Aalborg Universitet

AALBORG UNIVERSITY

DENMARK

Phylogeography of the forest-dwelling European pine marten (Martes Martes)

new insights into cryptic northern glacial refugia

Ruiz-González, Aritz; Madeira, María José; Randi, Ettore; Abramov, Alexei V.; Davoli, Francesca; Gómez-Moliner, Benjamín J.

Phylogeography of the forest-dwelling European pine marten (*Martes martes*): new insights into cryptic northern glacial refugia

ARITZ RUIZ-GONZÁLEZ[1,2,3]*, MARÍA JOSÉ MADEIRA[1,2], ETTORE RANDI[3,4], ALEXEI V. ABRAMOV[5], FRANCESCA DAVOLI[3] and BENJAMÍN J. GÓMEZ-MOLINER[1,2]

[1]*Department of Zoology and Animal Cell Biology, University of the Basque Country UPV/EHU, C/Paseo de la Universidad 7, 01006 Vitoria-Gasteiz, Spain*
[2]*Systematics, Biogeography and Population Dynamics Research Group, Lascaray Research Center, University of the Basque Country (UPV/EHU), Avda. Miguel de Unamuno, 3, 01006 Vitoria-Gasteiz, Spain*
[3]*Laboratorio di genetica, Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA), Via Cà Fornacetta 9, 40064, Ozzano dell'Emilia, Bologna, Italy*
[4]*Department 18/Section of Environmental Engineering, Aalborg University, Sohngårdsholmsvej 57, 9000 Aalborg, Denmark*
[5]*Laboratory of Mammalogy, Zoological Institute, Russian Academy of Sciences, Universitetskaya nab. 1, Saint-Petersburg 199034, Russia*

The role of southern European peninsulas as glacial refugia for temperate species has been widely established, but the role of cryptic northern refugia has only recently been addressed. Here, we describe the phylogeographic pattern of the forest-dwelling European pine marten (*Martes martes*), using a 1600-bp mitochondrial DNA (mtDNA) fragment from 287 individuals sampled across the entire distribution range of the species. To clarify the relationships between *M. martes* and its sister species the sable (*Martes zibellina*) in Fennoscandia and Russia, ten *M. zibellina* samples were also included in the analyses. Our results reveal the presence of 69 different haplotypes for *M. martes* and ten haplotypes for *M. zibellina*, which are split into three major assemblages: Mediterranean, central–northern European, and Fennoscandian–Russian clades, showing a global pattern of spatial segregation, with some area of overlap and genetic admixture. It is apparent that the Mediterranean phylogroup did not significantly contribute to the postglacial recolonization of most of the Palaearctic range of the species. Instead, most of Europe was colonized by the central–northern European phylogroup, which probably survived the last glaciations in northern cryptic refugia, as has previously been suggested by palaeontological studies. A highly divergent phylogroup has been discovered in Fennoscandia–Russia, which includes specimens from both Martes species. Calculations of divergence times suggest that the phylogroups split during the Pleistocene. Overall, our study indicates a complex phylogeographic history for *M. martes*, indicating a mixed pattern of recolonization of northern Europe from both Mediterranean and non-Mediterranean refugia, providing new insights into the existence of cryptic northern glacial refugia for temperate species in Europe. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, **109**, 1–18.

ADDITIONAL KEYWORDS: cryptic glacial refugia – *Martes martes* – *Martes zibellina* – mitochondrial DNA – phylogeography – quaternary glaciations.

*Corresponding author. E-mail: aritz.ruiz@ehu.es

INTRODUCTION

The dramatic climate changes during the Quaternary have had a heavy impact on the distributions of species within the Palaearctic region (Avise, 2000; Hewitt, 2004). The prevailing theory for most temperate species in Europe has been that populations were forced to shift their distribution range, surviving in Mediterranean refuge areas during the Last Glacial Maximum (LGM) (Taberlet *et al.*, 1998; Hewitt, 1999, 2001; Randi, 2007). Thus, interglacial and postglacial recolonizations of central and northern Europe could therefore have arisen from these Mediterranean refugial populations (Taberlet *et al.*, 1998; Hewitt, 2001). More recently, however, the additional existence of northern cryptic glacial refugia, that is, glacial refugia for temperate taxa located at higher latitudes than expected (Stewart & Lister, 2001; Stewart *et al.*, 2010), has received significant empirical support (Bhagwat & Willis, 2008; Provan & Bennett, 2008; Schmitt & Varga, 2012). A number of recent phylogeographic studies showed evidence for northern refugia in various temperate species (e.g. Jaarola & Searle, 2002; Deffontaine *et al.*, 2005; Kotlik *et al.*, 2006; Saarma *et al.*, 2007; Valdiosera *et al.*, 2007; Wojcik *et al.*, 2010; McDevitt *et al.*, 2012). Further evidence has come from the mammal fossil records (Sommer & Benecke, 2004; Sommer & Nadachowski, 2006), fossil pollen data, and macrofossil remains (Willis & van Andel, 2004), and from species distribution modelling (Svenning, Normand & Kageyama, 2008; Fløjgaard *et al.*, 2009). Altogether, these findings suggest that during the LGM some temperate species, which were formerly thought to have been completely restricted to Mediterranean core areas, could have survived at higher latitudes in extra-Mediterranean refugia, in addition to the typical Mediterranean refuge areas (Schmitt & Varga, 2012).

The European pine marten, *Martes martes* (Linnaeus, 1758), is a mid- sized mustelid that occurs throughout much of Europe and northern and central Asia, from northern Portugal to western Siberia, and is generally associated with forest habitats, mainly mature coniferous and mixed forests (Proulx *et al.*, 2004). *Martes martes* is either threatened or scarce in many countries where forest habitat loss and fragmentation are major threats (Kranz *et al.*, 2008). Recent reconstructions of the Quaternary distributions of mustelids, on the basis of fossil evidence, suggest the existence of a cryptic glacial refuge in the Carpathians for *M. martes*, in addition to the traditional Mediterranean refugia (Sommer & Benecke, 2004; Sommer & Nadachowski, 2006). Bhagwat & Willis (2008) suggest that species that have persisted in northern refugia have shared biogeographical traits that match those found in *M. martes*: a present-

day northern distribution, small body size, and cold tolerance. Moreover, the fossil record and recent molecular data evidence the existence of central European refugia for the bank vole, *Myodes glareolus* (Schreber, 1780), (Deffontaine *et al.*, 2005; Kotlik *et al.*, 2006; Wojcik *et al.*, 2010), which is one of the main prey sources of *M. martes* (Zalewski, 2004). Thus, taking into account all of this evidence, we hypothesized that populations of *M. martes* might have persisted either in southerly refugia or also further north in Europe, in northern cryptic glacial refugia.

The phylogeography of *M. martes* is only poorly known. Davison *et al.* (2001) suggested that *M. martes* populations currently occurring in central and northern Europe originated from several refugia, with subsequent admixture; however, this study was based on a small fragment of mitochondrial DNA (mtDNA; 325 bp) that was not sufficiently informative to resolve the species phylogeography. Moreover, a lack of samples from the main Mediterranean refuge areas (i.e. the Balkans, the Iberian Peninsula, and Italy) and the eastern Russian populations did not allow the identification of specific locations of refugia or the process of postglacial recolonization of Europe. Here, we present a more comprehensive study in terms of the number of specimens ($N = 287$) and length of the mtDNA sequence (1600 bp). The sampling also covered a broader geographic range, reaching as far as Fennoscandia in the north, European Russia in the east, and the Mediterranean peninsulas in the south. Sable, *Martes zibellina* (Linnaeus, 1758), samples were also included to better understand the relationships between *M. martes* and the closely related *M. zibellina* in Fennoscandia and Russia. Indeed, recent studies suggest that the subgenus *Martes* diversified during the Plio-Pleistocene, and recognized *M. martes* and *M. zibellina* as sister species within this subgenus (Koepfli *et al.*, 2008). Additional analyses, including population genetic-level sampling, are therefore needed to confidently resolve relationships among these recently evolved species (Koepfli *et al.*, 2008; Schwartz *et al.*, 2012). Thus, in this study we aim to: (1) identify the main phylogeographic patterns in *M. martes*; (2) reconstruct the postglacial colonization routes of central Europe; and (3) obtain the first data on the genetic structure of eastern European *Martes* populations, with special emphasis on the genetic relationship between *M. martes* and *M. zibellina*.

