



Universidad Autónoma  
de Madrid

**Biblos-e Archivo**  
Repositorio Institucional UAM

**Repositorio Institucional de la Universidad Autónoma de Madrid**

<https://repositorio.uam.es>

Esta es la **versión de autor** del artículo publicado en:  
This is an **author produced version** of a paper published in:

Journal of Zoological Systematics and Evolutionary Research 58.1 (2020): 459-474

**DOI:** <https://doi.org/10.1111/jzs.12323>

**Copyright:** © 2019 Blackwell Verlag GmbH

El acceso a la versión del editor puede requerir la suscripción del recurso

Access to the published version may require subscription

1 **A complex scenario of glacial survival in Mediterranean and continental refugia of a**  
2 **temperate continental vole species (*Microtus arvalis*) in Europe.**

3  
4 **Running Title:** Spatially explicit phylogeography of *M. arvalis*  
5

6  
7 Jesús T. García<sup>1</sup>, Julio Domínguez-Villaseñor<sup>1</sup>, Fernando Alda<sup>2</sup>, María Calero-Riestra<sup>1</sup>, Pedro  
8 Pérez Olea<sup>3</sup>, Juan Antonio Fargallo<sup>4</sup>, Jesús Martínez-Padilla<sup>5</sup>, Jesús Herranz<sup>3</sup>, Juan José  
9 Oñate<sup>3</sup>, Ana Santamaría<sup>1</sup>, Yoav Motro<sup>6</sup>, Carole Attie<sup>7</sup>, Vincent Bretagnolle<sup>8,9</sup>, Juan Delibes<sup>10</sup>,  
10 Javier Viñuela<sup>1</sup>  
11

12 1. IREC, Instituto de Investigación en Recursos Cinegéticos (CSIC-UCLM-JCCM), Av/ Ronda de Toledo 12,  
13 13071 Ciudad Real, Spain.

14  
15 2. Museum of Natural Science, Department of Biological Sciences, Louisiana State University, 119 Foster Hall,  
16 Baton Rouge, LA 70803, USA.

17 3. Terrestrial Ecology Group (TEG), Departamento de Ecología, Universidad Autónoma de Madrid, c/ Darwin,  
18 2, 28049 Madrid (Madrid), Spain.

19  
20 4. Department of Evolutionary Ecology, Museo Nacional de Ciencias Naturales MNCN-CSIC. C/ José Gutiérrez  
21 Abascal 2, 23006 Madrid, Spain.

22 5. Research Unit of Biodiversity, UMIB (CSIC, PA), University of Oviedo, C/Gonzalo Gutiérrez Quirós s/n,  
33600 Mieres, Spain

6. Plant Protection and Inspection Services, Ministry of Agriculture and Rural Development, POB 78, Bet  
Dagan, 50250, Israel.

23 7. 3 rue du puits, 79360 Vaubalier, France.

24  
25 8. Centre d'Etudes Biologiques de Chizé, UMR 7372, CNRS and Université de la Rochelle

26  
27 9. LTSER Zone Atelier Plaine and Val de Sèvre, CNRS, F-79360 Villiers-en-Bois

28  
29 10. C/ Pérez Cidón 28, 28027 Madrid

30  
31 Corresponding author: Jesús T. García (jesusgarcia.irec@gmail.com)

32  
33 **Keywords:** Glacial refugia, common vole, mitochondrial DNA, spatially-explicit  
34 phylogeography, “Continental” species.

35 **Abstract**

36

37 The role of glacial refugia in shaping contemporary species distribution is a long-standing  
38 question in phylogeography and evolutionary ecology. Recent studies are questioning  
39 previous paradigms on glacial refugia and postglacial recolonization pathways in Europe, and  
40 more flexible phylogeographic scenarios have been proposed. We used the widespread  
41 common vole *Microtus arvalis* as a model to investigate about the origin, locations of glacial  
42 refugia and dispersal pathways, in the group of “Continental” species in Europe. We used a  
43 Bayesian spatio-temporal diffusion analysis (relaxed random walk model) of cytochrome *b*  
44 sequences across the species range, including newly collected individuals from 10 Iberian  
45 localities and published sequences from 68 localities across 22 European countries. Our data  
46 suggest that the species originated in Central Europe, and we revealed the location of multiple  
47 refugia (in both southern peninsulas and continental regions) for this continental model  
48 species. Our results confirm the monophyly of Iberian voles and the pre-LGM divergence  
49 between Iberian and European voles. We found evidence of restricted post-glacial dispersal  
50 from refugia in Mediterranean peninsulas. We inferred a complex evolutionary and  
51 demographic history of *M. arvalis* in Europe over the last 50,000 years that does not  
52 adequately fit previous glacial refugia scenarios. The phylogeography of *M. arvalis* provides  
53 a paradigm of ice-age survival of a temperate continental species in western and eastern  
54 Mediterranean peninsulas (sources of endemism) and multiple continental regions (sources of  
55 postglacial spread). Our findings also provide support for a major role of large European river  
56 systems in shaping geographical boundaries of *M. arvalis* in Europe.

57

## 58 INTRODUCTION

59

60 It is widely understood that Pleistocene glacial refugia are a key factor shaping species  
61 evolutionary history and distribution worldwide. However, the number, size, geographical  
62 locations, duration of isolation, and role of glacial refugia as a source for postglacial  
63 recolonization remain subjects of intense scientific discussions (Fraser, Nikula, Ruzzante, &  
64 Waters, 2012; Hewitt, 2000; Schmitt, 2007). Recent studies have challenged traditional views  
65 such as temperate species sharing common glacial refugia in the European Mediterranean  
66 peninsulas (i.e. Iberia, Italy and the Balkans), or that these southern refugia are the only  
67 sources of postglacial colonization (Stewart, 2003). New and more complex phylogeographic  
68 hypotheses/scenarios have been proposed for different biogeographical groups (i.e.  
69 Mediterranean, continental and Arctic/Alpine species, *sensu* (De Lattin, 1967), such as the  
70 existence of extra-Mediterranean glacial refugia for Mediterranean species (reviewed in  
71 Schmitt & Varga, 2012) or steppe-adapted species (Garcia, Alda, et al., 2011; Garcia,  
72 Mañosa, et al., 2011), the evidence of cryptic refugia for cold-adapted species (Cruzan &  
73 Templeton, 2000; Stewart & Lister, 2001), the occurrence of both ‘macrorefugia’ and  
74 ‘microrefugia’ for some taxa (Joger et al., 2007; Rull, 2009, 2010), the existence of ‘refugia-  
75 within-refugia’ (Abellán & Svenning, 2014; Gómez & Lunt, 2007), or the consideration of  
76 the Oceanic-Continental gradient as a further biogeographic dimension, in addition to the  
77 traditional north-south axis, in defining the location of refugia for some biogeographical  
78 groups (Stewart, Lister, Barnes, & Dalén, 2010).

79

80 Recent advances in the refugial concept (Provan & Bennett, 2008), and the ample evidence  
81 for the individualistic (species- or group-specific) response to extreme climate events (see  
82 (Palmer et al., 2017), has also direct implications for the inferred scenarios of recolonization  
83 of many widespread species in the Palaearctic. Accordingly, new paradigms and conceptual  
84 frameworks about the extent and pathways of postglacial expansions from these refugia have  
85 arisen. Despite the classic scenario in Europe proposes northwards routes of colonization  
86 from accepted refugia in the three Mediterranean peninsulas (Hewitt, 1996), other studies  
87 have suggested a far more complex evolutionary history in which, for some species,  
88 Mediterranean peninsulas played a minor role in postglacial colonization (Bilton et al., 1998;  
89 Tougaard, Renvoisé, Petitjean, & Quéré, 2008; Tougaard, 2016). Overall, these investigations  
90 stress the need for additional continent-wide specific studies to contrast these models and  
91 really understand the complex biogeographical patterns of the European fauna (Schmitt, 2007;  
92 Stewart & Lister, 2001), especially in the group of continental species (Schmitt, 2007).

93

94 Palaearctic mammals (e.g. brown bear *Ursus arctos*, European hedgehog *Erinaceus europaeus*,  
95 bank vole *Myodes glareolus*, European brown hare *Lepus europaeus*) have proven excellent  
96 models for such phylogeographic studies (Hewitt, 1999; Randi, 2007), mostly due to their  
97 well-known biology, high population abundance or wide distributions, or relatively low  
98 dispersal rate (Beheregaray, 2008). Likewise, the common vole *Microtus arvalis* is becoming  
99 an increasingly popular model species to test evolutionary questions, including  
100 phylogeography (Martínková et al., 2013; Tougaard et al., 2008; Triant & DeWoody, 2006). It  
101 is one of the most studied species within the *Microtus* genus, which is by far the most diverse  
102 genus recognized in rodents today (Mahmoudi, Darvish, Aliabadian, Moghaddam, &  
103 Kryštufek, 2017; Wilson et al., 2017), indicative of recent radiation and ongoing speciation  
104 process (Barbosa, Paupério, Pavlova, Alves, & Searle, 2018; Jaarola et al., 2004). The species  
105 meets the criteria to be a useful model system for testing biogeographical patterns (see  
106 Schmitt, 2007): it has the ability to spread rapidly into new habitats and their populations are

107 sedentary and large enough to reflect phylogeographic signals. *M. arvalis* is also locally  
108 abundant and widely distributed across continental regions and Mediterranean peninsulas  
109 (Iberia, the Balkans, and the continental part of Italy) of the Western Palaearctic (Figure 1),  
110 and ranging in altitude from sea level up to 3000 m in the Alps (IUCN, 2016). Consequently,  
111 many and diverse regions through the range, including southern peninsulas and continental  
112 areas, are suitable for the species, making *M. arvalis* a valuable representative of the  
113 continental fauna for evaluating alternative refugial scenarios.  
114

115 Previous investigations of the phylogeography of the western arvalis form (*M. arvalis arvalis*)  
116 based on its mitochondrial DNA (mtDNA) variation have found several highly divergent  
117 lineages in Europe (Fink, Excoffier, & Heckel, 2004; Haynes, Jaarola, & Searle, 2003;  
118 Heckel, Burri, Fink, Desmet, & Excoffier, 2005; Martínková et al., 2013; Stojak, McDevitt,  
119 Herman, Searle, & Wójcik, 2015; Stojak, McDevitt, et al., 2016; Tougaard et al., 2008).  
120 Overall, five main evolutionary mitochondrial lineages have been identified: Western,  
121 Central, Eastern, Italian and Balkan (Figure 1). Some authors further distinguished between  
122 Western-North (France, Belgium and British Isles) and Western-South (Spain and Western  
123 France) lineages (Fink et al., 2004; Haynes et al., 2003; Tougaard et al., 2008; Bužan, Förster,  
124 Searle, & Kryštufek, 2010; Haynes et al., 2003; Martínková et al., 2013; Stojak et al., 2015).  
125 As the available *M. arvalis* population sequence data have increased and the analytical tools  
126 for phylogeographic inference have improved, different scenarios of isolation in refugia and  
127 postglacial colonization routes have been proposed. Some studies found support for the  
128 classical view, under which the species exclusively survived the ice ages in the southern  
129 peninsulas and expanded northward during the postglacial period (e.g. Haynes et al., 2003).  
130 While others provided evidence for the existence of extra-Mediterranean refugia located in  
131 Central and/or Eastern Europe (from Germany to the Carpathian Basin) (Fink et al., 2004;  
132 Heckel et al., 2005; Stojak et al., 2015; Tougaard et al., 2008). Some of these studies,  
133 therefore, contrast with the classical view in considering southern refugia as areas driving  
134 high endemism, where mountain ranges may have precluded north-south postglacial dispersal  
135 and gene flow. Instead, recolonization would have originated from cryptic —continental—  
136 glacial refugia, and rapidly expanded along a rough east-west axis (Stojak et al., 2015, 2016).  
137 However, whether one or more extra-Mediterranean refugia existed, their location, or if they  
138 coincided with classical Mediterranean refugia, are topics that remain largely unknown.  
139

