Multiple dispersal out of Anatolia: biogeography and evolution of oriental green lizards

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The oriental green lizards of the *Lacerta trilineata* group are widely distributed in Greece, Anatolia, the eastern Mediterranean, the southern Caucasus, and the Zagros mountains in Iran. We studied their phylogeography using three mitochondrial markers with comprehensive sampling from most representatives of the group. Their phylogeny and divergence times (implementing fossil-based molecular clock calibrations) were inferred using Bayesian methods, and haplotype networks were reconstructed to assess how genetic diversity and current distributional patterns were shaped. According to our phylogenetic analyses, the group constitutes a well-supported monophylum containing several distinct evolutionary lineages with high haplotype diversity. Vicariance might explain the divergences within most lineages that have accumulated by range restriction and expansion of populations as a result of Quaternary climate oscillations and glacial refugia. However, niche divergence appears to be a major force promoting speciation, and large scale distributional patterns between lineages were shaped earlier by multiple, independent dispersals out of Anatolia during the Pliocene and early Pleistocene. The results of the present study also suggest that the group is in need of a taxonomical revision because the identified lineages and genetic diversity are not congruent with the currently recognized subspecies. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, ••, ••–••.

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INTRODUCTION

Biogeographical patterns are often explained by opposing dispersal to vicariance as competing processes (Yoder & Nowak, 2006). Nevertheless, often both processes are responsible for shaping spatial patterns of diversity (Zink, Blackwell-Rago & Ronquist, 2000; Noonan & Chippindale, 2006), as has also been shown for lizards in the western Palearctic (Paulo *et al.*, 2008). On a more regional scale, this interplay is represented by isolation in refugia and subsequent spreading out. Topographical heterogeneity and lower latitude have made southern Europe,

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Turkey, and the southern Caucasus likely places for multiple glacial refugia, where genetic diversity may have accumulated over several ice ages leading to the origin of new lineages and ultimately new taxa (Taberlet *et al.*, 1998; Hewitt, 1999; Seddon *et al.*, 2002; Jakob, Ihlow & Blattner, 2007; Rossiter *et al.*, 2007). The bridge between Asia and Europe, also linking to the Ethiopian region via the Arabian Peninsula, provides a natural pathway for the spread of species both east–west and north–south (Tchernov, 1992; Kosswig, 1955; Mittermeier *et al.*, 2005). It has played an important role as a refugium during the Quaternary ice ages, holding populations during glacial periods that could move out from there to Europe via Thrace (European part of Turkey) and the Caucasus (Hewitt, 2001; Çıplak, 2003). The area was also one of the major corridors for postglacial invasions in the Palearctic (Steininger, Rabeder & Rögl, 1985; Veith *et al.*, 2003; Bilgin, 2011) and its biodiversity has been largely influenced by climatic fluctuations since the Pliocene. During the Pliocene, aridification and the establishment of the Mediterranean climate (Fauquette *et al.*, 1999; Cavazza & Wezel, 2003; Jiménez-Moreno, Fauquette & Suc, 2010) were major climatic changes that affected the distribution of species prior to glacial cycles. As broadly reviewed by Bilgin (2011), the geographical distribution of intraspecific genetic diversity of 29 species of plants and animals in Anatolia shows two major phylogeographical patterns: pattern I comprises genetically differentiated populations in the Balkans and in Anatolia (Stöck *et al.*, 2006; Stamatis *et al.*, 2009), whereas pattern II is characterized by subgroups within Anatolia (Schmidtler, 1998; Jakob *et al.*, 2007; Simonato *et al.*, 2007). Special cases include groups that show a juxtaposition of patterns I and II (Dubey *et al.*, 2008; Stöck *et al.*, 2008), suggesting that Anatolia is a centre of diversity for surrounding regions (Bilgin, 2011).

Within the lizard subfamily Lacertinae, the species-rich tribe Lacertini apparently originated in the western Palearctic (Arnold, Arribas & Carranza, 2007) and radiated in the late Eocene-Oligocene (Hipsley *et al.*, 2009), generating most of the extant genera. Rapid diversification during this period (Pavlicev & Mayer, 2009) led to their current allopatric and often disjunct ranges (Carranza, Arnold & Amat, 2004; Arnold *et al.*, 2007). However, some genera such as *Podarcis*, *Lacerta*, and *Timon* are widespread and the last two may have been among the first which considerably dispersed based on the mitochondrial sequence differences reported (Harris, Arnold & Thomas, 1998; Arnold *et al.*, 2007; Ahmadzadeh *et al.*, 2012). Today, green lizards (genus *Lacerta*) inhabit a large area extending from the Iberian Atlantic coast to Central Asia (Godinho *et al.*,

