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Precise paternal ancestry of hybrid unisexual ZW lizards (genus *Darevskia*: Lacertidae: Squamata) revealed by Z-linked genomic markers

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We genotyped multiple populations of all seven parthenogenetic species of rock lizards, genus *Darevskia*, as well as their putative sexual parental populations, using double digest RAD-sequencing genomic markers. Taking advantage of the conserved homology of the ZW/ZZ sex chromosomes among lacertid lizards, we aligned our short sequence reads to a reference Z chromosome assembly of the common wall lizard *Podarcis muralis*. This provided unique insight into the origin of all-female hybrid populations, which inherited their single Z chromosome exclusively from a paternal ancestor. The mapped Z-linked loci were used to construct a robust time-calibrated phylogeny. In each parent–offspring species pair, the geographically nearest population of the paternal species was identified as the most likely ancestor of the respective parthenogen, mirroring the trend observed previously on the maternal side in mitochondrial DNA. The estimated splits between the sampled paternal sexual ancestors and their daughter species occurred much earlier than suggested previously and during two narrow time periods: (1) the parthenogenetic *Darevskia armeniaca*, *D. dahli*, *D. uzzelli* and *D. rostombekowi* dated back to ~0.5 or ~0.9 Mya, depending on the calibration point used, while (2) *D. bendimahiensis*, *D. sapphirina* and *D. unisexualis* appear to have diverged ~1 or ~2 Mya.

ADDITIONAL KEYWORDS: hybridization – parthenogenesis – RAD-sequencing – rock lizards – ZZ/ZW species.

INTRODUCTION

Obligate parthenogenetic reproduction is widely documented in the order Squamata (lizards and snakes), and in the majority of cases is immediately preceded by hybridization between sexual representatives of phylogenetically distant lineages (Neaves & Baumann, 2011; Dedukh *et al.*, 2020). The majority of extant obligate parthenogenetic hybrid species investigated to date appear to be relatively young, suggesting that parthenogenesis is unlikely to be maintained for very long on the evolutionary timescale (Moreira *et al.*, 2021), despite demonstrated ecological advantages in the short term (Tarkhnishvili *et al.*, 2010).

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At the same time, to correctly estimate the time of origin of the parthenogenetic populations, one needs to (1) identify those populations of the sexual species that are most closely related to the true parents of the hybrid parthenogens, and (2) genotype both hybrid and parental populations with sufficient resolution in order to obtain robust age estimates (i.e. based on molecular clock or demographic simulation approaches). The difficulty in fulfilling these requirements leads to wide confidence intervals of age estimates in most studies on parthenogenetic reptiles (Tarkhnishvili *et al.*, 2010).

The rock lizards of the genus *Darevskia* Arribas, 1999 were the first example of hybrid parthenogenesis discovered in vertebrates (Darevsky, 1958, 1966), and remain one of the most well-studied systems (Murphy *et al.*, 2000; Badaeva *et al.*, 2008; Tarkhnishvili, 2012; Tarkhnishvili *et al.*, 2010, 2020; Freitas *et al.*, 2016; Galoyan *et al.*, 2019; Spangenberg *et al.*, 2020). In *Darevskia*, two maternal (*D. mixta* and *D. raddei*) and two paternal (*D. valentini* and *D. portschinskii*) sexual species have produced seven nominal parthenogenetic species (*D. armeniaca*, *D. dahli*, *D. unisexualis*, *D. rostombekowi*, *D. uzzelli*, *D. bendimahiensis* and *D. sapphirina*; Fig. 1). Their distribution is centred south of the Lesser Caucasus mountains in Georgia, Armenia and Turkey, as well as the Lake Van basin in eastern Turkey (Fig. 2). The hybrid origin of the parthenogenetic *Darevskia* and the identity of their parents at the species level was determined using only a few allozyme and mitochondrial DNA (mtDNA) markers (reviewed in Murphy *et al.*, 2000), and was broadly confirmed in all later genetic studies (Freitas *et al.*, 2016, 2019; Tarkhnishvili *et al.*, 2020). Notably, the parental sexual species themselves were later shown to harbour high levels of genetic diversity and complicated geographical structure (Tarkhnishvili *et al.*, 2020), and at least on the paternal side, they are characterized by widespread interspecific gene flow today as well as in the past (Tarkhnishvili *et al.*, 2013; Freitas *et al.*, 2019). Regarding the origin of the parthenogenetic species, these recent findings imply that the general view of reticulate evolution within *Darevskia* such as summarized two decades ago by Murphy *et al.* (2000) lacks sufficient detail, in terms