140 To gain a complete understanding of the phylogeographic history of this continental species  
141 model, and to contribute to our knowledge of European biogeographic patterns in general, it  
142 is necessary to explore the genetic structure of the species across its complete distribution  
143 range. In past studies, common vole populations at the extremes of the range, such as in the  
144 Iberian Peninsula, have been significantly underrepresented, limiting our capacity to make  
145 accurate inferences about its importance as a Mediterranean refugium and source of  
146 recolonization (Hewitt, 1999, 2001), as harbour of other refugia (i.e., ‘refugia-within-refugia’  
147 Abellán & Svenning, 2014; Gómez & Lunt, 2007), or potentially as driver of diversity and  
148 endemism. For instance, the Iberian peninsula is of particular biogeographical interest since  
149 some authors consider that the Iberian vole (*M. a. asturianus* Miller, 1908) shows a number  
150 of unique characters that warrant subspecies recognition (Delibes & Brunett-Lecomte, 1980;  
151 González-Esteban, Villate, & Gosálbez, 1995; Niethammer & Winking, 1971; Nesterova,  
152 Mazurok, Rubtsova, Isaenko, & Zakian, 1998; Rey, 1973). Iberian voles have larger teeth and  
153 body size, longer pregnancy, larger litters, slower development of the young, distinctive  
154 threatening calls, and lower degree of intraspecific aggressiveness than the European common  
155 vole (*M. a. arvalis*) (Frank, 1968). To date, however, there has been no attempt to evaluate the

156 Iberian common vole populations for subspecific status using molecular data and genetic  
157 methods.

158  
159 In this study, we carried out the widest geographically reconstruction of the demographic  
160 history and dispersal dynamics of the common vole in Europe, using a continuous Bayesian  
161 phylogeographic diffusion approach (Lemey, Rambaut, Welch, & Suchard, 2010), across the  
162 entire European distribution of the species. We aimed to assess the generality of existing  
163 models about the origin, putative refugia and dispersal of this continental model species in  
164 Europe by inferring the historical demography dynamic and spread of the species using  
165 spatially-explicit analyses. Specifically, we asked (1) where is the most probable geographic  
166 location of the root of the European lineages of *M. arvalis*; (2) whether the spatial distribution  
167 of genetic variation observed in this species reflect colonization from a single glacial  
168 refugium or from multiple refugia; (3) whether the pattern of genetic variation is consistent  
169 with the existence of one or more Mediterranean refugia, one or more continental refugia, or a  
170 combination of Mediterranean and extra-Mediterranean refugia; (4) whether the current  
171 geographic pattern observed in this continental mammal species is congruent with one of the  
172 common four paradigm patterns (Habel et al., 2005; Hewitt, 2000; Hewitt, 1999) of post-  
173 glacial range expansion from Mediterranean refugia (or, alternatively, if refugial populations  
174 have remained within Mediterranean refugia as geographical isolates).

175  
176

## 177 **MATERIALS AND METHODS**

178

### 179 **Population sampling and laboratory procedures**

180

181 We sampled 261 common vole specimens from 10 localities throughout its range in the  
182 Iberian peninsula (see details in Appendix 1 and Figure 1), using Sherman traps between 2012  
183 and 2014. From each individual, we took 2 mm tissue biopsies from the ear, and preserved  
184 them in 96% ethanol. Afterwards, all individuals were released at their respective collection  
185 site, and tissue and DNA samples were stored at the IREC (Instituto de Investigación en  
186 Recursos Cinegéticos) DNA data bank. All handling procedures were approved by the UCLM  
187 Ethics Committee (reference number CEEA: PR20170201) and in accordance with the  
188 Spanish and European policy for animal protection and experimentation. We completed our  
189 sampling with a compilation of 151 published cytochrome *b* sequences, collected in 68  
190 localities across 22 countries covering the entire European range of *M. arvalis* (references and  
191 GenBank accession numbers are indicated in Appendix 1).

192

193 The phylogeographic architecture of common vole has traditionally been studied mainly using  
194 the mtDNA cytochrome *b* gene (Fink et al., 2004; Haynes et al., 2003; Heckel et al., 2005;  
195 Martínková et al., 2013; Stojak et al., 2015, 2016; Tougaard et al., 2008) or in combination  
196 with microsatellite and Y chromosome data (e.g. Beysard & Heckel, 2014; Stojak, Borowik,  
197 Górný, McDevitt, & Wójcik, 2019; Stojak, McDevitt, et al., 2016). Although some  
198 discordances are expected among gene tree topologies at different markers and the species  
199 tree (Kubatko, Carstens, & Knowles, 2009), previous studies on the phylogeography of this  
200 and related species suggest that mtDNA is an excellent marker for identification of glacial  
201 refugia and study of range expansion (Herman et al., 2014; Martínková et al., 2013; Stojak et  
202 al., 2016). We are fully aware of the benefits of multi-locus versus approaches single-marker  
203 approaches, but multi-locus analyses would have constrained us to use a reduced number of  
204 populations. Moreover, former studies that have used autosomal and sex-chromosome

205 markers in *M. arvalis* have provided congruent phylogeographical topologies and very similar  
206 patterns of genetic structure and dating events to those found with mtDNA (Beysard &  
207 Heckel, 2014; Braaker & Heckel, 2009; Heckel et al., 2005; Martinková et al., 2013; Stojak et  
208 al., 2016), albeit much finer phylogenetic resolution was obtained with cytochrome *b* than  
209 with nuclear markers at the continental and regional scale (e.g. Heckel et al., 2005).  
210 Consequently, we used here this mitochondrial gene, given the need for sufficient resolution  
211 and direct comparison of our findings with earlier range-wide research on this species.

212  
213 We digested samples overnight in 250  $\mu$ L lysis buffer (0.1 M Tris-HCl, 0.005M EDTA, 2%  
214 SDS, 0.2 M NaCl, pH 8.5) and Proteinase K (10ng/ $\mu$ L) and extracted total genomic DNA  
215 from live specimens using a standard AcNH<sub>4</sub> protocol. We adjusted DNA extractions to a  
216 working dilution of 25 ng/ $\mu$ L and PCR-amplified the entire *cytochrome b* mitochondrial gene  
217 (1,143 bp) using primers L7 (5'-ACCAATGACATGAAAAATCATCGTT-3') and H6 (5'-  
218 TCTCCATTTCTGGTTTACAAGAC-3') (Tougaard et al., 2008). We cleaned up the PCR  
219 products using Exonuclease I and Shrimp Alkaline Phosphatase (Fermentas) and direct  
220 sequencing was performed with the same PCR primers and the BigDye Terminator v3.1  
221 Cycle Sequencing Kit (Applied Biosystems) on an ABI PRISM 3130xl Genetic Analyzer  
222 (Applied Biosystems). We manually checked chromatograms for stop codons and obvious  
223 sequencing errors, and aligned them using the software BioEdit 7.0.5.3 (Hall, 1999). All the  
224 sequences obtained are deposited in GenBank (MG874847-MG874883).

## 227 **Bayesian phylogeographic inference**

228  
229 We reconstructed the phylogeographic history of *M. arvalis* through continuous space and  
230 time, by modelling the spatial dispersion of its lineages using Bayesian phylogeographic  
231 inference in BEAST2 (Bouckaert et al., 2014; Lemey, Rambaut, Drummond, & Suchard,  
232 2009; Lemey et al., 2010). This model combines geographic and genetic data, using the  
233 geographical location of each specimen and accommodating branch-specific variation in  
234 dispersal rates. For this analysis we selected one sample per each haplotype found at each  
235 locality, totalling 197 sequences and 141 haplotypes, from 78 localities across 23 countries  
236 (Appendix 1). Our final alignment was restricted to a common length of 953 bp (Dataset S1,  
237 Supporting Information). We created a xml input file in BEAUTI v.2 (Bouckaert et al., 2014),  
238 and used a jitter option of 0.50 to create unique coordinates for individuals collected at  
239 identical sites.

240  
241 We used a Marginal Likelihood Estimates (MLE) model selection procedure to evaluate the  
242 relative fit of our data to (1) models that assume no branch-specific rate variation in dispersal  
243 rates (i.e. homogeneous Brownian diffusion), and (2) relaxed random walk (RRW) models  
244 that assume different distributions for rate variation among branches (i.e. Cauchy RRW,  
245 lognormal RRW and Gamma RRW). We generated posterior distributions of each model by  
246 running  $7 \times 10^7$  Markov Chain Monte Carlo (MCMC) generations that were sampled every  
247 7000 generations, after discarding the first 10% samples as burn-in. We assessed convergence  
248 by visualizing the outputs of posterior distributions and effective sampling sizes (ESS) in  
249 TRACER v.1.4.8. (Rambaut & Drummond, 2007).

250  
251 We then calculated Bayes Factors for each model, and used them to select the spatial  
252 diffusion model that is best supported by the data. Bayes Factors were derived from their  
253 MLE and based on path sampling (PS) method (Baele et al., 2012); MCMC chain of  $1 \times 10^6$

254 generations and 50 path steps. Our data fitted best the Cauchy-distributed RRW diffusion  
255 rates model (MLE = -5749.378), therefore, we applied this model to all subsequent analyses  
256 (Gamma-distributed RRW: MLE= -6468.140; lognormal-distributed RRW: MLE = -  
257 5897.870, and Homogeneous Brownian diffusion: MLE = -5962.448).

258  
259 To visualize the spatial diffusion of *M. arvalis* lineages, we inferred a maximum clade  
260 credibility (MCC) tree and projected it on a spatial map. We ran four independent MCMC  
261 chains for  $2.5 \times 10^7$  generations—using the BEAGLE library (Ayres et al., 2012) to improve  
262 computational performance—and sampled their posterior distributions every 2500  
263 generations. Following burn-in of the first 10% trees, we combined the four independent  
264 analyses using LogCombiner 2.4.1 (whereby all parameters reached ESS>200) and randomly  
265 subsampled 10,000 trees, that were used to construct a MCC tree in TreeAnnotator 2.4.1. We  
266 used the resulting MCC tree as input for the program Spread 1.0.4 (Bielejec, Rambaut,  
267 Suchard, & Lemey, 2011) to generate a keyhole mark-up language (KML) file for visualizing  
268 spatial diffusion in Google Earth over the complete posterior distribution of trees. We used  
269 the Time Slicer function in Spread 1.0.4 to estimate the 80% Highest Posterior Density (HPD)  
270 region of the unobserved ancestral locations for each branch that intersects ten specific time  
271 points, which roughly coincide with major internal nodes within the MCC tree: 50,700 years  
272 before present, ybp; 43,500 ybp; 36,300 ybp; 33,400 ybp; 23,700 ybp; 21,100 ybp  
273 (coincident with the last glacial maximum, LGM); 16,300 ybp; 12,600 ybp; 7500 ybp, and the  
274 present.

