



Phylogeography of the Siberian roe deer in Eurasia and origin of the Siberian MtDNA lineage in European roe deer populations

Kamila Plis¹ · Magdalena Niedziałkowska¹ · Aleksey Danilkin² · Marina Kholodova² · Elena Zvychnayaya² · Nadezhda Kashinina² · Aleksey Bunevich³ · Innokentiy Okhlopkov⁴ · Nikolay Mamaev⁴ · Szilvia Kusza⁵ · Ladislav Paule⁶ · Rauno Veeroja⁷ · Algimantas Paulauskas⁸ · Ana-Maria Krapal⁹ · Juha Tiainen^{10,11} · Bogumiła Jędrzejewska¹

Received: 5 February 2024 / Accepted: 2 September 2025 / Published online: 7 October 2025
© The Author(s) 2025

Abstract

The Siberian roe deer (*Capreolus pygargus*) is distributed throughout continental Asia. In Eastern Europe, individuals of the European roe deer species (*Capreolus capreolus*) displaying mitochondrial DNA (mtDNA) of *C. pygargus* have been recorded. However, it is not clear if the origin of this introgression is an effect of natural processes or a consequence of human-mediated translocation of the Siberian roe deer. Only a large-scale phylogeographic analysis, spanning both the natural range of *C. pygargus* and the introgression zone, can answer this question. To reveal the cause of the introgression and to provide the most comprehensive picture of Siberian roe deer phylogeography, we analysed the mtDNA control region fragment (610 bp) of 352 individuals combined with 132 sequences available in GenBank, covering a vast region extending from Eastern Europe to Eastern Asia. We detected 101 mtDNA haplotypes and 6 haplogroups. The proportion of different haplogroups, varying along longitudinal gradients, showed a notable shift in the central parts of Siberia. Haplogroups B and D were the most frequent in the introgression zone. Seven genetic clusters of Siberian roe deer, including two in the introgression zone, were detected. MtDNA diversity of Siberian roe deer proved to be greater than previously documented. Two genetically and evolutionarily distinct haplogroups, occurring predominantly in Europe, appear to be remnants of natural interbreeding between the two roe deer species that could have occurred during one hundred thousand years ago in at least two different time periods. Additionally, past human-mediated translocations of the Siberian roe deer contributed to the observed introgression.

Keywords Capreolus pygargus · Mitochondrial DNA · Genetic diversity · Siberian lineage · Introgression · Translocations

Communicated by Astrid V. Stronen

✉ Kamila Plis
kplis@ibs.bialowieza.pl

✉ Magdalena Niedziałkowska
mniedz@ibs.bialowieza.pl

¹ Mammal Research Institute, Polish Academy of Sciences, Stoczek 1, Białowieża 17-230, Poland

² A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii prosp. 33, Moscow 119071, Russia

³ State National Park Belovezhskaya Pushcha, Kamenyuki 225063, Republic of Belarus

⁴ Institute of Biological Problems of Cryolithozone, Siberian Branch of Russian Academy of Sciences, Yakutsk 677000, Russia

⁵ Centre for Agricultural Genomics and Biotechnology, University of Debrecen, Egyetem tér 1, Debrecen 4032, Hungary

⁶ Department of Phytology, Technical University in Zvolen, T. G. Masaryka 24, Zvolen 960 01, Slovak Republic

⁷ Department of Wildlife Monitoring, Estonian Environment Agency, Mustamäe tee 33, Tallin 10616, Estonia

⁸ Department of Biology, Vytautas Magnus University, Vileikos g. 8-802, Kaunas 44404, Lithuania

⁹ “Grigore Antipa” National Museum of Natural History, Șoseaua Pavel D. Kiseleff 1, Bucharest 011341, Romania

¹⁰ Natural Resources Institute Finland (LUKE), Latokartanonkaari 9, Helsinki 00790, Finland

¹¹ Lammi Biological Station, University of Helsinki, Pääjärventie 320, Lammi 16900, Finland

Introduction

The roe deer, *sensu lato* (*Capreolus* Gray, 1821), is one of the most widespread meso-mammals in *Cervidae*, consisting of two sister species: the European roe deer (*C. capreolus* Linnaeus, 1758) and the Siberian roe deer (*C. pygargus* Pallas, 1771). They are primarily distinguished by differences in morphology, karyotype, and geographic distribution. The Siberian roe deer has a larger body mass (32 to 49 kg) than the European species (18 to 32 kg) (Danilkin 1995). Siberian males also have larger antlers (>27 cm in length with a span between tips of main beams 17–20 cm) than the European ones (length 17–26 cm and span 7–14 cm) (Danilkin 1995). These morphological differences may influence interspecies interactions and the potential for hybridization in regions, where the ranges of the two species overlap.

The contemporary range of the Siberian roe deer is mainly distributed throughout continental Asia (Danilkin 1995), while the European species occurs in most of Europe except for northernmost Fennoscandia, Ireland, and the Mediterranean Sea islands. The two species co-occur in the Volga-Don rivers region (European part of the Russian Federation). Until the early 20th century, their ranges also overlapped in the Northern Caucasus (Dinnik 1910). Ecological niche modelling of species distribution by Matosiuk et al. (2014) suggested that during the Last Interglacial period (LiG; ca. 120–40 ka BP), *C. pygargus* could have extended its range as far as Central Europe. The distribution of *C. capreolus* in this area corresponds to the time of European roe deer expansion calculated based on genetic data, which probably took place after the Younger Dryas (10.8–10.0 ka BP). The range shifts most likely brought the two roe deer species into broader contact and led to interbreeding (Matosiuk et al. 2014), as their ranges overlapped in the Holocene, at least in Ukraine (Danilkin et al. 2017). Fossil records known to date have confirmed the presence of *C. pygargus* as far west as Kiev, Ukraine (Danilkin et al. 2017 and references therein).

Studies on karyotypes showed that all populations of the European roe deer have the same chromosome set: $2n=70$, whereas all populations of the Siberian roe deer are characterized by the presence of one or more microchromosomes belonging to a group of ancestral B-chromosomes: $2n=70 + (1-14)$ (Danilkin 1995). Various studies using mitochondrial and microsatellite markers in European roe deer populations revealed a geographic pattern in the population structure, with generally high levels of genetic variation. Traces of introgression between Siberian and European roe deer were found in large areas of Central and Eastern Europe: in Finland, Estonia, Hungary, Lithuania, Poland, Belarus, Ukraine, Romania, and Russia (Kashinina et al. 2018; Lorenzini et al. 2014; Markov et al. 2016; Matosiuk

et al. 2014; Olano-Marin et al. 2014; Plakhina et al. 2014; Plis et al. 2022a, b). This widespread introgression raises questions about the degree of hybridization and its historical drivers.

The most rigorous experiments to interbreed these two species in human-mediated conditions were carried out in Germany (Stubbe and Bruchholz 1979), when two Siberian bucks were mated with European does. Almost 60% of crossbreeds resulted in successful births, but in nearly 70% of them human assistance (caesarean section or manual removal of the foetus) was required due to the large size of the foetus. Crossbreeds of the F1 generation did not produce any offspring. In contrast, backcrosses between hybrid does and nonadmixed European or Siberian roe deer buck produced viable offspring. These experiments showed that hybrid males are sterile, and the European does mated by Siberian bucks cannot normally deliver the larger foetus (Stubbe and Bruchholz 1979). This suggests one-way hybridization between species under natural conditions: the European buck mating with Siberian does. Throughout the 19th and 20th centuries, numerous successful translocations of Siberian roe deer occurred within populations of European roe deer (Danilkin 1995) to “improve” the quality of roe deer trophy and body size (Figure S1). This factor could have been a secondary cause of introgression between species.

Studies on the phylogeography and genetic structure of the Siberian roe deer are limited and prior studies covered only portions of the species’ natural range. Earlier findings by Zvychainaya et al. (2011) and Lee et al. (2016) identified several haplogroups and clusters within restricted regions of Asia, highlighted the need for studies at a broader geographic scale. Vorobieva et al. (2011) expanded our understanding with analyses of both ancient and modern samples, revealing distinct but localized genetic clusters, yet this study was also limited in scope. Additionally, the diversity and frequency of occurrence of Siberian roe deer mtDNA within the European roe deer range remain unclear due to the limited and regionally focused sampling, resulting in inconclusive phylogeographic patterns.

Our study addressed those gaps by providing a comprehensive analysis across the whole range of the Siberian roe deer, including European regions, where the introgression between *C. capreolus* and *C. pygargus* had been recorded. Our goals were to assess the broader phylogenetic and phylogeographic patterns of *C. pygargus* across its range and to clarify the phylogenetic relationships of Siberian mtDNA haplotypes found in European roe deer populations. We tested the hypothesis of natural introgression during the European range expansion of Siberian roe deer by examining whether the distribution and demographic parameters of a defined group of *C. pygargus* mtDNA haplotypes

reflected an initial hybridization pattern in Central Europe. We assumed that in case of natural processes the frequency of those introgressed haplogroups would increase from the western part of the study area towards near-fixation in the easternmost part of the European roe deer range e.g. in the surroundings of Moscow. Furthermore, we attempted to answer the question to what extent the observed introgression is also an effect of human-mediated translocations. We hypothesised that it will be possible to detect some traces of such introductions only in local roe deer populations. We assumed that the restricted distribution of such introduced haplogroups at large spatial distances from their continuous range could indicate human-induced translocations.

