DOI: 10.1111/jbi.13349

RESEARCH PAPER

WILEY Journal of Biogeography

Phylogeography, evolutionary history and effects of glaciations in a species (*Zootoca vivipara*) inhabiting multiple biogeographic regions

Jose L. Horreo[1] | **Maria L. Pelaez**[1,2] | **Teresa Suárez**[2] | **Merel C. Breedveld**[1,3] | **Benoit Heulin**[4] | **Yann Surget-Groba**[5] | **Tuula A. Oksanen**[6] | **Patrick S. Fitze**[1]

[1]Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales (MNCN-CSIC), Madrid, Spain

[2]Department of Cellular and Molecular Physiopathology, Centro de Investigaciones Biologicas (CSIC), Madrid, Spain

[3]Animal Ecology, University of Potsdam, Potsdam, Germany

[4]Station Biologique, CNRS UMR 655zu3, Paimpont, France

[5]Institut des Sciences de la Fôret tempérée, Université du Québec en Outaouais, Ripon, QC, Canada

[6]Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland

Correspondence
Patrick S. Fitze, Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales (MNCN-CSIC), José Gutiérrez Abascal 2, 28006 Madrid, Spain.
Email: patrick.fitze@mncn.csic.es

Funding information
Spanish Ministry of Education and Science, Grant/Award Number: CGL2008-01522, CGL2012-32459, CGL2016-76918, FPDI-2013-16116 and IJCI-2015-23618; Swiss National Science Foundation, Grant/Award Number: PPOOP3_128375, PP00P3_152929/1; Academy of Finland, Grant/Award Number: 7108955

Editor: David Chapple

Abstract

Aim: During glaciations, the distribution of temperate species inhabiting the Northern Hemisphere generally contracts into southern refugia; and in boreo-alpine species of the Northern Hemisphere, expansion from Northern refugia is the general rule. Little is known about the drivers explaining vast distributions of species inhabiting multiple biogeographic regions (major biogeographic regions defined by the European Environmental Agency). Here we investigate the fine-scale phylogeography and evolutionary history of the Eurasian common lizard (*Zootoca vivipara*), the terrestrial reptile with the world's widest and highest latitudinal distribution, that inhabits multiple biogeographic regions.

Location: Eurasia.

Methods: We generated the largest molecular dataset to date of *Z. vivipara*, ran phylogenetic analyses, reconstructed its evolutionary history, determined the location of glacial refuges and reconstructed ancestral biogeographic regions.

Results: The phylogenetic analyses revealed a complex evolutionary history, driven by expansions and contractions of the distribution due to glacials and interglacials, and the colonization of new biogeographic regions by all lineages of *Z. vivipara*. Many glacial refugia were detected, most were located close to the southern limit of the Last Glacial Maximum. Two subclades recolonized large areas covered by permafrost during the last glaciation: namely, Western and Northern Europe and North-Eastern Europe and Asia.

Main conclusions: In *Z. vivipara*, most of the glacial refugia were located in the South of their current distribution. Previous studies suggested the existence of Northern refuges, but the species' inability to overwinter on permafrost and the lack of genetic support suggest that the presence of a refugia in the north of the Alps is unlikely. This species currently inhabits boreo-alpine climates and retracted during previous glaciations into southern refugia, as temperate species. Two clades exhibited enormous geographic expansion that started from two distinct glacial refugia. These phylogeographic patterns were highly congruent with those of *Vipera berus*. Together they suggest that glacial retraction, the location of the refugia and absence of competition may have promoted the enormous geographic expansion of two clades.

KEYWORDS
ancestral area reconstruction, ancestral biogeographic region reconstruction, biogeography, glacial refuges, last glacial maxima, molecular diversity, phylogeny, post-glacial recolonization

1 | INTRODUCTION

Current biogeographic patterns of many organisms are strongly determined by the past climatic, geographical and geological history (e.g. Mittelbach et al., 2007). In many regions of the world, glaciations played a major role in shaping diversity (e.g. Fernández-Palacios et al., 2016). In the Northern Hemisphere, glaciations typically led to south-northward contraction-expansion patterns (Hewitt, 1996), either due to the reduction in suitable habitat and the formation of cold stage refugia in the south during glaciation with subsequent expansion during interglacials, or vice versa (warm-stage refugia; Stewart, Lister, Barnes, & Dalen, 2010). Species inhabiting temperate conditions frequently exhibit contraction during glaciation and subsequent recolonization during interglacials (Hewitt, 1996). Instead, boreo-alpine and steppe species are suggested to exhibit contraction during interglacials and, at least the former, recolonization during glacial periods (Kajtoch et al., 2016). Consequently, species distributions will contract into Southern, Northern or Montane and extrazonal refugia, depending on the inhabited biogeographic region (Kajtoch et al., 2016; Stewart et al., 2010). However, not all species inhabit a single biogeographic region and thus predictions about the contraction-expansion patterns and the location of refugia may not be straight-forward. For example the distribution of the common European viper (*Vipera berus*, Linnaeus, 1758), the world's most widely distributed terrestrial snake species, ranges from Scotland in the west to Pacific Russia in the Far East, and from Albania and Greece in the south to north of the Arctic Circle (Ursenbacher, Carlsson, Helfer, Tegelström, & Fumagalli, 2006). It thus inhabits temperate, boreal, alpine, Atlantic and continental climates (Ursenbacher et al., 2006). Potential refugia have been reconstructed in South-Central France (West of the Alps; Western subclade), in the lowland plains in the Balkans, on the Northern Italian Peninsula, in the northeast and east of the Carpathian Mountains (Carpathian subclade), and in the North of the Alps (Ursenbacher et al., 2006). This points to refugia South and West of the glaciers (South-Central France, Balkans, Northern Italy), but as well in the North of the Alps and North of the Carpathians. The former refugia are in locations predicted for temperate zone species, whereas the latter are rather locations predicted for boreo-alpine species. Similarly, the Eurasian common lizard *Zootoca vivipara* (Lichtenstein, 1823), the terrestrial reptile with the world's widest distribution and the highest latitudinal distribution in the Northern Hemisphere (Hikida, 2002), inhabits temperate, boreal, alpine, Atlantic and continental climates (Surget-Groba et al., 2001). Potential refuges have been reconstructed in Southern France (North of Pyrenees), North-Western and Northern Spain (Mila, Surget-Groba, Heulin, Gosá, & Fitze, 2013; Surget-Groba et al., 2001), Italy (Surget-Groba, Heulin, Ghielmi, Guillaume, & Vogrin, 2002) and the Carnian Alps (Heulin, Guillaume, Vogrin, & Surget-Groba, 2000). This points to refugia South and West of the glaciers, but also North of the Pyrenees. The findings for both species suggest that the distribution of species can be limited by both glacial and interglacial periods and that responses of a single species to glacial and interglacial periods may be similar to those of species inhabiting temperate and boreo-alpine regions (Hewitt, 1996; Kajtoch et al., 2016). These different responses may exist if different lineages adapted to different climates. For example if some lineages behave as boreo-alpine species (with expansions during glaciations and contractions during post-glacials), and others as temperate climate species (with expansion during post-glacials and contractions during glacials). However, for both *Z. vivipara* and *V. berus*, the sampling used in previous studies was limited (Provan & Bennet, 2008; Ursenbacher et al., 2006). For instance, in *V. berus* the sampling was not sufficient to localize more precisely the putative Northern refugia (Ursenbacher et al., 2006). Consequently, fine-scale phylogenetic and phylogeographic knowledge from widely distributed species is required to understand whether species exhibit at the same time biogeographic patterns of temperate zone species and of boreo-alpine species.

Here we study the phylogeography and location of refugia of the Eurasian common lizard (*Z. vivipara*), and investigate whether different lineages exhibit different responses to glacials and interglacials. Earlier studies detected six major genetic clades (Surget-Groba et al., 2001, 2002, 2006), two with oviparous (A and B) and four with viviparous (C, D, E and F) reproduction. The viviparous populations are widely distributed along the Palaearctic area (from western Europe up to Sakhalin and Hokkaido Islands; Heulin & Guillaume, 1989) where their northern limit is closely associated with the southern limit of the permafrost (Berman, Bulakhova, Alfimov, & Meshcheryakova, 2016). The oviparous populations are present only in two areas of its southern distribution: first in Northern Spain and the Pyrenees (Heulin & Guillaume, 1989), and second, in Northern Italy and the Carnian Alps (Heulin et al., 2000; Surget-Groba et al., 2002). Two subclades (B1 and B2) have been described by Surget-Groba et al. (2001) and more recently, regional studies with more intense sampling have provided evidence for additional well-supported subclades (Mila et al., 2013). This suggests not only that independent species may exist (Cornetti, Ficetola, Hoban, & Vernesi, 2015), but the current phylogeny and phylogeography are more complex than believed.

