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ARTICLE

Glacial refugia, postglacial dynamics, and hybrid zones of Pinaceae in Eurasia captured from sedimentary ancient DNA

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Abstract

Boreal forests cover nearly one-third of global forest area. Glacial cycles have shaped the distribution and connectivity of modern Pinaceae genera, yet species-level refugia, postglacial migrations, and hybridization patterns remain

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unclear due to limited high-resolution taxonomic and temporal data. We applied a hybridization capture approach targeting complete chloroplast genomes of *Abies*, *Larix*, *Picea*, and *Pinus* to sedimentary ancient DNA (*sedaDNA*) from 238 samples across 19 sediment records spanning the last 40,000 years from Europe, Asia, and Alaska. Methodological improvements enhanced sequencing depth and genome coverage, enabling detection of low-abundance taxa and the establishment of semiquantitative conifer time series. Population dynamics were inferred from single-nucleotide-polymorphism (SNP)-based species identification, admixture analysis, and haplotype network reconstruction. Previously undocumented glacial refugial Pinaceae populations were identified in western Taymyr, southern Yakutia, and Far East Siberia. Postglacial recolonization began with *Larix* as a pioneer during the Late Glacial, followed by *Picea* and *Pinus* in subsequent warming phases. On the eastern Tibetan Plateau, *Picea asperata* and *Picea purpurea* were dominant. Postglacial Pinaceae dynamics across Europe showed early dominance of *Pinus sylvestris* in high latitude (Kola Peninsula) and *Pinus cembra* and *Pinus mugo* in alpine regions (Swiss and Dinaric Alps) from the Late Glacial, later replaced primarily by *Picea abies*, which dominated in Czechia, southwestern Russia, and possibly southern Sweden, while *Pin. sylvestris* remained dominant in northern Germany. We also identified undocumented populations, including a potential hybrid spruce, possibly *Picea fennica*, expanding in the Kola Peninsula during the Early Holocene, and scattered *Larix decidua* populations across Europe in the Early and Middle Holocene. In the Dinaric Alps, *Picea* populations shifted from eastern to western lineages during the Younger Dryas–Holocene transition. In south-central Alaska, *Picea glauca* colonized early and expanded in the Late Holocene. Additionally, we documented potential hybridization zones across Europe between *Pic. abies* and *Picea obovata*, *Abies sibirica* and *Abies alba*, and *L. decidua* and *Larix sibirica*, as well as admixture among *Larix* species and between *Pinus sibirica* and *Pinus pumila* in Siberia. In conclusion, hybridization capture of *sedaDNA* provided high-resolution insights into past Pinaceae dynamics in Eurasia, supporting future paleogenomic research and forest restoration management.

KEYWORDS

boreal forest, chloroplast genome, glacial refugia, hybrid zones, hybridization capture, paleogenomics, Pinaceae, postglacial migration, sedimentary ancient DNA (*sedaDNA*)

INTRODUCTION

Boreal forests account for approximately 27% of the world's forested area (FAO, 2025). Predominantly located between 45° and 70° latitude across Eurasia (Fennoscandia, Russia) and North America (Canada, Alaska), they are primarily composed of conifers, along with broadleaf species, such as *Betula* and *Populus* (Kuusela, 1990; Neale & Wheeler, 2019). Boreal forests provide essential ecosystem services, including climate

regulation, permafrost stabilization, and wildlife habitat preservation (Apps et al., 2006; Bonan & Shugart, 1989). However, boreal ecosystems are increasingly challenged by environmental changes, including increasing extreme winter cold and summer warmth, drought, wildfire danger, and permafrost degradation, alongside human activities, such as deforestation (Jones et al., 2022; Sidorova et al., 2021; Wang et al., 2022). In addition to these present-day challenges, the current distribution of boreal forests has been heavily shaped by glacial cycles,

particularly by the Last Glacial Maximum (LGM, 23–19 cal ka BP; Clark et al., 2009), rather than solely by modern climate (Herzschuh, 2019; Herzschuh et al., 2016). However, our understanding of long-term forest dynamics remains limited due to a lack of high-resolution, species-level time series data on past conifer forest composition.

Modern circumboreal forests of western Eurasia are predominantly composed of evergreen needle-leaf species, such as Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*), while black spruce (*Picea mariana*) and white spruce (*Picea glauca*) are prevalent across northern North America (Pan et al., 2013; Woodward et al., 2004). In contrast, deciduous needle-leaf larch trees, which are better adapted to extreme cold and less prone to winter damage than evergreens, dominate the forests of eastern Eurasia (Herzschuh, 2019; Herzschuh et al., 2016). Specifically, *Larix sibirica* is primarily found between the Ural Mountains and the Yenisei River, in regions without continuous permafrost, whereas *Larix gmelinii* and *Larix cajanderi*, with their shallow root systems adapted for permafrost conditions, dominate the permafrost landscapes of central and northeastern Siberia (Abaimov, 2010). However, a comprehensive understanding of the environmental and ecological drivers underlying these contrasting patterns of Pinaceae distribution and dominance across boreal forests remains elusive, limiting our perspective on how these ecosystems may respond to future environmental changes.