2005). Six out of the eight species of *Lacerta* occur in Anatolia and adjacent regions, making the area a hotspot of green lizard diversity. Based mostly on phenetic similarities, the genus has been subdivided into several groups. One of these groups, the *Lacerta trilineata* group, is comprised of the oriental species *Lacerta media* Lantz & Cyrén, 1920, *Lacerta trilineata* Bedriaga, 1886 and *Lacerta pamphylica* Schmidtler, 1975 (Peters, 1962; Schmidtler, 1986a, b). This group's centre of distribution is Anatolia, although it spreads across the Aegean to the Balkan peninsula in the west, to the southern Caucasus, the Alborz and Zagros mountains of Iran in the east, and to Jordan in the south (Peters, 1962; Schmidtler, 1986a; Leviton *et al.*, 1992; Anderson, 1999; Arnold *et al.*, 2007; Ahmadzadeh *et al.*, 2008; Sindaco & Jeremcenko, 2008).

Similar to the southern European peninsulas, Anatolia served as a major refugium for reptiles during ice ages. Their evolutionary lineages may have persisted within separated refugia during that time and also radiated within this area, which yielded high genetic diversity. However, comprehensive studies on the evolutionary history of oriental green lizards, which could provide a well suited model to infer the likely geographical positions of such refugia via detailed phylogeographical analyses, are still lacking. Most of the group's taxa occur in allopatry, although there are cases of partial range overlap between some species and even between currently considered subspecies, which may raise questions regarding their taxonomic status. Although some molecular data have already been analyzed (Harris *et al.*, 1998; Mayer & Beyerlein, 2001; Godinho *et al.*, 2005), more profound conclusions on the group's phylogeography are hampered by insufficient sampling coupled with high levels of genetic differentiation between species, and particularly between morphologically described subspecies.

In the present study, we conducted an extensive sampling including most of the representatives of the *Lacerta trilineata* group aiming to determine: (1) how genetic diversity is partitioned geographically in this widespread group; (2) when the different genetic lineages diverged; and (3) whether these patterns are concordant with the distribution of the morphologically delimited (sub)species and with general biogeographical patterns found in the region.

MATERIAL AND METHODS

SAMPLING, SEQUENCE PROCESSING, AND ANALYSIS

Sampling encompassed 65 specimens covering all described species and subspecies in the *Lacerta trilineata* group, except for *Lacerta media isaurica* and *Lacerta trilineata citrovittata*. Total genomic

DNA from each individual was extracted using standard saline methods (Sambrook, Fritsch & Maniatis, 1989). The primers GluDG/Peil (modified from Palumbi *et al.*, 1991; Engstrom *et al.*, 2007), 12Sa/12Sb (Kocher *et al.*, 1989), and 16SL/16SH (Palumbi *et al.*, 1991) were used to amplify and sequence parts of the mitochondrial cytochrome *b*, 12S and 16S ribosomal RNA genes, respectively. The polymerase chain reaction (PCR) cycling conditions have been described previously (Ahmadzadeh *et al.*, 2012). Purified PCR products were sequenced by a commercial company (Macrogen). Sequences were checked with the original chromatograph data using CODONCODE (CodonCode Corporation) and subsequently aligned with MUSCLE, version 3.6 (Edgar, 2004). The alignment was checked by eye and manually refined where necessary. Additional sequences including representatives of all species of *Lacerta* and the closely-related genus *Timon* (Ahmadzadeh *et al.*, 2012), as well as members of the genus *Gallotia*, were downloaded from GenBank to obtain calibration points (see below), to check for monophyly of the main study group, and to compare divergences within the *L. trilineata* group to other groups. Specimens used in this study, accession numbers, and respective localities are provided in the Supporting information (Table S1).

For each gene, GTR+G was chosen as the best-fitting model of nucleotide substitution by the Akaike's information criterion using MRMODELTEST, version 2.2 (Nylander, 2004). Phylogenetic trees were inferred by maximum likelihood (ML) for each of the three genes separately using RAXML, version 7.2.X (Stamatakis, 2006). Qualitative evaluation (Wiens, 1998) of the gene trees showed no incongruent evolutionary histories of the different genes involved and subsequent analyses were performed using a concatenated data set (1728 bp including gaps), which was partitioned to estimate substitution model parameters separately for each gene. ML bootstrap values were calculated with RAXML (1000 replicates).

