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Phylogenetics and phylogeography of red deer mtDNA lineages during the last 50 000 years in Eurasia

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The present phylogeographic pattern of red deer in Eurasia is not only a result of the contraction of their distribution range into glacial refugia and postglacial expansion, but probably also an effect of replacement of some red deer *s.l.* mtDNA lineages by others during the last 50 000 years. To better recognize this process, we analysed 501 sequences of mtDNA cytochrome *b*, including 194 ancient and 75 contemporary samples newly obtained for this study. The inclusion of 161 radiocarbon-dated samples enabled us to study the phylogeny in a temporal context and conduct divergence-time estimation and molecular dating. Depending on methodology, our estimate of divergence between *Cervus elaphus* and *Cervus canadensis* varied considerably (370 000 or 1.37 million years BP, respectively). The divergence times of genetic lineages and haplogroups corresponded to large environmental changes associated with stadials and interstadials of the Late Pleistocene. Due to the climatic oscillations, the distribution of *C. elaphus* and *C. canadensis* fluctuated in north–south and east–west directions. Some haplotypes dated to pre-Last Glacial Maximum periods were not detected afterwards, representing possibly extinct populations. We indicated with a high probability the presence of red deer *sensu lato* in south-eastern Europe and western Asia during the Last Glacial Maximum.

ADDITIONAL KEYWORDS: ancient DNA – *Cervus elaphus* – cytochrome *b* – Holocene – Last Glacial Maximum – Late Pleistocene – mtDNA – phylogenetic – phylogeography – postglacial recolonization.

INTRODUCTION

Climatic oscillations significantly influence the distribution and phylogeographic pattern of many species. Previously, it has been suggested that the main drivers of extinctions and divergences of many Pleistocene populations were stadials, e.g. the Last Glacial Maximum (LGM) (Taberlet *et al.*, 1998; Hewitt, 2000). Furthermore, the study of Cooper *et al.* (2015) showed that the extinctions and/or turnovers of a number of mammal species or their different genetic lineages in the Late Pleistocene were correlated with episodes of fast climate change. During such events, most rapid and extreme changes in temperature and precipitation took place in short time periods, while in the LGM [26.5–19.0 kya (thousand years ago); Clark *et al.*, 2009], the climatic conditions were relatively stable, and different mammalian populations shifted their ranges or were able to adapt to environmental changes (Cooper *et al.*, 2015). Moreover, different organisms responded in individual ways to the climatic oscillations (Baca *et al.*, 2017). During Quaternary glaciations, temperate species disappeared from large areas of northern and central parts of Europe and north of 57°N in western Asia and survived the stadials in restricted areas, mostly in more southern parts of the continents (Hewitt, 1996, 2000; Taberlet, 1998; Foronova, 1999, 2001; Stewart *et al.*, 2010; Shpansky, 2018). In contrast to that, the ranges and genetic diversities of cold-adapted organisms such as collared lemmings (*Dicrostonyx* Gloger, 1841 spp.) and muskox [*Ovibos moschatus* (Zimmermann, 1780)] were much larger during glacial times than during the interglacials (Palkopoulou *et al.*, 2016; Baca *et al.*, 2017; Malikov *et al.*, 2020; Stefaniak *et al.*, 2021). Besides the north–south oscillations in the Late Pleistocene, there were also pulsations of east–west distribution ranges of different species or their morphs, e.g. in the saiga antelope [*Saiga tatarica* (Linnaeus, 1766)] and the Siberian roe deer [*Capreolus pygargus* (Pallas, 1771)], according to their adaptation to various environmental conditions related to the oceanic–continental climate gradient (Matosiuk *et al.*, 2014; Nadachowski *et al.*, 2016; Ratajczak *et al.*, 2016).

The red deer (*Cervus elaphus* Linnaeus, 1758) is one of the most widespread and ecologically most important, large and wild mammal species in Europe. Its biogeographic history has been studied in great detail in large-scale phylogeographic (Ludt *et al.*, 2004; Skog *et al.*, 2009; Niedziałkowska *et al.*, 2011) and, increasingly, also ancient DNA studies (Meiri *et al.*, 2013; Stanton *et al.*, 2016; Doan *et al.*, 2017) combined with (sub-)fossil records (Sommer *et al.*, 2008), creating an increasingly precise picture of its spatiotemporal distribution dynamics. The European red deer (*C. elaphus*) is closely related to Asian and North American populations, as well as the sika deer (*C. nippon* Temminck, 1838). Classification of this complex has been contentious, but according to the presently favoured taxonomic consensus, the red deer complex comprises the sika deer and three species of red deer: European/West Asian red deer (*C. elaphus*), Central Asian red deer (*C. hanglu* Wagner, 1844) and East Asian/North American wapiti or elk [*C. canadensis* (Erxleben, 1777)] (see, for example: Lorenzini & Garofalo, 2015; Meiri *et al.*, 2018). *Cervus elaphus* and *C. hanglu* are often called the elaphoid red deer, while the *C. canadensis* populations are the wapitoids. Hereafter, the term red deer *sensu lato* (*s.l.*) is used when we refer to individuals of the red deer not assigned to any particular red deer species (*C. elaphus*, *C. hanglu* or *C. canadensis*). All terms concerning red deer species or genetic lineages used in the text are defined in Table 1.

According to phylogenetic data, red deer *s.l.* evolved in central Asia (Ludt *et al.*, 2004). In the late Early

Table 1. Glossary of the terms concerning the red deer complex used in the text

Term	Explanation
Cervus canadensis	Wapiti, East Asian/North American red deer
C. elaphus	European/West Asian or Western red deer
C. hanglu	Central Asian Red deer
C. nippon	Sika deer
Elaphoids (elaphoid deer)	Usually refers to *C. elaphus* + *C. hanglu* but in this study we use the term for *C. elaphus* only (see also Hu *et al.*, 2019)
Wapitoids (wapitoid deer)	*C. canadensis*
Red deer complex	Deer belonging to one of the following species: *C. canadensis*, *C. elaphus*, *C. hanglu* or *C. nippon*
Red deer *sensu lato* (*s.l.*)	Red deer belonging to *C. canadensis*, *C. elaphus* or *C. hanglu* (monophyletic lineage according to Hu *et al.*, 2019)

Pleistocene, it appeared in south-western Siberia (Alexeeva, 1980; Foronova, 1999, 2001) and Europe (Van der Made *et al.*, 2014, 2017; Stefaniak, 2015; Van der Made & Dimitrijević, 2015) and about 15 kya migrated to North America via the Bering Strait (Meiri *et al.*, 2018). According to the study of Doan *et al.* (2018), red deer *s.l.* migrated from Asia into Europe in several migration waves through south-eastern to western Europe.

The elaphoid deer, *C. elaphus*, in particular, has been the focus of phylogeographic studies based on mitochondrial DNA (mtDNA) (e.g. Ludt *et al.*, 2004; Skog *et al.*, 2009; Niedziałkowska *et al.*, 2011; Doan *et al.*, 2018; Meiri *et al.*, 2018). There are four main mtDNA haplogroups (called A to D in the most widely used nomenclature). The contemporary genetic pattern of *C. elaphus* in Europe is interpreted as a consequence of its survival in different refugia during the LGM and subsequent postglacial recolonization of the continent (Skog *et al.*, 2009; Niedziałkowska *et al.*, 2011; Meiri *et al.*, 2013). According to the fossil record, the LGM refugia of the species within Europe were located in Iberia, south-western France, Italy, the Balkans, Greece and Moldova east of the Carpathians (Sommer *et al.*, 2008). The Crimea, although harbouring many different mtDNA haplogroups of red deer *s.l.* during the last 50 kya, was not an LGM refugium for the species according to Doan *et al.* (2018). The LGM refugia for wapitoids were confined to the Urals (Niedziałkowska *et al.*, 2021) and eastern Asia (Meiri *et al.*, 2018).

Most European populations of *C. elaphus* belong to three haplogroups: A, B and C (Skog *et al.*, 2009; Niedziałkowska *et al.*, 2011). A signal concordant with the mitochondrial DNA has recently been identified in the nuclear genome (Zachos *et al.*, 2016). Haplogroup A is most widely distributed from Iberia in the south-west of the continent through western Europe and from the British Isles to Scandinavia and central/eastern Europe, where it co-occurs with haplogroup C that is otherwise distributed in south-eastern Europe, including the Balkans and the Carpathians. Haplogroup B is today confined to the introduced populations on the Tyrrhenian islands of Sardinia and Corsica and to North Africa (Algeria and Tunisia). Based on ancient DNA analyses, it has recently been identified as a refugial lineage on the Italian mainland during the LGM (Doan *et al.*, 2017), making the Tyrrhenian islands and North Africa 'genetic museums', where haplogroup B survived after *C. elaphus* had been introduced there, before they became extinct in Italy. Haplogroup D inhabits Turkey and Transcaucasia, but it was also found in the Polish Carpathians and the relict population of Mesola in the Po delta area in Italy (Borowski *et al.*, 2016).

We hypothesize that the present phylogeographic pattern of *C. elaphus* in Europe is not only a result of the contraction of elaphoid populations into glacial refugia and post-LGM expansion into formerly uninhabitable areas, but also an effect of replacement of some red deer *s.l.* mtDNA lineages. This does not necessarily mean that there was no genetic continuity between *C. elaphus* occurring before and after the LGM, but it indicates that different genetic lineages of red deer *s.l.* could have dominated prior to the LGM than after it. The aim of our study is to uncover the phylogenetic and phylogeographic patterns of red deer *s.l.* during the last 50 000 years using, according to the best of our knowledge, the largest dataset to date, comprising 194 ancient Eurasian red deer *s.l.* samples, including 161 radiocarbon-dated samples. The obtained data are used to estimate the divergence times of the main genetic lineages of red deer and compare them with the main environmental changes that occurred during the last 50 000 years.

MATERIAL AND METHODS

ANCIENT AND HISTORICAL SAMPLES

DNA extraction

DNA was extracted from 455 red deer *s.l.* samples from Eurasia obtained from archaeological or palaeontological collections and excavations (Supporting Information, Table S1; Fig. 1). Bone and tooth fragments were washed with bleach, rinsed with ddH_2O, UV-irradiated for 7 min on each side and pulverized in a cryogenic mill (Spex CentriPrep). Approximately 200 mg of powder was used for DNA extraction, which was performed using two methods: (1) phenol-chloroform as described in Doan *et al.* (2017) and (2) according to the protocol by Dabney *et al.* (2013). DNA extraction was performed at the Institute of Genetics and Biotechnology of the University of Warsaw laboratory (IGiB UW) (1) and Centre of New Technologies of the University of Warsaw laboratory (2), both laboratories dedicated to ancient DNA analyses.

Singleplex cytochrome b *amplification*

Short fragments of cytochrome *b* sequences (*Cytb*) were amplified from DNA extracts to check the success of DNA extraction and confirm that the analysed samples belong to red deer *s.l.* specimens. Amplifications were performed in a 25-µL reaction volume containing 2 µL mock or ancient DNA extracts, 0.2 µmol/L forward and reverse primers as described in Stanković *et al.* (2011) and 12.5 µL AmpliTaq Gold PCR Master Mix (Applied Biosystem). Amplification conditions consisted of a 12 min activation step at 95 °C, followed by 45 cycles (95 °C for 30 s, 45 °C for 30 s, 72 °C for 30 s); and a final extension at 72 °C for 7 min. Polymerase chain reaction products were purified with ExoI/FastAP (Thermo Scientific) and sequenced in both directions on an ABI PRISM 3730xl DNA Sequencer. The obtained sequences were compared with the GenBank database using BLASTn (Altschul *et al.*, 1997) to find homologous sequences in red deer *s.l.*

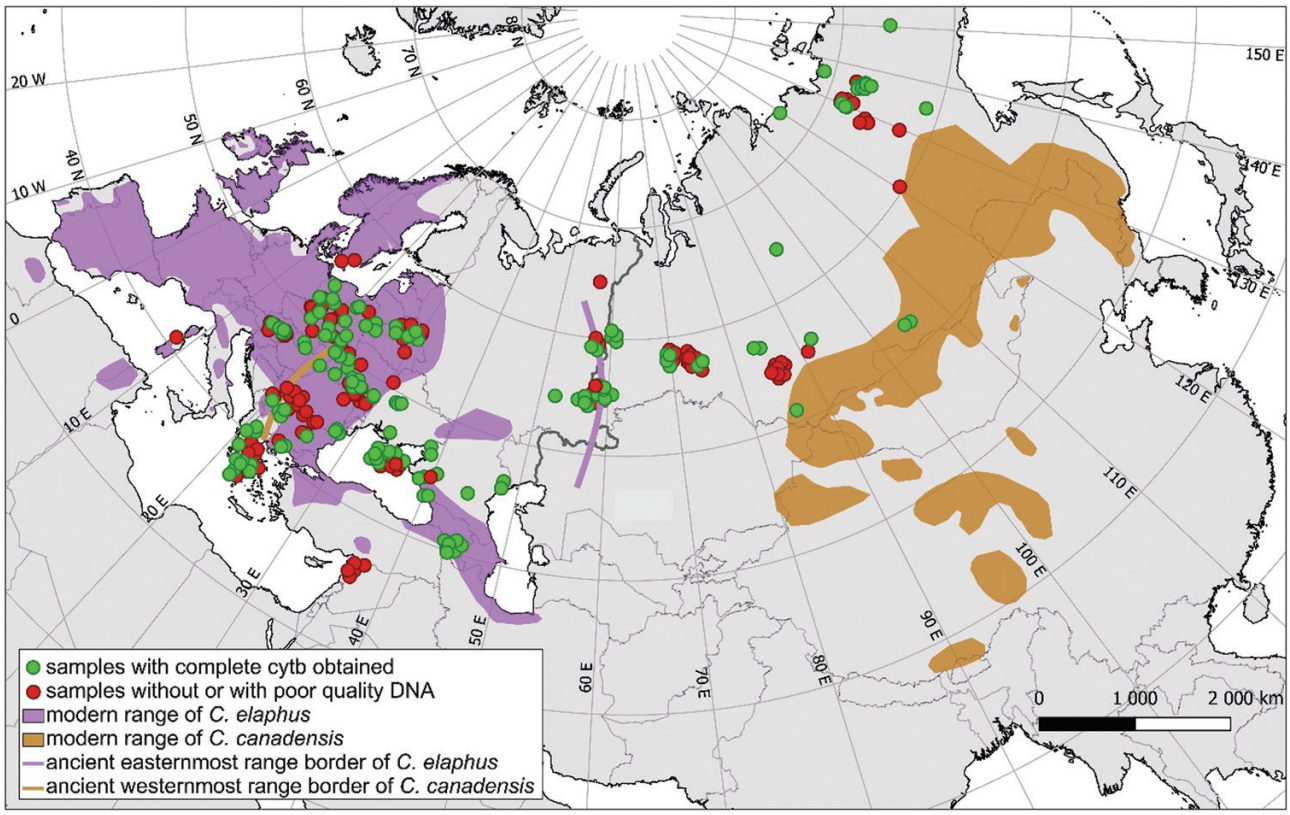

Figure 1. Geographic distribution of ancient and historical red deer *s.l.* samples analysed in this study. Modern range of *C. elaphus* was according to Niedziałkowska *et al.* (2021) and references therein. Modern range of *C. canadensis* refers to its range in Asia only and was created based on IUCN data and data from Stepanova (2010). The localization of borders of the easternmost range of elaphoids and the westernmost range of wapitiods is based on the results of the present study and published data (Meiri *et al.*, 2018).