The Scandinavian Ice Sheet in Europe reached its maximum extent asynchronously during the LGM, covering areas from Great Britain in the west to central Germany and Poland in the south, and nearly to the Moscow Basin in the East European Platform (Hughes et al., 2016). Indeed, previous studies utilizing pollen, plant macrofossils, and sedimentary ancient DNA (*sedaDNA*) indicate that coniferous taxa in Europe primarily survived the LGM in southern refugia, such as the Pyrenees, the southern Alps, the Carpathian Mountains, and the southern Ural (Binney et al., 2009, 2017; Tollefsrud et al., 2008; Willis et al., 2000). Limited evidence also suggests that scattered *Picea* populations likely survived in northern refugia, such as on the East European Plain near the southern margin of the Scandinavian Ice Sheet (Binney et al., 2009), or in ice-free regions in northwest Norway, but remains unconfirmed (Alsos et al., 2020; Birks et al., 2012; Parducci et al., 2012). In contrast, much of the Russian Arctic east of the Kola Peninsula remained ice-free, allowing *Pinaceae* to persist in numerous scattered high-latitude refugia, including *Larix* populations on the Taymyr Peninsula, near their present northern limits, even during the LGM (Payette et al., 2002; Schulte, Meucci,

et al., 2022; Semerikov et al., 2013). However, the larger LGM refugia for conifers in Siberia were located in the Ural Mountains (Shuvaev et al., 2023; Shuvaev & Ibe, 2021), southern and central Siberia (Altai-Sayan region, Yenisei valley, Baikal region and Amur River basin; Aleksić & Geburek, 2010; Bezrukova et al., 2005; Shuvaev et al., 2023), and Northeast Siberia (Lena River and Kolyma Highlands; Ashastina et al., 2018; Petrenko et al., 2024). In North America, the Cordilleran and Laurentide Ice Sheets covered parts of the northern United States and most of Canada, with forest trees mainly surviving in southern refugia and in eastern Beringia (Anderson et al., 2006; Payette et al., 2002; Zazula et al., 2006). Identifying previously unrecognized northern Eurasian refugia is crucial for evaluating their genetic contribution to postglacial population dynamics and their influence on migration rates. Yet, detecting low-abundance populations at the species level remains challenging, and the potential role of these refugia as contact zones between different Pinaceae species has rarely been explored. Such zones may have facilitated hybridization and increased genetic resilience during the LGM. Hybridization and introgression were likely frequent in multispecies refugia (Krutovskii & Bergmann, 1995; Neale & Wheeler, 2019; Tollefsrud et al., 2015), and subsequent expansion phases probably enhanced admixture through renewed gene flow (Neale & Wheeler, 2019). Recent work demonstrates that hybridization can broaden ecological niches (e.g., *Pic. abies* × *Picea obovata*; Karunarathne et al., 2024) and generate hybrids with increased tolerance to harsh conditions (Meucci et al., 2025).

Because chloroplast DNA (cpDNA) in Pinaceae is paternally inherited, it is particularly sensitive to introgressive hybridization and captures elevated intraspecific variation (Du et al., 2009; Neale & Wheeler, 2019). Traditionally, however, hybridization has been studied mainly using modern populations, as retrieving sufficiently complete *sedaDNA* for population-level analyses has been difficult (Twyford & Ennos, 2012). Targeting full chloroplast genomes in *sedaDNA* now provides a rare opportunity to assess hybridization directly in ancient populations and to investigate the role of hybrids in both glacial refugia and postglacial dynamics.

As the climate warmed during the Late Glacial (19–11.7 cal ka BP [calibrated 1000 years before present]), particularly during the Bølling/Allerød Interstadial (14.7–12.9 cal ka BP; INTIMATE project; Cohen & Gibbard, 2019), Pinaceae species recolonized areas from both large southern refugia and scattered northern refugia (Giesecke, 2013; Schulte, Meucci, et al., 2022). Early-successional genera, such as pines and larches, led this expansion, likely due to their

efficient long-distance dispersal capabilities (Edwards et al., 2014). Between 12.9 and 11.7 cal ka BP, the Younger Dryas stadial triggered abrupt Northern Hemisphere cooling, with the strongest effects in the North Atlantic and eastern high northern latitudes (60–90° N), while North America, particularly Alaska, experienced minimal to undetectable cooling (Carlson, 2013; Clark et al., 2012; Herzschuh et al., 2023; Kaufman et al., 2020). The extent to which the Younger Dryas influenced genetic patterns by shaping the encounter or isolation of Pinaceae populations—and consequently affecting population transitions at specific sites, shifts in species boundaries, and hybridization zones—remains poorly understood. Rapid warming at the onset of the Early Holocene (11.7–8.2 cal ka BP) progressed from west to east, advancing with the retreat of the Laurentide Ice Sheet. Alaska experienced the Holocene Thermal Maximum (HTM) between 11 and 9 ka, approximately 4 ka earlier than northeastern Canada (Clark et al., 2012; Heiri et al., 2014; Kaufman et al., 2004). This indicates that the HTM was highly time-transgressive. Nonetheless, evidence suggests that high-latitude Arctic temperatures peaked during the Middle Holocene (8.2–4.2 cal ka BP), at ca 7 ka BP, with an average of $1.6 \pm 0.8^\circ\text{C}$ above the 20th-century mean (Kaufman et al., 2004). This climatic transition from the Younger Dryas to the HTM likely caused the decline of some small, scattered Pinaceae populations, while others expanded as environmental conditions stabilized, potentially favoring late-successional species, such as spruces and firs, due to their superior competitive abilities in warmer climates (Feurdean et al., 2013; Giesecke, 2013; Tollefsrud et al., 2008). In the Late Holocene (4.2 ka BP–present), cooler and wetter conditions—likely influenced by reduced solar irradiance, increased surface albedo due to land cover changes, and intensified volcanic activity—led to a decline in Pinaceae abundance at northern latitudes (Kaufman & Broadman, 2023; Sjögren & Damm, 2019). Despite increasing paleoecological insight into postglacial Pinaceae dynamics, the roles of individual species and hybrids in competitive replacement remain poorly understood. Niche-specific dominance patterns and the distribution of low-abundance populations, such as *Larix* in Europe, are still largely unexplored across Eurasia. The response to postglacial environmental changes was likely shaped by both coordinated Pinaceae migration patterns—linked to shared climatic niches and joint refugia—and species-specific traits advantageous for particular environments and successional dynamics. However, comparable genetic data from multiple sites across broad geographic areas, which are necessary to study these processes, remain extremely rare.