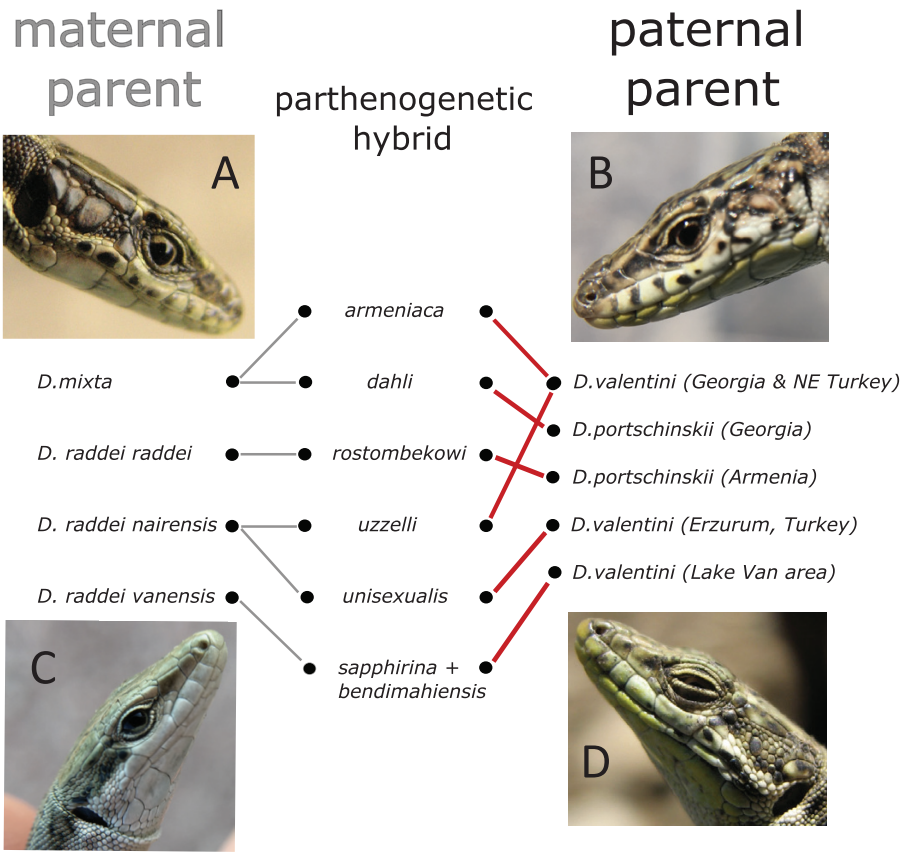

Figure 1. Schematic origin of parthenogenetic taxa in the genus *Darevskia*. The list of matrilinear ancestors on the left is taken unmodified from figure 10 in Tarkhnishvili *et al.* (2020), while details of the patrilinear ancestry on the right were updated using the results of the present study. The insert photographs indicate the sexual ancestral species: maternal *D. mixta* (A) and *D. raddei* (C), and paternal *D. valentini* (B) and *D. portschinskii* (D).

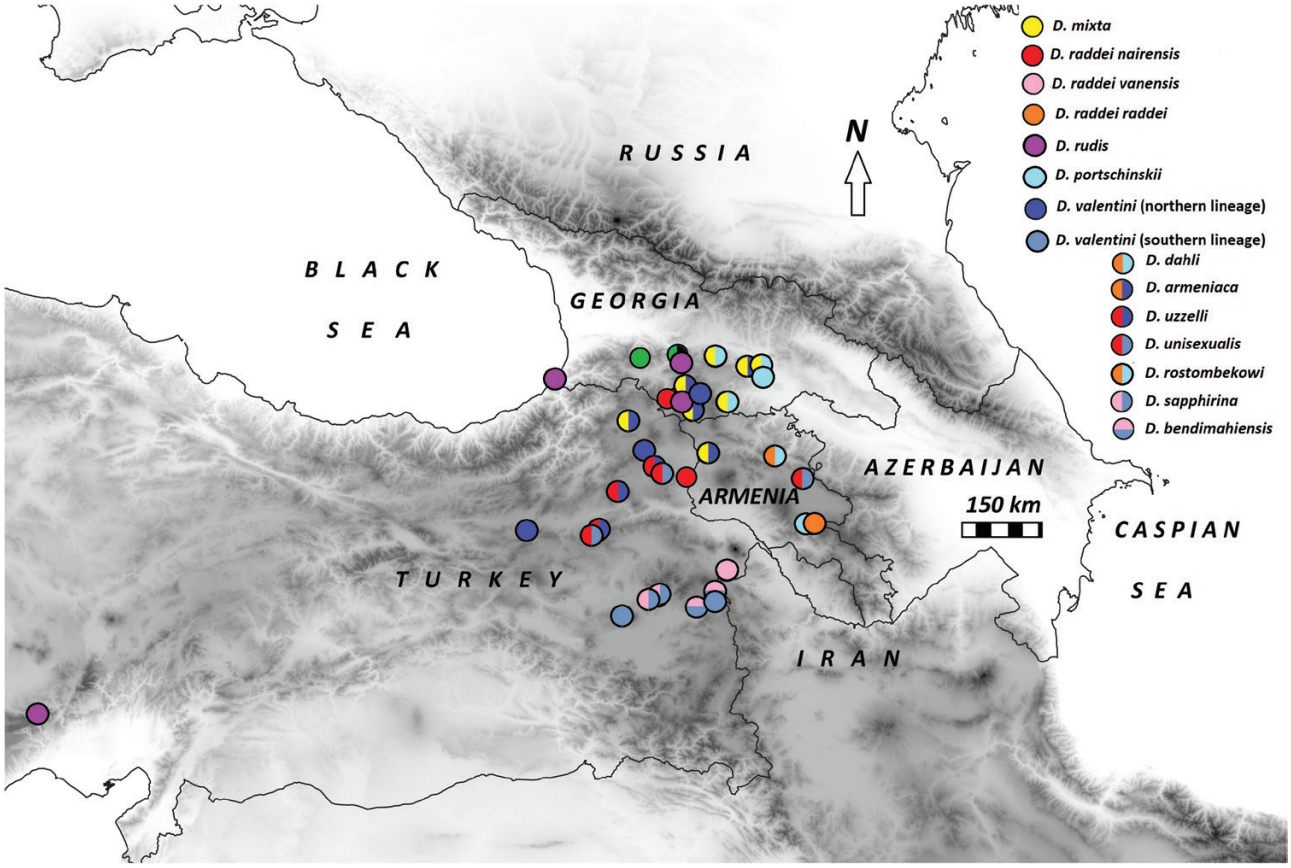

Figure 2. Sampling locations of seven parthenogenetic taxa, their presumed ancestral sexual species and their close relatives.

of both geographical sampling and genome resolution. While a number of recent studies have compared the genetic distances of various paternal and hybrid parthenogenetic populations using a handful of mitochondrial and nuclear DNA (nDNA) markers, their results lacked genomic resolution (Tarkhnishvili *et al.*, 2020), and only a few species were studied locally (Freitas *et al.*, 2016; Ryskov *et al.*, 2017; Girnyk *et al.*, 2018; Vergun *et al.*, 2020). Estimates of the time of the origin of some parthenogenetic species have been made (Murphy *et al.*, 2000; Freitas *et al.*, 2016; Murtskhvaladze *et al.*, 2020), and in all cases they were based on mtDNA sequences. This effectively measures the time of divergence of the parthenogetic lineage from its nearest maternal parent, but lacks validation based on analysis of the paternal genomes. Considering broad variation of rates of molecular evolution throughout the tree of life (Ho, 2020), this complicates true estimation of the time of origin of the pathenogenetic species.

For the first time in *Darevskia*, we use high-throughput genotyping [double-digest restriction site associated sequencing (ddRAD-seq)] to obtain genetic data in all parthenogenetic and their putative parental species over the entire area where parthenogenesis occurs. As far as our data allow, we attempt to resolve the exact paternal ancestry of all known parthenogenetic species, and estimate the respective divergence times between the extant parthenogenetic and paternal populations. To this end, we perform phylogenetic analysis using multiple short sequences from one region of the genome, which in parthenogenetic *Darevskia* has been inherited exclusively from the paternal side: the Z chromosome.

