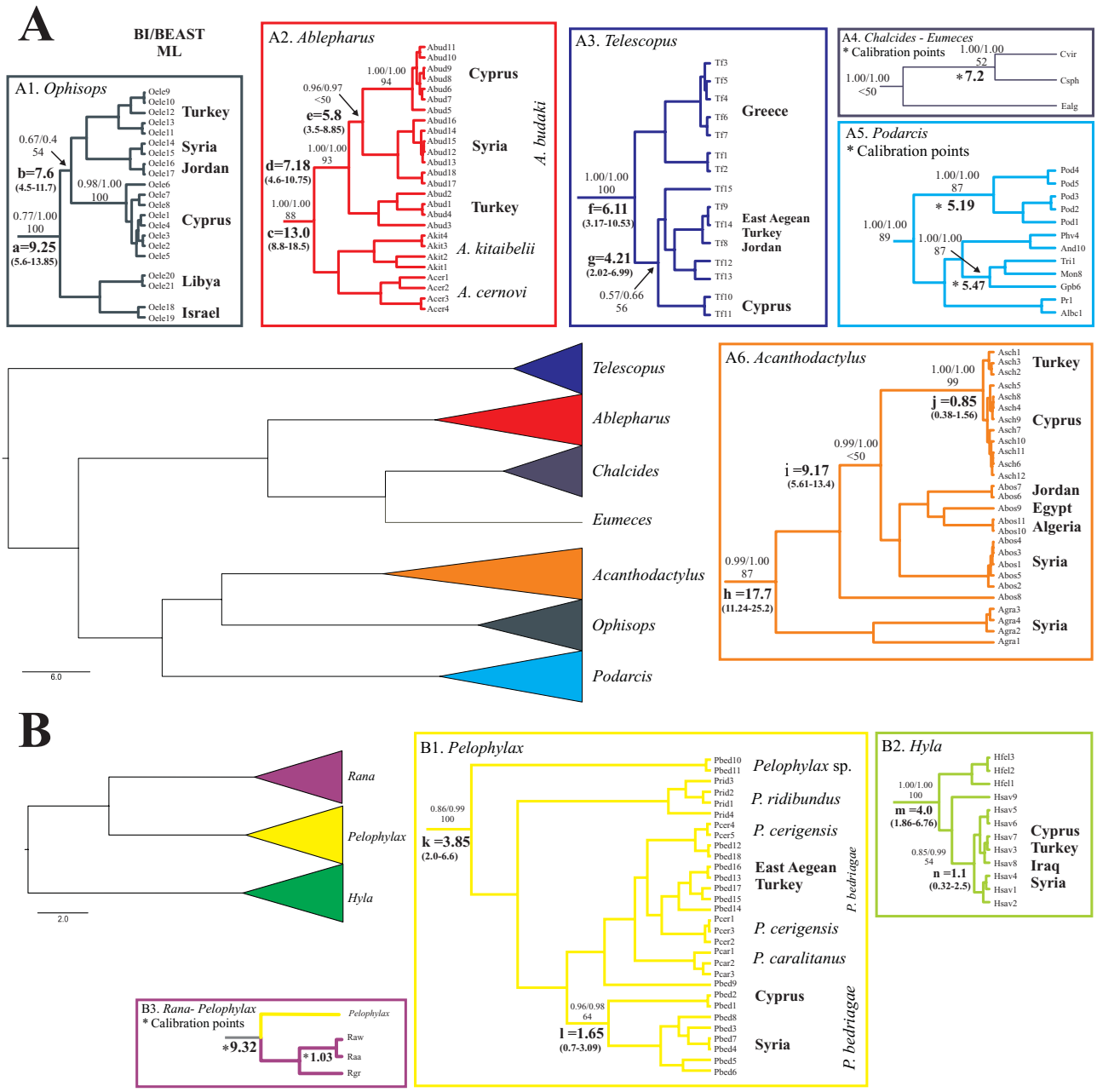


Figure 2. Bayesian inference (BI) tree and molecular timescale for Cypriote herptiles. A, phylogram of Cypriote reptiles. B, phylogram of Cypriote amphibians. Divergence dates resulting from analysis of the mtDNA sequence data through Bayesian relaxed molecular dating. Numbers on branches indicate posterior probabilities (BI and BEAST), and bootstrap support (ML). Only the statistical support for the monophyly of each species and for the Cypriote lineages are presented. Numbers below branches are the estimated times of divergence. Asterisks in the clades of *Podarcis*, *Rana*, and *Chalcides* indicate the age constraints (calibration points) used in this study.

Considering the ingroup taxa, two very well-supported clades were identified in *Ablepharus*. The first clade includes specimens of *A. kitaibelii* and *A. chernovi*, whereas the second comprises specimens of *A. budaki*. The latter clade is further subdivided into three monophyletic lineages that include specimens from different geographical regions: Cyprus, Syria, and Turkey.

In the case of *A. schreiberi*, the specimens from Cyprus have sister-group relationships with the homonym specimens from southern Turkey (Hatay Province), the taxonomic status of which has been