To reconstruct the historical dynamics of Pinaceae, including glacial refugia, postglacial range dynamics, and

hybridization events, and leading to their current distribution, it is crucial to identify populations at the species level. This requires a high-resolution, targeted approach capable of detecting even low-abundance groups that may have persisted in refugia or as scattered individuals along migration routes. Traditional paleoecological methods, such as pollen and plant macrofossil analyses, have been pivotal in identifying glacial refugia and reconstructing past vegetation dynamics (Binney et al., 2009, 2017). However, pollen data interpretation is often complicated by species-specific differences in production, dispersal, and preservation (Prach et al., 2023; Vondrák et al., 2019), while plant macrofossils, though taxonomically more precise, are often spatially and temporally discontinuous, particularly limiting their effectiveness for detecting rare, low-abundance, or geographically scattered populations (Hu et al., 2009). Methods such as target capture, metabarcoding, and shotgun sequencing applied to *sedaDNA* offer broad taxonomic coverage and have proven valuable for reconstructing past and present biodiversity, although with different levels of taxonomic resolution (Revéret et al., 2023). While all three methods in principle may be used to obtain within-species-level resolution (Lammers et al., 2021, 2024), only target capture is the most thoroughly explored method used for this approach (Capo et al., 2021; Meucci et al., 2021; Schulte, Meucci, et al., 2022). Hybridization capture targeting the complete cp genome has shown high efficiency in identifying *Larix* species in Siberia and estimating historical abundance when compared to pollen data and metabarcoding results from the same locations (Schulte, Li, et al., 2022; Schulte, Meucci, et al., 2022). By targeting the entire species-specific cpDNA and capturing fragments shorter than the 85 bp typically required for metabarcoding markers, this method increases the likelihood of recovering target DNA from ancient sediments, even under conditions of poor DNA preservation. Therefore, it potentially enables more sensitive detection of species-specific abundances in glacial refugia and recolonization processes, as well as ecological dynamics such as succession and competition, even among rare taxa. However, since *sedaDNA* is susceptible to contamination and variable preservation, comparing the results with pollen and plant macrofossil data remains crucial for robust ecological interpretations. Conversely, the greater number of single nucleotide polymorphisms (SNPs) retrieved across the complete cp genome compared to metabarcoding and shotgun methods facilitates the potential reconstruction of population haplotype networks and enables admixture analysis. These analyses were not attempted in previous studies using hybridization capture of Pinaceae *sedaDNA* (Schulte, Li, et al., 2022; Schulte, Meucci, et al., 2022), yet

they offer valuable spatial and temporal links between sites, contributing to the reconstruction of historical migration routes.

In this study, we conducted a *sedaDNA* analysis using hybridization capture to target the complete cp genomes of four Pinaceae genera (*Picea*, *Pinus*, *Larix*, and *Abies*). The DNA was extracted from 19 sediment records collected across Europe, Asia, and North America, spanning the last 40,000 years. We estimated absolute and relative read counts of each Pinaceae genus in each sample, conducted SNP-based species identification, and employed admixture analysis and haplotype networks to achieve three main objectives: (1) identify potential glacial refugia, where Pinaceae species may have persisted through the LGM; (2) investigate postglacial Pinaceae dynamics in Eurasia and the roles of successional stages among the four Pinaceae genera within postglacial expansion (linking populations across sites); and (3) explore the contact between previously isolated populations or species and the role of hybrid populations in glacial refugia and postglacial expansion dynamics.

MATERIALS AND METHODS

Sequencing and *de novo* assembly of *Pic. obovata* chloroplast genome

Fresh needles from one *Pic. obovata* Ledeb. (also known as *Pic. abies* subsp. *obovata* [Ledeb.] Hultén) with an individual tree height of 22.5 m were collected in filter bags and dried on silica gel during fieldwork expedition in Central Yakutia (Russia, EN18081; 59.97044801° N, 112.987204° E). The samples were then stored at the Alfred Wegener Institute (AWI) Helmholtz Centre for Polar and Marine Research, Potsdam. DNA extraction followed the procedure described in Meucci et al. (2021). DNA library preparation, shotgun sequencing, and demultiplexing were performed by the BGI Genomics sequencing service. Paired-end sequencing (2 × 150 bp) was performed on a BGISEQ (DNBseq technology) PE150 platform (BGI Group, Yantian, Shenzhen, China). The bioinformatic pipeline for *de novo* assembly and cp genome annotation followed the procedure described in Meucci et al. (2021) with the following modifications. The draft cp genome was annotated using GeSeq v2.03 (Tillich et al., 2017) and subsequent manual curation using Geneious Prime (<https://www.geneious.com>). The annotated cp genome assembly of *Pic. obovata* is available from the National Center for Biotechnology Information (NCBI) under GenBank sequence number PV631374.

Sediment records

Samples from 15 lakes, two ancient lakes, one cave, and one slump sediment record were included in the cp genome target enrichment study (Figure 1, Table 1; Meucci et al., 2026: tab. 1).

Core subsampling, DNA extraction, and sequencing library preparation

Subsampling of sediment cores (Potsdam, Germany) (Meucci et al., 2026: tab. 1) was conducted as outlined in Epp et al. (2018), in the climate chamber of the Helmholtz Centre for Geoscience (GFZ) in Potsdam with minor modifications (see Appendix S1: Supplemental material and methods). Age models for the sediment records were adopted from the studies cited in Meucci et al. (2026: tab. 1). Radiocarbon dates from the Otto Mörsch Cave samples were calibrated using the IntCal20 atmospheric calibration curve (Reimer et al., 2020) in OxCal v4.4 (Ramsey, 2021). Calibrated ranges are reported at their highest probability intervals, which in this dataset range from 91% to 95.4%. For interpretative purposes, a single representative date was calculated as the median of each calibrated range (Meucci et al., 2026: tab. 5 and 6).

sedaDNA was extracted from all sediment samples in the paleogenetic laboratory at the AWI (Potsdam, Germany) using 1–3 g of sediment and the DNeasy PowerMax Soil Kit (Qiagen, Germany), following the manufacturer's protocol with minor modifications (see Appendix S1: Supplemental material and methods).