Homologous and well-differentiated ZZ/ZW sex chromosomes with shared origin dating back as far as 85 Mya are characteristic for all lacertid lizards (Rovatsos *et al.*, 2016, 2019; Stöck *et al.*, 2021). The ZZ/ZW system possesses several attractive properties compared to XX/XY. The Z chromosome can show a signature of polymorphism similar to the faster evolving X relative to the autosomes (Mank *et al.*, 2007, 2010; but see Axelsson *et al.*, 2004). Assuming an even sex ratio and similar variance in reproductive success between the sexes, the effective population size on Z is expected to be three-quarters that of the autosomes.

The Z chromosome spends two-thirds of its time in the males, and therefore should experience a slightly higher mutation rate than autosomes. Hence, neutral substitutions on the Z should proceed slightly faster than on the autosomes and on the W (Wilson Sayres & Makova, 2011). At the same time, sex chromosomes in general are expected to be less prone to introgression following interspecific hybridization (i.e. due to reduced fitness of the heterogametic sex), which has been demonstrated empirically for X (Macholán *et al.*, 2011; Maroja *et al.*, 2015) as well as for Z (Storchová *et al.*, 2010). In short, the sex chromosomes and the Z chromosome in particular can be expected to more adequately reflect the history of divergence even in cases where incomplete reproductive isolation and secondary hybridization complicate phylogenetic analyses at other parts of the genome.

In *Darevskia*, all-female parthenogenetic populations are diploid and heterogametic (ZW). The presence of female heterogametic chromosomes has been directly confirmed in *Darevskia portschinskii*, *D. raddei* (Spangenberg *et al.*, 2019; Rovatsos *et al.*, 2019), *D. valentini*, *D. unisexualis* (Spangenberg *et al.*, 2017, 2020a), *D. armeniaca* (Kupriyanova, 2009) and *D. rostombekowi* (Spangenberg *et al.*, 2020b). Assuming that the hybrid species resulted instantly from the union between the paternal (Z) and maternal (W) gametes, the Z chromosome in parthenogens is then inherited directly and exclusively from the paternal parent. This eliminates the need to phase and correct the assignment of haplotypes to the respective parental genomes: a non-trivial task because many alleles will be shared by both parental populations due to ancestral polymorphism (Tarkhnishvili *et al.*, 2020). In contrast, the Z chromosome is expected to be completely free of maternally derived alleles and can be used in phylogenetic analysis as a single sequence unit – in contrast to the seemingly 'private' alleles on the autosomal sequences, which could still be inherited from the mother. The only differences from the ancestral paternal variant are therefore due to accumulation of novel mutations, which offers unique insight into the evolutionary history of the parthenogenetic populations after hybridization has occurred.

MATERIALS AND METHODS

Live lizards were collected in Turkey, Georgia and Armenia, and tissue samples (tail tips) were taken with negligible harm to the animals, as described previously (Tarkhnishvili *et al.*, 2020). The animals were released immediately following the procedure. Sex was identified based on external morphological characters and only adult females were included in the present study to avoid possible bias due to unequal dosage of Z chromosomes between sexes. In total, 99 individuals were analysed using ddRAD-seq and these represented all seven parthenogenetic species as well as their respective sexual parents from multiple localities (Fig. 2; Supporting Information Table S1). In particular, our sampling covered all currently identified, genetically distinct subspecies/geographical variants of the parental taxa *D. mixta*, *D. raddei*, *D. portschinskii* and *D. valentini* found near the parthenogens, as well as *D. rudis rudis* and *D. rudis obscura* from Georgia that are known to exchange genes with both *D. portschinskii* and *D. valentini* (Tarkhnishvili *et al.*, 2013), and a distant *D. rudis bolkardaghica* from the Taurus mountains in southern Turkey, representative of the large distribution range of *D. rudis* (Arribas *et al.*, 2013; Candan *et al.*, 2021). Eleven individuals of *D. raddei*, three of *D. mixta* and one of *D. derjugini*, all representing the maternal clade of parthenogenetic *Darevskia* (Murphy *et al.*, 2000), were used as outgroups in the phylogenetic analysis.

GENOMIC LIBRARY PREPARATION AND BIOINFORMATIC PROCESSING

Genomic DNA was extracted from alcohol-preserved tissues as described by Tarkhnishvili *et al.* (2020), and genomic ddRAD library preparation and sequencing was outsourced to a commercial facility (Floragenex Inc., OR, USA). Briefly, DNA samples were digested with restriction enzymes *Pst*I and *Mse*I (New England Biolabs), and genomic libraries were constructed and multiplexed using a standard protocol (see Supporting Information), and sequenced on two lanes of an Illumina HiSeq instrument (100-bp single-end reads). No differences were observed in the FASTA read quality between the two batches of samples. Reads with quality score < 30 were discarded. Raw reads were de-multiplexed and adaptors and indexes removed using Stacks 2.57 software (Rochette *et al.*, 2019), and quality checked in FastQC. We then aligned the reads to the reference genome of *Podarcis muralis* (Andrade *et al.*, 2019), NCBI assembly GCF_004329235.1, using the default mismatch settings in BowTie 2 (Langmead & Salzberg, 2012) and selecting the conservative end-to-end flag to ensure that the entire read matches the reference. Only those reads successfully mapped to the *Podarcis* Z chromosome (NCBI assembly CM014761.1) were retained for downstream analyses (Supporting Information Table S1). The remaining autosomal and unplaced sequences are outside of the scope of the present study but will be used in future analyses. The reference *P. muralis* genome does not include any annotations specific to the W chromosome. After alignment and converting of the individual sequences into BAM format with SAMtools (Li *et al.*, 2009) we

performed reference-based assembly in Stacks 2.57 using the software default settings for variant calling. To maximize the number of useful RAD loci per sample, we assumed that each individual comes from its own separate population with size = 1.