275  
276 We also explored temporal changes in the effective population size using a Bayesian skyride  
277 tree prior implemented in BEAST v. 1.8.1 (Drummond, Suchard, Xie, & Rambaut, 2012). We  
278 used the HKY model of sequence evolution and a strict molecular clock with an average  
279 substitution rate across the tree of  $\mu = 3.27 \times 10^{-7}$  substitutions/site/year (assuming a  
280 generation time of 1 year) (Martínková et al., 2013). We obtained this value from the most  
281 recently published—and likely most accurate—rate for cytochrome *b* of *M. arvalis*  
282 (Martínková et al., 2013; Stojak et al., 2015, 2016), estimated using ancient DNA sequences  
283 from radiocarbon-dated specimens. We ran four independent MCMC chains for  $3 \times 10^7$   
284 generations sampling every 3000 generations. Convergence was assessed in TRACER  
285 v.1.4.8. by confirming that the effective sampling size for all parameters was higher than 200,  
286 and that independent runs yielded similar posterior distributions. We combined the four runs  
287 using LogCombiner v1.8.1 (<http://beast.bio.ed.ac.uk/logcombiner>) after discarding the first  
288 10% of sampled generations as burn-in, and obtained estimates and credible intervals for each  
289 parameter and demographic reconstruction using TRACER v.1.4.8.

290  
291 With the aim to infer within- and between-lineage evolutionary relationships among  
292 haplotypes analysed, we constructed a median-joining network (Bandelt, Forster, & Röhl,  
293 1999) using the software NETWORK 5.0 (available at [http://www.fluxus-](http://www.fluxus-engineering.com/sharenet.htm)  
294 [engineering.com/sharenet.htm](http://www.fluxus-engineering.com/sharenet.htm)). Genetic distances were calculated through a pairwise distance  
295 matrix in MEGA v. 7.0.16 (Kumar, Stecher & Tamura, 2016).

296  
297

## 298 RESULTS

299

300 The MCC tree obtained using a Bayesian phylogeographic analysis with the Cauchy RRW  
301 model recovered with high support (all posterior probabilities = 1.0) all previously described  
302 mtDNA lineages of *M. arvalis* (Figure 2), and the network analysis provide congruent results



303 (Figure 3). The Western group (including North- and South-western lineages) was sister to  
304 the group that includes the Balkan, Italian, Central and Eastern lineages. However, the low  
305 support received for the node of the Balkan lineage bifurcation at the root of this group means  
306 that the existence of a polytomy at the root of *M. arvalis* diversification cannot be ruled out.  
307 Within the Western-South lineage, we found further phylogenetic structure, where all the  
308 Iberian specimens formed a monophyletic group that was sister to *M. arvalis* from the rest of  
309 the lineage (including populations from Pyrenees and SW France) (Figures 2 and 3).  
310 Sequence divergence between the Iberian and the rest of the Western-South clade (including  
311 French and Pyrenean populations) is comparable to the divergence levels found among other  
312 European lineages (Table 1).

313  
314 Based on the MCC tree, we inferred the most probable geographical location of the common  
315 ancestor of *M. arvalis* lineages in the alpine region of Switzerland-Liechtenstein, around  
316 51,000 ybp (Figure 4A). From this region of origin, the Central-European ancestor split into  
317 the two major ancestral lineages between 51,000-43,000 ybp. One ancestral lineage expanded  
318 to the southeast, resulting in the origin of the Balkan, Italian, Central and Eastern lineages,  
319 and the other one expanded to the north-west, resulting in the ancestor of the current Western  
320 lineages (Figures 4A and 4B). Diversification in extant lineages and expansion of the eastern  
321 ancestral lineage occurred between 43,000-21,000 ybp along the Dalmatian (Adriatic) coast,  
322 from the western Austria Alps to the north-western part of the Balkan Peninsula (Figures 4C–  
323 F). Over the same time period, the western ancestral lineage progressed towards the south-  
324 west of the continent, through central France to the Pyrenees. Colonization of the Iberian  
325 Peninsula was estimated to have occurred ~23,000-21,000 ybp (Figures 4E and 4F), following  
326 divergence of the Western-North and Western-South clades around 33,400 ybp (Figure 2).  
327 Finally, the Iberian clade split and further extended its geographic range during the last 9000  
328 years (Figures 2, 4I and 4J), coinciding with the main period of diversification in all other  
329 extant lineages.

330  
331 Our phylogeographic reconstruction also suggested that dispersal from central Europe (Alps)  
332 to the east was geographically restricted (Figures 4A-H) until 12,600-7,900 years ago, when  
333 expansion proceeded within the range of the extant Central lineage along two axes: to the  
334 north-east, reaching the easternmost limit of the current distribution of *M. arvalis* in Russia-  
335 Mongolia, and to the southeast, reaching the southern part of the Carpathians region (Figure  
336 4I). From then until present time, major northward range expansions occurred in populations  
337 of the Western, Central and Eastern regions (Figures 4I and 4J). These recent expansion  
338 events resulted in the current distribution of the species across the Atlantic, boreal and  
339 continental regions of Europe.

340  
341 The skyride plot showed a long period of demographic stasis from the time of the most recent  
342 common ancestor of *M. arvalis* lineages (~50,000 ybp) until 35,000 ybp (Figure 2). Then,  
343 effective population size slowly increased until 18,500 ybp, likely associated with the major  
344 cladogenetic events in the history of *M. arvalis*. Finally, we detected a sudden increase in  
345 effective population size during the last 12,000 years, coinciding with the diversification and  
346 expansion of the current lineages of *M. arvalis* after the Younger Dryas (12.9-11.7 kya) cold  
347 reversal (Figure 2).

348  
349

## 350 DISCUSSION

351

352 The present study aimed to understand how a continental small mammal species have  
353 responded to global climate changes in the past, especially through the late Pleistocene to  
354 Holocene transition. Accordingly, we used an extensive range-wide sampling of mtDNA  
355 variation and a Bayesian phylogeographic inference framework to reconstruct the  
356 evolutionary history of *M. arvalis* in continuous space and time.

357  
358 In general, previous phylogeographic studies on *M. arvalis* consistently distinguish six  
359 mitochondrial lineages, and evidence for multiple glacial refugia during the last glaciation  
360 together with existence of refugia further north than traditional Mediterranean ones is now  
361 substantial (Bužan et al., 2010; Fink et al., 2004; Haynes et al., 2003; Martínková et al., 2013;  
362 Stojak et al., 2015; Stojak et al. 2019; Tougaard et al., 2008). However, attempts to reconstruct  
363 the evolutionary history of each lineage (i.e. locate and circumscribe their origin, historical  
364 refugia and post-glacial colonization routes) have proved contentious (see below), with some  
365 discrepancy between inferences from studies made at different spatial resolutions and  
366 analytical methods.

367  
368 Our data suggest that the species originated in Central Europe, and that the most likely  
369 location of major glacial refugia were the peri-Alpine, peri-Pyrenean, and peri-Dinaric  
370 regions, confirming the existence of multiple glacial refugia for this continental species.  
371 Additionally, we found support for glacial survival in at least two classical Mediterranean  
372 refugia—in the Iberian and Balkan peninsulas— confirming a scenario of Pleistocene survival  
373 in multiple southern and northern refugia. But most interestingly, peninsular lineages are not  
374 found outside these areas and, consequently, southern peninsulas did not serve as sources of  
375 northward postglacial expansions, becoming centres of endemism for *M. arvalis*. Our results  
376 confirm the monophyly of Iberian common voles and the pre-LGM divergence between the  
377 Iberian and European phylogroups, which supports the status of the Iberian voles (*M. a.*  
378 *asturianus*) as a distinct subspecies in line with previous research (Delibes & Brunet-  
379 Lecomte, 1980; González-Esteban et al., 1994, 1995; Nesterova et al., 1998; Niethammer &  
380 Winking, 1971; Rey, 1973). However, this observation should be treated with caution while it  
381 is confirmed by a synthetic approach that combines an additional sampling of different genes  
382 and morphological and ecological data. Our results, therefore, are generally consistent with  
383 recent research on *M. arvalis* based on DNA sequences and nuclear loci, but provide  
384 additional insights, particularly in the spatial location of the cradle, glacial refugia and  
385 colonization routes.

386  
387 Altogether, we propose that the classical model of southern glacial refugia in Mediterranean  
388 peninsulas, and posterior colonization of northern deglaciated areas (Hewitt, 2000), does not  
389 fit the evolutionary and demographic history of this continental species, as recent findings  
390 seem to corroborate (e.g. Schmitt & Varga, 2012; Tougaard et al., 2008). Nor does this  
391 mammal species fit very well to the scenarios described so far for any of the biogeographical  
392 types of temperate continental species (Schmitt, 2007), which compared to our model tend to  
393 have greater geographical restrictions to the west in the location of refugia and lineage  
394 distributions. The species provide a paradigm of ice-age survival of temperate —  
395 continental— species in western and eastern Mediterranean peninsulas (sources of endemism)  
396 and multiple continental regions (sources of postglacial spread).

397  
398 **European glacial refugia and postglacial expansion**  
399

400 Our phylogenetic reconstruction was congruent with previous studies and recovered with high  
401 support six lineages of *M. arvalis* (Bužan et al., 2010; Fink et al., 2004; Haynes et al., 2003;  
402 Martínková et al., 2013; Stojak et al., 2015; Tougard et al., 2008). The well-delimited  
403 geographic distribution of these lineages exposes the importance of allopatric factors in  
404 shaping the genetic structure of this species at a continental scale, probably through multiple  
405 isolation events during the mid- and late Würm ice age (60,000-11,500 ybp). Our model-  
406 based phylogeographic analysis inferred the location of the most recent common ancestor of  
407 all haplotypes in a region between the Alps and southern Germany around 51,000 ybp (95%  
408 HPD: 62,400 -39,500 ybp). This location is consistent with the oldest fossil remains of *M.*  
409 *arvalis* found in SW Germany (Kowalski, 2001) and relates closely to what (Tougaard et al.,  
410 2013) proposed as the common cradle of *M. arvalis* in ‘western Central Europe’. Moreover,  
411 our date estimates for the root of the tree and of major lineages coincided with recent research  
412 (Stojak et al., 2016). This scenario is clearly not consistent with the fossil evidence (oldest  
413 fossil remains of *M. arvalis* in Central Europe from the Late Cromerian, 465,000 ybp;  
414 (Kowalski, 2001) although accurate species identification in fossil remains appear to be  
415 problematic and should always be considered cautiously for this and related species (Navarro  
416 et al., 2018, Tougaard et al, 2016). The extreme similarity between sibling vole species  
417 (Markova et al., 2010) and the morphological changes probably suffered by the species  
418 between the mid Holocene and the present day are known to be unfavourable for diagnosing  
419 *M. arvalis* in the fossil record (Markova, Beeren, van Kolfschoten, Strukova, & Vrieling,  
420 2012). Here, we focus on extant lineages and, therefore, we lack direct information about  
421 extinct lineages, which are often those found in the fossil record but that probably did not  
422 survive past periods (e.g. Tougaard 2016). This probably explains the large discrepancy in  
423 estimated origin and divergence times of major *M. arvalis* lineages between our study and the  
424 fossil evidence. In the future, research involving ancient DNA can contribute to shed light on  
425 these complex scenarios.

426  
427 Even though the Bayesian posterior mean estimate should provide a reasonable basis for  
428 inferences, it is unwise to use our data to make strong statements about a single origin and  
429 subsequent lineage diversification or that different lineages originated more or less at the  
430 same time, given that 95% HPD estimate for the root of the tree overlap with the origin of the  
431 Western, Italian, Balkan and Central+Eastern lineages. In any case, *M. arvalis* could have  
432 survived in the Alps during the LGM and Younger Dryas periods (YD; 12,900-11,700 ybp,  
433 (Rasmussen et al., 2006), maybe in regions of lower elevation between the northern slope of  
434 the Alps and the Danube river in southern Germany. Most importantly, this area could  
435 represent not only the origin of dispersal for the species in Europe, but also an extra-  
436 Mediterranean glacial refugium.