Multiplex cytochrome b *amplification and next generation sequencing*

The entire cytochrome *b* sequence (1140 bp) was amplified in multiplex PCRs using 12 overlapping primer pairs, as described in Stanković *et al.* (2011). Amplifications were performed in a 25-µL reaction volume containing 2 µL mock or ancient DNA extracts, 0.16–0.32 µM forward and reverse primers and 12.5 µL AmpliTaq Gold PCR Master Mix (Applied Biosystem). The amplification conditions were as follows: 12 min activation step at 95 °C; 30 cycles (95 °C for 30 s, 53 °C for 30 s, 72 °C for 30 s); and a final extension at 72 °C for 7 min. Multiplex PCR products were used to prepare libraries for next generation sequencing (NGS) on Roche 454 or MiSeq Illumina platforms following the protocols of Stiller *et al.* (2009) or Meyer & Kircher (2010), respectively. The concentration of DNA in each library was determined by real-time PCR using Library Quantification Kits (Kapa Biosystems). Libraries were pooled in equimolar ratios and sequenced on a 454 GS FLX Titanium (Roche) platform at the Institute of Biochemistry and Biophysics Polish Academy of Sciences (IBB PAS) or on the MiSeq Illumina platform using MiSeq Reagent Kit v.3 at the IBB PAS, DNA Research Center Ltd and the IGiB UW, Poland.

Next generation sequencing data processing

Obtained NGS reads were assembled with pandaseq (Masella *et al.*, 2012). Primer sequences were trimmed with mothur (Schloss *et al.*, 2009) and final sequences were assembled in contigs using SeqMan Pro (DNASTAR, Inc.). To ensure authenticity of the sequence data, each sample was amplified in two independent multiplex PCRs, and contigs from both replicates were used to create consensus sequences according to the guidelines proposed by Stiller *et al.* (2009).

Additional amplifications

Some of the primer pairs showed low amplification efficiency. In order to obtain the whole *Cytb* sequence for all individuals, additional amplifications were performed. Missing *Cytb* fragments were amplified in singleplex PCRs using the same primer pairs as in multiplex reactions. Amplification conditions were the same as described above in *Singleplex cytochrome* b *amplification* except for the annealing temperature, which was 53 °C. The PCR products were purified with ExoI/FastAP (Thermo Scientific) and sequenced in both directions on an ABI PRISM 3730xl DNA sequencer. We obtained at least four sequences for each sample from independent amplifications.

Modern samples

The modern *C. elaphus* samples came from the collection of tissue samples stored in the Mammal Research Institute Polish Academy of Sciences (MRI PAS). They were obtained during previous scientific projects conducted by the authors of this study. The samples were collected from officially culled individuals. No animals were killed for the purpose of this study. The samples were stored in 95% ethanol and kept at –20 ºC prior to DNA extraction. DNA was extracted using the Qiagen DNeasy blood and tissue kit following the manufacturer's protocol. The whole cytochrome *b* gene (1140 bp) was amplified by PCR using primers A1 and B2 designed by Ludt *et al.* (2004) and Cerv1, Cerv9F and Cerv12R described in Stanković *et al.* (2011) and following the protocols presented in these papers. Amplicons were then sequenced on an automated ABI 3100 sequencer (Applied Biosystems).

Radiocarbon and molecular dating

The AMS ^{14}C dating of 27 ancient samples, from which we successfully retrieved the complete cytochrome b (Cyt b) sequences, was conducted at the Gliwice Absolute Dating Methods Centre (GADAM, Poland) and Poznań Radiocarbon Laboratory (Supporting Information, Table S1). Collagen extraction was performed following the methods described in detail by Piotrowska *et al.* (2019). Radiocarbon dates were calibrated using the OxCal 4.2 software (Bronk Ramsey, 2009) and the IntCal13 calibration curve (Reimer *et al.*, 2013). Hereafter, the ages are provided as cal BP, i.e. calibrated age in years before AD 1950. Additional 134 radiocarbon dates of analysed samples were obtained from previously published studies and were included in the analyses (Supporting Information, Table S1). The age of undated samples or those with infinite radiocarbon dates was estimated using BEAST analyses (Shapiro *et al.*, 2011; more details in Molecular dating and Table S3 in Supporting Information).

Phylogenetic analyses

DNA sequences obtained in this study were aligned in Mafft 7 (Katoh & Standley, 2013; Katoh *et al.*, 2019) with 232 previously published *Cytb* sequences from modern, ancient and historical *C. canadensis*, *C. elaphus*, *C. hanglu* and *C. nippon* specimens obtained from GenBank (Supporting Information, Table S2). Visayan spotted deer *Rusa alfredi* (Sclater, 1870) (GenBank: JN632698) was used as an outgroup, because this species was shown to be sister to *Cervus* in other studies (e.g. Doan *et al.*, 2018). Our final alignment was 1131 bp long. We

excluded the first 9 bp from all *Cytb* sequences in order to include a maximal number of sequences with the same length in the analyses. We applied two phylogenetic methods: Bayesian tree and median-joining network reconstruction. The Bayesian haplotype tree was constructed in MrBayes 3.2.6 (Ronquist & Huelsenbeck, 2003) using partitioning into three codon positions and the reversible jumping nucleotide substitution model (nst = mixed) with rate variation across sites. The base frequencies, rate matrix and shape parameter were unlinked between the partitions. We ran four Markov chains in two independent analyses for 15 million generations, sampling every 100 generations. The first 25% of samples were discarded as burn-in. To investigate the unusual position of *C. hanglu*, we performed an additional Bayesian reconstruction tree analysis, in which we excluded most haplotypes that did not cluster with any of the recognized haplogroups and haplotypes from haplogroup F (more details in Supporting Information, Phylogenetic analysis with reduced dataset and Fig. S1). The median-joining network was constructed in PopART 1.7 (Leigh *et al.*, 2015). For better resolution in the network reconstruction, we excluded *C. nippon* sequences from the analyses.

Divergence-time estimation

To estimate the divergence times between red deer *s.l.* lineages we created a dataset consisting of 161 directly radiocarbon-dated samples, as well as 48 randomly chosen modern sequences. All modern sequences were divided into eight geographic regions (western Europe; eastern and central Europe; southern Europe; Sardinia, Corsica and northern Africa; the Crimea, the North Caucasus and the Middle East; southern Asia; eastern Asia; North America). From each region we randomly selected six sequences, considering the number of individuals. This resulted in a dataset in which modern sequences were not over-represented but still covered the genetic diversity of red deer *s. l*. We also created a second dataset that consisted only of sequences belonging to *C. elaphus* clades (141 sequences). Analyses were performed in BEAST 1.10.4 (Suchard *et al.*, 2018). For both datasets, we applied the TN+G nucleotide substitution model that was selected by jModelTest (Posada, 2008), partitioned into three codon positions with substitution rate, rate heterogeneity and base frequencies unlinked between positions, uncorrelated relaxed clock with log-normal distribution, and the Bayesian Skygrid population model that was chosen using path sampling and stepping-stone sampling analyses (Baele *et al.*, 2013). Median calibrated radiocarbon ages of samples were used as molecular clock calibration points, and the CTMC rate reference (Ferreira & Suchard, 2008) was used as substitution rate prior. In separate analysis, we used the earliest red deer fossil from Europe (0.9 Mya; Lister *et al.*, 2010) as the additional internal node calibration point, following the analyses of Meiri *et al.* (2018). For more details see Supporting Information.

For both alignments, we ran two Markov chain Monte Carlo (MCMC) simulations through 50 million iterations with a sampling frequency of 5000 steps. The convergence and ESS values were checked using TRACER v.1.6 (Rambaut *et al.*, 2014). Results from the two analyses were combined using LogCombiner 1.10.4 with 10% burn-in. The phylogenetic trees were summarized in TreeAnnotator 1.10.4. Skygrid analyses were performed in TRACER to check for the changes in female effective population size (Ne_f) during the past 50 000 years.

We performed date randomization tests (Firth *et al.*, 2010; Ho *et al.*, 2011) by repeating the BEAST analyses described above for ten datasets with randomly assigned sequence age.

Phylogeographic reconstruction

Results of the above-mentioned analyses were used to reconstruct the distribution of red deer *s.l.* mtDNA haplogroups over the last 50 000 years. All samples were divided into five time periods (> 41 kya BP; 41–26 kya BP; 18–9 kya BP; 9–4 kya BP; 4–0 kya BP) according to their median calibrated radiocarbon age, median estimated molecular age or archaeological/palaeontological estimates. This division into five time periods corresponds with significant climatic and environmental changes. The first period covers the time prior to the LGM; the second encloses the immediate pre-LGM period, when the temperature started to decrease; the third represents the time after the LGM, when the temperature started to increase; the fourth corresponds to the Early Holocene; and the fifth covers the time period with clear signs of human-caused deforestation across Europe (Fyfe *et al.*, 2015). No samples dated to the LGM were analysed in our study.

Results from MrBayes' analyses were used to assign each sample to a haplogroup. Phylogeographic maps were constructed in QGIS 3.4.15 (http://qgis.osgeo.org) with spatial data obtained from Natural Earth (https://www.naturalearthdata.com/). Additional ancient samples from Europe published by Meiri *et al.* (2013), and two samples from Romania (Meiri *et al.*, 2018) that were not analysed in this study due to too short *Cytb* sequences, were included only in the haplogroup distribution maps (Supporting Information, Table S2). Their haplogroup assignment was obtained from Doan *et al.* (2018), except for the Romanian samples, whose assignment followed the results in Meiri *et al.* (2018).

RESULTS

We successfully isolated DNA from 295 (65%) of the 455 ancient samples. Among them we identified 28 samples that belonged to other species (e.g. roe deer, cattle and moose) as revealed by sequencing the short *Cytb* fragment, and 73 that showed poor DNA quality. Consequently, in our final analyses we used the complete *Cytb* sequences obtained from 194 ancient red deer *s.l.* samples and 75 sequences obtained from modern samples (Supporting Information, Table S1). All DNA sequences were deposited in GenBank under accession numbers MT266562–MT266830 and MT427356.

PHYLOGENIES

Bayesian reconstruction of phylogeny based on 501 sequences grouped into 249 haplotypes yielded a well-resolved tree, although not all nodes were highly supported (> 0.9, Fig. 2). The red deer complex was divided into four well-supported mtDNA lineages representing four possible species: *Cervus canadensis*, *C. elaphus*, *C. hanglu* and *C. nippon*. All ancient red deer samples analysed in this study belonged to *C. elaphus* and *C. canadensis*, hereafter called elaphoids and wapitoids, respectively, whereas newly obtained sequences from modern samples, collected in Europe only, exclusively belonged to *C. elaphus*. According to our tree, *C. hanglu* is a sister-group to all other lineages, whereas *C. nippon* is a sister-taxon to *C. canadensis* (Fig. 2). Additional MrBayes analyses that excluded some of the extinct haplotypes showed that without them, *C. hanglu* was the sister-taxon to *C. elaphus*, whereas the relation between *C. nippon* and C. *canadensis* remained the same (Supporting Information, Phylogenetic analysis with reduced dataset and Fig. S1.).

Within *C. elaphus*, we found six clades called haplogroups A–F (following the nomenclature proposed by Skog *et al.*, 2009), although only A, B and D were highly supported (> 0.9), along with one (X) of the three haplogroups retrieved for *C. canadensis* and the lineages of *C. nippon* and *C. hanglu* (Fig. 2). Haplogroups D and E were sister-clades and together were sister to the other four haplogroups among which F was sister to A/B/C: ((DE)(F(A(BC)))). Within *C. canadensis*, we retrieved three clades called haplogroups X, Y and Z. Haplogroups X and Y were closely related and formed a sister sublineage to Z (Fig. 2).

Four divergent haplotypes (H131, H204, H227, H232) did not cluster with any of the haplogroups (Figs 2, 3). All of them were extinct haplotypes found in the Urals (H227 and H232), western Siberia (H204) or central Europe (modern-day Poland – H131). Haplotype H232 was sister to the whole *C. elaphus* lineage (Fig. 2) and showed an intermediate position between *C. elaphus* and *C. canadensis* in the network (Fig. 3). Moreover, it was genetically distinct from both of them: 25 mutational steps from the closest H123 in haplogroup A of *C. elaphus*, and 22 mutational steps from H141 in the *C. canadensis* haplogroup Y (Fig. 3). Haplotype H131 was sister to the whole *C. elaphus* lineage (minus H232) in the tree (Fig. 2) but part of the elaphoid lineage in the network, where it was closest to haplogroup F (separated by 16 mutational steps from H130, Fig. 3). In the tree, H227 was sister to haplogroups A–C (Fig. 2), while in the network it was located one mutational step from haplogroup F (H201) and two mutational steps from the most common haplotype in haplogroup C (Fig. 3). Haplotype H204 in the *C. canadensis* lineage was not part of haplogroups X–Z, but instead was sister to all of them, separated by 11 mutational steps from the closest haplotypes H231 and H233 in haplogroup Z (Fig 3). The genetic divergence between the elaphoid and wapitoid lineages was high as their two closest haplotypes, extinct H123 in haplogroup A and H141 in haplogroup Y, were separated by 41 mutational steps (Fig. 3). The haplotype network (Fig. 3) showed that haplogroups within the elaphoid and wapitoid lineages were more closely related and separated by fewer mutational steps: less than 20 in the former and less than five in the latter. Due to ignoring ambiguous sites and gaps in the alignment, some of the haplotypes presented in the tree (Fig. 2) collapsed into a single haplotype in the network (Fig. 3). Nevertheless, relationships among haplotypes presented in the network are similar to those found in the Bayesian tree. The network showed a star-like phylogeny within haplogroups A, C, E and Y, indicating a possible recent population expansion.

Among all identified haplotypes of the elaphoid and wapitoid deer (N = 241, excluding *C. hanglu*, *C. nippon* and *R. alfredi*; Figs 2, 3; Supporting Information, Tables S1, S2), 56% were found only among ancient samples, 37% only in modern samples and 6% (usually the most common and ancestral ones according to the network) were found both in modern and ancient individuals. The proportion of extinct haplotypes differed among haplogroups. In the case of haplogroup F, all haplotypes became extinct (based on our sample). A substantial fraction of extinct haplotypes was also found in haplogroups D (86%), Y (78%) and B (70%). The largest number of exclusively modern haplotypes in the analysed dataset was found in haplogroups A (41%) and E (59%). Moreover, haplogroup A contained the highest number of haplotypes (6, 12%) found both in modern and ancient samples.