MATERIAL AND METHODS

SAMPLES AND LABORATORY PROCEDURES

Tissue and hair samples were collected from 287 *M. martes* throughout 21 countries, correspond-
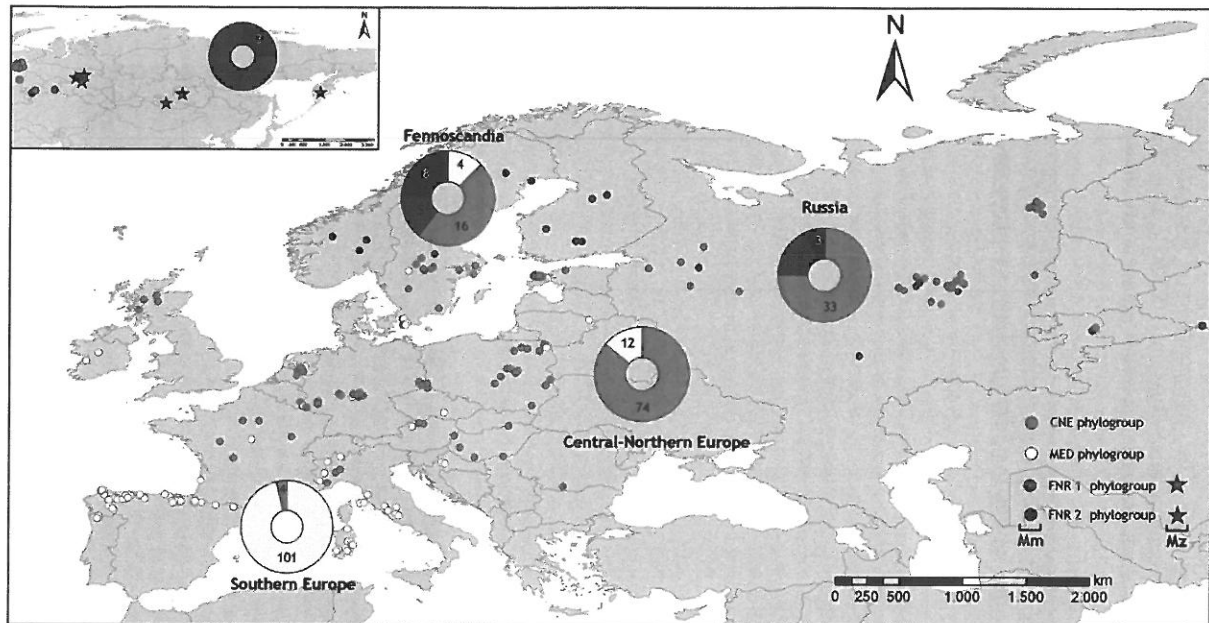
Figure 1. Geographical distribution of the European pine marten (*Martes martes*, Mm) (*N* = 287) and sable (*Martes zibellina*, Mz) (*N* = 10) samples, represented as dots and stars, respectively. The correspondence of each sample with the discovered phylogroups is also shown. Mediterranean (MED), central–northern European (CNE), Fennoscandian–Russian 1 (FNR1) and Fennoscandian–Russian 2 (FNR2) phylogroups are represented in white, light grey, black grey, and black, respectively. The proportion of each phylogroup at different geographical regions (southern Europe; central–northern Europe; Fennoscandia and Russia) is represented as open circles (see Table 1 for a detailed description of each geographical region).

ing to the main areas of their distribution range (Fig. 1; Table 1). We also added ten *M. zibellina* samples from Russia. These specimens were obtained from collaborators and museum collections (see Table S1 for additional locality and specimen information). DNA was isolated from tissue and hair using the Qiagen DNeasy Tissue DNA extraction kit (Qiagen Inc., Valencia, CA, USA), according to the manufacturer's instructions.

The mitochondrial DNA region selected in this study includes the final part of the cytochrome *b* gene, *tRNAPro*, *tRNAThr*, the control region (D-loop), and the initial part of *12S* rRNA used in a previous work on genetic variability of the European polecat, *Mustela putorius* Linnaeus, 1758, in Europe (Pertoldi *et al.*, 2006). This fragment of ~1600 bp in length was amplified using the forward primer LutbF (5′-AGAACACCCATTCATCATTATCG-3′) and the reverse primer LLU12SH91 (5′-CTAGAGGGAT GTAAAGCA CCG-3′) (Pertoldi *et al.*, 2006). The standard polymerase chain reaction (PCR) amplifications were conducted in 15-μL reactions containing 1 μL of diluted template DNA, 3.2 pmol of each primer, 1.75 μM deoxyribonucleotide triphosphate (dNTP), 1.33 μM MgCl$_2$, 1.56 μL of Gold ST★R 10X

buffer, and 0.6 U Taq DNA polymerase, using the following cycling conditions: an initial denaturing step at 94 °C for 5 min; 35 cycles of denaturing at 94 °C for 50 s, annealing at 58.5 °C for 45 s, and extending at 72 °C for 90 s, with a final extending step of 72 °C for 10 min.

The PCR products were purified using EXO-SAP IT (USB, Cleveland, OH, USA), and sequenced using the BigDye Terminator Kit V1.1 (Applied Biosystems, Foster City, CA, USA) in an ABI PRISM Model 3130 Genetic Analyzer (Applied Biosystems). Electropherograms were visually inspected using SEQSCAPE 2.5 (Applied Biosystems), and nucleotide sequences were aligned using the default parameters of CLUSTALX 2.0 (Larkin *et al.*, 2007) and manually checked in BIOEDIT 5.0.9 (Hall, 1999). The minisatellite repetition of the control region [(TACGCACACG)-*N*] was removed from the phylogenetic analysis to reduce ambiguous sites with the out-groups selected.

PHYLOGENETIC ANALYSES

The data set used for phylogenetic analysis includes the haplotype sequences of the selected mtDNA region obtained from 287 *M. martes* and ten

Table 1. Geographical distribution and frequency of the 69 mtDNA haplotypes found in the 287 *Martes martes* (Mm), and in the 11 haplotypes found in ten *Martes zibellina* (Mz) samples and two European Molecular Biology Laboratory (EMBL)/GenBank sequences. The correspondence of the haplotypes with the discovered phylogroups is also shown. Mediterranean (MED), central–northern European (CNE), Fennoscandian–Russian 1 (FNR1), and Fennoscandian–Russian 2 (FNR2) are shown in white, light grey, dark grey, and black, respectively. The EMBL/GenBank database accession numbers are indicated for each haplotype

GenBank accession no.	Haplotype	Southern Europe									Central–northern Europe											Fennoscandia			Russia
		SP_NE	SP_NW	POR	IT_CS	IT_IS	IT_N	CRO	IR	SC	FR	LUX	AU	NTH	GE	CR	PL	LT	EST	HU	RO	SW	FIN	NW	RUS
HM025990	Mm1	19																							
HM025991	Mm2	2																							
HM025992	Mm3	1																							
HM025993	Mm4	1																							
HM025994	Mm5		2																						
HM025995	Mm6		3																						
HM025996	Mm7		1																						
HM025997	Mm8		1																						
HM025998	Mm9		29	4																					
HM025999	Mm10				1	5									2	1									
HM026000	Mm11				6																				
HM026001	Mm12				2																				
HM026002	Mm13				1																				
HM026003	Mm14				2	4																			
HM026004	Mm15					5																			
HM026005	Mm16						2																		
HM026006	Mm17						2																		
HM026007	Mm18						4								1	1									
HM026008	Mm19							4															4		
HM026009	Mm20								6																
HM026010	Mm21										1														
HM026011	Mm22										1							1							
HM026012	Mm23																		1						
HM026013	Mm24												1												
HM026014	Mm25											1													
HM026015	Mm26																								
HM026016	Mm27															1									

		Southern Europe						Central–Northern Europe														Fennoscandia			Russia	
		SP_NE	SP_NW	POR	IT_CS	IT_IS	IT_N	CRO	IR	SC	FR	LUX	AU	NTH	GE	CR	PL	LT	EST	HU	RO	SW	FIN	NW	RUS	TOTAL
	n	23	36	4	12	14	12	4	6	12	7	6	3	10	21	1	16	1	15	5	1	23	7	3	57	299
HM026035	Mm46																								1	
HM026036	Mm47																								8	
HM026037	Mm48																								13	
HM026038	Mm49																								2	
HM026039	Mm50																								7	
HM026040	Mm51																								1	
HM026041	Mm52																								1	
HM026042	Mm53																									
HM026043	Mm54																									
HM026044	Mm55				2																		2				
HM026045	Mm56																							4		2	
HM026046	Mm57																							1		1	
HM026047	Mm58																										
HM026048	Mm59																									1	
HM026049	Mm60																									1	
HM026050	Mm61																									1	
HM026051	Mm62																									1	
HM026052	Mm63																									1	
HM026053	Mm64																									1	
HM026054	Mm65																										
HM026055	Mm66																						1	2		2	
HM026056	Mm67																										
HM026057	Mm68																						2				
HM026058	Mm69																						2		3		
HM026059	Mz1					2																					
HM026060	Mz2					2																					
HM026061	Mz3																									1	
HM026062	Mz4																									1	
HM026063	Mz5																									1	
HM026064	Mz6																									1	
HM026065	Mz7																									1	
HM026066	Mz8																									1	
HM026067	Mz9																									1	
HM026068	Mz10																									1	
FJ429093	Mz11																									2	

Geographical groups are indicated: southern Europe; central–northern Europe; Fennoscandia and Russia. Abbreviations for populations: AU, Austria; CR, Czech Republic; CRO, Croatia; EST, Estonia; FIN, Finland; FR, France; GE, Germany; HU, Hungary; It_CS, central-south Italy; It_IS, Island of Sardinia; It_N, North Italy; IR, Ireland; LT, Lithuania; LUX, Luxembourg; NTH, the Netherlands; NW, Norway; PL, Poland; POR, Portugal; RO, Romania; RUS; Russia; SC, Scotland; Sp_NE, Spain north-east; Sp_NW, Spain north-west; SW, Sweden.

M. zibellina samples. Two additional *M. zibellina* sequences obtained from GenBank (FJ429093 and NC011579) were also included. Three related species were selected as out-groups: *Mustela putorius*, (AY962040); the yellow-throated marten, *Martes flavigula* (Boddaert, 1785) (FJ719367); and the wolverine, *Gulo gulo* Linnaeus, 1758 (NC_009685). The number of polymorphic sites, transitions, and transversions, and haplotype (h) and nucleotide (π) diversities, were obtained with ARLEQUIN 3.0 (Excoffier, Laval & Schneider, 2005).

Phylogenetic reconstructions were performed by a distance method using the neighbour-joining algorithm (NJ) (Saitou & Nei, 1987), and using the maximum-parsimony criterion (MP) (Fitch, 1971) algorithm implemented in PAUP 4.0b10 (Swofford, 2002). For distance analyses, the Hasegawa, Kishino, and Yano (HKY) model with rate heterogeneity and invariable sites ($\alpha = 0.732$; $I = 0.8693$) was selected as the best-fitting model of nucleotide substitution for the molecular data set by the Akaike information criteria approach, using MODELTEST 3.6 (Posada & Crandall, 1998). We therefore used this model and these parameters for inferring distance matrices. The MP analysis was conducted with the heuristic search algorithm, tree bisection and reconnection (TBR) swapping, and the maximum number of trees constrained to 1000. Phylogenetic trees were rooted with a homologous region for the selected out-groups (*Mustela putorius*, *Martes flavigula*, and *Gulo gulo*). The robustness of the trees was assessed by bootstrap resampling (BS) (10 000 random replications for NJ analysis; 5000 random replications for MP analysis; Felsenstein, 1985).

