Molecular Phylogenetics and Evolution 102 (2016) 117–127

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

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

Parthenogenesis through the ice ages: A biogeographic analysis of Caucasian rock lizards (genus *Darevskia*)

Susana Freitas [a,b,*], Sara Rocha [c], João Campos [a], Faraham Ahmadzadeh [d], Claudia Corti [e], Neftali Sillero [f], Çetin Ilgaz [g], Yusuf Kumlutaş [g], Marine Arakelyan [h], D. James Harris [a], Miguel A. Carretero [a]

[a] CIBIO Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando Quintas, N° 7. 4485-661 Vairão, Vila do Conde, Portugal
[b] Department of Animal and Plant Sciences, The University of Sheffield, Sheffield S10 2TN, UK
[c] Department of Biochemistry, Genetics and Inmunology, University of Vigo, 36310 Vigo, Spain
[d] Department of Biodiversity and Ecosystem Management, Environmental Sciences Research Institute, Shahid Beheshti University, G.C., Evin, Tehran, Iran
[e] Museo di Storia Naturale dell'Università di Firenze, Sezione di Zoologia "La Specola", Via Romana, 17, 50125 Firenze, Italy
[f] CICGE Centro de Investigação em Ciências Geo-Espaciais, Faculdade de Ciências da Universidade do Porto (FCUP), Observatório Astronómico Prof. Manuel de Barros, Alameda do Monte da Virgem, 4430-146 Vila Nova de Gaia, Portugal
[g] Dokuz Eylül University, Faculty of Science, Department of Biology, 35160 Buca, İzmir, Turkey
[h] Yerevan State University, Alek Manoogian, 1, Yerevan 0025, Armenia

ARTICLE INFO

Article history:
Received 9 December 2015
Revised 23 May 2016
Accepted 26 May 2016
Available online 28 May 2016

Keywords:
Darevskia
Parthenogenesis
mtDNA
Phylogeny
Ecological niche models
Glaciations

ABSTRACT

Darevskia rock lizards include both sexual and parthenogenetic species, mostly distributed in the heterogeneous and ecologically diverse Caucasus. The parthenogenetic species originated via directional hybridogenesis, with only some of the sexual species known to serve as parentals. However, it remains unclear when and where these events happened and how many parental lineages were involved. A multilocus phylogeographic analysis was performed on the parthenogens *D. unisexualis*, *D. bendimahiensis* and *D. uzzeli*, and their putative maternal species *D. raddei*. Results show the parthenogenetic species all have relatively recent origins, approximately 200–70 kyr ago, and at least three hybridization events were involved in their formation. Ecological niche models identify the region where hybridization events leading to the formation of *D. unisexualis* took place, namely in the northeast of the current distribution. Models also suggest that the sexual *D. raddei* might have undergone a habitat shift between the Last Interglacial and the Last Glacial Maximum.

1. Introduction

The study of parthenogenetic organisms, which reproduce in the absence of sex, provides an opportunity to understand the significance of sexual reproduction and the evolution of sex. In particular, taxa that present both sexual and parthenogenetic reproduction within the same clade, provide an opportunity to compare both reproductive forms and analyse their eventual ecological interactions (Gilabert et al., 2014; Otto and Nuismer, 2004). Reptiles are good model organisms for such studies due to the wide variety of reproductive modes and life history strategies, and lizards in particular are recurrent models used in studies of speciation, phylogeography and adaptation (Camargo et al., 2010). Several lizard families include parthenogenetic and sexual species, making them especially interesting for studying the evolution and function of sexual reproduction (Avise, 2008). Indeed, parthenogenesis was first described in vertebrates in the lizard genus *Darevskia* (Darevsky, 1967). Since then, at least 43 other cases of parthenogenetic reproduction have been described in the Squamata (Kearney, 2003; Vrijenhoek, 1989). It is estimated that 0.6% of squamates (which comprise around 7000 species) can reproduce parthenogenetically, either obligatorily or facultatively (Kearney et al., 2009). Parthenogenesis is found across the squamate phylogeny and through a wide geographical range and ecological conditions. Most, but not all, parthenogenetic forms arose after hybridization between two related species, but the scenario for the origin of the parthenogenesis varies with the group and it is highly complex (Avise, 2008). Given this widespread distribution and the fact that parthenogenetic reproduction is frequently considered an "evolutionary dead-end" (Bell, 1982), it is still not fully

* Corresponding author at: CIBIO Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando Quintas, N° 7. 4485-661 Vairão, Vila do Conde, Portugal.
E-mail address: freitas.sn@gmail.com (S. Freitas).

http://dx.doi.org/10.1016/j.ympev.2016.05.035
1055-7903/

understood whether new parthenogenetic lineages regularly appear and how they compete with sexual forms.

In this study we focus on lizards of the genus *Darevskia* Arribas, 1997 of the family Lacertidae. This is a group of small lizards found across the Caucasus and adjacent regions, including Turkey, Iran and the Balkans (Arnold et al., 2007). Currently 32 species are recognised (Ahmadzadeh et al., 2013b; Uetz and Hošek, 2015) which occupy a wide diversity of habitats, from forest and meadows to rocky habitat. Initial estimates of phylogenetic relationships based on partial Cytochrome-*b* (Cyt-*b*) mitochondrial DNA sequences and protein electrophoretic data suggest parthenogenetic lineages result from successful directional hybridization events between sexual *Darevskia* species. Only four parent species are thought to have been involved, *D. raddei* (Boettger, 1892) and *D. mixta* (Méhely, 1909) as the maternal donors and *D. valentini* (Boettger, 1892) and *D. portschinskii* (Kessler, 1878) as the paternal donors (Fu et al., 1997; Murphy et al., 2000). The sexual *Darevskia* species that most commonly contributes as a parental for the parthenogenetic lineages is *D. raddei*, being the proposed maternal species for at least five of them: *D. unisexualis* (Darevsky, 1966) (Armenia, northeastern Turkey and southern Georgia), *D. uzzelli* (Darevsky & Danielyan, 1977) (northeastern Turkey), *D. bendimahiensis* (Schmidtler, Eiselt & Darevsky, 1994) (northeast of Lake Van), *D. sapphirina* (Schmidtler, Eiselt & Darevsky, 1994) (north of Lake Van in the vicinity of Erciş) and *D. rostombekowi* (Darevsky, 1957) (northern Armenia and western Azerbaijan) (Fu et al., 1997; Baran et al., 2012). Nevertheless, *D. raddei* itself has been suggested to be a species-complex containing the forms "*raddei*", "*nairensis*" and "*vanensis*" whose status and phylogenetic relationships are still a matter of debate (Grechko et al., 2007). As a consequence, it remains unclear if different *D. raddei* lineages may have been involved in the hybridization events that led to the parthenogenetic lineages. The form "*raddei*" is distributed throughout the south and northeast of Armenia and Nagorno-Karabakh (Arakelyan et al., 2011), Azerbaijan and the northern part of the east Azerbaijan and Ardabil provinces of Iran (Anderson, 1999). The form "*vanensis*" is found in easternmost Anatolia, east of Lake Van and the west Azerbaijan Province of Iran (Baran et al., 2012). The differences between them are based on quantitative morphological traits that are not fully diagnostic (Anderson, 1999). The third form "*nairensis*" is restricted to the northeastern part of Armenia, along the western margin of the Sevan Lake. It is noteworthy that sympatry of *D. raddei* "*nairensis*" with one of the parthenogenetic forms (*D. unisexualis*) has been described for a single locality: Lchap (Gegharkunik province), on the west margin of the Sevan Lake in Armenia (Arakelyan et al., 2011; M. Arakelyan and F. Danielyan, unpubl. com.). Examining the diversity of Cyt-*b* sequences within the *D. raddei* complex (except "*vanensis*"), MacCulloch et al. (2000) concluded that the forms "*raddei*" and "*nairensis*" were conspecific due to the paraphyletic relationships found. A fourth form, *D. raddei "chaldoranensis*" has been recently described based on scalation and coloration characters, from a single locality of northern Zagros, western Azerbaijan Province of Iran (Rastegar-Pouyani et al., 2011, 2012), falling within the putative range of the form "*vanensis*".

The region where these forms occur, the Caucasus, includes a remarkable habitat and topographical heterogeneity likely to have promoted the formation of important biological barriers, and harboured multiple glacial refugia for sedentary species, including reptiles, during the last cold period (Ahmadzadeh et al., 2013a,b; Tarkhnishvili et al., 2000, 2013). Nevertheless, evolutionary studies reveal heterogeneous biogeographic patterns for the biota in this region. While the Caucasus may have acted as a complex secondary contact zone for some species (Seddon et al., 2002), for others it appears to have acted as a barrier to expansion (Tarkhnishvili et al., 2000).

Here, we aim to infer the biogeographic patterns of parthenogenetic and bisexual rock lizards by addressing three questions: (1) Where and when did the parthenogenetic *Darevskia* species appear and could this be related to known biogeographic events? (2) How many parental lineages contributed for the parthenogenetic species under study? and (3) Have parthenogenetic species undergone identifiable periods of range expansion or contraction since their origin? We focus on the *Darevskia raddei* sensu lato sexual species and the hybrid parthenogenetic daughter lineages, *D. unisexualis, D. uzzelli and D. bendimahiensis*.

