Molecular Phylogenetics and Evolution 66 (2013) 992–1001

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

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

Molecular phylogeny and historical biogeography of the Anatolian lizard *Apathya* (Squamata, Lacertidae)

Paschalia Kapli [a,b,1], Dimitra Botoni [a,b,1], Çetin Ilgaz [c,1], Yusuf Kumlutaş [c], Aziz Avcı [d], Nasrullah Rastegar-Pouyani [e], Behzad Fathinia [e], Petros Lymberakis [a], Faraham Ahmadzadeh [f,g], Nikos Poulakakis [a,b,*]

[a] Molecular Systematics Lab, Natural History Museum of Crete, University of Crete, 71409 Iraklion, Crete, Greece
[b] Biology Department, University of Crete, 71409 Iraklion, Crete, Greece
[c] Dokuz Eylül University, Faculty of Science, Department of Biology, 35160 Buca/İzmir, Turkey
[d] Adnan Menderes University, Faculty of Science and Arts, Department of Biology, 09010 Aydın, Turkey
[e] Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran
[f] Department of Biodiversity and Ecosystem Management, Environmental Sciences Research Institute, Shahid Beheshti University, Evin, Tehran, Iran
[g] ZoologischesForschungsmuseum Alexander Koenig, Adenauerallee 160, 53113 Bonn, Germany

ARTICLE INFO

Article history:
Received 7 August 2012
Revised 23 November 2012
Accepted 5 December 2012
Available online 20 December 2012

Keywords:
Anatolian Diagonal
Divergence times
Mitochondrial DNA
Molecular systematics
Nuclear DNA
Phylogeography

ABSTRACT

Apathya is a lacertid genus occurring mainly in south-east Turkey and its adjacent regions (part of Iran and Iraq). So far two morphological species have been attributed to the genus; *A. cappadocica* (with five subspecies, *A. c. cappadocica*, *A. c. muhtari*, *A. c. schmidtlerorum*, *A. c. urmiana* and *A. c. wolteri*) and *A. yassujica*. The first species occupies most of the genus' distribution range, while *A. yassujica* is endemic of the Zagros Mountains. Here, we explored *Apathya*'s taxonomy and investigated the evolutionary history of the species by employing phylogenetic and phylogeographic approaches and using both mitochondrial (mtDNA) and nuclear markers. The phylogenetic relationships and the genetic distances retrieved, revealed that *Apathya* is a highly variable genus, which parallels its high morphological variation. Such levels of morphological and genetic differentiation often exceed those between species of other Lacertini genera that are already treated as full species, suggesting the necessity for a taxonomic revision of *Apathya*. The phylogeographical scenario emerging from the genetic data suggests that the present distribution of the genus was determined by a combination of dispersal and vicariance events between Anatolia and Southwest Asia dating back to the Miocene and continuing up to the Pleistocene. Key geological events for the understanding of the phylogeography of the genus are the movement of the Arabian plate that led to the configuration of Middle East (orogenesis of the mountain ranges of Turkey and Iran) and the formation of Anatolian Diagonal.

1. Introduction

The lizard family Lacertidae consists of about 280 species placed in 24–30 genera found throughout Eurasia and Africa (Arnold et al., 2007). Based on morphology most of the recognized genera appear to be included in monophyletic clades, with the exception of the paraphyletic central African *Adolfus* and *Lacerta* sensu lato of Europe, SW and N Asia and northwest Africa.

The genus *Lacerta*, a morphologically extremely diverse genus, was initially revised by Arnold (1973) who separated four tropical and southern African species from Lacerta and raised the subgenera *Podarcis* and *Gallotia* to the generic level. In this revision, *Lacerta* was divided into two groups: *Lacerta* part I which is more or less equivalent to *Lacerta sensu stricto* and *Lacerta* part II. The former encompasses large bodied *Lacerta* species, usually occurring in areas of dense and shrubby vegetation, while the latter consists of smaller bodied species, many of them saxicolous.

The systematics of Arnold's (1973) *Lacerta* part II is far from being resolved. Some species groups previously assigned to *Lacerta* are now grouped in different genera or subgenera (Arnold et al., 2007; Mayer and Arribas, 2003) with unknown relationships among them. One of them is the medium size lacertid lizard of the genus *Apathya*, first described from Erciyes Mountain in Kayseri, Turkey (Ilgaz et al., 2010).

Nowadays, *Apathya* Mehely, 1907 includes 2 species [*Apathya cappadocica* (Werner, 1902) and *A. yassujica* (Nilson et al., 2003)] and occurs (Fig. 1) in Central, East, and South Anatolia, Northern

* Corresponding author at: Molecular Systematics Lab, Natural History Museum of Crete and Biology Department, University of Crete, Iraklion, Crete, Greece.
E-mail address: poulakakis@nhmc.uoc.gr (N. Poulakakis).
[1] These author contributed equally to this work.

1055-7903/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ympev.2012.12.002

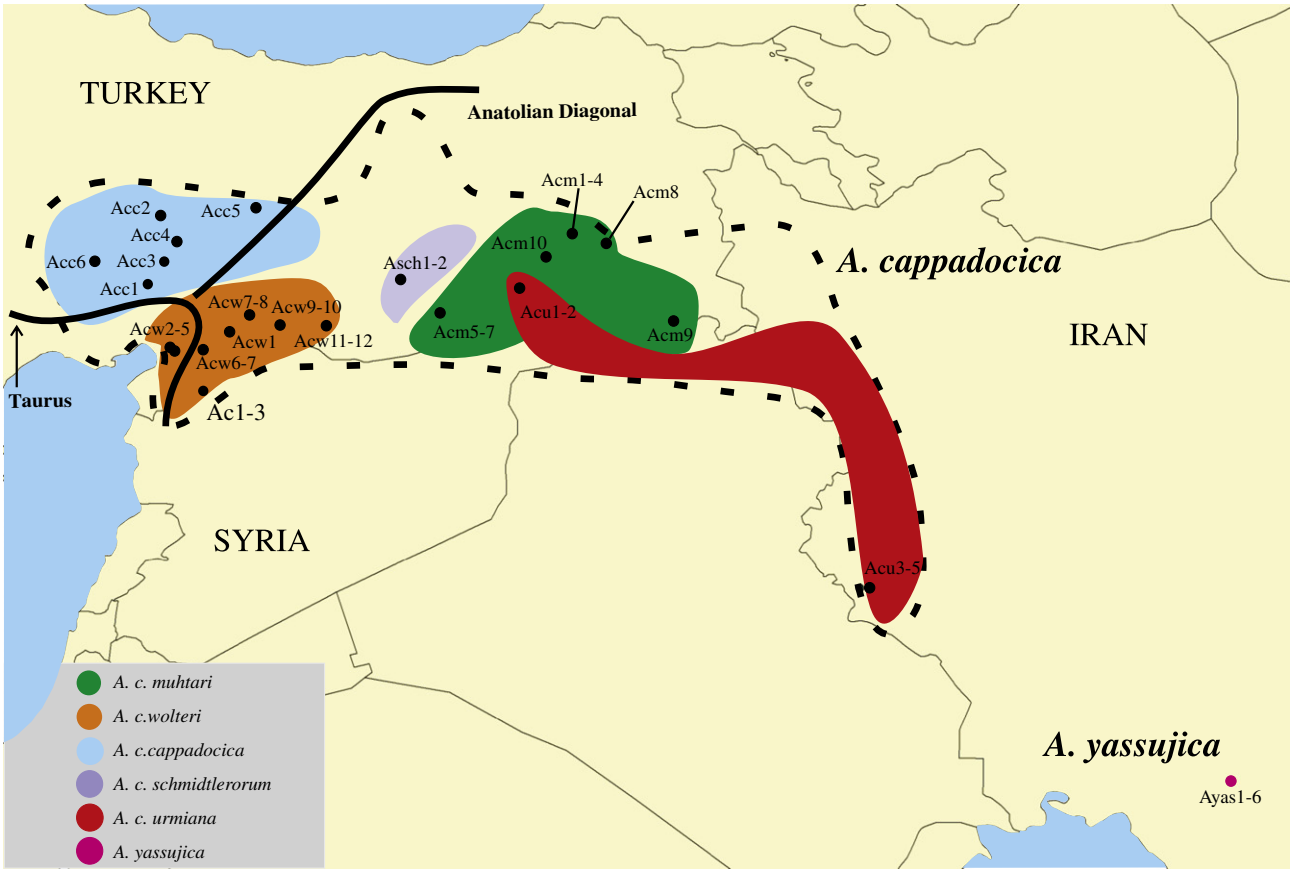

Fig. 1. Map showing the sampling localities of the specimens examined. Numbers refer to specimen codes given in the Table 1. The dashed black line shows the distribution of *A. cappadocica*. The shaded areas correspond to the subspecies distributions (Sindaco and Jeremčenko, 2008). The thick black line represents the "Anatolian Diagonal" which runs through northeast and central Turkish Anatolia (Nilson et al., 1990).