Extracted DNA was used for single-stranded DNA library preparation according to the protocols described in Gansauge et al. (2017) and Gansauge and Meyer (2013) with modifications described in Schulte, Meucci, et al. (2022). For each library, 30 ng of DNA extract was used. In each batch of seven libraries, a negative control (blank) library was included, using only diethylpyrocarbonate (DEPC) purified water instead of DNA. Library quantification was performed using quantitative polymerase chain reaction (Maricic et al., 2010), and fragment length distribution of the libraries was measured using the 4200 TapeStation System (Agilent, USA).

Hybridization bait construction

Four species-specific hybridization bait sets targeting the complete chloroplast genomes of *L. gmelinii*, *Pic. obovata*, *Pinus pumila*, and *Abies sibirica*, excluding the ribosomal RNA regions, were designed for hybridization capture. The bait sets targeting the cp genomes of *Pic. obovata*,

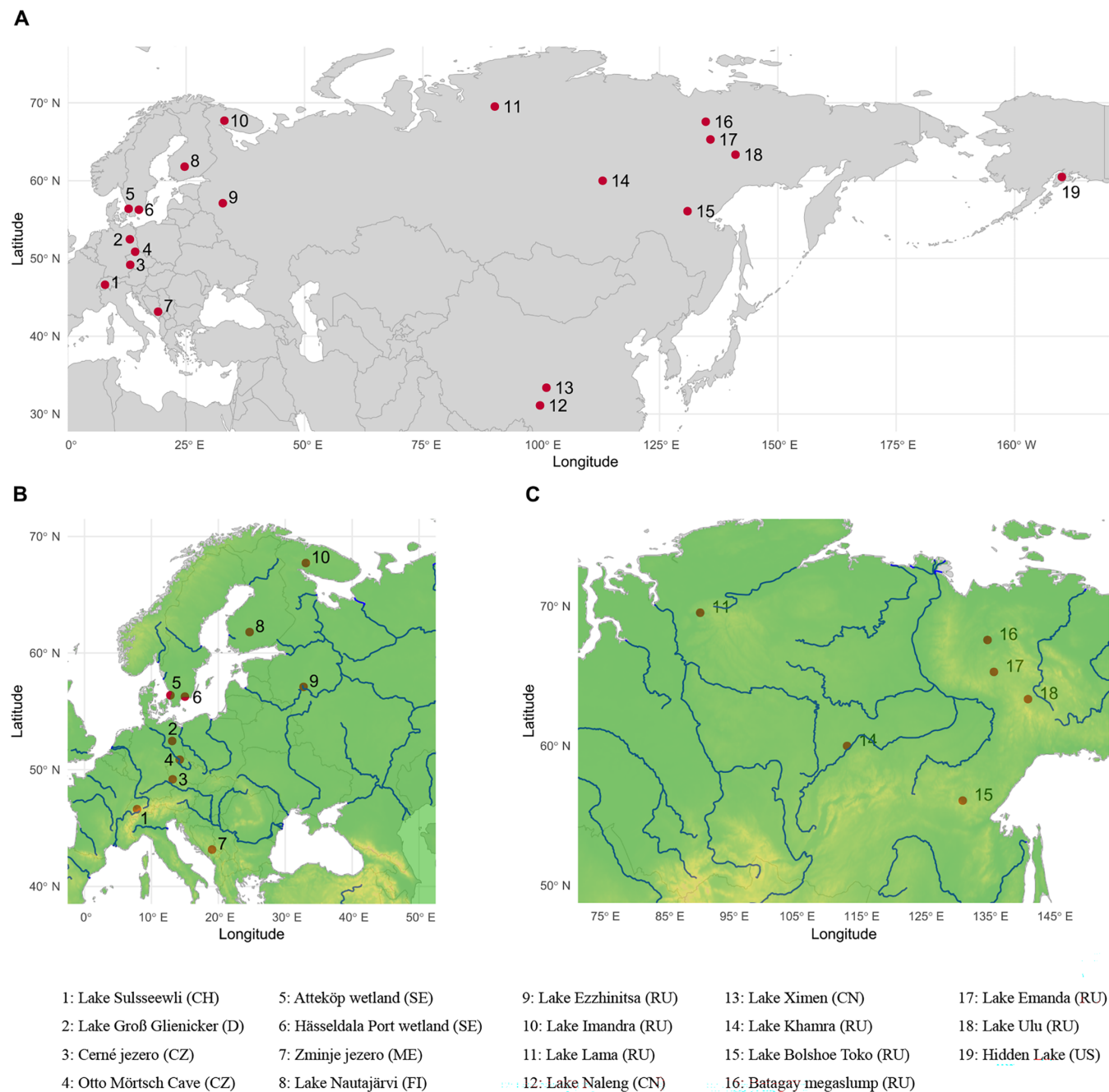


FIGURE 1 (A) Maps showing the locations of all sediment records included in this study, marked with red numbered dots corresponding to locations listed in the legend, and enlargements of (B) Europe and (C) central and eastern Siberia with color shading indicating altitude (green, low; yellow, high) and rivers shown in blue.

Pin. pumila, and *A. sibirica* were produced by long-range PCR using newly designed primers (Meucci et al., 2026: tab. 2) generated with the Primer3 2.3.7 plugin in Geneious Prime 2024.0.3 based on the species-specific cp reference genomes (NCBI GenBank: Meucci et al., 2026: tab. 3A). The bait set targeting the cp genome of *L. gmelinii* was produced by long-range PCR with previously designed primers (Meucci et al., 2026: tab. 2; Zimmermann et al., 2019). The bait sequence

construction is species-specific. However, the hybridization capture method can efficiently target also closely related yet phylogenetically divergent species within the same Pinaceae genus due to the highly conserved chloroplast genomes among them (Schulte et al., 2021). For this reason, we will refer to this as genus-specific targeting throughout the manuscript. Full experimental details of bait construction are provided in Appendix S1: Supplemental material and methods.