We also performed a Bayesian phylogeny estimation using MRBAYES 3.0b4 (Huelsenbeck & Ronquist, 2001). Metropolis-coupled Markov chain Monte Carlo (MCMC) sampling was performed using four chains run for 2 000 000 iterations and using the most suitable model, determined by MODELTEST. Bayesian posterior probabilities (BPPs) were picked from the 50% majority-rule consensus of trees sampled every 20 generations, after removing trees obtained before the chains reached an apparent plateau (the 'burn-in', determined by empirical checking of likelihood values). The whole procedure was repeated three times, starting from different random trees, and resulted in the same tree topologies.

PHYLOGEOGRAPHIC ANALYSES

NETWORK 4.1.0.6 (Bandelt, Forster & Röhl, 1999) was used to construct a median-joining (MJ) network in order to infer the relationships between haplotypes. The data matrix included the combined nucleotide sequences of the mtDNA region from all the *M. martes* and *M. zibellina* specimens sequenced.

The genetic structure of populations was examined using an analysis of molecular variance (AMOVA) performed in ARLEQUIN 3.0 (Excoffier *et al.*, 2005). The AMOVA was conducted at three hierarchical levels of population subdivisions: among geographical groups (Fig. 1; Table 1); among populations within regional groups; and within populations (see Table 1 for population designation). The significance of these parameters was estimated by 10 000 permutations of the distance matrix.

DEMOGRAPHIC ANALYSIS

Demographic histories of different phylogroups were inferred by a pairwise mismatch distribution analysis between individuals (Rogers & Harpending, 1992), computed under a population growth–decline model in DNASP 5.0 (Librado & Rozas, 2009). Multimodal distributions were consistent with demographic stability, whereas sudden expansion would generate a unimodal pattern (Slatkin & Hudson, 1991). Hypotheses of demographic expansion were tested using Fu and Li's F (Fu & Li, 1993) and Tajima's D statistics (Tajima, 1989). Significances for F statistics were obtained by means of coalescent simulations of a panmictic population of constant size, conditioned by the number of segregating sites. For each case, 1000 simulations were run in DNASP.

ESTIMATION OF DIVERGENCE TIMES

We estimated divergence times of splits using the Bayesian relaxed phylogenetic approach, implemented in BEAST 1.4.6 (Drummond & Rambaut, 2007). Analyses were performed using the HKY model of nucleotide substitution (previously estimated with MODELTEST). Rate variation among sites was modelled using a gamma distribution with four rate categories. The uncorrelated lognormal relaxed molecular clock model was used to estimate substitution rates for all nodes in the tree, with uniform priors on the mean (0, 100) and standard deviation (0, 10) of this clock model. We employed expansion growth as the coalescent prior, with the in-group constrained to be monophyletic with respect to the out-group.

Molecular dating was derived using the fossil record of the extinct species *Martes vetus* Kretzoi, 1956 as the calibration point (400 Ka; Wolsan, 1993), as this species has been considered ancestral to both *M. martes* and *M. zibellina* (Anderson, 1994). A lognormal distribution suitable for modelling fossil data (Ho, 2007) was used as a prior, with parameter values of 300 000 years as the minimum age (lower bound parameter) and 400 000 years as the mean, with a standard deviation of the distribution of 1000 years.

Additionally, we set the mean of the normal distribution of the root height prior to 4.8 Mya, assuming this time as the time of divergence between *M. flavigula* and the subgenus *Martes* (Koepfli *et al.*, 2008), with a standard deviation of 1.0 Myr. Three independent MCMC runs of 20 000 000 steps were performed. Samples from the three chains, which yielded similar results, were combined to estimate the posterior distribution of the substitution model and tree model parameters, as well as node ages. Analyses of these parameters in TRACER 1.4 (Rambaut & Drummond, 2007) suggested that the number of MCMC steps was more than adequate, with effective sample sizes of all parameters often exceeding 100, and TRACER plots showing strong equilibrium after discarding the burn-in.

To compare the genetic findings with subfossil records, spatial and temporal information on the distribution of *M. martes* during the Pleistocene was obtained from Sommer & Benecke (2004) and Sommer & Nadachowski (2006).

RESULTS

PATTERN OF SEQUENCE VARIATION

We identified a total of 69 haplotypes among the 287 *M. martes* specimens (Mm1–Mm69), and ten different haplotypes among the ten *M. zibellina* samples (Mz1–Mz10). We included two GenBank sequences that corresponded to an additional *M. zibellina* haplotype (Mz11). In the alignment comprising both species (1566 bp) there were 95 variable sites, of which 59 were parsimony informative. The average transitions/transversions ratio was 18.4. When excluding *M. martes* haplotypes belonging to the Fennoscandian–Russian phylogroup there were only 47 variable sites, of which 25 were parsimony informative.

PHYLOGENETIC AND PHYLOGEOGRAPHIC ANALYSES

The geographic distribution and frequency of the 69 *M. martes* and 11 *M. zibellina* mtDNA haplotypes are shown in Table 1. The haplotype distribution clearly differentiated sequences from three main geographic regions: (1) Southern Europe (i.e. the Mediterranean peninsulas); (2) central–northern Europe; and (3) Fennoscandia–Russia. The first group (haplotypes: Mm1–Mm27) included unique haplotypes discovered only in the three main Mediterranean peninsulas (Mm1–Mm8, Mm10–Mm17, and Mm19), some shared haplotypes between Southern Europe and central–northern Europe (Mm9 and Mm18), and some closely related haplotypes that have been discovered only in central–northern Europe (Mm21–Mm27) and Ireland (Mm20). The second group

(Mm28–Mm55) included haplotypes mainly distributed in central–northern Europe, some of them shared across a wide geographic range of this region (Mm29 and Mm31), in Fennoscandia (Mm31, and Mm45–Mm46), Russia (Mm49–Mm55), and Scotland (Mm28). The northern parts of Fennoscandia and Russia include unique haplotypes not found in any other regions, where both *M. martes* and *M. zibellina* haplotypes were mixed together (Mm51–Mm59 and Mz1–Mz8).

The NJ reconstruction of phylogenetic relationships between haplotypes is shown in Figure 2, with the MP and Bayesian trees showing identical topologies. The *M. martes* split into two major groups: a Fennoscandian–Russian (FNR) clade (BS, ≈96%; BPP, 1.0, including haplotypes Mm56–Mm65 and Mz1–Mz11), and a large clade grouping all other *M. martes* haplotypes (Mm1–Mm55; BS, 100%; BPP, 1.00). The latter group, which includes *M. martes* from nearly all the current European distribution of the species, is separated into two different phylogroups: the Mediterranean (MED) and central–northern European (CNE) phylogroups (Fig. 1).

The MED phylogroup (BS, ≈63%; BPP, 0.86) is predominantly made up of animals from the three main Mediterranean peninsulas (corresponding to 101 out of 123 samples in this group, i.e. 82.11%: Spain, Mm1–Mm9, $N = 59$; Portugal, Mm9, $N = 4$; Italy–Sardinia, Mm9–Mm19, $N = 34$; Croatia, Mm19 $N = 4$) and only a few individuals from central–northern Europe ($N = 12$), the Fennoscandian–Russian region ($N = 4$), and Ireland ($N = 6$) (Fig. 1; Table 1). The CNE group (BS, ≈67%; BPP, 1.00) is widely distributed throughout Europe, with the exceptions of the Mediterranean region and the northern area of Fennoscandia (see Figs 1 and 2; Table 1). The FNR phylogroup includes exclusively *M. martes* from northern Sweden (Mm67 and Mm69), Norway (Mm68), Finland (Mm58, Mm59, and Mm69), and Russia (Mm56, Mm57 Mm60–Mm66, and Mm69), which are grouped together with *M. zibellina* haplotypes (Mz1–Mz11) found east of the Urals. This phylogroup is subdivided into two mayor haplogroups: the first one (FNR1; BS, ≈80%; BPP, 1.00) is composed of *M. zibellina* specimens from Russia (Western Siberia, southern Transbaikalia, and Kamtchatka) and *M. martes* from Russia (Chelyabinsk Province and Leningrad Province), North Sweden, and Norway. The second haplogroup (FNR2; BS, ≈98%; BPP, 1.00) is composed of *M. zibellina* specimens from Russia (Western Siberia and North Transbaikalia) and *M. martes* from Finland and Russia (Penza Province, Kirov Province, and Tver Province) (Figs 1 and 2; Table 1).