Iraq, and West Iran (Arnold et al., 2007; Eiselt, 1979; Nilson et al., 2003). It is unique among Lacertini in having clear single keels on scales beneath toes, a transparent window in the lower eyelid consisting of several black-edged scales, and variable scaling on the side of the snout that may involve up to three postnasal and three loreal scales and sometimes an additional scale between the rostral and the nostril (Arnold et al., 2007).

The first species *(A. cappodocica)* is polytypic and includes five subspecies [*A. c. cappadocica* (Werner, 1902), *A. c. urmiana* (Lantz and Suchow, 1934), *A. c. wolteri* (Bird, 1936), *A. c. muhtari* (Eiselt, 1979), and *A. c. schmidtlerorum* (Eiselt, 1979)] with high protein (blood-serum) differentiation (Ilgaz et al., 2010; Nilson et al., 2003). On the other hand, *A. yassujica* with strongly keeled subdigital lamellae and eyelid with transparent window of several scales is a monotypic species. It differs from *A. cappadocica* in having a completely different color pattern, smaller size, fewer transverse ventral plate series, fewer dorsal, gular and collar scales, and smooth or obtusely keeled caudal scales (Nilson et al., 2003).

Anatolia, the region encapsulating most of the *Apathya*'s range (Fig. 1) has a complicated geological history that has also left an imprint on the biogeography of many other vertebrate taxa, especially amphibians and reptiles (see Bilgin, 2011 for review; Kornilios et al., 2012). Located in the Alpine–Himalayan Mountain belt between Eurasia, Africa and Arabia, Anatolia, its geomorphology is the result of the collision of the Arabian and African plates with the European plate, which promoted the closure of the Tethys Sea (Rögl, 1998). Due to its position and geological history, Anatolia has acted either as a bridge or as a barrier for species' dispersal between Asia, Europe, and the Ethiopian region, providing a natural pathway or acting as a vicariant agent (Tchernov, 1992). The Taurus Mountains, the Bitlis-Pötürge Massif, the Zagros Mountains, the Anatolian Diagonal, the Central Anatolian Plateau, and the Central Anatolian Lake system acted as most obvious barriers (Eronen et al., 2009; Nilson et al., 1990; Popov et al., 2006; Rögl, 1999). The formation of the mountain chains in Anatolia can be traced back to the Tertiary, when the northward movement of Europe resulted in the formation of the Alps. The Central Anatolian lake system that is located between the Taurus and the Black Sea Mountains, was also initially formed in that time and persisted cyclically until the end of the Pliocene (Bilgin, 2011 and references therein).

Severe climatic changes have also played a key role on the evolutionary and biogeographical history of animals inhabiting this area, since climatic oscillations between significantly wetter and drier conditions have produced repeated habitat changes and periodic modifications of major biota (Douady et al., 2003; Prentice and Jolly, 2000). The late Neogene represents a significant period in Cenozoic climatic history with major expansion of dry zones and replacement of forests by woodlands and grasslands, occurring particularly in the mid-latitudes in this time interval (as described by Kornilios et al. (2012)). The cause(s) of this global aridification trend are not yet fully understood, although it is commonly believed that there is a general relation with global Cenozoic cooling, since atmospheric moisture content decreased with decreasing air temperature (Kornilios et al., 2012). Due to their presumed limited natural-dispersal capacities and temperature dependence, reptiles are sensitive indicators of the palaeogeographical and palaeoclimatic events that have shaped their biogeographic history. Thus, studying the patterns and process that led to the diversification

of *Apathya* may shed light on the major speciation drivers in this interesting geological area which may provide insight in the evolutionary history of other taxa in the area.

In order to explore *Apathya*'s taxonomy and to assess the evolutionary history of the species, we sampled the distribution area of the species by analyzing 46 individuals form 24 sampling sites and performed several combined phylogenetic and phylogeographic reconstructions, using three gene fragments from two mtDNA (cytochrome oxidase; COI, and cytochrome; cyt *b*) and one nuclear (c-mos) genes.

2. Materials and methods

2.1. Samples and lab procedures

Tissue samples were available for 46 individuals representing all species and subspecies of *Apathya* (Table 1, and Fig. 1). Total genomic DNA was extracted using proteinase K digestion (10 mMTris–HCl pH 8.0, 50 mM EDTA pH 8.0, 10 mMNaCl, 0.5% SDS, 0.1 M DTT, and 10 ul of 20 mgr/mL proteinase K) followed by an ammonium acetate-extraction protocol. Partial segments of two mtDNA (COI and cyt *b*) and one nuclear (c-mos) genes were selected for the phylogenetic analyses. 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 ABI 377 automated sequencer. Both strands of the amplified PCR products were sequenced for all specimens. After sequencing the portions of COI and cyt *b* mitochondrial genes and producing a mitochondrial phylogeny, 14 specimens (marked with an asterisk in Table 1) from all the mtDNA defined clusters were also sequenced for the c-mos nuclear marker. For the c-mos gene, the nested primers (G73/G74) were used for reamplifications, as well as sequencing primers (Table S1). Gel-purified (QIAquick Gel extraction kit, Qiagen, Venlo, The Netherlands) amplified PCR fragments were cloned using the TA vector (TOPO TA Cloning Kit, Invitrogen, Carlsbad, CA).

Chromatographs were checked and sequences were edited and assembled using Codon-Code Aligner (v. 3.7.1, Codon Code Corporation). Coding gene fragments were translated into amino acids and no stop codons were observed. Specimens from several Lacertidae taxa were used for outgroups and age constraints comparisons (two specimens of *Helenolacerta graeca*, 10 specimens of *Podarcis* spp., and seven specimens of *Gallotia* spp., see Table 1). All newly determined sequences have been deposited in GenBank (accession numbers will be provided; Table 1).

2.2. Data analysis

The alignment of the sequences was performed separately for each gene with MAFFT v.6 (Katoh et al., 2002) with default parameters and FFT-NS-1 algorithm.

The best-fit model of DNA substitution were chosen according to the Akaike Information Criterion (Akaike, 1974; see Posada and 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 $\alpha \leqslant 1$ 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).

Sequence divergences were estimated in MEGA v.5.05 (Tamura et al., 2011), using the Tamura and Nei (TrN) (Tamura and Nei, 1993) model of evolution for the current taxonomic units of *Apathya*, and the main lineages produced through the phylogenetic analyses.

2.3. Gene tree estimation on mtDNA

Phylogenetic analyses were performed using Maximum Likelihood (ML) and Bayesian Inference (BI) methods.

Bayesian phylogenetic analysis was conducted in MrBAYES v.3.1.2 (Huelsenbeck and Ronquist, 2001) using the GTR + G, and HKY + G models of evolution for COI, and cyt *b*, respectively. The analysis was run four times with eight chains each run for 10^7 generations, sampling from the chain every 100 generations. This generated an output of 10^5 trees. In order to confirm that the chains had achieved stationarity, we evaluated "burn-in" by plotting log-likelihood scores and tree lengths against generation number using the Tracer v.1.5.0 (Rambaut and 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 $\geqslant$95% indicate significant support.

ML analyses were performed with RAxML v.7.2.8 (Stamatakis, 2006) with 100 random addition replicates in RAxML Black box (Stamatakis et al., 2008). A GTR + GAMMA model was used and parameters were estimated independently for each partition (i.e., gene). Reliability of the ML tree was assessed by rapid bootstrap analysis (Stamatakis et al., 2008) for 100 replications.

Topological constraints to test alternative topologies were constructed by hand and compared to the unconstrained (best) tree using the Shimodaira-Hasegawa (SH) (Shimodaira and Hasegawa, 1999) test using RELL bootstrap (1000 replicates) as implemented in PAUP* (v.4.0b10) (Swofford, 2002).

2.4. Species tree estimation and divergence times on mtDNA

Since no internal calibration points are available for *Apathya* to estimate the timing of its cladogenetic events, the substitution rate of the same mitochondrial regions from fully-calibrated phylogenies of various lizard groups were used as "external" calibration age constraints (Table 2). We used times calibration points based on the separation of the Canary Islands and the isolation of the island of Crete in the Aegean Sea as their geological history and island ages are very well known and it has been used to calibrate the phylogenies of other two lizards species, *Gallotia* spp. and *Podarcis* spp. (Cox et al., 2010; Poulakakis et al., 2003). Another calibration point was provided by the opening of the Gibraltar Strait at the end of Messinian Salinity Crisis (5.33 Mya), that acted as a vicariant event, giving rise to the two endemic lizards of the Balearic Islands: *P. lilfordii* in the Gymnesic Islands (Mallorca, Menorca and surrounding islets) and *P. pityusensis* in the Pityusic Islands (Ibiza, Formentera and surrounding islets) (Brown et al., 2008).

The divergence times within *Apathya* were estimated using BEAST v1.7.2 (Drummond and Rambaut, 2007). The input file was formatted with the BEAUti utility included in the software package. The analysis was run for 10^8 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): *COI* – GTR + G; *cyt b* – HKY + G; Relaxed Uncorrelated Lognormal Clock (estimate); Yule process of speciation; random starting tree.