The current biogeographic hypotheses for *Z. vivipara* suggest: (1) the existence of latitudinal contractions/expansions in the Western (French and Spanish) oviparous populations (clade B) during Pleistocene glaciations with refugia in Southern France (North of the

Pyrenees), in the south of the Pyrenees, and Northern Spain (i.e. South-Western Europe; Surget-Groba et al., 2001), (2) an Italian refuge of the oviparous clade A (Surget-Groba et al., 2002) and that (3) for clades C to F the location of potential refuges remains unknown and (4) clades D and E, respectively, may have exhibited post-glacial northwards expansion to Northern Europe and post-glacial north-east expansion to North-Eastern Europe and Asia (Surget-Groba et al., 2001). Taken together this information indicates (1) that *Z. vivipara* exhibits biogeographic patterns typical for temperate species, namely, Southern refuges during glaciations, and (2), its occurrence in high northern latitudes (at least up to 66° N; Surget-Groba et al., 2006; Berman et al., 2016) and the refuge North of the Pyrenees suggests that some lineages exhibit biogeographic patterns typical for boreo-alpine species. However, sample size, genetic methods and markers used in previous studies do not allow to draw robust conclusions (Surget-Groba et al., 2001) and the reason why some, but not all clades exhibit enormous geographical distributions remains unclear.

Here we generated the largest molecular dataset of *Z. vivipara* known to date. It covers almost the entire natural distribution and all known lineages, including fine-scale sampling of areas with limited sampling and of areas that have not been sampled. Genetic and biogeographic analyses are used to test the following hypotheses. First, are the locations of *Z. vivipara*'s refugia in areas commensurate with those predicted by temperate and/or boreo-alpine species? Second, are contraction/expansion patterns associated with glacials/interglacials? Third, do different clades/lineages inhabit different biogeographic regions? Fourth, do different clades/lineages inhabit areas with different climates? Fifth, are the results congruent with the idea that a single species exhibits at the same time biogeographic patterns typical for temperate and typical for boreo-alpine species?

2 | MATERIALS AND METHODS

2.1 | Used samples

The 231 samples used in this study (Figure 1) cover all described clades (clades A–F; Surget-Groba et al., 2001, 2006) of *Zootoca vivipara* and almost all the species distribution across Eurasia (specimen details, see Appendix S1 in Supporting Information). The number of samples per clade ranged between 24 and 63, and two outgroups were employed for the rooting of the phylogenetic tree, namely the common wall lizard (*Podarcis muralis*; Laurenti, 1768) and the western green lizard (*Lacerta bilineata*; Daudin, 1802). These outgroups were chosen because the genera are closely related to *Zootoca* and because both have been used in previous studies (Surget-Groba et al., 2001, 2002, 2006). DNA was extracted from lizard tissues using a commercial ChargeSwitch® gDNA Micro Tissue Kit (Invitrogen™, Carlsbad, USA) that provides better DNA-extraction quality compared to other methods (Horreo, Peláez, & Fitze, 2015). Tail, skin sheds or other tissues were obtained from collaborators (see Surget-Groba et al., 2006 and Acknowledgements) and from own sampling under licences from Instituto Aragonés de Gestión Ambiental LCE/Mp 24/2012/426 and 500201/24/2013/12572; Service Nature Languedoc-Roussilon arrêtés No. 2014304-004; DREAL Aquitaine; Préfecture Pyrénées Atlantiques: Arrêtés No. 02/2006, 47/2007, 03/2008, 06/2009, 08/2010, 07/2012; Parc National des Pyrénées 2012-55 & 2016_8; Xunta de Galicia 071/2014; Generalitat de Catalunya; Asturias; Parque Nacional Picos de Europa; Bizkaiko Foru Aldundia Zk/No. 1079; University of Jyväskylä 49/29.11.2004 and 21/22.5.2006; Finnish Ministry of the Environment 3/5714/2003 and 8/5714/2006; County Administrative Board of Norrbotten 522-1341-05; Finnish Environment Institute KSU-2004-L-535/254).

2.2 | Genetic markers

In all samples, three nuclear (nZV1, nZV2 and nZV3; 1,266 bp; Horreo, Peláez, Suárez, & Fitze, 2018) and three mitochondrial (ND2, CytB and 16S rRNA; 1,355 bp) gene fragments were amplified. Polymerase chain reaction (PCR) for gene amplification was done with 100 ng of template DNA in a total reaction volume of 25 μl employing a 5PRIME MasterMix Kit. PCRs cycles: initial denaturation (5 min) at 94°C; 35 cycles (30 s) at 94°C; annealing (30 s) at 59°C for the nuclear genes and at 53, 53 and 50°C for the ND2, CytB and 16S genes; extension (90 s) at 72°C; and a final extension at 72°C for 5 min. Five of the six genes amplified in all 231 *Z. vivipara* and the two outgroups, and ND2 amplified in 201 *Z. vivipara* and the outgroups. To amplify the remaining 30 *Z. vivipara* samples, a new reverse primer was designed (ND2-H; 5′–3′ sequence: GATGGAAGCCCACTGGTTAG) and amplified at 55°C together with the Met F6 primer of Macey, Larson, Ananjeva, Fang, and Papenfuss (1992). Heterozygote sites were coded with IUPAC codes. Fragments were concatenated following a total evidence approach (Kluge, 1998), leading to a dataset of 2,621 base pairs (bp) of 231 *Z. vivipara* and two outgroups. Alignments will be made available upon request.

2.3 | Phylogenetic and molecular clock analyses

For nuclear DNA, haplotypes of heterozygotes were inferred employing PHASE 2.1 (Stephens, Smith, & Donnelly, 2001), as well as SEQPHASE for creating input and output files (Flot, 2010). Recombination was tested on them with DNASP (Librado & Rozas, 2009) under the four-gamete test methodology (Hudson & Kaplan, 1985). This software was also employed to estimate the genetic variability in the different clades, measured as the number of variable sites, the haplotype diversity and the nucleotide diversity, in both nuclear (*n*) and mitochondrial (*mt*) DNA (in the case of nuclear DNA, each gene was analysed separately).

King and Lee (2015) showed that Bayesian analyses need to account for rate heterogeneity, because not accounting for variable evolutionary rates can cause highly anomalous and even incorrect results. Consequently, Bayesian inference with a Random Local Clock (RLC), implemented in BEAST 2.3.1 (Bouckaert et al., 2014) was used. Birth-Death model evolution and 100 million MCMC chains were run. jMODELTEST 2 (Posada, 2008) with the Akaike inference