TABLE 1 Overview of study sites with corresponding map number, site name, location, coordinates, and elevation.

Site ID on map	Site name	Location	Latitude, longitude	Elevation (m)
1	Lake Sulsseewli	Northern Swiss Alps, Bernese Alps, Switzerland	46.617639, 7.864028	1921
2	Lake Gross Glienicker	Berlin–Brandenburg, Germany	52.4617, 13.11114	31
3	Černé jezero	Plzeň Region, Czechia	49.179167, 13.1825	1008
4	Otto Mörtzsch Cave	Ústí nad Labem Region, Czechia	50.862354, 14.231624	220
5	Atteköp wetland	Hallandsåsen, Sweden	56.383333, 12.85	175–180
6	Hässeldala Port	Blekinge Province, Sweden	56.266667, 15.016667	63
7	Zminje jezero	Dinaric Alps, Montenegro	43.155833, 19.070556	1535
8	Lake Nautajärvi	Central-southern Finland	61.8, 24.683333	104
9	Lake Ezzhinita	Tver Oblast, Russia	57.10, 32.75	213
10	Lake Imandra	Kola Peninsula, Russia	67.715833, 33.085	127
11	Lake Lama	Taymyr Peninsula, Russia	69.533333, 90.2	53
12	Lake Naleng	Southeastern Tibetan Plateau, Sichuan Province, China	31.104460, 99.75346	4200
13	Lake Ximen	Northeastern Tibetan Plateau, Qinghai Province, China	33.3792, 101.1035	4030
14	Lake Khamra	Lensky district, Republic of Sakha (Yakutia), Russia	59.99095, 112.98345	340
15	Lake Bolshoe Toko	Neryungri district, Republic of Sakha (Yakutia), Russia	56.07637, 130.9158	903
16	Batagay megaslump	Verkhoyansky district, Russia	67.58, 134.77	60
17	Lake Emanda	Tomponsky district, Republic of Sakha (Yakutia), Russia	65.17, 135.45	671
18	Lake Ulu	Oymyakon district, Republic of Sakha (Yakutia), Russia	63.34189, 141.04611	950
19	Hidden Lake	Kenai Peninsula, Alaska	60.47226, −150.21169	86

Note: Latitude and longitude are given in decimal degrees.

Target enrichment by hybridization capture

DNA libraries from each sediment core were pooled in equimolar amounts. Extraction and library blanks (LBs; controls) were also pooled using a fixed volume of 1 μ L. The hybridization capture experiment, targeting the cp genomes of the four Pinaceae genera, was conducted following the protocol of Maricic et al. (2010) with modifications according to Schulte, Meucci, et al. (2022) and the following additional adjustments: (1) 3000 ng of the pooled site-specific DNA libraries were hybridized with 500 ng of baits (125 ng for each genus-specific bait set); (2) only a single round of hybridization capture was performed for each pool (details on the input concentration, number of amplification cycles, and concentration of captured material after each hybridization are

provided in Meucci et al., 2026: tab. 4); (3) to minimize duplicates, the number of amplification cycles for the captured material was optimized to achieve the minimal DNA amount necessary for sequencing following the formula: “cycle number at the inflection point minus 12.”

Sequencing of target enriched libraries

The final enriched site-specific library pools targeting the cp genomes of the four Pinaceae genera were pooled equimolarly in a sequencing pool of 10 nM. Three sequencing pools were prepared (Meucci et al., 2026: tab. 5) and sequenced at AWI (Bremerhaven, Germany) in one flow cell (1000 mio reads) on an Illumina NextSeq 2000 instrument with P3 Reagents (200 Cycles) and 2 $\times$ 100 bp paired-end sequencing cycles.

Taxonomic binning

Demultiplexed and adapter-trimmed FASTQ files provided by the sequencing company were quality-checked using FASTQC (Andrews, 2015) both before and after deduplication and trimming. Initial deduplication was performed with CLUMPIFY v. 39.01 using the *clumpify.sh* script (Bushnell, 2014), followed by trimming with FASTP v. 0.23.2 (Chen et al., 2018) using the following setting parameters: `--merge`, `--length_required = 30`, `--overlap_len_require = 5`, `--correction`, `--low_complexity_filter`, `--cut_front`, `--cut_tail`, `--cut_window_size = 4`, and `--cut_mean_quality = 10`. After merging, a second deduplication was carried out on both merged and unmerged reads using the “dedupe.sh” script from CLUMPIFY v. 39.01 (Bushnell, 2014). Chloroplast-enriched DNA sequence reads were analyzed and classified with KRAKEN2 v. 2.1.2 (Wood et al., 2019) using a confidence threshold of 0.5 against the plastid database (RefSeq plastid release of NCBI downloaded in September 2023; O’Leary et al., 2016) with the addition of the newly assembled cp genome of *Pic. obovata* generated in this study. The database was built using KRAKEN2’s standard parameters: k-mer length of 35 bp and minimizer length of 31 bp. Merged and unmerged reads classified at the family level to Pinaceae were extracted using KRAKENTOOLS `extract_kraken_reads.py` v. 1.2 (Lu et al., 2022). The extracted Pinaceae reads were counted and plotted for each sample and site using the R (R Core Team, 2022) package *tidyverse* (Meucci et al., 2026: tab. 6; Wickham et al., 2019; Appendix S1: Figures S1–S19).