From the geographic distribution of each phylogroup a global pattern of spatial segregation is
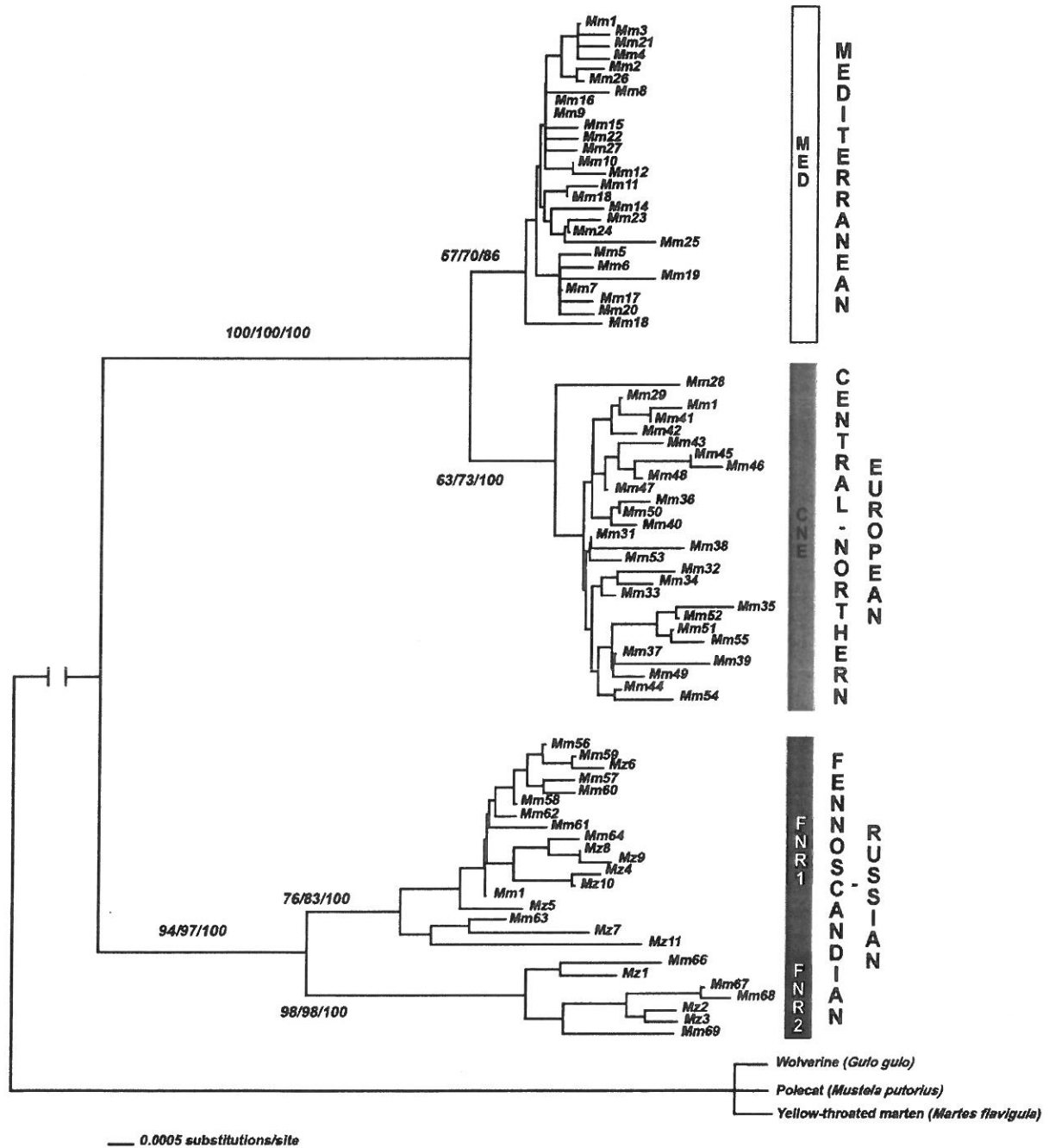
Figure 2. Neighbour-joining tree of the 69 *Martes martes* (Mm) and 11 *Martes zibellina* (Mz) mtDNA haplotypes. Bootstrap values (%) obtained by neighbour-joining (NJ) and maximum parsimony (MP) as well as Bayesian posterior probabilities are shown. See Table 1 for haplotype designations and geographical distribution.

observed, showing a south–north replacement, with some areas of overlap and genetic admixture (see Fig. 1; Table 1).

The median-joining network gave complementary information and confirmed the existence of these main three *Martes* phylogroups (Fig. 3). The distinction among phylogroups was supported, respectively, by six and > 30 mutations, which separated the MED and CNE phylogroups, and these phylogroups from the FNR phylogroup. In contrast, sequence
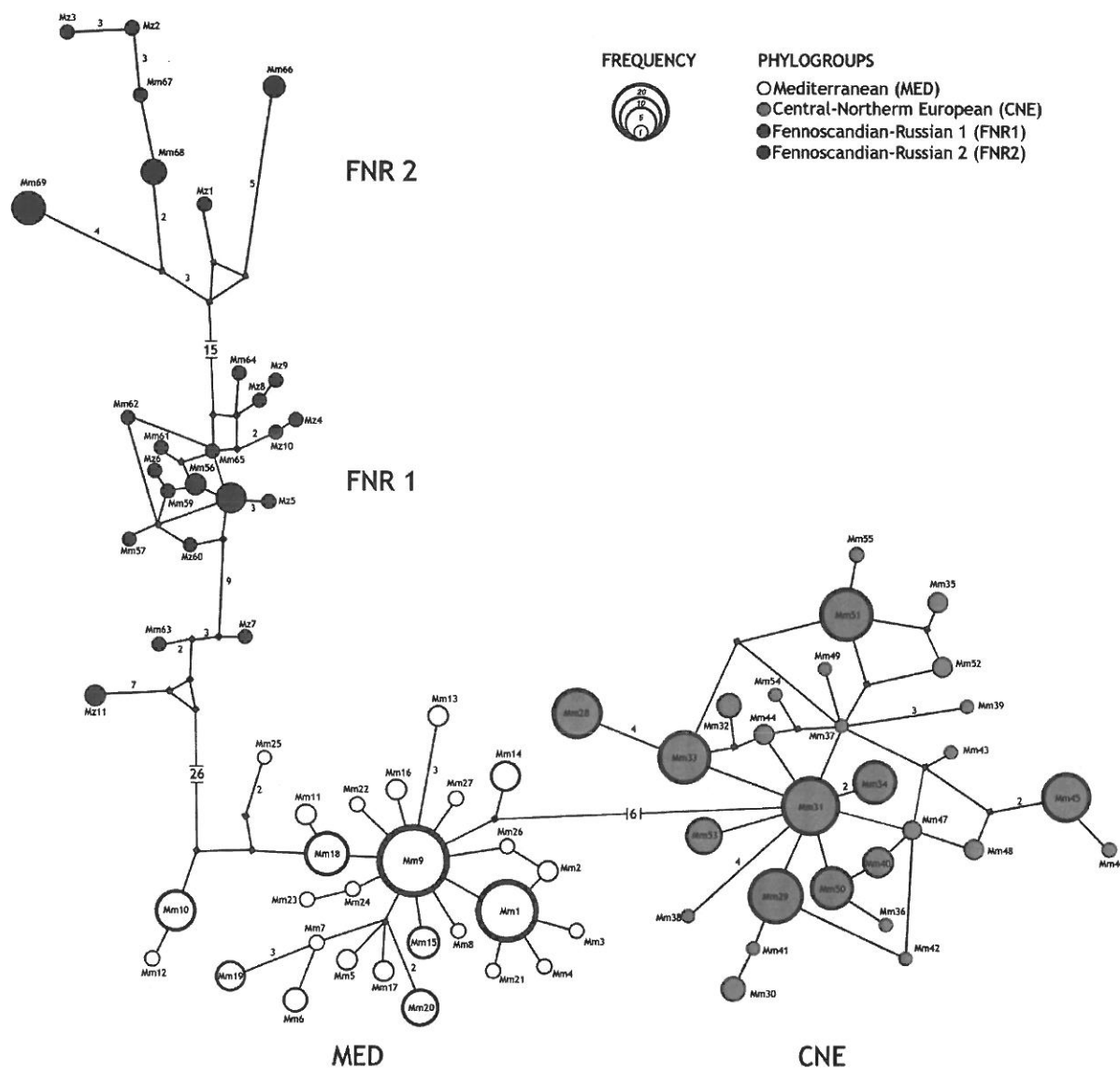
Figure 3. Median-joining network of the European pine marten (*Martes martes*, Mm) and sable (*Martes zibellina*, Mz) mtDNA haplotypes, coloured according to phylogroups. The numbers of mutations (greater than one) between haplotypes are indicated on the branches. The size of the circle is proportional to the frequency of each haplotype. See Table 1 for the haplotype designations and distribution.

divergence within each of these three haplogroups was low. The CNE phylogroup is organised around a dominant haplotype (Mm35). This group shows star-like topology, suggesting exponential growth of populations from a small number of individuals. The MED phylogroup is also organized around a dominant haplotype (Mm9), with star-like topology. The FNR haplogroup is also subdivided into two different subgroups (FNR1 and FNR2) separated by 15 mutations. In spite of the presence of two well-differentiated

subgroups within the Fennoscandian–Russian clade, we consider it as one unique phylogroup for the remaining analysis, taking into account the low number of samples and the admixture of *M. martes* and *M. zibellina* haplotypes within both subgroups.

The mismatch distribution (Fig. 4) and Tajima's D and Fu and Li's F statistics (Table 2) also suggest varied demographic histories for the *Martes* phylogroups. The negative and statistically significant values of Fu and Li's statistic (Table 2) and the bell-