To answer the first and second questions, a phylogenetic dating approach was employed. The molecular markers were used to determine the specific maternal lineage for each of the parthenogenetic forms analysed and, specifically, whether the parthenogenetic lineages come from single or multiple hybridization events. To try to infer the location of those events ecological niche modelling was performed based on the current environment and on projections to two different paleoscenarios, the Last Interglacial (LIG – 130 to 115 kyr ago) and the Last Glacial Maximum (LGM – 22 kyr ago), taking into account the age estimates for each species. If the origin of the parthenogenetic species occurred after the LIG, then comparisons of the potential distributions during the paleoscenarios analysed (LIG and LGM) with the present distribution model would allow inference regarding where these lineages could have been during the hybridization events. Regarding the last question, tests on population expansion/contraction were performed.

Furthermore, the current distribution ranges of the sexual species and of the parthenogen *D. unisexualis* were compared to the present habitat suitability model and to the projections for the estimated paleoscenarios as inferred by ecological niche modelling. With this we intend to infer how competition may influence the distribution of both parthenogenetic species and the sexual parentals. Due to their extremely restricted distribution, insufficient to infer ecological models, the other two parthenogenetic species, *D. uzzelli* and *D. bendimahinesis*, could not be included in this analysis.

2. Material and methods

2.1. Study area and datasets

A total of 235 samples collected across the whole species ranges were used for the molecular analyses (Supplementary Table 1). *D. raddei* sensu lato individuals were selected from 90 localities covering the whole distribution range of the complex, *D. unisexualis* from 15 localities (N = 32), and *D. uzzelli* (N = 5) and *D. bendimahiensis* (N = 3) from one locality each, due to their locally restricted distribution (Fig. 1, Supplementary Table 1). Presence records for 165 individuals (see Supplementary Table 1) were used to construct the ecological niche models (ENMs). In all cases, only records confirmed by molecular data were used. Geographic coordinates of sampling localities were geo-referenced with a Global Positioning System (GPS) receptor on the WGS84 datum. The study area is a polygon which includes the global distribution of both species (*D. raddei* and *D. unisexualis*) as provided by IUCN, defined by the coordinates xMin,yMin 37.8275,34.8814:xMax,yMax 53.126,45.1208. This area was chosen in order to detect suitable habitats outside the distribution ranges of both species and to analyse the overlap between both ENMs, but taking into account their limited dispersal rate. Outgroup species used were sampled (*D. portchinskii, D. rudis* Bedriaga, 1886, and *D. valentini*) or their sequences downloaded from Genbank (*Iranolacerta*). From all individuals sampled in the field, tail tips, photographs and basic mea-

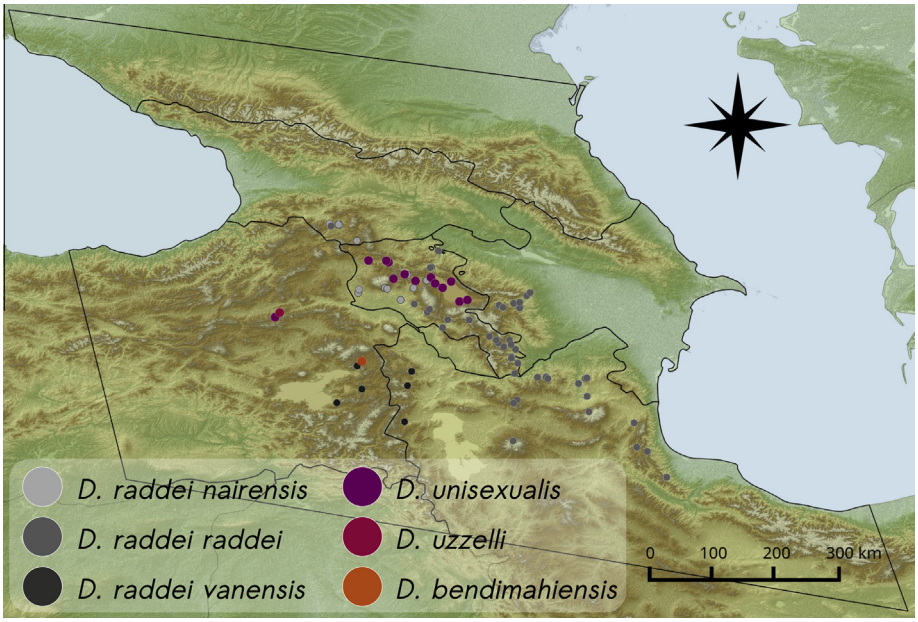

Fig. 1. Map with all individuals used in the study (for both Maxent model construction and genetic analyses) identified by species-specific colour codes. Ecotypes of sexual species *D. raddei* are in different tones of grey (light grey, "nairensis"; medium grey, "raddei"; dark grey, "vanensis"). Parthenogenetic species are represented in purple (*D. unisexualis*), dark pink (*D. uzzelli*) and orange (*D. bendimahiensis*). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

surements were also collected to associate to morphological descriptions of the species (Arakelyan et al., 2011).

2.2. Molecular data

Total genomic DNA was extracted from approximately 30 mg of each tail-tip following standard high-salt protocols (Sambrook and Russell, 2001). For phylogenetic analyses two partial mitochondrial genes; Cytochrome-*b* (Cyt-*b*) and NADH dehydrogenase-4 (ND4), and two partial nuclear genes; Melanocortin 1 receptor (MC1R) and oocyte maturation factor Mos (C-*mos*) were selected. Primers and PCR protocols are described elsewhere (Arévalo et al., 1994; Barata et al., 2012; Kocher et al., 1989; Pinho et al., 2007). Sequencing was conducted by a commercial facility (Macrogen Inc). Chromatograms were edited by eye in ChromasPro v1.7.4 (Technelysium), using ambiguity codes to represent heterozygous positions.

2.3. Phylogenetic analyses and divergence-time estimates

Sequence alignment was performed in MAFFT v6 (Katoh and Standley, 2013) using the automatic settings for the algorithm choice. For nuclear fragments, haplotypes phase was inferred with Phase version 2.1 (Stephens et al., 2001), and to reduce potential biases in downstream analyses only haplotype pairs with total posterior probabilities values above 0.6 were included in the analysis (Garrick et al., 2010) – this resulted in the exclusion of less than 1% of the sequences. Input files were prepared with SeqPHASE (Flot, 2010), which was also used to produce bi-allelic fasta files from PHASE outputs.

For the phylogenetic analyses, mtDNA fragments were concatenated but nuclear genes were analysed independently. Departing from an a-priori partitioning per coding position on each gene, PartitionFinder (Lanfear et al., 2012) was used to select the best-fit partitioning scheme and DNA substitution model(s). Under the corrected Akaike information criteria (AICc), the best partition set and models chosen and applied to the dataset are as follows: Cyt-*b*/position 1 = TVMef + I + G; ND4/position 3, Cyt-*b*/position 2 = K81uf + I + G; ND4/position 1, Cyt-*b*/position 3 = TIM + G; ND4/RNA subset, ND4/position 2 = GTR + G. Phylogenetic analyses were performed using Bayesian (MrBayes v. 3.2, Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and Maximum Likelihood (PhyML 3.0, Guindon and Gascuel, 2003) inferences (BI and ML, respectively). In ML, nodal support was estimated through 1000 bootstrap replicates (Felsenstein, 1985). In BI, all analyses started with randomly generated trees and ran for 30×10^6, with sampling at intervals of 1000 generations, producing 30,000 trees. Two independent runs were performed on each dataset. Burn-in was determined upon stabilisation of log likelihood using TRACER v1.5 (Drummond and Rambaut, 2007) and of the clades posterior probabilities with AWTY (Nylander et al., 2008). Individual mtDNA gene trees were also estimated with MrBayes, using the same strategy as with the concatenated mtDNA dataset. These gene trees were then compared to test for possible incongruences (data not shown).

The age of the most recent common ancestor (tMRCA) was estimated for all lineages of the mitochondrial DNA dataset on a "species-tree" analysis using *BEAST 2.3.1 (Bouckaert et al., 2014) with the mtDNA dataset. This approach was preferred since "species-tree" analysis can provide accurate gene-tree estimates (Drummond et al., 2012) and more realistic assessments of posterior clade supports (Drummond and Bouckaert, 2015). Even though only mtDNA markers were used the term "species-tree" analysis is used to identify the method in question. Both markers (ND4 and Cyt-*b*) were run with unlinked trees, sites and clock models so that the each marker and respective priors used would not constrain the calculation of the parameters for the other marker, such as mutation rate, tree topology or branch length. DNA substitution models for both markers were searched again with PartitionFinder (Lanfear et al., 2012), but this time unpartitioned schemes per marker were selected since mutation rates used (Pinho et al., 2007) were developed for a non-partitioned marker (ND4). The models selected for each marker were GTR + I + G (Cyt-*b*) and HKY + G (ND4). Individuals were assigned to "species" based on their mtDNA lineages. Four independent searches were run for 10^7 generations. A lognormal relaxed molecular clock was assumed, using

the mutation rate for ND4 estimated for the lacertid lizard genus *Podarcis* Wagler, 1830 (Pinho et al., 2007) and co-estimated for Cyt-*b*. Nuclear markers were not included in the tMRCA estimations given the hybrid origin of the parthenogens and respective uncertainty associated with phased haplotypes. The clock rate prior for the ucld.mean parameter for the ND4 dataset was set as a normal distribution with a mean of 0.0226 and a standard deviation of 0.0031, so that mutation rate varied between 0.0278 and 0.0174 mutation/site/million years. A uniform Yule prior was selected for the tree, with a random starting one. For the remaining parameters the default options were chosen. Convergence for all model parameters was determined in Tracer v.1.5. (Drummond and Rambaut, 2007) where high effective sample sizes (ESS) were observed for all parameters (>200 for the combined analyses). LogCombiner 2.3.0 (Bouckaert et al., 2014) was used to combine the log and tree files of the four runs, with 20% of the trees of each one discarded as burn-in, following an analysis of convergence of individual run parameters in Tracer v1.4 (Drummond and Rambaut, 2007). A maximum clade credibility (MCC) tree with mean tree heights and 95% highest probability densities (HPDs) was produced using Tree Annotator (Bouckaert et al., 2014).