Reads classified as Pinaceae were aligned against a concatenated genome composed of the *Pic. obovata* (this study, available in NCBI under GenBank accession number PV631374), *Pin. pumila* (NCBI GenBank: NC_041108.1), *L. gmelinii* (NCBI GenBank: MK468637.1), and *A. sibirica* (NCBI GenBank: NC_035067.1) cp reference genomes (Meucci et al., 2026: tab. 3A) using BWA *aln* v. 0.7.17 (Li & Durbin, 2009) with the parameters `-l 1024 -o 2 -n 0.01`, as recommended for ancient DNA read mapping by Oliva et al. (2021). Mapping quality and length filtering were performed using BAMTOOLS *filter* (Barnett et al., 2011), with the parameters: `mapQuality “≥25” length “≥35.”` Further processing of the alignment files, including conversion, sorting, and indexing, was conducted using SAMTOOLS v. 1.16.1 (Li et al., 2009). A third round of deduplication was performed, as deduplication is more effective on aligned reads. Deduplication for both merged and unmerged reads was performed using PICARD *MarkDuplicates* v.3.1.0 (Broad Institute, 2019). The alignments from the merged and unmerged reads were then combined into a single BAM file using SAMTOOLS *merge* (Li et al., 2009).

Mapped reads to the concatenated genome were subsequently extracted into genus-specific BAM files using SAMTOOLS *view* v. 1.16.1 (Li et al., 2009), by specifying the base pair region range (first and last base pair number) of the four cp reference genomes. The breadth (bases covered by at least one read) and depth of coverage of bait regions were calculated using SAMTOOLS *depth* followed by custom AWK scripts to compute the mean depth and breadth of coverage for each sample (Meucci et al., 2026: tab. 6). Genus-specific reads were counted from the genus-specific BAM files and plotted for each sample and site using the R (R Core Team, 2022) package *tidyverse* (Meucci et al., 2026: tab. 6; Wickham et al., 2019; Appendix S1: Figures S1–S19). Genus-specific reads were extracted from genus-specific BAM files and converted to FASTQ format using SAMTOOLS *fastq* v. 1.16.1 (Li et al., 2009).

SNP calling and SNP-based species identification

To identify species-specific SNPs of each Pinaceae genus, available cp reference genomes from species expected in the defined geographical regions (Meucci et al., 2026: tab. 3D) were mapped to cp reference genomes from species that are phylogenetically and geographically distant from those expected at our sites using BWA *mem* v. 0.7.17 (Li & Durbin, 2009). For example, *Picea pungens*, mostly located in central United States, was selected due to its significant geographic distance and its different phylogenetic clade from the species likely present at our study sites (Lockwood et al., 2013; Meucci et al., 2026: tab. 3C). Using a distant outgroup reduces reference bias that could occur if alleles of closely related species match the reference sequence, thus providing a more balanced and species-informative SNP dataset. Variations were called using FREEBAYES v. 1.3.6 (Garrison & Marth, 2012) with the following parameter settings: `--min-alternate-count 1 --min-alternate-fraction 0 --haplotype-length 0`, and `--pooled-continuous`. From the resulting SNP dataset, variant positions, reference alleles, and alternative alleles were extracted using R (R Core Team, 2022). Ascertained species-specific SNPs were labeled accordingly (e.g., *Pic. obovata*) (Meucci et al., 2026: tab. 7). If multiple reference genomes from the same species were present, their SNPs were merged under the species name, ensuring that only species-level distinctions were maintained. When multiple reference species shared the same alternative allele at a given position, the SNP was excluded from the species determination analysis, as it was not phylogenetically informative. The number of species-specific SNPs decreased as more cp reference

genomes were included, due to the increasing number of common SNPs that were subsequently excluded. Therefore, the selection of reference genomes was restricted to species expected within the defined geographical regions (Meucci et al., 2026: tab. 3D).

The genus-specific reads from sediment samples were mapped to the same cp reference genomes from the phylogenetically and geographically distant species (Meucci et al., 2026: tab. 3C) using BWA *aln* v. 0.7.17 (Li & Durbin, 2009) with the parameters: `-l 1024 -o 2 -n 0.01`. Alignment files were processed with SAMTOOLS v. 1.16.1 (Li & Durbin, 2009) for conversion, sorting, and indexing. Read groups were added with PICARD *AddOrReplaceReadGroups* v.3.1.0 (Broad Institute, 2019). Genus-specific SNPs were jointly called for all sediment samples using FREEBAYES v. 1.3.6 (Garrison & Marth, 2012) with the options: `--pooled-continuous` and `--min-base-quality 10`. The resulting variant call files (VCFs) were processed with PLINK v. 1.90 (Chang et al., 2015) to remove samples with >90% missing data, while VCFTOOLS v. 0.1.16 (Danecek et al., 2011) was used to filter out indels, sites with >70% missing data, and minor alleles with frequencies below 1%. Variant positions, reference and alternative alleles, and read depth counts per sample were extracted from the each genus-specific SNP dataset using R (R Core Team, 2022).

To compare the genus-specific SNPs from sediment samples with ascertained species-specific SNPs from the reference alignment, the two datasets were merged in R (R Core Team, 2022) based on genomic position and allele identity. Genus-specific SNPs that matched ascertained species-specific SNPs were assigned to the corresponding species (Meucci et al., 2026: tab. 7), whereas SNPs absent from all reference genomes were classified as “unknown.” Variants occurring at positions where all reference genomes were identical were excluded from further analysis, as they were not phylogenetically informative. The absolute read counts and the relative proportion of reads assigned to each reference species were calculated and visualized for each site (Appendix S1: Figures S20–S29). Data were analyzed and visualized in R (R Core Team, 2022) using the package *tidyverse* (Wickham et al., 2019).

Ancient damage patterns, admixture analysis, and population TCS haplotype networks

Ancient damage patterns (postmortem C > T substitutions at read ends) were assessed from the Pinaceae-classified reads mapped to the concatenated genome (Appendix S1: Figure S30, upper panels) and

from the genus-specific reads mapped to the distant species reference genomes (Appendix S1: Figure S30, lower panels). All sample alignment files (BAM) of each site were merged together using SAMTOOLS MERGE (Li & Durbin, 2009). Ancient damage patterns were assessed using MAPDAMAGE2 v. 2.2.1 (Jónsson et al., 2013) providing the concatenated genome and the distant species reference genomes used for sample alignments (Meucci et al., 2026: tab. 3A,C). The frequency of C > T substitutions across the first 25 positions at the 5' end of both Pinaceae-classified reads, and genus-specific reads were extracted from the 5pCtoT_freq output file and plotted for each site.