Mediterranean

Central-Northern European

Fennoscandian-Russian
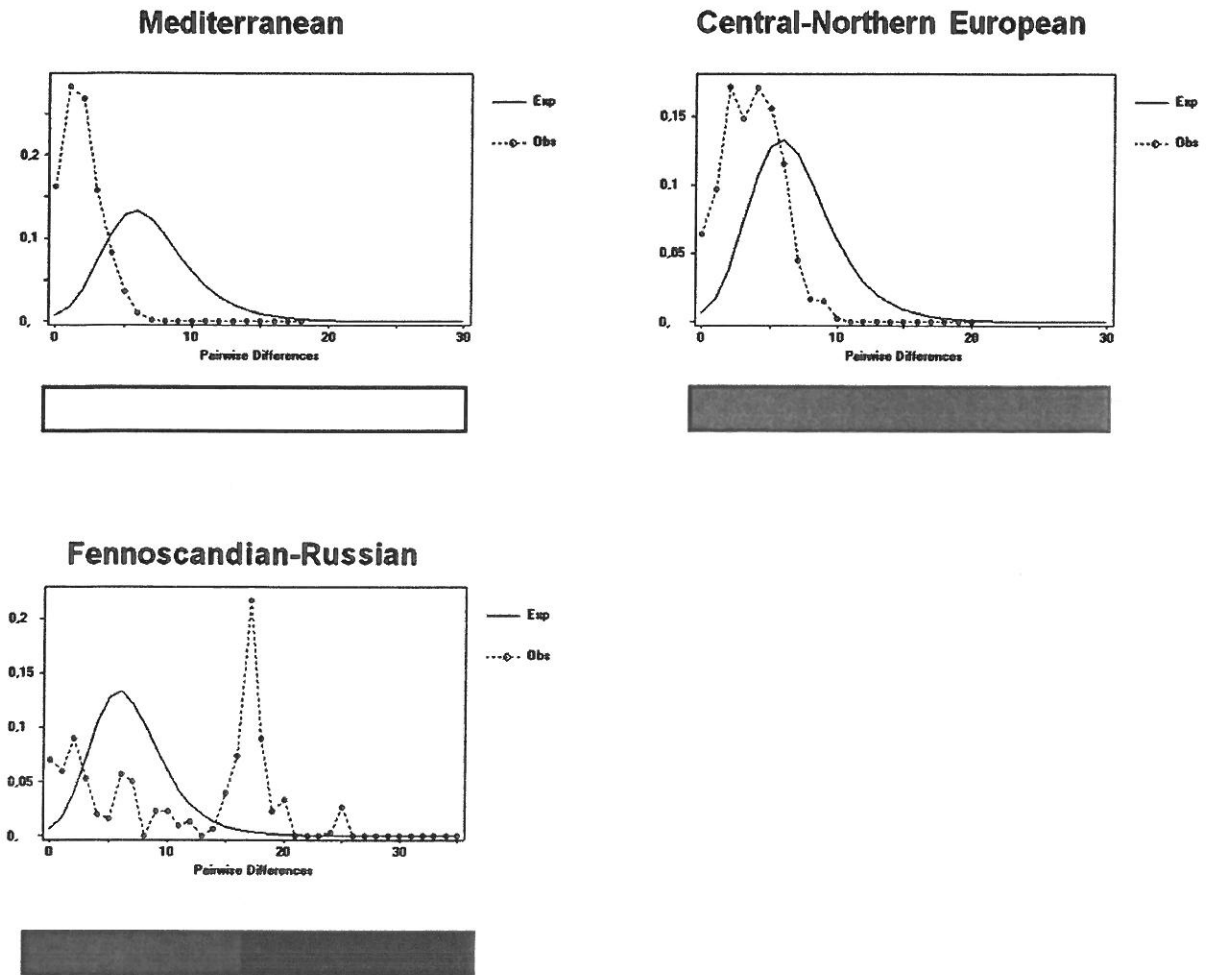
Figure 4. Mismatch distribution analysis for the three major phylogroups.

Table 2. Genetic variability observed within the main genetic phylogroups, and Tajima's *D* and Fu and Li's *F* statistics test

	N	Number of haplotypes	Nucleotide diversity π ($\pm$SD percentage)	Haplotype diversity (h $\pm$ SD)	Tajima's *D*	Fu and Li's *F*
Total	299	79	0.647 ± 0.048	0.955 ± 0.006	–	–
MED	123	26	0.120 ± 0.010	0.837 ± 0.027	−2.1672 ($P < 0.05$)	−3.340 ($P < 0.01$)
CNE	139	28	0.234 ± 0.012	0.936 ± 0.006	−1.68615 ($P > 0.05$)	−2.42733 ($P < 0.05$)
FNR	37	14	0.707 ± 0.054	0.930 ± 0.030	0.03059 ($P > 0.05$)	−0.09011 ($P > 0.05$)

CNE, central–northern European phylogroup; FNR, Fennoscandian–Russian phylogroup (FNR1 and FNR2); MED, Mediterranean phylogroup.

shaped mismatch distributions, are indicative of population expansions in the past within the MED and CNE phylogroups (Fig. 4). The FNR (FNR1 and FNR2) phylogroup showed a multimodal mismatch distribution (Fig. 4) that could indicate the admixture of two expanding populations, as also suggested by the positive result of the Tajima test, or long-term stability. In this regard, the non-significant result of

Table 3. Analyses of molecular variance based on mtDNA data from the main geographical groups

Source of variation	Variance components	Percentage of variation	P	φ statistics
Among groups	6.841	73.11	< 0.001	φCT = 0.859
Among populations/groups	1.201	12.83	< 0.001	φSC = 0.477
Within populations	1.315	14.06	< 0.001	φST = 0.731

Table 4. Matrix of distances between phylogeographic groups inferred from the data (below diagonal), assuming an HKY model with rate heterogeneity and invariable sites (α = 0.732, I = 0.8693). Values within regions and are shown in bold

	MED	CNE	FNR
MED	**0.001**		
CNE	0.006	**0.001**	
FNR	0.020	0.021	**0.008**

Fu and Li's test and the Tajima test are in agreement with this last possibility, although it could also be caused by the low number of samples. A larger number of samples is therefore needed to clarify the demographic history of this group.

POPULATION STRUCTURE AND GENETIC DIVERSITY STATISTICS

The AMOVA results were also consistent with the regional subdivision of samples into three main groups, as suggested by the MJ network and the phylogenetic trees. The most probable phylogeographic structures were those with maximum and statistically significant percentages of variation explained by differences among groups (Table 3). The AMOVA showed that the majority of the total mtDNA variation (73.11%) was distributed among geographical groups, whereas a low percentage (12.83%) was observed among populations within the groups. Moreover, the φ statistic suggests a low level of gene flow between populations (φCT = 0.859; $P < 0.001$).

Intra- and intergroup genetic distances were very low: between 0.001–0.008 and 0.006–0.021, respectively (Table 4). The genetic distance between CNE and MED phylogroups was 0.006. The genetic distances between the FNR and CNE phylogroups and the MED phylogroup were slightly higher: 0.021 and 0.020, respectively (Table 4).

The highest value of nucleotide diversity was found in the FNR phylogroup, and the lowest was found in the MED phylogroup (Table 2). On the other hand, haplotype diversity was similar in CNE and FNR

phylogroups, being higher than in the MED phylogroup (Table 2).

DIVERGENCE TIMES

Assuming the fossil record of *M. vetus* as a calibration for the divergence point of the *M. martes — M. zibellina* complex, different periods of diversification can be recognized for *Martes* populations, all of them falling within the Pleistocene period: (1) 0.29 Mya [95% highest posterior density (HPD) 0.12–0.48 Mya] for the estimate of the time to the most recent common ancestor (TMRCA) of the Fennoscandian–Russian phylogroup, which began to differentiate before the two main European phylogroups; (2) 0.15 Mya (95% HPD 0.06–0.26 Mya) and 0.13 Mya (95% HPD 0.04–0.22 Mya) for the timing of FNR1 and FNR2 groups, respectively; (3) 0.16 Mya (95% HPD 0.064–0.28 Mya) for the separation time between the two major European phylogroups (i.e. MED and CNE); and (4) 0.092 Mya (95% HPD 0.03–0.15 Mya) and 0.081 Mya (95% HPD 0.03–0.14 Mya) as the divergence times for CNE and MED phylogroups, respectively.

DISCUSSION

THE ROLE OF PLEISTOCENE GLACIATIONS ON PHYLOGEOGRAPHIC PATTERNS AND *MARTES* SPECIES DIVERSIFICATION

Recent studies suggest that the subgenus *Martes* [*Martes foina* (Erxleben, 1777), *Martes americana* (Turton, 1806), *Martes melampus* (Wagner, 1840), *M. zibellina*, and *M. martes*] diversified during the Plio-Pleistocene, and recognized *M. martes* and *M. zibellina* as sister species within this subgenus (Koepfli *et al.*, 2008). Our divergence analysis estimates that the separation time between the two major European phylogroups (i.e. MED and CNE) of *M. martes* took place during the middle–late Pleistocene, *c.* 160 000 BP (0.16 Mya; 95% HPD 0.064–0.28 Mya), probably during the late Riss or early Würm glaciations; however, the two different lineages of the FNR phylogroup, which comprises specimens of both morphospecies (*M. martes* and *M. zibellina*), began to differentiate earlier, *c.* 290 000 BP, but were also asso-

ciated with the glacial periods of the middle–late Pleistocene (0.29 Mya; 95% HPD 0.12–0.48 Mya). All divergence estimates obtained for the *M. martes–M. zibellina* complex fall within the Pleistocene, which is typical of mammalian intraspecific phylogroups (Avise, 2000). Our results suggest that Pleistocene climate conditions initiated phylogeographic differentiation, as well as contributing to sculpting pre-existing phylogeographic variation into today's considered sister species, *M. martes* and *M. zibellina*. Palaeontological data indicate that the earliest known records of *M. zibellina* are much more recent (late Pleistocene; Anderson, 1994) than the first *M. martes* fossil found in central Europe (Riss–Würm interglacial, *c.* 120 000 BP; Anderson, 1994). However, the divergence times obtained in the present work indicate that diversification of the FNR group pre-dated the divergence between MED and CNE phylogroups. Thus, it is possible that the absence of fossil record data from Asia limits the information on *M. zibellina*, and probably more ancient records of the species could exist in this region (Sommer & Benecke, 2004). We therefore hypothesize that isolation of the FNR phylogroup in a refugium, presumably located in Eastern Asia (Sommer & Benecke, 2004), may have played an important role in the origin of *M. zibellina*, which occurs eastwards of the Urals across all the Siberian coniferous taiga forests. However, the phylogeographic pattern of *M. zibellina* should be further explored with a more comprehensive sampling and the use of nuclear markers.

GEOGRAPHIC DISTRIBUTION OF THE PHYLOGROUPS: BIOGEOGRAPHICAL IMPLICATIONS

The current distribution of the three major mtDNA lineages described in this study strongly correlates with the main biogeographic regions in Europe (Roekaerts, 2002), showing a global pattern of spatial segregation and a south–north replacement, with some area of overlap and genetic admixture. The MED phylogroup is closely associated with the Mediterranean peninsulas, distributed in the Atlantic and Alpine areas where the temperate mixed forests are predominant, and with only a few MED individuals present in Continental Europe. The CNE phylogroup covers most of *M. martes* distribution range arriving up to the Ural Mountains, and is distributed across the continental biogeographic region. Finally, the FNR phylogroup occurs exclusively in the boreal region. These results suggest that populations belonging to these three haplogroups might be adapted to distinct environmental conditions. Indeed, there is a clear pattern of latitudinal variation in *M. martes* body size, decreasing from south to north (Reig, 1992), and also in diet composition, food niche

breadth, and prey size for martens in Europe (Zalewski, 2004). Geographic variation in habitat and diet probably played an important role in shaping *M. martes* evolutionary adaptations, life-history strategies, and ecological roles.