For all analyses implemented in BEAST, results were analyzed in Tracer to assess convergence and effective sample sizes

Table 1
Sample codes (Fig. 1), species names, sampling locations (locality, region, country) and collection/museum numbers of the specimens used in the phylogenetic analyses. (NHMC: Natural History Museum of Crete, ZDEU: Zoology Department, Ege University). GenBank accession numbers of sequence data for the segments of COI/*Cyt b*/*c-mos* respectively, are also shown. Asterisks indicate the specimens that were selected from GMYC analysis to sequence the c-mos nuclear marker.

Code	Collection No	Species	Locality		Country	Acc. No.
Acc1	DB12033	*A. c. cappadocica*	25 km SW Göksun	S Anatolia	Turkey	
Acc2	ZDEU 31/2007	*A. c. cappadocica*	Fakıekinciliği, Pınarbaşı, Kayseri	C Anatolia	Turkey	
Acc3	ZDEU 28/2006	*A. c. cappadocica*	Gebezeli mt, Adana	S Anatolia	Turkey	
Acc4	ZDEU 20/2007	*A. c. cappadocica*	Tarlaköy, Sarız, Kayseri	C Anatolia	Turkey	
Acc5*	ZDEU 5/2007	*A. c. cappadocica*	Ciritbelen Village, Kuluncak, Malatya	C Anatolia	Turkey	
Acc6	ZDEU 5/1995	*A. c. cappadocica*	Yahyalı, Kayseri	C Anatolia	Turkey	
Acm1	ZDEU 7/2010-1a	*A. c. muhtari*	13 km NW of Tatvan, Bitlis	E Anatolia	Turkey	
Acm2	ZDEU 7/2010-1b	*A. c. muhtari*	13 km NW of Tatvan, Bitlis	E Anatolia	Turkey	
Acm3	ZDEU 7/2010-1c	*A. c. muhtari*	13 km NW of Tatvan, Bitlis	E Anatolia	Turkey	
Acm4	ZDEU 7/2010-1d	*A. c. muhtari*	13 km NW of Tatvan, Bitlis	E Anatolia	Turkey	
Acm5	ZDEU 7/2010-2a	*A. c. muhtari*	2 km N of Derik, Mardin	SE Anatolia	Turkey	
Acm6*	ZDEU 7/2010-2b	*A. c. muhtari*	2 km N of Derik	SE Anatolia	Turkey	
Acm7*	ZDEU 7/2010-2c	*A. c. muhtari*	2 km N of Derik	SE Anatolia	Turkey	
Acm8*	ZDEU 7/2010-3	*A. c. muhtari*	47 km E of Tatvan, Bitlis	E Anatolia	Turkey	
Acm9*	ZDEU 17/2010	*A. c. muhtari*	Çukurca, Hakkari	SE Anatolia	Turkey	
Acm10*	ZDEU 18/2010	*A. c. muhtari*	Baykan, Siirt	SE Anatolia	Turkey	
Acm11*	ZDEU 6/2004	*A. c. muhtari*	Recep Village, Çelikhan/Adıyaman	SE Anatolia	Turkey	
Acsch1*	ZDEU 13/2010	*A. c. schmidtlerorum*	Between Diyarbakır and Siverek	SE Anatolia	Turkey	
Acsch2	ZDEU 14/2010	*A. c. schmidtlerorum*	Between Diyarbakır and Siverek	SE Anatolia	Turkey	
Acw1	ZDEU 7/2005	*A. c.wolteri*	Akçaburç-Şehitkamil, Gaziantep	SE Anatolia	Turkey	
Acw2	ZDEU 1/2010	*A. c.wolteri*	Çardak Valley, Hassa, Hatay	S Anatolia	Turkey	
Acw3*	ZDEU 2/2010	*A. c. wolteri*	Çardak Valley, Hassa, Hatay	S Anatolia	Turkey	
Acw4	ZDEU 3/2010	*A. c. wolteri*	Hassa, Hatay	S Anatolia	Turkey	
Acw5	ZDEU 4/2010	*A. c. wolteri*	Hassa, Hatay	S Anatolia	Turkey	
Acw6*	ZDEU 5/2010	*A. c. wolteri*	Between Kilis and Hassa	SE Anatolia	Turkey	
Acw7	ZDEU 6/2010	*A. c. wolteri*	Between Kilis and Hassa	SE Anatolia	Turkey	
Acw8	ZDEU 7/2010	*A. c. wolteri*	Ballık Village, Yavuzeli, Gaziantep	SE Anatolia	Turkey	
Acw9*	ZDEU 8/2010	*A. c. wolteri*	Ballık Village, Yavuzeli, Gaziantep	SE Anatolia	Turkey	
Acw10	ZDEU 9/2010	*A. c. wolteri*	Between Birecik and Halfeti	SE Anatolia	Turkey	
Acw11	ZDEU 10/2010	*A. c. wolteri*	Between Birecik and Halfeti	SE Anatolia	Turkey	
Acw12	ZDEU 11/2010	*A. c. wolteri*	Küçükalanlı Village, Şanlıurfa	SE Anatolia	Turkey	
Acw13*	ZDEU 12/2010	*A. c. wolteri*	Küçükalanlı Village, Şanlıurfa	SE Anatolia	Turkey	
Ac1	NHMC80.3.66.1	*A. c. cf. wolteri*	Samaan Mt., 40 km NW of Allepo		Syria	
Ac2	NHMC80.3.66.2	*A. c. cf. wolteri*	Samaan Mt., 40 km NW of Allepo		Syria	
Ac3*	NHMC80.3.66.3	*A. c. cf. wolteri*	Samaan Mt., 40 km NW of Allepo		Syria	
Acu1	ZDEU 15/2010	*A. c. urmiana*	Hasankeyf, Batman	SE Anatolia	Turkey	
Acu2*	ZDEU 16/2010	*A. c. urmiana*	Hasankeyf, Batman	SE Anatolia	Turkey	
Acu3*	NHMC80.3.66.5	*A. c. urmiana*	Manehst Mt, Shirvan & Chardavol	Ilam	Iran	
Acu4	NHMC80.3.66.6	*A. c. urmiana*	Manehst Mt, Shirvan & Chardavol	Ilam	Iran	
Acu5	NHMC80.3.66.7	*A. c. urmiana*	Manehst Mt, Shirvan & Chardavol	Ilam	Iran	
Ayas1*	DB6452	*A. yassujica*	30 km SE of Yassuj	Kohgiluyeh	Iran	
Ayas2	NHMC80.3.168.1	*A. yassujica*	30 km SE of Yassuj	Kohgiluyeh	Iran	
Ayas3	NHMC80.3.168.2	*A. yassujica*	30 km SE of Yassuj	Kohgiluyeh	Iran	
Ayas4	NHMC80.3.168.3	*A. yassujica*	30 km SE of Yassuj	Kohgiluyeh	Iran	
Ayas5	NHMC80.3.168.4	*A. yassujica*	30 km SE of Yassuj	Kohgiluyeh	Iran	
Ayas6	NHMC80.3.168.6	*A. yassujica*	30 km SE of Yassuj	Kohgiluyeh	Iran	
Hgr1	NHMC80.3.65.25	*H. graeca*	Polidroso	Peloponnesos	Greece	
Hgr2	NHMC80.3.65.26	*H. graeca*	Polidroso	Peloponnesos	Greece	
Pcr1	NHMC80.3.51.1	*P. cretensis*	Argiroupoli	Crete Isl.	Greece	/AF486216/
Pcr2	NHMC80.3.51.2	*P. cretensis*	Argiroupoli	Crete Isl.	Greece	
Pcr3	NHMC80.3.51.13	*P. cretensis*	Sougia	Crete Isl.	Greece	/AF486218/
Ppel1	NHMC80.3.54.2	*P. peloponnesiaca*	Kalavrita	Peloponnesos	Greece	/AY896121/
Ppel2	NHMC80.3.54.7	*P. peloponnesiaca*	Stoupa	Peloponnesos	Greece	/AY896124/
Ppel3	NHMC80.3.54.15	*P. peloponnesiaca*	Kalavrita	Peloponnesos	Greece	
Plil1	DB6653	*P. lilfordi*	Mallorca isl.	Balearic Isl.	Spain	//EF679300
Plil2	DB6807	*P. lilfordi*	Mallorca isl.	Balearic Isl.	Spain	//EF679303
Ppit1	DB10457	*P. pityusensis*	Palma de Mallorca, Mallorca isl.	Balearic Isl.	Spain	//EF679324
Ppit2	DB10468	*P. pityusensis*	Palma de Mallorca, Mallorca isl.	Balearic Isl.	Spain	//EF679326
Gatl1	DB1242	*G. atlantica mahoratae*	La Oliva, Fuerteventura isl.	Canary Isl.	Spain	//AF435106
Gatl2	DB1244	*G. atlantica atlantica*	Nazaret-Teguise, Lanzarote isl.	Canary Isl.	Spain	//AF435104
Ggal1	DB1263	*G. galloti palmae*	La Lomada, La Palma ils.	Canary Isl.	Spain	//AY152004
Ggal2	DB1277	*G. galloti eisentrauti*	La Laguna, Tenerife ils.	Canary Isl.	Spain	//AY152002
Gcae1	DB2091	*G. caesaris gomerae*	Los Casetas, La Gomera ils.	Canary Isl.	Spain	//AY152005
Gcae2	DB2457	*G. caesaris caesaris*	Valverde, El Hierro isl.	Canary Isl.	Spain	//AY152006
Gste1	DB2455	*G. stehlini*	Barranco de Mógan, Gran Canaria	Canary Isl.	Spain	//AY152001

(ESSs) for all parameters. The final tree (Species Tree or Tree with divergence estimates and their 95% highest posterior densities, HPD) was computed in TreeAnnotator v.1.7.2. Trees were visualized using the software FigTree v1.3.1 (Rambaut, 2006–2009).