Phylogenetic analysis

All Z-linked ddRAD loci were concatenated and used in the phylogenetic analysis. Large genome-wide datasets with a high proportion of missing loci typically perform better relative to small datasets where the number of loci has been sacrificed to increase cross-individual coverage (Wiens & Morrill, 2011; Roure *et al.*, 2013; Talavera *et al.*, 2021). Principal component analysis (PCA) was performed using the adegenet (Jombart 2008) R package, and the co-ancestry matrix was constructed in fineRADstructure (Malinsky *et al.*, 2018). A phylogeny of the Z chromosome was calculated using a Bayesian approach in BEAST v.2.6.3 (Bouckaert *et al.*, 2014) on the CIPRES Science gateway (Miller *et al.*, 2010). Prior to building the trees, the most plausible substitution models were selected using MEGA-X software (Kumar *et al.*, 2018). BEAST was run using the Yule process, and with a random distribution of the offspring number between individuals; Markov chain Monte Carlo iterations = 100 000 000. The strict as well as the relaxed exponential clock models were used to obtain a range of divergence time estimates.

Correlation analysis of the distances

We analysed the association between the geographical and genetic distances for all pairs of parthenogenetic and sexual individuals from the paternal clade. Geographical distances were measured as the Euclidean distance based on the exact geographical coordinates, between the locations of 60 studied parthenogenetic individuals and each of 21 individual-based locations of *D. portschinskii*, *D. valentini* and *D. rudis*. Genetic distances were measured as the proportion of nucleotide substitutions within each pair of parthenogenetic and sexual individuals. Since these only represented a subset of all possible individual pairs, the Mantel test could not be applied and Pearson's correlation coefficients, both direct and ln-transformed, were inferred between the geographical and genetic distances.

RESULTS

After alignment to the *Podarcis* genome with an average mapping success rate of 53%, and the reference-based assembly of the ddRAD loci in Stacks 2.57, a total of 14 588 loci were mapped to the Z chromosome in all 99 female individuals, of which 3659 loci contained 6806 informative single nucleotide polymorphisms (SNPs). Only 56 loci were genotyped in all individuals, but at least 47 (48%) of the studied individuals were genotyped at 12 143 (83%) loci, while at most two individuals were missing at 5302 (36%) loci (details in Supporting Information Table S2). Missing data per se do not constitute a major source of bias in phylogenetic analyses, especially when Bayesian methods are employed (Wiens & Morrill, 2011; Roure *et al.*, 2013), and thus our dataset contained sufficient information for further phylogenetic analysis.

The Bayesian tree topology (Fig. 3) clearly differentiated two major phylogenetic clades in *Darevskia*, previously designated by Murphy *et al.* (2000) as 'caucasica' (i.e. including the species *mixta*, *raddei* and *derjugini*) and 'rudis' (including nominal *rudis*, *portschinskii* and *valentini*). The sexual species *mixta* and *raddei* from the 'caucasica' clade, although known to be maternal parents of the parthenogenetic taxa, appeared just as distant from them on the Z chromosome as they were from the paternal sexual species of the 'rudis' clade. In contrast, the Z chromosomes of parthenogens clustered closely with their respective paternal species, down to the level of local geographical populations. In particular, *armeniaca* appeared in the same subclade with *valentini* samples from Georgia; *dahli* with *portschinskii* collected in Georgia; *rostombekowi* with *portschinskii* south of Lake Sevan in Armenia; *uzzelli* with *valentini* from Ardahan, Turkey; *unisexualis* with *valentini* from Erzurum, Turkey; and two parthenogenetic taxa, *bendimahiensis* and *sapphirina*, with *valentini* populations from Çaldıran (NE of Lake Van, Turkey). The same pairs of paternal sexual and daughter parthenogenetic populations separated clearly on the individual-based PCA and fineRADstructure plots (Supporting Information). In all analyses, *D. sapphirina* and *D. bendimahiensis* appear as a single undifferentiated clade, thus questioning their current status as two separate species (Schmidtler *et al.*, 1994; Tarkhnishvili *et al.*, 2020). The choice of the molecular clock substitution model (strict or relaxed exponential) had no effect on the tree topology.

We found a strong correspondence of the geographical and genetic distances between the parthenogenetic population and its closest paternal relative. For example, one population of *D. bendimahiensis* was found just 3 km from its closest paternal parent *D. valentini* in Çaldıran (Lake Van area), and the two geographically distant populations of *D. portschinskii* in Georgia and in Armenia clustered locally with their respective daughter species *D. dahli* and *D. rostombekowi* (Fig. 2). Overall, the correlation