HAPLOTYPE NETWORK

The genealogy of the study group was further assessed across its distributional range by building a haplotype network using a statistical parsimony network construction approach as implemented in TCS, version 1.21 (Clement, Posada & Crandall, 2000). For this, a concatenated data set of 12S and 16S (749 bp) was used with gaps coded as a fifth character state.

DIVERGENCE TIME ESTIMATION

Divergence times were estimated with BEAST, version 1.6.2, using a relaxed molecular clock approach with an estimated rate and uncorrelated lognormal distribution (Drummond *et al.*, 2006; Drummond & Rambaut, 2007). Analyses were performed on the concatenated dataset containing all three genes. BEAST also produces a phylogenetic tree using Bayesian inference (BI) and calculates posterior probabilities for nodes. The Yule speciation process was used as tree prior. Convergence of the Markov chain Monte Carlo chains was checked with TRACER, version 1.5 (Rambaut & Drummond, 2007). Results of the BEAST analyses were summarized from five independent runs with 10^7 trees each, sampling every 1000 and omitting the initial 10^3 trees of each run as burn-in, resulting in 45 000 trees. Node heights (i.e. node ages) were calculated as means of the posterior estimates and 95% highest posterior density intervals (HPD).

Three fossil records and one geological event were used for calibration. Fossils included *Lacerta ruscinensis* (5.3 Mya, used as minimum age of European *Timon* because it is indistinguishable from the extant European *Timon* spp.; Estes, 1983), *Lacerta* cf. *viridis* (8.7 Mya, used as minimum age of the clade containing *Lacerta viridis* and *Lacerta bilineata*, showing diagnostic characters of this group; Tempfer, 2004; Venczel, 2006), and *Lacerta* sp. (17.5 Mya, used as minimum age of *Lacerta* because it differs from *Timon* but exhibits features typical of *Lacerta* spp.; Cernansky, 2010) and were implemented as hard minimum bounds with a gamma-shaped prior distribution (shape = 1, scale = 1). Additionally, the formation of El Hierro island (1.05 Mya; Guillou *et al.*, 1996) was used to calibrate the split between *Gallotia caesaris caesaris* and *Gallotia caesaris gomerae* (Carranza *et al.*, 2004) using a soft bound (normal prior distribution, SD = 0.02). Our estimates of the age of key events in the phylogeny of the group were also compared with the expected values based on proposed rates of molecular evolution for these mitochondrial genes within lacertids (Carranza *et al.*, 2004; Paulo *et al.*, 2008) and fall within these ranges. Thus, although using these few and only partially identified fossil calibrations to date the tree is not ideal (Parham *et al.*, 2012), the dates proposed appear reasonable based on multiple sources of evidence.