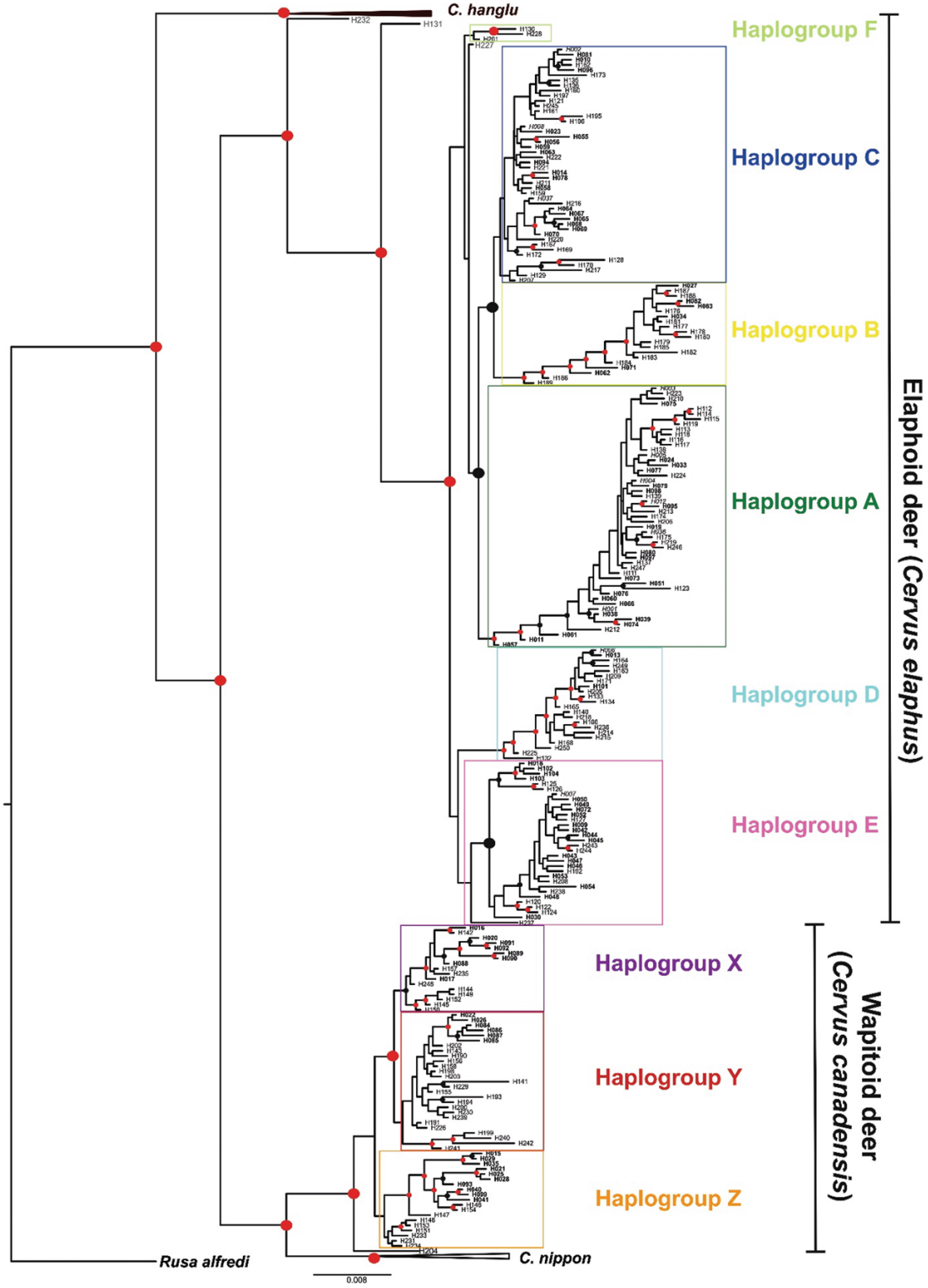

Figure 2. Bayesian phylogeny of *Cytb* haplotypes (1131 bp) from ancient and modern specimens calculated with MrBayes. Black circles at nodes indicate posterior probabilities above 0.7 and red circles posterior probabilities above 0.9. Names of haplotypes occurring only in modern samples are represented by haplotype names in bold, whereas those occurring in both modern and ancient samples are represented by italicized names, and those found only in ancient samples are in normal font. Names in bigger grey or black font indicate intermediate ancient haplotypes from elaphoid and wapitoid clades, respectively, that do not belong to any of the distinguished haplogroups. Detailed descriptions of haplotypes are presented in the Supporting Information, Tables S1, S2.

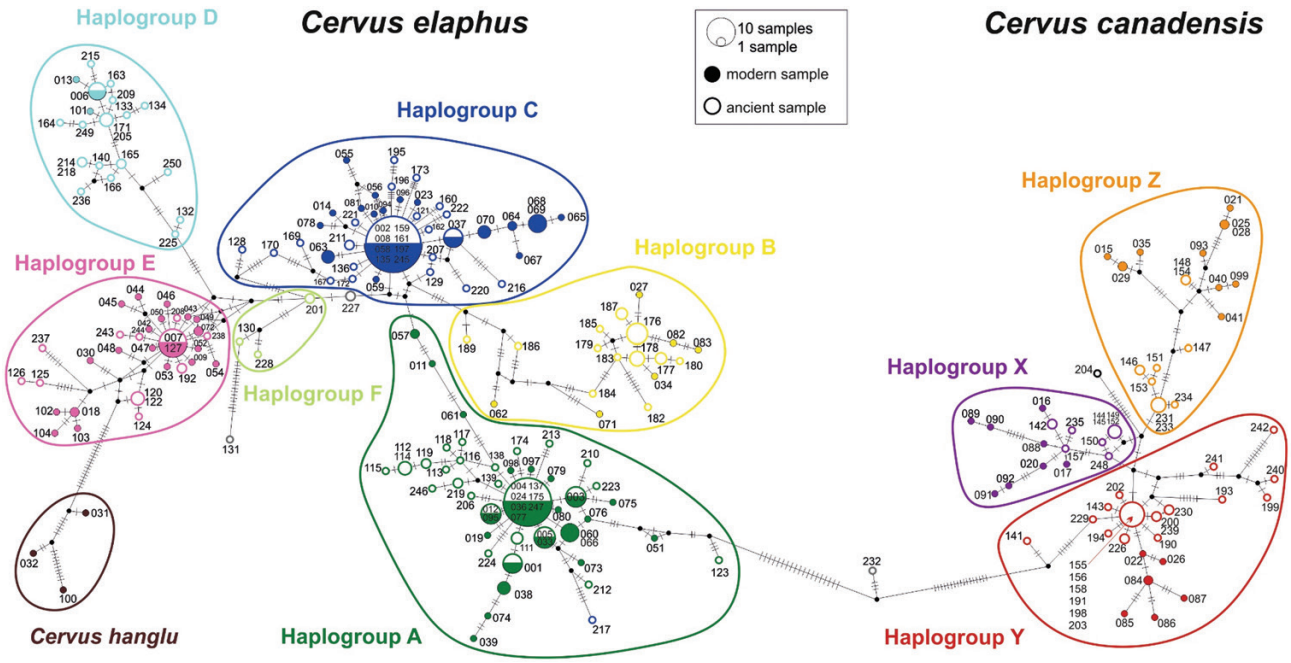

Figure 3. Median-joining haplotype network calculated with PopART. Black circles represent missing haplotypes. Hatch marks represent mutational steps between haplotypes. Haplotype names and haplogroup colours correspond to those in the Bayesian tree (Fig. 2).

Divergence times

The tree topology obtained in BEAST for the dated samples only (Fig. 4) showed some differences compared to the MrBayes tree shown in Fig. 2 (for details see Supporting Information, Table S4). For instance, haplogroup A was sister to haplogroups B and C in MrBayes, but it was a sister-clade to all remaining haplogroups of the elaphoids in the BEAST tree. Moreover, haplogroup F (represented by only two of its three haplotypes in this analysis) was not monophyletic on the BEAST tree. In total, six haplotypes were located in different positions in the MrBayes tree than in the BEAST tree, but the nodes of three of them had higher posterior probabilities in the MrBayes tree and the remaining nodes were not well supported in either of the two trees (see Figs 2 and 5). However, these six haplotypes had similar positions in the MrBayes tree and the network (Fig. 3), so that the differences between MrBayes and BEAST analyses might be a result of the lower number of sequences used in the latter.

According to our divergence-time estimations, the mean rate of the uncorrelated log-normal relaxed molecular clock was 0.165 substitutions per site per million years (sub/site/Myr) with 95% intervals of highest posterior density (HPD) of 0.104–0.254 sub/site/Myr.

The divergence time (95% intervals of HPD) of the elaphoid and wapitoid deer was estimated to 114–795 kya BP [mean age of most recent common ancestor (MRCA) – 374 kya BP, Table 2]. According to our estimates, the wapitoid lineage diversified into the retrieved haplogroups slightly earlier than the elaphoid lineage. Their most MRCA was dated to 174 (95% intervals of HPD: 75–284) and 144 (69–233) kya, respectively. Furthermore, the wapitoid haplogroups, on average, diversified earlier than the elaphoid haplogroups. Haplogroup Z was the first (the age of MRCA is about 90 ka BP), and in the elaphoid lineage haplogroups A, C and D diversified earlier (MRCA dated to 50–60 kya) than B and E (MRCA at about 30 kya, Table 2). Divergence-time estimations with an additional internal node calibration point of the earliest *C. elaphus* fossil in Europe (~0.9 Myr) showed that the mean substitution rate was similar (median 0.148 sub/site/Myr with 95% HPD: 0.089–0.229 sub/site/Myr) to the one calculated in the analysis with only tip node calibration. However, the MRCA of elaphoid and wapitoid deer was estimated to be three to four times older (1.37 Mya, 95% HPD: 0.88–2.32 Mya), whereas the age of the MRCA of the specific haplogroups was similar compared to the analysis without internal calibration point (Table 2; Supporting Information, Fig. S2 in Divergence-time estimation with internal node fossil calibration). Additional separate analyses of the elaphoid clades only showed that coalescent events of the majority of haplotypes within particular haplogroups (i.e. internal nodes) occurred 14–20 kya (Table 2; Fig. 5).

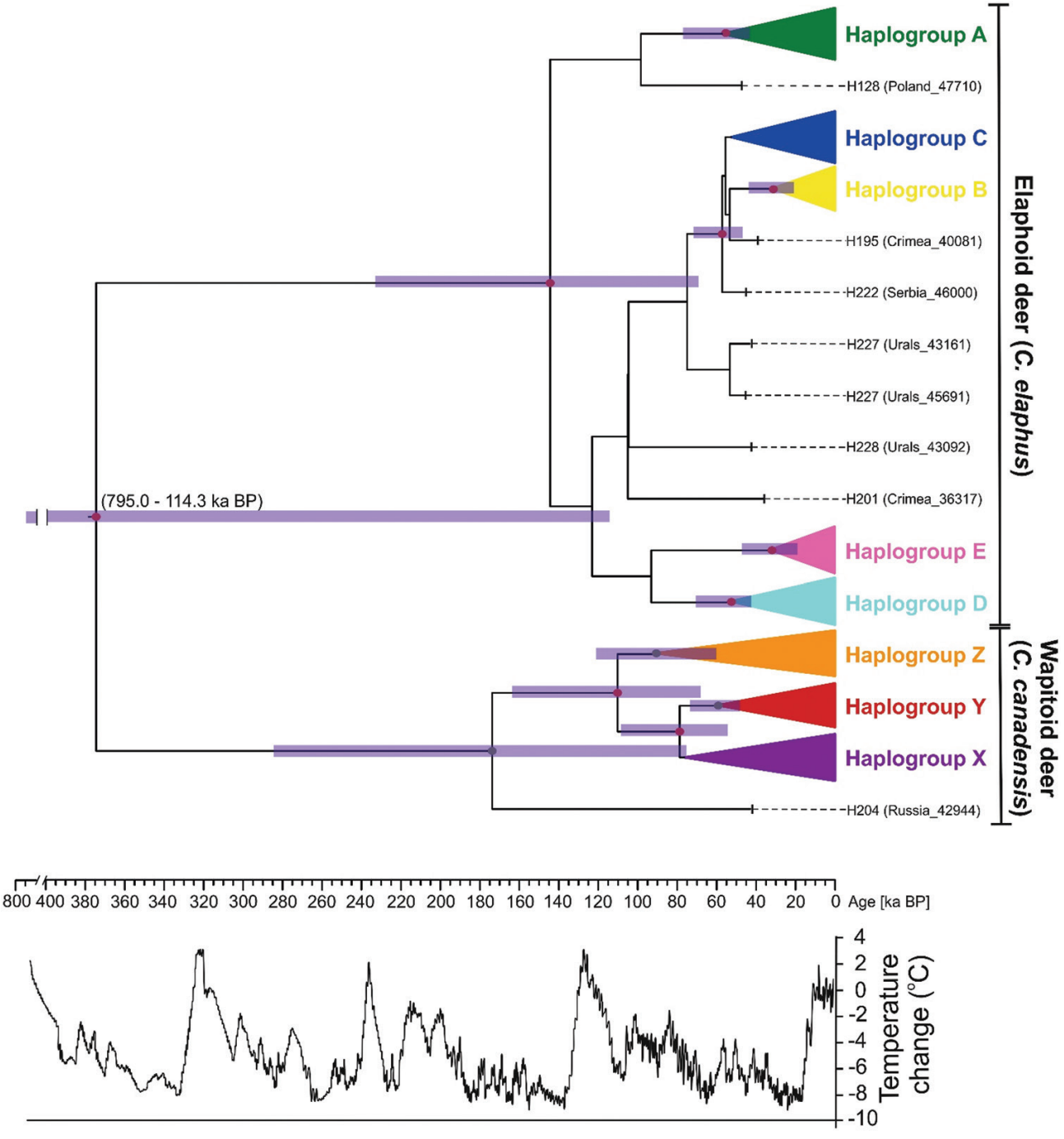

Figure 4. Dated phylogeny of the elaphoid and wapitoid deer obtained in BEAST from radiocarbon-dated ancient sequences and randomly chosen modern sequences, displayed in the context of temperature change. Black circles at nodes indicate posterior probabilities above 0.7. and red circles posterior probabilities above 0.9. Purple rectangles at well-supported nodes represent 95% HPD of estimated ages. For better resolution, the deepest node interval is not fully shown. Ancient samples placed outside haplogroups are described by sample name, geographic location and median radiocarbon age. The graph below the phylogeny shows temperature oscillations according to data from the Vostock ice core (Petit *et al.*, 1999).