Admixture analysis was conducted using genus-specific SNPs after excluding postmortem derived C > T substitutions, utilizing ADMIXTURE v. 1.3.0 (Alexander et al., 2009). To determine the optimal number of clusters (*K*), cross-validation analysis was performed (Appendix S1: Figure S31), evaluating up to 10 clusters. The results for the two values of (*K*) with the lowest cross-validation errors (the optimal [*K*] and the next best [*K*]) were plotted (Appendix S1: Figures S32–S35).

Population Templeton, Crandall, and Sing (TCS) haplotype networks were reconstructed for sediment samples using genus-specific SNPs. Postmortem derived C > T substitutions were removed from the genus-specific VCFs using BCFTOOLS v. 1.9 (Li, 2011). For each Pinaceae genus, the VCF files were converted into NEXUS format using PGDspider with haploid settings (Lischer & Excoffier, 2012). The NEXUS files were then used to construct TCS haplotype networks (Templeton et al., 1992) via POPART v. 1.7 (Clement et al., 2002) with default settings (Meucci et al., 2026: tab. 8). Only the SNPs with available data in all selected samples were included. Missing data for each sample were considered neutral, neither supporting nor contradicting the connections between nodes. Sample selection for each TCS network was guided by the research focus on specific species and their potential links across sites, while also accounting for the fact that including low-coverage samples would significantly reduce the number of informative segregating sites.

RESULTS AND DISCUSSION

Hybridization capture

This study implemented a target enrichment approach on *sedaDNA* extracted from sediment records retrieved from 15 lakes, 2 wetlands, 1 cave, and 1 permafrost soil archive across Europe, Asia, and western North America (Figure 1; Meucci et al., 2026: tab. 1). In total, DNA extraction and single-stranded library preparation were

conducted on 238 sediment samples, with an additional 45 DNA extraction blanks (EBs) and 45 LBs included as negative controls (Meucci et al., 2026: tab. 5). The hybridization capture experiment targeting the complete cp genomes of four Pinaceae genera (*Picea*, *Pinus*, *Larix*, and *Abies*) was conducted using equimolar pools of site-specific single-stranded DNA libraries, including a fixed volume for each negative control. Using equimolar DNA input enables site-specific comparisons of captured *sedaDNA* and interpretation without bias from DNA concentration differences. The captured *sedaDNA* was paired-end sequenced in three batches of equimolarly pooled samples, generating approximately 3610 million (M) raw reads (Meucci et al., 2026: tab. 6). Quality filtering and deduplication resulted in a total of 1298 M reads (~36% of total raw reads, in median 4.7 M per sample), with 99.98% originating from sediment samples and 0.01% from negative controls (the negative control IE119L-8 from Batagay megaslump was removed from this calculation because it generated an exceptionally high number of raw reads; however, only one read was classified as Pinaceae).

A median of 992 reads per sample (0–244,563) were classified at the family level of Pinaceae (see plots in Appendix S1: Figures S1–S19). To enable competitive mapping and taxonomic discrimination, these reads were aligned to a concatenated reference genome composed of complete cp genomes from the four Pinaceae genera used in the bait design (Meucci et al., 2026: tab. 3A). Applying a competitive mapping strategy, reads with low mapping quality (<25 Phred score) and short length (<35 bp), which are prone to unspecific mapping on the concatenated reference, were filtered out. This resulted in a median of approximately 50% high-quality mapped reads among those classified as Pinaceae. Specifically, a total of 1.4 M reads were mapped to the concatenated reference genome, with 99.95% derived from sediment samples and 0.05% from negative controls. An average of 6070 high-quality mapped reads was obtained for the sediment samples, while negative controls averaged 7.2 reads, with 76 controls below and 14 above this average (Meucci et al., 2026: tab. 6). Specifically, the average number of reads per species in negative controls was 5.4 for *Picea*, 1.0 for *Pinus*, 0.5 for *Larix*, and 0.3 for *Abies*. According to Schulte, Meucci, et al. (2022), the exclusion of reads from negative controls does not substantially affect the overall patterns observed in the sample data. However, we applied a conservative threshold of six reads—slightly above the highest species-specific average (for *Picea*) in the negative controls—as the minimum read count required for interpreting a sample's result. Each blank exceeding this threshold was individually evaluated by subtracting its read counts from the

corresponding samples to assess potential contamination. All data underlying these assessments are fully accessible in Meucci et al. (2026: tab. 6), where blanks and corresponding samples can be traced via their library numbers. Guidelines for locating these data are provided in Appendix S1: Supplemental materials and methods.

High-quality genus-specific reads, mapped to their respective cp reference genomes within the concatenated reference, were extracted, quantified, and visualized across all samples from each site (Appendix S1: Figures S1–S19). These genus-specific reads were subsequently mapped to one phylogenetically and geographically distant cp genome per genus (see *SNP calling and SNP-based species identification*), enabling consistent and comparative SNP calling across all samples from all sediment records within each Pinaceae genus. SNP-based species identification was conducted using only single-species variants located at positions derived from the alignment of available cp reference genomes of species likely present in the studied region (Meucci et al., 2026: tab. 3D). Reads assigned via these single-species SNPs were quantified and visualized for each sample (Appendix S1: Figures S20–S29). As 133 was the minimum number of genus-specific reads that allowed SNP-based species identification in our dataset, we considered counts between 6 and 133 to fall below the identification threshold, suggesting that interpretations based on such low read numbers should be approached with caution. Low counts may be influenced by very sparse local individuals, long-distance pollen transport, or differential DNA preservation related to sediment composition. By integrating independent published proxies and multiple analytical approaches, we interpreted these signals cautiously while still recognizing consistent ecological patterns where supported.