RESULTS

Both ML and BI analyses strongly support seven distinct evolutionary lineages within the monophyletic *Lacerta trilineata* group (Fig. 1; for the complete trees including all other species of *Lacerta* and outgroups, see also supporting information, Fig. S1). Concerning *L. media*, the four identified lineages correspond to the current subspecific taxonomy (Schmidtler, 1986a) and together form a monophyletic
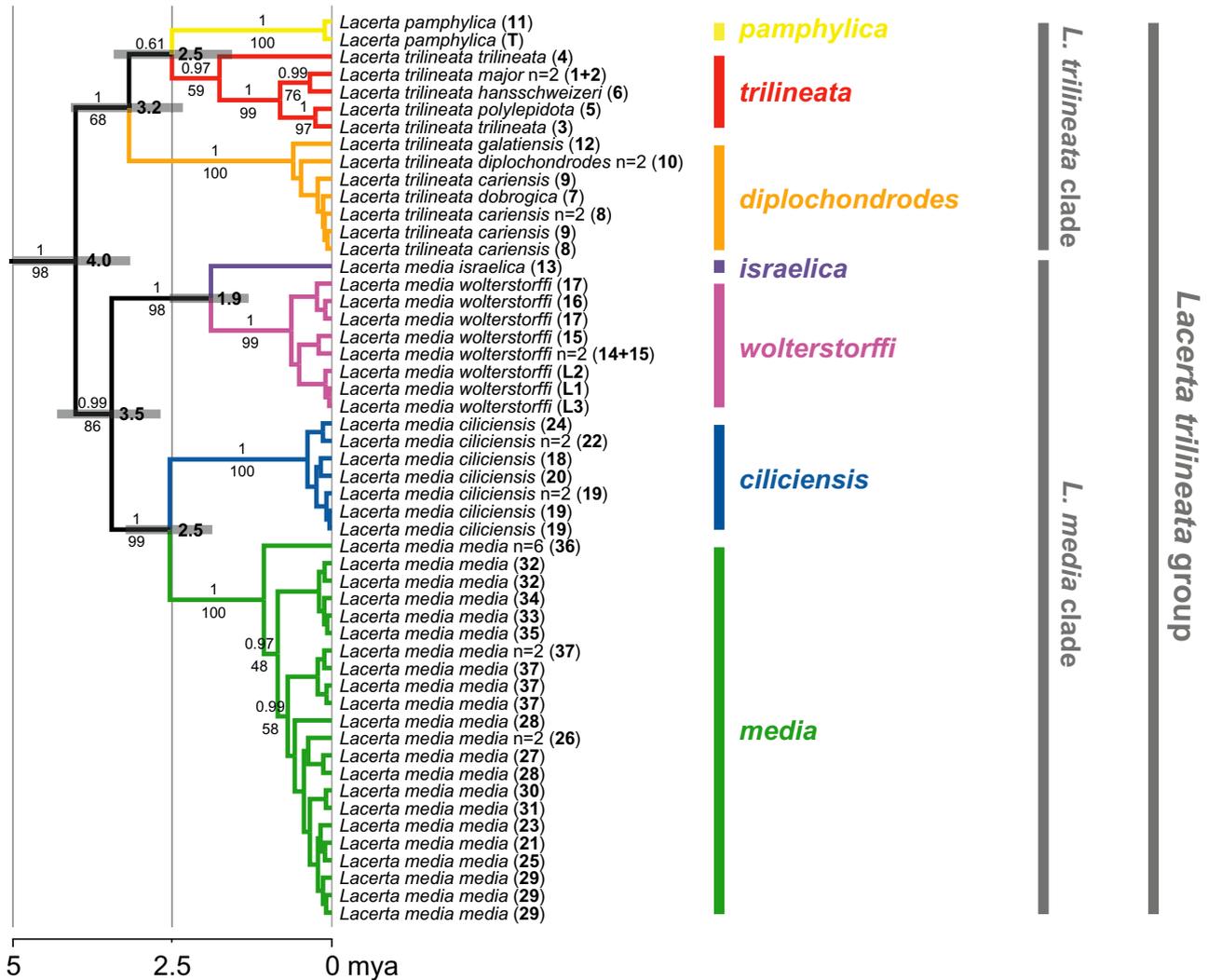
Figure 1. Dated Bayesian tree of the *Lacerta trilineata* group, inferred from 1728 bp of mitochondrial DNA (cytochrome *b*, 12S and 16S rRNA), revealing seven evolutionary lineages. Grey bars show 95% highest posterior density intervals of estimated node ages; bold numbers are mean node ages (Mya). Posterior probabilities are shown above and maximum likelihood bootstrap values are shown below branches (support values are only shown for nodes with posterior probabilities > 0.5). Numbers in parenthesis behind taxa refer to the localities shown in Fig. 3.

clade. This *L. media* clade can be divided into a northern subclade comprising the lineages *L. m. media* and *L. m. ciliciensis*, which are estimated to have split 2.5 Mya (HPD 1.9–3.2 Mya), and a southern subclade comprising *Lacerta media wolterstorffi* and *Lacerta media israelica*, which split ca. 1.9 Mya (HPD 1.3–2.5 Mya). The two subclades have diverged approximately 3.5 Mya (HPD 2.7–4.3 Mya).

The second major clade contains *L. trilineata* and *L. pamphylica*. The eight subspecies of *L. trilineata* included in our analyses constitute only two lineages, which can be termed as a western lineage comprising Adriatic and Greek taxa (*Lacerta trilineata hansschweizeri*, *Lacerta trilineata major*, *Lacerta*