The best-fitting population model in all BEAST analyses, regardless of the dataset used, was Bayesian Skygrid. This analysis did not reveal significant changes in female effective population size (Ne_f) during the past 50 000 years (data not shown). Separate skyline analyses for *C. elaphus* deer

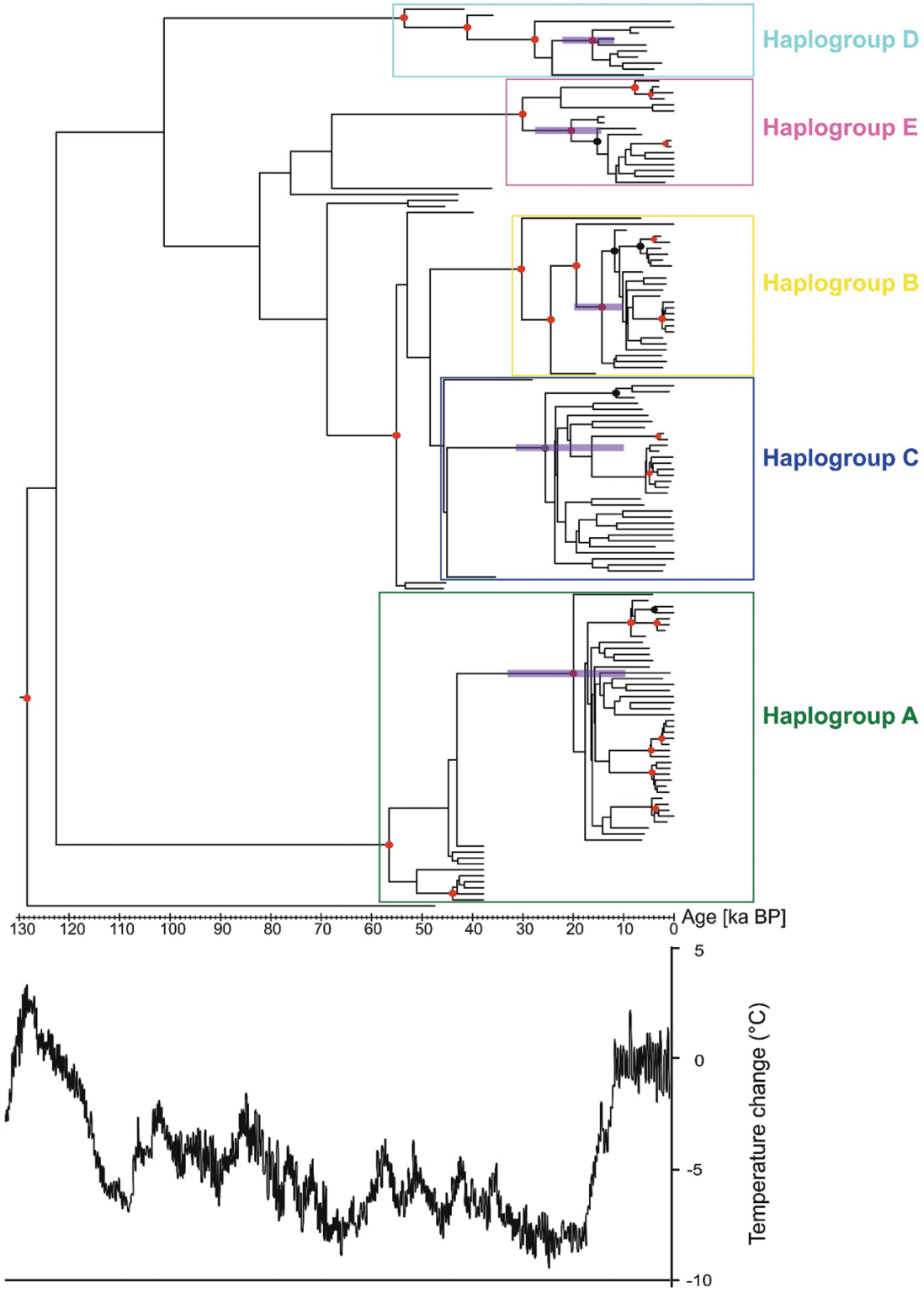

Figure 5. Dated phylogeny of the elaphoid deer obtained in BEAST from radiocarbon-dated ancient sequences and randomly chosen modern sequences. Black circles at nodes indicate posterior probabilities above 0.7 and red circles posterior probabilities above 0.9. Rectangles at nodes represent 95% HPD of selected node ages. The graph below the phylogeny shows temperature change according to data from the Vostock ice core (Petit *et al.*, 1999).

Table 2. Estimated coalescent times of the red deer *s.l.* clades and age of particular nodes in ka BP. Asterisks [*] indicate that the node has low support with a posterior probability < 0.7. Age shown in ka BP. MRCA – most recent common ancestor. HPD – intervals of the highest posterior density. Mean node age refers to ages of nodes of the elaphoid clades shown in Fig. 5

Clades	MRCA (95% HPD)	MRCA—analysis with internal node calibration (95% HPD)	Mean node age (95% HPD)
Elaphoid and Wapitoid deer	374.5 (114.3–795.0)	1373.1 (885.5–2316.1)	-
Wapitoid deer	173.8 (75.4–284.5)	208.9 (79.2–422.5)	-
Haplogroup Z	91.4 (60.1–121.1)	96.7 (61.5–139.6) *	-
Haplogroup X	76.3 (50.8–90.6) *	73.2 (51.2–102.8) *	-
Haplogroup Y	59.6 (48.1–73.4)	62.4 (49.0–80.7)	-
Elaphoid deer	144.4 (69.2–233.0)	899.2 (879.3–918.4)	-
Haplogroup A	57.2 (43.3–77.1)	69.7 (43.7–108.7)	20.0 (9.7–33.2)
Haplogroup D	53.2 (42.4–70.5)	60.1 (42.4–93.8)	16.2 (12.1–21.9)
Haplogroup C	52.7 (45.5–54.8) *	34.7 (28.3–43.1) *	25.6 (10.0–31.5)
Haplogroup E	32.6 (19.0–47.3)	34.9 (19.2–52.1)	20.5 (14.4–27.6)
Haplogroup B	32.0 (20.7–43.8)	33.9 (21.1–46.9)	14.3 (10.1–19.9)

haplogroups (A–E) showed that there were changes in Ne_f through time (Supporting Information, Fig. S3 in Effective population size reconstruction). For most of the haplogroups, effective population size was constant until 10 kya, after which population size increased. For haplogroup D, the increase started earlier – at about 17–15 kya, for C and E at about 10 kya, and for A at about 3 kya. A decrease of Ne_f was observed only in haplogroup B during the last 3 kya.

Spatiotemporal patterns of genetic lineages

In the period older than 41 000 years both lineages, elaphoids and wapitoids, co-existed together in eastern Europe (present-day Romania, the Crimea) and the Urals (Fig. 6). Just before the LGM (41–26 kya), *C. elaphus* disappeared from the Urals, and the contact zone between the wapitoid and elaphoid deer was confined to the Crimea. During and after the LGM, the wapitoids were no longer detected in Europe (compare Figs 6 and 7).

In the two oldest periods, the range of wapitoids covered vast areas from present-day Romania to north-eastern Asia, where their three haplogroups (X–Z) were detected (Fig. 6). Haplogroup X occurred only in north-eastern Asia. Before 41 kya, the distribution of haplogroup Y was restricted to the western part of the wapitoid range (the Crimea, the Urals and western Siberia), but later – prior to the LGM – haplogroup Y spread eastward and became the dominant haplogroup among the wapitoid deer (Fig. 6). Before 41 kya, haplogroup Z was widely distributed – it was present in the Urals and north-eastern Siberia. However, between 41 and 26 kya, we only found it in the Urals. Haplotype H204, which in the MrBayes tree was sister to all remaining wapitoid haplogroups, was detected only in one sample dated to ~43 kya and found in western Siberia (Fig. 6).

After the LGM, *C. canadensis* was detected only in Asia (Fig. 7). The oldest sample of this lineage (molecularly dated to 10.6 kya) belonged to haplogroup Y (Fig. 7; Supporting Information, Table S1). Similarly, in the next time period, only one wapitoid deer sample dated to the Middle Holocene was found: it belonged to haplogroup X and was detected in western Siberia (Fig. 7, middle panel). During the last 4000 years, the X clade was distributed from central Asia to north-eastern Siberia. In the most recent past the presence of this haplogroup has been confirmed only in central Asia (Fig. 7, lower panel).

In western Asia, the last wapitoid deer in our study, dated to *c.* 3.2 kya and belonging to haplogroup Y, was found in the eastern Urals. For the last 2000 years this clade has occurred only in central Asia (Supporting Information, Table S1; Fig. 7, lower panel). After the LGM, we did not detect haplogroup Z among our analysed samples. However, according to modern data, it occurs in southern and eastern Asia and in the Tien Shan, where also wapitoids of haplogroup X and *C. hanglu* occur. Today, the distribution of *C. canadensis* in Asia is restricted to an area from the central and southern parts of the continent (south Russia, Mongolia and China) to the coast of the Sea of Japan. The analysed modern samples covered almost the whole contemporary range of wapitoids in Asia (compare Figs 1 and 7, lower panel). Surprisingly, we also detected the presence of haplogroup A in western Sichuan in China, represented by its most common haplotype (H004, Figs 2 and 7, lower panel).

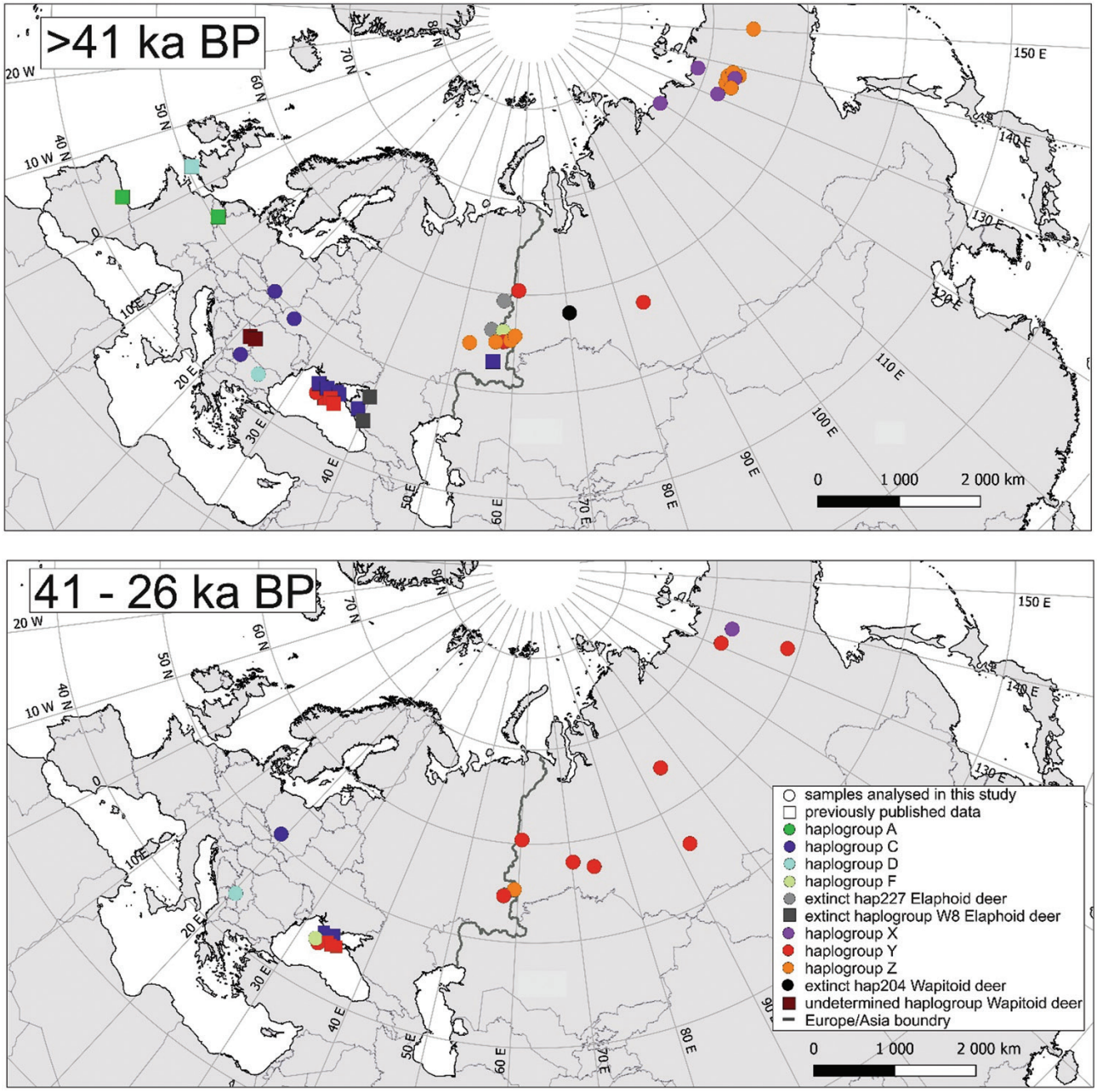

Figure 6. Distribution of mtDNA red deer *s.l.* haplogroups in Eurasia before the LGM. Upper panel, in the period older than 41 kya; Lower panel, between 41 and 26 kya. The colours of samples correspond to haplogroups as in previous figures. Red deer *s.l.* records are listed in the Supporting Information, Tables S1, S2.

Before 41 kya, elaphoid deer (haplogroups A, C, D, F in our samples from that time) ranged from the Atlantic coast to the Ural Mountains (Fig. 6). Haplogroup A occurred only in western Europe, and haplogroup C covered an area from the Carpathians through the Balkans and Crimea to the southern Urals, so further east than today (compare Figs 6 and 7). Haplogroup D was found only in two localities: the British Isles and the Balkans. Haplogroup F and H227 – the sister-haplotype to haplogroups A–C – were recorded in the southern Urals. In the Caucasus, another haplogroup called W8 [described in Doan *et al.* (2018)] occurred, a sister-clade to haplogroup B. In Doan *et al.* (2018) haplogroup B was considered to be part of W8 but we decided to separate haplogroup B from W8 in this paper as it is genetically distant from the rest of the W8 haplotypes. W8 and the haplotype H227 were not found in the following time periods after 41 kya.

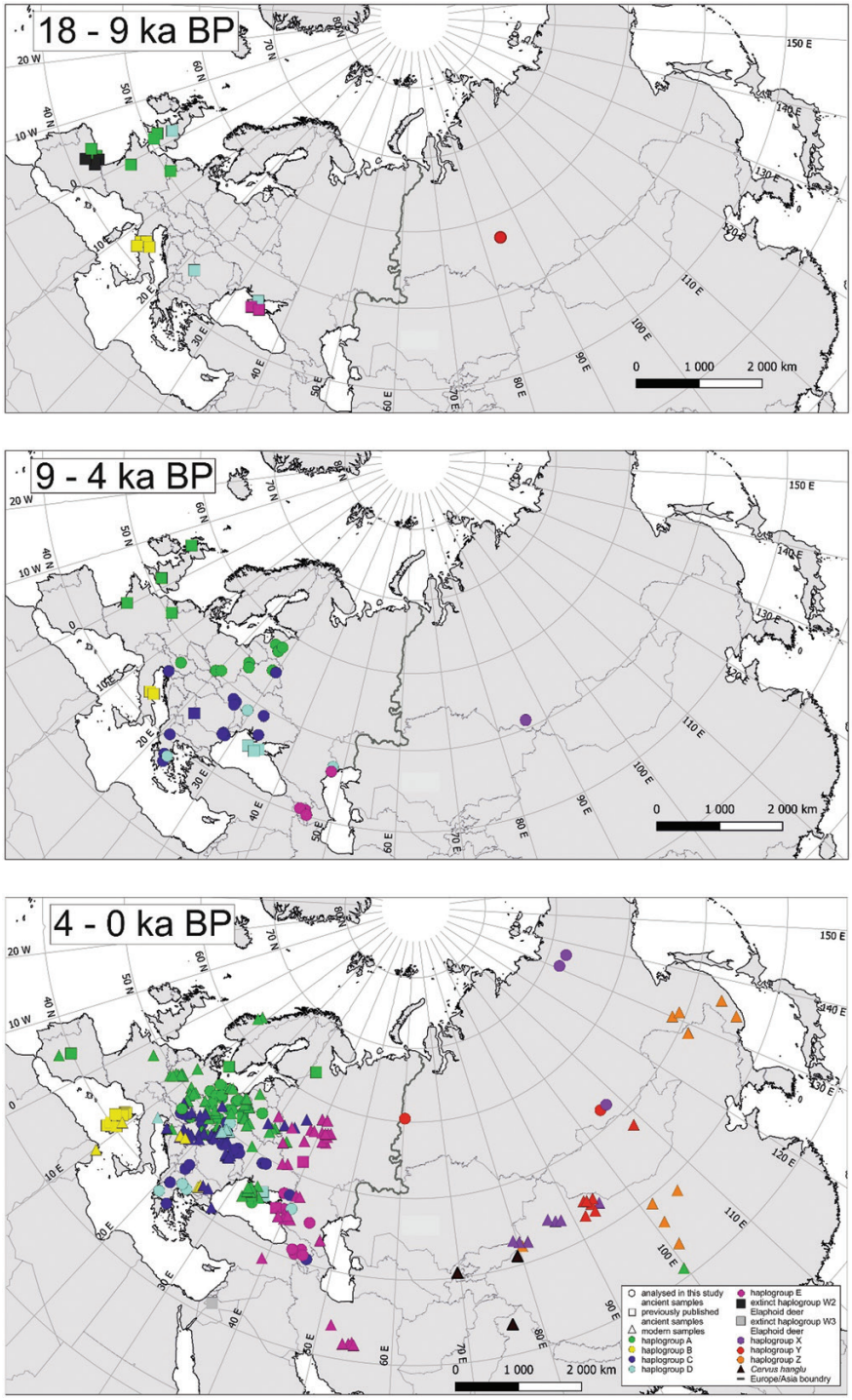

Figure 7. Distribution of mtDNA red deer *s.l.* haplogroups in Eurasia after the LGM. Upper panel, between 18 and 9 kya; Middle panel, between 9 and 4 kya; Lower panel, between 4 kya and today. The colours of samples correspond to haplogroups as in previous figures. Red deer *s.l.* records are listed in the Supporting Information, Tables S1, S2.