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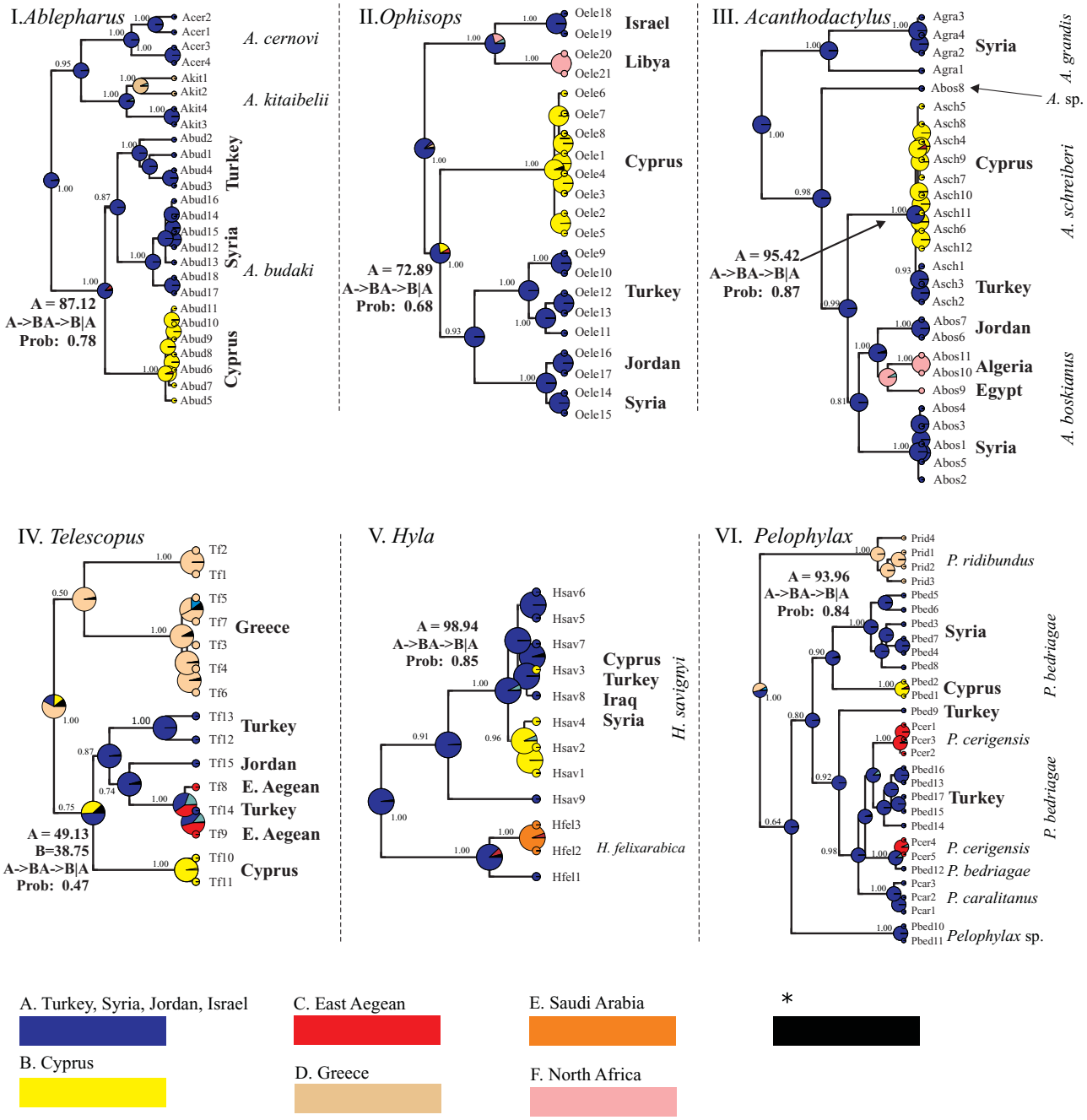


Figure 3. Graphical output from BBM analysis (exported from RASP). Graphical results of ancestral distributions at each node, emphasizing the node that led to Cypriote lineages obtained by BBM analysis. Pie charts at each node show probabilities of alternative ancestral ranges. The colour key indicates possible ancestral ranges at different nodes; black with an asterisk represents other ancestral ranges. Bayesian credibility values are indicated above the branches.

recently updated to subspecific level (*A. schreiberi ataturi*) (Yalçinkaya & Göçmen, 2012). The lineage that appears to be more closely related to *A. schreiberi* from Cyprus and Turkey is that of *A. boskianus*, which comprises specimens from Syria, Jordan, Israel, Egypt, Libya, Tunis, and Algeria. Note that the specimen from Israel that has been described as *A. schreiberi* (GU433292, unpublished data) clusters with *A. boskianus* from Syria, Israel, Jordan, and Egypt and not with the rest of *A. schreiberi* from Cyprus and Turkey (Fig. S1). This means that either the identification of this specimen was wrong or the

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Table 2. Sequence divergences (%) among the Cypriote and the main lineages of the six studied species for the cyt *b*/16S rRNA based on Tamura & Nei model of evolution; no values were calculated (n.c.) where no data were available

	Cyprus					
	Acanthodactylus schreiberi	*Ablepahrus budaki*	*Ophisops elegans*	*Telescopus fallax*	*Pelophylax bedriagae*	*Hyla savignyi*
A.schr Turkey	0.9/0.3					
A.bos Mid East	13.5/7.0					
A.gra Syria	22.0/12.5					
A.bud Syria		10.2/5.1				
A.bud Turkey		7.7/4.7				
A.cer Turkey		14.3/8.2				
A.kit Greece/Turkey		13.8/7.0				
O.ele Syria/Jordan			10.4/4.5			
O.ele Turkey			16.9/6.4			
O.ele Israel			10.1/6.1			
O.ele Libya			9.8/6.1			
T.fal Turkey/E Aegean				4.9/n.c.		
T.fal Greece				6.4/n.c.		
T.fal Jordan				4.5/n.c.		
P.bed Turkey					3.1/1.7	
P.bed Syria					3.5/1.8	
P.car Turkey					4.0/2.0	
P.cer Greece					4.1/2.0	
P.rid Greece					8.2/3.1	
P.sp Turkey					5.6/2.5	
H.sav Mid East						n.c/0.7
H.fel Mid East						n.c/4.6

A.schr, *A. schreiberi*; *A.bos*, *A. boskianus*; *A.gra*, *A. grandis*; *A.bud*, *A. budaki*; *A. cer*, *A. cernovi*; *A.kit*, *A. kitaibelii*; *O.ele*, *O. elegans*; *T.fal*, *T. fallax*; *P.ded*, *P. bedriagae*; *P.car*, *P. caralitanus*; *P.cer*, *P. cerigensis*; *P.rid*, *P. ridibundus*; *P.sp*, *Pelophylax* sp.; *H.sav*, *H. savignyi*; *H.fel*, *H. felixarabica*.

taxonomy and distribution of *A. schreiberi* may need revision.