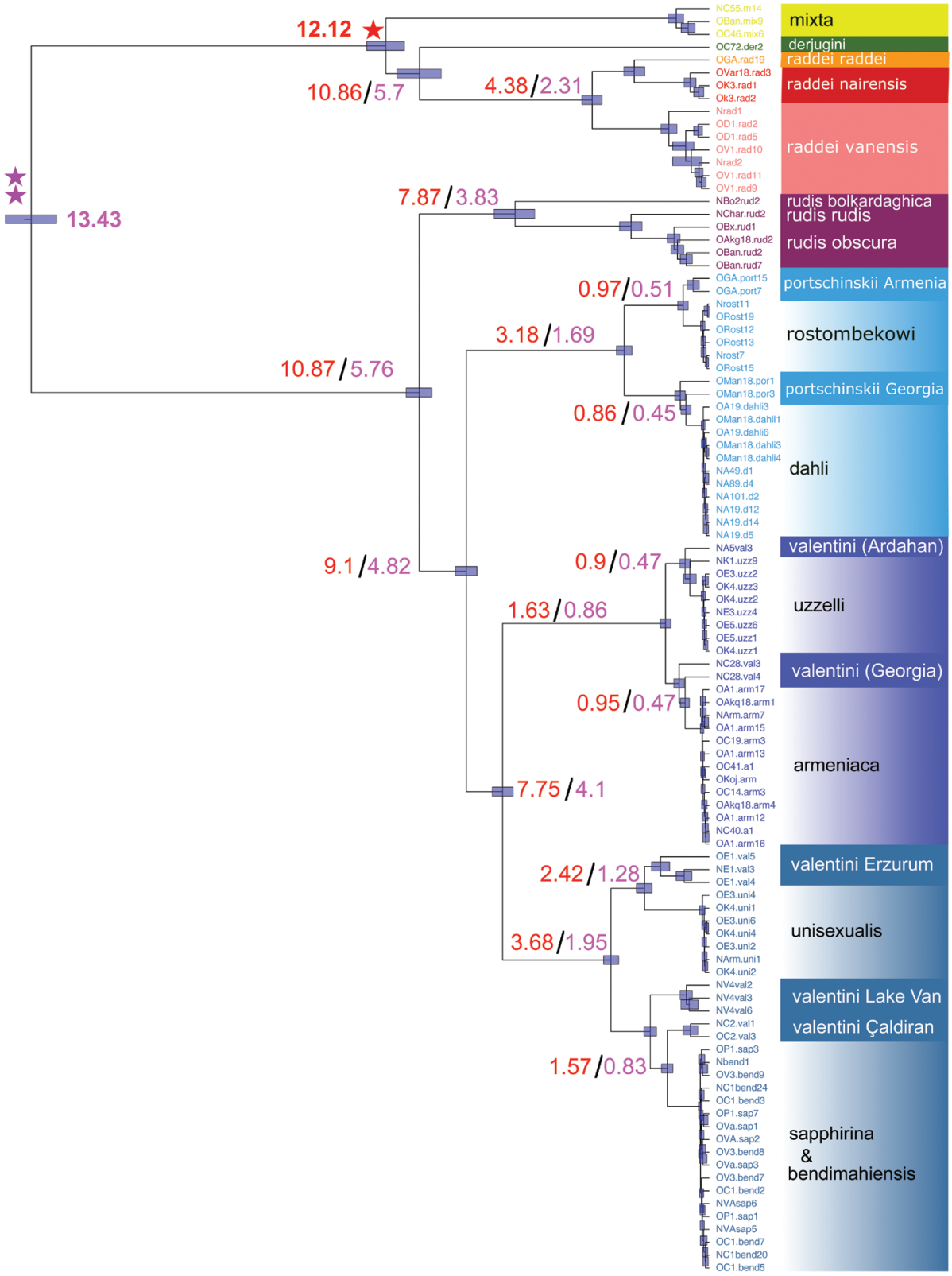

Figure 3. Bayesian phylogeny of parthenogenetic taxa and their parental sexual species constructed from Z-linked ddRAD loci. BEAST v.2.6.3 tree, calibrated by (i) the *raddei–mixta* split, indicated by the single red star symbol, at 12.12 Mya

coefficient between the geographical and genetic distance was 0.265 (the total number of distances compared was 1260). After log-transformation of both geographical and genetic distances, the correlation coefficient increased to 0.477, suggesting that genetic distance does not greatly increase for those sexual populations which are very remote from the target parthenogenetic locations (Fig. 4).

To estimate the node ages on our Z chromosome tree, we calibrated the molecular clock using (1) the previous divergence time estimates between *D. raddei* and *D. mixta* as reported in three different studies (14.4, 12.12 or 8.76 Mya: respectively Kumar *et al.*, 2017, Murtskhvaladze *et al.*, 2020 and Garcia-Porta *et al.*, 2019), or (2) divergence between *D. valentini* and *D. raddei* (20.6, 18.53 or 13.43 Mya), as reported in the same studies. The resulting divergence time estimates of the parthenogenetic species from their closest extant paternal relative are given in Table 1. The timings of four such events (i.e. the divergence of *armeniaca*, *dahli*, *rostombekowi* and *uzzelli*) grouped within roughly the same period, which we dated to as early as 1.16–1.84 Mya (median times) under the relaxed exponential clock model calibrated according to the TimeTree online resource (Kumar *et al.*, 2017), or to as recent as 0.45–0.51 Mya under the strict clock model calibrated according to Garcia-Porta *et al.* (2019). The divergence of *unisexualis* and *bendimahensis–sapphirina* took place within a broader and earlier time frame, i.e. from 2.18–3.43 to 0.83–1.28 Mya, respectively. The 95% probability intervals between these two major groups overlapped when the relaxed exponential clock model was used, but remained separate when the strict clock model was chosen (Table 1; Fig. 3).

DISCUSSION

The molecular phylogeny built on the basis of Z chromosome sequences provides insight into paternal ancestry, as well as the place and time of origin of parthenogenesis in *Darevskia* lizards. The first remarkable observation is that the splits of four parthenogenetic species (*armeniaca*, *dahli*, *uzzelli* and *rostombekowi*) from their respective paternal ancestor (local populations of *valentini* and *portschinskii*) all occurred within a short period of time. All species in this group are today found in a compact area centred in the Lesser Caucasus (Figs 2, 5), and could indeed point to a rapid series of hybridizations with the maternal species *mixta* and *raddei*, probably preceded by simultaneous range expansions due to a climatic event. While our estimates of the split times depend heavily on the choice of clock model and calibration points (Table 1), we argue that the strict clock model might be a more reasonable assumption for closely related species (Langley & Fitch, 1974; Tiley *et al.*, 2020). In this case, the middle Pleistocene Climatic Transition, i.e. glacial cycles increasing in amplitude and duration (Clark *et al.*, 2006), could have acted as a trigger for the origin of parthenogenesis around ~1 or ~0.5 Mya in the Lesser Caucasus. The geographical model of the last time (Last Glacial Maximum) when conditions were unsuitable for rock lizards over most of their current range clearly indicates the areas just north of the Lesser Caucasus as possible refugia for this group (Fig. 5), and the same refugia were probably used by *Darevskia* lizards during the earlier glacial maxima as well.

Shared geography (both occur on the south-western side of the Kars–Erzurum Plateau) coupled with the change in climate could also have been responsible for the origin of parthenogenetic *bendimahiensis–sapphirina* and *unisexualis* species. However, their divergence times from the closest extant paternal populations of *valentini* are considerably older compared to those in the Lesser Caucasus and not simultaneous (from 2.18–3.43 to 0.83–1.28 Mya; Table 1, Fig. 3). If the strict clock model is chosen, the divergence times overlap roughly with the earliest Pleistocene glacial cycles Gibbard *et al.* 2010. In fact, our 2.42 Mya estimate of the split of *D. unisexualis* from its paternal ancestor *D. valentini*, under the strict clock model and 12.12 Mya calibration at the *mixta–raddei* node according to Murtskhvaladze *et al.* (2020), corresponds very closely to its estimated divergence from the maternal ancestor *D. raddei* reported in the same study (2.59 Mya; Murtskhvaladze *et al.*, 2020). In summary, it seems plausible that the earlier origin of *unisexualis* and *bendimahienis–sapphirina* was triggered by a different climatic event(s), and could have followed the range expansion of the parental species from the refugial areas south of the Lesser Caucasus (Fig. 5).