Between 41 and 26 kya, the elaphoids disappeared from the Urals and were not detected further east than the Crimea (Fig. 6, lower panel). Interestingly, among others, haplogroup F was discovered in the Crimea. It was also present in central Europe (in what is now southern Poland, not shown in Fig. 6) during Marine isotope stage 3 (MIS 3) (according to the palaeontological context), although the exact time is

unclear due to lack of a radiocarbon date for the sample (Fig. 2; Supporting Information, Table S1; H130).

After the LGM, between 18 and 9 kya, new haplogroups of elaphoid deer appeared in Europe: haplogroup B in the Apennine Peninsula and haplogroup E in Crimea, where it co-existed with haplogroup D. Moreover, in Iberia, haplogroup W2 [now extinct, a sister-clade to all haplogroups of European elaphoids described in Doan *et al.* (2018)] was found and dated by Meiri *et al.* (2013) to 18 698 cal BP (Supporting Information, Table S2; Fig. 7, upper panel). The presence of haplogroup A in the British Isles about 13 kya (Supporting Information, Table S2) suggests that it expanded to the north after the LGM. The distribution of haplogroup D after the LGM was similar to the pre-LGM period: from Britain to the Balkans and Crimea.

In the Early and Middle Holocene (9 and 4 kya), haplogroup A, the most widely distributed one, was found in almost all of western and central-eastern Europe (Fig. 7, middle panel). It reached present-day Estonia at least by 6.2 kya, and in present-day Belarus it created a contact zone with haplogroup C approximately 4.3 kya. Between 9 and 4 kya, haplogroup C was mostly found in the Balkans, in some regions together with haplogroup D. Haplogroup E, which was only found in Crimea in older periods, expanded to present-day Armenia and southern Russia, adjacent to the Caspian Sea. The distribution of haplogroup B was restricted to the Apennine Peninsula (Fig. 7, middle panel).

Between 4 kya and the present, the distribution of all haplogroups in our dataset expanded (Fig. 7, lower panel). Haplogroup A reached Scandinavia, but also moved to south-eastern Europe. It occurred as far south as present-day Turkey, but the ancient sample (not shown in Fig. 7) belonging to this haplogroup (Fig. 2; Supporting Information, Table S1; H123 – sample AR4) was not radiocarbon-dated, therefore it is unclear when haplogroup A reached Turkey. Today haplogroup A is found in Ukraine, and it is the only haplogroup present in Crimea.

The oldest sample from Sardinia for which we have genetic information is dated to 2.7 kya and belonged to haplogroup B. By then, at the latest, haplogroup B was present on the island, and later elaphoid deer of haplogroup B were found on Corsica, in continental Europe (the Balkans) and North Africa. Haplogroup D was still present in many European localities before 1 kya, but today it is restricted to southern Poland and the Mesola red deer (*C. elaphus italicus*) in northern Italy (Fig. 7, lower panel; Zachos *et al.*, 2014). Haplogroup E expanded from the Caucasus to the north and south and today is found in eastern Europe, Turkey and Iran. Around 800 ya, in the Middle East (present-day Jordan), there was one more extinct elaphoid haplogroup referred to as W3 in Doan *et al.* (2018), sister to the clade consisting of all modern European elaphoids (Fig.7).

Before the LGM, the region with the highest diversity of different mtDNA lineages and haplotypes was the one stretching from Crimea along the northern coast of the Black Sea to the Ural Mountains and western Siberia (Fig. 6). During the late glacial period and the Early Holocene (18–9 kya), more haplogroups of red deer *s.l.* were found and detected in western than in eastern Europe (Fig. 7). However, within the last 4000 years, the highest genetic diversity of *C. elaphus* has been detected in central, eastern and south-eastern Europe (Fig. 7, lower panel).

DISCUSSION

Phylogeny and divergence times of red deer *s.l.* lineages

According to the results of our phylogenetic analyses, *C. hanglu* is a sister-group to both *C. elaphus* and *C. canadensis* lineages in contradiction to previous studies, where this species was sister to *C. elaphus* only (Lorenzini & Garofalo, 2015; Doan *et al.*, 2018; Meiri *et al.*, 2018). The reason for this could be the much larger dataset of red deer *s.l.* mtDNA sequences consisting of 194 ancient samples analysed in the present study, including also several haplotypes intermediate between *C. elaphus* and *C. canadensis,* probably representing extinct lineages. Our additional analysis on a reduced dataset supports this claim. We showed that without these extinct and genetically distinct haplotypes the position of *C. hanglu* changed to the sister-species of *C. elaphus*, in accordance with many previous studies. This could be also one of the reasons for the much later divergence time of *C. elaphus* and *C. canadensis* obtained in this study (mean age of MRCA: 374 kya; 95% intervals of HPD: 114–795 kya). The phylogenetic pattern obtained in this study may also be due to the analysis of a single gene, i.e. cytochrome *b* sequences, but the analyses performed by Doan *et al.* (2018) on the same single marker yielded *C. hanglu* and *C. elaphus* as sister-taxa. However, this relationship is not universally found: a recent nuclear phylogenomic study found *C. hanglu* (*C. e. yarkandensis* Blanford, 1892 in their classification) to be sister to *C. canadensis*; together, they were sister to *C. elaphus*, and all three of them were in turn sister to the sika deer *C. nippon* (Hu *et al.*, 2019). This is in line with morphology but in clear contrast to the well-known fact that virtually all mtDNA studies have yielded a sister-group relationship between sika and *C. canadensis* (e.g. Lorenzini & Garofalo, 2015; Doan *et al.*, 2018; Meiri *et al.*, 2018). Such phylogenetic discordances

between the nuclear and the mitochondrial genome could be due to incomplete lineage sorting and/or hybridization of mtDNA lineages and have been found in other species as well [see Wang *et al.* (2018) for the example of the European bison, *Bison bonasus* (Linnaeus, 1758)].

As mentioned above, the time of the MRCA of *C. elaphus* and *C. canadensis* calculated using our dataset was much younger than that calculated in other studies (6–7 Myr: Ludt *et al.*, 2004; Lorenzini & Garofalo, 2015; 0.5–1.5 Myr: Polziehn & Strobeck, 2002; Doan *et al.*, 2018; Meiri *et al.*, 2018). The mutation rate calculated in our study was also much faster than that estimated by others (μ = 0.0118 sub/site/Myr in Polziehn & Strobeck, 2002; 0.0039 in Ludt *et al.*, 2004). These differences in divergence times and mutation rates are due to the fact that most of the previous studies relied on deep calibration points estimated based on fossil materials, whereas we exclusively used the radiocarbon-dated sequences and modern samples. What is more, an additional analysis with combined molecular clock calibration using radiocarbon-dated samples and fossils showed that the estimation of mutation rates, as well as the estimation of the MRCA of each of the haplogroups, remained similar. The age constraints on the MRCA of the elaphoid lineage using fossil calibration was, therefore, the only factor that was responsible for pushing back the age of the MRCA of the elaphoid and wapitoid lineages and the divergence time between them to 1.37 Mya (95% HPD: 0.88–2.32 Mya). This MRCA corresponds well with the divergence times of the elaphoids and wapitoids found in previous studies (Polziehn & Strobeck, 2002; Doan *et al.*, 2018; Meiri *et al.*, 2018). The younger MRCA (374 kya) obtained using molecular clock calibration and radiocarbon-dated samples was calculated only based on lineages sampled today and back to *c.* 50 000 years. There were probably many unsampled older lineages, now extinct, which, if they had survived to today, could have pushed all these dates back. Moreover, we did not include *C. hanglu* sequences into the estimates of the divergence time of red deer *s.l.* lineages, as was done by other authors (e.g. Ludt *et al.*, 2004; Meiri *et al.*, 2018).

The earliest red deer [*C. elaphus acoronatus* (Beninde 1937)] found in Europe (in present-day Germany and Montenegro) lacked a 'crown' at the top of the antler (Lister *et al.*, 2010) and was dated to 0.936–1.00 Mya (MIS 21–MIS 25; Van der Made *et al.*, 2014, 2017; Stefaniak, 2015; Van der Made & Dimitrijević, 2015). The occurrence of *C. elaphus* was probably connected with well-known large mammal migration waves in Europe during the climate cooling between 1.1 and 0.6 Mya (the Early/Middle Pleistocene boundary; Kahlke *et al.*, 2011; Markova & Vislobokova, 2016; Strani, 2020). The earliest fossil coronate deer (characteristic of extant European *C. elaphus*) are from the Holsteinian of Germany (Steinheim) and the Hoxnian of Britain (Swanscombe) (Lister, 1986), both now dated to MIS 11 (*c.* 400 kya) (Schreve & Bridgland, 2002; Nitychoruk *et al.*, 2006; Penkman *et al.*, 2013; Van der Made *et al.*, 2014; Stefaniak, 2015). According to our study, the upper limit of the MRCA of *C. elaphus* was 233 kya, considerably younger than the time of the first red deer occurrence in the fossil record. However, the MRCA estimate refers to the crown group of our sampled *C. elaphus*. The MRCA of *C. elaphus* estimated using also the fossil calibration (MRCA ca. 900 kya) better fits the age of the oldest fossil records of the species found in Europe, but is much older than the earliest known remains of coronate deer (*c.* 400 kya). The age of these remains better corresponds to the divergence time of *C. elaphus* and *C. canadensis* calculated using only our genetic dataset (374 kya). Probably further studies, which should include genomic data, will be necessary to get a more precise insight into the divergence time of these two genetic lineages of red deer.

Further divergence of the wapitoid (mean age of MRCA: 174 kya, 95% HPD: 284.5–75.4 kya) and the elaphoid deer (144 kya, 233–69.2 kya) calculated using only our genetic dataset, took place from MIS 8 to MIS 4 (including several stadials and interglacials; Past Interglacial Working Group, 2016). The majority of coalescent events of the extant *C. elaphus* mtDNA haplogroups are dated to wide time ranges (95% HPD were between 77–10 kya, MIS 5–MIS 1). These time ranges (MIS 8–MIS 1) cover several Quaternary glacials and interglacials, including the time of post-LGM expansion in Europe, so resolution is not high enough to link the divergence times of different genetic lineages and haplogroups of the red deer *s.l.* to specific climatic events.

Although the genomic sequencing of ruminants (including Cervidae species) indicated that there was a significant decrease in their numbers between 100 000 and 50 000 years ago, concurrent with the increase of human activity (Chen *et al.*, 2019), and although there were significant range shifts of red deer *s.l.* in Eurasia during the last 50 000 years (e.g. Meiri *et al.*, 2013, 2018; Shpansky, 2018; Niedziałkowska *et al.*, 2021), our analyses did not reveal significant changes in female effective population size (Ne_f). This may result from the composition of our dataset, which contained samples from various populations subjected to different changes in their population size. Indeed, results of Skyline reconstructions performed for separate elaphoid haplogroups revealed a significant increase in Ne_f for all of them (except haplogroup B) after the LGM (starting from 17 kya). Meiri *et al.* (2014) also found a significant increase in Ne_f in wapitoids in north-east Siberia during the last 15 000 years. Our results correspond with the modern abundance of several ungulate

species, including *C. elaphus*, in many areas of Europe (Carpio *et al.*, 2021). However, there were also periods in historical times (e.g. in 17th–19th centuries), when numbers of *C. elaphus* significantly decreased and/or the species even disappeared from some European regions (Hartl *et al.*, 2003; Nielsen *et al.*, 2008; Niedziałkowska *et al.*, 2012).

Ranges of elaphoid and wapitoid deer

According to radiocarbon-dated fossils and samples of European red deer *s.l.* analysed in this study, before the LGM, i.e. between 50 and 26 kya, the range of the species covered western, central and south-eastern Europe, from the present-day British Isles and Spain to the southern and central Urals. No red deer *s.l.* were detected in northern Europe (Niedziałkowska *et al.*, 2021; this study), but this could be an effect of a poor Quaternary fossil record in this region. In Asia, red deer *s.l.* occurred in north-eastern (Meiri *et al.*, 2014; this study) and south-western Siberia (Gromov, 1948; Alexeeva, 1980; Foronova 1999, 2001) and in more southern parts of the continent – mainly in present-day China (Meiri *et al.*, 2018). Within western Siberia, red deer *s.l.* have never spread to north of 58°N (Shpansky, 2018). Around 50 kya, when the climate was warmer than in the period before and during the LGM, the ranges of elaphoids and wapitoids overlapped in south-eastern Europe (Crimea, Romania) and the Urals (Stanković *et al.*, 2011; Doan *et al.*, 2018; Meiri *et al.*, 2018; this study). As the climate was getting more severe, elaphoid deer disappeared from the Ural Mountains before 41 kya and their range shrank westward. The more cold-adapted wapitoid deer (Geist, 1999) probably still occurred in the westernmost part of their range in the Urals and in eastern Europe until the LGM or even longer. According to indirectly radiocarbon-dated remains of red deer antlers described by Croitor & Obadă (2018), wapitoid deer were present in the territory of present-day Moldova until about 24.5 kya cal (20 350 ± 230 kyr according to calibrations by Niedziałkowska *et al.*, 2021), and they may even have occurred further west in the Late Pleistocene of France (MIS 2; Croitor, 2020). Some remains of red deer *s.l.* radiocarbon-dated to the LGM were found in eastern Europe and the Urals in areas probably unsuitable for *C. elaphus*, so they probably belonged to *C. canadensis* (Niedziałkowska *et al.*, 2021 and references therein). Indeed 11 out of 15 ancient red deer samples from the Urals analysed in this study belonged to the wapitoids and, according to environmental niche modelling, there were no suitable conditions for elaphoids in this region during the last 54 000 years (Niedziałkowska *et al.*, 2021).

