Mitochondrial DNA

http://informahealthcare.com/mdn
ISSN: 1940-1736 (print), 1940-1744 (electronic)

Mitochondrial DNA, Early Online: 1–8
 DOI: 10.3109/19401736.2014.987238

informa
healthcare

FULL LENGTH RESEARCH PAPER

First assessment on the molecular phylogeny of *Anatololacerta* (Squamata, Lacertidae) distributed in Southern Anatolia: insights from mtDNA and nDNA markers

Kamil Candan[1,2], Tolga Kankılıç[3], Özgür Güçlü[4], Yusuf Kumlutaş[2], Salih Hakan Durmuş[5], Petros Lymberakis[6], Nikos Poulakakis[6,7], and Çetin Ilgaz[2]

[1]*Department of Biology, Faculty of Science, Ege University, Bornova-İzmir, Turkey,* [2]*Department of Biology, Faculty of Science, Dokuz Eylül University, Buca-İzmir, Turkey,* [3]*Department of Biology, Faculty of Science and Arts, Aksaray University, Aksaray, Turkey,* [4]*Department of Plant and Animal Production, Güzelyurt Vocational School, Güzelyurt-Aksaray, Turkey,* [5]*Department of Biology, Faculty of Education, Dokuz Eylül University, Buca-İzmir, Turkey,* [6]*Natural History Museum of Crete, University of Crete, Iraklio, Crete, Greece, and* [7]*Department of Biology, University of Crete, Iraklio, Crete, Greece*

Abstract

The genus *Anatololacerta* (Lacertidae) occurs mainly in Anatolia (western and southern Turkey) and on the Aegean islands Samos, Ikaria, and Rhodos. Although its taxonomy has long been debated and is currently nascent, three morphological species have been attributed to this genus: *Anatololacerta anatolica*, *Anatololacerta oertzeni,* and *Anatololacerta danfordi*. Here, we investigated the evolutionary history of *A. oertzeni* and *Anatololacerta danfordi* based on both mitochondrial and nuclear markers (16S rRNA and cmos). In total, 34 *Anatololacerta* specimens were analyzed using maximum likelihood (ML) and Bayesian inference (BI) methods. Our results supported the presence of four well-supported lineages: two belongs to *A. oertzeni* and two to *A. danfordi*. The temporal diversification of these lineages probably started with the divergence of the first *A. oertzeni* lineage from western Antalya at 7.9 Mya. The other two major splits may have occurred in early Pliocene (4.4 Mya: the divergence of the second *A. oertzeni* from *A. danfordi*) and in late Pliocene (2.7 Mya: the divergence of the two lineages of *A. danfordi*). The phylogeographical scenario suggests that the major diversification events (from late Miocene to late Pliocene) could be related with climatic oscillations (such as the late Miocene aridification and the Messinian Salinity Crisis) and tectonic movements (such as the uplift of the central Taurus mountain).

Keywords

Anatolia, *Anatololacerta*, biogeography, divergence times, phylogeny, molecular systematic, reptilia

History

Received 18 September 2014
Revised 5 November 2014
Accepted 10 November 2014
Published online 9 December 2014

Introduction

Lacertidae Oppel, 1811 – one of the largest families of squamate reptiles – consists of about 280 species, spreads throughout Eurasia and Africa (Arnold et al., 2007). It was revised by Arnold (1989), who proposed the division of the Lacertidae into two subgroups, the ''Primitive Palearctic and Oriental Lacertids'' and the ''Ethiopian and Advanced Saharo-Eurasian forms''. Two subfamilies were recognized in Lacertidae based on DNA sequence data: Gallotiinae and Lacertinae (Arnold et al., 2007). The latter comprises two monophyletic tribes: the Eremiadini with 21 genera that is found in Africa and southwest and central Asia, and the Lacertini with 19 genera (Arnold et al., 2007; Uetz, 2014) that is distributed in Europe, northwest Africa, and southwest and east Asia.

Although the phylogeny of Lacertidae have been thoroughly investigated (e.g. Arnold et al., 2007; Pavlicev & Mayer, 2009) their phylogenetic relationships remain unresolved and the phylogenetic position of several taxa is unknown. Among them the genus *Anatololacerta* (*Lacerta danfordi* group) includes three morphological species (*Anatololacerta anatolica*, *Anatololacerta danfordi*, and *Anatololacerta oertzeni*), which lived in Anatolia and on the Aegean islands Samos, Ikaria, and Rhodos. The type species of *Anatololacerta*, *A. danfordi* was described as *Zootoca danfordi* by Günther (1876) and *Lacerta danfordi* by Bedriaga (1879) (type locality: Bolkar Mountains, Turkey). Few years later, Werner (1900) described a new species (*L. anatolica*) from Gökçekısık, Eskişehir (western Anatolia). However, its taxonomic status was controversial, as it could be considered either as a distinct species (Cyren, 1941; Bodenheimer, 1944) or a subspecies of *L. danfordi* (Boulenger, 1920; Budak, 1976; Mertens, 1952; Wettstein, 1967). In a more detailed study (Budak, 1976), it was noted that three subspecies of *L. danfordi* (*L. d. anatolica*, *L. d. pelasgiana,* and *L. d. danfordi*) were distributed in Anatolia. The *L. danfordi* complex was divided based on morphological characters into three different species (*L. danfordi*, *L. anatolica*, and *L. oertzeni*). The parapatric distribution of these species (*A. anatolica* in northwest Turkey and adjacent islands, *A. oertzeni* in southwest Turkey and adjacent islands, and *A. danfordi* in southcentral Turkey) have led to two respective

Correspondence: Kamil Candan, Department of Biology, Faculty of Science, Ege University, TR-35040 Bornova-İzmir, Turkey. E-mail: kamilcandan@yahoo.com

contact zones (*anatolica/oertzeni* and *oertzeni/danfordi*) (Eiselt & Schmidtler, 1986). In the subspecies level, nine morphological subspecies have been recognized in these species (*L. d. danfordi*, *L. d. bileki*, *L. a. anatolica*, *L. a. aegaea*, *L. o. oertzeni*, *L. o. pelasgiana*, *L. o. budaki*, *L. o. finikensis*, and *L. o. ibrahimi*), but their taxonomic status is also controversial (Baran, 1990; Baran & Kumlutaş, 1999; Baran et al., 2001; Çevik et al., 2006; Güçlü & Olgun, 2008; Kapli et al., 2011; Kumlutaş et al., 2003; Mayer & Arribas, 2003, Mayer & Pavlicev, 2007; Pavlicev & Mayer, 2009; Tok, 1999).

Anatolia is considered a major biodiversity hotspot globally (Myers et al., 2000; Mittermeier et al., 2004). It is a largely mountainous region whose diverse geomorphology produces many different habitats and climatic regions that have played an important role as refugia during the Quaternary (Hewitt, 2001). Due to its geological history and position, Anatolia acted either as a bridge or barrier for the dispersal of animal species between Asia, Europe, and the region of Ethiopia (northeast Africa) through the Middle East (Tchernov, 1992). Due to their presumed limited dispersal capacities, at least compared with other vertebrates, and temperature dependence, ''herptiles'' are ideal models for the study of palaeogeographic and palaeoclimatic events, and it is revealed their genetic diversity contributes to understand biogeographical patterns.