In *O. elegans*, two major clades were identified; the first consists of *O. elegans* specimens from Cyprus and the Middle East (excluding Israel) and the second from North Africa and Israel. The first one could be further subdivided into three subclades that host *O. elegans* specimens from separate geographical regions as follows: (a) Cyprus, (b) Syria, Jordan, and (c) central, east and southern regions of Turkey. Although the monophyly of each of these subclades was supported by high bootstrap values and posterior probabilities, the relationships among them are considered unresolved.

In *T. fallax*, two clades were also recognized: the eastern subclade that includes specimens from Cyprus, Turkey, Jordan, and the east Aegean islands; and the western clade that consists of *T. fallax* specimens from Greece. However, the low statistical support does not permit us to be confident of the intra-clade relationships.

The four specimens of *H. savignyi* from Cyprus cluster in a clade with specimens from Syria, Turkey, and Iraq with high posterior probability, whereas the intra-clade phylogenetic relationships could be considered as unresolved (Fig. 2B2).

Finally, in *Pelophylax* the phylogenetic relationships of *P. ridibundus* and *P. bedriagae* from Greece, Turkey, Syria, and Cyprus support a sister-group relationship of Cyprus with Syria with high statistical support and the polyphyly of *P. bedriagae* due to position of *P. caralitanus* and *P. cerigensis* within the lineage of *P. bedriagae*.

Chronogram and biogeography

Convergence in the chosen chronophylogenetic analyses was reached prior to 10^8 generations, yielding very high effective sample sizes for all parameters. Estimates for all pairwise comparisons are reported in Figure 2 with 95% credibility intervals and highest posterior densities. The ingroup topologies obtained (lnL = –3533.608 for amphibians, and lnL = –10 843.433 for reptiles) are given in Figure 2. The produced divergence times for the Cypriote lineages ranged from 7.6 to 0.85 Mya.

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From a biogeographical point of view, BBM analysis suggest that the ancestors of all Cypriote lineages originated in Turkey and Syria (area A), suggesting six dispersal events to the island of Cyprus for each of the six studied species with high marginal probabilities (> 0.68) with the exception of *T. fallax* for which the probability was 0.47 (Fig. 3). The frequency of occurrence of this range was 87.12 for *A. budaki*, 72.89 for *O. elegans* (Fig. 3II), 95.42 for *A. schreiberi* (Fig. 3III), 49.13 for *T. fallax* (Fig. 3IV), 98.94 for *H. savignyi* (Fig. 3V), and 93.96 for *P. bedriagae* (Fig. 3VI).

DISCUSSION

Regarding the distribution of the genealogical lineages of the six studied herptile species of Cyprus, three basic patterns have been observed that correspond to three different geological periods (late Miocene, early Pliocene, and Pleistocene) (Fig. 2).

Old colonizers

Ophisops and *Ablepharus* were assessed to have colonized the island in the late Miocene. The diversification within *Ophisops* in south-west Asia has been estimated at late Miocene (9.0 Mya) and the lineage that led to the Cypriote populations of *O. elegans* split from the rest of *O. elegans* lineages at 7.6 Mya. The unresolved relationships of the *Ophisops* lineages are in agreement with the results of Kyriazi *et al*. (2008). The latter authors supported a scenario of simultaneous geographical dispersal of an ancestral lineage that occurred in south-west Asia towards the areas where *O. elegans* are distributed today. In *Ablepharus*, on the other hand, the divergence of *A. budaki* from Cyprus occurred within the MSC (5.8 Mya), whereas the origin of the cat-snake (*T. fallax*) is dated to the early Pliocene, very soon after the end of the MSC. In particular, the eastern subclade of *T. fallax* that includes specimens from Cyprus, Turkey, Jordan, and the eastern Aegean islands diversified from the other subclades at 4.21 Mya.

Young colonizers and new settlers

The biogeographical history of the other three taxa (*Acanthodactylus*, *Pelophylax*, and *Hyla*) was estimated to be more recent (Pleistocene; 0.85, 1.65, and 1.11 Mya, respectively).

In the tree frog genus *Hyla*, the present phylogeny is consistent with Gvoždík *et al*. (2010) and supports the very recent history of the Cypriote populations. The diversification of this clade is dated to 1.11 Mya. However, this time and the unresolved topology within this clade support the recent colonization of Cyprus by *H. savignyi*, indicating either a recent overseas or even an anthropogenic dispersal from southern Anatolia.

From a phylogeographical point of view, the Western Palearctic water frogs (*Pelophylax*) constitute a mystery despite the numerous studies concerning this taxon (Lymberakis & Poulakakis, 2010). Two recent studies have explored the diversity of the eastern distribution of the taxon (Greece, Anatolia, and Levant) based on mtDNA markers (Lymberakis *et al*., 2007; Akin *et al*., 2010). These studies differ considerably in the times of divergences estimated in each case, due to the choice of different calibration points: the isolation of Crete at 5.3 Mya in Lymberakis *et al*. (2007) and the separation of Cyprus after the flooding of the Mediterranean at the end of the MSC (~5.3 Mya) in Akin *et al*. (2010). Based on the first study, the island of Cyprus appears to have been inhabited by the ancestor of *P. bedriagae* during the early Pleistocene (2.4 Mya), while the second one suggested that this species reached the island through the land bridge that probably existed at the end of the Miocene connecting Cyprus with Anatolia (~5.3 Mya). However, the second approach suffers in two ways. The assumption that the population of Cyprus became isolated due to the flooding of the Mediterranean sea at the end of the Messinian produces a circularity issue, as this event was used as calibrator and dated gene tree then produced was used to establish that the main divergence within *Pelophylax* of Cyprus is associated with the end of the MSC. The second flaw is related with the fact that the colonization of Cyprus by *Pelophylax* might have occurred later than the MSC through oversea dispersal. This argument is strengthened given that Cyprus has been secondarily inhabited by *P. caralitanus* from the Turkish shore (Akin *et al*., 2010; Plötner *et al*., 2010). Consequently, as the existence of a land bridge connecting the island of Cyprus with Anatolia is uncertain and two independent lineages of *Pelophylax* are present on the island, it is risky to use the isolation of Cyprus at the end of the MSC as a calibration point.