trilineata polylepidota, and the nominate subspecies; summarized as '*trilineata*' henceforth) and an eastern lineage comprising the taxa occurring from the Danube delta to Anatolia (*Lacerta trilineata cariensis*, *Lacerta trilineata diplochondrodes*, *Lacerta trilineata dobrogica*, and *Lacerta trilineata galatiensis*; summarized as '*diplochondrodes*' henceforth). A third lineage matches *L. pamphylica*, which is nested within this clade forming a sister relationship with the western lineage. Support for this relationship is weak and only inferred by BI, although none of our analyses evoked a sister relationship of the western and eastern lineages (see Supporting information, Fig. S1). Estimated divergence time is 2.5 Mya (HPD
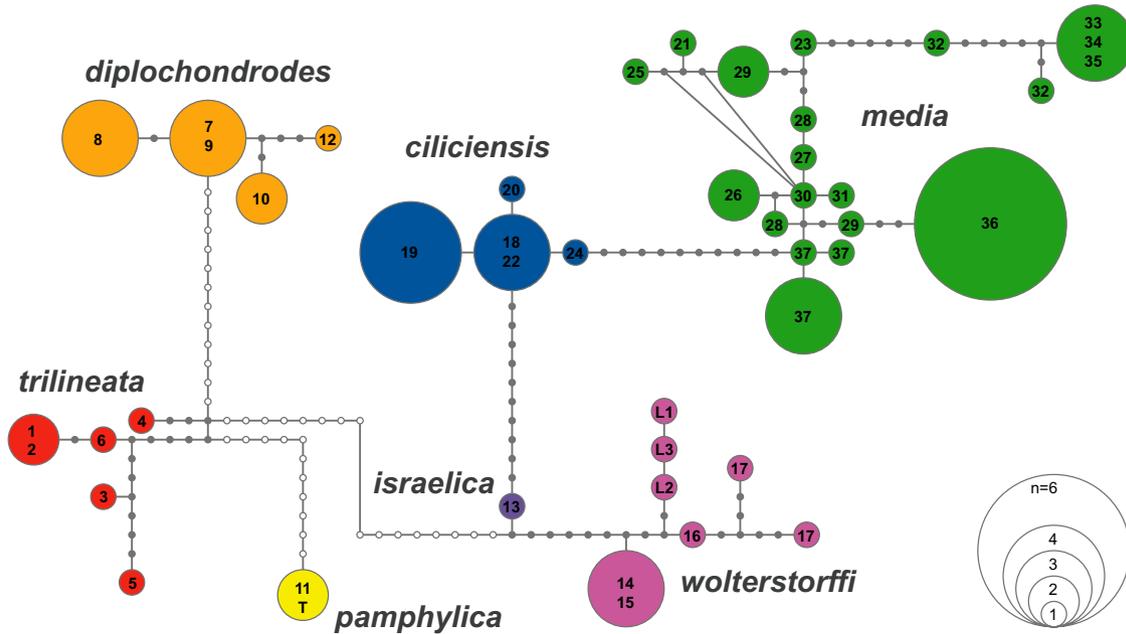
Figure 2. Unrooted haplotype network constructed with statistical parsimony based on 749 bp of mitochondrial 12S and 16S rRNA of the *Lacerta trilineata* group. Circle size is relative to haplotype frequency; numbers in circles refer to the localities in Fig. 3 (samples without precise locality data are labelled with 'L' and 'T' and originate from Lebanon and Turkey, respectively). Dots represent extinct or unsampled haplotypes. The four independent subnetworks (95% cut-off) are connected by empty dots.

1.6–3.4 Mya) between *L. pamphylica* and the western lineage and 3.2 Mya (HPD 2.3–4.1 Mya) between this pair and the eastern lineage.

A total of 40 different haplotypes were identified among the 65 samples using 749 bp of the combined 12S and 16S rRNA (Fig. 2). Statistical parsimony produced four independent subnetworks when applying a 95% connection limit (i.e. the maximum number of mutational steps between pairs of sequences associated with the probability of parsimony is used as cut-off); one for each of the three lineages of the *L. trilineata* clade and another for the whole *L. media* clade. However, the lineages belonging to the latter clade each form a distinct haplogroup within the subnetwork.

DISCUSSION

BIOGEOGRAPHY AND EVOLUTION

In the present study, we provide the first comprehensive and robust assessment of the phylogenetic relationships within the widespread group of oriental green lizards, based on mitochondrial DNA sequence data. Regarding the phylogeographical patterns proposed for the Anatolian region by Bilgin (2011), the *L. trilineata* group as a whole exhibits pattern II, with the two main clades having split within Anatolia in the Pliocene (mean 4.0 Mya, HPD 3.2–5.0 Mya).

Geological events in the area of the Taurus and the western Anatolian mountains during the Pliocene (Davis, 1971; Kosswig, 1955) formed barriers to gene flow, isolating populations of the ancestral species in several areas (Kornilios *et al.*, 2011). Anatolian mountains, especially in the south, have played an important role in speciation and definition of biogeographical subregions (Çıplak, 2003, 2004). However, when taking a closer look at each of the clades, our results suggest different (and more complex) patterns.