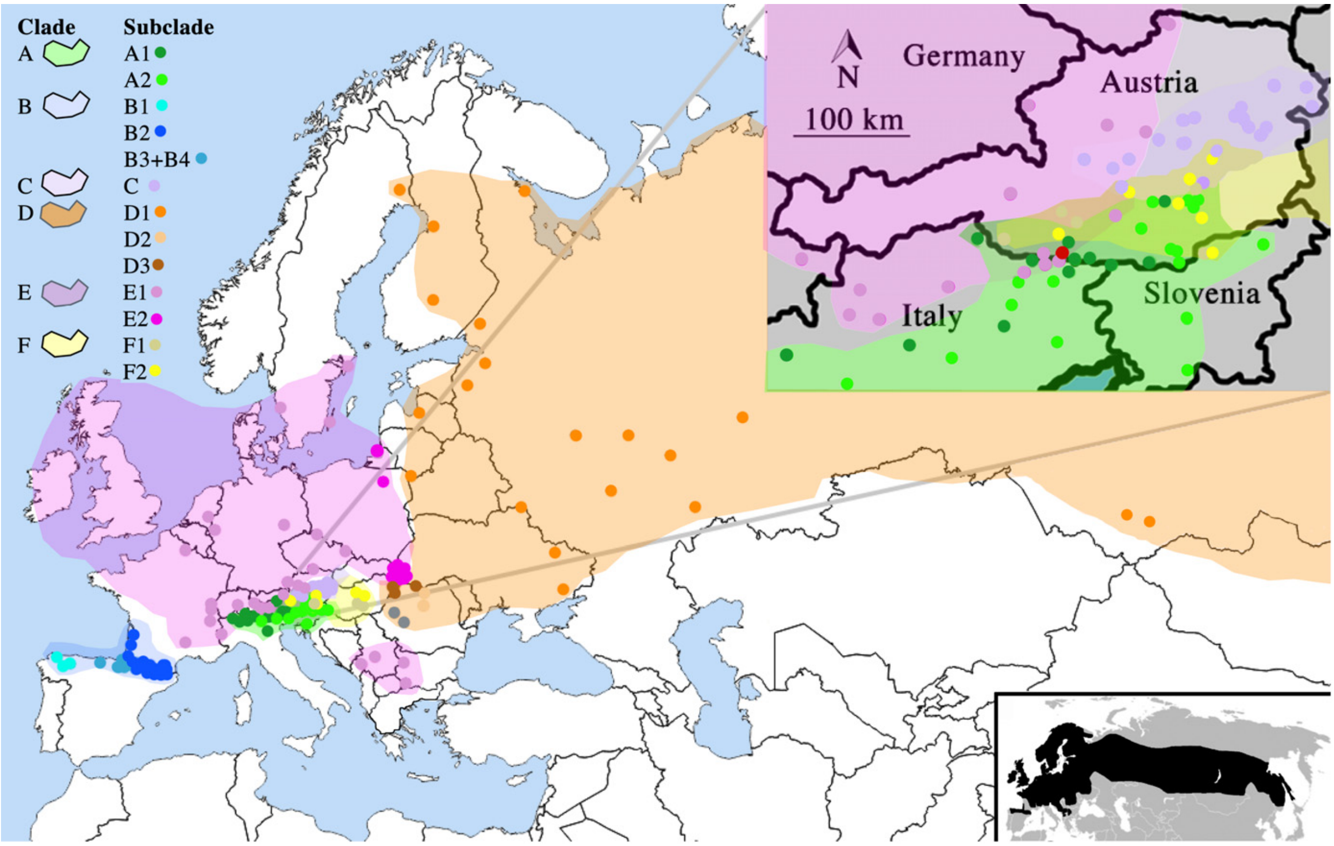

FIGURE 1 Map showing sampled populations (dots) belonging to all described *Zootoca vivipara* clades across Eurasia (Sakhalin is not shown). Dot and shade colour represent different clades and subclades unravelled by the phylogenetic analyses (Figure 3) and shaded areas the approximate area occupied by them. The zoomed map represents the central European contact zone (in Austria, Italy and Slovenia) and the red population a confirmed secondary contact between clade E and A (Lindtke et al., 2010). The map in the lower right corner shows the species' range following IUCN Red List (http://maps.iucnredlist.org/)

criteria (AIC) was used to evaluate different models of nucleotide substitution. The GTR model fitted best in *mt* DNA, *n* DNA and in the concatenated dataset. Consequently, GTR with no partitions was used for all analyses. Phylogenetic Maximum Likelihood analyses were done with MEGA 7 (Kumar, Stecher, & Tamura, 2016), using the GTR substitution model with 100 bootstrap replications. Molecular calibration of the tree was done using the dating of the node between the oviparous clade A of *Z. vivipara* and the other clades (4.5 Ma; 95% confidence interval (CI) = 6.1–2.6 Ma; Cornetti, Menegon, Giovine, Heulin, & Vernesi, 2014), since the estimate of this node was based on precise geological calibration (Cornetti et al., 2014), by means of the divergence between the Peloponnese wall lizard (*Podarcis peloponnesiaca*; Bibron & Bory, 1833) and the Cretan wall lizard (*P. cretensis*; Wettstein, 1952) when Crete became isolated from Peloponnese during the Messinian (5.2 ± 0.1 Ma; Beerli, Hotz, & Uzzell, 1996).

2.4 | Phylogeographic and biogeographic inferences

Ancestral area reconstruction was done with RASP 3.2 software (Yu, Harris, Blair, & He, 2015), which implements the two most widely and accepted phylogeographic reconstruction methods. Both methods were used: (1) the Lagrange Dispersal-Extinction-Cladogenesis method (DEC; Ree & Smith, 2008) and (2) the Statistical-Dispersal Vicariance Analysis with options allow extinctions and allow reconstruction (maximum = 100) checked (S-DIVA; Yu, Harris, & He, 2010), and individual and combined statistics were derived. Four major phylogeographic areas were defined representing the current phylogeographic distribution of *Z. vivipara*'s clades: Italy (including the areas covered by clade A), Northern and Central Europe and southern Balkan (covered by the clades C, E and F; hereafter referred to as European phylogeographic area), Asia (clade D) and South-Western Europe (clade B), and nodes were allowed to be attributed to a maximum of two phylogeographic areas. The same approach was used to reconstruct ancestral biogeographic regions. The biogeographic region (EEA, 2012) in which a specimen was captured was determined and nodes were allowed to be attributed to a maximum of two biogeographic regions. Refugia during Last Glacial Maximum (LGM: 21,000 years ago) were inferred based on the current clade distributions and locations where the species could not survive during the last glaciation, due to continental ice sheet cover and permafrost.

3 | RESULTS

3.1 | Genetic variability

No recombination was found within nuclear genes (minimum number of recombination events, Rm, was zero in all cases). Haplotype diversity ranged between 0.056 (clade B, nZV1) and 0.913 (clade C, *mt* DNA), and nucleotide diversity between 0.001 (clade B, nZV1 and nZV2, clade C, nZV3) and 0.053 (clade D, *mt* DNA; Table 1). The TCS haplotype network (Figure 2) showed a clear separation of all extant clades (A, B, C, D, E, F) by several mutation steps, and star-like patterns in approximately half of the haplotype groups (i.e. subclades).

3.2 | Phylogenetic analyses

Most ancestral nodes of the *Z. vivipara* clades (A, B, D, E and F) had a posterior probability (pp) of ≥0.99 (Figure 3; Appendix S2, S3). Clade B, D, E and F contained statistically well-supported and geographically separated subclades (Table 2) and clade A contained two subclades with moderate statistical support (A1, A2, Figure 1). Clade B consisted of three subclades with pp = 1 and one sample with node support of pp = 0.85 (OF25, Figure 3). Individuals belonging to the NC Spain and the NE Spain subclade (Mila et al., 2013) grouped together in the same subclade (B3 + B4), exhibited monophyly, and were not statistically supported (pp = 0.74, Appendix S2). Subclades B2 and B1 included individuals belonging to the Southern France (Mila et al., 2013; B2 in Surget-Groba et al., 2006) and NW Spain subclade (Mila et al., 2013; B1 in Surget-Groba et al., 2006; note: all except one individual of B1, belong to the NW Spain subclade). Clade D consisted of three statistically well-supported subclades (pp = 1): D1, D2 and D3. Two samples (VRO1B and VRO5D) belonging to clade D were isolated from the rest (pp = 0.89 and 1) by >10 mutational steps (Figure 2), and both stem from two distinct geographical locations (Western Romanian Carpathians and Southern Carpathians) represented by only one sample (Figure 3), suggesting that additional subclades may exist. Clade E consisted of two subclades with pp = 1: E1 and E2. Clade F consisted of two subclades with pp = 1: F1 and F2 (Figure 1). Within clade C, no evidence for subclades existed. The molecular dating is shown in Figure 3, and the age of ancestral nodes in Appendix S3. Maximum likelihood phylogenetic tree (Appendix S4) exhibited the same clade topology as the Bayesian trees.

3.3 | Phylogeography

Over large areas the distribution of the subclades is allopatric. A1 inhabits NW Italy and A2 NE Italy (Figure 1). B1 inhabits NW Spain, B2, Southern France (North of the Pyrenees), B3 and B4 NC Spain and the NE Spain respectively (Mila et al., 2013). D1 inhabits the North and East of Eurasia (Northern Sweden, Finland, Latvia, Lithuania, Belarus, Ukraine, Russia including Altai and Tuva Regions and Mongolia, Table 2), D2 the Central East-Carpathian Mountains (Romania) and D3 the NW of and the central Inner East-Carpathian Mountains (Theiss region in Hungary, Romania, Slovakia and Ukraine; Table 2). VRO1B and VRO5D, belonging to clade D, originated from the Southern and Western Romanian Carpathians (Romania) respectively. E1 inhabited Western, Central and Northern Europe, and the southern Balkans, and E2 the North of the East-Carpathian Mountains (frontier between Slovakia, Ukraine and Hungary), Eastern Poland and Kaliningrad Oblast (Table 2). F1 is mainly distributed in SW Austria and F2 mainly in SE Austria (Figure 1), whereas clade C inhabits an area south of clade E and north of clade F in central Austria.

Geographical overlap among clades existed in two areas (Figure 1): in the Eastern Alps among clades A, C, E and F (in Austria, NE Italia and Northern Slovenia), and in the Central East-Carpathian Mountains between clades D and E (in Northern Romania, North-Eastern Hungary and Eastern Slovakia). In the Central European Contact zone geographical overlap existed among clades A and E (in NE Italy, and Kärnten), among A and F (in Northern Slovenia, Kärnten and SW Steiermark), among E and F (in East Tirol and Kärnten) and among C and F (in Steiermark). In subclades, evidence for geographical overlap existed between subclades F1 and F2 (in Southern Austria, Northern Slovenia and Hungary), between subclades B2 and B3 + B4, and to a lesser extent between the subclades A1 and A2 (in Northern Italy and in Southern-central Austria).