2.4. Population structure

Haplotype networks were constructed for both nuclear loci (MC1R and C-*mos*) using the statistical parsimony algorithm in TCS 1.21 (Clement et al., 2000). Analyses were performed with phased nuclear data of the species in study and additional sexual species expected to have acted as, or be closely related to, the paternal species. These were included to compare alleles of the parthenogens to the sexual species from which they potentially originated.

Diversity parameters for each gene were estimated only for the sexual species *D. raddei* and the parthenogen *D. unisexualis,* since sample sizes for the remaining (parthenogenetic) species were insufficient. Estimates of haplotype diversity (Hd), nucleotide diversity (π), neutrality tests Tajima's *D* and Fu's *Fs*, as well as Harpending's raggedness index (r) were calculated, as well as the significance of Tajima's *D* and Fu's *Fs* statistics, tested by generating 1000 random samples under the null hypothesis of selective neutrality and population equilibrium, using a coalescent simulation algorithm adapted from Hudson (1991) in DNAsp. Significance of r was tested using a parametric bootstrap approach (Schneider and Excoffier, 1999).

2.5. Environmental data

Climatic variables were retrieved from the WorldClim online data (Hijmans et al., 2005). The spatial resolution for current climate variables was 30 arc-seconds (approximately 1 km^2) and for past climate variables 2.5 arc-minutes (approximately 5 km^2). From the 19 Bioclim variables, those with a correlation lower than 0.7 and considered biologically relevant for both species were selected.

Three past climate scenarios were used: one scenario for the Last Interglacial (LIG: ~120–140 kyr years BP; Otto-Bliesner et al. 2008); and two scenarios (CCSM - the Community Climate System Model, and MIROC - the Model for Interdisciplinary Research on Climate) for the Last Glacial Maximum (LGM: ~22 kyr years BP) (Hijmans et al., 2005).

2.6. Ecological niche models

The realised niches (*sensu* Hutchinson, 1957) of *D. raddei* sensu lato and *D. unisexualis* were estimated using the Maximum Entropy method implemented in Maxent 3.3.2 (Phillips et al., 2004, 2006). All forms within *D. raddei* were considered as a single group given the low phylogenetic distance between them ("*vanensis*" vs "*raddei*") or the lack of differentiation ("*nairensis*" and "*raddei*"; see Results). The ecological niche models for the present were then projected to the three past climate scenarios selected.

Maxent runs were performed with autofeatures, selecting randomly 70% (number of points) of the presence records as training data and 30% (number of points) as test data for *D. raddei* locations, and all the presence records (number of points) as training data for *D. unisexualis*, due to the limited number of records for this species. Default parameters were used in order to compare the different models.

Models were evaluated with receiver operated characteristics (ROC) plots. The area under the curve (AUC) of the ROC plot was taken as a measure of the overall fit of the Maxent model (Liu et al., 2005) (random models have an AUC equal to 0.5). AUC was selected because it is independent of prevalence (the proportion of presence in relation to the total dataset size; see VanDerWal et al. (2009)). The importance of each climate variable for explaining the species distribution was determined by: (1) jackknife analysis of the average AUC with training and test data; and (2) average percentage contribution of each environmental factor to the models. The mean realised niche model and its projections to past scenarios were reclassified in presence-absence maps using the average value of the 10 percentile training presence logistic as the threshold. This would decrease the potential error associated to the dataset. So that we defined suitable habitat to include 90% of the data used to develop the model. Cells with values higher and lower than the threshold were considered either suitable or unsuitable for the presence of the species (in the latter case species were considered to be absent from these cells). Identification of areas of probable sympatry between species was determined by overlap analysis, multiplying the distribution model of each species in the "Raster Calculator" function of QGIS.

3. Results

3.1. Phylogenetic analyses

Two mitochondrial DNA markers were analysed in this study comprising 270 concatenated sequences and 110 unique haplotypes within the concatenated dataset. In total, mtDNA markers correspond to 1753 bp (Cyt-*b*: 919 bp, 143 parsimony informative sites; ND4: 834 bp, 98 parsimony informative sites).

The mtDNA gene markers do not show any indels or stop codons when translated. The individual gene trees recovered from both mtDNA gene regions are topologically concordant with no well supported conflict and both Bayesian and Maximum Likelihood analyses result in the same overall tree topology (data not shown). A previous study found evidence for a nuclear copy of the Cyt-*b* in another species of *Darevskia* (Freitas et al., 2016). However, given that both mtDNA markers produced concordant individual tree topologies for the major lineages, and the lack of stop codons and indels in these sequences, we have no reason to consider this issue further here. The mtDNA gene genealogy shows *D. raddei* sensu lato as monophyletic with maximum support (Fig. 2), with the three parthenogens analysed being placed within the *D. raddei* lineage. According to our results, the form *"nairensis"* (Fig. 2, light grey) does not correspond to a monophyletic lineage, and haplotypes from individuals morphologically assigned to this form are shared with individuals recognised as *"raddei"* (haplotype number 5 is shared by *D. raddei raddei* from Gosh and Pzorak and *D. raddei nairensis* from Hovk, all in Armenia). In contrast, the form *"vanensis"* (Fig. 2, dark grey) does corresponded to a single lineage, which appears distinct from the rest of the *D. raddei* individuals

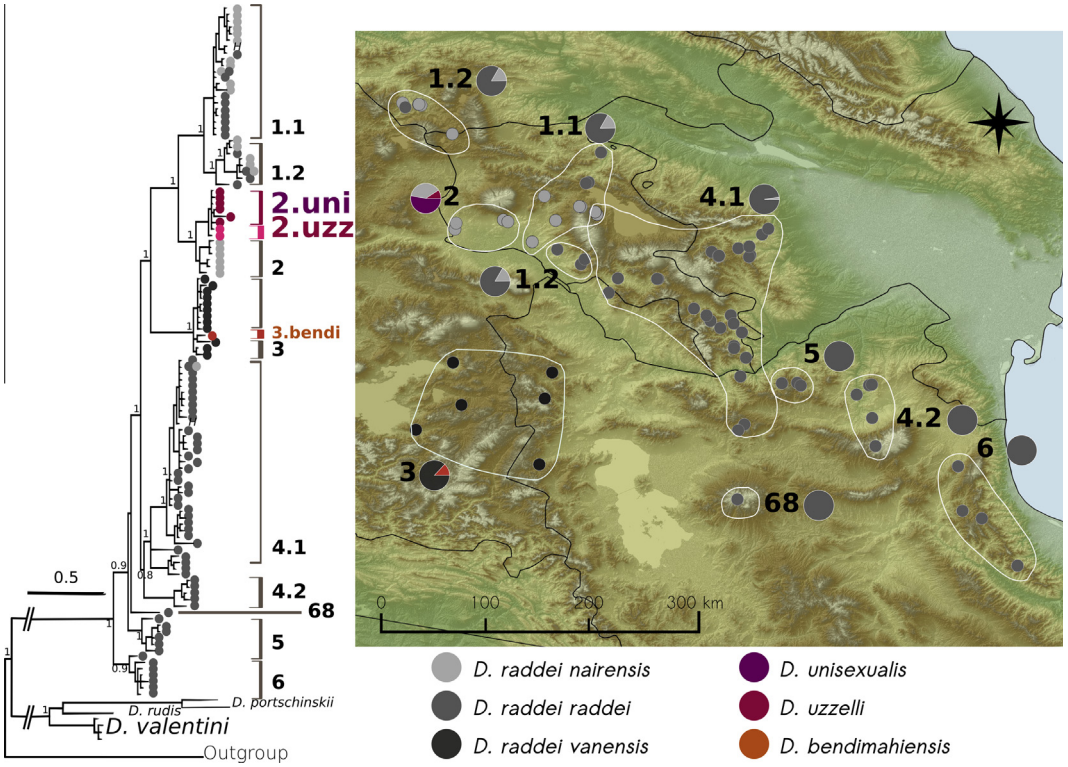

Fig. 2. 50% majority rule consensus of bayesian estimates of mtDNA (Cyt-*b* and ND4) trees for the *D. raddei* "complex", parthenogenetic species and outgroups. Tip-labels correspond to the lineages on the map (on the right) and are the same as in Fig. 3. Pie charts represent the frequency of the different taxa clustered within each mtDNA lineage. Samples of parthenogenetic individuals are not shown in the map for ease of understanding (dark grey: *D. raddei "vanensis"*, medium grey: *D. raddei "raddei"*, and light grey: *D. raddei "nairensis"*; orange: *D. bendimahiensis*, purple: *D. unisexualis*, dark pink: *D. uzzelli*). Only posterior probability values above 0.8 are presented. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

analysed ("*raddei*" *and "nairensis*") although still nested within *D. raddeii* sensu lato. Its haplotypes are shared only with the parthenogen *D. bendimahiensis.*

Both Bayesian and ML mtDNA phylogenetic analyses show a Southeast-Northwest differentiation within the *D. raddei* group (Fig. 2): the basal lineages 68 (a single haplotype), 5 and 6 contain the individuals from the southernmost part of the distribution, in the region of Ardabil and east Azerbaijan provinces of Iran (Fig. 2). Lineages 4.1 and 4.2 include individuals from South Armenia and Ardabil region in Iran and are found South and North of the lineages 5 and 6. Lineage 3 contains the individuals located around Lake Van in Turkey and lineages 1.1 and 1.2, and 2 are found Northeast of the Geghama Mountains. Even though there is a clear Southeast-Northwest differentiation, most mtDNA lineages are in contact and geographical structure is only detected in some cases, such as lineage 3 whose samples are geographically isolated and genetically differentiated from the rest.