Within the *L. trilineata* clade in particular, the first split occurred in western Anatolia during the late Pliocene (3.2 Mya), separating the *diplochondrodes* lineage from the ancestor of *trilineata* and *pamphylica*. The last two lineages finally diverged during the late Pliocene/early Pleistocene (2.5 Mya). The three lineages have been genetically isolated for a long time because each of them constitutes a subnetwork in the haplotype analyses under statistical parsimony (Fig. 2). Their current distributional ranges are disjoined by the zoogeographical break of the mid-Aegean trench (Fig. 3), which constitutes a barrier for many taxa (Lymberakis & Poulakakis, 2010), including lizards (Poulakakis *et al.*, 2005b, c), although its formation 12–9 Mya predates (Papadopoulou, Anastasiou & Vogler, 2010) the divergence times estimated within *L. trilineata*,
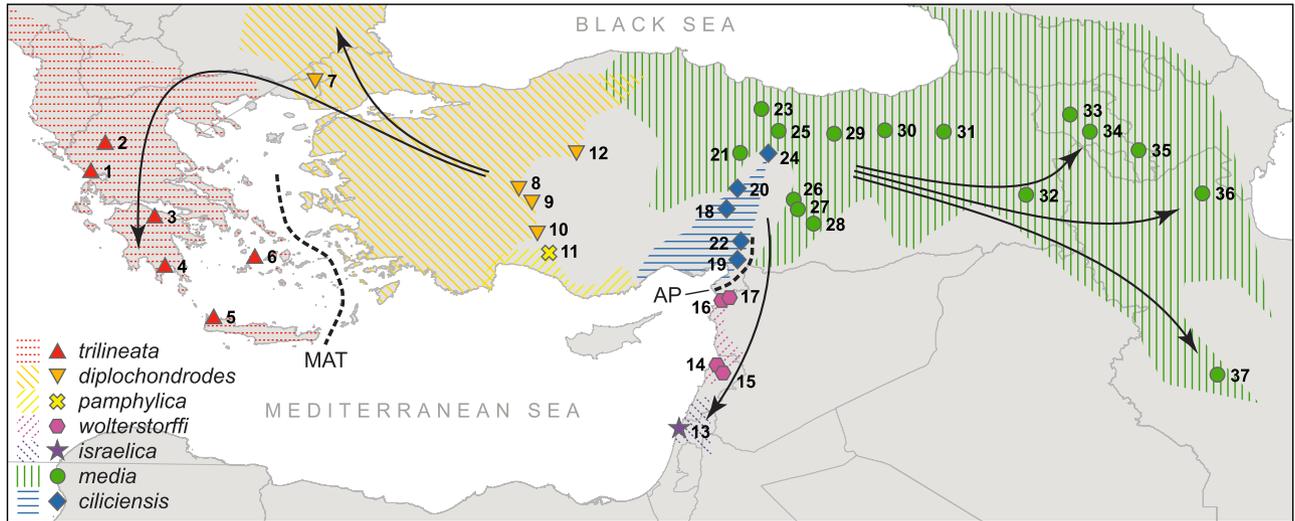
Figure 3. Map of the distributional ranges (hatched areas; based on point-wise occurrences from Ahmadzadeh *et al*., 2013) and sampled localities (symbols) for the lineages detected within the *Lacerta trilineata* group. The dashed lines mark the mid-Aegean trench (MAT) and the Amik plain (AP), which both form major barriers for lizard dispersal. The independent dispersal routes out of Anatolia are indicated by arrows.

as does the Messinian salinity crisis (Krijgsman *et al*., 1999) during which dispersal across the Aegean would have been possible over land as a result of a lower sea level (Lymberakis & Poulakakis, 2010). Thus, if vicariance caused by the mid-Aegean trench and dispersal during the Messinian salinity crisis did not generate the pattern observed here, how was this pattern shaped?