For comparison, the relaxed molecular clock Bayesian method implemented in MCMCTREE program in PAML v.4.5 (Yang, 2007) was also performed. The constrained BEAST tree was used for divergence time estimation. The MCMCTREE program allows for minimum (lower) and maximum (upper) time constraints. Markov

Table 2
Calibration points used for the dating of *Apathya* divergences.

	Split	Age in Mya	Reference
1	*P. cretensis – P. peloponnesiaca*	5.0–5.5	Poulakakis et al. (2003)
2	*P. lilfordi – P. pityusensis*	5.33	Brown et al. (2008)
3	*Gallotia* root	10.72–12.61	Cox et al. (2010)
4	*Gallotia caesaris – G. galloti*	3.14–3.73	Cox et al. (2010)

chain Monte Carlo (MCMC) approximation with a burn-in period of 50,000 cycles was obtained, and every 50 cycles was taken to create a total of 10,000 samples. To diagnose possible failure of the Markov chains to converge to their stationary distribution, two replicate MCMC runs were performed with two different random seeds for each analysis. Also distributions of parameter values from MCMC samples were visualized using Tracer to check mixing, choose a suitable burn-in, and look for trends that might suggest problems with convergence. The number of samples (10,000) was large enough to reach effective sample sizes (>200) for all parameters estimated in this study.

The coalescent-based species-tree approach implemented in *BEAST (Heled and Drummond, 2010), an extension of BEAST, was used to test the origin and diversification patterns in *Apathya*, and to compare these results to those obtained from the ML and BI analyses of the concatenated dataset. In order to reconstruct the topology of the species tree this analysis needs a priori information regarding the species/populations delimitation and the species/populations assignation of the individuals. We used the results obtained from the phylogenetic analyses outlined above to define the groups of individuals to be used as "species" (populations) in *BEAST. The analysis was run for 10^8 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): *COI* – GTR + G; *cyt b* – HKY + G; Relaxed Uncorrelated Lognormal Clock (estimate); Yule process of speciation; random starting tree.

2.5. Phylogenetic analyses on nDNA

Mitochondrial genetic clusters that represent "independently evolving" entities were selected using the method of Pons et al. (2006), which identifies genetic clusters using a generalized mixed Yule coalescent (GMYC) model in R package SPLITS (SPecies'LImits by Threshold Statistics). The method is available as part of the R package 'splits' (http://r-forge.r-project.org/projects/splits/). This analysis was conducted on the BEAST ultrametric tree. For the phylogenetic analysis at the species level, a single exemplar representing each GMYC group in the mtDNA analysis was selected for sequencing of nuclear marker (c-mos).

The genealogical relationships among c-mos sequences were estimated with the statistical parsimony, implemented in TCS v1.21 (Clement et al., 2000).

2.6. Phylogenetic analyses on complete dataset (mt and nDNA)

Maximum Likelihood (ML) and Bayesian Inference (BI) phylogenetic analyses were also performed on a concatenated dataset, containing the two mitochondrial (COI and cyt *b*) and the nuclear gene (c-mos). Maximum Likelihood analysis was performed as describe before. The BI analysis was run four times with eight chains each run for 10^7 generations, sampling from the chain every 100 generations, from which 10% were discarded as burn-in. Models and prior specifications applied were as follows: *COI* – GTR + G; *cyt b* – HKY + G; and *c-mos* – TrN.

2.7. Detecting long branch attraction (LBA)

Long-branch attraction (LBA) may arise in "any situation in which similarity due to convergent or parallel changes produces an artifactual phylogenetic grouping of taxa due to an inherent bias in the estimation process" (Anderson and Swofford, 2004). A number of methods have been suggested and applied to reduce the risk of having an outcome affected by LBA, including excluding long-branch taxa and faster evolving third codon positions, using inference methods less sensitive to LBA such as likelihood, and sampling more taxa to break up long branches and sampling more characters (Bergsten, 2005). Differences in the results from different inference methods, such as parsimony vs. likelihood vs. Bayesian, are sometimes taken as evidence of LBA (methodological discordance). Siddall and Whiting (1999) noted the obvious fact that for a long branch to be able to attract or be attracted another long branch need to be included simultaneously in the analysis. So, in a case where two long branched taxa are grouped together, removing one while keeping the other in and vice versa would allow them to find their correct position in separate analyses. If the clade was correct, then the separate analyses would not alter the position of the long branches in the tree. If however, one branch moves to another part of the tree, then one could argue that the clade including both taxa was an LBA artifact. It has been pointed out that, since outgroup taxa almost always represent long branches and are as such a hazard towards misplacing long branched ingroup taxa, phylogenetic analyses should always be run with and without the outgroups included (Bergsten, 2005).

To test for LBA we applied four approaches that might detect this phenomenon: (i) independent analysis of different gene partitions, (ii) long-branch extraction (for LBA to occur we need at least one other branch that attracted the potentially misplaced branch), (iii) outgroup extraction, and (iv) methodological discordance. A Maximum Parsimony (MP) analysis was performed in PAUP* with heuristic searches using stepwise addition and performing tree-bisection reconnection (TBR) branch swapping and the resulted topology was compared with the corresponding trees from ML and BI analyses. Moreover, another MP analysis was carried out (i) excluding the third codon positions in COI and cyt *b*, (ii) with and without the outgroups; (iii) with and without outgroup, excluding the lineage of *A. yassujica* in the clade of which is suspected to show LBA problem; (iv) with and without outgroup, excluding the lineage of *A. c. urmiana* (sister group relationship with *A. yassujica*).

2.8. Biogeographic analysis

The mtDNA species tree obtained using *BEAST were turned into NEWICK format and used for the biogeography analysis. In order to determine the broad scale geographic evolution of the clade, a parametric likelihood analyses using Lagrange was conducted (Ree and Smith, 2008), which implements the likelihood-based geographic range reconstruction method, using biogeographic speciation scenarios and parameters for dispersal and extinction (Ree et al., 2005). We considered species/subspecies/populations to be distributed within five broad areas: central Anatolia (cAN), eastern Anatolia (eAN), southern Anatolia and northern Syria (sAN), western Iran (wIR), and southern Iran (sIR).

3. Results

3.1. Mitochondrial lineages

A total of 1074 base pairs (bp) of concatenated mtDNA (*COI* 660 bp, and *cyt b* 414 bp) were obtained for 46 *Apathya* spp. The

concatenated alignment of the ingroup sequences revealed 46 haplotypes and contained 319 variable sites (299 parsimony informative).

The TrN genetic distances between the main lineages of *Apathya* spp. and the outgroup taxa (*Podarcis, Hellenolacerta, Gallotia*) varied from 4.7% to 29% in cyt *b* and 4.0–26.8% in COI (Table 3). Of interest is the high sequence divergence (17.9% in cyt *b* and 13.2% in COI) between the *urmiana* lineages, which include specimens from eastern Anatolia and Iran.

Maximum Likelihood ($-\ln L = 7980.46$) and BI ($-\ln L = 8004.63$) analyses of the concatenated mtDNA data produced identical topologies (Fig. 2A). Five very-well supported lineages were recovered from these analyses (posterior probability; pp = 1.00, and bootstrap support, bs > 99), corresponding to the populations of *A. c. urmiana* and *A. yassujica* from eastern Anatolia and Iran (Iranian group, B with 1.00/97 statistical support), *A. c. muhtari* from eastern and southeastern Anatolia, *A. c.* cf. *wolteri* from Syria, *A. c. wolteri* from southern Anatolia, and *A. c. cappadocica* from central Anatolia (Anatolian group, A with 0.96/78 statistical support) (Fig. 2A).

The topological constraint analyses indicate that the alternative hypotheses of (a) monophyly of *A. cappadocica* and (b) monophyly of *A. c. muhtari* were rejected by the SH test ($P = 0.000$ for a, and $P = 0.000$ for b; $P < 0.05$) suggesting that the two alternative cases are significantly different.