TABLE 1 Genetic variability in the mitochondrial and nuclear genes per *Zootoca vivipara* clade (A, B, C, D, E and F). Given are the number of individuals (*N*), haplotype and nucleotide diversity (Hd and Nd respectively)

	Clade	A	B	C	D	E	F
DNA type	N	38	63	24	30	52	24
Nuclear	Hd—nZV1	0.555	0.056	0.513	0.645	0.262	0.653
	Hd—nZV2	0.764	0.347	0.809	0.658	0.401	0.595
	Hd—nZV3	0.642	0.252	0.198	0.778	0.110	0.411
	Nd—nZV1	0.006	0.001	0.004	0.007	0.002	0.007
	Nd—nZV2	0.003	0.001	0.007	0.005	0.004	0.004
	Nd—nZV3	0.008	0.002	0.001	0.003	0.002	0.005
Mitochondrial	Hd—*mt*	0.859	0.859	0.913	0.685	0.854	0.866
	Nd—*mt*	0.006	0.011	0.003	0.053	0.005	0.007

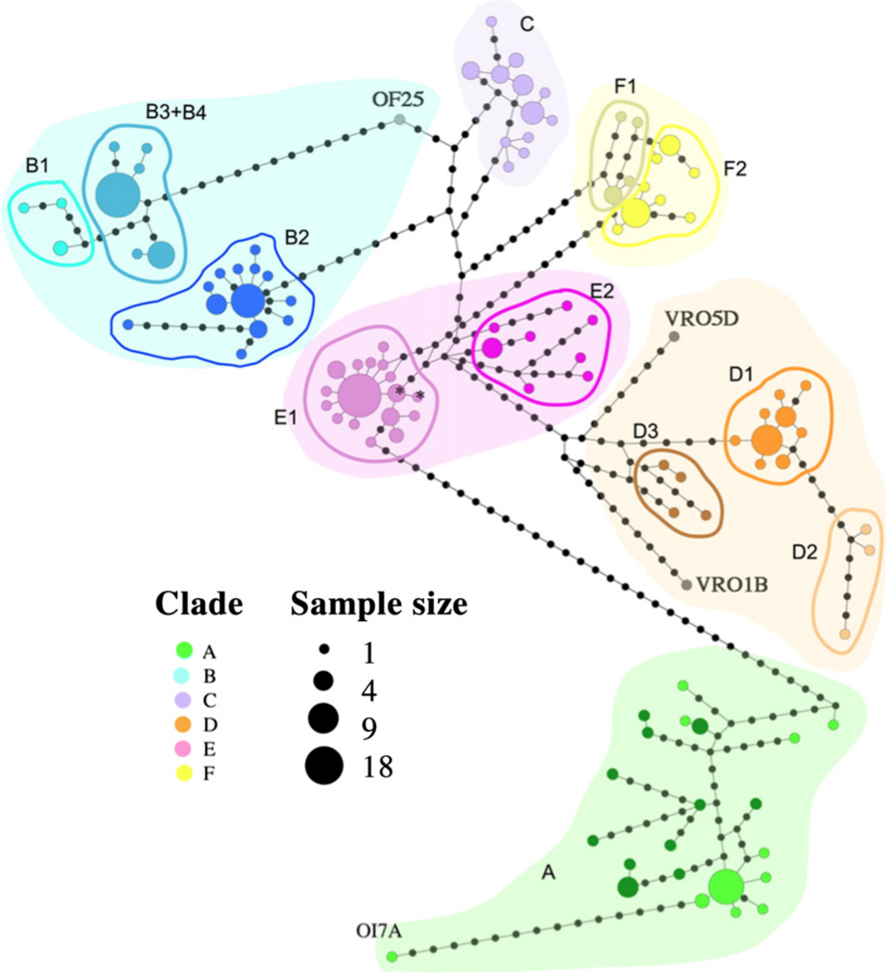

FIGURE 2 Haplotype network (TCS) of all described extant *Zootoca vivipara* clades (Surget-Groba et al., 2001; see within figure legend). Each colour circle represents a haplotype and its size is proportional to the haplotype's frequency. Black dots represent mutation steps and connecting line length is proportional to their number. Dots within shaded areas correspond to the same previously described clade (A, B, C, D, E, F; Surget-Groba et al., 2001; colour code see within-figure legend), circled haplotypes correspond to subclades with statistical support on the phylogeny (Figure 3). Asterisk in haplotypes belonging to E1 indicate haplotypes located on the Balkans. Grey haplotypes correspond to isolated haplotypes (in the phylogeny; Figure 3)

Two subclades exhibit a very large geographical distribution: subclade E1 prevails from the Alps to Scandinavia and from Ireland to Poland and it also inhabits the Southern Balkans (Figure 1), and subclade D1 prevails from Southern Ukraine to Northern Scandinavia and from Lithuania/Latvia and Ukraine to Mongolia and Japan. The distribution of the other clades and subclades was much smaller and some occupy areas smaller than 20,000 km^2 (clade C and subclades B1, B2, B3 + B4, D3, F1 and probably D2).

3.4 | Biogeography

The ancestral area reconstruction suggested that the ancestor of *Z. vivipara* lived in the Italian/European phylogeographic area. Clade A remained in this area, whereas the ancestor of clades B to F was reconstructed in the European phylogeographic area. The ancestor of clades E and D colonized the Asian phylogeographic area, where clade D diversified. Clade E remained in the European and Asian phylogeographic area until today. The ancestor of clades B, C and F was reconstructed with high probability in the European phylogeographic area, from where the ancestor of clade B and C colonized the Southwestern European area (Figure 3). Later on the ancestor of clade C remained only in the European and the ancestor of clade B diversified in the Southwestern European phylogeographic area.

The reconstruction of the ancestral biogeographic regions shows that the ancestor of *Zootoca* inhabited the alpine biogeographic region (Figure 3). Clade A and the ancestors of the other clades remained in the alpine region with the exception of the ancestor of clade D that colonized the continental biogeographic region (Figure 3). Within clades, the ancestor of B1 and B3 + B4 colonized the Atlantic, the ancestor of F1 the pannonian, the ancestor of D1 the boreal, and the ancestor of D3 the pannonian biogeographic region. Seven subclades recently colonized one (B3 + B4, B2, C, F2, E2, D1) to three (E1) new biogegeographic regions. Only two subclades changed biogeographic region (B1, D1), all others still inhabit the alpine region, and D1 and E1 inhabit 3 and 4 biogeographic regions respectively.

4 | DISCUSSION

4.1 | Genetic structure

The phylogenetic analyses unravelled six major clades (Figure 3). These clades coincided with the major groups of the TCS haplotype network (Figure 2). The positions of the clades in the phylogenetic tree (Figure 3) were identical to those of an earlier phylogenetic maximum parsimony tree based on mitochondrial DNA (Surget-