437  
438 The timing and pattern of cladogenetic events also point to geographical isolation during the  
439 last glaciation as the major driver of lineage divergence, as well as to the existence of multiple  
440 refugia for *M. arvalis*. Most of the major splits between lineages occur around the time of the  
441 LGM, such as the split between Western-North and Western-South lineages (mean: 34,028  
442 ybp. 95% HPD: 45,447 – 23,882 ybp), the split between Eastern and Central lineages (mean:  
443 24,072 ybp. 95% HPD: 15,823 – 32,275 ybp), or the split between the Iberian and Western-  
444 South lineages (mean: 21,069 ybp. 95% HPD: 29,743 – 14,087 ybp). For instance, the most  
445 recent common ancestor of the Eastern lineage was located west of the Danube river around  
446 13,639 ybp (95% HPD: 18,809 – 8,820 ybp), which could imply that a glacial refugium in the  
447 eastern Alps was the origin of this lineage. However, based on the molecular data alone, we  
448 cannot rule out the existence of a Carpathian refugium, as it has been proposed for numerous

449 species (Pazonyi, 2004; Kotlik et al., 2006; Sommer & Nadachowski, 2006) including *M.*  
450 *arvalis* (Stojak et al., 2015, 2019).

451  
452 Similarly, the high differentiation of the Balkan lineage relative to the other Central-Eastern  
453 lineages could be the result of allopatric divergence, following isolation in a Ponto-  
454 Mediterranean refugium in the north-western Dalmatian coast (Dinaric Alps). Furthermore,  
455 and despite the co-occurrence of both Eastern and Balkan haplotypes in that region at the  
456 present time (Bužan et al., 2010; Stojak et al., 2015), it appears that the Balkan lineage has  
457 historically maintained a very restricted distribution, and has not expanded nor contributed to  
458 the gene pool of northern or eastern European populations. The large and recent expansion  
459 detected (Figure 4) in populations of the Eastern lineage to the centre and north-western  
460 Balkan Peninsula and south of the Danube river would explain their presence there. Based on  
461 these, we could first consider the Balkan Peninsula as a region of endemism of *M. arvalis*,  
462 and second, refute previous hypotheses suggesting that the Eastern lineage originated from a  
463 refugium in the Balkans (Haynes et al., 2003; Heckel et al., 2005).

464  
465 Within the Western-South lineage, the split between the Iberian and the French clades around  
466 the LGM suggests divergence in two separate refugia. One refugium might have existed near  
467 the region of Aquitaine, in southwestern France (i.e. Pyrénées-Atlantiques), from where the  
468 species likely expanded to northern Europe, and another one south of Pyrenees—or south of  
469 the Ebro river Valley—that further extended to the centre and north-west of the Iberian  
470 peninsula. According with the temporal-spatial diffusion pattern, the Iberian peninsula served  
471 as a glacial (Mediterranean) refugium for extant populations of common vole during the last  
472 cold stage of the Pleistocene, including the LGM. This Iberian clade, however, did not back-  
473 colonize the Pyrenees or France during the present interglacial period, as could be deduced by  
474 the absence of Iberian haplotypes in populations of these two regions. The Holocene  
475 expansion of Iberian voles could have been limited ecologically—maybe by predators or  
476 competition with closely related species—or geographically, by physical barriers such as the  
477 Ebro river following deglaciation (see below). Consequently, Iberian voles can be considered  
478 as a long-term isolated endemism of the Iberian Peninsula (see also Tougaard et al., 2008)  
479 without admixture with other mitochondrial lineages, and concordant with previous  
480 subspecific designation. The most recent common ancestor of tentative *M. a. asturianus*  
481 specimens was estimated around 9,000 ybp in north-central Spain, and the more recent split  
482 within this clade were dated 8,500 – 4,000 years ago. The geographical expansion of the  
483 Iberian clade during the late Holocene (i.e. last 5,000 years) seems to be a key factor shaping  
484 its current population genetic structure.

485  
486 In central Europe, the presence of a glacial refugium has already been suggested for *M.*  
487 *arvalis* (Fink et al., 2004; Tougaard et al., 2008), and for other species (reviewed in Schmitt &  
488 Varga, 2012), but for the first time we were able to pinpoint its geographic location using  
489 model-based phylogeographic inference. There is good evidence that several continental  
490 species had multiple differentiation centres around the European high mountain systems  
491 (Schmitt, 2007; Stewart & Lister, 2001), possibly due to the existence of more humid  
492 conditions around these mountains in a context of increased dryness westwards during the  
493 coldest phases of the Pleistocene in Europe. For this group of species it has been argued that  
494 water-limited conditions, rather than temperature, may have limited the species' glacial  
495 distributions along an east–west gradient in response to moisture (Schmitt, 2007), and there is  
496 recent evidence of the importance of climate for the genetic structure of this and related  
497 species (Stojak, 2019).

498  
499 Our analyses, however, failed to conclude whether only one or multiple refugia occurred  
500 scattered along the Alps and adjacent areas. Multiple glacial refugia have been proposed  
501 along the northern, southern, south-western, and eastern slopes of the Alps (e.g.  
502 (Schönswetter, Stehlik, Holderegger, & Tribsch, 2005). Particularly for *M. arvalis*, the Alpine  
503 region is a well-known contact zone between three evolutionary lineages (Western-North,  
504 Central and Italian), and both the northern and southern slopes of the Alps have been  
505 proposed as sources of post-LGM recolonization (Braaker & Heckel, 2009). Interestingly, the  
506 inferred location and time for the origin of expansion of the current lineages of *M. arvalis*  
507 coincide with the main routes of westward expansion of agriculture in Europe during the  
508 Neolithic period ~10,200-2000 ybp (e.g. Larson et al., 2007; Rowley-Conwy, 2011). The  
509 treeless tundra or steppe that had previously extended over much of Europe was progressively  
510 replaced by woodlands from the beginning of the Holocene, eleven thousand years ago (e.g.  
511 (Hejcman, Hejcmanová, Pavlů, & Beneš, 2013; Huntley & Birks, 1983; Turner & Hannon,  
512 1988). Thus, it could be argued, that the clearance of woodlands to turn them into cultivated  
513 lands (Roberts et al., 2018), and the progressive anthropogenic transformations of the  
514 landscape (Bouma, Varralyay, & Batjes, 1998; Garcia, Alda, et al., 2011; Garcia, Mañosa, et  
515 al., 2011; Novenko, Volkova, Nosova, & Zukanova, 2009; O'Connor & Shrubbs, 1986;  
516 Ruddiman, 2003), could have facilitated the expansion and connectivity of populations of  
517 steppe and grassland species in a postglacial environment (Bouma et al., 1998; Garcia, Alda,  
518 et al., 2011; Garcia, Mañosa, et al., 2011; O'Connor & Shrubbs, 1986; Pärtel, Bruun, &  
519 Sammul, 2005).

520  
521 The geographical structure of the lineages of *M. arvalis*, also hints at factors limiting  
522 postglacial recolonization and/or current dispersal of the species. The major mtDNA lineages  
523 of *M. arvalis* in Central Europe are well delimited by the major river systems (Figure 1). For  
524 example, the Loire and Rhone rivers in France divide the Western-North and Western-South  
525 lineages, something already indicated by (Tougard et al., 2008). Likewise, the Ebro river in  
526 Spain separates the tentative *M. a. asturianus* specimens and *M. a. arvalis* from the Western-  
527 South clade, which includes populations from the Pyrenees and southern France. Overall, the  
528 distribution of *M. a. asturianus* in Iberia is limited to the north and south by the Ebro and  
529 Tajo rivers, respectively. The range of the Italian lineage is also limited to the south by the  
530 Po, and the Central lineage is roughly delimited by the transitional zone between Oder and  
531 Vistula to the west, and by the Danube to the south (Stojak et al., 2016a,b). Furthermore, the  
532 part of the Danube basin in the eastern Balkan region seems to represent a geographic  
533 boundary between the Balkan and Eastern lineages. Similarly, and in view of the current  
534 geographic distribution (Figure 1), the Don-Volga river system marks the eastern limit of the  
535 *arvalis* range (Bulatova et al., 2007; Jaarola et al., 2004; Tougard et al., 2013). As could be  
536 expected, these barriers are porous, and most lineages show haplotypes that have leaked to the  
537 other side of these rivers, somewhat blurring the boundaries between lineages near the  
538 headwaters. This could either be a consequence of recent dispersal during periods of reduced  
539 runoff in the Holocene (Bernárdez et al., 2008; Combourieu-Nebout et al., 2013), or by  
540 passage through river headwaters, where lineages often come into contact (e.g. Fouquet et al.,  
541 2012). In fact, most European lineages of *M. arvalis* meet in a Central European region near  
542 de Alps (Figure 1), which coincides also with the headwaters of the major European rivers.  
543 Even more recently, river bridges and other human infrastructures also allow voles to cross  
544 these barriers. More populations from putative areas of secondary contact and lineage  
545 admixture, as well as the use of additional nuclear genetic markers, will be used in future  
546 studies to assess patterns of hybridization and introgression between lineages.

547  
548 Our findings, therefore, provide support for a major role of large European river systems in  
549 shaping geographical boundaries of *M. arvalis* (Stojak et al., 2016). In contrast to the widely  
550 accepted role of mountain systems (e.g. Alps, Pyrenees) precluding post-glacial expansion of  
551 many temperate species in southern Mediterranean peninsulas (Habel et al., 2005; Hewitt,  
552 1999; Schmitt, 2007), or the role of humans as responsible for the colonization of some  
553 islands (e.g. Martinkova et al. 2013), very little is known about a similar potential role of  
554 rivers.

555  
556 It is expected that during the deglaciation of Europe, the distribution and extent of freshwater  
557 systems suffered dramatic changes; and these had a significant effect in the post-glacial  
558 recolonization of terrestrial species. When ice sheets started to retreat from their maximum  
559 extent, between 22,000 and 17,000 ybp, there was an increase meltwater production and river  
560 water runoff, causing a sudden reactivation of the hydrological cycle (Mangerud et al., 2004;  
561 Ménot et al., 2006). Therefore, these water bodies may have played a dual role, either as  
562 barriers for dispersal, and/or as corridors, along water banks, consequently affecting gene  
563 flow and genetic differentiation, especially in small mammals or species with low vagility  
564 such as voles.

565  
566 In conclusion, *Microtus arvalis* represents a remarkable study organism that combines  
567 attributes of continental and Mediterranean faunal elements (De Lattin, 1967; Schmitt &  
568 Varga, 2012). On one hand, central Europe acted as a glacial refugium and as a source for  
569 other European populations, and on the other hand, additional Mediterranean refugia acted as  
570 drivers of endemism. Although there is abundant literature on Mediterranean species that  
571 found shelter in extra-Mediterranean glacial retreats (Provan & Bennett, 2008; Schmitt &  
572 Varga, 2012), very few studies report continental species in both Mediterranean and extra-  
573 Mediterranean refugia. The pattern we describe for the common vole is, however, highly  
574 congruent with the phylogeographical pattern in the bank vole *Myodes glareolus*, a European  
575 forest vole species with Mediterranean and continental refugia, and in which the  
576 Mediterranean phylogroups did not contribute to the postglacial recolonization of much of  
577 their range (Deffontaine et al., 2005). To the best of our knowledge, so far, the co-occurrence  
578 among Mediterranean and extra-Mediterranean glacial refugia for continental species is  
579 mainly restricted to eastern Europe, with few examples referred to continental species with  
580 refugial areas in the Balkan Peninsula, such as butterflies (Gratton, Konopiński, & Sbordoni,  
581 2008; Junker et al., 2015; Schmitt, Rákósy, Abadjiev, & Müller, 2007), reptiles (Ursenbacher,  
582 Carlsson, Helfer, Tegelström, & Fumagalli, 2006) and invertebrates (Pinceel, Jordaens,  
583 Pfenninger, & Backeljau, 2005). Whether *M. arvalis* majorly follows a continental or a  
584 Mediterranean specific biogeographic pattern (Schmitt, 2007) is still unclear, in part because  
585 of the difficulty to classify ecologically diverse and widely distributed species. More research  
586 on continental species is needed to test the generality of the biogeographic pattern of *M.*  
587 *arvalis* in Europe.