In our case, it appears that the colonization of Cyprus by *P. bedriagae* occurred during the Pleistocene (~1.65 Mya) when the island was not joined by a land bridge to the mainland (Hadjisterkotis *et al*., 2000; Jolivet *et al*., 2006; Bache *et al*., 2012). Unfortunately, specimens and/or data of *P. caralitanus* from Cyprus were not available. Thus, it is impossible to discuss the time of colonization of the island by this species. However, the inner position of *P. caralitanus* of Cyprus and Turkey (subclade MHG6a of Akin *et al*., 2010) in the phylogenetic clade of *P. bedriagae* (Akin *et al*., 2010) is an indication of a more recent history of *P. caralitanus* on Cyprus than that of *P. bedriagae*.

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It is worth noting here that from a taxonomic point of view, the current phylogenetic tree supports the polyphyly of *P. bedriagae*, indicating either an introgression between *P. bedriagae* and *P. caralitanus* or that the *P. bedriagae* complex needs taxonomic re-evaluation.

Biogeographical identity of Cyprus

In general, frogs and lizards are terrestrial animals with poor overseas dispersal ability. The estimated dates for the colonization of the six taxa of this study produced three distinct patterns that could be considered for the origin of those animals in Cyprus. *Ablepharus* and *Ophisops* appear to have reached the island by the late Miocene (Messinian), *Telescopus* at the beginning of the Pliocene, and *Pelophylax*, *Acanthodactylus*, and *Hyla* at different times during the Pleistocene. The results of the biogeographical analysis (BBM) support that the colonization of Cyprus by these taxa occurred by dispersal.

Thus, the question is how these animal species reached the island of Cyprus? Did they use, should it have existed, a land bridge that joined the island with surrounding continental regions (geodispersal) or did they disperse over the sea (transmarine dispersal or human-mediated)? In two recent geological studies (Jolivet *et al*., 2006; Bache *et al*., 2012), the palaeogeographical representation of the eastern Mediterranean region showed the connection of the island of Cyprus with the continental areas of Turkey and Syria (Fig. 4). Although this is in disagreement with the suggestion of several researchers (Hadjisterkotis *et al*., 2000, and references therein) that the island was never connected to the mainland, it seems to fit well with the estimated time of divergence for *A. budaki*. This taxon appears to have dispersed to Cyprus before 5.8 Mya within the MSC. A similar history has been described for the Eurasian blind-snake (*Typhlops vermicularis*) for which the isolation of Cyprus' lineage from the mainland was estimated at 5.2 Mya (Kornilios *et al*., 2012) and it was considered to the result of a geodispersal event (the authors characterized it as a vicarianistic diversification event) that occurred between Cyprus and Syria or eastern Mediterranean Turkey. A very close time of divergence was estimated for *Cyrtopodion kotschyi* by Kasapidis *et al*. (2005). However, this case should be taken with caution, as the authors estimated four different values (3.1, 3.5, 4.6, and 6.1 Mya) using four different phylogenetic models and using as calibration point the separation of the island of Crete at 10 Mya. Thus, the evolutionary history of *C. kotschyi* in Cyprus could be considered as problematic. The same is true for *T. fallax*; the isolation of the eastern clade that includes the Cypriote specimens was estimated at 4.21 Mya (early Pliocene). However, the low statistical support of the intra-clade relationships and the small number of analysed specimens (only four specimens from the Middle East) do not permit us to be accurate regarding when the cat-snake reached the island.

On the other hand, *Ophisops* showed an earlier invasion, during the early Miocene (7.6 Mya). At that time a land bridge did not exist (Fig. 4). This means that the colonization history of *O. elegans* could be due to transmarine dispersal.

During the Pliocene and Pleistocene, Cyprus was never joined by a land bridge to the mainland shoreline (Fig. 4). Even at times of minimum sea levels during Pleistocene glacial maxima, when the sea

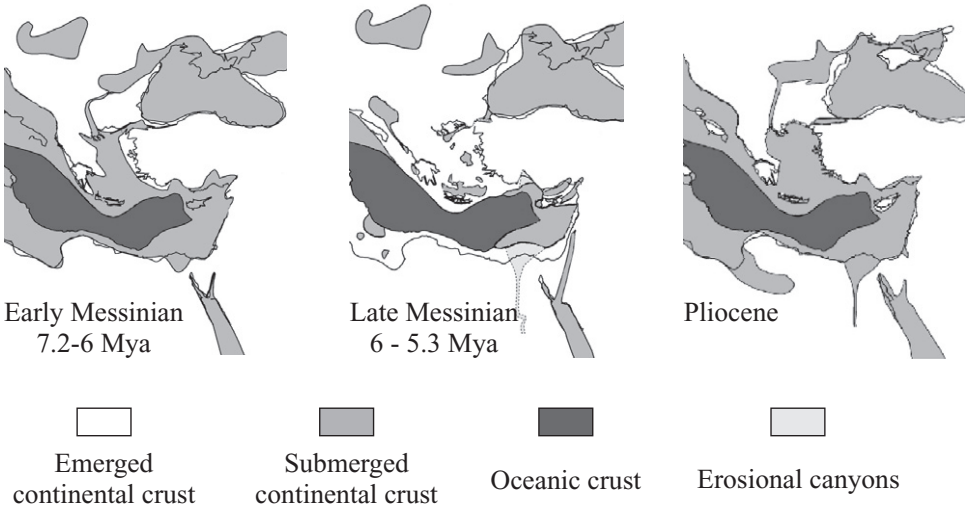


Figure 4. Palaeogeographical maps of the eastern Mediterranean from the late Miocene to Pliocene (redrawn after Jolivet *et al*., 2006).