While we have high confidence in the derived topology of our Z chomosome tree, our ability to

(Murtskhvaladze *et al.*, 2020) or (ii) by the *valentini–raddei* split, indicated by the double purple star, at 13.43 Mya (Garcia-Porta *et al.*, 2019). The names of two major clades in the genus *Darevskia* (maternal 'caucasica' and paternal 'rudis') that participated in hybridization and produced parthenogenetic taxa are given according to Murphy *et al.* (2000). Alternative divergence time estimates (Mya) that followed from the two different calibration points (i–ii) are shown next to the selected nodes in red (i) and purple (ii) font. Bars at the nodes represent 95% highest posterior density intervals. All nodes with ages shown had very high Bayesian support values (>0.99).

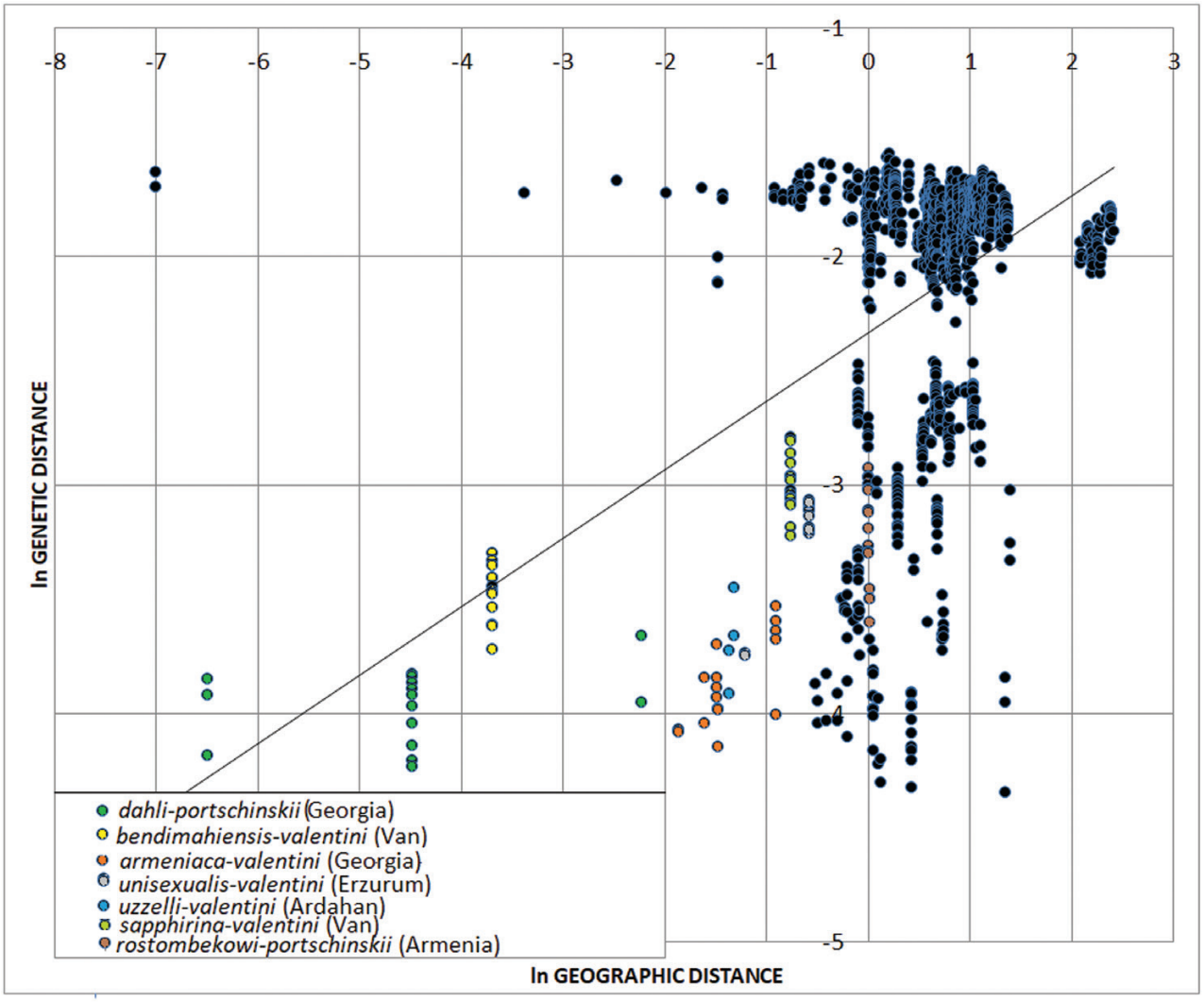

Figure 4. Genetic distances between the parthenogenetic and sexual individuals plotted against straight geographical distances between their locations. Numbers are ln-transformed. Distances between the individual parthenogens and their presumed patrilineal ancestral populations are shown with different symbols (see key).

estimate the true divergence times could be limited by a number of factors. First, the sexual populations examined might not necessarily include the 'true' paternal ancestral lineages, due to them either being extinct or not sampled. This is less of a concern in the nominal subspecies *D. v. valentini* (Georgia and Ardahan in Turkey) and all *portschinskii* samples, which are have been studied phylogeographically (Tarkhnishvili *et al.*, 2013; Rato *et al.*, 2021), but is more relevant to *D. valentini* from Erzurum and the Lake Van basin, where more ancient phylogenetic lineages have recently been discovered (Candan *et al.*, 2021). Second, while using the molecular clock, it is hard to avoid relative bias in the estimation of the divergence time between the old and recently diverged lineages. Our calibration is based on different timescales, with the earliest datings of the most basal split between the 'rudis' and 'caucasica' clades (Murphy *et al.*, 2000) around 20 Mya (Kumar *et al.*, 2017; Murtskhvalandze *et al.*, 2020), which corresponds to those from a number of earlier publications (Pyron & Burbrink, 2014; Roquet *et al.*, 2014; Zheng and Wiens, 2016), although we also used the third calibration based on a more recent divergence (13.43 Mya) suggested by Garcia-Porta *et al.* (2019). If calibrations are placed on the ancient nodes, the ages of young nodes are likely to be overestimated (Tiley *et al.*, 2020). In addition, the largest posterior density intervals for the inferred time tend to show positive skewness for tip branches taxa and negative skewness for basal branches (Beavan *et al.*, 2021). This also explains the unexpectedly long inferred average divergence time of the Z

Table 1. Estimated divergence times (Mya) of parthenogenetic species from their closest extant paternal population. Two combinations of the calibration point + model used to construct the tree in Figure 3 are highlighted in bold.