588  
589

## 590 **ACKNOWLEDGEMENTS**

591  
592 We wish to thank students and friends for their help in the field, and especially to Iván García  
593 Egea and Jorge Piñeiro Álvarez for invaluable assistance collecting samples. Also, we would  
594 like to also thank the numerous landowners who allowed us access to their properties, and  
595 acknowledge the support and cooperation of GREFA, and the communities from the villages

596 of Villalar de los Comuneros, Boada de Campos, San Martín de Valderaduey and Fuentes de  
597 Nava. We also thank Elisabeth Haring (Editor-in-Chief of JZSER) and two reviewers for  
598 constructive and helpful comments of this manuscript. This work was supported by I+D  
599 National Plan Projects of the Spanish Ministry of Economy, Industry and Competitiveness  
600 (CGL2011-30274, CGL2015-71255-P, CGL2013-42451-P), and the Fundación BBVA  
601 Research Project TOPIGEPLA (2014 call).  
602

- 603  
604 **REFERENCES**  
605  
606 Abellán, P., & Svenning, J. C. (2014). Refugia within refugia—patterns in endemism and  
607 genetic divergence are linked to Late Quaternary climate stability in the Iberian Peninsula.  
608 *Biological Journal of the Linnean Society*, **113**, 13–28.  
609  
610 Ayres, D. L., Darling, A., Zwickl, D. J., Beerli, P., Holder, M. T., Lewis, P. O., Huelsenbeck,  
611 J. P., Ronquist, F., Swofford, D. L., Cummings, M. P., Rambaut, A., & Suchard, M. A.  
612 (2012). BEAGLE: An Application Programming Interface and High-Performance Computing  
613 Library for Statistical Phylogenetics. *Systematic Biology*, **61**, 170–173.  
614  
615 Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M. A., & Alekseyenko, A. V.  
616 (2012). Improving the accuracy of demographic and molecular clock model comparison while  
617 accommodating phylogenetic uncertainty. *Molecular Biology and Evolution*, **29**, 2157–2167.  
618  
619 Bandelt, H. J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring  
620 intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.  
621  
622 Barbosa, S., Paupério, J., Pavlova, S. V., Alves, P. C., & Searle, J. B. (2018). The *Microtus*  
623 voles: Resolving the phylogeny of one of the most speciose mammalian genera using  
624 genomics. *Molecular Phylogenetics and Evolution*, **125**, 85–92.  
625  
626 Barker, R. J., Van Den Bussche, R. A., Wright, A. J., Wiggins, L. E., Hamilton, M. J., Reat,  
627 E. P., Smith, M. H., Lomakin, M. D., & Chesser, R. K. (1996). High levels of genetic change  
628 in rodents of Chernobyl. *Nature*, **380**, 707–708.  
629  
630 Beheregaray, L. B. (2008). Twenty years of phylogeography: the state of the field and the  
631 challenges for the Southern Hemisphere. *Molecular Ecology*, **17**, 3754–3774.  
632  
633 Bernárdez, P., González-Álvarez, R., Francés, G., Prego, R., Bárcena, M. A., & Romero, O.  
634 E. (2008). Late Holocene history of the rainfall in the NW Iberian peninsula—Evidence from  
635 a marine record. *Journal of Marine Systems*, **72**, 366–382.  
636  
637 Beysard, M., & Heckel, G. (2014). Structure and dynamics of hybrid zones at different stages  
638 of speciation in the common vole (*Microtus arvalis*). *Molecular Ecology*, **23**, 673–687.  
639  
640 Bielejec, F., Rambaut, A., Suchard, M. A., & Lemey, P. (2011). SPREAD: spatial  
641 phylogenetic reconstruction of evolutionary dynamics. *Bioinformatics*, **27**, 2910–2912.  
642  
643 Bilton, D. T., Mirol, P. M., Mascheretti, S., Fredga, K., Zima, J., & Searle, J. B. (1998).  
644 Mediterranean Europe as an area of endemism for small mammals rather than a source for  
645 northwards postglacial colonization. *Proceedings of the Royal Society of London B:*  
646 *Biological Sciences*, **265**, 1219–1226.  
647  
648 Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C-H., Xie, D., Suchard, M. A.,  
649 Rambaut, A., & Drummond, A. J. (2014). BEAST 2: a software platform for Bayesian  
650 evolutionary analysis. *PLoS Computational Biology*, **10**, e1003537.  
651



652 Bouma, J., Varralyay, G., & Batjes, N. (1998). Principal land use changes anticipated in  
653 Europe. *Agriculture, Ecosystems and Environment*, **67**, 103–119.

654

655 Braaker, S., & Heckel, G. (2009). Transalpine colonisation and partial phylogeographic  
656 erosion by dispersal in the common vole (*Microtus arvalis*). *Molecular Ecology*, **18**, 2518–  
657 2531.

658

659 Bulatova, N., Golenishchev, F., Bystrakova, N., Pavlova, S., Koval'skaya, J., Tikhonov, I.,  
660 Didorchuk, M., & Ivanov, K. (2007). Distribution and geographic limits of the alternative  
661 cytotypes of two *Microtus* voles in European Russia. *Hystrix, the Italian Journal of*  
662 *Mammalogy*, **18**, 99–109.

663

664 Bužan, E. V., Förster, D. W., Searle, J. B., & Kryštufek, B. (2010). A new cytochrome b  
665 phylogroup of the common vole (*Microtus arvalis*) endemic to the Balkans and its  
666 implications for the evolutionary history of the species. *Biological Journal of the Linnean*  
667 *Society*, **100**, 788–796.

668

669 Combourieu-Nebout, N., Peyron, O., Bout-Roumazeilles, V., Goring, S., Dormoy, I., Joannin,  
670 S., Sadori, L., Siani, G., & Magny, M. (2013). Holocene vegetation and climate changes in  
671 central Mediterranean inferred from a high-resolution marine pollen record (Adriatic Sea).  
672 *Climate of the Past Discussions*, **9**, 1969–2014. <https://doi.org/10.5194/cpd-9-1969-2013>  
673

674 Cruzan, M. B., & Templeton, A. R. (2000). Paleoecology and coalescence: phylogeographic  
675 analysis of hypotheses from the fossil record. *Trends in Ecology & Evolution*, **15**, 491–496.  
676

677 De Lattin, G. (1967). *Grunddriss der Zoogeographie*. Gustav Fischer, Jena.

678

679 Deffontaine, V., Libois, R., Kotlík, P., Sommer, R., Nieberding, C., Paradis, E., Searle, J. B.  
680 & Michaux, J. R. (2005). Beyond the Mediterranean peninsulas: evidence of central European  
681 glacial refugia for a temperate forest mammal species, the bank vole (*Clethrionomys*  
682 *glareolus*). *Molecular Ecology*, **14**, 1727–1739.

683

684 Delibes, M., & Brunett-Lecomte, P. (1980). Presencia del topillo campesino ibérico *Microtus*  
685 *arvalis asturianus* Millar 1908, en la meseta del Duero. *Doñana, Acta Vertebrata*, **7**, 120–123.  
686

687 Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics  
688 with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.  
689

690 Fink, S., Excoffier, L., & Heckel, G. (2004). Mitochondrial gene diversity in the common vole  
691 *Microtus arvalis* shaped by historical divergence and local adaptations. *Molecular Ecology*,  
692 **13**, 3501–3514.

693

694 Fouquet, A., Noonan, B. P., Rodrigues, M. T., Pech, N., Gilles, A., & Gemmell, N. J. (2012).  
695 Multiple Quaternary Refugia in the Eastern Guiana Shield Revealed by Comparative  
696 Phylogeography of 12 Frog Species. *Systematic Biology*, **61**, 461–461.

697

698 Frank, V. F. (1968). Zur Kenntnis der spanischen Feldmaus (*Microtus arvalis asturianus*  
699 Miller, 1908). *Bonner Zoologische Beiträge*, **19**, 189–197.

700

701 Garcia, J. T., Alda, F., Terraube, J., Mougeot, F., Sternalski, A., Bretagnolle, V., & Arroyo,  
702 B. (2011). Demographic history, genetic structure and gene flow in a steppe-associated raptor  
703 species. *BMC Evolutionary Biology*, *11*, 333.

704

705 Garcia, J. T., Mañosa, S., Morales, M. B., Ponjoan, A., García de la Morena, E. L., Bota, G.,  
706 Bretagnolle, V., & Davila, J. A. (2011). Genetic consequences of interglacial isolation in a  
707 steppe bird. *Molecular Phylogenetics and Evolution*, *61*, 671–676.

708

709 Gómez, A., & Lunt, D. H. (2007). Refugia within refugia: patterns of phylogeographic  
710 concordance in the Iberian Peninsula. In S. Weiss & N. Ferrand. (Eds.), *Phylogeography of*  
711 *southern European refugia* (pp. 155–188). Dordrecht, Springer.

712

713 González-Esteban, J., Villate, I., & Gosálbez, J. (1994). Requerimientos ambientales de  
714 *Microtus arvalis asturianus* Miller, 1908 (Rodentia, Arvicolidae) en la Península Ibérica.  
715 *Doñana, Acta Vertebrata*, *21*, 109–118.

716

717 González-Esteban, J., Villate, I., & Gosálbez, J. (1995). Expansión del área de distribución de  
718 *Microtus arvalis asturianus* Miller, 1908 (Rodentia, Arvicolidae) en la Meseta Norte (España).  
719 *Doñana Acta Vertebr.*, *22*, 106–10.

720

721 Gratton, P., Konopiński, M. K., & Sbordoni, V. (2008). Pleistocene evolutionary history of  
722 the Clouded Apollo (*Parnassius mnemosyne*): genetic signatures of climate cycles and a  
723 ‘time-dependent’ mitochondrial substitution rate. *Molecular Ecology*, *17*, 4248–4262.

724

725 Habel, J. C., Schmitt, T., & Müller, P. (2005). The fourth paradigm pattern of post-glacial  
726 range expansion of European terrestrial species: the phylogeography of the Marbled White  
727 butterfly (Satyrinae, Lepidoptera). *Journal of Biogeography*, *32*, 1489–1497.

728

729 Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis  
730 program for Windows 95/98/NT. *Nucleic Acid Symposium Series*, *41*, 95–98.

731

732 Haynes, S., Jaarola, M., & Searle, J. (2003). Phylogeography of the common vole (*Microtus*  
733 *arvalis*) with particular emphasis on the colonization of the Orkney archipelago. *Molecular*  
734 *Ecology*, *12*, 951–956.