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dropped to at least 120 m below its present level, the island remained isolated from the now submerged Gulf of Alexandretta on the coast of Asia Minor by at least 30 km (Hadjisterkotis *et al*., 2000). Thus, the most probable scenario for the colonization of Cyprus by Pleistocene animals (water frogs, tree frogs, Schreiber's lizards) is based on overseas dispersal (active or passive). This kind of dispersal is not uncommon for Cypriote biota. Several palaeontologists have stated that few, now extinct, mammals of Cyprus (elephants and hippos) settled in this island from Anatolia following the so-called island sweepstakes model (Sondaar, 1986). The absence of this kind of land bridge during the Pliocene and Pleistocene is also supported by the occurrence of a comparatively large number of island endemics, indicating the long span of time (the last 5 Myr; Hadjisterkotis *et al*., 2000; Jolivet *et al*., 2006; Bache *et al*., 2012) during which the island has been separated from the mainland (Hadjisterkotis *et al*., 2000).

Yet, human-aided dispersal of biota cannot be ignored, as Cyprus, like many other Mediterranean islands, has undergone intensive human influence for more than 10 000 years (Whittaker & Fernández-Palacios, 2007). Many researchers have expressed the opinion that several herptiles might have reached the island of Cyprus via human-induced translocations [ocellated skink (*Chalcides ocellatus*), the house gecko (*Hemidactylus turcicus*), green toad (*Bufo variabilis*), and probably the Balkan terrapin (*Mauremys rivulata*)] (Mantziou, 2006; Stöck *et al*., 2006; Kornilios *et al*., 2010; Rato, Carranza & Harris, 2011). This assumption was based on the low morphological and genetic differentiation among some of its east Mediterranean and Cypriote populations, a sign of a founder effect, probably due to very recent settlement.

CONCLUSIONS

Old colonizers arrived on Cyprus in the late Miocene or early Pliocene either by a land bridge (geodispersal) that connected Cyprus with the mainland during the MSC (*A. budaki*, *T. vermicularis*, and *C. kotschyi* could probably have followed this route) or by transmarine dispersal (*O. elegans* during the early Miocene and *T. fallax* during the early Pliocene). On the other hand, the younger colonizers arrived through transmarine dispersal from the Middle East (*P. bedriagae*, *H. savignyi*, *A. schreiberi*, *M. rivulata*, and probably *B. variabilis*), whereas the new settlers arrived via human-induced (voluntary or not) introductions (*C. ocellatus*, *H. turcicus*, and probably *B. variabilis*, *M. rivulata*, and *H. savignyi*). Consequently, the colonization history of Cyprus is complex and has been defined by several geological events and human activities. The transmarine dispersal that occurred independently during the Miocene, Pliocene, and Pleistocene appears to have had a major role in the shaping of the Cypriote biodiversity. Geodispersal, if it ever existed, would have occurred during the MSC when a land bridge could probably have joined the island with Syria and/or Turkey, while humans have also had an effect on Cypriote biodiversity.

Despite the increased volume of evolutionary research that has been undertaken in Cyprus, several species groups remain relatively unstudied in terms of diversification within Cyprus and their relationship to possible sources of colonists. The present work contributes to our knowledge of biogeography in the oceanic island of Cyprus, especially regarding the herpetofauna of the island.

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APPENDIX 1

Samples used in this study. Identifier (as in Figs 1, 2), species names, sampling locations (locality/country), accession numbers, references, and museum number (NHMC: Natural History Museum of Crete) of the specimens analysed in the current study. Asterisks indicate samples that were used only in a simple neighbour-joining analysis (see Fig. S1) due to missing information (only 16S rRNA was available). PS, present study.

Identifier	Species	Locality	Country	Cyt *b*	16S rRNA	Reference	NHMC
Asch1	*A. schreiberi*	Yumurtalik-Adana	Turkey	JX847535	JX847500	PS	80.3.11.1
Asch2	*A. schreiberi*	Yumurtalik-Adana	Turkey	JX847536	JX847501	PS	80.3.11.2
Asch3	*A. schreiberi*	Yumurtalik-Adana	Turkey	JX847537	JX847502	PS	80.3.11.3
Asch4	*A. schreiberi*	Ammochostos	Cyprus	JX847538	JX847503	PS	80.3.11.13
Asch5	*A. schreiberi*	Ammochostos	Cyprus	JX847539	JX847504	PS	80.3.11.15
Asch6	*A. schreiberi*	Derineia Larnakas	Cyprus	JX847540	JX847505	PS	80.3.11.17
Asch7	*A. schreiberi*	Lefkosia	Cyprus	JX847541	JX847506	PS	80.3.11.18
Asch8	*A. schreiberi*	Salamina	Cyprus	JX847542	JX847507	PS	80.3.11.19
Asch9	*A. schreiberi*	Goneli Lefkosias	Cyprus	JX847543	JX847508	PS	80.3.11.28
Asch10	*A. schreiberi*	Kato Moni	Cyprus	JX847559	JX847524	PS	80.3.11.29
Asch11	*A. schreiberi*	Kato Moni	Cyprus	JX847560	JX847525	PS	80.3.11.30
Asch12	*A. schreiberi*	Kato Moni	Cyprus	JX847561	JX847526	PS	80.3.11.31
*Asch13	*A. schreiberi*		Israel	n.a.	GU433292	Tikochinski *et al*., unpubl. data	
Abos1	*A. boskianus*	Palmyra to Hamah	Syria	JX847544	JX847509	PS	80.3.76.1
Abos2	*A. boskianus*	Palmyra to Hamah	Syria	JX847545	JX847510	PS	80.3.76.2
Abos3	*A. boskianus*	Palmyra to Hamah	Syria	JX847546	JX847511	PS	80.3.76.5
Abos4	*A. boskianus*	Palmyra to Hamah	Syria	JX847547	JX847512	PS	80.3.76.6
Abos5	*A. boskianus*	Palmyra to Hamah	Syria	JX847548	JX847513	PS	80.3.76.7
Abos6	*A. boskianus*	Wadi Rum spring	Jordan	JX847549	JX847514	PS	80.3.76.13
Abos7	*A. boskianus*	Wadi Rum spring	Jordan	JX847550	JX847515	PS	80.3.76.14
Abos8	*Acanthodactylus* sp.	Wadi Rum	Jordan	JX847551	JX847516	PS	80.3.76.55
Abos9	*A. boskianus*	Wadi Sudr	Egypt	JX847552	JX847517	PS	80.3.76.30
Abos10	*A. boskianus*	Natural reserve of Mergheb	Algeria	JX847557	JX847522	PS	80.3.76.76
Abos11	*A. boskianus*	Natural reserve of Mergheb	Algeria	JX847558	JX847523	PS	80.3.76.77
*Abos12	*A. boskianus*		Israel	n.a.	GU433289	Tikochinski *et al*., unpubl. data	
*Abos13	*A. boskianus*		Israel	n.a.	GU433290	Tikochinski *et al*., unpubl. data	