Materials and methods

Study area, sample and data collection

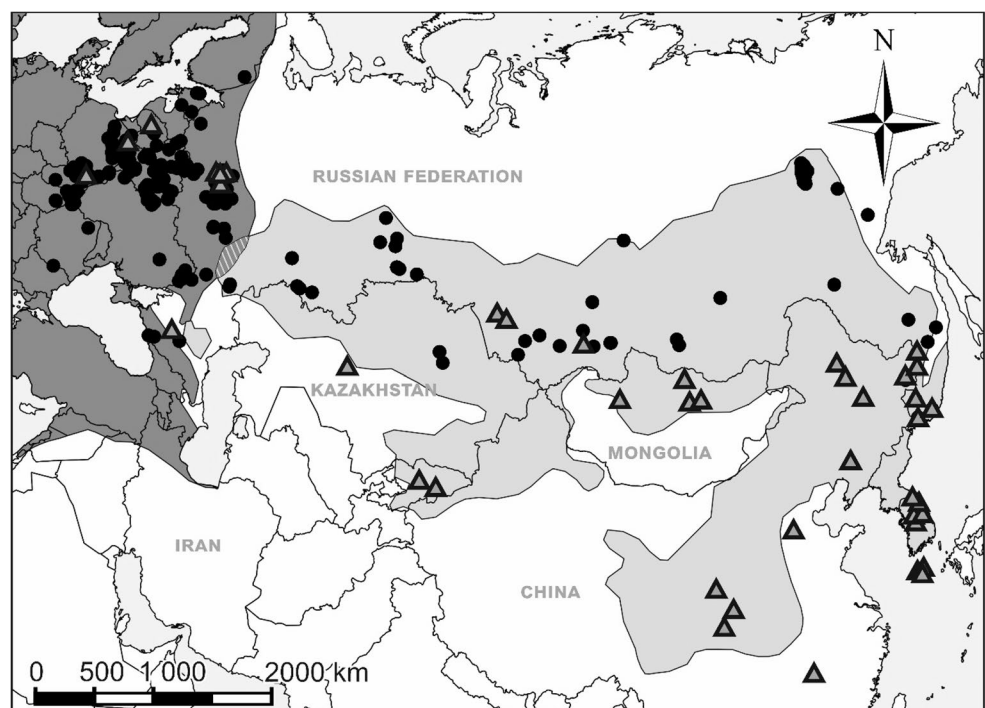
Between 2013 and 2017 we gathered 85 samples of Siberian roe deer from the natural range of this species across various locations in the Russian Federation (both European and Asian parts) and Kazakhstan. To cover a broader range across the species distribution, we incorporated 132 mtDNA sequences of roe deer available in GenBank, along with their frequencies reported in publications (Ao et al. 2020; Bayarlkhagva et al. 2013; Kim et al. 2017; Koh and Randi 2001; Koh et al. 2013; Li et al. 2020; Lorenzini et al. 2014; Matosiuk et al. 2014; Xiao et al. 2007), and by Kharitonov

et al. (unpublished). To provide insights into the range of hybridization, we included 267 samples from our previous studies (Plis et al. 2022a, b) that identified Siberian mtDNA introgression in European roe deer populations. These samples were collected from nine European countries: Belarus, Estonia, Finland, Hungary, Lithuania, Poland, Romania, Slovakia, and Ukraine. The entire study area (Fig. 1) ranged from Slovakia to South Korea ($19^{\circ}4'58''$ – $126^{\circ}38'59''$ E) and from the Republic of Sakha (Yakutia) in the Russian Federation to China ($61^{\circ}1'36''$ – $27^{\circ}52'10''$ N). Samples, consisting of fresh fragments of skin or muscle from legally hunted animals, were predominantly collected by hunters. All samples were obtained legally, and sample collection did not require ethics approval. Before DNA extraction, all samples were stored in 96% ethanol at -20°C . The geographic coordinates of the samples were assigned based on information on culling location provided by the hunters.

DNA extraction and sequencing

Total genomic DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Germany) following the manufacturer's guidelines. A fragment of the mtDNA control region was amplified by PCR with the primers L-Pro and H-Phe (Randi et al. 1998) and Taq PCR Master Mix Kit (Qiagen, Germany). Cycling conditions were 95°C for 15 min; 35 cycles of 94°C for 15 s, 56°C for 15 s, and 72°C for 1 min; and a final step of 72°C for 10 min. PCR products were purified using CleanUp (A&A Biotechnology,

Fig. 1 Map showing the distribution of roe deer sampling locations used in this study. Black dots indicate samples collected specifically for this study, while triangles represent data obtained from literature sources. The geographic ranges of the Siberian roe deer and European roe deer are depicted in light grey and dark grey, respectively. Areas where both species are reported to overlap, according to IUCN data, are represented with alternating dark and light stripes



Gdańsk, Poland). Sequencing reactions were carried out in a 10 µl volume using the Big Dye sequencing kit v.3.1 (Applied Biosystems) with the forward primer. Products were purified with the Exterminator kit (A&A Biotechnology) and sequenced on an ABI 3130 xl Genetic Analyser (Applied Biosystems). Sequencing resulted in good-quality mitochondrial control region fragments of 610 bp, which were analysed with the ABI DNA Sequencing Analysis software. All obtained mtDNA fragments were aligned against the reference sequence of the Siberian roe deer (GenBank KF724447.1; Lorenzini et al. 2014) and manually edited in BioEdit. v.7.0.5.3 (Hall 1999).

Sequences and data analyses

Reference data from GenBank were combined with the DNA fragments obtained in this study and shortened to maintain the same length for all sequences. The consensus mtDNA control region of 610 bp was used for subsequent analyses. We assigned the obtained sequences to haplotypes using Arlequin 3.5.1.3 software (Excoffier and Lischer 2010). The internal structure of subclades was defined based on haplotype genealogy constructed in HapView (Salzburger et al. 2011).

We conducted Bayesian phylogenetic analyses using BEAST X (Suchard et al. 2018) to estimate the time to the most recent common ancestor (TMRCA) for each roe deer mtDNA lineage. The HKY substitution model was selected based on the Bayesian Information Criterion (BIC) scores obtained from jModelTest v2.1 (Darriba et al. 2012). To infer divergence times in our BEAST analysis, we applied a calibration prior on the divergence between *C. capreolus* and *C. pygargus*, using a mean age of 1.125 Ma (95% highest posterior density [HPD]: 0.9–1.35 Ma), as reported by de Jong et al. (2020), without an a priori definition of the substitution rate. Additionally, we conducted two parallel analyses under a strict molecular clock model: (1) using the same divergence prior (mean 1.125 Ma, 95% HPD: 0.9–1.35 Ma) combined with a fixed substitution rate of 8.91×10^{-9} substitutions per site per million years, modeled as a normal distribution (95% HPD: $7.71\text{--}10.01 \times 10^{-9}$), following Deng et al. (2022); and (2) using a divergence prior of 2.25 Ma (95% HPD: 1.87–2.63 Ma), as reported by Deng et al. (2022), along with the same fixed substitution rate. MCMC chains were run for 30,000,000 generations with parameters sampled every 3,000 generations. Convergence and effective sample size (ESS > 200) values were assessed using Tracer (Rambaut et al. 2018). The resulting trees were summarized in TreeAnnotator (Drummond et al. 2012) with 20% of trees discarded as burn-in. Results were visualized using FigTree (Rambaut 2014). The maximum

clade credibility (MCC) tree was chosen to represent the phylogenetic relationships. An additional phylogenetic tree was constructed using MEGA version 7.0.14 (Kumar et al. 2016) and phylogenetic relationships were inferred based on the Neighbor-Joining (NJ) method with 10,000 bootstrap replicates to assess branch support. The resulting tree was visualized and annotated within MEGA to highlight key haplogroups and assess the phylogenetic structure of the samples. Posterior probability (the Bayesian trees) or Bootstrap (in case of NJ tree) values higher than 50 were annotated above the nodes.

Spatial genetic analyses were performed using GENELAND (Guillot et al. 2005, 2008). Multiple runs were conducted with different values of the number of genetic clusters (K) to assess convergence and stability. The MCMC chain was run 50 times for 1,000,000,000 iterations with 10% burn-in. Posterior distributions of individual assignments to genetic clusters and spatial coordinates were obtained from the MCMC output. These results were visualized using maps generated by GENELAND, illustrating the spatial distribution of inferred genetic clusters among Siberian roe deer populations. Convergence of the MCMC chains was assessed using diagnostic tools provided by GENELAND. The final results, including spatial clustering patterns and individual cluster assignments, were further visualized using Geographic Information System software QGIS version 3.12.3 (QGIS Development Team 2024) to enhance the interpretation of the spatial genetic structure. The allelic (F_{ST}) and the nucleotide (Φ_{ST}) fixation indices with permutation tests were calculated using Arlequin 3.5.1.3. to assess genetic differentiation among clusters defined by GENELAND. Summary statistics were calculated in DnaSP 5.10.01 (Librado and Rozas 2009). The following statistics were computed: the number of unique haplotypes (h), the number of segregating (polymorphic) sites (S), haplotype diversity (H_d), nucleotide diversity (π), and the average number of pairwise nucleotide differences (k). The figures presenting the distribution of pairwise differences were also created in DnaSP. Additionally, we included Levins's index (B ; Levins 1968) to express the diversity of haplotypes, using the formula:

$$B = \frac{1}{\sum p_i^2}$$

where p_i is the proportion of samples with haplotype i in a deme. We calculated Levins's index (B) according to the number of haplotypes and haplogroups in the sample. The B index minimum value is 1, and its upper bound is equal to the maximum number of the calculated parameters in the sample.