Wapitoids most probably disappeared finally from Europe when the climate became warmer in the late glacial and the Holocene, resulting in today's large gap between the geographic ranges of *C. elaphus* in the west and *C. hanglu* and *C. canadensis* in the east (Fig. 7).

Phylogeographic pattern of red deer *s.l.* haplogroups during the last 50 000 years in Eurasia

Although some authors have suggested that there was a lack of phylogeographic structure in European mammals prior to the LGM (Hofreiter *et al.*, 2004), our results suggest a somewhat different picture. On the one hand, the range of some *C. elaphus* haplogroups was indeed much wider and covered larger parts of Europe (e.g. haplogroups C and D; Meiri *et al.*, 2013; this study) or Asia (haplogroup Z) before 41 kya compared to later periods prior to the LGM (41–26 kya), when the climate started to be colder. On the other hand, the distribution of haplogroup A was restricted to western and south-western Europe in the Late Pleistocene even before the LGM and has covered large parts of the continent in the Holocene. Although we cannot rule out that the changes in haplogroup distribution that we found are an artefact of small sample sizes for the time just before and during the LGM (41–18 kya), the number of analysed samples was large enough for the detection of several genetically distinct haplogroups or haplotypes that only occurred in eastern Europe and western Asia before the LGM (> 26 kya) (haplogroups F as well as haplotypes 204 and 227). The phylogenetic position of haplogroup F and haplotypes 204 and 227 would be in line with them being the result of earlier migration waves into Europe and western Asia from central Asia in the Pleistocene (compare: Meiri *et al.*, 2013; Doan *et al.*, 2018), but at this point this remains speculative.

The Urals were not an LGM refugium for the elaphoids, but it is possible that wapitoids survived there during the LGM (Niedziałkowska *et al.*, 2021). There is one red deer *s.l.* fossil record dated to the LGM (25 416 median cal years ago) and found in the southern Urals (Kosintsev & Bachura, 2014; for calibration see: Niedziałkowska *et al.*, 2021), but it was not analysed genetically, so it is unclear to which of the haplogroups it belonged. However, haplotype H230 (haplogroup Y) was found in the Urals and dated to 42 kya and 3 kya, suggesting genetic continuity that, if true, would imply that wapitoid deer were present in the Urals also during the LGM.

Cervus canadensis samples dated to the time period within the LGM (21–22 kya median cal) were found also in north-eastern Siberia and in south-eastern Asia (present-day China; Meiri *et al.*, 2018). The ages of several Asian *C. canadensis* samples analysed in

this study were dated to about 27–28 kya cal, so to the time period just before the LGM. Three of them were found in south-western and central Siberia and two in north-eastern Siberia (Supporting Information, Table S1). Moreover, one sample from south-western Siberia, molecularly dated to the early Holocene (10 657 ya), belonged to haplogroup Y. Since this haplogroup was found in the region before the LGM, and is still found there today, this clade could have been present continuously in southern Siberia for at least 44 000 years. It is possible that the wapitoids survived the LGM not only in present-day China and in the Urals but also in central and south-western Siberia, since the southern ice sheet limit in the western part of Asia was above 70°N latitude (Svendsen *et al.*, 2004). Red deer *s.l.* remains have been continuously found in south-western Siberia (in the Kuznetsk Basin) among the interglacial, interstadial, as well as periglacial faunas since at least the Middle Pleistocene (Foronova, 1999, 2001). A recent study has suggested that Siberia (the Altai-Sayan region) was also an important Late Pleistocene refuge area for brown bears (Anijalg *et al.*, 2018). Notably, during the LGM, the ice cover in Asia was mainly limited to mountain areas (Margold *et al.*, 2016) and large northern areas were covered by tundra and periglacial steppes (Kuzmin, 2008). As for south-western Siberia (the Kuznetsk Basin), this area represented vast steppes and forest-steppes (Foronova, 2001) and was probably available to a diverse fauna of mammals, including such cold-adapted animals as wapitoid deer, but further studies are needed to resolve this issue.

Data on the distribution of the phylogenetic lineages of red deer *s.l.* in Asia during the late glacial are scarce. About 15 kya, *C. canadensis* expanded from north-eastern Asia into North America via the Bering Strait (Meiri *et al.*, 2014). These expanding wapitoid deer belonged to two mtDNA clades (Meiri *et al.*, 2014) and one of them was haplogroup Y (cf. Doan *et al.*, 2018), which, according to our study, had the largest range in Eurasia before the LGM, as far west as Crimea.

According to the fossil record, *C. canadensis* was present in north-eastern Siberia until about 500 years ago (Meiri *et al.*, 2014) and probably in the Urals until the mid-19th century (Niedziałkowska *et al.*, 2021 and references therein). In Asia, the range of *C. candensis* shifted to the east and south over the last centuries. The reason why red deer *s.l.* became extinct in the Urals and large areas of Asia is not clear, but may be related to climate and habitat changes, as discussed in Niedziałkowska *et al.* (2021). The disappearance of red deer *s.l.* from large areas of western Siberia (except its southern part) may be associated with aridification and cooling of the climate during and after the LGM. In the Holocene, the return of red deer *s.l.* did not occur due to intensive waterlogging of large areas of the south-eastern and central parts of the West Siberian Plain (Shpansky, 2018). Today wapitoid deer occur in the mountains of southern Siberia, central and south-eastern Asia (present-day China; Meiri *et al.*, 2018), slowly expanding their range to the north in eastern Siberia as a consequence of global warming (Stepanova, 2010).

Red deer occurring today in central Asia are wapitoids of haplogroups X–Z and *C. hanglu*. The single individual of haplogroup A (belonging to *C. elaphus*) that we found among contemporary red deer in China is probably a result of introduction by humans, as no more individuals of that haplogroup were found in Asia.

Based on the distribution of *C. elaphus* records dated to the LGM and the results of the phylogenetic analysis from this study, as well as environmental niche modelling (Queirós *et al.*, 2019; Niedziałkowska *et al.*, 2021), we reconstructed the possible LGM refuge areas for different elaphoid haplogroups and their post-LGM migration routes in Europe (Fig. 8). Although Doan *et al.* (2018) concluded that the Crimea was not an LGM refugium for the species, according to our study the most common haplotype (H008) of haplogroup C has been present in south-eastern Europe for 47 kyr. The oldest Late Pleistocene samples displaying this haplotype were found in Crimea, while the oldest sample dated to the Holocene (7.2 kya) was discovered in its closest proximity on the southern Ukrainian mainland. Therefore, it seems at least possible that elaphoids were present continuously in this region also during the LGM.

After the LGM new haplogroups such as B, E, W2 and W3 [the latter two described by Doan *et al.* (2018)] were found for the first time. When the climate was getting warmer, *C. elaphus* rapidly recolonized Europe (Fig. 8; Sommer *et al.*, 2008). According to the fossil record, the species reached western, central and north-western Europe, including Scandinavia, by about 10 kya (Niedziałkowska *et al.*, 2021 and references therein). The majority of elaphoid deer recolonizing western, northern and partly central Europe were those of haplogroup A (Skog *et al.*, 2009; Niedziałkowska *et al.*, 2011; Meiri *et al.*, 2013; Fig. 8). The expanding haplogroup A possibly replaced other elaphoid deer haplogroups that could have survived the LGM in western Europe, for instance W2 in the Iberian Peninsula and haplogroup D in the present-day British Isles (Meiri *et al.*, 2013, 2018; Fig. 8). The dominance today of haplogroup A in Europe could be the result of a rapid expansion from south-western Europe to the northern, central and eastern parts of the continent in the wake of the retreat of the ice sheet after the LGM, as indicated by Niedziałkowska *et al.* (2021). Similarly, Borowski *et al.* (2016) explained the dominance of haplogroup A in Poland as an effect

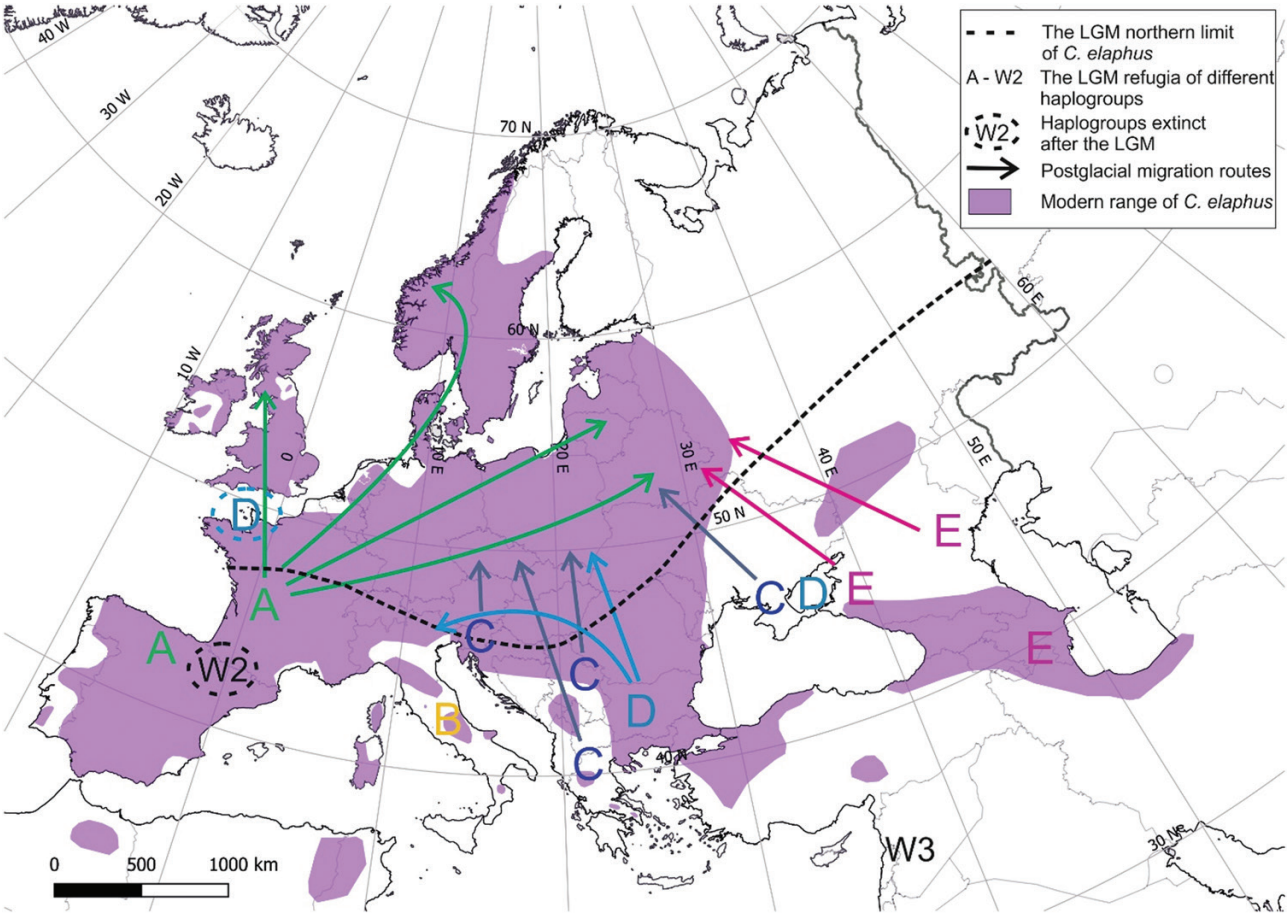

Figure 8. Potential refugial areas of different mtDNA haplogroups of *Cervus elaphus* and their post-LGM migration routes hypothesised based on the results of this study. The northern limit of the range of *C. elaphus* during the LGM is determined based on the study of Niedziałkowska *et al.* (2021). *C. elaphus* records are listed in the Supporting Information, Tables S1, S2.

of density blocking of haplogroup C by this rapidly expanding haplogroup from south-western refugia.

The south-east and parts of central Europe were mostly recolonized by haplogroup C (Fig. 8). Other samples from the eastern and south-eastern parts of Europe dated to the late glacial and Holocene periods belonged to haplogroups D and E, which together are sister to the other haplogroups found in *C. elaphus*. Similar to the situation in the British Isles (Meiri *et al.*, 2013), haplogroup D was replaced also in eastern Europe, this time by the expanding haplogroup C. Today, D is a relict haplogroup that is only found in the Carpathians (southern Poland) and the Mesola Forest in Italy (Borowski *et al.*, 2016; Doan *et al.*, 2017, 2018; this study). After the LGM, haplogroup D also occurred in areas where the closely related haplogroup E was found, e.g. in the Crimea and present-day southern Russia and the Caucasus. Extant elaphoid deer of haplogroup E were described as the subspecies *C. elaphus maral* Gray, 1850 and differ morphologically from western European *C. elaphus* (e.g. from haplogroup A and C; Meiri *et al.*, 2018). Some authors also include red deer of haplogroup D into the maral subspecies (*C. e. maral*), based on their morphology and size [see Meiri *et al.* (2018); except for the Mesola deer, which have been described as a separate subspecies: Zachos *et al.* (2014)]. Maral subfossils dated to the Holocene were described in north-eastern Romania by Croitor & Cojocaru (2016). Today haplogroup E occurs in eastern and south-eastern Europe, in Asia Minor and Iran (Meiri *et al.*, 2018). According to genetic data, this haplogroup is also closely related to the extinct haplogroup W2, which was found in Spain just after the LGM and was hypothesized to be the *C. elaphus* lineage which came to Europe in one of the earliest migration waves from Asia (Meiri *et al.*, 2013; Doan *et al.*, 2018). Therefore, haplogroup E could have been present in eastern Europe/western Asia continuously for much longer and survived the LGM there, although, in our study, it was found only in post-LGM times (Fig. 8, see also: Meiri *et al.*, 2018).

Haplogroup B – the clade most closely related to the Caucasian sequences from the W8 haplogroup, which occurred there before the LGM (Doan *et al.*, 2018) – had the smallest distribution range in comparison to other post-LGM haplogroups of *C. elaphus* in Europe. It was restricted to mainland Italy (now extinct there), the Tyrrhenian islands (Sardinia and Corsica) and North Africa, and single individuals elsewhere (the Alps, Hungary and Bulgaria). No ancient deer carrying B haplotypes have been found outside the Apennine Peninsula, and the populations on the Tyrrhenian islands (classified as *C. e. corsicanus* Erxleben, 1777) and in North Africa (*C. e. barbarus* Bennett, 1833) are descendants of animals introduced by humans (Doan *et al.*, 2017). In other words, haplogroup B is the Italian LGM refugial lineage that today only survives *ex situ*.