735

736 Heckel, G., Burri, R., Fink, S., Desmet, J-F., & Excoffier, L. (2005). Genetic structure and  
737 colonization processes in European populations of the common vole, *Microtus arvalis*.  
738 *Evolution*, *59*, 2231–2242.

739

740 Hejzman, M., Hejzmanová, P., Pavlů, V., & Beneš, J. (2013). Origin and history of grasslands  
741 in Central Europe—a review. *Grass and Forage Science*, *68*, 345–363.

742

743 Herman, J. S., McDevitt, A. D., Kawalko, A., Jaarola, M., Wójcik, J. M., & Searle, J. B.  
744 (2014). Land-bridge calibration of molecular clocks and the post-glacial colonization of  
745 Scandinavia by the Eurasian field vole *Microtus agrestis*. *PloS One*, *9*, e103949.

746

747 Hewitt, G.M. (1996). Some genetic consequences of ice ages, and their role, in divergence  
748 and speciation. *Biological Journal of the Linnean Society*, *58*, 247–276.

749

- 750 Hewitt, G.M. (1999) Post-glacial re-colonization of European biota. *Biological Journal of the*  
751 *Linnean Society*, **68**, 87–112.
- 752
- 753 Hewitt, G. (2000). The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.
- 754
- 755 Hewitt, G. M. (2001). Speciation, hybrid zones and phylogeography—or seeing genes in  
756 space and time. *Molecular Ecology*, **10**, 537–549.
- 757
- 758 Hewitt, G. M. (2004). Genetic consequences of climatic oscillations in the Quaternary.  
759 *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **359**, 183–  
760 195.
- 761
- 762 Huntley, B. & Birks, H. J. B. (1983). *An atlas of past and present pollen maps for Europe, 0–*  
763 *13,000 years ago*. Cambridge University Press, Cambridge.
- 764
- 765 IUCN (2016). *Microtus arvalis*: Yigit, N., Hutterer, R., Kryštufek, B. & Amori, G.: The  
766 IUCN Red List of Threatened Species 2016: e.T13488A22351133.
- 767
- 768 Jaarola, M., Martínková, N., Gündüz, İ., Brunhoff, C., Zima, J., Nadachowski, A., Amori, G.,  
769 Bulatova, N. S., Chondropoulos, B., Fraguédakis-Tsolis, S., González-Esteban, J., López-  
770 Fuster, M. J., Kandaurov, A. S., Kefelioğlu, H., Mathias, M da L., Villate, I., & Searle, J. B.  
771 (2004). Molecular phylogeny of the speciose vole genus *Microtus* (Arvicolinae, Rodentia)  
772 inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, **33**,  
773 647–663.
- 774
- 775 Joger, U., Fritz, U., Guicking, D., Kalyabina-Hauf, S., Nagy, Z. T., & Wink, M. (2007).  
776 Phylogeography of western Palaearctic reptiles—Spatial and temporal speciation patterns.  
777 *Zoologischer Anzeiger-A Journal of Comparative Zoology*, *246*(4), 293–313.
- 778
- 779 Junker, M., Zimmermann, M., Ramos, A.A., Gros, P., Konvicka, M., Neve, G., Rakosy, L.,  
780 Tammaru, T., Castilho, R., & Schmitt, T. (2015) Three in One-Multiple Faunal Elements  
781 within an Endangered European Butterfly Species. *PLoS ONE*, **10**, e 0142282.
- 782
- 783 Kotlik, P., Deffontaine, V., Mascheretti, S., Zima, J., Michaux, J. R., & Searle, J. B. (2006). A  
784 northern glacial refugium for bank voles (*Clethrionomys glareolus*). *Proceedings of the*  
785 *National Academy of Sciences*, **103**, 14860–14864.
- 786
- 787 Kowalski, K. (2001). Pleistocene rodents of Europe. *Folia Quaternaria*, **72**, 3–389.
- 788
- 789 Kubatko, L. S., Carstens, B. C., & Knowles, L. L. (2009). STEM: species tree estimation  
790 using maximum likelihood for gene trees under coalescence. *Bioinformatics*, **25**, 971–973.
- 791
- 792 Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics  
793 Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**, 1870-1874.
- 794
- 795 Larson, G., Albarella, U., Dobney, K., Rowley-Conwy, P., Schibler, J., Tresset, A., Vigne, J-  
796 D., Ceiridwen, J. E., Schlumbaum, A., Dinu, A., Bălăşescu, A., Dolman, G., Tagliacozzo,  
797 A., Manaseryan, N., Miracle, P., Van Wijngaarden-Bakker, L., Masseti, M., Bradley, D. G.,  
798 & Cooper, A. (2007). Ancient DNA, pig domestication, and the spread of the Neolithic into

799 Europe. *Proceedings of the National Academy of Sciences*, **104**, 15276–15281.  
800  
801 Lemey, P., Rambaut, A., Drummond, A. J., & Suchard, M. A. (2009). Bayesian  
802 phylogeography finds its roots. *PLoS Computational Biology*, **5**, e1000520.  
803 Lemey, P., Rambaut, A., Welch, J. J., & Suchard, M. A. (2010). Phylogeography takes a  
804 relaxed random walk in continuous space and time. *Molecular Biology and Evolution*, **27**,  
805 1877–1885.  
806  
807 Mahmoudi, A., Darvish, J., Aliabadian, M., Moghaddam, F. Y., & Kryštufek, B. (2017). New  
808 insight into the cradle of the grey voles (subgenus *Microtus*) inferred from mitochondrial  
809 cytochrome b sequences. *Mammalia*, **81**, 583–593.  
810  
811 Mangerud, J., Jakobsson, M., Alexanderson, H., Astakhov, V., Clarke, G. K., Henriksen, M.,  
812 Hjort, C., Krinner, G., Lunkka, J-P., Möller, P., Murray, A., Nikolskaya, O., Saarnisto, M.,  
813 Svendsen, J. I. (2004). Ice-dammed lakes and rerouting of the drainage of northern Eurasia  
814 during the Last Glaciation. *Quaternary Science Reviews*, **23**, 1313–1332.  
815  
816 Markova, E., Malygin, V., Montuire, S., Nadachowski, A., Quéré, J.-P., & Ochman, K.  
817 (2010). Dental variation in sibling species *Microtus arvalis* and *M. rossiaemeridionalis*  
818 (Arvicolinae, Rodentia): between-species comparisons and geography of morphotype dental  
819 patterns. *Journal of Mammalian Evolution*, **17**, 121–139.  
820  
821 Markova, E., Beeren, Z., van Kolfschoten, T., Strukova, T., & Vrieling, K. (2012).  
822 Differentiating sibling species in the Quaternary fossil record: a comparison of morphological  
823 and molecular methods to identify *Microtus arvalis* and *M. rossiaemeridionalis* (Arvicolinae,  
824 Rodentia). *Journal of Systematic Palaeontology*, **10**, 585–597.  
825  
826 Martínková, N., Barnett, R., Cucchi, T., Struchen, R., Pascal, M., Fischer, M. C.,  
827 Higham, T., Brace, S., Ho, S. Y., Qu'er'e, J-P., O'Higgins, P., Excoffier, L., Heckel, G.,  
828 Hoelzel, A. R., Dobney, K. M., & Searle J. B. (2013). Divergent evolutionary processes  
829 associated with colonization of offshore islands. *Molecular Ecology*, **22**, 5205–5220.  
830  
831 Ménot, G., Bard, E., Rostek, F., Weijers, J. W., Hopmans, E. C., Schouten, S., & Damsté, J.  
832 S. S. (2006). Early reactivation of European rivers during the last deglaciation. *Science*, **313**,  
833 1623–1625.  
834  
835 Navarro, N., Montuire, S., Laffont, R., Steimetz, E., Onofrei, C., & Royer, A. (2018).  
836 Identifying Past Remains of Morphologically Similar Vole Species Using Molar Shapes.  
837 *Quaternary*, **1**, 20.  
838  
839 Nesterova, T. B., Mazurok, N. A., Rubtsova, N. V., Isaenko, A. A., & Zakian, S. M. (1998).  
840 The vole gene map. *ILAR Journal*, **39**, 138–144.  
841  
842 Niethammer, J., & Winking, H. (1971). Die spanische Feldmaus (*Microtus arvalis asturianus*  
843 Miller, 1908). *Bonner Zoologisches Beitrage*, **19**, 189–197.  
844  
845 Novenko, Ey., Volkova, E., Nosova, N., & Zugarova, I. (2009). Late Glacial and Holocene  
846 landscape dynamics in the southern taiga zone of East European Plain according to pollen and  
847 macrofossil records from the Central Forest State Reserve (Valdai Hills, Russia). *Quaternary*

848 *International*, **207**, 93–103.  
849  
850 O'Connor, R., & Shrubbs, M. (1986). *Farming and Birds*. Cambridge, Cambridge University  
851 Press.  
852 Palmer, G., Platts, P. J., Brereton, T., Chapman, J. W., Dytham, C., Fox, R., Pearce-Higgins,  
853 J. W., Roy, D. B., Hill, J. K., & Thomas, C. D. (2017). Climate change, climatic variation and  
854 extreme biological responses. *Philosophical Transactions of the Royal Society of London B:  
855 Biological Sciences*, **372**, 20160144.  
856  
857 Pärtel, M., Bruun, H. H., & Sammuli, M. (2005). Biodiversity in temperate European  
858 grasslands: origin and conservation. *Grassland Science in Europe*, **10**, 14.  
859  
860 Pazonyi, P. (2004). Mammalian ecosystem dynamics in the Carpathian Basin during the last  
861 27,000 years. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **212**, 295–314.  
862  
863 Pinceel, J., Jordaens, K., Pfenninger, M., & Backeljau, T. (2005) Rangewide phylogeography  
864 of a terrestrial slug in Europe: evidence for Alpine refugia and rapid colonization after the  
865 Pleistocene glaciations. *Molecular Ecology*, **14**, 1133–1150.  
866  
867 Provan, J., & Bennett, K. (2008). Phylogeographic insights into cryptic glacial refugia. *Trends  
868 in Ecology & Evolution*, **23**, 564–571.  
869  
870 Rambaut, A., & Drummond, A. (2007). *Tracer v1.4*. Available from:  
871 <http://beast.bio.ed.ac.uk/Tracer>.  
872  
873 Randi, E. (2007). *Phylogeography of South European mammals*. In S. Weiss & N. Ferrand  
874 (Eds.), *Phylogeography of Southern European Refugia* (pp. 101–126). Dordrecht, Springer.  
875  
876 Rasmussen, S. O., Andersen, K. K., Svensson, A. M., Steffensen, J. P., Vinther, B. M.,  
877 Clausen, H. B., Siggaard-Andersen, M-L., Johnsen, S. J., Larsen, L. B., Dahl-Jensen, D.,  
878 Bigler, M., Röthlisberger, R., Fischer, H., Goto-Azuma, K., Hansson, M. E., & Ruth, U.  
879 (2006). A new Greenland ice core chronology for the last glacial termination. *Journal of  
880 Geophysical Research*, **111**, D06102.  
881  
882 Rey, J. M. (1973). Las características biométricas y morfológicas del topillo campesino,  
883 *Microtus arvalis asturianus*, del Sistema Ibérico. *Boletín de La Real Sociedad Española de  
884 Historia Natural–Biología*, **71**, 283–297.  
885  
886 Roberts, N., Fyfe, R. M., Woodbridge, J., Gaillard, M-J., Davis, B. A. S., Kaplan, J. O.,  
887 Marquer, L., Mazier, F., Nielsen, A. B., Sugita, S., Trondman, A-K., & Leydet, M. (2018).  
888 Europe's lost forests: a pollen-based synthesis for the last 11,000 years. *Scientific Reports*, **8**,  
889 716.  
890  
891 Rowe, K. C., Heske, E. J., Brown, P. W., & Paige, K. N. (2004). Surviving the ice: Northern  
892 refugia and postglacial colonization. *Proceedings of the National Academy of Sciences of the  
893 United States of America*, **101**, 10355–10359.  
894  
895 Rowley-Conwy, P. (2011). Westward Ho!: The Spread of Agriculture from Central Europe to  
896 the Atlantic. *Current Anthropology*, **52**(S4), S431–S451.