The "species-tree" inference (Fig. 3) largely matches the Bayesian concatenated "gene-trees" except for the position of lineage 3. However, given the low posterior probability values, the position of this lineage relative to the other *D. raddei* lineages remains unclear. The estimated 95% HPD intervals of node heights show the differentiation in the *D. raddei* group dates back to around 0.7 Myr (1.1–0.5) and its split from *D. clarkorum* (the closest sexual species included in this study) dates from 3 to 1.2 Myr. The closest *D. raddei* lineage to the parthenogens *D. unisexualis* and *D. uzzelli* - lineage 2 - splits from these parthenogens 290–75 kyr ago while the differentiation within these two parthenogenetic species dated 61–2 kyrs ago. The origin of *D. bendimahiensis*, or the split between this parthenogenetic species and its closest *D. raddei* lineage, dates to 204–18 kyr.

3.2. Population structure

Analyses of nuclear DNA show all parthenogenetic individuals are heterozygous for both markers, with each allele shared with a different species. Some sexual individuals are also found to be heterozygous but their alleles are never shared between species as it is always the case with the parthenogens. To assess the maternal and paternal genomic contribution in the parthenogenetic individuals, samples of *D. portschinskii, D. valentini* and *D. rudis* individuals were incorporated to the analyses and haplotype networks were constructed (Fig. 4). Each of the alleles found was expected to group with each parental group. Thus, the allele corresponding to the maternal contribution is considered to be the allele shared with the *D. raddei* individuals, the other allele corresponding to the paternal contribution (shared or closer to *D. portschinskii, D. rudis* and *D. valentini*). The expected paternal species for the three parthenogenetic species was *D. valentini* (Darevsky, 1967), although it was still pending confirmation by genetic data. In order to include other species closely related to *D. valentini* (Fu et al., 1997) and the diversity within the putative paternal group, we have also added samples of *D. portschinskii* and *D. rudis*.

Within *D. raddei* sensu lato, networks of nuclear haplotypes show a weak geographic structure, even though MC1R (644 bp, 43 variable positions), faster evolving than C-*mos* (550 bp, 14 variable positions) (Fig. 4), shows a higher degree of diversity. However, some of the mitochondrial lineages have corresponding haplotype groups in the MC1R network: haplotypes 16, 17, 32 and 33 (of MC1R) is a group formed by samples found in the southernmost part of the species distribution, in the west Alborz region (Asalem, Hir, Khalkhal, Meskin Shahr) which corresponds to mtDNA lineage six. Haplotypes 9, 6, 20, 36, 39 correspond to individuals from South Armenia and

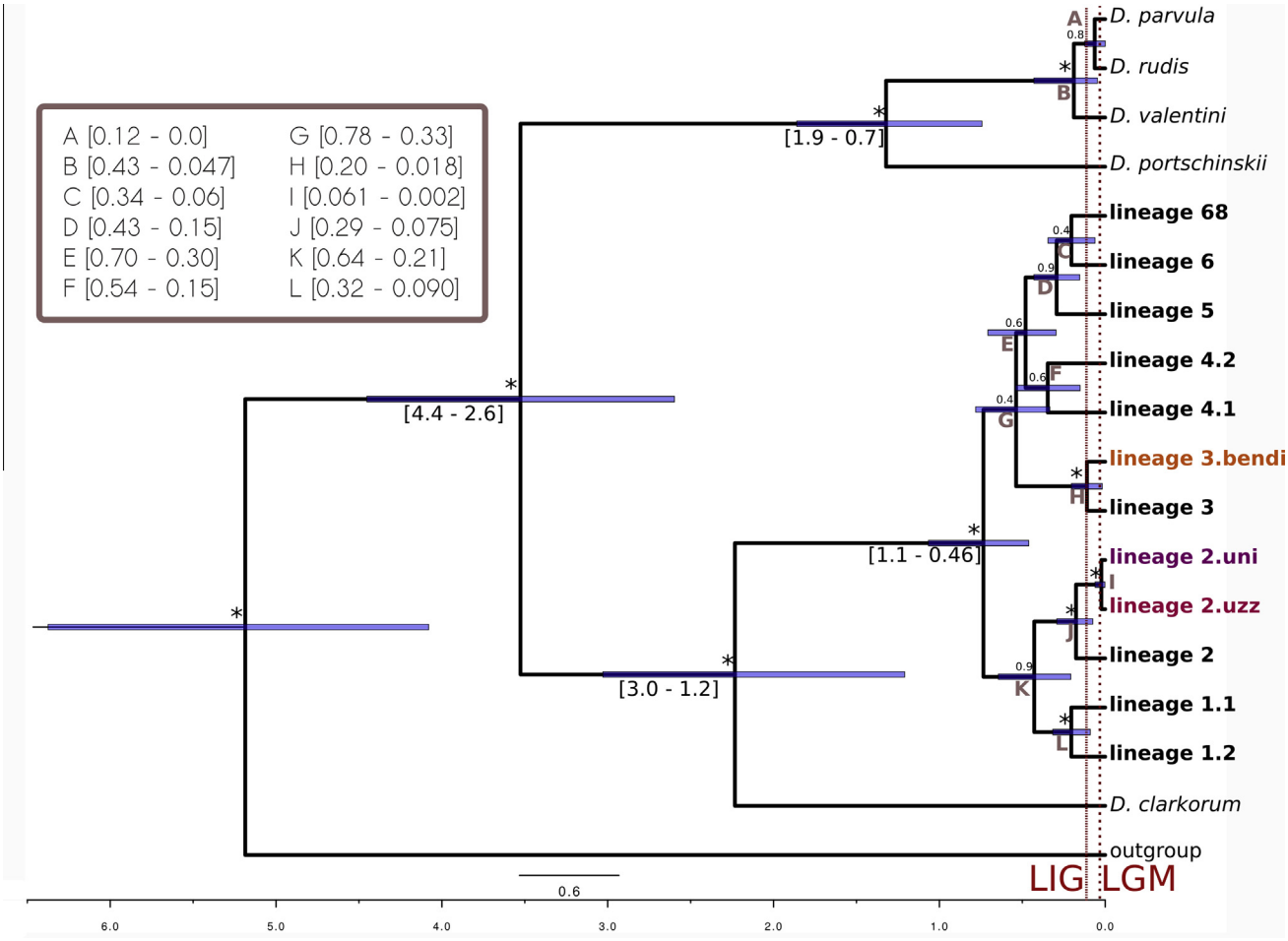

Fig. 3. Species-tree estimate (MCC) of *D. raddei* sensu lato and the parthenogenetic species *D. unisexualis* (lineage 2.uni), *D. uzzelli* (lineage 2.uzz) and *D. bendimahiensis* (lineage 3.bendi). Divergence time intervals in Myrs. Posterior probabilities are presented for each split, stars represents posterior probability of 1. Parthenogenetic species are shown in different colours, similar as in other figures (orange: *D. bendimahiensis*, purple: *D. unisexualis*, dark pink: *D. uzzelli*). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

NKR (mtDNA lineage 4.1 in Fig. 2). Haplotypes 26, 27 and 29 are only found in individuals from the Lake Van area in Turkey, and western Azerbaijan (province of Iran) and Gollodja in the adjacent Iranian Azerbaijan (near the borderwith Turkey and the Lake Van region), which define mtDNA lineage three. The remaining haplotypes correspond to individuals ascribed either to *D. raddei "nairensis"* or to *D. raddei "raddei"*, distributed North and South of Mount Aragats in central Armenia, respectively.

Regarding the maternal contribution (*D. raddei* sensu lato), all of the parthenogenetic species present two haplotypes only, 1 and 27. Specimens identified as *D. unisexualis* and *D. uzzelli* share haplotype 1 with individuals from Mount Aragats, specifically Amberd Castle and Lchaschen. The only homozygous individuals for this haplotype are *D. raddei nairensis* found in Amberd Castle, where the frequency of this haplotype is likely higher. Haplotype 27 is shared by *D. bendimahiensis* and individuals identified as *D. raddei "vanensis"*, located around Lake Van in Turkey and Iranian Azerbaijan. Haplotypes 5, 38, 25, 22, 23 and 24 corresponded to the putative paternal species. Alleles 4, 21 and 25 are found in the parthenogenetic species, and were therefore inherited from the paternal species that contributed to the original hybridization event. Regarding the paternal contribution, *D. unisexualis* presents two different haplotypes, and *D. bendimahiensis* and *D. uzzelli* only one each. While *D. unisexualis* and *D. uzzelli* share the same maternal haplotype, for the paternal contribution *D. unisexualis* shares its most common haplotype with *D. bendimahiensis*, while *D. uzzelli* shares its haplotype with individuals of *D. valentini*.