After the LGM, the environmental conditions changed significantly, and different genetic clades of red deer *s.l.* occurred and dominated in Eurasia. Results of several palaeontological studies have indicated that red deer *s.l.* body size was larger during colder periods than in warmer times (Stefaniak, 2015; Meiri *et al.*, 2018; Musil, 2018; Stefaniak *et al.*, 2020). The stags of maral red deer are among the largest *C. elaphus* in Europe (Geist, 1999). We do not know if they are better adapted to colder or more continental conditions than the majority of elaphoid deer found in Europe today, but this could be an explanation why haplogroup D disappeared from large parts of their range in times of climatic warming. However, this cannot be claimed with any certainty, as *C. elaphus* of significantly different body sizes are described even in the same modern populations of elaphoids (Borowik & Jędrzejewska, 2017; Fløjgaard *et al.*, 2017). Moreover, marals today inhabit one of the warmest regions of eastern Europe and western Asia, so further genomic studies are needed to test the hypothesis of their being adapted to colder or more continental climatic conditions.

CONCLUSIONS

The combination of ancient and modern red deer *s.l.* mtDNA sequences helps us to obtain a more highly resolved picture of the phylogeographic pattern of red deer *s.l.* in Eurasia during the last 50 000 years. For example, the inclusion of ancient DNA enabled us to detect extinct haplogroups no longer present in extant populations. Our divergence estimates are younger than in other studies (95% HPD of MRCA: 114.3–795 kya), but the mean age of the elaphoid/wapitoid split, calculated at *c.* 374 kya, agrees with the age of the earliest fossils of coronate *C. elaphus* in Europe (*c.* 400 kya). The divergence time of *C. elaphus* and *C. canadensis* estimated, including a fossil calibration (1.37 Mya, 95% HPD: 0.88–2.32 Mya), has a better fit with the age of the oldest fossil records of the species found in Europe (*c.* 0.9–1.0 Mya) and divergence time between these red deer lineages obtained in previous studies.

The distribution range of red deer *s.l.* and their lineages oscillated not only in a north–south direction but also changed in an east–west direction. Some haplogroups or haplotypes dated to the pre-LGM period were no longer detected after it. It is possible that these individuals represented populations adapted to different environmental conditions than those present after the LGM. To definitively decide whether it was differential adaptation or genetic drift that produced this pattern, adaptive genomic studies need to be carried out on both ancient and modern red deer *s.l.*

The analyses of a large number of ancient red deer *s.l.* samples allowed us to confirm with high probability their presence in south-eastern Europe and western Asia during the LGM. After the LGM, the range of *C. elaphus* expanded towards the north in Europe, while *C. canadensis* moved to the south and east in Asia, as compared to pre-LGM periods. The changes in range of elaphoids were significantly different from the shift in distribution of wapitoids, as the environmental conditions occurring on both continents varied substantially during the last 50 000 years. Although red deer *s.l.* have extensively and repeatedly been translocated in the past by humans, it seems that its contemporary phylogeographic pattern in Eurasia is mostly an effect of natural processes, a response of the species to Quaternary environmental changes and replacement of some mtDNA lineages by other expanding mtDNA lineages.

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REFERENCES

Alexeeva EV. 1980. *Mkelopitayuschie pleistocena yugo-vostoka Zapadnoi Sibiri.* Moscow: Nauka, 187 [in Russian].

Altschul SF, **Madden TL**, **Schäffer AA**, **Zhang J**, **Zhang Z**, **Miller W**, **Lipman DJ. 1997.** Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25:** 3389–3402.

Anijalg P, **Ho SY**, **Davison J**, **Keis M**, **Tammeleht E**, **Bobowik K**, **Tumanov IL**, **Saveljev AP**, **Lyapunova EA**, **Vorobiev AA. 2018.** Large-scale migrations of brown bears in Eurasia and to North America during the Late Pleistocene. *Journal of Biogeography* **45:** 394–405.

Baca M, **Nadachowski A**, **Lipecki G**, **Mackiewicz P**, **Marciszak A**, **Popović D**, **Socha P**, **Stefaniak K**, **Wojtal P. 2017.** Impact of climatic changes in the Late Pleistocene on migrations and extinction of mammals in Europe: four case studies. *Geological Quarterly* **61:** 291–304.

Baele G, **Li WLS**, **Drummond AJ**, **Suchard MA**, **Lemey P. 2013.** Accurate model selection of relaxed molecular clocks in Bayesian phylogenetics. *Molecular Biology and Evolution* **30:** 239–243.

Borowik T, Jędrzejewska B. 2017. Heavier females produce more sons in a low-density population of red deer. *Journal of Zoology* **302**: 57–62.

Borowski Z, **Swislocka M**, **Matosiuk M**, **Mirski P**, **Krysiuk K**, **Czajkowska M**, **Borkowska A**, **Ratkiewicz M. 2016.** Purifying selection, density blocking and unnoticed mitochondrial DNA diversity in the red deer, *Cervus elaphus*. *PLoS One* **11:** e0163191.

Bronk Ramsey CB. 2009. Bayesian analysis of radiocarbon dates. *Radiocarbon* **51:** 337–360.

Carpio AJ, **Apollonio M**, **Acevedo P. 2021.** Wild ungulate overabundance in Europe: contexts, causes, monitoring and management recommendations. *Mammal Review* **51:** 95–108.

Chen L, **Qiu Q**, **Jiang Y**, **Wang K**, **Lin Z**, **Li Z**, **Bibi F**, **Yang Y**, **Wang J**, **Nie W**, **Su W**, **Liu G**, **Li Q**, **Fu W**, **Pan X**, **Liu C**, **Yang J**, **Zhang C**, **Yin Y**, **Wang Y**, **Zhao Y**, **Zhang C**, **Wang Z**, **Qin Y**, **Liu W**, **Wang B**, **Ren Y**, **Zhang R**, **Zeng Y**, **da Fonseca RR**, **Wei B**, **Li R**, **Wan W**, **Zhao R**, **Zhu W**, **Wang Y**, **Duan S**, **Gao Y**, **Zhang YE**, **Chen C**, **Hvilsom C**, **Epps CW**, **Chemnick LG**, **Dong Y**, **Mirarab S**, **Siegismund HR**, **Ryder OA**, **Gilbert MTP**, **Lewin HA**, **Zhang G**, **Heller R**, **Wang W. 2019.** Large-scale ruminant genome sequencing provides insights into their evolution and distinct traits. *Science* **364:** eaav6202.

Clark PU, **Dyke AS**, **Shakun JD**, **Carlson AE**, **Clark J**, **Wohlfarth B**, **Mitrovica JX**, **Hostetler SW**, **McCabe AM. 2009.** The Last Glacial Maximum. *Science (New York, N.Y.)* **325:** 710–714.

Cooper A, **Turney C**, **Hughen KA**, **Brook BW**, **McDonald HG**, **Bradshaw CJA. 2015.** Abrupt warming events drove Late Pleistocene Holarctic megafaunal turnover. *Science* **349:** 602–606.

Croitor R. 2020. A new form of wapiti *Cervus canadensis* Erxleben, 1777 (Cervidae, Mammalia) from the Late Pleistocene of France. *Palaeoworld* **29:** 789–806.

Croitor R, **Cojocaru I. 2016.** An antlered skull of a subfossil red deer, *Cervus elaphus* L., 1758 (Mammalia: Cervidae), from Eastern Romania. *Acta Zoologica Bulgarica* **68:** 407–414.

Croitor R, **Obada T. 2018.** On the presence of Late Pleistocene wapiti, *Cervus canadensis* Erxleben, 1777 (Cervidae, Iklantinalia) in the Palaeolithic site Climauti II (Moldova). *Contributions to Zoology* **87:** 1–10.

Dabney J, **Knapp M**, **Glocke I**, **Gansauge M-T**, **Weihmann A**, **Nickel B**, **Valdiosera C**, **García N**, **Pääbo S**, **Arsuaga J-L**, **Meyer M. 2013.** Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proceedings of the National Academy of Sciences of the USA* **110:** 15758–15763.

Doan K, **Zachos FE**, **Wilkens B**, **Vigne J-D**, **Piotrowska N**, **Stanković A**, **Jędrzejewska M. 2017.** Phylogeography of the Tyrrhenian red deer (*Cervus elaphus corsicanus*) resolved using ancient DNA of radiocarbon-dated subfossils. *Scientific Reports* **7:** 2331.

Doan K, **Mackiewicz P**, **Sandoval-Castellanos E**, **Stefaniak K**, **Ridush B**, **Dalen L**, **Weglenski P**, **Stankovic A. 2018.** The history of the Crimean red deer population and *Cervus* phylogeography in Eurasia. *Zoological Journal of the Linnean Society* **183:** 208–225.

Ferreira MAR, **Suchard MA. 2008.** Bayesian analysis of elapsed times in continuous-time Markov chains. *Canadian Journal of Statistics* **36:** 355–368.

Firth C, **Kitchen A**, **Shapiro B**, **Suchard MA**, **Holmes EC. 2010.** Using time-structured data to estimate evolutionary

rates of double-stranded DNA viruses. *Molecular Biology and Evolution* **27:** 2038–2051.

Fløjgaard C, **De Barba M**, **Taberlet P**, **Ejrnæs R. 2017.** Body condition, diet and ecosystem function of red deer (*Cervus elaphus*) in a fenced nature reserve. *Global Ecology and Conservation* **11:** 312–323.

Foronova IV. 1999. Quaternary mammals and stratigraphy of the Kuznetsk Basin (southwestern Siberia). *Antropozoikum «Quaternary of Siberia»* **23:** 71–98.

Foronova IV. 2001. *Quaternary mammals of the south-east of Western Siberia (Kuznetsk Basin): phylogeny, biostratigraphy, and palaeoecology.* Novosibirsk: Publishing House of Siberian Branch, Russian Academy of Sciences, 243 [in Russian].

Fyfe RM, **Woodbridge J**, **Roberts N. 2015.** From forest to farmland: pollen-inferred land cover change across Europe using the pseudobiomization approach. *Global Change Biology* **21:** 1197–1212.

Geist V. 1999. *Deer of the world: their evolution, behaviour and ecology*. Mechanicsburg: Stackpole Books.

Gromov VI. 1948. *Paleontologitcheskoe i arkheologitcheskoe obosnovanie stratigrafii kontinental'nykh otlozheniy chetvertichnogo perioda na territorii SSSR (mlekopitayuschie, paleolit). Trudy Instituta geologicheskikh nauk, vol. 64, geologicheskaya seriya, 17.* Moscow: Izdatel'stvo Akademii Nauk SSSR, 524 [in Russian].

Hartl GB, **Zachos F**, **Nadlinger K. 2003.** Genetic diversity in European red deer (*Cervus elaphus* L.): anthropogenic influences on natural populations. *Comptes Rendus Biologies* **326:** 37–42.

Hewitt MH. 1996. Some genetic consequences of ice ages, and their role, in divergence and speciation. *Biological Journal of the Linnean Society* **58:** 247–276.

Hewitt G. 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405:** 907–913.

Ho SYW, **Lanfear R**, **Phillips MJ**, **Barnes I**, **Thomas JA**, **Kolokotronis SO**, **Shapiro B. 2011.** Bayesian estimation of substitution rates from ancient DNA sequences with low information content. *Systematic Biology* **60:** 366–375.

Hofreiter M, **Serre D**, **Rohland N**, **Rabeder G**, **Nagel D**, **Conard N**, **Munzel S**, **Pääbo S. 2004.** Lack of phylogeography in European mammals before the last glaciation. *Proceedings of the National Academy of Sciences of the USA* **101:** 12963–12968.

Hu PF, **Shao YC**, **Xu JP**, **Wang TJ**, **Li YQ**, **Liu HM**, **Rong M**, **Su WL**, **Chen BX**, **Cui SH**, **Cui XZ**, **Yang FH**, **Tamate H**, **Xing XM. 2019.** Genome-wide study on genetic diversity and phylogeny of five species in the genus *Cervus*. *BMC Genomics* **20:** 384.

Kahlke R-D, **García N**, **Kostopoulos DS**, **Lacombat F**, **Lister AM**, **Mazza PPA**, **Spassov N**, **Titov VV. 2011.** Western Palaearctic palaeoenvironmental conditions during the Early and early Middle Pleistocene inferred from large mammal communities, and implications for hominin dispersal in Europe. *Quaternary Science Reviews* **30:** 1368–1395.

Katoh K, **Standley DM. 2013.** MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30:** 772–780.

Katoh K, **Rozewicki J**, **Yamada KD. 2019.** MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* **20:** 1160–1166.

Kosintsev PA, **Bachura OP. 2014.** Formation of recent ranges of mammals in the Urals during the Holocene. *Biology Bulletin* **41:** 629–637.

Kuzmin YV. 2008. Siberia at the Last Glacial Maximum: environment and archaeology. *Journal of Archaeological Research* **16:** 163–221.

Leigh JW, **Bryant D. 2015.** Popart: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* **6:** 1110–1116.

Lister AM. 1986. New results on deer from Swanscombe, and the stratigraphical significance of deer remains in the Middle and Upper Pleistocene of Europe. *Journal of Archaeological Science* **13:** 319–338.

Lister AM, **Parfitt SA**, **Owen FJ**, **Collinge SE**, **Breda M. 2010.** Metric analysis of ungulate mammals in the early Middle Pleistocene of Britain, in relation to taxonomy and biostratigraphy II: Cervidae, Equidae and Suidae. *Quaternary International* **228:** 157–179.

Lorenzini R, **Garofalo L. 2015.** Insights into the evolutionary history of *Cervus* (Cervidae, tribe Cervini) based on Bayesian analysis of mitochondrial marker sequences, with first indications for a new species. *Journal of Zoological Systematics and Evolutionary Research* **53:** 340–349.

Ludt CJ, **Schroeder W**, **Rottmann O**, **Kuehn R. 2004.** Mitochondrial DNA phylogeography of red deer (*Cervus elaphus*). *Molecular Phylogenetics and Evolution* **31:** 1064–1083.

Malikov DG, **Shpansky AV**, **Svyatko SV. 2020.** New data on distribution of musk ox *Ovibos moschatus* in the Late Neopleistocene in the south-east of Western Siberia and the Minusinsk Depression. *Russian Journal of Theriology* **19:** 183–192.

Margold M, **Jansen JD**, **Gurinov AL**, **Codilean AT**, **Fink D**, **Preusser F**, **Reznichenko NV**, **Mifsud C. 2016.** Extensive glaciation in Transbaikalia, Siberia, at the Last Glacial Maximum. *Quaternary Science Reviews* **132:** 161–174.

Markova AK, **Vislobokova IA. 2016.** Mammal faunas in Europe at the end of the Early–Beginning of the Middle Pleistocene. *Quaternary International* **420:** 363–377.

Masella AP, **Bartram AK**, **Truszkowski JM**, **Brown DG**, **Neufeld JD. 2012.** PANDAseq: paired-end assembler for illumina sequences. *BMC Bioinformatics* **13:** 31.

Matosiuk M, **Borkowska A**, **Świsłocka M**, **Mirski P**, **Borowski Z**, **Krysiuk K**, **Danilkin AA**, **Zvychaynaya EY**, **Saveljev AP**, **Ratkiewicz M. 2014.** Unexpected population genetic structure of European roe deer in Poland: an invasion of the mtDNA genome from Siberian roe deer. *Molecular Ecology* **23:** 2559–2572.