3.2. Species tree estimation and divergence times on mtDNA

In the estimation of the divergence times, high effective sample sizes were observed for all parameters in all BEAST analysis (posterior ESS values >300) and assessment of convergence statistics in Tracer indicated that all analyses had converged. According to the inferred dates resulted from BEAST and MCMCTREE (Fig. 2A), the diversification of *Apathya* lineages dates back to middle Miocene (13.66 and 14.24 Mya in BEAST and MCMCTREE, respectively) with the splitting of the *A. c. urmiana* and *A. yassujica* lineages from the rest of *A. cappadocica* lineages (Table 4). Within this latter group the diversification seems to have occurred during the late Miocene (8.38 and 10.39 Mya, respectively).

The resulting species tree with information from mitochondrial markers (Fig. 2B) recovered the relationships between the lineages supported by BI and ML analyses (Fig. 2A).

3.3. Gene tree estimation on nDNA

In GMYC analysis, the single threshold option ($\log L_{null} = 291.98$, $\log L_{GMYC} = 297.61$, $p = 0.01^{*}$) was statistically preferred over the multiple threshold (Chi-square = 0.83, d.f. = 6, $p = 0.991$, n.s.) and identified 16 distinct evolutionary entities within the *Apathya* lineages (Fig. 2A). One specimen from each one of the 16 distinct evolutionary entities was amplified and sequenced for the nuclear gene (c-mos). The parsimony network analysis resulted in a single network (Fig. 3A).

3.4. Phylogenetic analyses on complete dataset (mt and nDNA)

The produced dataset was concatenated with the mitochondrial DNA dataset, producing a dataset of 1425 bp (*COI* 660 bp, *cyt b* 414 bp, and *c-mos* 351 bp). Maximum Likelihood ($-\ln L = 8506.50$) and BI ($-\ln L = 8675.92$) analyses of the concatenated data produced phylogenies in agreement with the mitochondrial phylogenies (Fig. 3B).

3.5. Long branch attraction

In all cases (including and excluding outgroups and including and excluding taxa with long branches, excluding the fast evolving third codon positions), Parsimony, Bayesian, and Likelihood analyses recovered tree topologies (trees not shown) that are identical in the placement of the two putatively long branches (*A. yassujica*, and *A. c. urmiana*), suggesting that the sister group relationship of *A. yassujica* and *A. c. urmiana* is not likely to be a LBA artifact.

3.6. Biogeographic analysis

Biogeographic reconstructions for six major nodes are presented in Fig. 2B. The root of *Apathya* lineages was found to be widespread across all eastern areas (eastern Anatolia and Iran). The ancestor of the Iranian group (B) clade was reconstructed to have been located eastern Anatolia and Iran, while the ancestor of the Anatolian clade was inferred to have originated from eastern and central or southern Anatolia. In general, it seems that the biogeographical history of *Apathya* in the region is the result of several vicariance and dispersal events.

4. Discussion

4.1. Phylogeny of Apathya spp

The data and analyses presented here stem from a complete taxon sampling composed by 46 specimens with representatives of all recognized species and subspecies of the genus *Apathya*. This has enabled a robust phylogenetic reconstruction, the uncovering of intraspecific diversity, and the identification of interesting biogeographical patterns. Importantly, the phylogenetic results show high level of nodal support and a striking agreement with the morphological taxonomic analyses of *Apathya* (Arnold et al., 2007; Eiselt, 1979; Ilgaz et al., 2010; Nilson et al., 2003), increasing our confidence that the recovered topology represents the true evolutionary history of the genus.

Table 3
Sequence divergences (%) among the main lineages of *Apathya* and the outgroups (*Podarcis, Hellenolacerta, Gallotia*) for the cyt *b* (below diagonal) and COI (above diagonal) based on Tamura and Nei model of evolution. Values in diagonal are within lineages sequence divergences [cyt *b* (COI)]. No values were calculated (*n*/*c*) where no data was available.

Lineage	1	2	3	4	5	6	7	8	9	10	11
1. *A. c. wolteri*	2.3 (2.4)	5.9	7.4	12.0	11.4	21.7	17.7	16.9	20.4	21.7	21.7
2. *A. c. cappadocica*	4.7	0.5 (0.6)	7.1	12.0	12.1	22.0	17.1	17.0	19.8	20.9	19.8
3. *A. c. cf wolteri* – Syria	8.2	7.1	0.0 (0.3)	11.2	10.9	19.8	16.5	16.6	19.1	20.0	21.6
4. *A. c. muhtari*	11.7	12.4	11.2	5.2 (3.2)	4.0	19.0	16.5	16.1	19.3	21.8	21.1
5. *A. c. schmidtlerorum*	12.2	12.6	13.6	5.2	0.2 (0)	18.4	16.3	15.7	20.1	21.7	20.7
6. *A. c.urmiana* – Turkey	18.8	18.2	20.7	18.6	19.4	0.3 (0.2)	13.2	13.6	21.8	20.8	26.8
7. *A. c. urmiana* – Iran	14.5	15.3	18.1	17.4	17.1	17.9	0.0(0.1)	9.9	22.9	19.7	23.5
8. *A. yassujica*	16.1	16.3	18.4	19.8	17.7	16.8	11.1	0.6 (0.1)	22.9	19.9	25.1
9. *Hellenolacerta*	20.9	21.0	21.6	21.0	20.4	23.9	22.1	21.8	0.7(0.2)	21.5	21.1
10. *Podarcis*	22.1	20.7	22.6	23.0	22.5	23.5	22.2	20.9	22.1	11.8 (10.2)	23.0
11. *Gallotia*	26.0	24.4	25.7	24.6	23.4	27.0	24.9	28.1	29.0	25.7	12.6 (13.5)

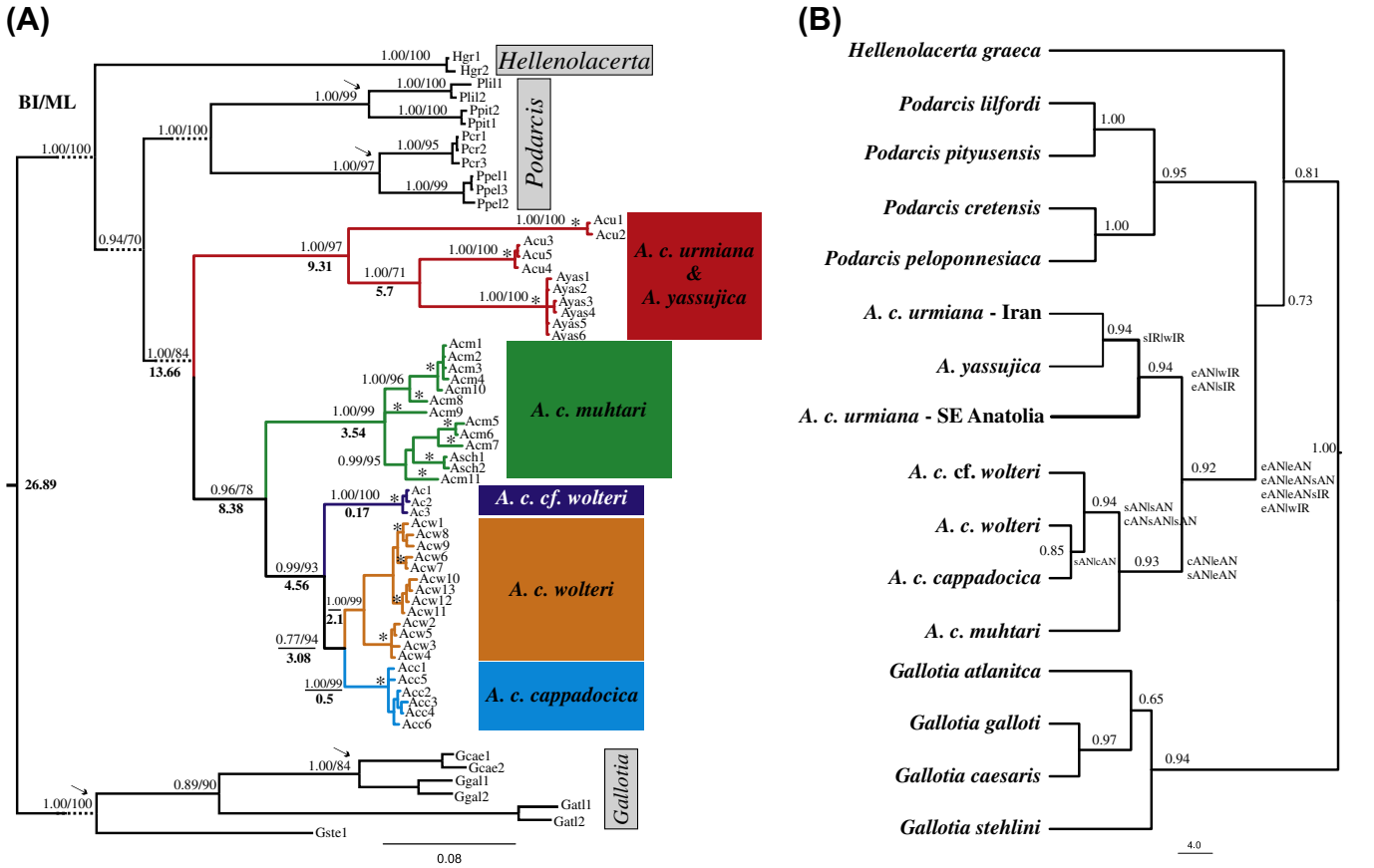

Fig. 2. (A) Bayesian Inference (BI) tree reconstructed from the mtDNA dataset. Numbers on branches indicate posterior probabilities and bootstrap supports (BI/ML). The numbers below the branches are the estimated time of divergences (BEAST). The asterisks (*) on branches represent the 16 entities of *Apathya* that have been identified by GMYC analysis under the single threshold option. The arrows indicate the calibration points. (B) The coalescent-based species tree implemented in *BEAST. Numbers on branches indicate posterior probabilities. The results of lagrange biogeographic analysis are presented in the major nodes of *Apathya*'s phylogeny.