Results

Phylogenetics of the Siberian roe deer

Analyses of 484 Siberian roe deer sequences of the mtDNA control region fragment (610 bp) revealed 101 haplotypes (Table 1) with 78 polymorphic sites. The numbers of samples representing each haplotype ranged from 1 to 91; 60 sequences were singletons (see Table S1). We identified 43 new haplotypes, not described in the literature so far. The haplotypes obtained in this study were deposited in GenBank under accession numbers PP810042 - PP810141.

Phylogenetic analyses indicated the division of the sequences into 6 haplogroups, which were named with consecutive letters of the alphabet starting with A (Figs. 2, S2–S4 and 3). Samples not assigned to any specific group were marked as “Other”.

Assuming 1.125 Ma (de Jong et al. 2020) as the divergence time between *C. capreolus* and *C. pygargus*, the two oldest mtDNA haplogroups B and A evolved around 0.8 Ma BP and 0.7 Ma BP, respectively (Figs. 2, S2). Subsequently, differentiation into haplogroups C and D occurred around 0.6–0.5 Ma BP, and into haplogroups E and F around 0.4 Ma BP (Figs. 2, S2). If the assumed divergence time between the two species was older (2.25 Deng et al. 2022), the divergence time of different haplogroups ranged from 1.7 to 0.6 Ma BP (Figure S3). Comparison of the haplogroup divergence time with climatic oscillations showed

that the majority of haplogroups appeared in warmer periods (Figs. 2, S2 and S3).

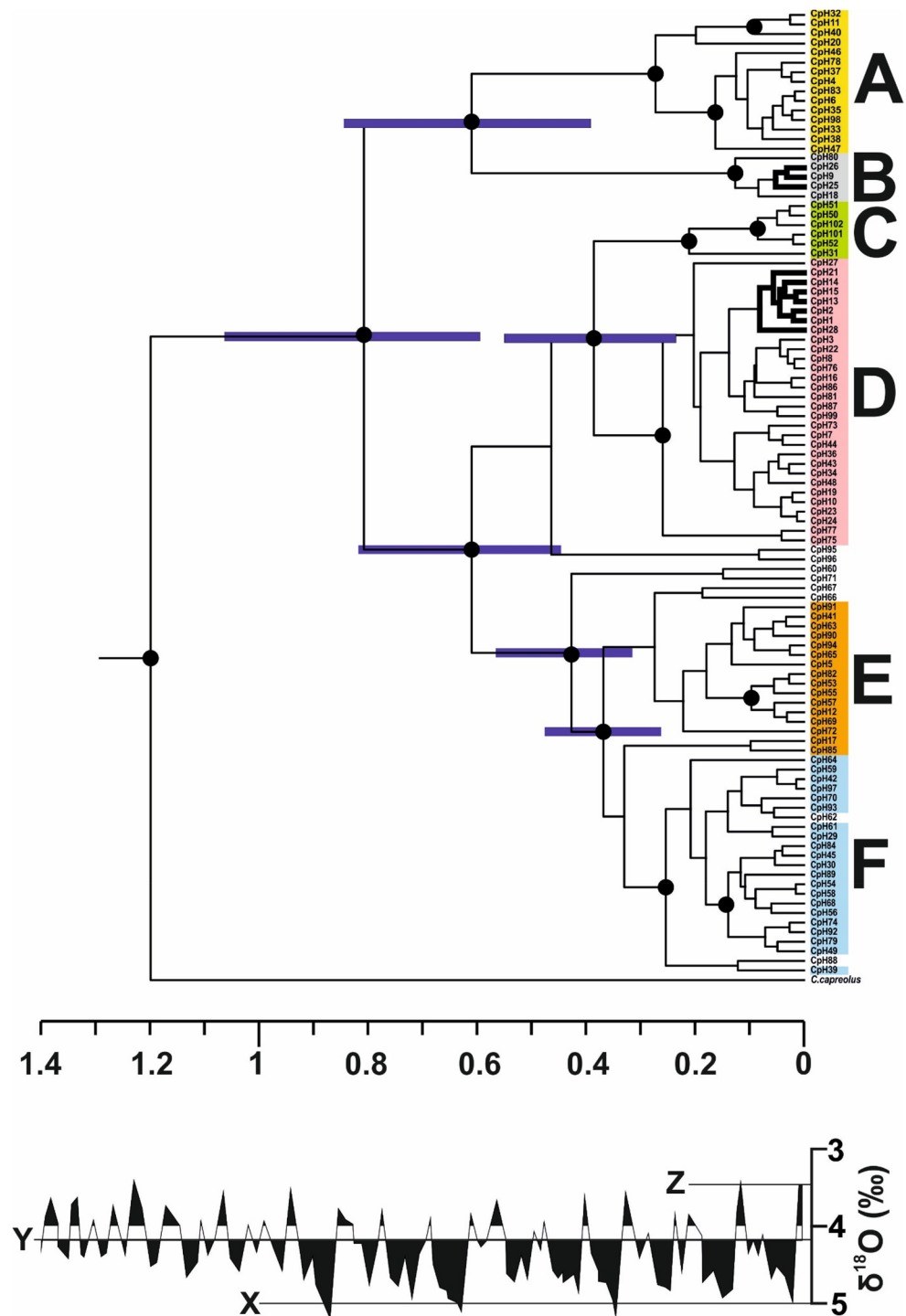
There are differences in phylogenetic positions of some haplogroups among the four obtained phylogenetic trees (Figs. 2, S2–S4). In the Bayesian tree with younger divergence times and with substitution rate as well as in the NJ tree (Figures S2 and S4) haplogroup B appears to diverge earlier than the rest of the haplogroups (A, C–F). In the tree without substitution rate (Fig. 2) and in the tree S3 (older divergence time and with substitution rate) haplogroups A and B are indicated as sister haplogroups. In the Figure S2, haplogroup B seems to be the oldest haplogroup (it split about 800 ka BP) and haplogroup A evolved later, about 700 ka year BP. Similar topology of the tree was indicated in the NJ tree (Figure S4), further suggesting that haplogroups A and B may represent an early diverging Lineages. Molecular dating analyses suggest that haplogroups C and D diverged from E and F around 600–500 ka BP, depending on the applied calibration, though these estimates should be viewed as approximate. Removing the substitution rate from the Bayesian analyses shifted the divergence times further back. Also, the positions of the haplotypes belonging to the “Other” group differed among the trees (Fig. 2, S2–S4). However, given the limited number of informative sites in the control region fragment (610 bp), the relationships among most haplogroups and their estimated divergence times should be treated with caution and require further studies including longer fragments of mtDNA sequences.

Table 1 Estimates of genetic diversity of the MtDNA control region (610 bp) in the Siberian roe deer (*C. pygargus*) haplogroups (see Fig. 2) and clusters defined by GENELAND (see Fig. 4)

Group	<i>n</i>	<i>h</i>	<i>S</i>	H_d	π	<i>k</i>	B_h	B_L
Haplogroup								
A	41 (23)	15	17	0.844	0.008	3.368	5.66	–
B	26 (23)	5	6	0.548	0.002	0.935	2.11	–
C	13 (7)	6	9	0.718	0.006	3.436	2.97	–
D	321 (273)	30	30	0.843	0.005	2.919	6.31	–
E	27 (3)	15	21	0.943	0.005	2.957	10.24	–
F	48 (22)	23	31	0.847	0.007	3.988	5.23	–
Other	8 (1)	7	18	0.964	0.011	6.393	8.33	–
GENELAND cluster								
1	72 (72)	7	23	0.649	0.014	8.539	2.78	1.74
2	190 (163)	18	34	0.742	0.005	3.232	3.82	1.10
3	100 (79)	19	36	0.861	0.012	7.069	6.83	1.62
4	34 (5)	23	42	0.978	0.019	11.264	20.64	4.31
5	36 (30)	9	31	0.757	0.014	8.576	3.79	2.02
6	27 (3)	23	44	0.986	0.013	8.191	19.70	3.70
7	25 (0)	19	28	0.978	0.013	7.812	14.53	4.63
Total	484 (352)	101	78	0.926	0.014	8.463	13.62	2.14

Number of samples is indicated by *n* (numbers in parentheses correspond to samples collected and analysed in this study), *h* – number of haplotypes, *S* – number of polymorphic sites, H_d – haplotype diversity, π – nucleotide diversity, *k* – average number of pairwise differences, B_h – Levin’s index (*B*) on the haplotype level, B_L – Levin’s index (*B*) on the haplogroup level. Haplotypes which were not assigned to any clade are marked as “Other”