Numerous ''herptile'' species have been studied from this region, using molecular data (e.g. *Apathya cappadocica*, *Ablepharus kitaibelii*, *Typhlops vermicularis*, *Blanus strauchi*, *Chalcides ocellatus*, *Ophisops elegans*, *Vipera anatolica*, *Pelophylax bedriagae*, *Bufotes variabilis*, *Hyla arborea*, *Lacerta media*, *Zamenis hohenackeri,* etc.), revealing many cases of hidden diversity and biogeographic patterns that have been affected by Anatolia's complicated geological and climatological history (Ahmadzadeh et al., 2013; Akın et al., 2010; Gül et al., 2012; Gvozdik et al., 2010b; Jandzik et al., 2013; Kapli et al., 2012; Kornilios et al., 2010, 2011, 2012; Kyriazi et al., 2008; Nilson & Andren, 2001; Özdemir et al., 2014; Poulakakis et al., 2005b; Sindaco et al., 2014). In most of the cases, southwestern Anatolia seems to harbor cryptic phylogenetic lineages.

This study is the first attempt to evaluate the evolutionary history of *Anatololacerta* in southwestern Anatolia. We aimed to investigate the phylogenetic relationships of *Anatololacerta* populations collected from the western part of the Taurus Mountains, including the contact zone of *A. o. ibrahimi* and *A. d. bileki*, using both mitochondrial and nuclear DNA data, aiming to investigate the case of hybridazation among *A. o. ibrahimi* and *A. d. bileki*.

Materials and methods

Sample collection

A total of 34 *Anatololacerta* specimens (collected from 2012 to 2013) were used in this study: 28 *A. danfordi* and six *A. oertzeni* from 19 localities. The samples were identified in species level based on their morphological characters. *Anatololacerta oertzeni* is distinguished from *A. danfordi* by having higher number of temporal and gular scales, longer first supratemporal plate, and lower number of elongated marginals as well as typical color-pattern features (Eiselt & Schmidtler, 1986). All specimens were preserved in 95% ethanol. Sequences from two species (*Podarcis muralis* and *Darevskia valentini*) were retrieved from Genbank and were used as outgroups. Specimen data and GenBank accession numbers are given in Table 1. The sampling localities are shown in Figure 1.

DNA extraction, PCR amplification, and sequencing

For all specimens, total genomic DNA was extracted using the Invitrogen DNA extraction kit (Invitrogen Inc., Grand Island,

Table 1. List of specimens used in this study with the localities, the collection numbers, number of specimens, and the corresponding accession numbers for the 16S and cmos sequences.

Species	(*n*)	Haplotype code 16S/cmos	Locality	Museum number	Accession number 16S	Accession number cmos
A. oertzeni	1	ADS2/ADM2	Küçükçaltıcak (Antalya)	26	KF870838	KF870861
A. oertzeni	1	ADS3/ADM3	Sögütcuması (Antalya)	35	KF870839	KF870862
A. oertzeni	1	ADS1/ADM1	Termessos (Antalya)	20	KF870837	KF870860
A. oertzeni	1	ADS4/ADM4	Perge (Antalya)	42	KF870840	KF870863
A. oertzeni	1	ADS5/ADM5	Korkuteli (Antalya)	133	KF870841	KF870864
A. oertzeni	1	ADS5/ADM6	Korkuteli (Antalya)	134	KF870841	KF870865
A. danfordi	1	ADS16/ADM17	Gazipaşa (Antalya)	268	KF870852	KF870876
A. danfordi	1	ADS17/ADM17	Gazipaşa (Antalya)	269	KF870853	KF870876
A. danfordi	2	ADS6/ADM8	Bucak (Burdur)	49–144	KF870842	KF870867
A. danfordi	2	ADS13/ADM12	Konaklı (Antalya)	60–61	KF870849	KF870871
A. danfordi	4	ADS12/ADM11	Avsallar (Antalya)	56–57–58–59	KF870848	KF870870
A. danfordi	1	ADS9/ADM14	Beşkonak (Antalya)	146	KF870845	KF870873
A. danfordi	2	ADS11/ADM9	Oymapınar Dam (Manavgat-Antalya)	41–54	KF870847	KF870868
A. danfordi	2	ADS11/ADM10	Oymapınar Dam (Manavgat-Antalya)	53–55	KF870847	KF870869
A. danfordi	1	ADS10/ADM9	Güzelyalı Village (Manavgat-Antalya)	52	KF870846	KF870868
A. danfordi	2	ADS19/ADM20	Menekşe Stream (Gülnar-Mersin)	91–105	KF870855	KF870879
A. danfordi	1	ADS21/ADM22	Menekşe Stream (Gülnar-Mersin)	108	KF870857	KF870881
A. danfordi	1	ADS19/ADM22	Menekşe Stream (Gülnar-Mersin)	109	KF870855	KF870881
A. danfordi	2	ADS19/ADM22	Çamlıca Village (Gülnar-Mersin)	130–132	KF870855	KF870881
A. danfordi	1	ADS7/ADM7	Kurna (Burdur)	95	KF870843	KF870866
A. danfordi	2	ADS14/ADM15	Akseki (Antalya)	147–150	KF870850	KF870874
A. danfordi	1	ADS23/ADM24	Sertavul Pass (Karaman)	162	KF870859	KF870883
A. danfordi	1	ADS8/ADM13	Eğirdir (Isparta)	182	KF870844	KF870872
A. danfordi	1	ADS15/ADM16	Beyşehir (Konya)	180	KF870851	KF870875
		Darevskia valentini			AF206597	EF632257
		Podarcis muralis			FJ460597	EF632282

NY). Partial segments of mtDNA (16 S rRNA) and nuclear (cmos) genes were targeted as markers. Primers used in PCR amplifications and in the cycle-sequencing reactions are shown in Table 2. PCR amplifications were performed in 50 µL reactions, containing 1 × PCR Buffer (ABM Inc., Ontario, Canada), 1.5 mM $MgCl_2$ (ABM Inc., Ontario, Canada), 2.5 mM dNTP, 0.5 mM each primer, 1.5 unite Taq polymerase (ABM Inc., Ontario, Canada), and 2–3 µL (50 ng DNA) of template DNA. For 16S, the profile of the PCR amplification consisted of an initial denaturation step at 95 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min, and a final 10-min extension step at 72 °C. For cmos, the profile of the PCR amplification consisted of an initial denaturation step at 95 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min, and a final 10-min extension step at 72 °C. All amplicons were purified using the Column-Pure PCR Clean-up Kit (ABM Inc., Ontario, Canada) and sequenced with BigDye Terminator cycle sequencing chemistry (v.3.1) (Applied Biosystems, Waltham, MA) in an AB3700 or 3730xl automatic sequencer (Life Technologies, Carlsbad, CA).