The C-*mos* haplotype network (Fig. 4, bottom) shows little variation and most *D. raddei* sensu lato individuals share haplotype 1 or one derived from it by one or two mutation steps. There is no geographic structure reflected in this network. As with MC1R, C-*mos* sequences of the putative paternal species (*D. valentini, D. rudis* and *D. portschinskii*) were used to allocate the paternal contribution and to differentiate the maternal from the paternal alleles. Regarding the maternal contribution, all three parthenogenetic lineages analysed share the same allele. This is the most common allele, found also in all the individuals ascribed to *D. raddei "nairensis"* (and all homozygous), but also in those identified as *D. raddei "vanensis"* and most of the *D. raddei "raddei"*. Regarding the paternal contribution, the three parthenogens share the same haplotype, which is not found in any of the putative paternal species used. This network shows a slight star-like shape. Neutrality tests were calculated for each species (Table 1). Tajima's *D* shows significant negative values for *D. raddei* (ND4) and *D. unisexualis* (Cyt-*b* and ND4). Fu's *Fs* also shows negative values for all markers analysed in both species, even though none is significant. Both tests R_2 and raggedness r detected significant low positive values for all markers.

3.3. Ecological niche models

Maxent models were generated only for species with a sufficient number of geographic records, *D. raddei* sensu lato and *D. unisexualis*. Given their restricted distribution range, *D. uzzelli* and *D. bendimahiensis* could not be included in this analysis.

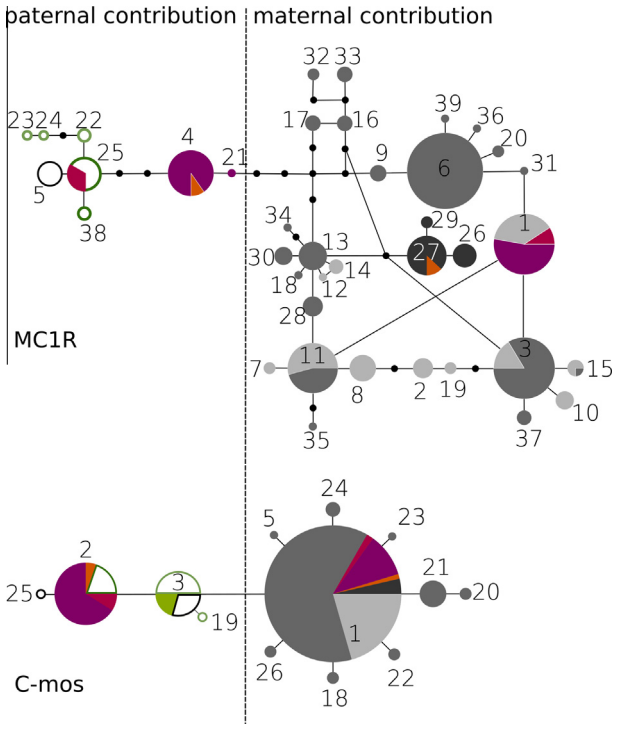

Fig. 4. Statistical parsimony networks for MC1R and C-*mos* in *D. raddei* group, parthenogenic descendant species and some individuals of the putative paternal species of those parthenogens. Small black circles represent missing or unsampled haplotypes. Grey colours correspond to the *D. raddei* sensu lato (dark grey: *D. raddei* "*vanensis*", medium grey: *D. raddei* "*raddei*", and light grey: *D. raddei* "*nairensis*"; orange: *D. bendimahiensis*, purple: *D. unisexualis*, dark pink: *D. uzzelli*; white with dark green outline: *D. valentini*; white with light green outline: *D. rudis*; green: *D. parvula*; white with black outline: *D. portschinskii*). Different parental contributions were identified with the position on the network of the parental species: *D. raddei* sensu lato as the maternal genomic contribution and *D. valentini*/*D. portschinskii* group as the paternal contribution. Circles correspond to haplotypes, numbered as in Supplementary Table 1, with size proportional to their frequency. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Both Maxent ensemble models have mean AUC values higher than 0.9, for training data (*D. raddei*: 0.9131; *D. unisexualis*: 0.9792) and close to 0.9 for test data (*D. raddei*: 0.8714; *D. unisexualis*: 0.9734): thus, training AUC and test AUC are within the same value range meaning the model is dependent on the record data but not on which subset of the record data is used. The variables that more strongly contribute to the model of *D. raddei* are BIO18, BIO17, BIO4 and BIO112 (Precipitation of the Warmest Quarter, Precipitation of the Driest Quarter, Temperature Seasonality (standard deviation ∗ 100) and Mean Temperature of the Coldest Quarter, respectively) and mostly revolve around the availability of water in the warmest (and driest) months of the year. The model of *D. unisexualis* is more strongly affected by BIO9 and BIO1 (Annual Mean Temperature and Mean Temperature of the Driest Quarter) and similarly to *D. raddei*'s model, BIO17 and BIO18 equally affected by similar variables except for the BIO2 and BIO4 (Supplementary Fig. 1). These patterns are concordant with the jack-knife analysis of AUC and gain values of training and test data, for models calculated with only one variable and models calculated without that variable (Supplementary Fig. 1).

The present area suitable for *D. raddei* (Fig. 5) mostly overlaps with its current distribution range, although some suitable unoccupied areas (Arakelyan et al., 2011) are identified as suitable habitat, especially towards the west (Turkey) and northeast (Georgia-Azerbaijan). *D. raddei vanensis* individuals (located next to Lake Van in Turkey) fall outside the suitable habitat for *D. raddei*. When projected to the LGM most suitable habitat for *D. raddei* is shifted to the east of its current distribution, does not include mountain tops, and tends to be restricted to valleys and plains. Interestingly, no suitable habitat was found when projecting the distribution of *D. raddei* to the LIG.

The *D. unisexualis* model (Fig. 5) occupies a much more reduced area, concentrating within Armenia. *D. unisexualis* individuals found in Turkey (Horasan) do not fall inside the area predicted by the model. When projected to the LGM, both scenarios (CCSM and MIROC) produced slightly different results in terms of predicted suitable area, even though they both tend to find more suitable habitat to the east, as with the projection for *D. raddei*. No suitable habitat was found when projecting to the LIG. In all cases, when comparing the models of distribution for both species, the potential habitat of *D. unisexualis* falls within that of *D. raddei*.

4. Discussion

Parthenogenesis is a rare reproductive mode that, despite being found in most animal groups, is observed in less than 0.5% of known species (Vrijenhoek, 1989). Given the low number of species where it is observed, the switch from sexual to parthenogenetic reproduction is expected to happen only rarely. Also, the twiggy distribution of parthenogenetic species in the tree of life suggests independent sexual to asexual events (Butlin, 2002), with most appearing to be recent species and with few deep branches - a measure of longevity - of the parthenogenetic forms.

Our results show multiple origins of parthenogenetic species resulting from recurrent hybridization events in a very short time interval. Parthenogenetic species are expected to be short lived, with severe evolutionary constraints and the change to parthenogenesis is expected to happen rarely (Vrijenhoek, 1989). However, parthenogenetic species in *Darevskia* evolved multiple times in a reticulate pattern and different sexual lineages participated in the hybridization events that led to their origin. This had already

Table 1
Summary statistics, tests of neutrality and growth for the sexual species *D. raddei* sensu lato and the parthenogen *D. unisexualis*.

Species	Marker	n	Sites	π	Tajima's D	F_S	H_d	θ W	Raggedness *r*	R_2
D. raddei	Cyt-*b*	169	921	29.27	−0.12119	−1.1866	0.96758	29.867	0.00595*	0.08399*
	ND4	169	839	20.47941	−0.10468*	−1.05767	0.95241	20.304	0.00984*	0.8582*
	MC1R	328	695	2.79	−0.049	−4.575	0.819	2.86997	0.054*	0.078*
	C-*mos*	248	552	0.00045	−0.04419	−0.21047	0.20499	0.248	0.49367*	0.09042*
D. unisexualis	Cyt-*b*	30	921	0.14073	−0.013*	0.10483	0.11468	0.13151	0.41563*	0.15*
	ND4	30	841	0.71221	−0.02719*	0.057	0.427	0.74666	0.298*	0.139*
	MC1R	56	695	3.765	−0.06668	−0.13597	0.7857	3.77	0.081	0.105*
	C-*mos*	54	552	0.983	0.024	0.01565	0.496	1.01	0.25*	0.11*

m, Number of sequences, Sites, number of sites analysed per sequence, π, nucleotide diversity, F_s, Fu's (1997) F_s, H_d, Haplotype diversity, R_2, Ramos-Onsins and Rozas' (2002) R_2.

* Significant at $P < 0.05$.