SOUTHERN EUROPEAN GLACIAL REFUGIA FOR THE MEDITERRANEAN PHYLOGROUP

The role of the southern European peninsulas (i.e. Iberia, Italy, and the Balkans) as a glacial refuge for temperate species has been widely established (Taberlet *et al.*, 1998; Hewitt, 2001; Randi, 2007). Our phylogeographic analyses reveal a mtDNA phylogroup joining all *M. martes* populations from these three regions (Figs 1 and 2), suggesting a large Mediterranean population during the late Pleistocene, where gene flow between populations was possible. This pattern of continuous gene flow across southern Europe has been also reported in brown bear (*Ursus arctos*) populations (Valdiosera *et al.*, 2007). Additionally, as fossil remains of *M. martes* have been reported from southern Europe during the LGM (Sommer & Benecke, 2004; Sommer & Nadachowski, 2006), it seems likely that the Mediterranean peninsulas played an important role as glacial refugia for *M. martes*, as has previously been proposed for other temperate species (Randi, 2007).

Even though southern refugial areas are currently expected to exhibit high genetic diversity (Hewitt, 2004), the Mediterranean phylogroup shows the lowest genetic variability of the three identified phylogroups. The low nucleotide and haplotype diversities characterizing this phylogroup could be associated with population fragmentation, followed by severe population bottlenecks during the Quaternary glaciations.

CENTRAL–NORTHERN EUROPEAN PHYLOGROUP: NEW INSIGHTS INTO THE CRYPTIC NORTHERN GLACIAL REFUGIA

The low proportion of *M. martes* from the Mediterranean lineage (18 out of 123, i.e. 14.6%) identified in central–northern Europe strongly suggests that this lineage has not been the source of major postglacial recolonizations of this region. Moreover, no haplotypes from the CNE phylogroup were found in any of the known southern refuges of Europe (except two samples near the contact zone in the Italian Alps, which may come from a recent recolonization from CNE lineage populations in the Alps; Balestrieri *et al.*, 2010). Rather, our data suggest that central–northern Europe was recolonized by a *M. martes* phylogroup that survived the last glaciations in a central European glacial refugium, as has been

previously proposed by palaeontological data (Sommer & Benecke, 2004; Sommer & Nadachowski, 2006). Indeed, fossil records of *M. martes* were found during the LGM in the east of the Carpathians, in Moldova (Markova *et al.*, 1995; Sommer & Benecke, 2004), and in the Deszczowa and Mamutova caves of southern Poland (Sommer & Nadachowski, 2006). Moreover, the Carpathian region was covered with suitable habitat for *M. martes* during the LGM, with patches of mixed coniferous and deciduous forests, instead of a uniform, steppe-like landscape (Willis, Rudner & Sümegi, 2000).

Schmitt & Varga (2012) points out that populations surviving in northern refugia may have an even higher genetic diversity and expansive power than populations restricted to the more southern 'classical' refugia, which in fact matches with the phylogeographic pattern outlined in this study. The CNE phylogroup presently covers most of the *M. martes* distribution range in the Palaearctic region (Fig. 1), and was subjected to a recent population expansion (Figs 3 and 4). Additionally, this phylogroup is characterized by high haplotype and nucleotide diversity, in comparison with the MED phylogroup, suggesting that these populations have been affected by less severe population bottlenecks.

The existence of a non-Mediterranean refugium would also be consistent with the past and present ecological traits of *M. martes*. The current range distribution of *M. martes* includes coniferous forests and cold environments (Proulx *et al.*, 2004). As a cold-tolerant species, they would have been able to survive at northern latitudes, even at the former boundary between woodland and tundra. Indeed, the northernmost subfossil record from the Late Glacial was situated in Denmark, assigned to the Younger Dryas (14 000 BP). Bhagwat & Willis (2008) suggest that the persistence of species in northerly glacial refugia is closely related with some biological and biogeographical traits, such as small body size, a present-day northerly distribution, and cold-tolerance, which in fact strongly matches with the traits found in *M. martes*.

Consequently, as suggested for several other species (e.g. Deffontaine *et al.*, 2005; Kotlik *et al.*, 2006; Valdiosera *et al.*, 2007; McDevitt *et al.*, 2012), the present genetic study in conjunction with the available fossil data (Sommer & Benecke, 2004; Sommer & Nadachowski, 2006) strongly indicate that a temperate forest species such as *M. martes* did not respond to the last glaciation by simply shifting their distributions to the Mediterranean region, but also survived at higher latitudes previously considered inhospitable, providing new insights into the existence of cryptic northern glacial refugia in Europe

(Bhagwat & Willis, 2008; Provan & Bennett, 2008; Stewart *et al.*, 2010).

THE FENNOSCANDIAN–RUSSIAN GROUP

In our analysis we identified the existence of a third continental phylogroup that joined *M. martes* from Fennoscandia and several regions of Russia together with *M. zibellina* specimens collected from a wide geographic area eastwards of the Urals. The FNR group is characterized by a very different demographic history, genetic diversity, and genetic divergence compared with the other European phylogroups (Figs 2, 3; Tables 2, 3). Moreover, this phylogroup is subdivided into two subgroups, suggesting two different mtDNA lineages present in the *Martes* populations of Fennoscandia–Russia (Fig. 2). However, we have not found any correspondence between these two subgroups, neither with geographic distribution nor with the two morphospecies considered. Thus, in the FNR phylogroup the morphological species concept does not correspond to the phylogenetic species concept (De Queiroz, 2007). According to our results it seems likely that this ancient phylogeographic lineage could have evolved to give rise to the origin of *M. zibellina* east of the Urals, with a secondary contact, followed by a subsequent genetic introgression between *M. martes* and *M. zibellina* in Fennoscandia and the Russian region.

Previous studies have found that *M. martes* and *M. zibellina* formed a monophyletic group, with *M. martes* paraphyletic with respect to *M. zibellina* (Stone & Cook, 2002; Marmi, Lopez-Giraldez & Domingo-Roura, 2004). Although incomplete lineage sorting can result in paraphyletic relationships (Davison *et al.*, 1999), these two species are not reproductively isolated, and successful hybridization (Grakov, 1994) or mtDNA introgression between them is known (Davison *et al.*, 2001; Rozhnov *et al.*, 2010). Thus, the most likely explanation for the existence of this divergent group in the Fennoscandian *M. martes* is that it originated by introgression from *M. zibellina*, because the same type was found in *M. zibellina* from the Russian Far East. The microsatellite data also provide support for a distinct Fennoscandian group, with elevated genetic distance values between Fennoscandia and the central European populations (Kyle, Davison & Strobeck, 2003).

The origin of postglacial recolonization during the Holocene probably lies in one or more Asian glacial refugia (Sommer & Benecke, 2004) that could be located in the southern or eastern Urals, or even in the Caucasus; however, this phylogroup should be investigated further by the combined use of nuclear markers and a more extensive sampling from these regions. Moreover, a comparison with the phylogeo-

graphic pattern of *M. zibellina* (currently underway) will probably provide valuable information concerning the evolutionary history of this complex marten phylogroup, and whether refugial isolation could have led to speciation between these species.

POSTGLACIAL RECOLONIZATION OF
M. MARTES POPULATIONS

The impact of Quaternary glaciations, and the identification of the main postglacial colonization routes from glacial refugia, has been widely studied in different mammalian species across Eurasia (Taberlet *et al.*, 1998; Hewitt, 2004; Stewart *et al.*, 2010).

In this study, the evidence of population structuring into three different phylogroups found within European *M. martes* populations is a clear sign of postglacial recolonization from different refuge areas, with posterior intermixing. The high levels of haplotype diversity and low levels of nucleotide diversity found (Table 2) may suggest rapid demographic expansions from small effective population sizes, multiple refuges, and secondary contact between populations from different refuges (Avise, 2000). The geographic distribution of some shared haplotypes and phylogeographic groups (Fig. 1; Table 2) agrees with this last possibility. The study by Davison *et al.* (2001) found a similar population structure in Europe, with the existence of the same three groups inferred in the present study; however, the low support values obtained for each of the groups detected, because of the small mtDNA fragment used (325 bp), and the limited sampling from Mediterranean and from the eastern Russian *Martes* populations, did not provide clear clues for the postglacial recolonization of central Europe.

As previously discussed, the MED and CNE lineages retreated in separate refugia during the LGM (southern European peninsulas for the MED lineage and the Carpathians for the CNE lineage). Populations of the MED lineage probably went through a bottleneck during the last glaciation, and after the LGM the population expansion from the Mediterranean peninsulas was likely to have been associated with haplotype diversification, as suggested by the star-like phylogeny (Fig. 3) and the low nucleotide and high haplotype diversities (Table 2). The MED lineage expanded north up to southern Sweden, but the low proportion of *M. martes* of the MED lineage discovered in central–northern Europe demonstrated that this lineage has not been the source of major postglacial recolonizations of this region.

The persistence of the CNE *M. martes* populations in central European refugia must have significantly reduced the time by which recolonizing animals reached the northern parts of Europe after the LGM

(Sommer & Nadachowski, 2006; Schmitt & Varga, 2012). Indeed, although the MED phylogroup is more restricted to the southern European areas, the CNE phylogroup stretches up to the northern Urals. The rapid recolonization of the CNE populations, which survived in northern refugia, could be the reason for the presence of *M. martes* in regions like Denmark and the Czech Republic during the Younger Dryas and the Magdalennian: (*c.* 17 000–9 000 BP), respectively (Sommer & Benecke, 2004). Thus, the most likely explanation is that these ice-age rear-edge CNE populations probably became the leading edges of the postglacial northwards range expansions, thus strongly impacting the genetic constitution of central and northern Europe, and limiting the expansion of MED populations surviving in southern refugia (Schmitt & Varga, 2012).