Sequence analyses

All DNA sequences were aligned using BioEdit v7.0.9 (Promega, Carlsbad, CA) (Hall, 1999). The number of haplotypes, haplotype (Hd) diversity, and nucleotide (Π) diversity were calculated in DnaSP v5 (Promega, Carlsbad, CA) (Librado & Rozas, 2009). For nuDNA, RDP4 v4.3 (Promega, Carlsbad, CA) (Martin et al., 2010) was used to detect recombination in cmos sequences.

Phylogenetic analyses

The genetic distances among the major phylogenetic clades that have high statistical values in terminal nodes in our phylogeny were calculated based on the TrN model of evolution (Tamura & Nei, 1993) in MEGA v6 (Tamura et al., 2011).

Phylogenetic analyses based on mtDNA (16S) were performed with the neighbor joining (NJ), maximum likelihood (ML), and Bayesian inference (BI) methods. The most suitable model of DNA substitution was chosen using jModelTest v2 (Ambion, Foster City CA) (Darriba et al., 2012; Guindon & Gascuel, 2003), under the Bayesian information criterion (Luo et al., 2010). The model was GTR+G ($\ln L = -2796.787$) (Tavaré, 1986).

NJ was performed in MEGA using the calculated TrN genetic distances. Bootstrapping with 1000 pseudo-replicates was used to examine the robustness of the nodes in the resulting tree (Felsenstein, 1985).

ML (Felsenstein, 1981) was performed with PHYML v.3.0 (Graphpad, La Jolla, CA) (Guindon et al., 2010), with the application of the SPR method and the model parameters fitted to the data by likelihood maximization. Reliability of the ML tree was based on 1000 bootstrap replicates. An ML tree was also constructed using TreePuzzle v. 5.2 (NCBI, Bethesda, MD) (Schmidt et al., 2002) and involved the tree algorithm quartet puzzling (Strimmer & von Haeseler, 1996). Bootstrapping was performed using 100 replicates.

BI was conducted in MrBAYES v3.2.1 (MrBayes, Tallahassee, FL) (Huelsenbeck & Ronquist, 2001) using the GTR+G model of evolution for 16S. The analysis was run four times with eight chains, each run for 5×10^6 generations, and sampling from the chain every 100 generations. In order to confirm that the analyses had achieved stationarity and to evaluate ''burn-in'', we plotted log-likelihood scores and tree lengths against generation number using Tracer v1.6 (BoatTrader, Norfolk, VI) (Rambaut et al., 2014). After verifying that stationarity has been reached, the first 5×10^3 trees (10% ''burnin'' in Bayesian terms) were discarded, and a majority-rule consensus tree was generated from the remaining 4.5×10^4 trees. Posterior probabilities were calculated

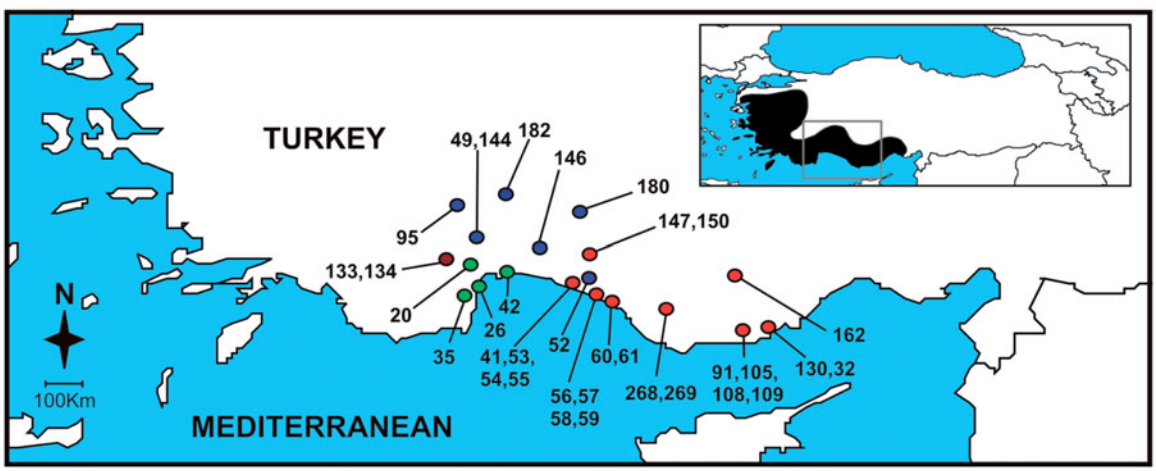

Figure 1. Sample localities of *Anatololacerta* populations in south Anatolia. Numbers refer to museum codes given in Table 1.

Table 2. List of 16S and cmos primer sequences, codes, and sources used in this study.

Name	Gene	Primer sequence	Source
L1921	16S rRNA	5′-cccgaaaccaaacgagcaa-3′	Murphy et al. (2000)
H2568	1st segment	5′-ctacctttgcacggttaggataccgcggc-3′	Murphy et al. (2000)
16Sar	16S rRNA	5′-cgcctgtttatcaaaaacat-3′	Palumbi (1996)
16Sbr	2nd segment	5′-ccggtctgaactcagatcacgtt-3′	Palumbi (1996)
L1zmos	cmos	5′-ctagcttggtgttctatagactgg-3′	Mayer & Pavlicev (2007)*
Hcmos3		5′-ggtgatggcaaatgagtagat-3′	Mayer & Pavlicev (2007)

*The referred primer was modified from Whiting et al. (2003)

Table 3. Sequence divergences, as % genetic distance values, among the main clades of our phylogeny for 16S (above diagonal) and cmos (below diagonal).

Main clades	A	B	C	D
Clade A	**0.0/0.3**	3.3	3.5	3.3
Clade B	0.5	**0.0/0.0**	1.7	1.7
Clade C	0.5	0.6	**0.3/0.5**	0.9
Clade D	0.3	0.5	0.3	**0.3/0.1**

Values in diagonal are genetic divergences within each clade (16S/cmos).

as the percentage of samples recovering any particular clade, where probabilities ≥95% indicate significant support.

The genealogical relationships among the 16S and cmos haplotypes, separately, were constructed using TCS v.1.21 (Irvine, CA) (Clement et al., 2000) with statistical parsimony (Templeton et al., 1992) and a connection limit of 95%.

Estimation of divergence times

There are no internal calibration points available for the genus *Anatololacerta* to estimate the timing of cladogenetic events. For this reason, the substitution rate of the same mitochondrial region (16S) of another member of the Lacertini, i.e. *Podarcis*, was used. This rate was estimated to be 0.46% per million years (Poulakakis et al., 2005a). The divergence times within *Anatololacerta* were estimated using BEAST v1.7.2 (Drummond & Rambaut, 2007) based on the 16S rRNA data. 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): 16S – GTR+G; Strict Clock; Yule process of speciation; random starting tree. For all BEAST analyses, results were analyzed in Tracer v1.6 to assess convergence and effective sample sizes (ESSs) for all parameters. The final tree was computed in TreeAnnotator v1.7.2 (Global Viral Forecasting Initiative, San Francisco, CA).