	Calibrated with *mixta—raddei* split		Calibrated with *valentini—raddei* split	
	T strict clock	Relaxed exponential	T strict clock	Relaxed exponential
Kumar *et al.* (2017)	*14.4 ± 0.55*	*14.4 (6.0–30.1)*	*20.6 ± 0.8*	*20.6 (9.7–41.0)*
dahli–portsch (Georgia)	1.02 ± 0.02	1.45 (0.24–1.7)	0.69 ± 0.01	1.03 (0.21–1.43)
rostom–portsch (Ardahan)	1.16 ± 0.02	1.16 (0.22–1.6)	0.79 ± 0.01	0.82 (0.2–1.5)
armen–val (Georgia)	1.06 ± 0.02	1.60 (0.25–2.0)	0.72 ± 0.01	0.96 (0.2–1.68)
uzzelli–val (Ardahan)	1.05 ± 0.02	1.84 (0.21–2.49)	0.72 ± 0.01	1.30 (0.2–2.46)
unisex–val (Lake Van)	2.88 ± 0.06	3.43 (0.8–4.0)	1.96 ± 0.04	2.42 (0.6–3.2)
bendi–val (Lake Van)	1.87 ± 0.02	2.18 (0.5–2.8)	1.27 ± 0.02	1.53 (0.4–2.5)
sapph–val (Lake Van)	1.87 ± 0.02	2.18 (0.5–2.8)	1.27 ± 0.02	1.53 (0.4–2.5)
Murtskhvaladze *et al.* (2020)	***12.12 ± 0.55***	*12.12 (5.1–25.0)*	*18.53 ± 0.7*	*18.53 (8.0–36.9)*
dahli–portsch (Georgia)	**0.86 ± 0.02**	1.22 (0.23–1.5)	0.62 ± 0.02	0.92 (0.21–1.43)
rostom–portsch (Ardahan)	**0.97 ± 0.02**	0.98 (0.2–1.5)	0.71 ± 0.02	0.74 (0.2–1.5)
armen–val (Georgia)	**0.89 ± 0.02**	1.14 (0.23–1.8)	0.65 ± 0.02	0.86 (0.2–1.67)
uzzelli–val (Ardahan)	**0.89 ± 0.02**	1.55 (0.2–2.47)	0.64 ± 0.02	1.17 (0.19–2.45)
unisex–val (Lake Van)	**2.43 ± 0.06**	2.88 (0.6–3.3)	1.77 ± 0.04	2.17 (0.59–3.1)
bendi–val (Lake Van)	**1.57 ± 0.02**	1.83 (0.41–2.5)	1.14 ± 0.02	1.38 (0.39–2.4)
sapph–val (Lake Van)	**1.57 ± 0.02**	1.83 (0.41–2.5)	1.14 ± 0.02	1.38 (0.39–2.4)
Garsia-Porta *et al.* (2019)	*8.76 ± 0.47*	*8.76 (4.8–21.0)*	***13.43 ± 0.53***	*13.43 (5.6–26.9)*
dahli–portsch (Georgia)	0.62 ± 0.02	0.88 (0.21–1.42)	**0.45 ± 0.01**	0.67 (0.2–1.4)
rostom–portsch (Ardahan)	0.70 ± 0.02	0.71 (0.2–1.5)	**0.51 ± 0.01**	0.53 (0.19–1.62)
armen–val (Georgia)	0.64 ± 0.02	0.72 (0.2–1.66)	**0.47 ± 0.01**	0.62 (0.2–1.65)
uzzelli–val (Ardahan)	0.64 ± 0.02	1.12 (0.19–2.45)	**0.47 ± 0.01**	0.85 (0.18–2.43)
unisex–val (Lake Van)	1.75 ± 0.06	2.08 (0.59–3.1)	**1.28 ± 0.03**	1.58 (0.56–2.8)
bendi – val (Lake Van)	1.14 ± 0.02	1.32 (0.39–2.4)	**0.83 ± 0.01**	1.00 (0.37–2.1)
sapph–val (Lake Van)	1.14 ± 0.02	1.32 (0.39–2.4)	**0.83 ± 0.01**	1.00 (0.37–2.1)

chromosome of some conspecific individuals from the same population, but does not undermine conclusions on the divergence time between the parthenogens and their closest patrilineal ancestor populations. While our results raise the possibility of much older continuous existence of all obligate parthenogenetic forms in *Darevskia* than previously suggested (Moritz *et al.*, 1992; Freitas *et al.*, 2016), achieving more robust conclusions is possible by combining the molecular clock analysis with other approaches. In particular, demographic modelling based on genomic data with large sample sizes in parthenogenetic populations, recently performed on another parthenogenetic lizard species, *Aspidoscellis laredoensis*, produced a maximum age estimate of ~500 kya (Barley *et al.*, 2022), which coincides with our most recent estimates (0.45–0.51 Mya).