According to our results, it is noticeable that the current island populations of Britain and Ireland are represented by the two main European phylogroups: CNE and MED, respectively. Interestingly, a similar phylogeographic pattern has been found in other mustelid species inhabiting the Bristish Isles (Martínková *et al.*, 2007; O'Meara *et al.*, 2012). However, the recent discovery of one haplotype that is representative of martens from the Iberian Peninsula in museum specimens from Wales indicates that both phylogroups were present in Britain (Jordan *et al.*, 2012). There are two main hypotheses for explaining the postglacial re-establishment of martens on the British Isles according to these data, which are not mutually exclusive: a natural postglacial recolonization of the MED group from the Iberian Peninsula, tracking the coastline for both Ireland and Britain; and a natural colonization of the CNE phylogroup from continental Europe to Britain. However, we cannot disregard an anthropic origin of these populations, which is also congruent with the early trade routes that were established between south-west Europe and Ireland from the Mesolithic, as has previously been proposed (Searle, 2008).

The presence of the three major phylogroups in Fennoscandia suggests that *M. martes* recolonized this area from the north-east, by the FNR phylogroup, and from the south, by the MED and CNE phylogroups. Similar north–south phylogeographical patterns with a suture zone in central Fennoscandia have been described for several other mammals, strongly corroborating this recolonization model (e.g. Taberlet *et al.*, 1998; Jaarola, Tegelstrom & Fredga, 1999; Brunhoff *et al.*, 2003).

At present, it is impossible to know exactly how hybridization occurred between martens in Fennoscandia–Russia, or where the FNR phylogroup refugium was located; however, as *M. zibellina* is an eastern species that is more cold-adapted than

M. martes, it might have been the first to colonize north-east Fennoscandia from an undetermined Eastern refugium in Asia (Sommer & Benecke, 2004). As the climate became warmer, *M. martes* could also colonize Fennoscandia, replacing *M. zibellina* with mitochondrial introgression as the dwindling *M. zibellina* population mated with *M. martes* (Grakov, 1994; Rozhnov *et al.*, 2010). An alternative scenario is suggested by Davison *et al.* (2001). *Martes zibellina* is generally limited by the Ural Mountains to the west, but during the 'Little Ice Age' (*c.* 1550–1850) there is evidence that *M. zibellina* penetrated deep into Europe, providing a suitable scenario for mating with *M. martes*. As the temperature increased again, the range of *M. zibellina* was restricted once again to the Urals. Currently, although the FNR phylogroup stretches up to central Sweden, the CNE phylogroup is restricted eastwards up to the Ural Mountains.

PREY–PREDATOR RELATIONSHIPS: A LINKED PHYLOGEOGRAPHIC PATTERN BETWEEN *MYODES GLAREOLUS* AND *MARTES MARTES*

Co-evolved relationships may lead to a high level of congruence in distributional history, which could be strong between predators and their potential prey species (Abrams, 2000). *Myodes glareolus* is one of the main prey species of *Martes martes* across its entire distribution area (Zalewski, 2004). Consequently, a linked phylogeographic pattern could be expected for prey–predators that inhabit the same forest habitats. Indeed, recent phylogeographic studies conducted with *Myodes glareolus* have found a close pattern to that shown by *Martes martes* (Deffontaine *et al.*, 2005; Kotlik *et al.*, 2006). Interestingly, *Myodes glareolus* has three different Mediterranean lineages in southern Europe, belonging to each of the Mediterranean peninsulas (Deffontaine *et al.*, 2005), whereas we found a unique Mediterranean lineage for *Martes martes*. These traits are congruent with the restricted dispersal capabilities of *Myodes glareolus* in comparison with a highly mobile mid-sized carnivore such as *Martes martes*. Moreover, Kotlik *et al.* (2006) provided the clearest phylogeographic evidence of a northern glacial refugium for temperate species in central Europe. Thus, the same location of a glacial refugium for the main predator of *Myodes glareolus* gives strong support for central European refugia in temperate forest mammals. Regarding the Fennoscandian–Russian region (west of the Urals), a Ural phylogroup was also identified for *Myodes glareolus* that is closely related to the red-backed vole, *Myodes rutilus* (Pallas, 1779), a species found east of the Urals. These data are congruent with the FNR phylogroup, where *M. martes* and *M. zibellina* haplotypes are admixed together. Thus, similar patterns between closely related species (*Martes martes* and *Martes zibellina*; *Myodes glareolus* and *Myodes rutilus*) in the Fennoscandian–Russian region could therefore highlight the importance of the Ural mountains on species diversification processes, bearing in mind that this suture zone is supposed to constitute the distribution limit between some related taxa or genetic lineages in the Palaearctic region (Hewitt, 2001; Deffontaine *et al.*, 2005; Korsten *et al.*, 2009; Del Cerro *et al.*, 2010).

CONCLUSIONS

The role of glacial refugia in intraspecific evolution has been widely addressed for many different taxa through phylogeographic analysis (Avise, 2000); however, the response of each species to the climatic changes of the Quaternary depends largely on their adaptations and environmental tolerances (Stewart *et al.*, 2010). The mtDNA groups inferred in this study show a strict phylogeographic pattern throughout the species range, with the presence of three major phylogroups: MED, CNE, and FNR. With each of these related to specific biogeographic regions: Alpine–Atlantic, Continental, and Boreal, respectively (Roekaerts, 2002). On the whole, our study indicates a complex phylogeographic history for *M. martes*, indicating a mixed pattern of recolonization of northern Europe from both Mediterranean and non-Mediterranean refugia. The presence of the CNE lineage, widespread across central and northern Europe, which does not correspond to the lineages present in any of the three peninsular refugia, suggests that the source of this lineage lies elsewhere, possibly in a northern cryptic refugia located in the Carpathians, as it has been previously proposed by palaeontological data. These results provide new insights into the evidence that cryptic refugia existed in Central Europe during glaciations; however, this does not exclude the importance of Mediterranean peninsulas as a relevant source of diversity for *M. martes*. Moreover, a highly divergent phylogroup has been discovered in Fennoscandia–Russia, which appears to be characterized by a very different demographic history compared with the other European phylogroups, and comprises specimens of both *M. martes* and *M. zibellina* morphospecies. Our results on the latter phylogroup suggest that Pleistocene conditions played a major role in initiating phylogeographic differentiation, as well as in the speciation processes of today's sister species *M. martes* and *M. zibellina*.

Finally, the linked phylogeographic patterns found between *Martes martes* and *Myodes glareolus* provided clear evidence about the Quaternary effects on the evolution of forest-dwelling species, and suggest

that co-evolved prey–predator relationships lead to a stronger congruence of their phylogeographic histories.

ACKNOWLEDGEMENTS

REFERENCES

Abrams PA. 2000. The evolution of predator-prey interactions: theory and evidence. *Annual Review of Ecology and Systematics* **31:** 79–105.

Anderson E. 1994. Evolution, prehistoric distribution, and systematics of *Martes*. In: Buskirk SW, Harestad AS, Raphael MG, Powell RA, eds. *Martens, sables, and fishers: biology and conservation.* Ithaca, NY: Cornell University Press, 13–25.

Avise JC. 2000. *Phylogeography: the history and formation of species.* Cambridge, MA: Harvard University Press.

Balestrieri A, Remonti L, Ruiz-Gonzalez A, Gomez-Moliner BJ, Vergara M, Prigioni C. 2010. Range expansion of the pine marten (*Martes martes*) in an agricultural landscape matrix (NW Italy). *Mammalian Biology* **75:** 412–419.

Bandelt HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16:** 37–48.

Bhagwat SA, Willis KJ. 2008. Species persistence in northerly glacial refugia of Europe: a matter of chance or biogeographical traits? *Journal of Biogeography* **35:** 464–482.

Brunhoff C, Galbreath KE, Fedorov VB, Cook JA, Jaarola M. 2003. Holarctic phylogeography of the root vole (*Microtus oeconomus*): implications for late Quaternary biogeography of high latitudes. *Molecular Ecology* **12:** 957–968.

Davison A, Birks JDS, Brookes RC, Messenger JE, Griffiths HI. 2001. Mitochondrial phylogeography and population history of pine martens *Martes martes* compared with polecats *Mustela putorius*. *Molecular Ecology* **10:** 2479–2488.

Davison A, Birks JDS, Griffiths HI, Kitchener AC, Biggins D, Butlin RK. 1999. Hybridization and the phylogenetic relationship between polecats and domestic ferrets in Britain. *Biological Conservation* **87:** 155–161.

De Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* **56:** 879–886.

Deffontaine V, Libois R, Kotlik P, Sommer R, Nieberding C, Paradis E, Searle JB, Michaux J. 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.

Del Cerro I, Marmi J, Ferrando A, Chashcin P, Taberlet P, Bosch M. 2010. Nuclear and mitochondrial phylogenetics provide evidence for four species of Eurasian badgers (Carnivora). *Zoologica Scripta* **39:** 415–425.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7:** 214.

Excoffier L, Laval G, Schneider S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* **1:** 47–50.

Felsenstein J. 1985. Confidence-limits on phylogenies with a molecular clock. *Systematic Zoology* **34:** 152–161.

Fitch WM. 1971. Toward defining course of evolution – minimum change for a specific tree topology. *Systematic Zoology* **20:** 406–416.

Fløjgaard C, Normand S, Skov F, Svenning JC. 2009. Ice age distributions of European small mammals: insights from species distribution modelling. *Journal of Biogeography* **36:** 1152–1163.

Fu YX, Li WH. 1993. Statistical tests of neutrality of mutations. *Genetics* **133:** 693–709.

Grakov NN. 1994. Kidus – a hybrid of the sable and the pine marten. *Lutreola* **1:** 1–4.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. *Nucleic Acids Symposium Series* **41:** 95–98.

Hewitt GM. 1999. Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* **68:** 1–2.

Hewitt GM. 2001. Speciation, hybrid zones and phylogeography – or seeing genes in space and time. *Molecular Ecology* **10:** 537–549.

Hewitt GM. 2004. Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **359:** 183–195.

Ho SYW. 2007. Calibrating molecular estimates of substitution rates and divergence times in birds. *Journal of Avian Biology* **38:** 409–414.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17:** 754–755.

Jaarola M, Searle JB. 2002. Phylogeography of field voles (*Microtus agrestis*) in Eurasia inferred from mitochondrial DNA sequences. *Molecular Ecology* **11:** 2613–2621.