Results

In total, 1074 bp of 16S and 559 bp of cmos were obtained. For 16S, 62 (5.7%) and 54 (5.0%) were variable and parsimony informative sites, respectively. The corresponding values for cmos were nine (1.6%) and eight (1.4%), respectively.

Eighteen haplotypes with $H_d = 0.94$ and $\bar{I} = 0.011 \pm 0.0019$ were found for 16S and eight haplotypes with $H_d = 0.74$ and $\bar{I} = 0.003 \pm 0.0006$ were estimated for cmos. For *cmos* gene, we did not find any evidence of recombination.

The genetic distances, within and among the main lineages of our ingroup, were given in Table 3. These values ranged from 0.9 to 3.5% for 16S and 0.3 to 0.6% for cmos.

Phylogenetic analyses (NJ, ML, and BI) produced trees with similar topologies and maximum posterior tree on the basis of BI was shown (Figure 2). According to the produced tree, *Anatololacerta* populations are assigned to four very well-supported clades (A, B, C, and D). The relationships among these clades are to some extent unresolved, although some relationships (nodes) are supported in some of the analyses. In general, *A. oertzeni* populations from two clades, A and B, of which A branches off first in our phylogeny. Clades C and D include *A. danfordi* populations and seem to have a sister–clade relationship.

High ESS values were observed for all parameters in all BEAST analyses (posterior ESS values >200) and assessment of convergence statistics in Tracer indicated that all analyses had converged. According to the inferred dates, major diversification events may have occurred in the Late Miocene (mean: 7.9 Mya, 95% intervals: 5.7–10.3 Mya), in the Early Pliocene or Miocene/Pliocene boundary (mean: 4.4 Mya, 95% intervals: 3.0–6.0 Mya) and the Pliocene/Pleistocene transition (mean: 2.7 Mya, 95% intervals: 1.7–3.7 Mya), as shown in Figure 2.

Finally, three independent 16S haplotype networks (Figure 3) were identified from the statistical parsimony analysis, corresponding to clades A, B, and C+D. The respective network for cmos gave no similar results (Figure 3).

Discussion

Southwest Anatolia is a mountainous (3000 m) and densely forested area, representing the western edge of the Taurus Mountain ridge. Our analyses on *Anatololacerta* specimens collected from this region revealed four distinct lineages, three of which (clades A, B, and C) are supported by high bootstrap (bs) values and posterior probabilities (pp) (Figure 2). However, the interclade relationships are rather ambiguous due to the low statistical support. Ignoring this, it seems that clades C and D, which are currently recognized as *A. danfordi*, are grouped together, but that clades A and B that belong to *A. oertzeni* appear to be more closely related to *A. danfordi* (clades C and D) than to conspecific clade A.

The mean level of mtDNA divergences recorded among the major lineages vary from 0.9% between clade C and D to 3.5% between clade A and C. The corresponding values in nuclear DNA (cmos) are 0.3% between clades C and D and 0.6% between clades B and C. Of particular note is the relatively high genetic distance between the clade B and the conspecific clade A (*A. oertzeni*) considering mtDNA data, indicating an inconsistency between the genetic data (genetic distances and tree topology) and the morphological species. But it is not supported by nuDNA due to inadequate genetic diversity. In contrast, the genetic divergence values within each of the four clades are very low. Although we cannot exclude restricted sampling as a possible explanation, this could be due to high levels of gene flow between the respective populations or to genetic drift (bottleneck or founder effects). Moreover, the high genetic divergence between the main clades, especially between clade A and the others, probably indicates restricted gene flow due to external (physical or ecological) or intrinsic barriers. This is not an uncommon observation, since it has been recorded in previous studies on reptiles in this particular region, such as the Greek blind snake (*Typhlops vermicularis*) (Kornilios et al., 2011, 2012), the worm-lizard (*Blanus strauchi*) (Sindaco et al., 2014), the snake-eyed lizard (*Ophisops elegans*) (Kyriazi et al., 2008), and Transcaucasian rat-snake (*Zamenis hohenackeri*) (Jandzik et al., 2013).

The most basal clade of our phylogenetic tree, clade A, includes specimens from the area of Korkuteli (the western part of the studied area). The phylogenetic position of this clade renders *A. oertzeni* as a non-monophyletic species highlighting the need for taxonomic reconsideration. However, the small number of samples analyzed from this clade (only two) and the relatively reduced posterior probability do not permit us to draw safe conclusions without adding more specimens and data (other genetic markers) to the analyses.

The occurrence of cryptic diversity (cryptic genetic lineages) has been observed in several of the studied ''herptile'' species that have continuous geographic distributions in Anatolia, such as *O. elegans* with four distinct genetic lineages in Anatolia (Kyriazi et al., 2008), water frogs of the genus *Pelophylax* (Akın et al., 2010; Plötner et al., 2001), several forms of *Testudo graeca* that