Table 4
Estimated tMRCA for the major splits. Dates given in millions of years before present, with mean and 95% Highest Posterior Density (HPD).

Node	BEAST		MCMCTREE	
	Mean	HPD	Mean	HPD
Root of the tree	26.89	20.8–34.3	20.8	18.8–23.6
A. c. urmiana and *A. yassijica*/rest of *A. cappadocica*	13.66	10.3–17.5	14.24	10.5–18.2
A. c. urmiana Anatolia/*A. c. urmiana* Iran	9.31	6.7–12.47	9.01	5.3–13.1
A. c. urmiana Iran/*A. yassijica*	5.7	3.7–7.9	6.12	2.9–9.9
A. c. muhtari/*A. c. capadocia* and *A. c. wolteri*	8.38	6.1–11.0	10.39	7.9–14.3
A. c. cf wolteri Syria/*A. c. capadocia* and *A. c. wolteri*	4.56	3.1–6.3	5.83	3.1–9.6
A. c. capadocia/*A. c. wolteri*	3.08	2.1–4.2	3.89	2.4–6.2

All the analyses supports the existence of five lineages (Fig. 2) that could be divided in two major clusters (Anatolian group, A; and Iranian group, B). The first cluster includes four lineages, all originating from Anatolia, whereas the second one consists of one lineage found in both Iran and Anatolia. In the Anatolian group A, three out of the four lineages correspond to distinct taxonomic units (subspecies) of *A. cappadocica* (*A. c. cappadocica*, *A. c. wolteri*, and *A. c. muhtari*). The fourth lineage is found in northern Syria. The specimens of this lineage have been recognized here as *A. c.* cf. *wolteri*, indicating their problematic taxonomic status. The genetic-distinctiveness of this lineage combined with the weakness of its assignment to one of the five currently recognized subspecies of *A. cappadocica* suggests that it could represent a distinct taxonomic unit (i.e., subspecies) within *A. cappadocica*.

Despite the general concordance between morphological and phylogenetic conclusions, two important discrepancies were observed. First, although the morphology supports the inclusion of the specimens Asch1 and Asch2 from eastern Anatolia in the subspecies *A. c. schmidtlerorum*, the results of our molecular analyses indicate that *schmidtlerorum* and *muhtari* were not genetically differentiated. The second and the most impressive is the phylogenetic position of *A. c. urmiana* and its relationship with *A. yassujica.* The close relationship of *A. c. urmiana* with *A. yassujica* (pp = 1.00, bs = 97) renders *A. cappadocica* paraphyletic with respect to *A. yassujica*. Moreover within the lineage of *A. c. urmiana* and *A. yassujica,* the specimens of *urmiana* do not form a monophyletic clade. The specimens of *A. c. urmiana* from southeastern Anatolia branch off first, whereas the three specimens of *A. c. urmiana* from southwestern Iran cluster with *A. yassujica* from its type locality. It is worth noticing here that the *urmiana* populations also differ in morphology. Specimens of the southern fraction of *urmiana* distribution are bigger, have more dorsals and supraciliary granulae, fewer gulars,

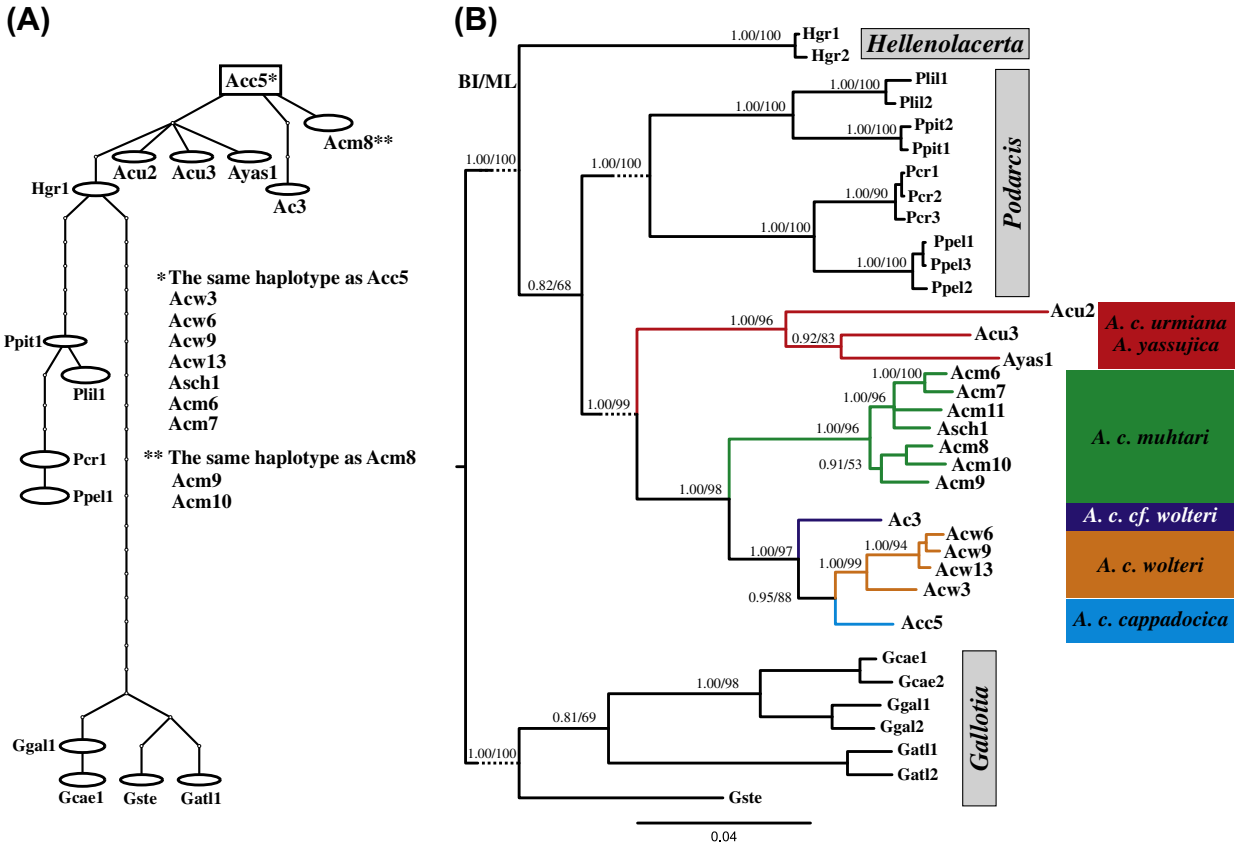

Fig. 3. (A) Statistical parsimony networks corresponding to nDNA sequence variation calculated with TCS with fix connection limit at 25 steps. (B) Bayesian Inference (BI) tree reconstructed from the complete dataset (mtDNA and nDNA genes). Numbers on branches indicate posterior probabilities and bootstrap supports (BI/ML). For one GMYC entity (Acm11) we failed to amplify the c-mos.

and more temporals and a fragmented preanal plate (Nilson et al., 2003). Given this and considering the fact that our data reject the alternative hypotheses (monophyly of *A. cappadocica* and the monophyly of *A. c. muhtari*), the taxonomy of those taxa in species and subspecies level needs reevaluation.

4.2. Extreme mtDNA diversity and systematics in Apathya spp.

Several recently published analyses of Middle East herpetofauna have revealed high levels of genetic differentiation and cryptic species (Ahmadzadeh et al., 2012; Kapli et al., 2008; Kornilios et al., 2011; Kornilios et al., 2012; Kyriazi et al., 2008). In the present analyses, the surprising result was the extreme diversity of mitochondrial DNA found among the lineages of *Apathya* species and subspecies analyzed (up to 20.7% and 22.% in cyt *b* and COI, respectively).