897  
898 Ruddiman, W. (2003). The anthropogenic greenhouse era began thousands of years ago.  
899 *Climate Change*, **61**, 261–293.  
900  
901 Rull, V. (2009). Microrefugia. *Journal of Biogeography*, **36**, 481–484.  
902  
903 Rull, V. (2010). On microrefugia and cryptic refugia. *Journal of Biogeography*, **37**, 1623–  
904 1625.  
905  
906 Schmitt, T. (2007). Molecular biogeography of Europe: Pleistocene cycles and postglacial  
907 trends. *Frontiers in Zoology*, **4**, 11.  
908  
909 Schmitt, T., Rákosy, L., Abadjev, S., & Müller, P. (2007). Multiple differentiation centres of  
910 a non-Mediterranean butterfly species in south-eastern Europe. *Journal of Biogeography*, **34**,  
911 939–950.  
912  
913 Schmitt, T., & Varga, Z. (2012). Extra-Mediterranean refugia: The rule and not the  
914 exception? *Frontiers in Zoology*, **9**, 22.  
915  
916 Schönswetter, P., Stehlik, I., Holderegger, R., & Tribsch, A. (2005). Molecular evidence for  
917 glacial refugia of mountain plants in the European Alps. *Molecular Ecology*, **14**, 3547–3555.  
918  
919 Sommer, R. S., & Nadachowski, A. (2006). Glacial refugia of mammals in Europe: evidence  
920 from fossil records. *Mammal Review*, **36**, 251–265.  
921  
922 Stewart, J. R., & Lister, A. (2001). Cryptic northern refugia and the origins of the modern  
923 biota. *Trends in Ecology & Evolution*, **16**, 608–613.  
924  
925 Stewart, J. R. (2003). Comment on ‘Buffered Tree Population Changes in a Quaternary  
926 Refugium: Evolutionary Implications’. *Science*, **299**, 825.  
927  
928 Stewart, J. R., Lister, A.M., Barnes, I., & Dalén, L. (2010) Refugia revisited: individualistic  
929 responses of species in space and time. *Proceedings of the Royal Society of London B:*  
930 *Biological Sciences*, **277**, 661–671.  
931  
932 Stojak, J., McDevitt, A. D., Herman, J. S., Searle, J. B., & Wójcik, J. M. (2015). Post-glacial  
933 colonization of eastern Europe from the Carpathian refugium: evidence from mitochondrial  
934 DNA of the common vole *Microtus arvalis*. *Biological Journal of the Linnean Society*, **115**,  
935 927–939.  
936  
937 Stojak, J., McDevitt, A. D., Herman, J. S., Kryštufek, B., Uhlíková, J., Purger, J. J.,  
938 Lavrenchenko, L. A., Searle, J. B., & Wójcik, J. M. (2016). Between the Balkans and the  
939 Baltic: Phylogeography of a common vole Mitochondrial DNA lineage limited to Central  
940 Europe. *PloS One*, **11**, e0168621.  
941  
942 Stojak, J., Wójcik, J. M., Ruczyńska, I., Searle, J. B., & McDevitt, A. D. (2016). Contrasting  
943 and congruent patterns of genetic structuring in two *Microtus* vole species using museum  
944 specimens. *Mammal Research*, **61**, 141–152.  
945

946 Stojak, J., Borowik, T., Górny, M., McDevitt, A. D., & Wójcik, J. M. (2019). Climatic  
947 influences on the genetic structure and distribution of the common vole and field vole in  
948 Europe. *Mammal Research*, **64**, 19–29.  
949  
950  
951 Tougard, C., Renvoisé, E., Petitjean, A., & Quéré, J-P. (2008). New insight into the  
952 colonization processes of common voles: inferences from molecular and fossil evidence.  
953 *PLoS One*, **3**, e3532.  
954  
955 Tougard, C., Montuire, S., Volobouev, V., Markova, E., Contet, J., Aniskin, V., & Quere, J-P.  
956 (2013). Exploring phylogeography and species limits in the Altai vole (Rodentia: Cricetidae).  
957 *Biological Journal of the Linnean Society*, **108**, 434–452.  
958  
959 Tougard, C. (2016). Did the Quaternary climatic fluctuations really influence the tempo and  
960 mode of diversification in European rodents? *Journal of Zoological Systematics and*  
961 *Evolutionary Research*, **55**, 46–56.  
962  
963 Triant, D., & DeWoody, J. (2006). Accelerated molecular evolution in *Microtus* (Rodentia) as  
964 assessed via complete mitochondrial genome sequences. *Genetica*, **128**, 95–108.  
965  
966 Turner, C., & Hannon, G. E. (1988). Vegetational evidence for late Quaternary climatic  
967 changes in southwest Europe in relation to the influence of the North Atlantic Ocean.  
968 *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **318**, 451–  
969 485.  
970  
971 Ursenbacher, S., Carlsson, M., Helfer, V., Tegelström, H., & Fumagalli, L. (2006).  
972 Phylogeography and Pleistocene refugia of the adder (*Vipera berus*) as inferred from  
973 mitochondrial DNA sequence data. *Molecular Ecology*, **15**, 3425–3437.  
974  
975 Willis, K. J., & Whittaker, R. J. (2000). The refugial debate. *Science*, **287**, 1406–1407.  
976  
977 Wilson, D. E., Lacher, T. E., Mittermeier, R. A., Martínez-Vilalta, A., Leslie, D. M., Elliott,  
978 A., Christie, D.A., Llobet, T., Hoyo, J., & Copete, J. L. (2017). *Handbook of the Mammals of*  
979 *the World: 7: rodents II*. Lynx Edicions, Barcelona.  
980  
981  
982

## Figure Legends

**Figure 1. Current distribution and sampling locations for *Microtus arvalis* in Europe.** The blue-shaded zone corresponds to the distribution range of the common vole and the solid black line marks the suture zone between the western (*arvalis*) and eastern (*obscurus*) subspecies. The approximate geographic boundaries of major phylogenetic lineages described in the literature are indicated with dashed lines. Sampling localities are coloured according to the cytochrome *b* lineage (red: Iberian; orange: Western–South; dark blue: Western–North; light blue: Balkan; light green: Italian; dark green: Central; pink: Eastern). The numbers refer to sample locality numbers listed in Appendix 1. Major European rivers are shown with light blue lines and names in black.

**Figure 2. Maximum clade credibility (MCC) tree of *Microtus arvalis*.** Bayesian analysis of the cytochrome *b* gene (141 haplotypes sampled in 78 different localities from 23 countries across the species range; Appendix 1) with the best fit model (Cauchy RRW). The 95% highest probability density (95%HPD) for the most internal nodes and major lineages are shown as blue bars. Bayesian posterior probability values are included for major nodes. Coloured branches represent each of the major lineages, color-coded as in Figure 1. Grey background shaded range (95% HPD) and white line (median) indicates the effective population size trajectory based on Bayesian skyride analysis of mtDNA. The abscissa shows the time in years before present.

**Figure 3. Median-joining network of *Microtus arvalis* haplotypes.** Each node corresponds to a haplotype determined for a unique cytochrome *b* sequence. Numbers in circles show the numbers of nucleotide differences between major lineages. Black dots represent missing haplotypes not observed in the analyzed individuals. Haplotypes were broadly grouped into their respective lineages (see also Figure 1). Haplotypes under the previously described Western-South lineage are sub-grouped into samples from Iberian (tentative *M. a. asturianus* specimens), and samples from Pyrenees and France (*M. arvalis*).

**Figure 4. Spatial projection of the diffusion pattern in *Microtus arvalis* through time.** Results based on the maximum clade credibility (MCC) tree, estimated with a Bayesian phylogeographic analysis in BEAST (Cauchy RRW model) at ten time slices defined to coincide with major internal nodes within the MCC tree. The yellow lines represent the branches of the MCC tree. The polygons in the map (panels B to J) represent the 80% HPD uncertainty in the geographic locations of internal nodes in the MCC tree. The white-red gradient of the polygons represents the relative age of the dispersal events from white (old) to red (recent). The inferred location of the most recent common ancestor of *M. arvalis* lineages in Europe is shown in panel A (contours represent the 80% [yellow], 60% [orange] and 40% [red] HPD regions, based on kernel density estimates).



1028 **Appendix 1.** List of all *M. arvalis* haplotypes used for Bayesian analysis of the cytochrome *b*  
 1029 gene in this study, geographic location and number of different haplotypes in each locality  
 1030 (NH). The numbers in parentheses correspond with localities in Figure 1.  
 1031

Geographic origin	Ref.	NH	Lat.	Long.	GenBank Accession Number
Kranj; Slovenia (1)	<i>a</i>	1	46.24	14.36	GU187381
Ljubljana; Slovenia (2)	<i>a</i>	2	46.04	14.52	GU187382-3
Gacko; Bosnia (3)	<i>a</i>	4	43.10	18.33	GU187368, GU187373, GU187376-7
Kupres, BH; Bosnia (4)	<i>a</i>	4	43.56	17.11	GU187365-7, GU187369
Mt. Zelengora; Bosnia (5)	<i>a</i>	4	43.15	18.35	GU187368, GU187373-5
Bosanski Petrovac; Bosnia (6)	<i>a</i>	1	44.35	16.21	GU187384
Mt.Šator; Bosnia (7)	<i>a</i>	1	44.09	16.37	GU187385
Mt. Komovi; Montenegro (8)	<i>a</i>	2	42.42	19.39	GU187378-9
Slano Kopovo; Serbia (9)	<i>a</i>	2	45.20	20.10	GU187384, GU187362
Mt. Suva planina; Serbia (10)	<i>a</i>	1	43.07	22.16	GU187380
Białowieża; Poland (11)	<i>b</i>	3	52.42	23.52	KP255605-7
Popówka; Poland (12)	<i>b</i>	3	52.04	23.26	KP255614-6
Sochaczew; Poland (13)	<i>b</i>	3	52.13	20.14	KP255599-601
Poddębice; Poland (14)	<i>b</i>	3	51.54	18.58	KP255587-9
Łowicz; Poland (15)	<i>b</i>	3	52.06	19.56	KP255602-4
Chernobyl; Ukraine (16)	<i>c</i>	1	51.27	30.22	U54488
Buchak; Ukraine (17)	<i>b</i>	3	49.87	31.43	KP255618-20
Vienna; Austria (18)	<i>d</i>	2	48.38	16.35	AY708460-1
Brussels; Belgium (19)	<i>d</i>	4	50.80	4.35	AY708508-10, AY708462
Stalhille; Belgium (20)	<i>e</i>	2	51.21	3.07	GU190540, GU190536
Vetřkovice; Czech Republic (21)	<i>d</i>	4	49.76	17.80	AY708471-3, AY708505
Drnholec; Czech Republic (22)	<i>f</i>	2	48.85	16.48	FR865434
Nosislav; Czech Republic (23)	<i>f</i>	2	49.00	16.65	FR865432-3
Studeneć; Czech Republic (24)	<i>f</i>	1	50.55	15.53	FR865434
Freiburg; Germany (25)	<i>g</i>	7	48.00	7.80	FJ789987, FJ789989, FJ790014-8
Heilsbronn; Germany (26)	<i>d</i>	3	49.32	10.80	AY708476-8
Alflen; Germany (27)	<i>e</i>	2	50.18	7.04	GU190616-7
NE Hamburg; Germany (28)	<i>e</i>	2	53.60	10.05	GU190662-3
Schiltach; Germany (29)	<i>e</i>	1	48.30	8.34	GU190618
Rosenheim; Germany (30)	<i>g</i>	2	47.85	12.12	FJ790019-20
Aigle; Switzerland (31)	<i>g</i>	4	46.35	6.92	FJ789990-1, AY708519, FJ790029
Lausanne; Switzerland (32)	<i>g</i>	4	46.57	6.55	AY708514, AY708467-9
Walenstadt; Switzerland (33)	<i>g</i>	3	47.12	9.30	AY708519, FJ790011-2
Chur; Switzerland (34)	<i>g</i>	2	46.83	9.48	AY708512, AY708466
Mont-la-ville; Switzerland (35)	<i>g</i>	3	46.63	6.40	AY708510, FJ790005, FJ790029
Trento; Italy (36)	<i>h</i>	1	46.06	11.11	AY220766
Laas; Italy (37)	<i>g</i>	1	46.62	10.70	FJ789995