Jaarola M, Tegelstrom H, Fredga K. 1999. Colonization history in Fennoscandian rodents. *Biological Journal of the Linnean Society* **68:** 113–127.

Jordan NR, Messenger J, Turner P, Croose E, Birks J, O'Reilly C. 2012. Molecular comparison of historical and contemporary pine marten (*Martes martes*) populations in the British Isles: evidence of differing origins and fates, and implications for conservation management. *Conservation Genetics* **13:** 1195–1212.

Koepfli KP, Deere KA, Slater GJ, Begg C, Begg K, Grassman L, Lucherini M, Veron G, Wayne RK. 2008. Multigene phylogeny of the Mustelidae: resolving relationships, tempo and biogeographic history of a mammalian adaptive radiation. *BMC Biology* **6:** 10.

Korsten M, Ho SYW, Davison J, Pahn B, Vulla E, Roht M, Tumanov IL, Kojola I, Andersone-Lilley Z, Ozolins J, Pilot M, Mertzanis Y, Giannakopoulos A, Vorobiev AA, Markov NI, Saveljev AP, Lyapunova EA, Abramov AV, Mannil P, Valdmann H, Pazetnov SV, Pazetnov VS, Rokov AM, Saarma U. 2009. Sudden expansion of a single brown bear maternal lineage across northern continental Eurasia after the last ice age: a general demographic model for mammals? *Molecular Ecology* **18:** 1963–1979.

Kotlik P, Deffontaine V, Mascheretti S, Zima J, Michaux JR, Searle JB. 2006. A northern glacial refugium for bank voles (*Clethrionomys glareolus*). *Proceedings of the National Academy of Sciences of the United States of America* **103:** 14860–14864.

Kranz A, Tikhonov A, Conroy J, Cavallini P, Herrero J, Stubbe M, Maran T, Abramov A. 2008. Martes martes. In: *IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2.* Available at: http://www.iucnredlist.org Downloaded on 17 January 2012.

Kyle CJ, Davison A, Strobeck C. 2003. Genetic structure of European pine martens (*Martes martes*), and evidence for introgression with *M. americana* in England. *Conservation Genetics* **4:** 179–188.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. Clustal W and clustal X version 2.0. *Bioinformatics* **23:** 2947–2948.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25:** 1451–1452.

Markova AK, Smirnov NG, Kozharinov AV, Kazantseva NE, Simakova NEK, Kitaev LM. 1995. Late pleistocene distribution and diversity of mammals in Northern Eurasia. *Paleontologia i Evolucio* **29:** 5–143.

Marmi J, Lopez-Giraldez JF, Domingo-Roura X. 2004. Phylogeny, evolutionary history and taxonomy of the Mustelidae based on sequences of the cytochrome b gene and a complex repetitive flanking region. *Zoologica Scripta* **33:** 481–499.

Martínková N, McDonald RA, Searle JB. 2007. Stoats (*Mustela erminea*) provide evidence of natural overland colonization of Ireland. *Proceedings of the Royal Society B – Biological Sciences* **274:** 387–1393.

McDevitt AD, Zub K, Kawalko A, Oliver MK, Herman JS, Wojcik JM. 2012. Climate and refugial origin influence the mitochondrial lineage distribution of weasels (*Mustela nivalis*) in a phylogeographic suture zone. *Biological Journal of the Linnean Society* **106:** 57–69.

O'Meara DB, Edwards CJ, Sleeman DP, Cross TF, Statham MJ, McDowell JR, Dillane E, Coughlan JP, O'Leary D, O'Reilly C, Bradley DG, Carlsson J. 2012. Genetic structure of Eurasian badgers *Meles meles* (Carnivora: Mustelidae) and the colonization history of Ireland. *Biological Journal of the Linnean Society* **106:** 893–909.

Pertoldi C, Breyne P, Cabria MT, Halfmaerten D, Jansman HAH, Van Den Berge K, Madsen AB, Loeschcke V. 2006. Genetic structure of the European polecat (*Mustela putorius*) and its implication for conservation strategies. *Journal of Zoology* **270:** 102–115.

Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14:** 817–818.

Proulx G, Aubry KB, Birks J, Buskirk SW, Fortin C, Frost HC, Krohn WB, Mayo L, Monakhov V, Payer D, Saeki M, Santos-Reis M, Weir R, Zielinski WJ. 2004. World distribution and status of the genus *Martes* in 2000. In: Harrison DJ, Fuller AK, Proulx G, eds. *Martens and fishers (Martes) in human- altered environments: an international perspective.* New York: Springer-Verlag, 77–98.

Provan J, Bennett KD. 2008. Phylogeographic insights into cryptic glacial refugia. *Trends in Ecology & Evolution* **23:** 564–571.

Rambaut A, Drummond AJ. 2007. Tracer v1.4, Available at: http://beast.bio.ed.ac.uk/Tracer

Randi E. 2007. Phylogeography of South European mammals. In: Weiss S, Ferrand N, eds. *Phylogeography of Southern European Refugia.* Dordrecht: Springer, 101–126.

Reig S. 1992. Geographic-variation in pine marten (*Martes martes*) and beech marten (*M. foina*) in Europe. *Journal of Mammalogy* **73:** 744–769.

Roekaerts M. 2002. *The biogeographical regions map of Europe. Basic principles of its creation and overview of its development.* Copenhagen: European Environment Agency.

Rogers AR, Harpending H. 1992. Population-growth makes waves in the distribution of pairwise genetic-differences. *Molecular Biology and Evolution* **9:** 552–569.

Rozhnov VV, Meschersky IG, Pishchulina SL, Simakin LV. 2010. Genetic analysis of sable (*Martes zibellina*) and pine marten (*M. martes*) populations in sympatric part of distribution area in the northern Urals. *Russian Journal of Genetics* **46:** 488–492.

Saarma U, Ho SYW, Pybus OG, Kaljuste M, Tumanov IL, Kojola I, Vorobiev AA, Markov NI, Saveljev AP, Valdmann H, Lyapunova EA, Abramov AV, Mannil P, Korsten M, Vulla E, Pazetnov SV, Pazetnov VS, Putchkovskiy SV, Rokov AM. 2007. Mitogenetic structure of brown bears (*Ursus arctos* L.) in northeastern Europe and a new time frame for the formation of European brown bear lineages. *Molecular Ecology* **16:** 401–413.

Saitou N, Nei M. 1987. The neighbor-joining method – a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4:** 406–425.

Schmitt T, Varga Z. 2012. Extra-Mediterranean refugia: the rule and not the exception? *Frontiers in Zoology* **9:** 22.

Schwartz M, Ruiz-González A, Pertoldi C, Masuda R. 2012. *Martes* conservation genetics: assessing within-species movements, units to conserve and connectivity cross ecological and evolutionary time. In: Aubry KB, Zielinski WJ, Raphael MG, Proulx G, Buskirk SW, eds. *Biology and conservation of marten, sables, and fisher: a new synthesis.* New York: Cornell University Press, 398–428.

Searle JB. 2008. The colonization of Ireland by mammals. In: Davenport JL, Sleeman DP, Woodman PC, eds. *Mind the gap: postglacial colonization of Ireland.* Ireland: Irish Naturalists' Journal Special Supplement, 109–111.

Slatkin M, Hudson RR. 1991. Pairwise comparisons of mitochondrial-dna sequences in stable and exponentially growing populations. *Genetics* **129:** 555–562.

Sommer R, Benecke N. 2004. Late- and Post-Glacial history of the Mustelidae in Europe. *Mammal Review* **34:** 249–284.

Sommer RS, Nadachowski A. 2006. Glacial refugia of mammals in Europe: evidence from fossil records. *Mammal Review* **36:** 251–265.

Stewart JR, Lister AM. 2001. Cryptic northern refugia and the origins of the modern biota. *Trends in Ecology & Evolution* **16:** 608–613.

Stewart JR, Lister AM, 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.

Stone KD, Cook JA. 2002. Molecular evolution of Holarctic martens (genus *Martes*, Mammalia: Carnivora: Mustelidae). *Molecular Phylogenetics and Evolution* **24:** 169–179.

Svenning JC, Normand S, Kageyama M. 2008. Glacial refugia of temperate trees in Europe: insights from species distribution modelling. *Journal of Ecology* **96:** 1117–1127.

Swofford DL. 2002. *PAUP*: phylogenetic analysis using parsimony (* and other methods), version 4.0b10.* Sunderland, MA: Sinauer Associates.

Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* **7:** 453–464.

Tajima F. 1989. Statistical-method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123:** 585–595.

Valdiosera CE, Garcia N, Anderung C, Dalen L, Creput-Bonnoure E, Kahlke RD, Stiller M, Brandstrom M, Thomas MG, Arsuaga JL, Gotherstrom A, Barnes I. 2007. Staying out in the cold: glacial refugia and mitochondrial DNA phylogeography in ancient European brown bears. *Molecular Ecology* **16:** 5140–5148.

Willis KJ, van Andel TH. 2004. Trees or no trees? The environments of central and eastern Europe during the Last Glaciation. *Quaternary Science Reviews* **23:** 2369–2387.

Willis KJ, Rudner E, Sümegi P. 2000. The full-glacial forests of central and southeastern Europe. *Quaternary Research* **53:** 203–213.

Wojcik JM, Kawalko A, Markova S, Searle JB, Kotlik P. 2010. Phylogeographic signatures of northward post-glacial colonization from high-latitude refugia: a case study of bank voles using museum specimens. *Journal of Zoology* **281:** 249–262.

Wolsan M. 1993. Phylogeny and classification of early European Mustelidae (Mammalia, Carnivora). *Acta Theriologica* **38:** 345–384.

Zalewski A. 2004. Geographical and seasonal variation in food habits and prey size of European pine martens. In: Buskirk SW, Harestad AS, Raphael MG, Powell RA, eds. *Martens, sables, and fishers: biology and conservation.* Ithaca, NY: Cornell University Press, 13–25.

SUPPORTING INFORMATION

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

Table S1. Locality and specimen information of *Martes* samples.