Fig. 2 Phylogenetic tree of the Siberian roe deer mtDNA (based on 610 bp control region fragment) obtained in BEAST using Hasegawa-Kishino-Yano (HKY) substitution model with a prior calibration point for split of *C. capreolus* and *C. pygargus* set at 1.125 Ma. Posterior probabilities with values above 50% are shown as bullet points on nodes. Bars at selected nodes represent 95% HPD of node age. A–F mtDNA haplogroups. Branches in haplogroups B and D marked in bold denotes haplotypes occurring exclusively in Europe as introgression into *C. capreolus* populations. Lower panel: climatic oscillations (as measured by oxygen isotope changes) of the last 1.4 million years. Y – isotope ratio some 2.5 Ma BP; X – isotope ratio near 0.9 Ma BP; Z – the Holocene isotope ratio (from Andersen and Borns 1994 after Hewitt 2011; modified)

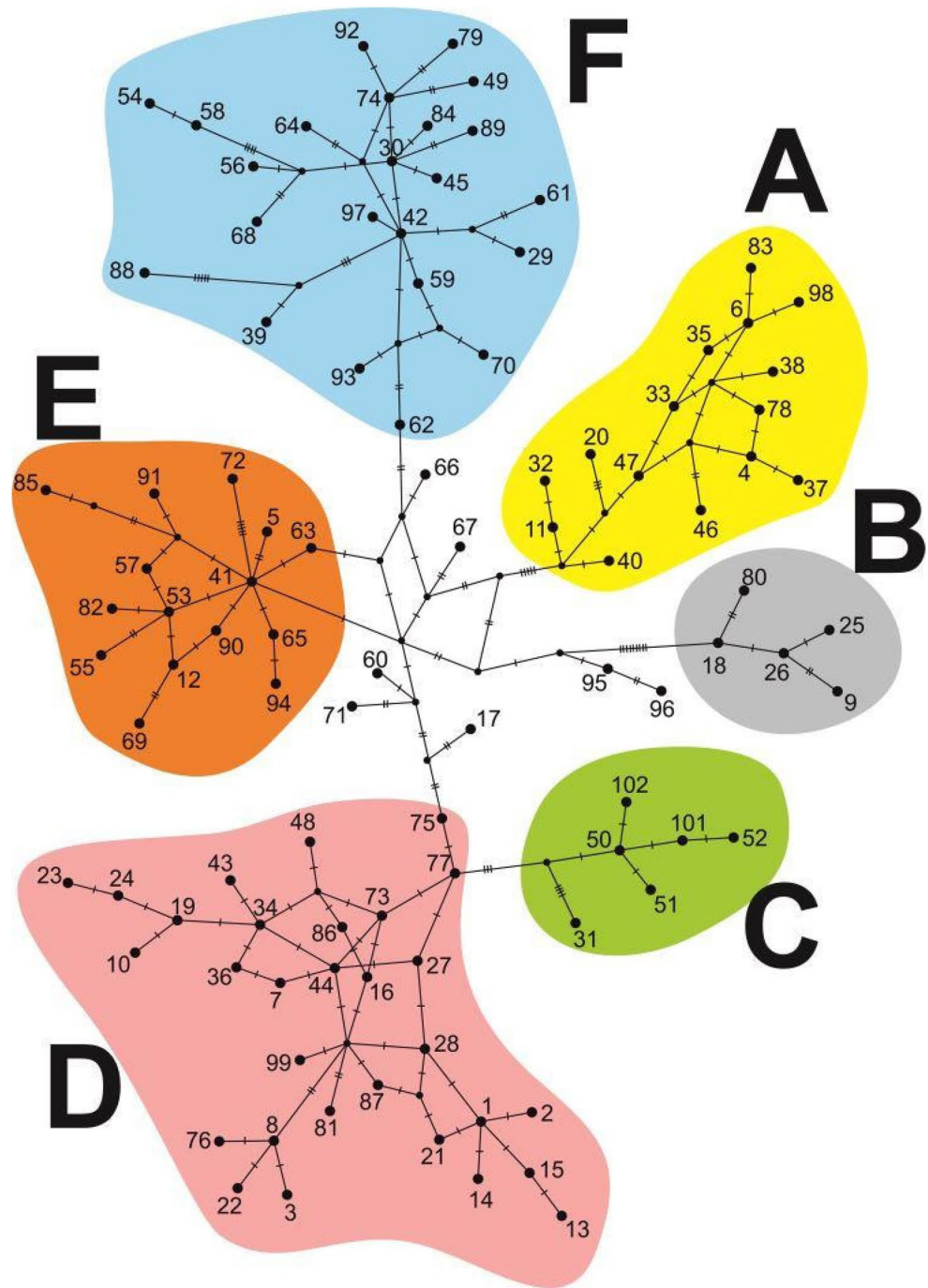


The number of individuals carrying haplotypes, belonging to each of the haplogroups ranged from 13 in haplogroup C to 321 in haplogroup D, while the number of distinct haplotypes within each haplogroup varied from 5 in haplogroup B to 30 in haplogroup D. Only 8 samples (from Volgograd oblast in southwestern Russia, Khabarovsk Krai in the easternmost Russia, and from China and South Korea) representing 7 haplotypes were not assigned to any

monophyletic group (Table S1, Figs. 2 and 3, S2, S3). The number of haplotypes revealed in haplogroups A–F was positively correlated with the number of analysed samples ($r=0.84$, $p<0.05$).

The number of segregating sites ranged from 6 to 30. Roe deer showed overall high values of both haplotype (H_d) and nucleotide diversity (π), i.e., 0.93 and 0.014 respectively, but these indices varied across haplogroups (Table 1). The

Fig. 3 Phylogenetic median-joining network presenting the genetic relationships among mtDNA haplotypes of the Siberian roe deer. Mutation steps that distinguish individual haplotypes are indicated by vertical lines on the branches between haplotypes. Colours denote different haplogroups, small dots show missing haplotypes and numbers represent different haplotypes (e.g., 55=CpH55 in Table S1 and Fig. 2)



highest haplotype diversity was in haplogroup E ($H_d = 0.94$), while the lowest one was in haplogroup B ($H_d = 0.55$). Similarly, the highest value of nucleotide diversity was recorded in haplogroup A ($\pi = 0.008$), and the lowest one in haplogroup B ($\pi = 0.002$). The average number of pairwise differences also differed by haplogroup, with the lowest value in haplogroup B. Despite being separated by a high number of mutation steps from other clusters, haplogroup B was composed of highly similar haplotypes with only minor differences among them (Fig. 3). Only haplogroup B showed

a unimodal distribution of pairwise differences, aligning with expectations from the sudden expansion model (Figure S5). A similar pattern was observed for a subset of seven haplotypes within haplogroup D, found exclusively in introgressed individuals and forming a monophyletic group (Figs. 2, S2–S4). All other haplogroups showed multimodal distributions of pairwise differences, consistent with more complex demographic histories (Figure S3). Levins's B index at the haplotype level (Bh) ranged from 2.11 in haplogroup B to 10.24 in haplogroup E (Table 1). None of the

diversity indices calculated for haplogroups were significantly correlated with the number of samples analysed per group (Pearson's $r = -0.22$ to 0.43 , $p > 0.10$).

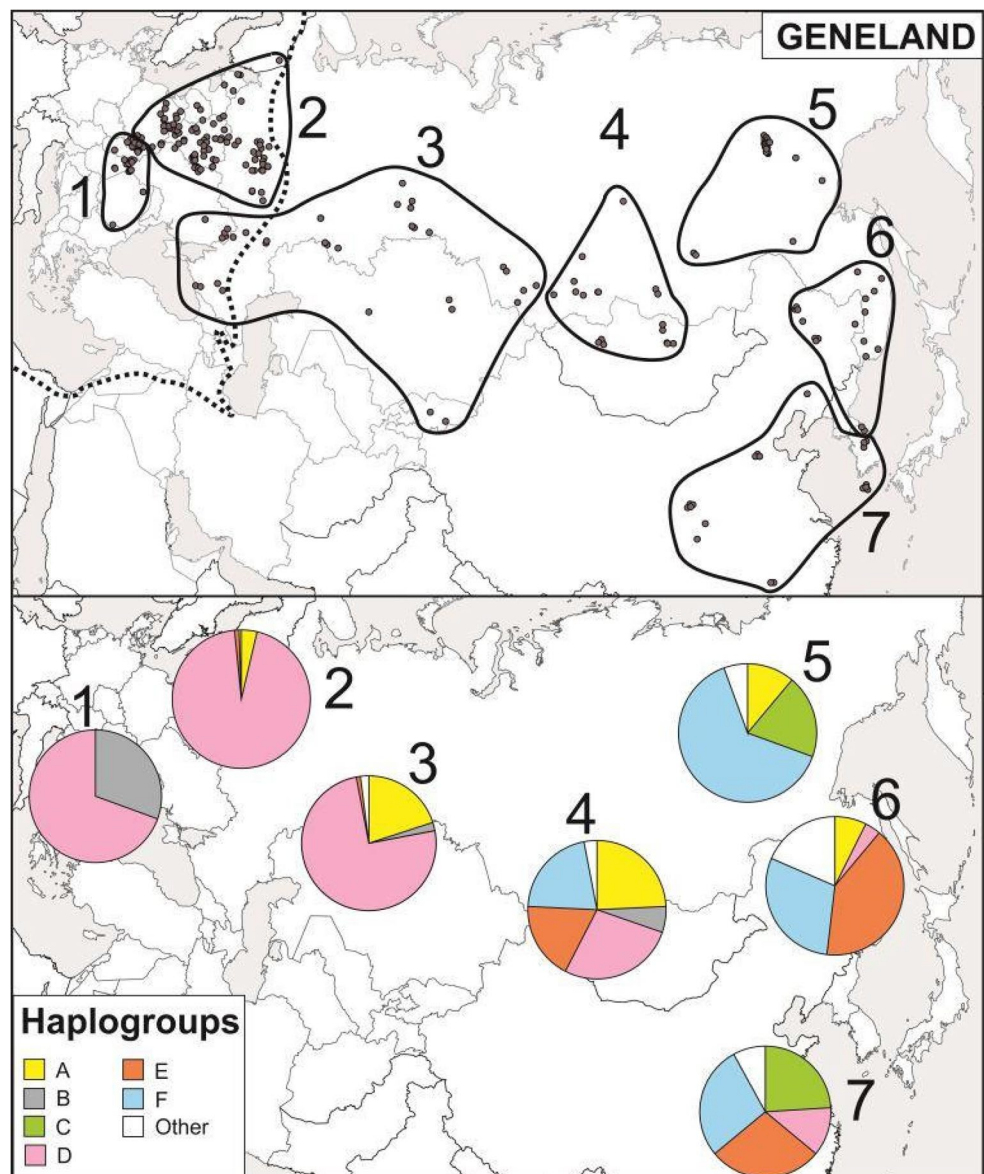
Population structure and genetic diversity