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APPENDIX 1 *Continued*

Identifier	Species	Locality	Country	Cyt *b*	16S rRNA	Reference	NHMC
*Abos14	*A. boskianus*		Israel	n.a.	GU433291	Tikochinski *et al*., unpubl. data	
Agra1	*A. grandis*	As Sukhnah	Syria	JX847553	JX847518	PS	80.3.77.1
Agra2	*A. grandis*	Dayr az Zawr 60 km west	Syria	JX847554	JX847519	PS	80.3.77.2
Agra3	*A. grandis*	Sapkha	Syria	JX847555	JX847520	PS	80.3.77.3
Agra4	*A. grandis*	Sapkha	Syria	JX847556	JX847521	PS	80.3.77.4
Abud1	*A. budaki*	Kas/Antalya	SE Turkey	JX847562	JX847527	PS	80.3.131.51
Abud2	*A. budaki*	Akseki and Cevizli	SE Turkey	JX847563	JX847528	PS	80.3.131.52
Abud3	*A. budaki*	Akseki	SE Turkey	JX847564	JX847529	PS	80.3.131.53
Abud4	*A. budaki*	Harbiye	SE Turkey	JX847565	JX847530	PS	80.3.131.54
Abud5	*A. budaki*	Rizocarpaso	Cyprus	JX847566	JX847531	PS	80.3.131.44
Abud6	*A. budaki*	Agros	Cyprus	AY561366	AY561420	Poulakakis *et al*. (2005a)	
Abud7	*A. budaki*	Kiverniti beach	Cyprus	AY561367	AY561421	Poulakakis *et al*. (2005a)	
Abud8	*A. budaki*	Kamares	Cyprus	AY561368	AY561422	Poulakakis *et al*. (2005a)	
Abud9	*A. budaki*	Germasogeia	Cyprus	AY561369	AY561423	Poulakakis *et al*. (2005a)	
Abud10	*A. budaki*	Vavla	Cyprus	AY561370	AY561424	Poulakakis *et al*. (2005a)	
Abud11	*A. budaki*	Roudia bridge	Cyprus	AY561371	AY561425	Poulakakis *et al*. (2005a)	
Abud12	*A. budaki*	Alawit mountain	Syria	AY561372	AY561426	Poulakakis *et al*. (2005a)	
Abud13	*A. budaki*	Alawit mountain	Syria	AY561373	AY561427	Poulakakis *et al*. (2005a)	
Abud14	*A. budaki*	Alawit mountain	Syria	AY561374	AY561428	Poulakakis *et al*. (2005a)	
Abud15	*A. budaki*	Alawit mountain	Syria	AY561375	AY561429	Poulakakis *et al*. (2005a)	
Abud16	*A. budaki*	Lattakia	Syria	AY561376	AY561430	Poulakakis *et al*. (2005a)	
Abud17	*A. budaki*	Allepo	Syria	AY561377	AY561431	Poulakakis *et al*. (2005a)	
Abud18	*A. budaki*	Allepo	Syria	AY561378	AY561432	Poulakakis *et al*. (2005a)	
Akit1	*A. kitaibelii*	Peloponnesos	Greece	AY561334	AY561388	Poulakakis *et al*. (2005a)	
Akit2	*A. kitaibelii*	Kithira isl.	Greece	AY561336	AY561390	Poulakakis *et al*. (2005a)	
Akit3	*A. kitaibelii*	Izmir	Turkey	AY561361	AY561415	Poulakakis *et al*. (2005a)	
Akit4	*A. kitaibelii*	Izmir	Turkey	AY561362	AY561416	Poulakakis *et al*. (2005a)	
Acer1	*A. cernovi*	Homs	Syria	AY561379	AY561433	Poulakakis *et al*. (2005a)	
Acer2	*A. cernovi*	Yoncali, Arguva	E Turkey	JX847567	JX847532	PS	80.3.79.31
Acer3	*A. cernovi*	north of Saimbeyli	SE Turkey	JX847568	JX847533	PS	80.3.79.25
Acer4	*A. cernovi*	Sariz	C Turkey	JX847569	JX847534	PS	80.3.79.35
Oele1	*O. elegans*	Larnaka Kamares	Cyprus	JX847570	EU081733	PS and Kyriazi *et al*. (2008)	80.3.70.124
Oele2	*O. elegans*	Larnaka	Cyprus	JX847571	EU081734	PS and Kyriazi *et al*. (2008)	80.3.70.127
Oele3	*O. elegans*	Lemesos	Cyprus	JX847572	EU081735	PS and Kyriazi *et al*. (2008)	80.3.70.128
Oele4	*O. elegans*	Athalassa	Cyprus	JX847573	EU081736	PS and Kyriazi *et al*. (2008)	80.3.70.131
Oele5	*O. elegans*	Arkounta	Cyprus	JX847574	EU081737	PS and Kyriazi *et al*. (2008)	80.3.70.132
Oele6	*O. elegans*	Pareklisia	Cyprus	JX847575	EU081738	PS and Kyriazi *et al*. (2008)	80.3.70.133
Oele7	*O. elegans*	Stavros Psokas	Cyprus	JX847576	EU081739	PS and Kyriazi *et al*. (2008)	80.3.70.135
Oele8	*O. elegans*	Roudia	Cyprus	JX847577	EU081740	PS and Kyriazi *et al*. (2008)	80.3.70.139
Oele9	*O. elegans*	Kayseri	Turkey	EU081629	EU081698	Kyriazi *et al*. (2008)	
Oele10	*O. elegans*	Karahamzeli	Turkey	EU081630	EU081699	Kyriazi *et al*. (2008)	
Oele11	*O. elegans*	Karakurt	Turkey	EU081635	EU081704	Kyriazi *et al*. (2008)	
Oele12	*O. elegans*	Saz Golu	Turkey	EU081637	EU081705	Kyriazi *et al*. (2008)	
Oele13	*O. elegans*	Kuskukiran Gecidi	Turkey	EU081638	EU081706	Kyriazi *et al*. (2008)	
Oele14	*O. elegans*	Nizran	Syria	EU081642	EU081711	Kyriazi *et al*. (2008)	
Oele15	*O. elegans*	Rocky desert	Syria	EU081644	EU081716	Kyriazi *et al*. (2008)	
Oele16	*O. elegans*	Jerash	Jordan	EU081650	EU081723	Kyriazi *et al*. (2008)	
Oele17	*O. elegans*	Zai park	Jordan	EU081655	EU081728	Kyriazi *et al*. (2008)	
Oele18	*O. elegans*	Mezudat Nimrod	Israel	EU081660	EU081731	Kyriazi *et al*. (2008)	
Oele19	*O. elegans*	Nahal Zin	Israel	EU081663	EU081732	Kyriazi *et al*. (2008)	
Oele20	*O. elegans*	Kyrinis-Apolonias	Libya	EU081673	EU08148	Kyriazi *et al*. (2008)	
Oele21	*O. elegans*	canyon after National Park	Libya	EU081674	EU08149	Kyriazi *et al*. (2008)	
Pbed1	*P. bedriagae*	Cha river	Cyprus	DQ474136	DQ474188	Lymberakis *et al*. (2007)	
Pbed2	*P. bedriagae*	Cha river	Cyprus	DQ474137	DQ474189	Lymberakis *et al*. (2007)	
Pbed3	*P. bedriagae*	Al Jaboul L.	Syria	DQ474129	DQ474181	Lymberakis *et al*. (2007)	
Pbed4	*P. bedriagae*	Hawaig Gorge	Syria	DQ474130	DQ474182	Lymberakis *et al*. (2007)	
Pbed5	*P. bedriagae*	Krak des Chevaliers	Syria	DQ474131	DQ474183	Lymberakis *et al*. (2007)	
Pbed6	*P. bedriagae*	Lattakia beach	Syria	DQ474132	DQ474184	Lymberakis *et al*. (2007)	
Pbed7	*P. bedriagae*	Qal' at Al Rahbeh castle	Syria	DQ474133	DQ474185	Lymberakis *et al*. (2007)	
Pbed8	*P. bedriagae*	Maquam Assayedh	Syria	DQ474134	DQ474186	Lymberakis *et al*. (2007)	
Pbed9	*P. bedriagae*	Sanlıurfa, Bozova	SE Turkey	AB640991	AB640944	Bülbül *et al*. (2011)	