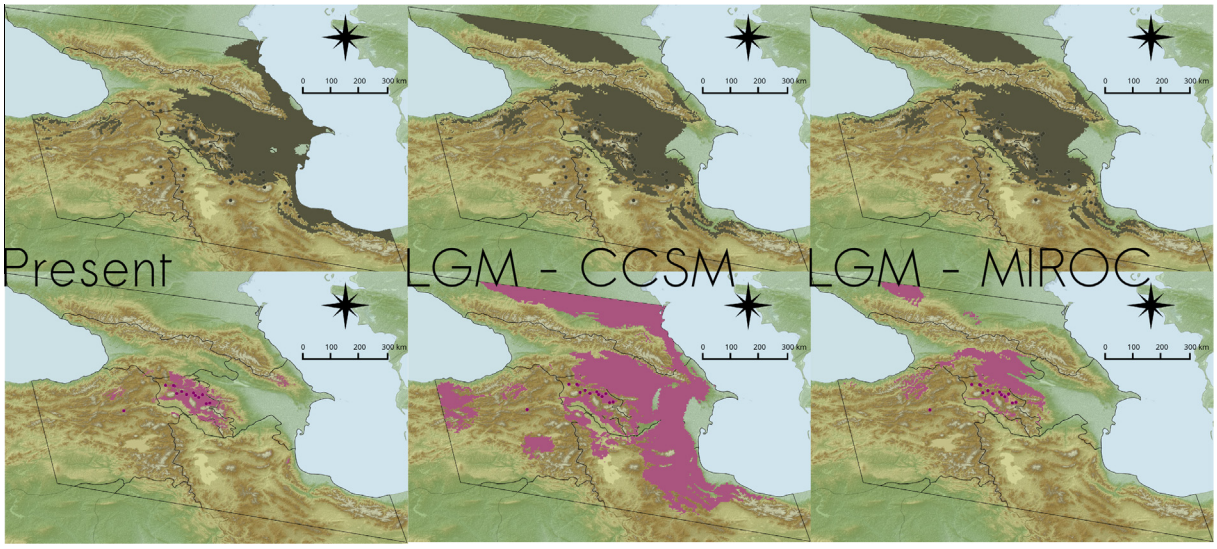

Fig. 5. Ecological niche models of the present distribution for *D. raddei* (grey) and *D. unisexualis* (pink) and projections to the past (Last Glacial Maximum, LGM). Projections to the past were performed using two scenarios, MIROC and CCSM. Details of the individuals used are in Supplementary Table 1. Points of the individuals used are in the maps in grey (*D. raddei*) and pink (*D. unisexualis*). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

been suggested from the limited evidence based on Cyt-*b* and proteins (Murphy et al., 2000) but is now placed in a robust spatiotemporal context by our multilocus analyses for those parthenogens that have *D. raddei* as the maternal species.

4.1. Parthenogenetic species origin

All parthenogenetic species analysed here are young in age (Fig. 3). Despite this recent origin, *D. unisexualis* is distributed across a considerable range (Fig. 1) and is rarely found in sympatry with its maternal species, *D. raddei* (Arakelyan et al., 2011). This is even more surprising considering that the suitable area of *D. unisexualis* predicted by the ecological niche modelling widely overlaps with that of *D. raddei* sensu lato. This niche overlap (Fig. 5), together with the wide distribution of *D. unisexualis* despite its recent origin (Figs. 1 and 2) and the obtained signals of population expansion (Table 1), suggest this parthenogen may even outcompete its maternal species within its range. Evidence that parthenogenetic *Darevskia* can outcompete their sexual parental species has already been shown for the parthenogenetic *D. dahli* (Tarkhnishvili et al., 2000).

In this study, the mtDNA was used to analyse the maternal ancestry of the parthenogenetic species and nuclear markers to assess their maternal and paternal contributions. For both nuclear markers, as expected, all parthenogens presented two different alleles, one representing the maternal ancestry (shared with *D. raddei*) and the other representing the paternal contribution (shared or closer to *D. portschinskii, D. rudis* and *D. valentini*). The parthenogens analysed here are allocated in two different lineages in the mtDNA tree. Thus, two different *D. raddei* lineages were apparently involved in the hybridization events that led to the origin of these parthenogens. *D. unisexualis* and *D. uzzelli* belong to the same mtDNA clade as individuals identified as *D. raddei "nairensis"* (Fig. 2: mtDNA lineage 2), and it is likely that this is the maternal lineage for both *D. unisexualis* and *D. uzzelli*. On the other hand, *D. bendimahiensis* shares the same mtDNA haplotype with individuals identified as *D. raddei "vanensis"* found in Turkey east of Lake Van and adjacent Iran (Fig. 2: mtDNA lineage 3), and therefore this lineage is the most likely one to have contributed in situ as the maternal parental for this parthenogen.

Considering the maternal ancestry, two contributing maternal lineages for the three parthenogenetic species analysed are confirmed with the nuclear markers. *D. unisexualis* and *D. uzzelli* share the same MC1R haplotype with individuals from Mount Aragats. This haplotype was only found in homozygosity in individuals from Amberd Castle, and it is probable that this population reflects the original genetic maternal ancestry of both parthenogenetic species (*D. unisexualis* and *D. uzzelli*). The inferred maternal ancestry of *D. bendimahiensis* with nuclear markers is concordant with the mtDNA phylogenetic tree.

D valentini and *D. portschinskii* were used to allocate the paternal ancestry in the analysis of the nuclear markers. In the MC1R network, *D. unisexualis* shares its most common haplotype with *D. bendimahiensis*, while *D. uzzelli* shares its haplotype with individuals of *D. valentini*. This contrasts with the maternal ancestry where *D. unisexualis* and *D. uzzelli* share the same haplotype, and *D. bendimahiensis* presents a different maternal allele. Therefore, even though the maternal lineage was the same, two different paternal alleles are identified and, hence, at least two different hybridization events were responsible for the (independent) origin of *D. unisexualis* and *D. uzzelli*. It is noteworthy that contrary to what had been previously reported by Fu et al. (2000a), we did not find evidence of reciprocal hybridization in *D. uzzelli*. In their work, Fu and collaborators suggested the initial hybridization leading to the origin of *D. uzzelli* was most likely reciprocal, since they found mtDNA of both parental species in these parthenogens. However, all individuals analysed in this study showed the same combination of haplotypes both for mtDNA and for nuclear markers, so it is unlikely that a reciprocal hybridization is at the origin of *D. uzzelli*.

Parthenogenetic reproduction can be performed via two ways: apomictic parthenogenesis or automictic parthenogenesis (Simon et al., 2003). While in the first the meiosis is suppressed and clonal offspring are produced under a mitosis-like cell division, the second retains meiosis (and recombination) and ploidy is restored by the duplication or fusion of the maternal gametes (Simon et al., 2003). Since all parthenogens analysed are heterozygous for the nuclear markers and considering the high number of individuals tested, apomictic parthenogenesis is here favoured. However, in some cases of automixis, the chromosomes are replicated prior to the normal meiosis, so diploidy and heterozygosity are

restored in the egg (Simon et al., 2003). In such cases heterozygosity will only be lost in some parts of the genome and after some time. Thus, their consistent heterozygosity can also be explained by their recent origin, and it would be interesting to perform a genome-wide analysis to clarify this question.

According to the placement of *D. unisexualis* and *D. uzzelli* within the phylogeny of the *D. raddei* complex, we estimate that these parthenogens split from the closest *D. raddei* lineage around 170 kyr (291–75 kyr), very close to or even during the LIG (130–115 kyr). Very likely, mild climate conditions may have facilitated population expansions of parental species increasing the probability of secondary contacts and opening the opportunity for the hybridization between the parental species. For *D. bendimahiensis*, the split with its closest *D. raddei* lineage (lineage 3) should have happened between 204 and 78 k yrs ago. This time interval practically overlaps with the split between *D. unisexualis* + *D. uzzelli* with *D. raddei* lineage 2. This could suggest the hybridization mediating the origin of *D. bendimahiensis* was concurrent with the hybridization event which led to the *D. unisexualis* + *D. uzzelli* lineage. The split between *D. uzzelli* and *D. unisexualis*, on the other hand, appeared to have happened later while the LGM was taking place. Given they share the same mitochondrial lineage and maternal alleles and differ only in the paternal allele it is not clear whether *D. uzzelli* and *D. unisexualis* originated from two different hybridization events between *D. raddei* and *D. valentini*, or if one was first originated and then backcrossed with a *D. valentini* male giving origin to the other. Since only MC1R could differentiate different paternal lineages, nuclear markers across the genome need to be analysed in order to understand the complex reticulate evolution history of these parthenogens and the relationship between them.

4.2. Phylogenetic relationships and historical range shifts

Even though the maternal contributions for the parthenogenetic species studied here were already proposed (Fu et al., 2000b), phylogenetic relationships between the parental species were obscure. Here, a phylogeographic analysis of *D. raddei* with mtDNA and nuclear markers is performed, and the intraspecific diversity compared to the possible biogeographic barriers, either current or past, within the range of this species complex. Currently, *D. raddei* sensu lato is distributed along the mountain ranges in the Central Caucasus. Given the interconnectivity of these mountain ranges and the prevalence of these species in mountain habitats, mountains are not expected to represent current barriers to dispersal, but instead act as bridges facilitating expansion. However, arid lowlands and possibly deep river beds may act as geographic barriers to dispersal for these species.

Under the current climatic conditions, no obvious strong barriers to dispersal are found within the occupied range with the possible exception of the Aras River. The Aras valley, the political border between Armenia, Azerbaijan, Iran and Turkey, has a temperate arid mountain climate and is likely to be a current barrier to dispersal between lineages 1, 2 and 4 (in Armenia) and 3 (Turkey). This barrier may have caused the lineage formed by the individuals morphologically identified as *D. raddei "vanensis"* to be geographically isolated and to have evolved in allopatry. This group is monophyletic in the mtDNA tree and also harbours a distinct group of MC1R haplotypes. This indicates at least a certain degree of isolation, and provides some support for the subspecies *D. raddei vanensis*. In contrast, *D. raddei "raddei"* and *D. raddei "nairensis"* are found to be paraphyletic. Not only do they form part of the same mtDNA lineages but they also share haplotypes (both nuclear and mitochondrial). Therefore these two taxa lack phylogenetic support. Given the divergence time estimates (Fig. 3), *D. raddei* divergence started no earlier than 1.5 Myrs ago [1.53–0.0116]. Hence, the semi-isolated pattern found for the mtDNA lineages likely originated during the Pleistocenic ice-ages.