Analyses done in GENELAND revealed 7 clusters (Fig. 4, upper panel). Two westernmost clusters (1 and 2) included only the European roe deer individuals with introgression of the Siberian deer mtDNA. Cluster 3, spanning from Ukraine to southern Russia and Kazakhstan, included both individuals with introgression and nonadmixed *C. pygargus*. The central-eastern parts of Asia harboured four clusters (Fig. 4, upper panel). Cluster 4 inhabited the central parts of Siberia and Mongolia, cluster 5 – the Republic of Sakha (Yakutia, Russia), cluster 6 – Khabarovsk and Primorsky

Krai (Russia), northeast China, and the northern part of the Korean Peninsula, and cluster 7 – eastern China, southern part of the Korean Peninsula, and Jeju Island (South Korea).

The number of samples in each cluster varied between 25 and 190, while the number of haplotypes ranged from 7 to 23 (Table 1, S1). The number of haplotypes found in the GENELAND clusters was not correlated with the number of samples in each cluster ($r = 0.083$, $p > 0.10$). Interestingly, the prevalence of *C. pygargus* mtDNA haplogroups among specific European roe deer populations varied notably. Among all roe deer samples collected and analysed within the range of *C. capreolus* (data from Plis et al. 2022a) the proportion of introgressed individuals reached 26.6% in Cluster 1 and 22.6% in Cluster 2. In the western part of Cluster 3 (in the range of European roe deer, only) the introgression rate was 35.6%.

Fig. 4 Upper panel: Spatial distribution of samples divided into 7 genetic clusters according to the GENELAND analysis. Dashed line represents the putative range of the European roe deer lineage. (source: IUCN <https://www.iucnredlist.org/species/42395/22161386>). Lower panel: The proportions of haplogroups belonging to the Siberian lineage (see Figs. 2 and 3) in each of the identified clusters.



The deepest divide in the phylogeographic structure of the Siberian roe deer occurred in central parts of Siberia, approximately along the meridian 85°E, which separated clusters 1–3 to the west from clusters 4–7 to the east (Fig. 4, upper panel). The western clusters (including introgressed clusters 1 and 2) are strongly dominated by haplogroup D (69–92% of samples), and cluster 1 is the only one with a substantial share (31% of samples) of haplogroup B. The eastern clusters are more diversified with haplogroups A, C, E, and F having fair shares in their composition (Fig. 4, lower panel). Clusters 1 and 2 found in the European part of the study area tended to have the lowest values of genetic diversity of mtDNA, whereas clusters 4, 6, and 7 located in central and eastern Asia were characterized by very high haplotype (H_d , B_h) and nucleotide (B_L) diversity (Table 1, S1). The largest genetic differentiation was recorded between the most distant clusters located in the eastern parts of Europe (cluster 1 for F_{ST} and cluster 2 for Φ_{ST}) and eastern Asia (cluster 5; $F_{ST} = 0.303$; $\Phi_{ST} = 0.619$) (Table 2).

MtDNA haplotype composition of the Siberian clade of *C. capreolus* compared to *C. pygargus* population

We analysed the overall geographical distribution of the Siberian roe deer haplotypes found in the populations of the European roe deer (the Siberian clade of *C. capreolus*; see Plis et al. 2022a, b). Five haplogroups of the Siberian roe deer (A, B, D, E, F) were found to have introgressed into *C. capreolus* populations in Eastern Europe (Figs. 5, S6). In haplogroup B, three out of five haplotypes have survived only as introgressed mtDNA variants into European roe deer and now occur as far west as Slovakia, Hungary, and southern Poland (Figure S6).

In a widely occurring haplogroup A, the same (or closely related) haplotypes were found in both Siberian and European roe deer populations, yet in the latter species not far from the current western edge of *C. pygargus* range (Figure S6). The most complicated spatial pattern of haplotype distribution was found in haplogroup D, where one subgroup containing 7 haplotypes (marked in green in Fig. 5, upper panel) was recorded exclusively and abundantly in

C. capreolus populations in Central and Eastern Europe. Six other haplotypes occurred in both the European and the Siberian roe deer ranges (Fig. 5, upper panel). Finally, two haplotypes from haplogroup E, which was generally restricted to Eastern Asia, were found within or near the geographic range of *C. capreolus* (Fig. 5, lower panel).

In total, among 88 Siberian roe deer haplotypes from haplogroups A, B, D, E, and F (see Table S1), 27 (31%) were found to have introgressed into *C. capreolus* populations, including 16 haplotypes (18%) that were recorded exclusively in the European roe deer range. They consisted of the following haplotypes: haplogroup A (1): CpH4; haplogroup B (3): CpH26, CpH25, CpH9; haplogroup D (11): CpH28, CpH13, CpH15, CpH1, CpH2, CpH21, CpH14, CpH10, CpH22, CpH27, CpH3; and haplogroup F (1): CpH79 (Table S1). In the evolutionary oldest haplogroup B (Figure S2), three out of five haplotypes were no longer found in the range of *C. pygargus* (Figure S6, lower panel).

Discussion

Phylogeny and pan-Eurasian phylogeography of Siberian roe deer MtDNA lineage

Our study provides the most comprehensive analysis of the phylogenetic diversity of the Siberian roe deer on a Eurasian scale, encompassing the entire species range and areas with detected introgression of Siberian mtDNA into the population of European roe deer. The entire mtDNA lineage of *C. pygargus* exhibited high genetic variability, represented by six haplogroups and a substantial number of haplotypes. Across the region from Central Europe to the Far East, we observed a gradient in haplogroup frequencies within regional populations, with the highest rate of exchange in the central parts of Siberia. Out of 101 detected haplotypes, 27 were identified in the European roe deer range in Central and Eastern Europe, with 16 being exclusive to that area.

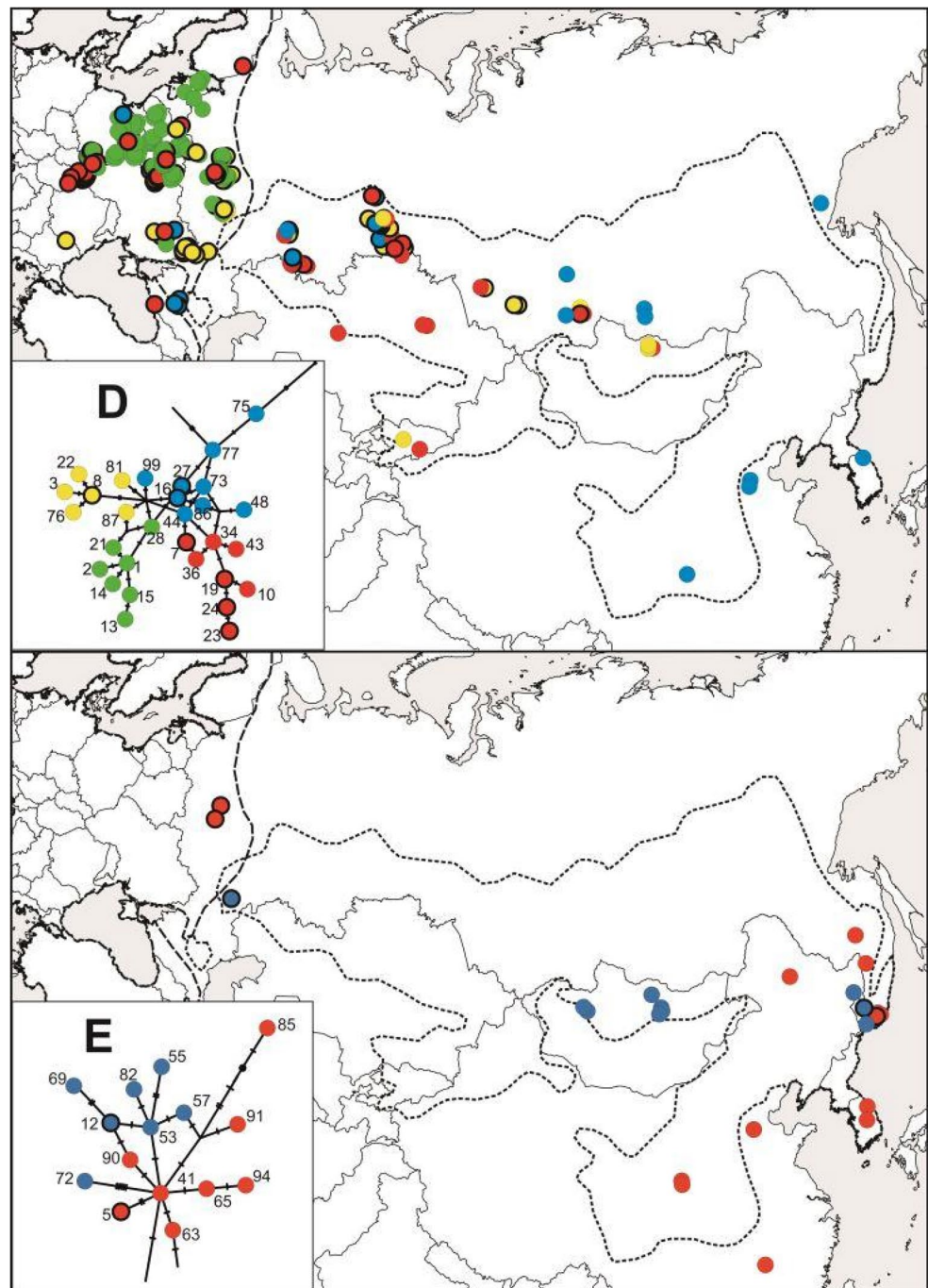
Consistent with earlier studies, we identified two widespread haplogroups (A and D) and three haplogroups (C, E, and F) predominantly occurring in the Eastern part of