A possible scenario includes several waves of dispersal from Anatolia to the Balkan peninsula via Thrace after divergence of the three lineages. In the first wave of dispersal, the *trilineata* lineage colonized the southern Balkan with subsequent extinction in areas in between. Southern Greece, where the most distinct population of the *trilineata* lineage is found, served as a glacial refugium (Taberlet *et al*., 1998; Joger *et al*., 2007), from which the lineage later spread to northern Greece and along the Adriatic coast. A second wave where the *diplochondrodes* lineage colonized Thrace and the Black Sea coast northwards to the Danube Delta then hampered re-colonization of these areas by the *trilineata* lineage (Fig. 3). Differences in the climatic niches of the two lineages (Ahmadzadeh *et al*., 2013) can explain the time-shift between the dispersal events, as well as why *trilineata* was unable to spread back into Anatolia because populations can only disperse into suitable environments. The Mediterranean climate (i.e. temperate with dry summers) arose during the Pliocene and is currently occupied by *trilineata* (Ahmadzadeh *et al*., 2013). Alternatively, overseas dispersal across the Aegean, which has been proposed for other reptiles (e.g. skinks; Poulakakis *et al*.,

2005a), might be considerable, although the Aegean islands did not function as stepping stones for green lizards according to the phylogenetic relationships revealed in the present study (Fig. 1) and were rather colonized multiple times with Greek and Anatolian mainland as source areas for western and eastern Aegean islands, respectively (*L. t. polylepidota* from Crete is the sister taxon of the central Greek populations, *L. t. hansschweizeri* from the Cyclades is the sister taxon of north-western Greek populations, and eastern Aegean islands are inhabited by *L. t. cariensis* and *L. t. diplochondrodes*).

The phylogeographical pattern of the *L. media* clade should be considered a special case, as Bilgin (2011) discussed for the white-breasted hedgehog (Filippucci & Simson, 1996; Santucci, Emerson & Hewitt, 1998; Seddon *et al*., 2002). Apparently, the geological events caused the fragmentation of the hypothesized ancestral taxa and, later, the process of cladogenesis was completed by the late Pliocene aridification and Quaternary climate oscillations, as suggested for other taxa (Veith *et al*., 2003). Taking into account the deep genetic distance between the northern and southern subclades of the *L. media* clade, this scenario appears to be reasonable. In other words, the split between these two subclades, which is estimated to have occurred 3.5 Mya, started with geological events (formation of the Anatolian mountain ranges) and climate fluctuations formed the current distributional patterns of the lineages. Both subclades occur in close proximity, although they are strictly allopatric, with the Amik plain in southern Turkey constituting a current barrier for gene flow

(Fig. 3). This appears to be a vicariant pattern, although the haplotypes found in the northern subclade are more similar to the haplotype of the southernmost lineage of the southern subclade (i.e. *L. m. israelica*) than to *L. m. wolterstorffi* (Fig. 2), which is distributed in between these taxa. This suggests southwards dispersal out of Anatolia by the ancestor of *L. m. israelica* and *L. m. wolterstorffi* followed by divergence of these two taxa 1.9 Mya (probably, they became isolated in mesic areas as a result of the postglacial aridification in the eastern Mediterranean) and subsequent re-colonization of the northern areas by *L. m. wolterstorffi* towards the emerging barrier of the Amik plain.

Strong environmental changes including geological events, emergence of mountain ranges (Taurus and Amanos in the south, Pontic mountains in the north, and the Anatolian Diagonal in the east), which were considered primary current and past barriers to gene flow (Davis, 1971; Kosswig, 1955; Schmidtler, 1998), and later Pleistocene climate oscillations resulting in southward progression of ice sheets during phases of cooling (Avise, 2000; Hewitt, 2001; Hewitt, 2004), have affected the distribution of the ancestor of *L. m. media* and *L. m. ciliciensis*, causing the evolution of the lineages and of divergences within the lineages in Anatolia. In addition, the central Anatolian lake system had considerable effects on the fauna and flora (Demirsoy, 1996). Anatolian populations are the most genetically diverse within *L. m. media*. The haplotype network (Fig. 2) supports Anatolia as the source area, from where multiple independent dispersal events to Armenia (including the easternmost Turkish populations in the Van area), to the Alborz mountains in north-western Iran, and to the Zagros mountains in western Iran are suggested (Fig. 3). As in the *L. trilineata* clade (see above), early divergence does not involve early dispersal. The population from north-western Iran split approximately 1.0 Mya from the ancestor of the remaining populations of *L. m. media*, although it is genetically homogeneous and might have spread much later than, for example, the population from western Iran, which features considerable haplotype diversity and is the sister group to the Anatolian populations (Fig. 1). Concerning the distinctive position of the north-western Iranian population, a similar pattern was detected in Anatolian mountain frogs and re-colonization from a refugium in the Alborz Mountains was proposed (Veith *et al.*, 2003). In general, both *L. m. ciliciensis* and *L. m. media* occur in continental and humid climates (Ahmadzadeh *et al.*, 2013), which, in contrast to previous assumptions (see above), suggests that the emerging mountains in Anatolia were interglacial refugia and not glacial barriers.