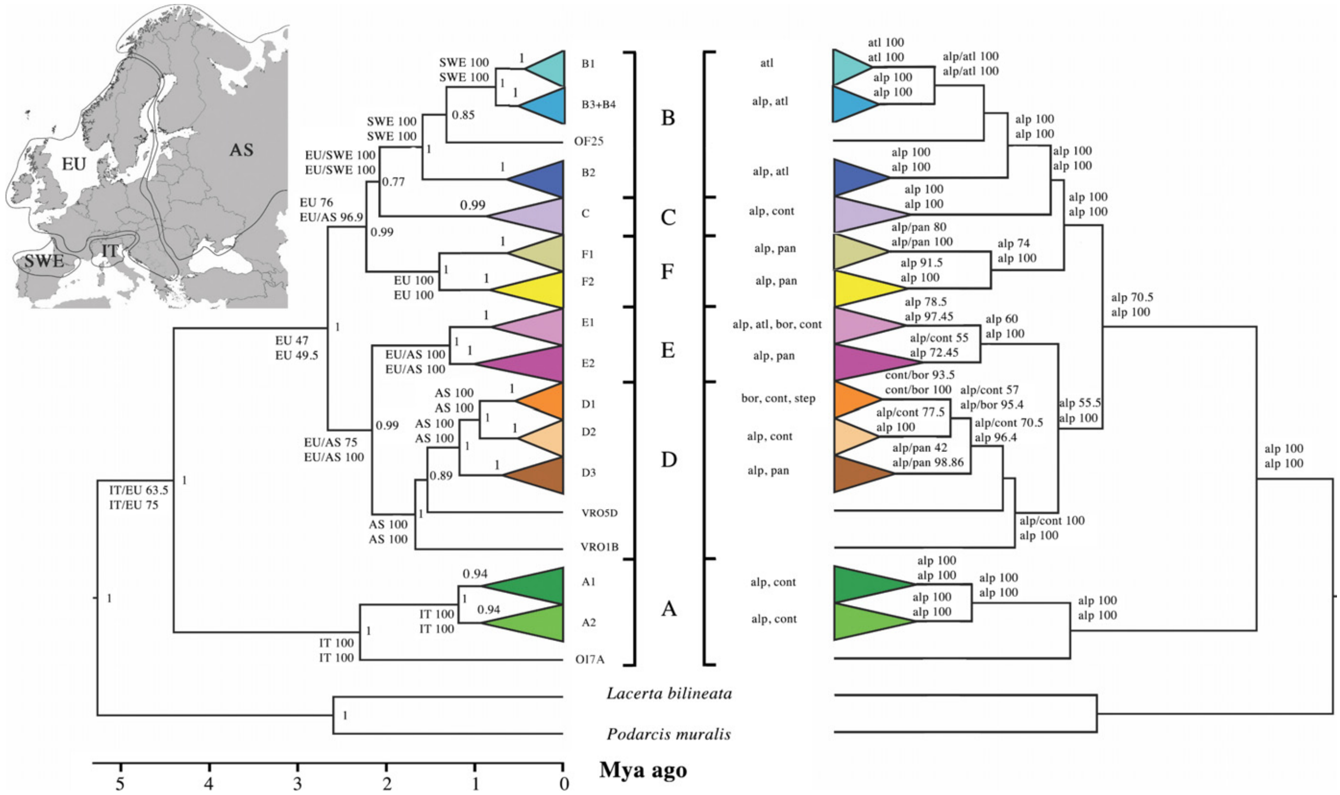

FIGURE 3 Bayesian phylogenetic tree of the 231 *Zootoca vivipara* samples and the outgroups included in this study (see Appendix 2 for a tree showing all 231 specimens). Node numbers are posterior probabilities. Different colours represent different clades and different tonalities different subclades. Subclade, clade and currently inhabited biogeographic regions are given in the middle of the two trees. The map shows the major phylogeographic areas and their nomenclature. Reconstructions of ancestral area (left tree, above the lines) and ancestral biogeographic region (right tree) are given for each node. Numbers beside the reconstructed area/biogeographic region correspond to the attribution probability (in %), with upper numbers corresponding to the combined statistic and lower numbers to the reconstruction method with the highest support. Acronyms correspond to phylogeographic areas: Italy (IT), Northern Europe and Balkan (EU), Asia (AS) and South-Western Europe (SWE) and biogeographic regions (EEA, 2012): alpine (alp), atlantic (atl), continental (cont), boreal (bor), pannonian (pan) and steppic (step)

Groba et al., 2006), and they differed from the positions of another tree based on mitochondrial DNA and a tree based on nuclear DNA (Cornetti et al., 2014). Our phylogenetic Bayesian tree included more taxa and more genetic information than the previous ones, and all but one of the nodes grouping different clades had high statistical support ($pp \geq 0.99$, Figure 3). Several subclades (Figure 3, Table 2) were detected and they correspond to separated groups in the TCS haplotype network (Figure 2; exception subclades of clade A). Our analyses provide robust evidence for 9 and moderate evidence for 2 subclades (Figure 3, Table 2), and they suggest that at least another 2 subclades (Western Romanian Carpathians, and Southern Carpathians) may exist. However, their validity needs confirmation with more extensive sampling.

4.2 | Current geographical distribution

Clades and subclades exhibit current allopatry of over large areas (Figure 1), with strong geographical overlap of clades only occurring in the Central European Contact Zone located in the East of the Eastern Alps (mainly in Kärnten, Steiermark, Northern Slovenia and North-Eastern Italy; Figure 1). Moderate overlap of three subclades existed in the Pyrenees and in the Carpathian Mountains and no overlap existed among subclades of D and E, and between B1 and the other subclades of B. However, given the close geographical proximity of D2, D3 and E2, more precise fine-scale sampling is required to clarify whether geographical overlap may exist at a smaller geographical scale. Secondary contacts among clades or subclades have been described in the Pyrenees (subclades B2 and B3 + B4; Mila et al., 2013) and in the Central European contact zone (clades A and D; Lindtke, Mayer, & Böhme, 2010). In this study, populations of the two contact locations were not included and our study provided no evidence for clade mixing outside these two contact locations, since in the TCS network (Figure 2) clades were clearly separated by many mutational steps and no intermediate haplotypes existed. The strong genetic structure and the described mixing in narrow contact zones (Lindtke et al., 2010; Mila et al., 2013) suggest that the observed contacts are rather recent, what points to future clade mixing due to current climate change. In contrast to these contacts, a study investigating a contact zone between clade A and E located in Northern Italy (*c.* 270 km east of the contact zone

TABLE 2 Clades and subclades of *Z. vivipara* and their distribution. Clade and subclade abbreviations, inhabited biogeographic region (EEA, 2012) and number and abbreviation of countries in which their occurrence has been confirmed are given

Clade	Subclade	Distribution range	Biogeographic region	Countries (*N*/abbreviations[a])	
A	A1	North-West Italy	Alpine, continental	2	A, I
A	A2	North-East Italy	Alpine, continental	3	A, I, SLO
B	B4	North-East Spain	Alpine	1	E
B	B3	North-Central Spain	Alpine, atlantic	1	E
B	B1	North-West Spain	Atlantic	1	E
B	B2	Southern France	Alpine, atlantic	3	AND, E, F
C	None	Austria	Alpine, continental	1	A
D	D1	North and East Eurasia	Boreal, continental, steppic	9	BY, FIN, J, LT, LV, RUS, S, UA
D	D2	Central East-Carpathians	Alpine, continental	1	RO
D	D3	North-West and Central Inner East-Carpathians	Alpine, pannonian	4	H, RO, SK, UA
E	E1	Western Europe and Southern Balkan	Alpine, atlantic, boreal, continental	15	A, B, BG, CH, D, F, GB, I, IRL, L, MNE, N, NL, S, SRB
E	E2	Eastern Europe	Alpine, pannonian	5	H, PL, RUS, SK, UA
F	F1	South-West Austria	Alpine, pannonian	3	A, H, SLO
F	F2	South-East Austria	Alpine, pannonian	2	A, H

[a] Acronyms: Austria (A), Andorra (AND), Belgium (B), Bulgaria (BG), Belarus (BY), Switzerland (CH), Germany (D), Spain (E), France (F), Finland (FIN), Great Britain (GB), Hungary (H), Italy (I), Irland (IRL), Japan (J), Luxemburg (L), Lithuania (LT), Latvia (LV), Montenegro (MNE), Norway (N), Netherlands (NL), Romania (RO), Russia (RUS), Sweden (S), Slovakia (Sk), Slovenia (SLO), Serbia (SRB), Ukraine (UA).

described by Lindtke et al., 2010) detected no introgression and hypothesized that reproductive isolation may exist (Cornetti et al., 2014). The absence of clade mixing over most of the distribution and the fact that in the Central European contact zone the colonization of the different locations is difficult to explain without contacts among clades, is in agreement with reproductive isolation. However, detailed fine-scale sampling and experimental studies are required to provide robust evidence for or against these hypotheses.

4.3 | Evolutionary history and refugia

Phylogenetic analyses and molecular dating indicate that the oviparous clade A and the other *Z. vivipara* clades split during the Pliocene 4.4 (4.2–4.6; 95% CI, Appendix S3) Ma, and the ancestral area reconstruction (Figure 3) suggested that the origin of *Z. vivipara* was in the Italian or the European phylogeographic area (Figure 4a). Given that earlier diverging genera (*Phoenicolacerta*, *Atlantolacerta*, *Gallotia*; Pyron, Burbrink, & Wiens, 2013) inhabit the middle east and Cyprus (*Phoenicolacerta*), Northern Africa (*Atlantolacerta*) and the Canary Islands (*Gallotia*), the most likely scenario is that the ancestor of *Z. vivipara* inhabited the Italian or the south of the European phylogeographic area (Figure 4a) and that the ancestor of the clades C, F and B moved Northwards (most likely through the Vienna Bassin). The ancestral area reconstruction and the high haplotype diversity in the Carpathians (Figures 2 and 3), suggest that the common ancestor of clade D and E colonized the Carpathians, where diversification into clade D and E happened (Figure 4a), and from where clade D (i.e. subclade D1) and E (i.e. subclade E1) colonized Asia and Northern to Western Europe (see below) respectively. The ancestor of clade B colonized South Western Europe, where differentiation into B2 and the other subclades happened 1.6 (1.2–2.0; 95% CI) Ma (Figure 3; Appendix S3).