Table 2 Pairwise Φ_{ST} (upper-right panel) and F_{ST} (lower-left panel) values estimated for the 7 clusters of Siberian roe deer identified by GENE-
LAND

Cluster	1	2	3	4	5	6	7
1		0.284	0.148	0.189	0.390	0.353	0.277
2	0.233		0.198	0.436	0.619	0.599	0.528
3	0.202	0.175		0.170	0.394	0.370	0.295
4	0.199	0.154	0.076		0.129	0.068	0.072
5	0.303	0.252	0.185	0.105		0.127	0.184
6	0.201	0.153	0.079	0.016	0.132		0.0745
7	0.211	0.162	0.088	0.025	0.140	0.018	

Numbers and distribution of the clusters as in Fig. 4. All values were statistically significant at $p < 0.005$

Fig. 5 Geographical distribution of the Siberian roe deer haplotypes belonging to haplogroups D (upper panel) and E (lower panel). The small insert panels show fragments of the haplotype network presented in Fig. 3. Colours denote subgroups of haplotypes, and numbers represent different haplotypes. Haplotypes that were recorded in the ranges of both European and Siberian roe deer are encircled in black. Broken lines mark contemporary ranges of the European and Siberian roe deer (see Fig. 1)



Asia. Additionally, we discovered a new (identified for the first time) haplogroup (B), which is rare and primarily found in introgressed populations in Europe. Haplogroup B appears to be among the earliest diverging lineages within *C. pygargus*, supporting its distinct evolutionary position according to the performed analyses. This discovery provides new insights into the genetic history and diversity of *C. pygargus*, suggesting previously unknown complexity of its mtDNA lineages. Nonetheless, the relationships among haplogroups should be treated as tentative due to the limited

resolving power of the control region fragment. Phylogenetic reconstruction based on short mtDNA sequences can be confounded by stochastic variation and phenomena such as long-branch attraction (Roch et al. 2018). Therefore, further studies involving complete mitogenomes or additional mtDNA genes from selected representative haplotypes are needed to clarify the basal relationships and evolutionary status of the detected haplogroups.

Furthermore, the presence of haplogroup B provided some further clues to past hybridization events and the

introgression of Siberian roe deer genes into European roe deer populations. Furthermore, we demonstrated that the population of European roe deer with introgressed mtDNA of *C. pygargus* has been divided into two genetic subpopulations, with the southern one having a high proportion of the rare and evolutionary older haplogroup B. Therefore, our study indicated that the mtDNA variability of the Siberian roe deer was much larger and the history of hybridization between the two *Capreolus* species was far more complex than previously thought.

However, there is still a lack of data concerning the introgression of nuclear genes of *C. pygargus* in the population of *C. capreolus*. Plakhina et al. (2014) detected introgression of Siberian roe deer DNA into nuclear genes of European roe deer in a limited number of individuals in the easternmost part of the European roe deer range in Russia. Also, a small separate population in the easternmost part of European roe deer range (near the Volga-Don River region, where the range of the two *Capreolus* species overlapped) was indicated in the microsatellite DNA study conducted by Niedziałkowska et al. (2024). However, further genomic study are needed to confirm if the distinctiveness of this population is an effect of hybridization of these two species as individuals with Siberian roe deer mtDNA were not identified as a separate genetic population of the species by microsatellite DNA analyses in other regions of Europe (Olano-Marin et al. 2014; Niedziałkowska et al. 2024). Moreover, Lee et al. (2015) identified only one microsatellite cluster of the Siberian roe deer in the Western part of the species range (covering the Urals and Western Siberia). Therefore, it is possible that population genetic structure of *C. pygargus* in the western part of its range is more difficult to detect based on nuclear DNA than by mtDNA data.

Lee et al. (2016) estimated a divergence age of 1.42–1.58 million years ago (Ma BP) between European and Siberian roe deer based on analyses of >2000-bp mtDNA fragment of 112 haplotypes, but did not sample populations to the west of the Ural Mts. Similarly, Deng et al. (2022) estimated that *C. pygargus* and *C. capreolus* diverged around 2.25 Ma BP, with major haplogroups diverging at 0.27–0.34 Ma BP, based on a limited sample of 15 haplotypes belonging to three or four haplogroups. Yet, they did not detect the genetically distinct haplogroup (haplogroup B in our study) prevalent in Central Europe. Both Lee et al. (2016) and Deng et al. (2022) identified haplogroups D and E but did not find out other haplogroups in their studies. Our comprehensive analyses including large dataset and the Eurasian scale sampling provided more detailed information concerning the phylogenetic and phylogeographic pattern of the species and the diversity of mtDNA variants from Siberian roe deer introgressed into European roe deer populations. The glacial cycles have affected the evolutionary

history of *C. pygargus* haplogroups, appear to have their roots 800–700 ka BP. Moreover, based on the obtained results, we suppose that ranges overlapped and hybridization between *C. pygargus* and *C. capreolus* occurred at least twice as the introgressed haplotypes belonged to haplogroups of significantly different divergence times (e.g. B and D).

Origins of the introgression of Siberian roe deer MtDNA in European roe deer populations

The presence of Siberian roe deer mtDNA in European roe deer populations was initially reported in Russia by Zvychainaya et al. (2011) and Danilkin (2014), followed by findings in eastern Poland by Matosiuk et al. (2014) and Olano-Marin et al. (2014), and in Lithuania and eastern Poland by Lorenzini et al. (2014). Matosiuk et al. (2014) and Lorenzini et al. (2014) suggested that the primary cause for introgression was natural interbreeding between the two species around 10.8–10 ka BP when their ranges overlapped in Eastern Europe. Their conclusion was based on the absence of the introgressed Siberian roe deer haplotypes in the modern population of the species. On the other hand, Zvychainaya et al. (2011) speculated that haplotypes shared between contemporary Siberian and European roe deer populations might be a result of successful relocations of *C. pygargus* to Europe in the 19th and 20th centuries.

Our study, utilizing a substantial dataset from Eurasia (Plis et al. 2022a, b; and this study), established that five Siberian roe deer haplogroups now present in European roe deer populations were also found in modern Siberian roe deer populations. However, we also identified 16 haplotypes of *C. pygargus* exclusive to *C. capreolus* populations in Europe. Both groups of haplotypes (the first consisting of mtDNA variants present only in Europe and the second found in the whole range of *C. pygargus*) were present in the defined clusters 1 and 2, indicating that the hybridization between the two species could have been an effect of different processes, both natural and caused by anthropogenic factors. European roe deer with Siberian mtDNA exhibited a clear genetic structure, forming a continuum with the genetic structure of nonadmixed Siberian roe deer populations. This structure demonstrated west–east changes in the frequencies of different haplogroups. The frequency of introgressed haplotypes was higher in the easternmost part of the European roe deer range. Notably, 85% of the early diverged haplogroup B occurred as an introgressed population in Central Europe (southern Poland, Slovakia and Hungary), despite its range extending to Siberia (although this haplogroup was rather rare in Asia). A similar wide, yet non continuous Eurasian distribution was observed in haplogroup D, the frequency of which was highest in

populations with introgression. One of the evolutionary branches of haplogroup D featuring seven haplotypes, was found exclusively in Europe. Interestingly, two out of those seven haplotypes (CpH1 and CpH28) had been previously reported by Lorenzini et al. (2014) and Matusiuk et al. (2014). Our identification of additional closely related haplotypes within haplogroup D, suggests larger genetic diversity of introgressed variants of mtDNA of Siberian roe deer, although it can also be a result of mutations appearing after initial introgression as the unimodal distribution of pairwise differences of these haplotypes corresponded to sudden expansion model. Furthermore, the presence of such closely related haplotypes could indicate that they are relatively young or have spread recently, especially if they are diverged from those present in the natural range of the Siberian roe deer. The distinction of mtDNA variants and their limited genetic diversity in the introgression zone may also be an effect of genetic drift, where closely related haplotypes are maintained due to restricted gene flow or strong selective pressures preserving genetic variants in areas isolated from the continuous range of *C. pygargus*.