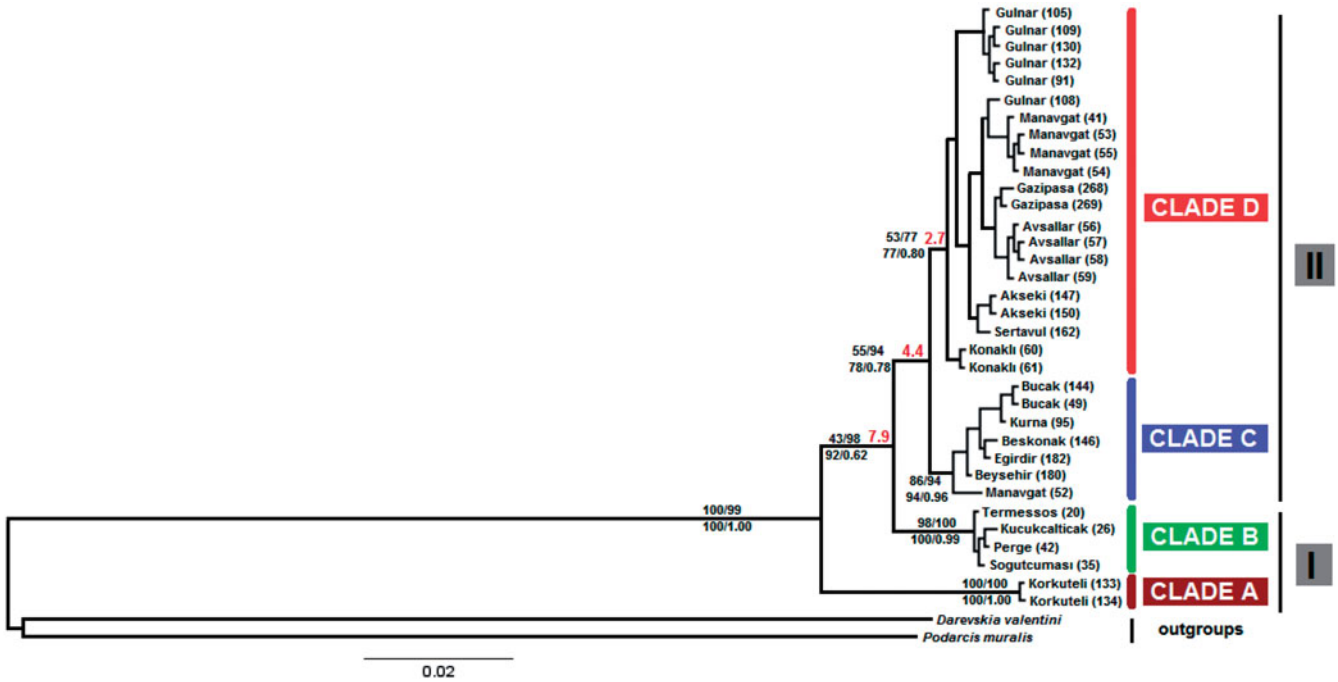

Figure 2. Phylogenetic relationships among the specimens of the present study, based on the 16 S sequences. Numbers above indicate the ML bootstrap values (PhyML/TreePuzzle) and below NJ bootstrap/BI posterior probabilities. Codes are those shown in Table 1. Colors indicate different groups and correspond to Figure 1. Divergence times (in Mya) are indicated on branches with red color. I indicates samples that belong to *A. oertzeni* and II indicates samples that belong to *A. danfordi*.

have been assigned to different species (Fritz et al., 2007) or the blind snake *Typhlops vermicularis* (Kornilios et al., 2011, 2012).

For the *A. danfordi* group, the two allopatric clades (C and D) show a very close phylogenetic relationship, which is confirmed by the network analyses since these clades are joined in the same network under the 95% connection limit. The allopatric distribution of these lineages combined with the relatively low interclade divergence (0.9%) suggests that they could probably be assigned to different taxa at the subspecies level. It is worth noticing that the samples from Akseki (147,150) that have been collected from the type locality of *A. danfordi bileki* are clustered in clade D. Although the geographic affinity of these clades could make someone suspect, the presence of a contact zone in the region of Manavgat River, this is not obvious in our data.

Regarding the contact zone between *A. danfordi* and *A. oertzeni* identified by Eiselt & Schmidtler (1986), our field observations did not verify its presence. As we stated above in the case of clade A, the restricted sampling could be a possible explanation. However, the analysis of specimens from the localities of Avsallar, Konaklı, and Gülnar which are in the contact zone of these species, and the produced phylogenetic relationships of *A. oertzeni* and *A. danfordi* indicate that a holistic re-evaluation on the number of species and the species distribution is necessary.

South-west Anatolia, especially the Taurus Mountain, has played an important role in the speciation and the biogeographic history of several organisms (Çıplak, 2003, 2004; Kornilios et al., 2011; Medail & Quezel, 1999). This region presents many physical (e.g. geographical) and ecological barriers such as mountainous and densely forested areas that reach altitudes of 3000–3500 m and valleys with major rivers (Kornilios et al., 2011). Several vicariant events may have occurred in this region, resulting from climatic oscillations that occurred during the Miocene, Pliocene, and Pleistocene, causing the isolation and allopatric distribution of several populations (Kornilios et al., 2011). Several studies of Anatolian ''herptiles'' showed that populations survived probably in suitable refugial habitats during times of unfavorable conditions (Akın et al., 2010; Fritz et al., 2007; Gvozdik et al., 2010a,b; Kornilios et al., 2010, 2011; Kyriazi et al., 2008; Plötner et al., 2001; Wielstra et al., 2010). In the case of southwest *Anatololacerta* populations, the major diversification events may have occurred in the Late Miocene with the divergence of the clade A (7.9 Mya), in the Early Pliocene with the divergence of clade B (4.4 Mya) and the Late Pliocene with the divergence of clades C and D (2.7 Mya). These time periods coincide with important diversification events in other organisms, linked to the palaeoclimate and palaeogeography.

In the Late Miocene, the diversification of many animal species was affected by (a) Late Miocene aridification (Fortelius et al., 2006; Guo et al., 2004) that was caused by the retreat of the Paratethys (7–8 Mya; Ramstein et al., 1997) and (b) the significant climate changes (Fortelius et al., 2006; He et al., 2010; Janis, 1993; Kornilios et al., 2011 and references therein).

The divergence of the second clade of *A. oertzeni* (B) dates back to the Early Pliocene (4.4 Mya). At the end of the Miocene, which marked the end of the aridification of the Asian inland (Guo et al., 2004) and the end of the Messinian Salinity Crisis (Krijgsman et al., 1999), a global cooling and drying trend around the Miocene/Pliocene boundary (Garcia-Alix et al., 2008) caused a significant vegetation and habitat turnover (Cerling et al., 1997). This event combined with the uplift of the central Taurus Mountain might be among the most important reasons for a wave of species' radiations (Akın et al., 2010; Gvozdik et al., 2010b; He et al., 2010; Jaffey & Robertson, 2005; Kornilios et al., 2012; Wielstra et al., 2010).

The diversification of clades C and D seemed to occur during the Late Pliocene (2.7 Mya). At the end of the Pliocene, the hotter and the wetter climate of the Late Pliocene (3.6–2.5 Mya; Willis et al., 1999) became colder and drier (Webb & Bartlein, 1992). This significant climatic oscillation was followed by widespread extinctions, except within refugia where the climate remained