Bozen; Italy (38)	<i>g</i>	1	46.50	11.30	FJ790024
Marling; Italy (39)	<i>g</i>	2	46.65	11.13	FJ790025, AY220766
Neumarkt; Italy (40)	<i>g</i>	1	46.32	11.27	FJ790026
Bendern; Liechtenstein (41)	<i>g</i>	1	47.22	9.50	FJ790031
Ruggell; Liechtenstein (42)	<i>g</i>	2	47.23	9.52	FJ790030, FJ790032
Nagycsány; Hungary (43)	<i>h</i>	1	45.86	17.95	AY220769
Velké Kosihi; Slovakia (44)	<i>h</i>	1	47.76	17.88	AY220767
Stebník; Slovakia (45)	<i>h</i>	1	49.38	21.27	AY220768
Hjerl Hede; Denmark (46)	<i>h</i>	1	56.48	8.87	AY220776
Nørre Farup; Denmark (47)	<i>e</i>	2	55.35	8.73	GU190660-1
Lauwersee; Netherlands (48)	<i>h</i>	1	53.38	6.18	AY220778
Pumerend; Netherlands (49)	<i>e</i>	2	52.51	4.94	GU190512, GU190516
Oostburg; Netherlands (50)	<i>e</i>	1	51.33	3.49	GU190531
Mantet; Pyrenees (51)	<i>h</i>	1	42.48	2.30	AY220789
Vernet les Bains; Pyrenees (52)	<i>e</i>	1	42.50	2.35	GU190383
Plà de Beret; Pyrenees (53)	<i>e</i>	3	42.72	0.84	GU190385, GU190388-9
Luumäki; Finland (54)	<i>h</i>	1	60.91	27.56	AY220770
Nuijamaa; Finland (55)	<i>h</i>	1	60.95	28.57	AY220770
Vladimir; Russia (56)	<i>h</i>	1	56.13	40.42	AY220771
Zvenigorod; Russia (57)	<i>h</i>	1	55.73	36.85	AY220770
Luxemburg; Luxemburg (58)	<i>e</i>	2	49.61	6.13	GU190395-6
Aiffres; France (59)	<i>e</i>	4	46.29	-0.41	GU190547-50
Armendarits; France (60)	<i>e</i>	1	43.30	-1.17	GU190634
Baie de l'Aiguillon; France (61)	<i>e</i>	3	46.30	-1.17	GU190417-8, GU190421
Cissé; France (62)	<i>e</i>	4	46.64	0.23	GU190552-5
Ste. Marie du Mont; France (63)	<i>e</i>	4	49.38	-1.23	GU190566-7, GU190569-70
Pihen lès Guînes; France (64)	<i>e</i>	2	50.87	1.79	GU190604, GU190606
Outre; France (65)	<i>e</i>	4	46.08	3.03	GU190411-3, GU190415
Les Forts; France (66)	<i>e</i>	3	48.33	1.18	GU190557-8, GU190560
Crépaillat; France (67)	<i>e</i>	3	46.14	2.74	GU190405-6, GU190408
Arbejal; Spain (68)	<i>i</i>	5	42.88	-4.50	MG874847, MG874849, MG474853-5,
Bello; Spain (69)	<i>i</i>	5	40.92	-1.50	MG874851, MG874856-9
Ventosa del Río Almar; Spain (70)	<i>i</i>	5	40.94	-5.31	MG874854, MG874863, MG874870, MG874872, MG874877
Campo Azálvaro; Spain (71)	<i>i</i>	5	40.68	-4.38	MG874847, MG874849, MG874854, MG874865-6
Chañe; Spain (72)	<i>i</i>	3	41.33	-4.39	MG874849, MG874854, MG874870
San Emiliano; Spain (73)	<i>i</i>	4	42.96	-6.02	MG874860, MG874873-5
Grañón; Spain (75)	<i>i</i>	5	42.46	-3.02	MG874850, MG874854, MG874860, MG874867, MG874871
Milles de la Polvorosa; Spain (76)	<i>i</i>	2	41.92	-5.73	MG874860, MG874863

Revilla de Campos; Spain (77)	<i>i</i>	5	42.01	-4.71	MG8741847, MG874863, MG874873, MG874860-1
San Martín de Valderaduey; Spain (78)	<i>i</i>	7	41.81	-5.47	MG8741847, MG874860-1, MG874868, MG874863-4, MG874876
Nofels; Austria (79)	<i>g</i>	2	47.15	9.34	FJ790031-2

1032 *a*>Bužan et al., 2010; *b*>Stojak et al., 2015; *c*>Barker et al., 1996; *d*>Fink et al., 2004;  
1033 *e*>Martínková et al., 2013; *f*>Tougaard et al., 2013; *g*>Braaker & Heckel, 2009; *h*>Haynes et  
1034 al., 2003; *i*>This study

1035  
1036  
1037  
1038  
1039  
1040  
1041

**Table 1.** Average pairwise genetic distance (uncorrected, *p*) of cytochrome *b* sequences for within (diagonal) and between (below diagonal) clades of *M. arvalis*. The Iberian clade was separated from the rest of the Western-South (W-S) clade (France and Pyrenees).

1042

	W-S (Iberian)	W-S (FR+Py)	Western-North	Balcan	Italian	Central	Eastern
W-S (Iberian)	<b>0,0035</b>						
W-S (FR+Py)	0,0159	<b>0,0037</b>					
Western-North	0,0219	0,0210	<b>0,0043</b>				
Balcan	0,0269	0,0259	0,0264	<b>0,0061</b>			
Italian	0,0344	0,0322	0,0313	0,0241	<b>0,0057</b>		
Central	0,0310	0,0276	0,0268	0,0267	0,0247	<b>0,0048</b>	
Eastern	0,0293	0,0300	0,0294	0,0264	0,0248	0,0149	<b>0,0080</b>

1043

Figure 1. Current distribution and sampling locations for *Microtus arvalis* in Europe.



Figure 2. Maximum clade credibility (MCC) tree of *Microtus arvalis*

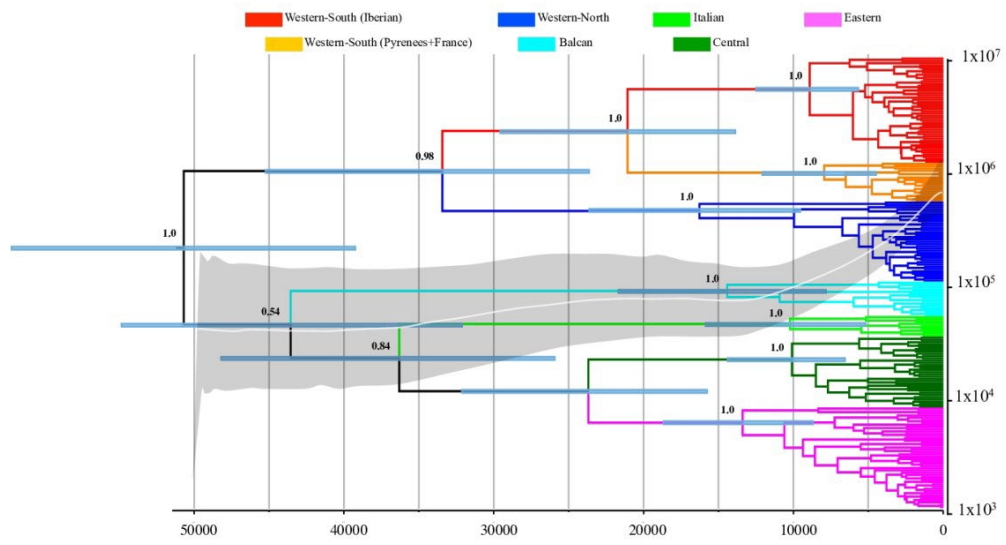


Figure 3. Median-joining network of *Microtus arvalis* haplotypes

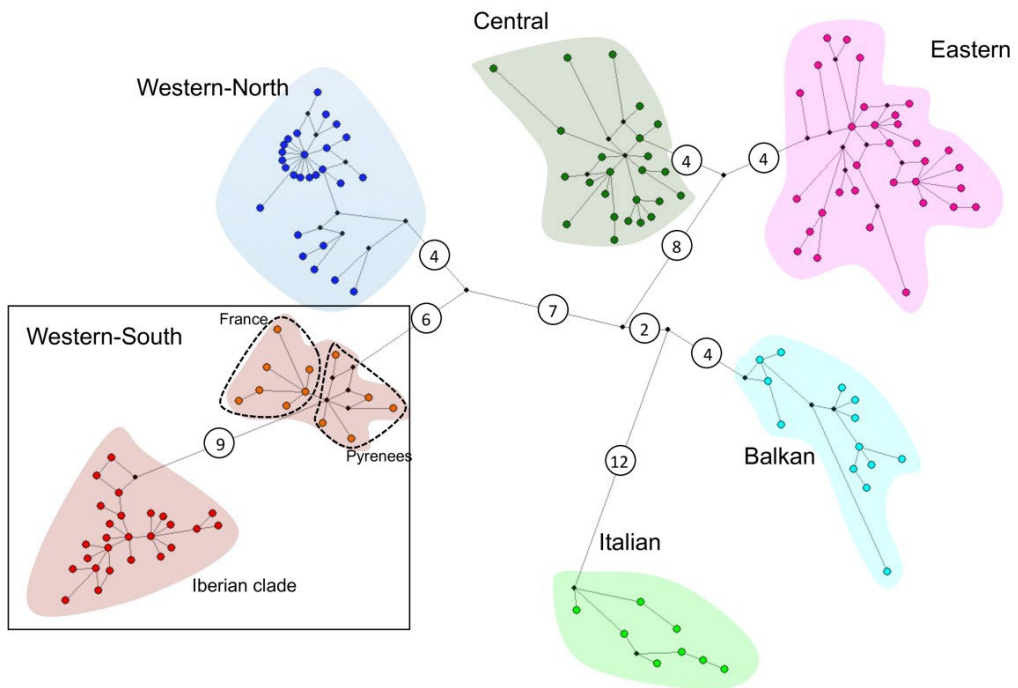


Figure 4. Spatial projection of the diffusion pattern in *Microtus arvalis* through time