The observed genetic differentiation between different lineages that correspond to different subspecies (4.7–20.7% in cyt *b* and 4.0–22.0%) is similar and, in some cases, higher than the divergence found in other lacertid at the species level (17–20% in cyt *b* of *Mesalina* spp., <17.6% in COI of *Ophisops* spp., 7.4–8.2% in cyt *b* of *Iberolacerta* spp., ca. 15% in cyt *b* of *Timon* spp.) (Ahmadzadeh et al., 2012; Crochet et al., 2004; Kapli et al., 2008; Kyriazi et al., 2008). Notably, for *Iberolacerta*, seven species are now recognized (Crochet et al., 2004), which are less genetically diverged than the different populations/subspecies of *A. cappadocica.* For the species within the genus *Timon* the authors recommended erection of the two former subspecies of *T. princeps* as full species (Ahmadzadeh et al., 2012).

The high level of genetic differentiation observed between the *A. c. urmiana* populations from southeastern Anatolia and Iran and the rest of *A. cappadocica* samples from Anatolia and Syria, and its phylogenetic position, raises questions regarding the inclusion of the currently recognized as *urmiana* specimens within *A. cappadocica*. The easiest solution to avoid the paraphyly of *A. cappadocica* could be the consideration of *A. yassujica* as a synonym of *A. cappadocica*. However, these species satisfy the prerequisites for delimiting species (Wiens and Penkrot, 2002). Given enough time, distinct species should: (1) have exclusive DNA haplotype phylogenies relative to other species; (2) have one or more diagnostic morphological characters (either fixed or at high frequency); and (3) form strongly supported morphologically based population clades. Our estimation of the timing of divergence indicates that the *A. c. urmiana* lineage from southeastern Anatolia diverged from the rest *A. c. urmiana* and *A. yassujica* before 9.31 Mya, which is approximately equal to two thirds of the taxons' existence, beginning by their common ancestor. The second and the third criterion are also satisfied as shown by the morphological analysis of previous work (Nilson et al., 2003). Seeing the above, the first solution for raising the observed paraphyly was rejected. Since morphologically *A. yassujica* is a well defined species and accepted today as such (Nilson et al., 2003), *A. c. urmiana* from southeastern Anatolia and Iran would need to be raised to specific rank to avoid the paraphyly of *A. cappadocica*.

Within *A. cappadocica*, the specimens from southeastern Anatolia could be described as new species, whereas the situation of the populations from western Iran is more complicated. The time of divergence between *A. c. urmiana* from western Iran and *A. yassujica* is 5.7 Mya that is a long time in which the populations have

been isolated from gene flow. Additionally, as pointed out by Nilson et al. (2003), *A. yassujica* differs from *A. c. urmiana* by several morphological characters, such as size and color patterns. These suggests that as for *A. c. urmiana* from Anatolia, the populations of *A. c. urmiana* from western Iran belong to a different taxon, although its ranking as different species or subspecies is debatable. A thorough molecular and morphological study with more specimens from Iran is required before a definitive statement can be made.

4.3. Diversification and tectonic changes

All the lineages of *Apathya* are grouped in two main clusters, the Anatolian group composed by *A. cappadocica* without *urmiana* (group A), and the Iranian group (B) that includes *A. c. urmiana* from southeastern Anatolia, western Iran and *A. yassujica* from Iran. These two clusters diverged around 13.66 Mya in middle Miocene, during which a sharp drop in global temperatures took place (Middle Miocene Climate Transition).

The results of the Lagrange biogeography analysis show that a vicariant event occurred in the eastern Middle East resulting in the split of group A from B. This event coincides with the opening of the eastern Anatolia to the central Paratethys in the early Serravallian (~13.6 Mya), creating an east/west sea barrier in this area (Rögl, 1999). The land connection between Anatolia and Iran possibly in late Langhian and early Serravallian allowed for ancestral form of *Apathya* to disperse in Iran. However, the disruption of this land in middle Serravallian (reconnected in Tortonian) led to the vicarianistic split of the genus into Anatolian (group A) and Iranian (group B) lineages.

However, the presence of some members of the Iranian group in the territory of the Anatolian group is a mystery. This divergence took place at 9.31 Mya in Tortonian. Although, this is not supported by Lagrange, it could be considered as a more recent invasion from Iran to Anatolia when these areas rejoined in Tortonian (Rögl, 1999).

Within the Anatolian group (A), the first split that led to *A. c. muhtari*/*A. c. schmidtlerorum* lineage occurred before 8.38 Mya. After that the population from Syria (*A. c.* cf. *wolteri*) diverged about 4.56 Mya and finally, another divergence event seems to have caused an almost synchronous diversification in *A. c. cappadocica* and *A. c. wolteri* (3.08 Mya).

One of the most important paleogeological changes in the structure of Anatolia that helps in understanding Anatolian faunal synthesis is the Anatolian Diagonal Line (see Fig. 1), which was originally suggested by Davis (1971 from Çiplak et al., 1993). The Anatolian Diagonal is a line of mountain ranges that run from the south of Gümüşhane – Bayburt in the north, southwest across Turkey to the Taurus Mountains (Mutun, 2010). It has been proposed as a significant geographic barrier shaping the current composition of various species across Turkey and dividing species/lineage distribution into east and west (Bilgin, 2011; Çiplak et al., 1993; Mutun, 2010; Rokas et al., 2003; Sengor et al., 2003), just from the beginning of geological origination since Miocene–Pliocene (Bilgin, 2011). Returning to the phylogeography of *A. cappadocica* (Anatolian group, A), the divergence of *A. muhtari* clade in eastern Anatolia from *A. c. cappadocica* and *A. c. wolteri* in central and southern Anatolia could be explained as the result of the Anatolian Diagonal formation.

The other two divergent events that occurred in early Pliocene could be explained by two other major tectonic movements in the central and south Anatolia. The first is the uplift of the Amanos mountains which occurred during late Pliocene that seems to explain the isolation of the Syrian populations. The second is the main orogenesis in the mountain chains bordering the Eastern Paratethys from the south (Pontides, Lesser Caucasus, and Taurus) that took place in the Pliocene (late Kimmerian, 3.3 Mya) (Popov et al., 2006) and could be consider as the vicarianistic event that isolated *A. c. cappadocica* from *A. c. wolteri*. The Lagrange biogeography analysis supports these hypotheses, since three independent vicarianistic events occurred in Anatolia resulting in the divergence of the currently recognized lineages of Anatolian group. The first with relative probability (Rel Pro) equal to 0.78 between eastern Anatolia and central/southern Anatolia, the second (Rel Pro = 0.95) between southern and central/southern Anatolia, and the third (Rel Pro = 0.82) between central and southern Anatolia.

5. Conclusions

Phylogeographic assessments of several taxa in Anatolia and Middle East have indicated the presence of cryptic diversity, especially in reptiles (Kapli et al., 2008; Kornilios et al., 2011; Kornilios et al., 2012; Kyriazi et al., 2008). What is exceptional in the case of *Apathya* is the high level of mitochondrial divergence among almost every sampled population, ranging from 4.7% to 20.7% in cyt b and 4.0% to 22.0% in COI. This raises the issue of how many species may occur within the group. From a biogeographical point of view, the estimated diversification dates and the respective palaeogeographical events that roughly occurred during these timeperiods, provide clues to resolve the phylogeographic history of the genus. They indicate that several vicarianistic events that are related with the formation of Anatolian Diagonal and the orogenesis of the mountain chains in southern and eastern Anatolia led to current distribution pattern of *A. cappadocica* and *A. yassujica* in Anatolia, Syria and Iran.

Acknowledgments

We are grateful to Miguel Angel Carretero (CIBIO, Portugal) for sharing samples and to Adalgisa Caccone (Yale University, USA) for critically reading the manuscript and the linguistic support of text.

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.2012.12.002.

References

Ahmadzadeh, F., Carretero, M.A., Harris, D.J., Perera, A., hme, W., 2012. A molecular phylogeny of the eastern group of ocellated lizard genus *Timon* (Sauria: Lacertidae) based on mitochondrial and nuclear DNA sequences. Amphibia-Reptilia 33, 1–10.

Akaike, H., 1974. New Look at Statistical-Model Identification. IEEE Transactions on Automatic Control Ac19, pp. 716–723.

Anderson, F.E., Swofford, D.L., 2004. Should we be worried about long-branch attraction in real data sets? Investigations using metazoan 18S rDNA. Molecular Phylogenetics and Evolution 33, 440–451.

Arnold, E.N., 1973. Relationships of the Palearctic lizards assigned to the genera *Lacerta*, *Algyroides* and *Psammodromus* (Reptilia: Lacertidae). Bulletin of The British Museum (Natural History) Zoology 25, 291–366.

Arnold, E.N., Arribas, O., Carranza, S., 2007. Systematics of the palaearctic and oriental lizard tribe Lacertini (Squamata: Lacertidae: Lacertinae), with descriptions of eight new genera. Zootaxa 1430, 3–86.

Bergsten, J., 2005. A review of long-branch attraction. Cladistics 21, 163–193.

Bilgin, R., 2011. Back to the suture: the distribution of intraspecific genetic diversity in and around anatolia. International Journal of Molecular Sciences 12, 4080–4103.