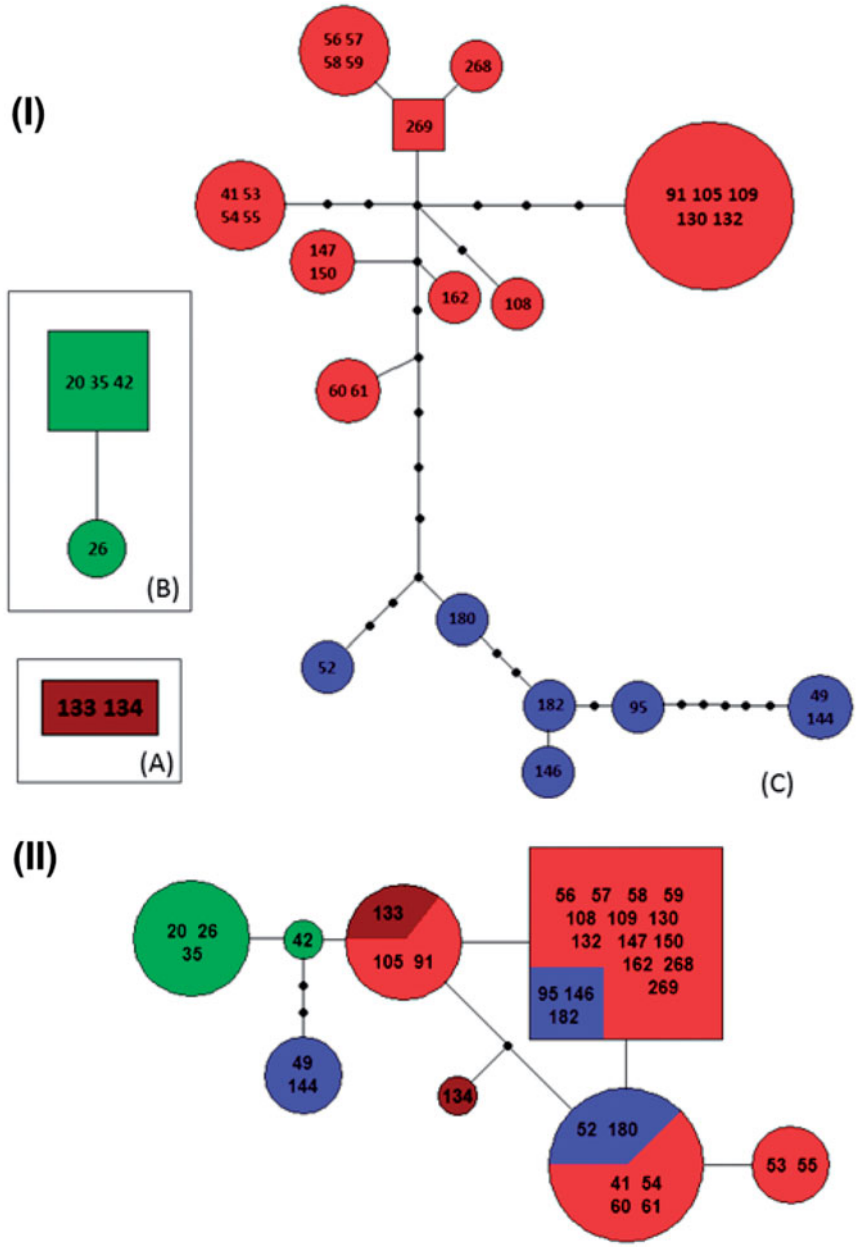

Figure 3. Parsimony networks corresponding to 16S (I) and cmos (II) sequences variation calculated with TCS with a 95% connection limit. Lines represent a mutational step, black circles missing haplotypes, and colored circles haplotypes. The circle area is proportional to the number of individuals. Rectangular shapes represent probable ancestral haplotypes. For correspondences of sample codes and locations, see Table 1 and Figure 1. (A) Clade A, (B) Clade B, and (C) Clades C and D.

within tolerance-limits for a species (Hewitt, 1996, 2000). This transition coincided with major diversification events in several animal species such as *Chalcides* (Carranza et al., 2008; Kornilios et al., 2010), *Typhlops* (Kornilios et al., 2011, 2012), and *Mastomys* (Mouline et al., 2008).

This study revealed that the *Anatololacerta* complex in southwest Anatolia exhibits great genetic diversity and cryptic phylogenetic lineages, and possibly needs a significant taxonomic revision (i.e. new lineages might represent distinct species or subspecies). The current phylogenetic and phylogeographic information can be added to the knowledge of their morphology and distribution, producing a more accurate taxonomy for the studied species. The inclusion of *A. anatolica* and the remaining *Anatololacerta* subspecies and additional data (i.e. analyses of more mitochondrial and nuclear markers, morphological characters) will be critical in elucidating the evolutionary history of the *Anatololacerta* group.

Acknowledgements

This study was based on Kamil Candan's M.Sc. Thesis supervised by Tolga Kankılıç and Özgür Güçlü.

Declaration of interest

This study was a part of the project supported by TÜBİTAK (The Scientific and Technological Research Council of Turkey) with Project number 112T269.

References

Ahmadzadeh F, Fleck M, Rödder D, Böhme W, Ilgaz Ç, Harris DJ, Engler JO, et al. (2013). Multiple dispersal out of Anatolia: Biogeography and evolution of oriental green lizards. Biol J Linn Soc 110:398–408.

Akın Ç, Bilgin C, Beerli P, Westaway R, Ohst T, Litvinchuk SN, Uzzell T, et al. (2010). Phylogeographic patterns of genetic diversity in eastern Mediterranean water frogs were determined by geological processes and climate change in the Late Cenozoic. J Biogeogr 37: 2111–24.

Arnold EN, Arribas O, Carranza S. (2007). Systematics of the palaearctic and oriental lizard tribe Lacertini (Squamata: Lacertidae: Lacertinae), with descriptions of eight new genera. Zootaxa 1430:1–86.

Arnold EN. (1989). Towards a phylogeny and bigeography of the Lacertidae: Relationships within an Old-world family of lizards derived from morphology. Brit Mus London 55:209–57.

Baran İ, Kumlutaş Y, Olgun K, Ilgaz Ç, Kaska Y. (2001). The herpetofauna of the vicinity of Silifke. Turk J Zool 25:245–9.

Baran İ, Kumlutaş Y. (1999). Köyceğiz Özel Koruma Bölgesi *Lacerta danfordi* (Reptilia: Lacertidae) populasyonu hakkında, [On *Lacerta danfordi* (Reptilia: Lacertidae) population of the Köyceğiz Specially Protected Area]. Turk J Zool 23:7–13.

Baran İ. (1990). Marmaris ve İskenderun arasındaki adalarımızın herpetofaunası [The herpetofauna of the islands between Marmaris and İskenderun]. Turk J Zool 14:113–26.

Bedriaga J. (1879). Verzeichnis der Amphibien und Reptilien Vorderasien. Bull Soc Imp Nat 54:22–52.

Bodenheimer FS. (1944). Introduction into the knowledge of the amphibia and reptilia of Turkey. Rev Fac Sci, İstanbul, Seri B 9:1–78.

Boulenger GA. (1920). Monograph of the lacertidae. Vol. I. British Museum (natural history). London: Department of Zoology. 352 pp.

Budak A. (1976). Anadolu'da yaşayan *Lacerta laevis*, *L. danfordi* ve *L. anatolica*'nın taksonomik durumları ve coğrafi yayılışları üzerinde araştırmalar [Taxonomic status and geographic distribution of *Lacerta laevis*, *L. danfordi* and *L. anatolica* distributed in Anatolia]. Ege Üniv Fen Fak İlmi Rap Ser 214:1–59.

Carranza S, Arnold EN, Geniez PH, Roca JL, Mateo JA. (2008). Radiation, multiple dispersal and parallelism in Moroccan skinks, *Chalcides* and *Sphenops* (Squamata: Scincidae), with comments on Scincus and Scincopus and the age of the Sahara Desert. Mol Phylogenet Evol 46:1071–94.

Cerling TE, Harris JM, MacFadden BJ, Leakey MG, Quade J, Eisenmann V, Ehleringer JR. (1997). Global vegetation change through the Miocene/Pliocene boundary. Nature 389:153–8.

Çevik E, Başkale E, Kaya U, Turgay F. (2006). Taxonomic status of some *Lacerta danfordi* (Günther, 1876) populations. Russ J Herpet 13: 89–92.

Çıplak B. (2003). Distribution of Tettigoniinae (Orthoptera, Tettigoniidae) bush-crickets in Turkey: The importance of the Anatolian Taurus Mountains in biodiversity and implications for conservation. Biodivers Conserv 12:47–64.