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APPENDIX 1 *Continued*

Identifier	Species	Locality	Country	Cyt *b*	16S rRNA	Reference	NHMC
Pbed10	*Pelophylax* sp.	Mersin, Mezitli	SE Turkey	AB640979	AB640934	Bülbül *et al*. (2011)	
Pbed11	*Pelophylax* sp.	Hatay, Asi Stream	SE Turkey	AB640979	AB640950	Bülbül *et al*. (2011)	
Pbed12	*P. bedriagae*	Antalya	SW Turkey	AY014392	AF215422	Kosuch *et al*. (2001)	
Pbed13	*P. bedriagae*	Kırıkkale, Bahsılı	C Turkey	AB640986	AB640972	Bülbül *et al*. (2011)	
Pbed14	*P. bedriagae*	Konya, Aksehir Lake	C Turkey	AB640983	AB640938	Bülbül *et al*. (2011)	
Pbed15	*P. bedriagae*	Canakkale, Kepez	NW Turkey	AB640996	AB640949	Bülbül *et al*. (2011)	
Pbed16	*P. bedriagae*	Sinop, Erfelek	NC Turkey	AB640986	AB640970	Bülbül *et al*. (2011)	
Pbed17	*P. bedriagae*	Artvin, Savsat	NE Turkey	AB640995	AB640948	Bülbül *et al*. (2011)	
Pbed18	*P. bedriagae*	Marmaris	Turkey	AY147957	AY147937	Veith, Kosuch & Vences (2003)	
Pcar1	*P. caralitanus*	Konya, Dineksaray	C Turkey	AB640981	AB640953	Bülbül *et al*. (2011)	
Pcar2	*P. caralitanus*	Konya, Dineksaray	C Turkey	AB640981	AB640936	Bülbül *et al*. (2011)	
Pcar3	*P. caralitanus*	Antalya, Manavgat	SW Turkey	AB640980	AB640935	Bülbül *et al*. (2011)	
Pcer1	*P. cerigensis*	Karpathos isl.	E Aegean	DQ474142	DQ474194	Lymberakis *et al*. (2007)	
Pcer2	*P. cerigensis*	Karpathos isl.	E Aegean	DQ474143	DQ474195	Lymberakis *et al*. (2007)	
Pcer3	*P. cerigensis*	Karpathos isl.	E Aegean	DQ474144	DQ474196	Lymberakis *et al*. (2007)	
Pcer4	*P. cerigensis*	Rodos isl.	E Aegean	n.a.	AF215420	Vences (2000)	
Pcer5	*P. cerigensis*	Rodos isl.	E Aegean	n.a.	AY147979	Veith *et al*. (2003)	
Prid1	*P. ridibundus*	Dadia	NE Greece	DQ474163	DQ474215	Lymberakis *et al*. (2007)	
Prid2	*P. ridibundus*	Therma	NE Greece	DQ474162	DQ474214	Lymberakis *et al*. (2007)	
Prid3	*P. ridibundus*	Kotili	NE Greece	DQ474160	DQ474212	Lymberakis *et al*. (2007)	
Prid4	*P. ridibundus*	Nestos	NE Greece	DQ474161	DQ474213	Lymberakis *et al*. (2007)	
Tf1	*T. fallax*	Milos	Greece	JX315517	n.a.	Kyriazi *et al*. (accepted)	
Tf2	*T. fallax*	Siros	Greece	JX315519	n.a.	Kyriazi *et al*. (accepted)	
Tf3	*T. fallax*	Santorini	Greece	JX315505	n.a.	Kyriazi *et al*. (accepted)	
Tf4	*T. fallax*	Santorini	Greece	JX315506	n.a.	Kyriazi *et al*. (accepted)	
Tf5	*T. fallax*	Crete	Greece	JX315509	n.a.	Kyriazi *et al*. (accepted)	
Tf6	*T. fallax*	Crete	Greece	JX315513	n.a.	Kyriazi *et al*. (accepted)	
Tf7	*T. fallax*	Anikithira	Greece	JX315507	n.a.	Kyriazi *et al*. (accepted)	
Tf8	*T. fallax*	Kastelorizo	Greece	JX315521	n.a.	Kyriazi *et al*. (accepted)	
Tf9	*T. fallax*	Lesvos	Greece	JX315520	n.a.	Kyriazi *et al*. (accepted)	
Tf10	*T. fallax*	Cyprus	Cyprus	JX315528	n.a.	Kyriazi *et al*. (accepted)	
Tf11	*T. fallax*	Germasogia, Lemesos	Cyprus	JX315527	n.a.	Kyriazi *et al*. (accepted)	
Tf12	*T. fallax*	Kupluce	Turkey	JX315524	n.a.	Kyriazi *et al*. (accepted)	