The divergence of clades B to E occurred between 2.7 (2.2–3.1; 95% CI) and 2.0 (1.6–2.4; 95% CI) Ma (Figure 3), at the Pliocene/Pleistocene boundary, when major climatic changes existed that led to habitat fragmentation (Bennet, 1990), segregation into refugia and diversification of several ectothermic species in the Mediterranean region and in the Carpathian Mountains (e.g. Ursenbacher et al., 2006). In contrast, the evolution of the subclades is younger and happened between 1.6 Ma (1.2–2.0; 95% CI) and 0.8 Ma (0.5–1.0; 95% CI) (see Appendix S3), most likely as a consequence of glaciations and diversification due to genetic drift in different refugia (Figure 4b).

Star-like haplotype networks, typical for fast population expansion (Hewitt, 1996), existed in clade A and in subclades B2, B3 + B4, F2, D1 and E1. Moreover, two subclades (D1 and E1) exhibit vast current distributions in areas covered by ice during the Last Glacial Maximum (LGM: 21,000 years ago, Figure 4). These patterns are congruent with glacial refugia and post-glacial geographical expansion in Southern France (B2; Surget-Groba et al., 2001), North-East and North-Central Spain (B3 + B4) and Northern Italy/Slovenia (Surget-Groba et al., 2002). This points to the typical geographical south-northward contraction-expansion due to glaciations and interglacials (Hewitt, 1996) in B and A, and to a refugia of clade B2 in the North of the glacial shield covering the Pyrenees, and subsequent southward expansion (Figure 4b). E1 and D1 exhibited

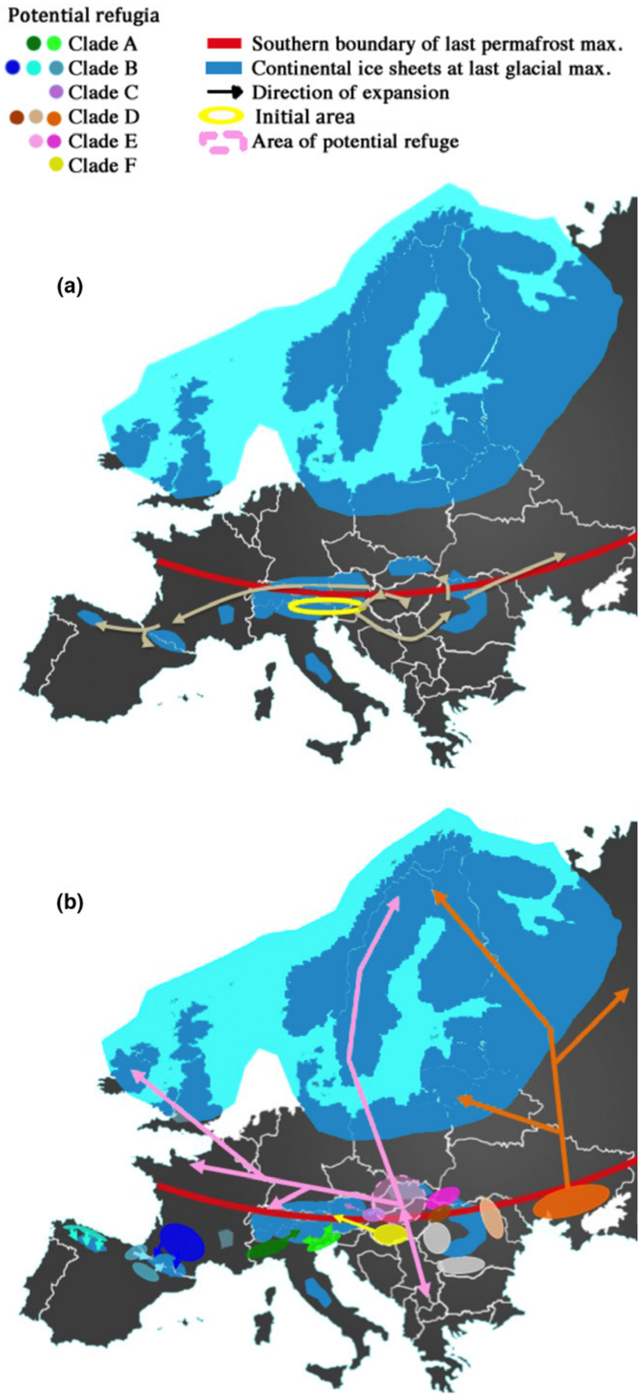

FIGURE 4 Map showing the reconstructed evolutionary history of *Zootoca vivipara* in Eurasia. (a) Suggested colonization routes during Pliocene and starting Pleistocene of the different clades, subclades and their ancestors and (b) glacial refugia during Pleistocene glaciations and post-glacial expansion patterns. In (a), the species' reconstructed initial area (around 4.4 Ma) is delimitated by a yellow ellipse and the most likely colonization routes are indicated with arrows. In (b) potential location of glacial refugia of the different clades and subclades are delimitated with ellipsis and directions of post-glacial expansion with arrows; the colours correspond to those of the clades/subclades in Figures 2 and 3. Potential, yet to be confirmed refugia are indicated in grey. The limit of the southern boundary of the permafrost limit during the LGM (around 21,000 years ago) is indicated in red

longitudinal expansion as well as northward expansion (Figure 4). The former colonizing Western and Northern Europe, as well as Southern Europe (Bulgaria, Montenegro, Serbia) and the later colonizing Eastern Europe and Asia as well as North-Eastern Europe and Northern Asia. The ancestral area reconstruction, haplotype network and current distribution (Figures 1–3) suggest that subclade D1 had a refugia in the Asian region, most likely close to the black sea given the extent of the glaciers and the permafrost during the last glacials (see below; Figure 3B; Vandenberghe et al., 2014). The location of the refugia of subclade E1 was reconstructed in the European or Asian area (Figure 3), and the genetic results do not allow to draw more precise conclusions about its location.

Subclade E1 (Figure 2) inhabits two isolated geographical areas (Northwestern Europe and the Southern half of the Balkans). The phylogenetic analyses unravelled that two haplotypes prevailed exclusively on the Balkans (one in the Pirin Mountains, and the other one in Montenegro, Serbia and NW Bulgaria; Figure 2). Moreover, no evidence for the existence of subclades or a monophyletic origin of the Balkan specimens existed and the TCS network showed that the genetically closest haplotypes located in Northern Europe differed by one base pair (Figure 2). This points to the existence of historic connections between the two geographical areas (Heulin, Surget-Groba, Sinervo, Miles, & Guiller, 2011), allowing the colonization of the Balkans from the north and subsequent northward migration (Figures 2 and 4b).

While several subclades and clades suggest fast colonization patterns, others exhibit high diversity, no star-like patterns and many mutational steps among haplotypes (Figure 2, clade C, subclades D3, E2, F1), suggesting that they diverged in different subrefugia, i.e. refugia within refugia (e.g. Hewitt, 1996), from where no important expansion happened. Clade F consisted of two statistically supported subclades (F1 and F2), and in clade C no clusters existed, suggesting two potential refugia of clade F and one of clade C, all of them most likely located in the Pannonian/Vienna basin (Figure 4b).

4.4 | Biogeographic implications

The results of the phylogeographic analyses point to refuges located in Western Europe (North, South and West to the Pyrenees), Central Europe (most likely in the Pannonian/Vienna Basin), Eastern Europe (East, West and probably as well South of the Carpathian Mountains), North to the black Sea and in Southern Europe (Northern Italy). No explicit evidence existed for refuges North of the Alps, but the precise location of the refuge of the ancestor of subclade E1 could not be reconstructed and the ancestral area reconstruction points to the European or Asian area and thus to a refuge north, south or east of the Alps.

The existence of permafrost even during Würm (115,000–10,000 years ago) down to the46° N latitude (Figure 4b), is incongruent with Northern refuges, since *Z. vivipara* cannot endure temperatures in hibernacula (5–20 cm below ground) below −10°C and since permafrost temperatures at this depth are frequently below −15°C (Berman et al., 2016). More likely and more congruent with the TCS network is a refuge South-East or in the East of the Alps, colonization of the Balkan and subsequent colonization of Northern and Western Europe (Figures 2 and 4). The fast and recent geographical expansion of subclades E1 and D1 is in line with the northward-shift of the southern permafrost limit and the rapid colonization of newly available habitat. Southern refuges of clade C and F in the Pannonian/Vienna Basin, of clade A in Northern Italy, and of D1 in the north of the black sea, is in line with permafrost forcing *Z. vivipara* into areas south to the southern permafrost limit during the last glaciations (Figure 4b). The split of clade B from the rest of *Z. vivipara*, its location in the Pyrenees, and the absence in the rest of Europe of individuals belonging to this clade, could be explained by early Pleistocene glaciations during which populations of *Z. vivipara* located North of the Alps may have gone extinct, thus disconnecting SW European populations from the rest of the distribution of *Z. vivipara*. Finally, the haplotype network (Figure 2) and the current geographical distributions (Figure 1) also suggest the existence of current admixture among subclades of clades F and A, most likely as a consequence of post-glacial colonization of areas previously covered by permafrost, in line with interbreeding observed between clades A and E in the central European contact zone (Lindtke et al., 2010), and between subclades of the clade B in the Pyrenean suture zone (Mila et al., 2013).