Çıplak B. (2004). Biogeography of Anatolia: The marker group Orthoptera. Mem Soc Entomol Ital 82:357–72.

Clement M, Posada D, Crandall KA. (2000). TCS: A computer program to estimate gene genealogies. Mol Ecol 9:1657–60.

Cyren O. (1941). Beitrage zur Herpetologie der Balkan-halbinsel. Mitt Königl Naturw Inst Sofia XIV 289–328.

Darriba D, Taboada GL, Doallo R, Posada R. (2012). jModelTest 2: More models, new heuristics and parallel computing. Nat Methods 9:772.

Drummond AJ, Rambaut A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7:214–21.

Eiselt J, Schmidtler JF. (1986). Der Lacerta danfordi Komplex (Reptilia: Lacertidae). Spixiana 9:289–328.

Felsenstein J. (1981). Evolutionary trees from DNA sequences – A maximum-likelihood approach. J Mol Evol 17:368–76.

Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–91.

Fortelius M, Eronen J, Liu L, Pushkina D, Tesakov A, Vislobokova I, Zhang Z. (2006). Late Miocene and Pliocene large land mammals and climatic changes in Eurasia. Palaeogeogr Palaeoclimatol Palaeoecol 238:219–27.

Fritz U, Hundsdorfer AK, Sirokyi P, Auer M, Kami H, Lehmann J, Mazanaeva LF, et al. (2007). Phenotypic plasticity leads to incongruence between morphology-based taxonomy and genetic differentiation in western Palaearctic tortoises (*Testudo graeca* complex; Testudines, Testudinidae). Amphibia-Reptilia 28:97–121.

Garcia-Alix A, Minwer-Barakat R, Martmn Suarez E, Freudenthal M, Martmn JM. (2008). Late Miocene–Early Pliocene climatic evolution of the Granada Basin (southern Spain) deduced from the paleoecology of the micromammal associations. Palaeogeogr Palaeocl 265:214–25.

Güçlü Ö, Olgun K. (2008). Present status of *Lacerta danfordi* Complex (Günther, 1876) in Southwestern Anatolia, Turkey. Russ J Herpet 15: 179–88.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. Syst Biol 59: 307–21.

Guindon S, Gascuel O. (2003). A simple, fast accurate method to estimate large phylogenies by maximum-likelihood. Syst Biol 52:696–704.

Gül S, Kutrup B, Özdemir N. (2012). Patterns of distribution of tree frogs in Turkey based on molecular data. Amphibia-Reptilia 33:95–10.

Günther A. (1876). Description of a new species of lizard from Asia Minor. Proc Zool Soc Lond 818.

Guo Z, Peng S, Hao Q, Biscaye PE, An Z, Liu T. (2004). Late Miocene-Pliocene development of Asian aridification as recorded in the red-earth formation in northern China. Glob Planet Change 41:135–45.

Gvozdik V, Jandzik D, Lymberakis P, Jablonski D, Moravec J. (2010a). Slow worm, *Anguis fragilis* (Reptilia: Anguidae) as a species complex: Genetic structure reveals deep divergences. Mol Phylogenet Evol 55: 460–72.

Gvozdik V, Moravec J, Klütsch C, Kotlík P. (2010b). Phylogeography of the Middle Eastern tree frogs (Hyla, Hylidae, Amphibia) as inferred from nuclear and mitochondrial DNA variation, with a description of a new species. Mol Phylogenet Evol 55:1146–66.

Hall TA. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41:95–8.

He K, Li YJ, Brandley MC, Lin LK, Wang YX, Zhang YP, Jiang XL. (2010). A multi-locus phylogeny of Nectogalini shrews and influences of the paleoclimate on speciation and evolution. Mol Phylogenet Evol 56:734–46.

Hewitt GM. (1996). Some genetic consequences of ice ages, and their role in divergence and speciation. Biol J Linn Soc 58:247–76.

Hewitt GM. (2000). The genetic legacy of the Quaternary ice ages. Nature 405:907–13.

Hewitt GM. (2001). Speciation, hybrid zones and phylogeography – or seeing genes in space and time. Mol Ecol 10:537–49.

Huelsenbeck JP, Ronquist F. (2001). MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754–5.

Jaffey N, Robertson A. (2005). Non-marine sedimentation associated with Oligocene – Recent exhumation and uplift of the Central Taurus Mountains, S Turkey. Sedimentary. Geology 173:53–89.

Jandzik D, Avcı A, Gvozdik V. (2013). Incongruence between taxonomy and genetics: Three divergent lineages within two subspecies of the rare Transcaucasian Rat Snake (*Zamenis hohenackeri*). Amphibia-Reptilia 34:579–84.

Janis CM. (1993). Tertiary mammal evolution in the context of changing climates, vegetation, and tectonic events. Annu Rev Ecol Syst 24: 467–500.

Kapli P, Botoni D, Ilgaz Ç, Kumlutaş Y, Avci A, Rastegar-Pouyani N, Fathinia B, et al. (2012). Molecular phylogeny and historical biogeography of the Anatolian lizard *Apathya* (Squamata, Lacertidae). Mol Phylogenet Evol 66:992–1001.

Kapli P, Poulakakis N, Lymberakis P, Mylonas M. (2011). A re-analysis of the molecular phylogeny of Lacertidae with currently available data. Basic Appl Herp 25:97–104.

Kornilios P, Ilgaz Ç, Kumlutaş 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: 351–63.

Kornilios P, Ilgaz Ç, Kumlutaş Y, Lymberakis P, Moravec J, Sindaco R, Rastegar-Pouyani N, et al. (2012). Neogene climatic oscillations shape the biogeography and evolutionary history of the Eurasian blindsnake. Mol Phylogenet Evol 62:856–73.

Kornilios P, Kyriazi P, Poulakakis N, Kumlutas Y, Ilgaz Ç, Mylonas M, Lymberakis P. (2010). Phylogeography of the ocellated skink *Chalcides ocellatus* (Squamata, Scincidae), with the use of mtDNA sequences: A hitch-hiker's guide to the Mediterranean. Mol Phylogenet Evol 54:445–56.

Krijgsman W, Hilgen FJ, Raffi I, Sierro FJ, Wilson DS. (1999). Chronology, causes and progression of the Messinian salinity crisis. Nature 400:652–5.

Kumlutaş Y, Öz M, Tunç MR, Düşen S, Özdemir A. (2003). Güneybatı Anadolu (29°–33° boylamlar arası) *Lacerta danfordi* (Reptilia: Lacertidae) populasyonları hakkında, [On the populations of (between east longitudes 29°–33°) *Lacerta danfordi* (Reptilia: Lacertidae) from south-western Anatolia]. Anadolu Univ J Sci Tech 4:165–72.

Kyriazi P, Poulakakis N, Parmakelis A, Crochet PA, Moravec J, Rastegar-Pouyani N, Tsigenopoulos CS, et al. (2008). Mitochondrial DNA reveals the genealogical history of the snake-eyed lizards (*Ophisops elegans* and *O. occidentalis*) (Sauria: Lacertidae). Mol Phylogenet Evol 49:795–805.