To estimate if *D. raddei* and *D. unisexualis* show deviations from neutrality and signals of population expansion, diversity parameters were calculated. Both species showed significant R_2 for all markers while Tajima's *D* was significantly negative only for ND4 (*D. raddei and D. unisexualis*) and Cyt-*b (D. unisexualis*). Negative values of Tajima's *D* (and Fu's *Fs*) and small positive values of R_2 are indicative of population growth (Aris-Brosou and Excoffier, 1996; Tajima, 1989). While Tajima's *D* uses information on mutation frequency, Fu's *Fs* test relies on haplotype distribution and has been shown under simulation to be the more powerful when analysing small populations (Ramos-Onsins and Rozas, 2002). Given the high number of samples for each "population", or in this case, species, this could explain why this test did not detect significant departures from neutrality while Tajima's *D* did.

Considering the recent origin of the parthenogenetic species analysed here (*D. unisexualis*), a recent expansion of this species is to be expected. Currently, *D. unisexualis* has a large distribution area resulting from expansion since its origin. The mtDNA lineages with short branches and several closely related mtDNA haplotypes of *D. raddei* sensu lato are indicative of potentially recent expansions which could match the deviations from neutrality found.

4.3. Trends from the past to the present

In the phylogenetic analysis of *D. raddei* sensu lato, we found very little geographical structure at both nuclear markers and sympatry of the mtDNA lineages. This suggests cyclical contact-isolation events, concordant with the complex biogeography of the Caucasus. During the Pleistocenic Glacial Periods, contrary to the current situation, the mountain ranges, (i.e. Geghama Mountains, a volcanic mountain range west to Lake Sevan spanning North to South and attaining 3567 m), may have acted as a barrier between the lineage from Northern Armenia and Georgia, and all the others. The mtDNA lineage 1 reaches this topographic barrier and is found in Northwest Geghama, while lineages 4, 5, 68 and 6 are found Southeast of this Mountain. A similar picture emerges for lineages 1 and 2. Mount Aragats, which currently represents a suitable habitat for these species, did not do so during the LGM. Therefore, lineage 2 may have been trapped on the western side of Aragats, and lineage 1 on the eastern side during this period.

After the LGM temperatures started to increase and the mountain environments became again suitable for these lizards, with lineages that had been previously separated able to come into contact, as currently is the case for lineage 1 and lineage 4. This pattern of isolation can be observed in the projections of the *D. raddei* models for the LGM. Here, both scenarios (MIROC and CCSM) show there was a decrease of suitable habitat around the mountain tops (Aragats and Geghama) and a general increase of potential distribution area and a geographical shift of the suitable habitat to the Azerbaijan lowlands, when compared with the present distribution model (Fig. 5).

The cyclical ice ages and subsequent expansion-contraction of organisms in a habitat relatively small but with heterogeneous topography must have allowed for the secondary contact of sexual *Darevskia* lineages in incomplete stages of reproductive isolation (Vrijenhoek, 1989). This likely allowed repeated hybridization events in separate geographical areas that originated hybrids that could not cross-back with the parental groups (or species) but instead were able to reproduce parthenogenetically.

Since its origin, there was a decrease of the potential habitat of *D. unisexualis* from the LGM to the present day, according to both scenarios (Fig. 5). However, considering that this parthenogen is likely expanding, and hence, not in equilibrium with the environ-

ment, the predicted model and its consequent projections will be probably underestimations (Wiens et al., 2009).

Given the present ecological model estimated in this study for *D. raddei* and *D. unisexualis*, one may conclude that there was no appropriate habitat available for either of them across the whole region in the LIG projections. Since the origin of *D. unisexualis* was estimated to have happened after the LIG, this species simply would not have been present. On the other side, and given its present distribution, the lack of suitable habitat for *D. raddei* during the LIG may suggest this species may have suffered a recent niche shift or, alternatively, that the scale of the model was inappropriate to detect suitable habitat during this period.

The models for the present cover a much larger area than the known distribution for both *D. raddei* and *D. unisexualis*. Ecological niche models are estimated based on a dataset of presence points collected and have an error rate associated with them. Additionally, given the limited dispersal abilities of these species, their absence from the potential habitat could be due to a separation by unsuitable habitat or an incomplete expansion process, or even because of competitive exclusion by other *Darevskia* species with similar ecological niches.

5. Conclusion

Given the scarcity and distribution of parthenogenetic species in the tree of life, the switch from sexual to parthenogenetic reproduction is expected to arise rarely and independently (Butlin, 2002). Most parthenogenetic forms appear to have originated recently, as shown by the lack of parthenogenetic deep phylogenetic branches.

Most sexual-parthenogenetic complexes show a polyphyletic origin of parthenogenetic lineages (Crease et al., 1989; Grismer et al., 2014; Simon et al., 2003), where parthenogenesis has evolved more than once. Our results clearly support a polyphyletic origin of parthenogenesis in *Darevskia* lizards as well, dated back to the Pleistocene, with different parental lineages contributing to the hybridization events occurring several times in different geographical regions. The origin of the polyphyletic parthenogenetic *Darevskia* has to be interpreted as resulting from repeated secondary contacts between groups that did not developed complete reproductive isolation. The distribution of the different *Darevskia* groups (or species) likely underwent repeated contraction-expansion events in response to the Pleistocenic climate oscillations, colder periods interspersed with warmer interglacials, promoting secondary contacts. Thus, some lineages were divergent enough to produce hybrids with disrupted meiosis, yet not so divergent as to compromise hybrid viability and fertility (Vrijenhoek, 1989).

We also show that even though sexual species and parthenogens overlap in their ecological niche, *D. unisexualis* is not found in sympatry with *D. raddei*. Since *D. unisexualis* derived from *D. raddei* and is therefore younger, this suggests that the first is outcompeting the second, as has been shown for other parthenogenetic *Darevskia* (Tarkhnishvili et al., 2010). Parthenogenetic species have some advantages over sexual species; they avoid the two-fold cost of males (Maynard Smith, 1978), having twice the reproductive output if other factors are excluded, they are not affected by the associated costs of sex as male-male competition, search and choice of mates (Galoyan, 2013) and in some cases the mechanics of meiosis (Lehtonen et al., 2012). In the short term, this may provide an advantage when in sympatry with sexual species (Burke et al., 2015; Tarkhnishvili et al., 2000). On the other hand, sexual reproduction is known as a driver of evolution and speciation. As such, parthenogenetic species, which lack the recombination benefits of sexual reproduction, are expected to be at disadvantage when in competition with sexual species in changing environments. The different stages of parthenogenetic species can help to understand the effect of asexuality (or the absence of sex) on the genome. Given the different ages and the polyphyletic and hybrid origin of their parthenogenetic species, *Darevskia* lizards provide a promising model for the study of the evolution of asexuality and why sexual reproduction is so widespread in the tree of life.

Acknowledgements

S. Drovetski, F. Jorge and A. Vardanyan assisted with fieldwork. We are especially grateful to two anonymous reviewers for their constructive comments on the manuscript. This research was partially funded by FCOMP-01-0124-FEDER-007062FCT – Portugal through the project PTDC/BIA-BEC/101256/2008, by the project "Biodiversity, Ecology and Global Change" co-financed by North Portugal Regional Operational Program 2007/2013 (ON.2 – O Novo Norte), under the National Strategic Reference Framework (NSRF), through the European Regional Development Fund (ERDF) and by the project "Preserving Armenian biodiversity: Joint Portuguese – Armenian program for training in modern conservation biology." of Gulbenkian Foundation (Portugal). Field work in Turkey was funded by the project supported by Dokuz Eylül University, Turkey (Project No. 2009.KB.FEN.003). NS was supported by a research contract (IF2013) from FCT.

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.2016.05.035.

References

Ahmadzadeh, F., Flecks, M., Carretero, M.A., Böhme, W., Ilgaz, C., Engler, J.O., Harris, J.D., Üzüm, N., Rödder, D., 2013a. Rapid lizard radiation lacking niche conservatism: ecological diversification within a complex landscape. J. Biogeogr. 40, 1807–1818.

Ahmadzadeh, F., Flecks, M., Rödder, D., Böhme, W., Ilgaz, Ç., Harris, D.J., Engler, J.O., Üzüm, N., Carretero, M.A., 2013b. Multiple dispersal out of Anatolia: biogeography and evolution of oriental green lizards. Biol. J. Linn. Soc. Lond. 110, 398–408.

Anderson, S.C., 1999. The Lizards of Iran. Society for the Study of Amphibians and Reptiles), Ithaca, NY.

Arakelyan, M., Danielyan, F.D., Corti, C., Sindaco, R., Levinton, A.E., 2011. Herpetofauna of Armenia and Nagorno Karabakh. Society for the Study of Amphibians and Reptiles, San Francisco.

Arévalo, E., Davis, S.K., Sites, J.W., 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (phrynosomatidae) in Central Mexico. Syst. Biol. 43, 387–418.

Aris-Brosou, S., Excoffier, L., 1996. The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism. Mol. Biol. Evol. 13, 494–504.

Arnold, E.N., Arribas, Ó., Carranza, S., 2007. Systematics of the Palaearctic and Oriental lizard tribe Lacertini (Squamata:Lacertidae:Lacertinae), with descriptions of eight new genera. Zootaxa 1474, 1–86.

Avise, J.C., 2008. Clonality: The Genetics, Ecology, and Evolution of Sexual Abstinence in Vertebrate Animals. Oxford University Press, Oxford, New York.

Barata, M., Carranza, S., Harris, D.J., 2012. Extreme genetic diversity in the lizard *Atlantolacerta andreanskyi* (Werner, 1929): a montane cryptic species complex. BMC Evol. Biol. 12, 167.