All detected refugia were south of the southern permafrost limit of the last glaciation (Figure 4b), and thus *Z. vivipara*'s refuges are in locations predicted for temperate zone species (Hewitt, 1996). This suggests that species inhabiting the boreo-alpine region do not necessarily exhibit Northern refuges. Interestingly, the reconstruction of ancestral biogeographic region shows that *Z. vivipara* originally inhabited the alpine region and that all 13 subclades colonized new biogeographic regions in the last 0.9 Ma (Figure 3), suggesting adaptation to different climates. The two most widely distributed subclades (D1, E1) colonized the continental and boreal region (Figure 3) after glacial retraction and E1 also colonized the Atlantic region, i.e. the biogeographic regions covering the biggest part of their current distribution. Since in the other subclades the colonization of new biogeographic regions happened approximately at the same time (Figure 3) and since none of the biogeographic regions mainly inhabited by D1 or E1 is exclusively inhabited by one subclade (the continental biogeographic region is inhabited by: A1, A2, C, D1, D2, E1; boreal by D1, E1; Atlantic, by B1, B2, B3 + B4, E1; Table 2), adaptation of D1 and E1 to different climates cannot explain their success.

The reconstructed refugia of D1 and E1 were in the East and North of the refugia of the other clades/subclades (Figure 4b) and thus at the forefront where new habitat became available due to glacial retraction. This suggests that the refugias' location may have allowed for colonization with low or inexistent competition with other clades/subclades, potentially explaining the large and recent expansion of the two clades. This idea is supported by the lack of large geographical expansion of the clades/subclades that retracted into refuges more south- or more west-wards (A, B, C, F, D2, D3). These clades/subclades colonized the same biogeographic regions approximately at the same time as D1 and E1 (Figure 3) and in contrast to D1 and E1, their refugia were in close geographical proximity. This suggests that they may have been exposed to intense competition with other clades (see also Central European contact zone, Figure 1), what may have hindered the colonization of newly available habitat.

5 | GENERAL CONCLUSIONS

The European common lizard (*Z. vivipara*) exhibits a complex evolutionary history that was mainly driven by glacial expansion and retraction patterns. The biogeographic patterns are congruent with those observed in pure temperate zone species and the current distribution is very similar to the distribution of *V. berus*, a terrestrial snake species that preys on *Z. vivipara* (e.g. Sebela, 1989) and inhabits the same biogeographic regions. Detected refugia were in similar locations as in *Z. vivipara* (Ursenbacher et al., 2006) and a Northern refuge of a subclade of *V. berus* has been proposed. However, *V. berus* has been classified as non-freeze tolerant (Andersson & Johansson, 2001), suggesting that in both species the subclade with potential Northern refugia may have had a refugia south of the southern permafrost limit. In both species, the clade for which a Northern refuge has been suggested colonized Sweden and Norway, and similarly, in both species the same clade that colonized Asia, also colonized Finland and was present in the Carpathian Mountains. In both species, the glacial refuges of the two clades with rapid geographical expansion have been reconstructed in the same geographical locations. This points to similar evolutionary patterns, potentially due to the colonization of areas previously covered by permafrost. Colonization of areas previously covered by permafrost also resulted in similar colonization patterns outside non-avian sauropsids, for example in the bank vole *Myodes glareolus* (Deffontaine et al., 2005). *Zootoca vivipara* and *V. berus* inhabit quite similar ecological niches, and the ecological niches of all other European terrestrial Sauropsids include either more thermophile habitats (the largest amount of species), or are restricted to narrow alpine areas (e.g. *Iberolacerta*, and *Vipera walser*), suggesting that only species with relatively good cold tolerance and inhabiting an ample thermal niche may benefit from glacial retraction and colonize vast areas. In both species, the refuge in the East of the Carpathian Mountains is the most eastward, and the refuge of the other fast expanding subclade (E1, Central European subclade; Ursenbacher et al., 2006) the most northward glacial refuge. This suggests that glacial retraction, the location of the refugia and absence of competition, may have been important for fast geographical expansion and thus for colonization success of both species. The results further show that although inhabiting the boreo-alpine region, both species and all their subclades/lineages exhibit responses to glaciations as do temperate zone species, suggesting that adaptations leading to real boreo-alpine behaviour (southward expansion from Northern refugia) may take many million years to evolve.

ACKNOWLEDGEMENTS

We thank M. Urieta for help during sampling of *Z. vivipara*. J.L. Horreo was supported by two Spanish MINECO post-doc grants (FPDI-2013-16116 and IJCI-2015-23618). W. Mayer and M.L.A. Ceirans provided samples. Project funds were provided by the Swiss National Science Foundation (PPOOP3_128375, PP00P3_152929/1 to P.S.F.), the Spanish Ministry of Education and Science (CGL2008-01522, CGL2012-32459, CGL2016-76918 to P.S.F.), and the Academy of Finland (grant no. 7108955 to T. Oksanen).

ORCID

Jose L. Horreo http://orcid.org/0000-0002-9851-7651
Teresa Suárez http://orcid.org/0000-0002-4553-3818
Merel C. Breedveld http://orcid.org/0000-0002-3198-7683
Yann Surget-Groba http://orcid.org/0000-0001-7612-4892
Tuula A. Oksanen http://orcid.org/0000-0003-3481-6158
Patrick S. Fitze http://orcid.org/0000-0002-6298-2471

REFERENCES

Andersson, S., & Johansson, L. (2001). Cold hardiness in the boreal adder, *Vipera berus*. *Cryo Letters*, *22*, 151–156.

Beerli, P., Hotz, H., & Uzzell, T. (1996). Geologically dated sea barriers calibrate protein clock for Aegean water frogs. *Evolution*, *50*, 1676–1687. https://doi.org/10.1111/j.1558-5646.1996.tb03939.x

Bennet, K. D. (1990). Milankovitch cycles and their effects on species in ecological and evolutionary time. *Palaeobiology*, *16*, 11–21. https://doi.org/10.1017/S0094837300009684

Berman, D. L., Bulakhova, N. A., Alfimov, A. V., & Meshcheryakova, E. N. (2016). How the most northern lizard, *Zootoca vivipara*, overwinters in Siberia. *Polar Biology*, *39*, 2411–2425. https://doi.org/10.1007/s00300-016-1916-z

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., . . . Drummond, A. J. (2014). BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *10*, e1003537. https://doi.org/10.1371/journal.pcbi.1003537

Cornetti, L., Ficetola, G. F., Hoban, S., & Vernesi, C. (2015). Genetic and ecological data reveal species boundaries between viviparous and oviparous lizard lineages. *Heredity*, *115*, 517–526. https://doi.org/10.1038/hdy.2015.54

Cornetti, L., Menegon, M., Giovine, G., Heulin, B., & Vernesi, C. (2014). Mitochondrial and nuclear DNA survey of *Zootoca vivipara* across the eastern italian Alps: Evolutionary relationships, historical demography and conservation implications. *PLoS ONE*, *9*, e85912. https://doi.org/10.1371/journal.pone.0085912

Deffontaine, V., Libois, R., Kotlik, P., Sommer, R., Nieberding, C., Paradis, E., . . . Michaux, J. R. (2005). Beyond the Mediterranean peninsulas: Evidence of central European glacial refugia for a temperate forest mammal species, the bank vole (*Clethrionomys glareolus*). *Molecular Ecology*, *14*, 1727–1739. https://doi.org/10.1111/j.1365-294X.2005.02506.x

EEA. (2012). *Protected areas in Europe - An overview*. (EEA Report No 5/2012). Copenhagen: European Environmental Agency.

Fernández-Palacios, J. M., Rijsdijk, K. F., Norder, S. J., Otto, R., Nascimiento, L., Fernández-Lugo, S., . . . Whittaker, R. J. (2016). Towards a glacial-sensitive model of island biogeography. *Global Ecology and Biogeography*, *25*, 817–830. https://doi.org/10.1111/geb.12320

Flot, J. F. (2010). SeqPHASE: A web tool for interconverting PHASE input/output files and FASTA sequence alignments. *Molecular Ecology Ressources*, *10*, 162–166. https://doi.org/10.1111/j.1755-0998.2009.02732.x

Heulin, B., & Guillaume, C. P. (1989). Extension géographique des populations ovipares de *Lacerta vivipara*. *Revue d'Ecologie*, *44*, 39–45.