Based on detected diversity of Siberian roe deer mtDNA, we suppose that the currently observed genetic structure and composition of unique and regionally restricted *C. pygargus* genes preserved in European populations reflect a relatively long-term presence of Siberian roe deer mtDNA in European roe deer populations. Contrary to Lorenzini et al. (2014) and Matusiuk et al. (2014), we suppose that the hybridization process took place more than once. Hewitt (2011) concluded, based on diverse species and continents, that hybrid-zone roots are often complex and deep, forming more than once as species or genetic lineages contracted and expanded in response to glacial cycles. Habitat modeling by Matusiuk et al. (2014) suggested that *C. pygargus* could have reached the Carpathians, contemporary Hungary, and Poland during the Last Interglacial (LiG) 0.12–0.14 Ma BP. Given their ancient evolutionary origin, the haplotypes of haplogroup B, exclusive to the Carpathian Mts. and the Carpathian Basin, might have survived the Last Glacial period in the Carpathian refugium, eventually becoming ‘endemic’ to that region. A branch of seven haplotypes in haplogroup D, which split before the Last Glacial Maximum (LGM, 19–26 ka BP, Clark et al. 2009) or even earlier (the exact divergence time should be taken with caution as we analysed only a fragment of mtDNA) and now occurs in Central and Eastern Europe, predominantly north of the Carpathians, might have experienced a range shifts in stadial and interstadial periods (see Figs. 2, S2, lower panel). Such a conclusion corresponds with the results of studies by Pavelková Řičánková et al. (2014), who demonstrated that the

Siberian roe deer range retracted eastwards into the inner Asian continent during the Last Glacial.

We propose that populations of *C. capreolus* with introgression of Siberian roe deer mtDNA could have survived the LGM in the Carpathian region (including Moldova) and the plains between the Dniester and Don rivers (the Ukrainian-Russian borderland). From these refugial areas, post-glacial colonization towards the north and west likely took place. Markova and Puzachenko (2019) found that the two refugial areas harboured a high number of mammals during the LGM and showed wide ecological connectivity. The dynamic changes in ranges of roe deer inhabiting Europe in response to climatic oscillations (Sommer et al. 2009) have also been reflected in the genetic diversity of roe deer with introgression of Siberian roe deer mtDNA. Clusters 1 and 2 found in the European part of the study area had the lowest values of mtDNA diversity, whereas clusters 4, 6, and 7 located in Central and Eastern Asia were characterized by very high haplotype and nucleotide diversity. Such patterns is in agreement with different demographic history and changes in effective population size of roe deer in Europe and eastern Asia since the Eemian Interglacial (130–115 ka BP, Dahl-Jensen et al. 2013) as inferred by de Jong et al. (2020).

The second proposed mechanism of introgression involves the relocation of Siberian roe deer from Siberia and the Far East to the European part of Russia and Eastern Europe during the 19th and 20th centuries. Figure S1 illustrates that most relocations were long-distance events, with Siberian roe deer repeatedly live-captured in the Russian Far East and southern Siberia, then released into the European part of Russia. These were regions where *C. capreolus* populations already possessed the Siberian lineage of mtDNA. There were also reverse translocations of both roe deer species from West to East (Figure S1). This complicates the distinction between natural (ancient) and human-driven (modern) origins of introgression. Nevertheless, we speculate that the presence of a few individuals belonging to haplogroup E in Eastern Europe might be the result of translocation efforts. First, the range of haplogroup E of the Siberian roe deer spans the Russian Far East, China, and Mongolia. Secondly, the two haplotypes found in Eastern Europe occurred in the Primorsky Krai region, where most live-capturing for relocation efforts took place (see Figure S1). Despite the need for caution in drawing conclusions about ancient natural versus modern human-related hybridization between Siberian and European roe deer, it appears that recent relocations had limited success compared to the efforts invested. The reproductive barrier between the two species likely contributed to this outcome.

Conclusions

The mitochondrial genetic population of the Siberian roe deer comprises six haplogroups distributed from Central Europe to the Pacific coast of Asia. The east-west gradient in haplogroup frequencies, with the highest exchange rate in the central parts of Siberia, resulted in seven genetic clusters of the Siberian roe deer, including two clusters in the range of the European roe deer belonging to the Siberian Lineage of mtDNA. Sixteen haplotypes from four haplogroups occur exclusively in Europe, Likely remnants of past natural interbreeding between Siberian and European roe deer. The results concurred with our expectation that both natural and local human-mediated processes have affected hybridization between the two roe deer species in Europe. The introgression could have occurred before the LGM, at least two times during 100 000 years. Translocations of Siberian roe deer to Eastern Europe in the 19th and 20th centuries have also left a genetic signal in the phylogeography of European roe deer. Although our study covered the vast area of the Palearctic, there are still regions requiring additional sampling and study efforts, especially the Caucasus region, Siberia, Mongolia, and China. Finally, more detailed genetic studies, including the analysis of ancient DNA and additional genetic markers (such as single nucleotide polymorphisms), are necessary to assess the relative contributions of past natural interbreeding and translocations to the contemporary presence of the Siberian mtDNA lineages in European roe deer populations.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13364-025-00820-2>.

Acknowledgements The study was financed by the National Science Centre in Poland under the project no. 2013/11/B/NZ8/00884 for BJ. The research was also supported by the European Commission project BIOGEAST, no. PIRSES-GA-2009-247652. The authors thank the Polish Hunting Association, V. Sidarovich, I. Dykiy, D. Vishnevsky, M. Kolesnikov, V. Lobkov, and many hunters for their help in collecting samples. Special gratitude to Maryna Shkvyria for her invaluable support throughout this study, her profound knowledge, and generous provision of samples. This paper formed a part of the PhD thesis of KP.

Author contributions KP, MN, AD and BJ conceived the ideas and designed methodology; KP, AD, MK, EZ, NK, AB, IO, NM, SzK, MS, LP, RV, AP, A-MK and JT collected the data; KP, MN, AD, LP and BJ analysed the data; KP, MN and BJ led the writing and revision of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Declarations

Competing interests The authors declare that they have no known competing financial or non-financial interests that could have appeared to influence the work reported in this paper. MN is the Associate Editor of Mammal Research.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Andersen BG, Borns HW (1994) The ice age world. Scandinavian University, Oslo
- Ao D, Yao Y, Li D, Xie M, Ni Q, Zhang M, Xu H (2020) The complete mitochondrial genome of Siberian roe deer (*Capreolus pygargus bedfordi*) and its phylogenetic analysis. Mitochondr DNA B 5(1):1122–1123. <https://doi.org/10.1080/23802359.2019.1711232>
- Bayarlkhagva D, Bayarlkhagva M, Gun-Aajav B, Batsuuri S (2013) Genetic diversity of mitochondrial DNA D-loop sequence of Siberian roe deer (*Capreolus pygargus*) inhabiting Mongolia. Int J Sci 2(3):105–108
- Clark PU et al (2009) The last glacial maximum. Science 325:710–714. <https://doi.org/10.1126/science.1172873>
- Dahl-Jensen D, Albert MR, Aldahan A, Azuma N, Balslev-Clausen D, Baumgartner M et al (2013) Eemian interglacial reconstructed from a Greenland folded ice core. Nature 493:489–494
- Danilkin A (1995) Behavioural ecology of Siberian and European roe deer. Springer Netherlands. <https://www.springer.com/gp/book/9780412638800>
- Danilkin A (2014) Roe deer (biological bases of resource management). KMK Scientific, Moscow
- Danilkin AA, Plakhina DA, Zvychnaya EY, Domnich AV, Kholodova MV, Sorokin PA, Volokh AM (2017) Siberian roe deer (*Capreolus pygargus* Pallas, 1771) in Ukraine: analysis of the mitochondrial and nuclear DNA. Biol Bull 44(6):575–583. <https://doi.org/10.1134/S106235901706005X>
- Darriba D, Taboada GL, Doallo R, Posada D (2012) Jmodeltest 2: more models, new heuristics and parallel computing. Nat Methods 9(8):772. <https://doi.org/10.1038/nmeth.2109>
- Deng MX, Xiao B, Yuan JX, Hu JM, Kim KS, Westbury MV, Lai XL, Sheng GL (2022) Ancient mitogenomes suggest stable mitochondrial clades of the Siberian roe deer. Genes 13(1):114. <https://doi.org/10.3390/genes13010114>
- Dinnik NY (1910) Mammals of the Caucasus. Pt. 1. Cetaceans and ungulates. Trans Cauc Branch Imperial Russ Geogr Soc 27(1):1–246
- Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with beauti and the beast 1.7. Mol Biol Evol 29:1969–1973. <https://doi.org/10.1093/molbev/mss075>
- Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and windows. Mol Ecol Resour 10(3):564–567. <https://doi.org/10.1111/j.1755-0998.2010.02847.x>
- Guillot G, Mortier F, Estoup A (2005) Geneland: a computer package for landscape genetics. Mol Ecol Notes 5(3):712–715
- Guillot G, Santos F, Estoup A (2008) Analyzing georeferenced population genetics data with geneland: a new algorithm to deal with