Bell, G., 1982. The masterpiece of nature: The evolution and genetics of sexuality (Berkeley).

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. BEAST 2: a software platform for bayesian evolutionary analysis. PLoS Comput. Biol. 10, e1003537.

Burke, N.W., Crean, A.J., Bonduriansky, R., 2015. The role of sexual conflict in the evolution of facultative parthenogenesis: a study on the spiny leaf stick insect. Anim. Behav. 101, 117–127.

Butlin, R., 2002. The costs and benefits of sex: new insights from old asexual lineages. Nat. Rev. Genet. 3, 311–317.

Camargo, A., Sinervo, B., Sites, J.W., 2010. Lizards as model organisms for linking phylogeographic and speciation studies. Mol. Ecol. 19, 3250–3270.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1659.
Crease, T.J., Stanton, D.J., Hebert, P.D.N., 1989. Polyphyletic origins of asexuality in *Daphnia pulex*. II. Mitochondrial-DNA variation. Evolution 43, 1016–1026.
Darevsky, I.S., 1967. Rock Lizards of the Caucasus: Systematics, Ecology and Phylogenesis of the Polymorphic Groups of Caucasian Rock Lizards of the Subgenus *Archaeolacerta*. Nauka, Leningrad.
Drummond, A.J., Bouckaert, R.R., 2015. Bayesian Evolutionary Analysis with BEAST. Cambridge University Press.
Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.
Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29 (8), 1969–1973.
Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783.
Flot, J.-F., 2010. SeqPhase: a web tool for interconverting PHASE input/output files and FASTA sequence alignments. Mol. Ecol. Resources 10, 162–166.
Freitas, S., Vavakou, A., Arakelyan, M., Drovetski, S.V., Crnobrnja-isailović, J., Kidov, A.A., Cogălniceanu, D., Corti, C., Lymberakis, P., Harris, D.J., et al., 2016. Cryptic diversity and unexpected evolutionary patterns in the meadow lizard, *Darevskia praticola* (Eversmann, 1834). Syst. Biodivers. 14, 184–197.
Fu, J., Murphy, R.W., Darevsky, I.S., 1997. Toward the phylogeny of Caucasian rock lizards: implications from mitochondrial DNA gene sequences (Reptilia: Lacertidae). Zool. J. Linnean Soc. 120, 463–477.
Fu, J., MacCulloch, R.D., Murphy, R.W., Darevsky, I.S., Tuniyev, B.S., 2000a. Allozyme variation patterns and multiple hybridization origins: clonal variation among four sibling parthenogenetic caucasian rock lizards. Genetica 108, 107–112.
Fu, J., Murphy, R.W., Darevsky, I.S., McEachran, J.D., 2000b. Divergence of the cytochrome b gene in the *Lacerta raddei* complex and its parthenogenetic daughter species: evidence for recent multiple origins. Copeia 2000, 432–440.
Garrick, R.C., Sunnucks, P., Dyer, R.J., 2010. Nuclear gene phylogeography using PHASE: dealing with unresolved genotypes, lost alleles, and systematic bias in parameter estimation. BMC Evol. Biol. 10, 118.
Gilabert, A., Simon, J.-C., Dedryver, C.-A., Plantegenest, M., 2014. Do ecological niches differ between sexual and asexual lineages of an aphid species? Evol. Ecol. 28, 1095–1104.
Grechko, V.V., Bannikova, A.A., Kosushkin, S.A., Ryabinina, N.L., Milto, K.D., Darevsky, I.S., Kramerov, D.A., 2007. Molecular genetic diversification of the lizard complex *Darevskia raddei* (Sauria: Lacertidae): early stages of speciation. Mol. Biol. 41, 764–775.
Grismer, J.L., Bauer, A.M., Grismer, L.L., Thirakhupt, K., Aowphol, A., Oaks, J.R., Wood, P.L., Onn, C.K., Thy, N., Cota, M., et al., 2014. Multiple origins of parthenogenesis, and a revised species phylogeny for the Southeast Asian butterfly lizards, *Leiolepis*. Biol. J. Linn. Soc. Lond., n/a–n/a
Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.
Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25, 1965–1978.
Hudson, R.R., 1991. Gene genealogies and the coalescent process. Oxford Surveys Evol. Biol. 7, 1–44.
Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
Hutchinson, G.E., 1957. Cold Spring Harbor Symposium on Quantitative Biology. Conclud. Remarks 22, 415–427.
Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment Software Version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.
Kearney, M.R., 2003. Why is sex so unpopular in the Australian desert? Trends Ecol. Evol. 18, 605–607.
Kearney, M., Fujita, M.K., Ridenour, J., 2009. Lost sex in the reptiles: constraints and correlations. In: Schön, I., Martens, K., Dijk, P. (Eds.), Lost Sex. Springer, Netherlands, pp. 447–474.
Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. PNAS 86, 6196–6200.
Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29, 1695–1701.
Liu, C., Berry, P.M., Dawson, T.P., Pearson, R.G., 2005. Selecting thresholds of occurrence in the prediction of species distributions. Ecography 28, 385–393.
MacCulloch, R.D., Fu, J., Darevsky, I.S., Murphy, R.W., 2000. Genetic evidence for species status of some Caucasian rock lizards in the *Darevskia saxicola* group. Amphibia-Reptilia 21, 169–176.
Maynard Smith, J., 1978. The Evolution of Sex. Cambridge University Press, New York.
Murphy, R.W., Fu, J., MacCulloch, R.D., Darevsky, I.S., Kupriyanova, L.A., 2000. A fine line between sex and unisexuality: the phylogenetic constraints on parthenogenesis in lacertid lizards. Zool. J. Linnean Soc. 130, 527–549.
Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L., Swofford, D.L., 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24, 581–583.
Otto, S.P., Nuismer, S.L., 2004. Species interactions and the evolution of sex. Science 304, 1018–1020.
Phillips, S.J., Dudík, M., Schapire, R.E., 2004. A maximum entropy approach to species distribution modeling. In: Proceedings of the Twenty-First International Conference on Machine Learning, (ACM), p. 83.
Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecol. Model. 190, 231–259.
Pinho, C., Harris, D.J., Ferrand, N., 2007. Contrasting patterns of population subdivision and historical demography in three western Mediterranean lizard species inferred from mitochondrial DNA variation: historical demography in a latitudinal gradient. Mol. Ecol. 16, 1191–1205.
Ramos-Onsins, S.E., Rozas, J., 2002. Statistical properties of new neutrality tests against population growth. Mol. Biol. Evol. 19, 2092–2100.
Rastegar-Pouyani, N., Faizi, H., Oraei, H., Khosravani, A., Fathinia, B., Heidari, N., Karamiani, R., Rastegar-Pouyani, E., 2011. A brief history and current status of herpetology in Iran.
Rastegar-Pouyani, N., Karamiani, R., Oraei, H., Khosrawani, A., Rastegar-Pouyani, E., 2012. A new subspecies of *Darevskia raddei* (Boettger, 1892) (Sauria: Lacertidae) from the West Azerbaijan Province, Iran: a new subspecies of *Darevskia raddei* (Boettger, 1892) (Sauria: Lacertidae) from the West Azerbaijan Province, Iran. Asian Herpetol. Res. 2, 216–222.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Sambrook, J., Russell, D.W., 2001. Molecular Cloning: A Laboratory Manual. CSHL Press.
Schneider, S., Excoffier, L., 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152, 1079–1089.
Seddon, J.M., Santucci, F., Reeve, N., Hewitt, G.M., 2002. Caucasus Mountains divide postulated postglacial colonization routes in the white-breasted hedgehog, Erinaceus concolor. J. Evol. Biol. 15, 463–467.
Simon, J.-C., Delmotte, F., Rispe, C., Crease, T., 2003. Phylogenetic relationships between parthenogens and their sexual relatives: the possible routes to parthenogenesis in animals. Biol. J. Linn. Soc. 79, 151–163.
Stephens, M., Smith, N.J., Donnelly, P., 2001. A new statistical method for haplotype reconstruction from population data. Am. J. Human Genet. 68, 978–989.
Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585–595.
Tarkhnishvili, D., Thorpe, R.S., Arntzen, J.W., 2000. Pre-pleistocene refugia and differentiation between populations of the Caucasian salamander (*Mertensiella caucasica*). Mol. Phylogenet. Evol. 14, 414–422.
Tarkhnishvili, D., Gavashelishvili, A., Avaliani, A., Murtskhvaladze, M., Mumladze, L., 2010. Unisexual rock lizard might be outcompeting its bisexual progenitors in the Caucasus. Biol. J. Linn. Soc. 101, 447–460.
Tarkhnishvili, D.N., Murtskhvaladze, M., Gavashelishvili, A., 2013. Speciation in Caucasian lizards: climatic dissimilarity of the habitats is more important than isolation time. Biol. J. Linn. Soc. 109, 876–892.
Uetz, P., Hošek, J., 2015. The Reptile Database, <http://www.reptile-database.org> accessed December 4.
VanDerWal, J., Shoo, L.P., Johnson, C.N., Williams, S.E., 2009. Abundance and the environmental niche: environmental suitability estimated from niche models predicts the upper limit of local abundance. Am. Nat. 174, 282–291.
Vrijenhoek, R.C., 1989. Genetic and ecological constraints on the origins and establishment of unisexual vertebrates. Evol. Ecol. Unisexual Vertebrates 466, 24–31.
Wiens, J.A., Stralberg, D., Jongsomjit, D., Howell, C.A., Snyder, M.A., 2009. Niches, models, and climate change: assessing the assumptions and uncertainties. PNAS 106, 19729–19736.