Heulin, B., Guillaume, C. P., Vogrin, N., & Surget-Groba, Y. (2000). Further evidence of the existence of oviparous populations of *Lacerta (Zootoca) vivipara* in the NW of the Balkan Peninsula. *Comptes rendus de l'Académie des sciences. Série III, Sciences de la vie.*, *323*, 461–468.

Heulin, B., Surget-Groba, Y., Sinervo, B., Miles, D., & Guiller, A. (2011). Dynamics of haplogroup frequencies and survival rates in a contact zone of two mtDNA lineages of the lizard *Lacerta vivipara*. *Ecography*, *34*, 436–447. https://doi.org/10.1111/j.1600-0587.2010.06540.x

Hewitt, G. M. (1996). Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, *58*, 247–276. https://doi.org/10.1111/j.1095-8312.1996.tb01434.x

Hikida, H. (2002). *Natural history of the reptiles*. Tokyo: University of Tokyo Press.

Horreo, J. L., Peláez, M. L., & Fitze, P. S. (2015). Skin sheds as a useful DNA source for lizard conservation. *Phyllomedusa*, *14*, 73–77. https://doi.org/10.11606/issn.2316-9079.v14i1p73-77

Horreo, J. L., Peláez, M. L., Suárez, T., & Fitze, P. S. (2018). Development and characterization of 79 nuclear markers amplifying in viviparous and oviparous clades of the European common lizard. *Genetica*, *146*, 115–121. https://doi.org/10.1007/s10709-017-0002-y

Hudson, R. R., & Kaplan, N. L. (1985). Statistical properties of the number of recombination events in the history of a sample of DNA sequences. *Genetics*, *111*, 147–164.

Kajtoch, L., Cieslak, E., Varga, Z., Paul, W., Mazur, M. A., Sramkó, G., & Kubisz, D. (2016). Phylogeographic patterns of steppe species in Eastern Central Europe: A review and the implications for conservation. *Biodiversity and Conservation*, *25*, 2309–2339. https://doi.org/10.1007/s10531-016-1065-2

King, B., & Lee, M. S. Y. (2015). Ancestral state reconstruction, rate heterogeneity, and the evolution of reptile viviparity. *Systematic Biology*, *64*, 632–544. https://doi.org/10.1093/sysbio/syv005

Kluge, A. G. (1998). Total evidence or taxonomix congruence: Cladistics or consensus classification. *Cladistics*, *14*, 151–158. https://doi.org/10.1111/j.1096-0031.1998.tb00328.x

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*, 1870–1874. https://doi.org/10.1093/molbev/msw054

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*, 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Lindtke, D., Mayer, W., & Böhme, W. (2010). Identification of a contact zone between oviparous and viviparous common lizards (*Zootoca vivipara*) in central Europe: Reproductive strategies and natural hybridization. *Salamandra*, *46*, 73–82.

Macey, J. R., Larson, A., Ananjeva, N. B., Fang, Z., & Papenfuss, T. J. (1992). Two novel gene Orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. *Molecular Biology and Evolution*, *14*, 91–104.

Mila, B., Surget-Groba, Y., Heulin, B., Gosá, A., & Fitze, P. S. (2013). Multilocus phylogeography of the common lizard *Zootoca vivipara* at the Ibero-Pyrenean suture zone reveals lowland barriers and high-elevation introgression. *BMC Evolutionary Biology*, *13*, 192. https://doi.org/10.1186/1471-2148-13-192

Mittelbach, G. G., Schemske, D. W., Cornell, H. V., Allen, A. P., Borwn, J. M., Bush, M. B., . . . Turelli, M. (2007). Evolution and the latitudinal diversity gradient: Speciation, extinction and biogeography. *Ecology*

Letters, *10*, 315–331. https://doi.org/10.1111/j.1461-0248.2007.01020.x

Posada, D. (2008). jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution*, *25*, 1253–1256. https://doi.org/10.1093/molbev/msn083

Provan, J., & Bennet, K. D. (2008). Phylogeographic insights into cryptic glacial refugia. *Trends in Ecology & Evolution*, *23*, 564–571. https://doi.org/10.1016/j.tree.2008.06.010

Pyron, R. A., Burbrink, T. B., & Wiens, J. J. (2013). A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology*, *13*, 93. https://doi.org/10.1186/1471-2148-13-93

Ree, R. H., & Smith, S. A. (2008). Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, *57*, 4–14. https://doi.org/10.1080/10635150701883881

Sebela, M. (1989). The diet of the common viper (*Vipera berus*) in the area of Mt Královicky Snèzník. *Folia Zoologica*, *29*, 117–123.

Stephens, M., Smith, N. J., & Donnelly, P. (2001). A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics*, *68*, 978–989. https://doi.org/10.1086/319501

Stewart, J. R., Lister, A. M., Barnes, I., & Dalen, L. (2010). Refugia revisited: Individualistic responses of species in space and time. *Proceedings of the Royal Society B-Biological Sciences*, *277*, 661–671. https://doi.org/10.1098/rspb.2009.1272

Surget-Groba, Y., Heulin, B., Ghielmi, S., Guillaume, C. P., & Vogrin, N. (2002). Phylogeography and conservation of the populations of *Zootoca vivipara* carniolica. *Biological Conservation*, *106*, 365–372. https://doi.org/10.1016/S0006-3207(01)00263-4

Surget-Groba, Y., Heulin, B., Guillaume, C. P., Puky, M., Semenov, D., Orlova, V., … Smajda, B. (2006). Multiple origins of viviparity, or reversal from viviparity to oviparity? The European Common Lizard (*Zootoca vivipara*, Lacertidae) and the evolution of parity. *Biological Journal of the Linnean Society*, *87*, 1–11. https://doi.org/10.1111/j.1095-8312.2006.00552.x

Surget-Groba, Y., Heulin, B., Guillaume, C. P., Thorpe, R. S., Kupriyanova, L., Bogrin, N., … Smith, N. (2001). Intraspecific phylogeography of *Lacerta vivipara* and the evolution of viviparity. *Molecular Phylogenetics and Evolution*, *18*, 449–459. https://doi.org/10.1006/mpev.2000.0896

Ursenbacher, S., Carlsson, M., Helfer, V., Tegelström, H., & Fumagalli, L. (2006). Phylogeography and Pleistocene refugia of the adder (*Vipera berus*) as inferred from mitochondrial DNA sequence data. *Molecular Ecology*, *15*, 3425–3437. https://doi.org/10.1111/j.1365-294X.2006.03031.x

Vandenberghe, J., French, H. M., Gorbunov, A., Marchenko, S., Velichko, A. A., Jin, H., … Wan, X. (2014). The Last Permafrost Maximum (LPM) map of the Northern Hemisphere: Permafrost extent and mean annual air temperatures, 25–17 ka BP. *Boreas*, *43*, 652–666. https://doi.org/10.1111/bor.12070

Yu, Y., Harris, A. J., Blair, C., & He, X. J. (2015). RASP (Reconstruct Ancestral State in Phylogenies): A tool for historical biogeography. *Molecular Phylogenetics and Evolution*, *87*, 46–49. https://doi.org/10.1016/j.ympev.2015.03.008

Yu, Y., Harris, A. J., & He, X. J. (2010). S-DIVA (statistical dispersal-vicariance analysis): A tool for inferring biogeographic histories. *Molecular Phylogenetics and Evolution*, *56*, 848–850. https://doi.org/10.1016/j.ympev.2010.04.011

BIOSKETCH

J.L.H. and P.S.F. are interested in evolutionary biology, ecology and population genetics, and in short- and long-term population dynamics. They are particularly interested in how ecology drives genetic evolution and adaptation.

J.L.H. and P.S.F. designed the study. J.L.H., M.C.B., B.H. and P.S.F. sampled Spanish populations and populations in Southern France. B.H., Y.S.G. and T.A.O. provided tissue samples. J.L.H., M.L.P., T.S. and P.S.F. developed genetic markers, amplified the gene fragments, and prepared the alignments. J.L.H. and P.S.F. analysed the data and wrote the article, and all authors commented the last version of the manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Horreo JL, Pelaez ML, Suárez T, et al. Phylogeography, evolutionary history and effects of glaciations in a species (*Zootoca vivipara*) inhabiting multiple biogeographic regions. *J Biogeogr*. 2018;00:1–12. https://doi.org/10.1111/jbi.13349