- null alleles and a friendly graphical user interface. *Bioinformatics* 24:1406–1407
- Hall TA (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98. https://doi.org/10.14601/Phytopathol_Mediterr-14998u1.29
- Hewitt GM (2011) Quarternary phylogeography: the roots of hybrid zones. *Genetica* 139:617–638
- Kashinina NV, Danilkin AA, Zvy chaynaya EY, Kholodova MV, Kiryakulov VM (2018) On the gene pool of roe deer (*Capreolus*) of Eastern Europe: analysis of the cyt b gene sequence variability. *Russ J Genet* 54(7):825–831. <https://doi.org/10.1134/S1022795418070049>
- Kim HR, Jeon MG, Min JH, Kim HJ, Park YC (2017) Complete mitochondrial genome of the roe deer *Capreolus pygargus tianschanicus* (Cervidae) from Korea. *Mitochondrial DNA Part B* 2(2):558–559. <https://doi.org/10.1080/23802359.2017.1365645>
- Koh HS, Randi E (2001) Genetic distinction of roe deer (*Capreolus pygargus* Pallas) sampled in Korea. *Mamm Biol* 66:371–375
- Koh HS, Bayarlkhagva D, Jang KH, Han ED, Jo JE, Ham EJ, Jeong SK, Lee JH, Kim KS, Kweon GH, In ST (2013) Genetic divergence of the Siberian roe deer from Korean Jeju Island (*Capreolus pygargus sochraceus*), reexamined from nuclear IRBP and mitochondrial cytochrome b and control region sequences of *C. pygargus*. *J Biol Res (Greece)* 19:46–55
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33(7):1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Lee YS, Markov N, Voloshina I, Argunov A, Bayarlkhagva D, Oh JG, Park YS, Min MS, Lee H, Kim KS (2015) Genetic diversity and genetic structure of the Siberian roe deer (*Capreolus pygargus*) populations from Asia. *BMC Genet* 16:100. <https://doi.org/10.1186/s12863-015-0244-6>
- Lee YS, Markov N, Argunov A, Voloshina I, Bayarlkhagva D, Kim BJ, Min MS, Lee H, Kim KS (2016) Genetic diversity and phylogeography of Siberian roe deer, *Capreolus pygargus*, in central and peripheral populations. *Ecol Evol* 6(20):7286–7297. <https://doi.org/10.1002/ece3.2458>
- Levins R (1968) Evolution in changing environments. Princeton University Press, Princeton
- Li Y, Li N, Chen L, Li Y, Xiong Z, Hu Y (2020) Estimating abundance of Siberian roe deer using fecal-DNA capture-mark-recapture in Northeast China. *Animals* 10(7):1135. <https://doi.org/10.3390/an110071135>
- Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25(11):1451–1452. <https://doi.org/10.1093/bioinformatics/btp187>
- Lorenzini R, Garofalo L, Qin X, Voloshina I, Lovari S (2014) Global phylogeography of the genus *Capreolus* (Artiodactyla: Cervidae), a palaeartic meso-mammal. *Zool J Linn Soc* 170(1):209–221. <https://doi.org/10.1111/zooj.12091>
- Markov G, Zvy chaynaya E, Danilkin A, Kholodova M, Sugar L (2016) Genetic diversity and phylogeography of roe deer (*Capreolus capreolus* L.) in different biogeographical regions in Europe. *C R Acad Bulg Sci* 69:579–584
- Markova A, Puzachenko A (2019) Mammal assemblages during the last glacial maximum (LGM) ($\leq 24 \geq 17$ Kyr BP). In: Markova A, Kolfschoten T, Puzachenko A (eds) Evolution of European ecosystems during Pleistocene–Holocene transition (24–8 Kyr BP). GEOS, Moscow, pp 44–56
- Matosiuk M, Borkowska A, Świsłocka M, Mirski P, Borowski Z, Krysiuk K, Danilkin AA, Zvy chaynaya 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. *Mol Ecol* 23(10):2559–2572. <https://doi.org/10.1111/mec.12745>
- Niedziałkowska M, Plis K, Marczuk B, Lang J, Heddergott M, Tainen J, Danilkin A, Kholodova M, Zvy chaynaya E, Kashinina N, Bunevich A, Paule L, Shkvyria M, Šprem N, Kusza S, Paulauskas A, Novák L, Kutal M, Miller C, Tsaparis D, Stoyanov S, Pokorny B, Flajšman K, Lavadinović V, Suchentrunk F, Krapal A-M, Dániľá G, Veeroja R, Jędrzejewska B (2024) Genetic diversity and complex structure of the European roe deer population at a continental scale. *J Mammal* 105:73–84
- Olano-Marin J, Plis K, Sönnichsen L, Borowik T, Niedziałkowska M, Jędrzejewska B (2014) Weak population structure in European roe deer (*Capreolus capreolus*) and evidence of introgressive hybridization with Siberian roe deer (*C. pygargus*) in northeastern Poland. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0109147>
- Pavelková Řičánková V, Robovský J, Riegert J (2014) Ecological structure of recent and last glacial mammalian faunas in Northern Eurasia: the case of Altai-Sayan refugium. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0085056>
- Plakhina DA, Zvy chaynaya EY, Kholodova MV, Danilkin AA (2014) Identification of European (*Capreolus capreolus* L.) and Siberian (*C. pygargus* Pall.) roe deer hybrids by microsatellite marker analysis. *Russ J Genet* 50:757–762. <https://doi.org/10.1134/S1022795414070151>
- Plis K, Niedziałkowska M, Borowik T, Lang J, Heddergott M, Tainen J, Bunevich A, Šprem N, Paule L, Danilkin A, Kholodova M, Zvy chaynaya E, Kashinina N, Pokorny B, Flajšman K, Paulauskas A, Djan M, Ristić Z, Novák L, Sz K, Ch M, Tsaparis D, Stoyanov S, Shkvyria M, Suchentrunk F, Kutal M, Lavadinovic V, Snjegota D, Krapal A-M, Danila G, Veeroja R, Dulko E, Jędrzejewska B (2022b) Mitochondrial DNA diversity and the population genetic structure of contemporary roe deer (*Capreolus capreolus*) in Europe. *Mamm Biol*. <https://doi.org/10.1007/s42991-022-00274-y>
- QGIS Development Team (2024) QGIS Geographic Information System. Open Source Geospatial Foundation Project. <http://qgis.org>
- Rambaut A (2014) FigTree, ver. 1.4.2. Program distributed by the author. <https://github.com/rambaut/figtree/releases>
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in bayesian phylogenetics using tracer 1.7. *Syst Biol* 67:901–904
- Randi E, Pierpaoli M, Danilkin A (1998) Mitochondrial DNA polymorphism in populations of Siberian and European roe deer (*Capreolus pygargus* and *C. capreolus*). *Heredity* 80:429–437. <https://doi.org/10.1046/j.1365-2540.1998.00318.x>
- Roch S, Nute M, Warnow T (2018) Long-branch attraction in species tree estimation: inconsistency of partitioned likelihood and topology-based summary methods. *Syst Biol* 68:281–97
- Salzburger W, Ewing GB, Haeseler AV (2011) The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. *Mol Ecol* 20(9):1952–1963. <https://doi.org/10.1111/j.1365-294X.2011.05066.x>
- Sommer R, Fahlke J, Schmölcke U, Benecke N, Zachos F (2009) Quarternary history of the European roe deer *Capreolus capreolus*. *Mammal Rev* 39:1–16. <https://doi.org/10.1111/j.1365-2907.2008.00137.x>
- Stubbe H, Bruchholz S (1979) Experiments in the hybridization of the European and Siberian roe deer (*Capreolus capreolus capreolus* L., 1758 x *Capreolus capreolus pygargus* Pall., 1771). *Zool Zh* 58(9):1398–1403

- Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A (2018) Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evol* vey016. <https://doi.org/10.1093/ve/vey016>
- Vorobieva NV, Sherbakov DY, Druzhkova AS, Stanyon R, Tsybankov AA, Vasil'ev SK, Shunkov MV, Trifonov VA, Graphodatsky AS (2011) Genotyping of *Capreolus pygargus* fossil DNA from Denisova cave reveals phylogenetic relationships between ancient and modern populations. *PLoS ONE* 6(8). <https://doi.org/10.1371/journal.pone.0024045>
- Xiao CT, Zhang MH, Fu Y, Koh HS (2007) Mitochondrial DNA distinction of Northeastern China roe deer, Siberian roe deer, and European roe deer, to clarify the taxonomic status of Northeastern China roe deer. *Biochem Genet* 45(1):93–102. <https://doi.org/10.1007/s10528-006-9052-z>
- Zvychainaya EY, Danilkin AA, Kholodova MV, Sipko TP, Berber AP (2011) Analysis of the variability of the control region and cytochrome b gene of MtDNA of *Capreolus pygargus* pall. *Biol Bull* 38(5):434–439. <https://doi.org/10.1134/S1062359011050189>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.