As far as the same sexual species are concerned, the topology of our Z chromosome tree shows two differences compared to the previously suggested phylogenies of *Darevskia*. In particular, *D. derjugini*, which is a sister taxon to *D. mixta* on the full mitogenome tree in Murtskhvaladze *et al.* (2020), as well as on the transcriptomic data-based tree in Garcia-Porta *et al.* (2019), is instead found on the same branch with *D. raddei* on our Z chromosome tree (Fig. 3). In the second case, the discrepancy includes our results and Murtskhvaladze *et al.* (2020), on the one side, and Garcia-Porta *et al.* (2019), on the other, regarding the positions of *D. rudis*, *D. valentini* and *D. portschinskii* (i.e. the 'rudis' group; Murphy *et al.*, 2000). Our Z chromosome results agree with the mitogenome tree, placing *D. rudis* as a sister taxon to both *D. valentini* and *D. portschinskii*, while the transcriptome data, as well as some earlier mtDNA-based studies, suggest that *D. portschinskii* is an outgroup (Tarkhnishvili *et al.*, 2013; Garcia-Porta *et al.*, 2019; Candan *et al.*, 2021). Further complicating the picture, the node datings within this subclade are drastically different between the mitochondrial and nuclear/genomic DNA-based phylogenies. In particular, while the entire 'rudis' group appears to have diverged from the common ancestor <1 Mya on the mtDNA tree, the most basal of the three species is separated by at least 4–5 Myr (our most recent median time estimates) according to our results (Fig. 3; Table 1) and to those of Garcia Porta *et al.* (2019). We suggest that these differences could be explained by hybridization among the three species,

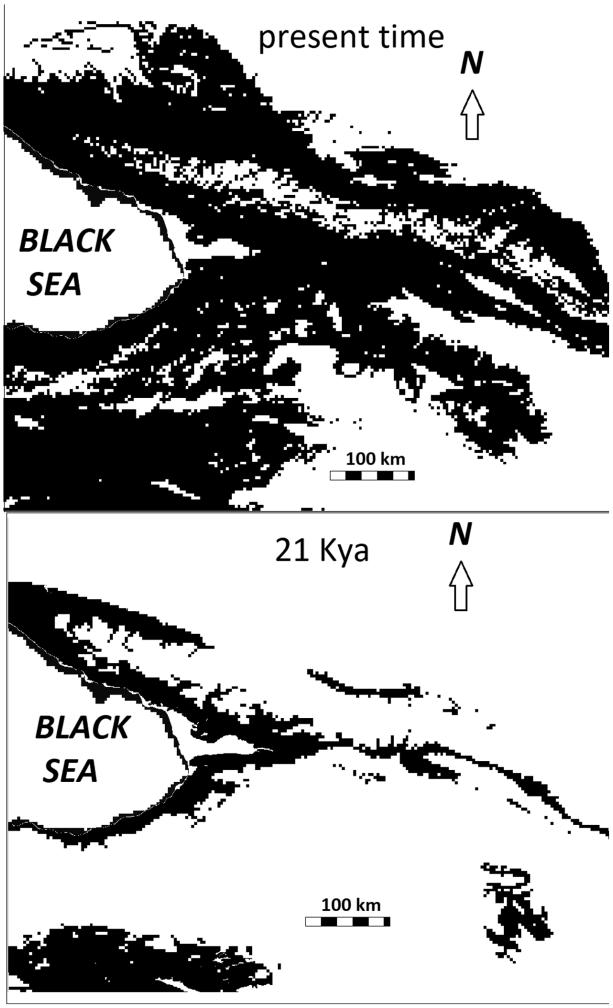

Figure 5. Geographical area with annual mean temperature > 0 °C and annual precipitation > 500 mm at present (upper panel) and 21 kya (Last Glacial Maximum), according to Hijmans *et al.* (2005) and the MIROC (Braconnot *et al.*, 2007) palaeoclimatic model.

as well as by differences in introgression among different parts of the genome. Introgression of mtDNA (Tarkhnishvili *et al.*, 2013) as well as microsatellite genotypes (Freitas *et al.*, 2019) has been demonstrated for all three species of the 'rudis' group. Moreover, the populations which were sampled in different studies could have possessed introgressed vs. non-introgressed haplotypes. In particular, *D. portschinskii* from some Georgian populations, used in the study of Murtskhvaladze *et al.* (2020), has mitochondrial haplotypes more closely related to *D. rudis obscura* and *D. valentini* from southern Georgia than to the populations from Armenia and south-eastern Georgia. Both mtDNA and autosomal elements may introgress more readily compared to genes located on sex chromosomes (Babik *et al.*, 2005; Macholan *et al.*, 2011; Zieliński *et al.*, 2013; Hassanin, 2015). One could speculate that the introgression of Z chromosomal sequences occurs much more rarely, and populations of *D. portschinskii* and *D. valentini* that are paraphyletic at some mtDNA and nuclear sequences (Tarkhnishvili *et al.*, 2013; Candan *et al.*, 2021, Garcia-Porta *et al.*, 2019) are monophyletic when the Z chromosome is considered.

In conclusion, our approach to constructing the phylogeny using Z-linked genomic markers proved to be very useful in identifying the genetically closest paternal sexual populations of each parthenogenetic species in *Darevskia* rock lizards. We have also pinpointed at least one major period when the hybridization and switch to parthenogenesis occurred almost simultaneously in four different instances, all seemingly confined to the same geographical area in the Lesser Caucasus, while the origin of two other parthenogenetic taxa must have taken place earlier in geological time. In addition, we found that the Z chromosome-based phylogeny of the sexual paternal species themselves also presents different divergence time estimates compared to previous studies based on mtDNA. Further analysis of autosomal genomic markers will provide more resolution into the very complex history of the origin and evolution of parthenogenesis in this group.

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and F.M. took part in the fieldwork; M.A., E.G., Ç.I. and Y.K. provided additional samples; M.M., M.G. and F.Ç. performed DNA extraction; A.Y., M.E. and D.T. analysed the data. A.Y. and D.T. wrote the paper and all authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

All authors declare no conflicts of interest. Following manuscript acceptance, the last co-author asked about disavowing his affiliation, but this request was not granted given where the research was conducted, in keeping with industry standards.

DATA AVAILABILITY

All individual sequences aligned to the Z chromosome of *Podarcis muralis* are available in BAM format from NCBI Sequence Read Archive accessible at https://www.ncbi.nlm.nih.gov/sra/PRJNA769575.

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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:

Methods. ddRAD library preparation protocol (perfomed by Floragenex, Inc., OR. USA).

Figure S1. Principal component analysis on individual Z-linked genotypes. Ellipses indicate pairs of parthenogenetic species + their inferred paternal bisexual population.

Figure S2. fineRADstructure co-ancestry plot constructed from the individual Z-linked genotypes. Species/population names are indicated on the left and individual samples names are listed along the *x*-axis. The scale bar on the right represents the absolute co-ancestry values inferred by fineRADstructure.

Table S1. Individual lizard samples used in the study.

Table S2. Distributions of ddRAD loci across samples and SNPs across loci (from populations.log.distribs file following the Stacks 2 assembly).