Meiri M, **Lister AM**, **Higham TFG**, **Stewart JR**, **Straus LG**, **Obermaier H**, **Morales MRG**, **Marin-Arroyo AB**, **Barnes I. 2013.** Late-glacial recolonization and phylogeography of European red deer (*Cervus elaphus* L.). *Molecular Ecology* **22:** 4711–4722.

Meiri M, **Lister AM**, **Collins MJ**, **Tuross N**, **Goebel T**, **Blockley S**, **Zazula GD**, **van Doorn N**, **Dale Guthrie R**, **Boeskorov GG**, **Baryshnikov GF**, **Sher A**, **Barnes I. 2014.** Faunal record identifies Bering isthmus conditions as

constraint to end-Pleistocene migration to the New World. *Proceedings of the Royal Society B: Biological Sciences* **281:** 20132167.

Meiri M, **Kosintsev P**, **Conroy K**, **Meiri S**, **Barnes I**, **Lister A. 2018.** Subspecies dynamics in space and time: a study of the red deer complex using ancient and modern DNA and morphology. *Journal of Biogeography* **45:** 367–380.

Meyer M, **Kircher M. 2010.** Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harbor Protocols* **2010:** pdb.prot5448.

Musil R. 2018. Phenological analysis of the Last Glacial vertebrates from the territory of Moravia (the Czech Republic) – continuity and change in faunistic communities. *Fossil Imprint* **74:** 199–236.

Nadachowski A, **Lipecki G**, **Ratajczak U**, **Stefaniak K**, **Wojtal P. 2016.** Dispersal events of the saiga antelope (*Saiga tatarica*) in Central Europe in response to the climatic fluctuations in MIS 2 and the early part of MIS 1. *Quaternary International* **420:** 357–362.

Niedziałkowska M, **Jędrzejewska B**, **Honnen AC**, **Otto T**, **Sidorovich VE**, **Perzanowski K**, **Skog A**, **Hartl GB**, **Borowik T**, **Bunevich AN**, **Lang J**, **Zachos FE. 2011.** Molecular biogeography of red deer *Cervus elaphus* from eastern Europe: insights from mitochondrial DNA sequences. *Acta Theriologica* **56:** 1–12.

Niedziałkowska M, **Jędrzejewska B**, **Wójcik JM**, **Goodman SJ. 2012.** Genetic structure of red deer population in northeastern Poland in relation to the history of human interventions. *Journal of Wildlife Management* **76:** 1264–1276.

Niedziałkowska M, **Doan K**, **Górny M**, **Sykut M**, **Stefaniak K**, **Piotrowska N**, **Jędrzejewska B**, **Ridush B**, **Pawełczyk S**, **Mackiewicz P**, **Schmölcke U**, **Kosintsev P**, **Makowiecki D**, **Charniauski M**, **Krasnodębski D**, **Rannamäe E**, **Saarma U**, **Arakelyan M**, **Manaseryan N**, **Titov VV**, **Hulva P**, **Bălăşescu A**, **Fyfe R**, **Woodbridge J**, **Trantalidou K**, **Dimitrijević V**, **Kovalchuk O**, **Wilczyński J**, **Obadă T**, **Lipecki G**, **Arabey A**, **Stanković A. 2021.** Winter temperature and forest cover have shaped red deer distribution in Europe and the Ural Mountains since the Late Pleistocene. *Journal of Biogeography* **48:** 147–159.

Nielsen K, **Olesen E**, **Pertoldi C. 2008.** Genetic structure of the Danish red deer (*Cervus elaphus*). *Biological Journal of the Linnean Society* **95:** 688–701.

Nitychoruk J, **Bińka K**, **Ruppert H**, **Schneider J. 2006.** Holsteinian interglacial = marine isotope stage 11? *Quaternary Science Reviews* **25:** 2678–2681.

Palkopoulou E, **Baca M**, **Abramson NI**, **Sablin M**, **Socha P**, **Nadachowski A**, **Prost S**, **Germonpré M**, **Kosintsev P**, **Smirnov NG**, **Vartanyan S**, **Ponomarev D**, **Nyström J**, **Nikolskiy P**, **Jass CN**, **Litvinov YN**, **Kalthoff DC**, **Grigoriev S**, **Fadeeva T**, **Douka A**, **Higham TF**, **Ersmark E**, **Pitulko V**, **Pavlova E**, **Stewart JR**, **Węgleński P**, **Stankovic A**, **Dalén L. 2016.** Synchronous genetic turnovers across Western Eurasia in Late Pleistocene collared lemmings. *Global Change Biology* **22:** 1710–1721.

Past Interglacials Working Group of PAGES. 2016. Interglacials of the last 800 000 years. *Reviews of Geophysics* **54:** 162–219.

Penkman KEH, **Preece RC**, **Bridgland DR**, **Keen DH**, **Meijer T**, **Parfitt SA**, **White TS**, **Collins MJ. 2013.** An aminostratigraphy for the British Quaternary based on Bithynia opercula. *Quaternary Science Reviews* **61:** 111–134.

Petit JR, **Jouzel J**, **Raynaud D**, **Barkov NI**, **Barnola JM**, **Basile I**, **Bender M**, **Chappellaz J**, **Davis M**, **Delaygue G**, **Delmotte M**, **Kotlyakov VM**, **Legrand M**, **Lipenkov VY**, **Lorius C**, **Pépin L**, **Ritz C**, **Saltzman E**, **Stievenard M. 1999.** Climate and atmospheric history of the past 420 000 years from the Vostok ice core, Antarctica. *Nature* **399:** 429–436.

Piotrowska N, **Tomczyk J**, **Pawełczyk S**, **Stanaszek ŁM. 2019.** Radiocarbon AMS dating of Mesolithic human remains from Poland. *Radiocarbon* **61:** 991–1007.

Polziehn RO, **Strobeck C. 2002.** A phylogenetic comparison of red deer and wapiti using mitochondrial DNA. *Molecular Phylogenetics and Evolution* **22:** 342–356.

Posada D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25:** 1253–1256.

Queirós J, **Acevedo P**, **Santos JPV**, **Barasona J**, **Beltran-Beck B**, **González-Barrio D**, **Armenteros JA**, **Diez-Delgado I**, **Boadella M**, **Fernandéz de Mera I**, **Ruiz-Fons JF**, **Vicente J**, **de la Fuente J**, **Gortázar C**, **Searle JB**, **Alves PC. 2019.** Red deer in Iberia: Molecular ecological studies in a southern refugium and inferences on European postglacial colonization history. *PLoS One* **14:** e0210282.

Rambaut A, **Drummond AJ**, **Suchard M. 2014.** Tracer v.1.6. Software available at: http://beast. bio. ed. ac.uk. Accessed 30 September 2016.

Ratajczak U, **Shpansky AV**, **Malikov DG**, **Stefaniak K**, **Nadachowski A**, **Wojtal P**, **Ridush B**, **Krakhmalnaya TV**, **Stepanchuk V**, **Mackiewicz P. 2016.** Quaternary skulls of the saiga antelope from eastern Europe and Siberia: *Saiga borealis* versus *Saiga tatarica* – one species or two? *Quaternary International* **420:** 329–347.

Reimer PJ, **Bard E**, **Bayliss A**, **Beck JW**, **Blackwell PG**, **Ramsey CB**, **Buck CE**, **Cheng H**, **Edwards RL**, **Friedrich M**, **Grootes PM**, **Guilderson TP**, **Haflidason H**, **Hajdas I**, **Hatté C**, **Heaton TJ**, **Hoffmann DL**, **Hogg AG**, **Hughen KA**, **Kaiser KF**, **Kromer B**, **Manning SW**, **Niu M**, **Reimer RW**, **Richards DA**, **Scott EM**, **Southon JR**, **Staff RA**, **Turney CSM**, **Van der Plicht J. 2013.** IntCal13 and Marine13 radiocarbon age calibration curves 0–50 000 Years cal BP. *Radiocarbon* **55:** 1869–1887.

Ronquist F, **Huelsenbeck JP. 2003.** MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Schloss PD, **Westcott SL**, **Ryabin T**, **Hall JR**, **Hartmann M**, **Hollister EB**, **Lesniewski RA**, **Oakley BB**, **Parks DH**, **Robinson CJ**, **Sahl JW**, **Stres B**, **Thallinger GG**, **Van Horn DJ**, **Weber CF. 2009.** Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75:** 7537–7541.

Schreve DC, **Bridgland DR. 2002.** Correlation of English and German Middle Pleistocene fluvial sequences based on mammalian biostratigraphy. *Geologie en Mijnbouw / Netherlands Journal of Geosciences* **81:** 357–373.

Shapiro B, **Ho SY**, **Drummond AJ**, **Suchard MA**, **Pybus OG**, **Rambaut A. 2011.** A Bayesian phylogenetic method to estimate unknown sequence ages. *Molecular Biology and Evolution* **28:** 879–887.

Shpansky AV. 2018. *Quaternary large mammals of the West Siberian Plain: habitat conditions and stratigraphic significance.* Unpublished D. Phil. Thesis, Tomsk State University. Tomsk, Russia.

Skog A, **Zachos FE**, **Rueness EK**, **Feulner PGD**, **Mysterud A**, **Langvatn R**, **Lorenzini R**, **Hmwe SS**, **Lehoczky I**, **Hartl GB**, **Stenseth NC**, **Jakobsen KS. 2009.** Phylogeography of red deer (*Cervus elaphus*) in Europe. *Journal of Biogeography* **36:** 66–77.

Sommer RS, **Zachos FE**, **Street M**, **Joris O**, **Skog A**, **Benecke N. 2008.** Late Quaternary distribution dynamics and phylogeography of the red deer (*Cervus elaphus*) in Europe. *Quaternary Science Reviews* **27:** 714–733.

Stanković A, **Doan K**, **Mackiewicz P**, **Ridush B**, **Baca M**, **Gromadka R**, **Socha P**, **Weglenski P**, **Nadachowski A**, **Stefaniak K. 2011.** First ancient DNA sequences of the Late Pleistocene red deer (*Cervus elaphus*) from the Crimea, Ukraine. *Quaternary International* **245:** 262–267.

Stanton DWG, **Mulville JA**, **Bruford MW. 2016.** Colonization of the Scottish islands via long-distance Neolithic transport of red deer (Cervus elaphus). *Proceedings of the Royal Society B: Biological Sciences* **283:** 20160095.

Stefaniak K. 2015. *Neogene and quaternary cervidae from Poland*. Habilitation thesis, Kraków, Poland: Institute of Systematics and Evolution of Animals Polish Academy of Sciences.

Stefaniak K, **Ratajczak U**, **Kotowski A**, **Kozłowska M**, **Mackiewicz P. 2020.** Polish Pliocene and Quaternary deer and their biochronological implications. *Quaternary International* **546:** 64–83.

Stefaniak K, **Lipecki G**, **Nadachowski A**, **Semba A**, **Ratajczak U**, **Kotowski A**, **Roblíčková M**, **Wojtal P**, **Shpansky AV**, **Malikov DG**, **Krakhmalnaya TV**, **Kovalchuk OM**, **Boeskorov GG**, **Nikolskiy PA**, **Baryshnikov GF**, **Ridush B**, **Jakubowski G**, **Pawłowska K**, **Cyrek K**, **Sudoł-Procyk M**, **Czyżewski Ł**, **Krajcarz M**, **Krajcarz MT**, **Żeromska A**, **Gagat P**, **Mackiewicz P. 2021.** Diversity of muskox *Ovibos moschatus* (Zimmerman, 1780) (Bovidae, Mammalia) in time and space based on cranial morphometry. *Historical Biology* **33:** 62–77.

Stepanova VV. 2010. Expansion of geographic range of red deer in Yakutia. *Russian Journal of Biological Invasions* **1:** 30–36.

Stewart JR, **Lister AM**, **Barnes I**, **Dalén L. 2010.** Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological Sciences* **277:** 661–671.

Stiller M, **Knapp M**, **Stenzel U**, **Hofreiter M. 2009.** Direct multiplex sequencing (DMPS): a novel method for targeted high-throughput sequencing of ancient and highly degraded DNA. *Genome Research* **19:** 1843–1848.

Strani F. 2020. Impact of Early and Middle Pleistocene major climatic events on the palaeoecology of Southern European ungulates. *Historical Biology* doi:10.1080/08912963.2020.1782898.

Suchard MA, **Lemey P**, **Baele G**, **Ayres DL**, **Drummond AJ**, **Rambaut A. 2018.** Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution* **4:** vey016.

Svendsen JI, **Alexanderson H**, **Astakhov VI**, **Demidov I**, **Dowdeswell JA**, **Funder S**, **Gataullin V**, **Henriksen M**, **Hjort C**, **Houmark-Nielsen M**, **Hubberten HW**, **Ingólfsson Ó**, **Jakobsson M**, **Kjær KH**, **Larsen E**, **Lokrantz H**, **Lunkka JP**, **Lysså A**, **Mangerud J**, **Matiouchkov A**, **Murray A**, **Möller P**, **Niessen F**, **Nikolskaya O**, **Polyak L**, **Saarnisto M**, **Siegert C**, **Siegert MJ**, **Spielhagen RF**, **Stein R. 2004.** Late Quaternary ice sheet history of northern Eurasia. *Quaternary Science Reviews* **23:** 1229–1271.

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

Van der Made J, **Dimetrijević V. 2015.** *Eucladoceros montenegrensis* n. sp. and other Cervidae from the Lower Pleistocene of Trlica (Montenegro). *Quaternary International* **389:** 90–118.

Van der Made J, **Stefaniak K**, **Marciszak A. 2014.** The Polish fossil record of the wolf *Canis* and the deer *Alces*, *Capreolus*, *Megaloceros*, *Dama* and *Cervus* in an evolutionary perspective. *Quaternary International* **326–327:** 406–430.

Van der Made J, **Rosell J**, **Blasco R. 2017.** Faunas from Atapuerca at the EarlyMiddle Pleistocene limit: the ungulates from level TD8 in the context of climatic change. *Quaternary International* **433:** 296–346.

Wang K, **Lenstra JA**, **Liu L**, **Hu Q**, **Ma T**, **Qiu Q**, **Liu J. 2018.** Incomplete lineage sorting rather than hybridization explains the inconsistent phylogeny of the wisent. *Communications Biology* **1:** 169.

Zachos FE, **Mattioli S**, **Ferretti F**, **Lorenzini R. 2014.** The unique Mesola red deer of Italy: taxonomic recognition (*Cervus elaphus italicus* nova ssp., Cervidae) would endorse conservation. *Italian Journal of Zoology* **81:** 136–143.

Zachos FE, **Frantz AC**, **Kuehn R**, **Bertouille S**, **Colyn M**, **Niedzialkowska M**, **Perez-Gonzalez J**, **Skog A**, **Sprem N**, **Flamand MC. 2016.** Genetic structure and effective population sizes in European red deer (*Cervus elaphus*) at a continental scale: insights from microsatellite DNA. *Journal of Heredity* **107:** 318–326.

SUPPORTING INFORMATION

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

Table S1. List of analysed ancient and modern samples.
Table S2. List of sequences previously published or obtained from other studies that we used in our analyses.
Molecular dating and Table S3.
Table S4. Differences in haplotype positions on the MrBayes and BEAST trees.
Phylogenetic analysis with reduced dataset and Fig S1.
Divergence-time estimation with internal node fossil calibration and Fig S2.
Effective population size reconstruction and Fig S3.