Brown, R.P., Terrasa, B., Pérez-Mellado, V., Castro, J.A., Hoskisson, P.A., Picornell, A., Ramon, M.M., 2008. Bayesian estimation of post-Messinian divergence times in Balearic Island lizards. Molecular Phylogenetics and Evolution 48, 350–358.

Çiplak, B., Demirsoy, A., Bozcuk, A.N., 1993. Distribution of orthoptera in relation to the anatolian diagonal in Turkey. Articulata 8, 1–20.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9, 1657–1659.
Cox, S.C., Carranza, S., Brown, R.P., 2010. Divergence times and colonization of the Canary Islands by *Gallotia* lizards. Molecular Phylogenetics and Evolution 56, 747–757.
Crochet, P.A., Chaline, O., Surget-Groba, Y., Debain, C., Cheylan, M., 2004. Speciation in mountains: phylogeography and phylogeny of the rock lizards genus *Iberolacerta* (Reptilia: Lacertidae). Molecular Phylogenetics and Evolution 30, 860–866.
Douady, C.J., Catzeflis, F., Raman, J., Springer, M.S., Stanhope, M.J., 2003. The Sahara as a vicariant agent, and the role of Miocene climatic events, in the diversification of the mammalian order Macroscelidea (elephant shrews). Proceedings of the National academy of Sciences of the United States of America 100, 8325–8330.
Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7, 214.
Eiselt, J., 1979. Ergebnisse zoologischer Sammelreisen in der Türkei *Lacerta cappadocica* Werner, 1902 (Lacertidae, Reptilia). Annalen des Naturhistorischen Museums inWien 82, 387–421.
Eronen, J.T., Ataabadia, M.M., Micheelsb, A., Karme, A., Bernor, R.L., Fortelius, M., 2009. Distribution history and climatic controls of the Late Miocene Pikermian chronofauna. Proceedings of the National academy of Sciences of the United States of America 106, 11867–11871.
Heled, J., Drummond, A.J., 2010. Bayesian inference of species trees from Multilocus data. Molecular Biology and Evolution 27, 570–580.
Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
Ilgaz, C., Arikan, H., Kumlutas, Y., Avci, A., 2010. Electrophoretic comparison of blood-serum proteins of *Apathya cappadocica* (Sauria, Lacertidae) subspecies from Anatolia. Acta Herpetol 5, 207–215.
Kapli, P., Lymberakis, P., Poulakakis, N., Mantziou, G., Parmakelis, A., Mylonas, M., 2008. Molecular phylogeny of three *Mesalina* (Reptilia: Lacertidae) species (*M. guttulata, M. brevirostris* and *M. bahaeldini*) from North Africa and the Middle East: another case of paraphyly? Molecular Phylogenetics and Evolution 49, 102–110.
Katoh, K., Misawa, K., Kuma, K.i., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30, 3059–3066.
Kornilios, P., Ilgaz, C., Kumlutas, Y., Giokas, S., Fraguedakis-Tsolis, S., Chondropoulos, B., 2011. The role of Anatolian refugia in herpetofaunal diversity: an mtDNA analysis of *Typhlops vermicularis* Merrem, 1820 (Squamata, Typhlopidae). Amphibia-Reptilia 32, 351–363.
Kornilios, P., Ilgaz, Ç., Kumlutaş, Y., Lymberakis, P., Moravec, J., Sindaco, R., Rastegar-Pouyani, N., Afroosheh, M., Giokas, S., Fraguedakis-Tsolis, S., Chondropoulos, B., 2012. Neogene climatic oscillations shape the biogeography and evolutionary history of the Eurasian blindsnake. Molecular Phylogenetics and Evolution 62, 856–873.
Kyriazi, P., Poulakakis, N., Parmakelis, A., Crochet, P.A., Moravec, J., Rastegar-Pouyani, N., Tsigenopoulos, C.S., Magoulas, A., Mylonas, M., Lymberakis, P., 2008. Mitochondrial DNA reveals the genealogical history of the snake-eyed lizards (*Ophisops elegans* and *O. occidentalis*) (Sauria: Lacertidae). Molecular Phylogenetics and Evolution 49, 795–805.
Mayer, W., Arribas, O., 2003. Phylogenetic relationships of the European lacertid genera *Archaeolacerta* and *Iberolacerta* and their relationships to some other 'Archaeolacertae' (sensu lato) from Near East, derived from mitochondrial DNA sequences. Journal of Zoological Systematics and Evolutionary Research 41, 157–161.
Mutun, S., 2010. Intraspecific Genetic Variation and Phylogeography of the Oak Gallwasp *Andricus caputmedusae* (Hymenoptera: Cynipidae): Effects of the Anatolian Diagonal. Acta Zoologica Academiae Scientiarum Hungaricae 56, 153–172.
Nilson, G., Andrén, C., Flärdh, B., 1990. Vipera albizona, a new mountain viper from central Turkey, with comments on isolating effects of the Anatolian "Diagonal". Amphibia-Reptilia, 285–294.
Nilson, G., Rastegar-Pouyani, N., Rastegar-Pouyani, E., Andrén, C., 2003. Lacertas of South and Central Zagros Mountains, Iran, with descriptions of two new taxa. Russian Journal of Herpetology 10, 11–24.
Pons, J., Barraclough, T.G., Gomez-Zurita, J., Cardoso, A., Duran, D.P., Hazell, S., Kamoun, S., Sumlin, W.D., Vogler, A.P., 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Systematic Biology 55, 595–609.
Popov, S.V., Shcherba, I.G., Ilyina, L.B., Nevesskaya, L.A., Paramonova, N.P., Khondkarian, S.O., Magyar, I., 2006. Late Miocene to Pliocene palaeogeography of the paratethys and its relation to the Mediterranean. Palaeogeography, Palaeoclimatology, Palaeoecology 238, 91–106.
Posada, D., 2008. JModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25, 1253–1256.
Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of akaike information criterion and Bayesian approaches over likelihood ratio tests. Systematic Biology 53, 793–808.
Poulakakis, N., Lymberakis, P., Antoniou, A., Chalkia, D., Zouros, E., Mylonas, M., Valakos, E., 2003. Molecular phylogeny and biogeography of the wall-lizard *Podarcis erhardii* (Squamata: Lacertidae). Molecular Phylogenetics and Evolution 28, 38–46.
Prentice, I.C., Jolly, D., 2000. Mid-Holocene and glacial-maximum vegetation geography of the northern continents and Africa. Journal of Biogeography 27, 507–519.
Rambaut, A., 2006–2009. FigTree v. 1.3.1. Tree Figure Drawing Tool. Institute of Evolutionary Biology (University of Edinburgh, UK).
Rambaut, A., Drummond, A.J., 2008. MCMC Trace Analysis Tool. Version v1.5.0, 2003–2009. <http://beast.bio.ed.ac.uk/Tracer>.
Ree, R.H., Moore, B.R., Webb, C.O., Donoghue, M.J., Crandall, K., 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. Evolution 59, 2299–2311.
Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Systematic Biology 57, 4–14.
Rögl, F., 1998. Paleogeographic considerations for mediterranean and paratethys seaways (oligocene to miocene). Annalen des Naturhistorischen Museums Wien 99, 279–310.
Rögl, F., 1999. Palaeogeographic considerations for Mediterranean and Paratethys seaways. Annalen des Naturhistorischen Museums Wien 99A, 279–310.
Rokas, A., Atkinson, R.J., Webster, L., Csoka, G., Stone, G.N., 2003. Out of anatolia: longitudinal gradients in genetic diversity support an eastern origin for a circum-Mediterranean oak gallwasp *Andricus quercustozae*. Molecular Ecology 12, 2153–2174.
Sengor, A.M.C., Ozeren, S., Genc, T., Zor, E., 2003. East Anatolian high plateau as a mantle-supported, north–south shortened domal structure. Geophysical Research Letters, 30.
Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Molecular Biology and Evolution 16, 1114–1116.
Siddall, M.E., Whiting, M.F., 1999. Long-branch abstractions. Cladistics-the International Journal of the Willi Hennig Society 15, 9–24.
Sindaco, R., Jeremčenko, V.K., 2008. The Reptiles of the Western Palearctic 1: Annotated checklist and distributional atlas of the Turtles, Crocodiles, Amphisbaenians and Lizards of Europe, North Africa. Middle East and Central Asia, Edizioni Belvedere, Latina, Italy.
Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.
Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 57, 758–771.
Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis using Parsimony (* and other methods). Version 4. Sinauer Associates, Sunderland, MA.
Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial-DNA in humans and chimpanzees. Molecular Biology and Evolution 10, 512–526.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution.
Tchernov, E., 1992. The Afro-Arabian component in the Levantine mammalian fauna – a short biogeographical review. Israel Journal of Zoology 38, 155–192.
Wiens, J.J., Penkrot, T.A., 2002. Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (Sceloporus). Systematic Biology 51, 69–91.
Yang, Z., 2006. Computational Molecular Evolution. Oxford University Press, Oxford, New York.
Yang, Z.H., 2007. PAML 4: phylogenetic analysis by maximum likelihood. Molecular Biology and Evolution 24, 1586–1591.