Librado P, Rozas J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–2.

Luo A, Qiao H, Zhang Y, Shi W, Ho SYW, Xu W, Zhang A, Zhu C. (2010). Performance of criteria for selecting evolutionary models in

phylogenetics: A comprehensive study based on simulated datasets. Evol Biol 10:242.
Martin DP, Lemey P, Lott M, Moulton V, Posada D, Lefeuvre P. (2010). RDP3: A flexible and fast computer program for analyzing recombination. Bioinformatics 26:2462–3.
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. J Zool Syst Evol Res 41:157–61.
Mayer W, Pavlicev M. (2007). The phylogeny of the family Lacertidae (Reptilia) based on nuclear DNA sequences: Convergent adaptations to arid habitats within the subfamily Eremiainae. Mol Phylogenet Evol 44:1155–63.
Medail F, Quezel P. (1999). Biodiversity hotspots in the Mediterranean basin: Setting global conservation priorities. Conserv Biol 13:1510–3.
Mertens R. (1952). Amphibien und Reptilien aus der Türkei. Rev Fac Sci Univ İstanbul Ser B 17:41–75.
Mittermeier RA, Gil PR, Hoffmann M, Pilgrim J, Brooks T, Mittermeier CG, Lamoreux J, Fonseca GAB. (2004). Hotspots revisited: Earth's biologically richest and most endangered terrestrial ecoregions. Mexico City: CEMEX.
Mouline K, Granjon L, Galan M. (2008). Phylogeography of a Sahelian rodent species Mastomys huberti: A Plio-Pleistocene story of emergence and colonisation of humid habitats. Mol Ecol 17:1036–53.
Murphy WR, Fu J, Macculloch DR, Darevsky IS, Kupriyanova LA. (2000). A fine line between sex and unisexuality: The phylogenetic constraints on parthenogenesis in lacertid lizards. Zool J Linn Soc 130: 527–49.
Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J. (2000). Biodiversity hotspots for conservation priorities. Nature 403: 853–8.
Nilson G, Andren C. (2001). The meadow and steppe vipers of Europe and Asia – The Vipera (Acridophaga) ursinii complex. Acta Zool 47: 87–267.
Özdemir N, Gül S, Poyarkov JRNA, Kutrup B, Tosunoğlu M, Doglio S. (2014). Molecular systematics and phylogeography of *Bufotes variabilis* (syn. Pseudepidalea variabilis) (Pallas, 1769) in Turkey. Turk J Zool 38:412–20.
Palumbi SR. (1996). Nucleic acids II: The polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK, editors. Molecular systematics, 2nd. ed. Sunderland: Sinauer Associates. p 205–47.
Pavlicev M, Mayer W. (2009). Fast radiation of the subfamily Lacertinae (Reptilia: Lacertidae): History or methodical artefact? Mol Phylogenet Evol 52:727–34.
Plötner J, Ohst T, Bohme W, Schreiber R. (2001). Divergence in mitochondrial DNA of Near Eastern water frogs with special reference to the systematic status of Cypriote and Anatolian populations (Anura, Ranidae). Amphibia-Reptilia 22:397–412.
Poulakakis N, Lymberakis P, Pafilis P, Valakos E, Mylonas M. (2005a). Phylogeography of Balkan wall lizard (*Podarcis taurica*) and its relatives inferred from mitochondrial DNA sequences. Mol Ecol 14: 2433–43.
Poulakakis N, Lymberakis P, Tsigenopoulos CS, Magoulas A, Mylonas M. (2005b). Phylogenetic relationships and evolutionary history of snake-eyed skink *Ablepharus kitaibelii* (Sauria: Scincidae). Mol Phylogenet Evol 34:245–56.
Rambaut A, Suchard MA, Xie D, Drummond AJ. (2014). Tracer v1.6. Available at: http://beast.bio.ed.ac.uk/Tracer
Ramstein G, Fluteau F, Besse J, Joussaume S. (1997). Effect of orogeny, plate motion and land-sea distribution on Eurasian climate change over the past 30 million years. Nature 386:788–95.
Schmidt HA, Strimmer K, Vingron M, von Haeseler A. (2002). TREE-PUZZLE: Maximum likelihood phylogenetic analysis using quartets and parallel computing. Bioinformatics 18:502–4.
Sindaco R, Kornilios P, Sacchi R, Lymberakis P. (2014). Taxonomic reassessment of *Blanus strauchi* (Bedriaga, 1884) (Squamata: Amphisbaenia: Blanidae), with the description of a new species from south-east Anatolia (Turkey). Zootaxa 3795:311–26.
Strimmer K, von Haeseler A. (1996). Quartet puzzling: A quartet maximum likelihood method for reconstructing tree topologies. Mol Biol Evol 13:964–9.
Tamura K, Nei M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–26.
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. Mol Biol Evol 28:2731–9.
Tavaré S. (1986). Some probabilistic and statistical problems in the analysis of DNA sequences. Lect Notes Math 17:57–86.
Tchernov E. (1992). The Afro-Arabian component in the Levantine mammalian fauna – A short biogeographical review. Israel J Zool 38: 155–92.
Templeton AR, Crandall KA, Sing CF. (1992). A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132:619–33.
Tok CV. (1999). Resadiye (Datça) yarımadası kertenkeleleri hakkında (Gekkonidae, Agamidae, Chamaeleonidae, Lacertidae, Scincidae, Amphisbaenidae), [On lizard species of Datça (Gekkonidae, Agamidae, Chamaeleonidae, Lacertidae, Scincidae, Amphisbaenidae)]. Turk J Zool 23:157–75.
Uetz P. (2014). The Reptile Database, Available at: http://www.reptile-database.org (Accessed on 1 August 2014).
Webb T, Bartlein PJ. (1992). Global changes during the last 3 million years: Climatic controls and biotic responses. Annu Rev Ecol Syst 23: 141–73.
Werner F. (1900). Beschreibung einer bisher noch unbekannter Eidechse aus Kleinasien *Lacerta anatolica*. Anz Akad Wiss Wien 37:269–71.
Wettstein O. (1967). Ergebnisse Zoologischer Sammelreisen in der Türkei, Versuch einer Klarung des Rassenkreisses von *Lacerta danfordi*, GTHR. 1876. Ann Naturhist Mus Wien 70:345–56.
Whiting AS, Bauer AM, Sites JM. (2003). Phylogenetic relationships and limb loss in sub-Saharan African and scincine lizards (Squamata: Scincine). Mol Phylogenet Evol 29:582–98.
Wielstra B, Themudo GE, Guclu O, Olgun K, Poyarkov NA, Arntzen JW. (2010). Cryptic crested newt diversity at the Eurasian transition: The mitochondrial DNA phylogeography of Near Eastern Triturus newts. Mol Phylogenet Evol 56:888–96.
Willis KJ, Kleczkowski A, Crowhurst SJ. (1999). 124,000-Year periodicity in terrestrial vegetation change during the late Pliocene epoch. Nature 397:685–8.