Rather than vicariance, which was used to explain most biogeographical patterns observed in this region, our results indicate that dispersal plays an important role in forming species' distributions. Such dispersal is associated with (or even fueled by) niche divergence (Ahmadzadeh *et al.*, 2013). On the other hand, the predominant climate fluctuations during the past three million years have strongly affected the distribution of most lineages and significantly shaped their genetic structure, especially in the Pliocene. Glaciations were only shifting ranges and promoting some geographical variation within lineages (Gómez & Lunt, 2007). The area is not exceptional regarding these effects and was repeatedly affected by fauna and flora exchanges (Hrbek & Meyer, 2003; Veith *et al.*, 2003; Dubey *et al.*, 2008; Rajabi-Maham, Orth & Bonhomme, 2008; Bilgin, 2011). Although vicariance might explain the divergences within most lineages that have accumulated by range restriction and expansion of populations as a result of Quaternary climate oscillations and glacial refugia, large scale distributional patterns between lineages were shaped earlier by multiple, independent dispersals out of Anatolia during the Pliocene and early Pleistocene.

TAXONOMIC IMPLICATIONS

The taxonomic definition and content of the genus *Lacerta* s.s. has been discussed in several studies (Arnold *et al.*, 2007). Within this genus, the *L. trilineata* group (as defined above) constitutes a well-supported monophyletic group (see Supporting information, Fig. S1) containing two distinct main clades, which correspond to the currently recognized species *L. media* and *L. trilineata* (Fig. 1). As already reported by Godinho *et al.* (2005), *L. pamphylica* is nested within the latter. The resulting paraphyly of *L. trilineata* can be taxonomically resolved by either elevating each lineage to species rank or by considering *L. pamphylica* as a subspecies of *L. trilineata*, as reported in a previous study (Schmidtler, 1975). However, subspecific taxonomy of this clade appears to be inflated because comparatively little (see *L. media* below; Böhme *et al.*, 2007) genetic diversity is found within lineages, especially in the *diplochondrodes* lineage, which still comprises four different morphologically defined subspecies (Peters, 1964). An uncertain classification based on the morphology of some western Anatolian populations in previous studies and clinal variation of morphological characters (Schmidtler, 1975, 1986a, b) provides further support for our assumption. On the other hand, southern Peloponnesian *L. trilineata* were assigned to the nominate subspecies, although they constitute the most divergent population within

the *trilineata* lineage and feature some morphological differences from other Greek populations (Buchholz, 1960; Böhme, 1974). It becomes obvious that current taxonomy does not properly reflect phylogenetic relationships and genetic diversity within this clade. Further studies incorporating nuclear DNA and a more comprehensive sampling of Greek and western Anatolian populations are needed to completely clarify this inconsistency.

Although phylogenetic patterns of the *L. media* clade suggested by our analyses are in concordance with the current subspecies described solely on the basis of morphology (Peters, 1964; Schmidtler, 1986a), their genetic diversity and relatively old divergence times (1.9–3.5 Mya) demand a critical revision of their taxonomy. In particular, the divergences between populations within *L. m. ciliciensis* and *L. m. media* are comparable to (or even larger than) those found between the distinguishable subspecies of *L. trilineata*. However, not only the topology and divergence times revealed by our phylogenetic analyses, but also the chorological patterns of related taxa can provide useful arguments for determining the group's taxonomy. For example, the range overlap of *L. pamphylica* and *L. t diplochondrodes* in south-western Turkey suggests that they may represent different species, although no definite contact zone was detected (Geniez, Geniez & Viglione, 2004). Consequently, they appear to occur in small-scale parapatry rather than sympatry. The same might apply to *L. m. ciliciensis* and *L. m. media*. Ecological diversification also plays an important role in speciation processes within this group (Ahmadzadeh *et al.*, 2013), indicating that vicariant events and resulting allopatry may not be the only force inducing diversification in green lizards. Alternative approaches on taxonomy might be necessary to reflect the phylogenetic diversity of this group, considering the influence of ecological factors on speciation described above, such as the ecological species (Schluter, 2001; Schluter, 2009; Schluter & Conte, 2009) that account for the processes eventually leading to speciation.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Taxa used for phylogenetic analyses, with respective localities and GenBank accession numbers.
Figure S1. Complete trees (dated Bayesian inference and maximum likelihood) including all species of *Lacerta* and outgroups.