Tf13	*T. fallax*	Adana	Turkey	JX315525	n.a.	Kyriazi *et al*. (accepted)	
Tf14	*T. fallax*	Gulnar, Mersin	Turkey	JX315522	n.a.	Kyriazi *et al*. (accepted)	
Tf15	*T. fallax*	Tasan	Jordan	AY188039	n.a.	Nagy *et al*. (2003)	
Hsav1	*H. savignyi*	Paramali, 3 km SE	Cyprus	n.a.	GQ916759	Gvoždík *et al*. (2010)	
Hsav2	*H. savignyi*	Paramali, 3 km SE	Cyprus	n.a.	GQ916777	Gvoždík *et al*. (2010)	
Hsav3	*H. savignyi*	Panagra	Cyprus	n.a.	GQ916754	Gvoždík *et al*. (2010)	
Hsav4	*H. savignyi*	Ammochostos	Cyprus	n.a.	GQ916758	Gvoždík *et al*. (2010)	
Hsav5	*H. savignyi*	Bagdad	Iraq	n.a.	GQ916767	Gvoždík *et al*. (2010)	
Hsav6	*H. savignyi*	Al Hasakah	Syria	n.a.	GQ916767	Gvoždík *et al*. (2010)	
Hsav7	*H. savignyi*	Kazanli, 10 km E of Mersin	Turkey	n.a.	GQ916754	Gvoždík *et al*. (2010)	
Hsav8	*H. savignyi*	Anamurium	Turkey	n.a.	GQ916754	Gvoždík *et al*. (2010)	
Hsav9	*H. savignyi*	Choqa Zanbil	Iran	n.a.	GQ916766	Gvoždík *et al*. (2010)	
Hfel1	*H. felixarabica*	Shayzar	Syria	n.a.	GQ916787	Gvoždík *et al*. (2010)	
Hfel2	*H. felixarabica*	Amran	Yemen	n.a.	GQ916785	Gvoždík *et al*. (2010)	
Hfel3	*H. felixarabica*	Sana'a	Yemen	n.a.	GQ916785	Gvoždík *et al*. (2010)	
Pod1	*P. cretensis*	Chrisi island, Crete	Greece	AF486212	AY896148	Poulakakis *et al*. (2005b)	
Pod2	*P. cretensis*	Lafonisi, Crete	Greece	AF486193	AY896157	Poulakakis *et al*. (2005b)	
Pod3	*P. cretensis*	Samaria, Crete	Greece	AF486204	AY896161	Poulakakis *et al*. (2005b)	
Pod4	*P. peloponnesiaca*	Feneos	Peloponnesos	AY896116	AY896173	Poulakakis *et al*. (2005b)	
Pod5	*P. peloponnesiaca*	Kalavrita	Peloponnesos	AY896121	AY896177	Poulakakis *et al*. (2005b)	
Gpb6	*P. bocagei*	Malpica, Galicia	Spain	AF469426	DQ081075	Pinho, Ferrand & Harris (2006)	
Albc1	*P. carbonelli*	La Alberca, Castilla y León	Spain	DQ081142	DQ081080	Pinho *et al*. (2006)	
PR1	*P. carbonelli*	Playa del Rompeculos	Spain	DQ081141	DQ081079	Pinho *et al*. (2006)	
Mon8	*P. hispanica*	Montesinho	Portugal	AF469447	DQ081086	Pinho *et al*. (2006)	
Trj1	*P. hispanica*	Trujillo Extremadura	Spain	AF469451	DQ081088	Pinho *et al*. (2006)	
And10	*P. hispanica*	Benatae, Andalucia	Spain	DQ081143	DQ081084	Pinho *et al*. (2006)	
Phv4	*P. hispanica*	Beja	Portugal	AF469455	DQ081083	Pinho *et al*. (2006)	
Raw	*R. a. wolterstorffi*			AY147959	AY147939	Veith *et al*. (2003)	
Raa	*R. a. arvalis*			AY147958	AY147938	Veith *et al*. (2003)	
Rgr	*R. graeca*			AY147963	AY147942	Veith *et al*. (2003)	
Cvir	*C.viridanus*			EU278117	EU278036	Carranza *et al*. (2008)	
Csph	*C. sphenopsiformis*			EU278107	EU278131	Carranza *et al*. (2008)	
Ealg	*E. algeriensis*			EU278253	EU278086	Carranza *et al*. (2008)	

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Figure S1. Phylogenetic analysis on *Acanthodactylus* that shows the position of *A. schreiberi* from Israel.
Table S1. Detailed description of primers and PCR conditions used in this study.

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