The analysis of ancient DNA damage patterns was conducted on both Pinaceae-classified reads mapped to the concatenated genome and the genus-specific reads mapped to the distant species reference genomes, merging all samples for each site. All records exhibited elevated frequencies of C > T substitutions at the first 25 read positions, consistent with characteristic postmortem damage and supporting the authenticity of *sedaDNA* (Appendix S1: Figure S30).

In comparison to the study of Schulte, Meucci, et al. (2022), which performed hybridization capture targeting *Larix* cp genome, this study used increased DNA library input, reduced amplification cycles for captured libraries, and optimized read deduplication procedures. Additionally, we lowered the confidence threshold for taxonomic classification to increase the number of reads available for competitive mapping to the concatenated genome of four genus-specific cp reference genomes.

Despite this, we applied stricter filtering for mapping quality and short read length. In summary, we achieved a similar percentage of raw reads remaining after filtering and deduplication (36% in this study compared to 35% in Schulte, Meucci, et al., 2022) with a higher percentage of reads taxonomically assigned to the target species (~8% of Pinaceae, averaging 12% per sample, compared to 1% of *Larix* in Schulte, Meucci, et al., 2022). We also achieved a higher maximum breadth of coverage of bait regions by high-quality mapped reads (89% vs. 71.5%) and a higher maximum read depth (81 vs. 6 reads). In summary, the adopted modifications to the wet lab experiment and data analysis pipeline led to improved target enrichment, characterized by highly deduplicated, diverse, and more comprehensive coverage of bait regions in the targeted captured sequences.

Early migration of pine and spruce in northwestern Russia

In summary, the captured Pinaceae record from the Lake Imandra sediment core (western Kola Peninsula) indicates a potential signal of Pinaceae presence during the Younger Dryas, followed by an Early Holocene expansion of *Pin. sylvestris* and a distinct hybrid spruce population potentially representing *Picea fennica*.

Our results showed a very low number of captured sequences from *Picea* and *Pinus* (8–33 reads) from 13.1 to 11.7 cal ka BP (Appendix S1: Figure S1; Meucci et al., 2026: tab. 6), which was insufficient for species determination. Similar uncertain occurrences during this interval have also been reported from *sedaDNA* metabarcoding studies (Poliakova et al., 2025), which concluded that regional vegetation was dominated by open tundra and that forest development was unlikely under cold, dry conditions. Accordingly, the low read counts in our data require cautious interpretation due to the potential contribution of modern contamination, long-distance input of cpDNA via extra-regional pollen, as well as methodological and chemical biases associated with *sedaDNA* analyses (Strandberg et al., 2026). Nevertheless, these signals raise the question of whether Pinaceae may have reached the Kola Peninsula during the Younger Dryas, potentially earlier than suggested by pollen records alone (Lenz et al., 2021).

A gradual expansion of Pinaceae began at 10.5 cal ka BP, with *Pin. sylvestris* (Figure 2; Appendix S1: Figure S24) experiencing a significant increase, peaking at 5.7 cal ka BP and remaining dominant over other taxa until 5.1 cal ka BP. This was followed by a gradual decline until 0.2 cal ka BP. Our data suggest that *Pin. sylvestris* displayed early-successional characteristics,

likely due to its rapid growth and efficient seed dispersal (Edwards et al., 2014; Feurdean et al., 2013), allowing it to take advantage of the warm Early Holocene climate and expanding rapidly at high latitudes, such as the Kola Peninsula. A low-density expansion of *Picea* on the Kola Peninsula was observed between 10.5 and 5.1 cal ka BP (31–1290 reads). SNP-based species identification revealed an admixed pattern dominated by *Pic. obovata* variants (Figure 3A), although *Picea* in this region is generally considered *Pic. abies* today (Tollefsrud et al., 2008). However, admixture analysis identified a distinct ancestry, distinguished from the patterns attributed to *Pic. abies* and *Pic. obovata* (Figure 4A), suggesting the presence of a hybrid population between the two species. This population may potentially represent *Pic. fennica*, a putative hybrid taxon with intermediate morphology previously reported across the Russian Plain and Scandinavia (Potokina et al., 2013; Volkova et al., 2014). This interpretation aligns with morphological studies of cone and seed scale traits, which identified three distinct spruce groups in northwestern Russia: southern populations resembling *Pic. abies*, intermediate populations with features of both species (referred to as *Pic. fennica*), and northern populations more similar to *Pic. obovata* (Popov, 2010). This same ancestry was also detected in low proportions at Lake Nautajärvi (central-southern Finland) during the Middle and Late Holocene, at Lake Lama (western Taymyr Peninsula) during the Late Glacial, and at Zminje jezero (Dinaric Alps, Montenegro). The TCS haplotype network based on *Picea* variants further supported this pattern, revealing a distinct cluster of the Middle and Late Holocene samples from Lake Imandra (Figure 5A), which showed to be closer to samples from Siberian sites such as Lake Khamra and Lake Lama than to the southwestern Russian sample from Lake Ezzhinita. This was confirmed when retaining only the 5.1 cal ka BP sample, which allowed a higher number of shared segregating sites with other samples (Appendix S1: Figures S36A,B). We note, however, that admixture signals in *sedaDNA* represent pooled genetic information from potentially multiple individuals and catchment sources; thus, the observed co-occurrence of alleles should be interpreted cautiously as consistent with, but not conclusive evidence for, local hybridization.

In conclusion, our findings suggest that spruce populations from east of the Urals or Central Siberia migrated westward to the Russian Plain, where contact with northward-expanding *Pic. abies* may have led to hybridization, potentially resulting in hybrids described as *Pic. fennica*. This population subsequently spread at low density to the Kola Peninsula during the Early Holocene, within the already advanced establishment of

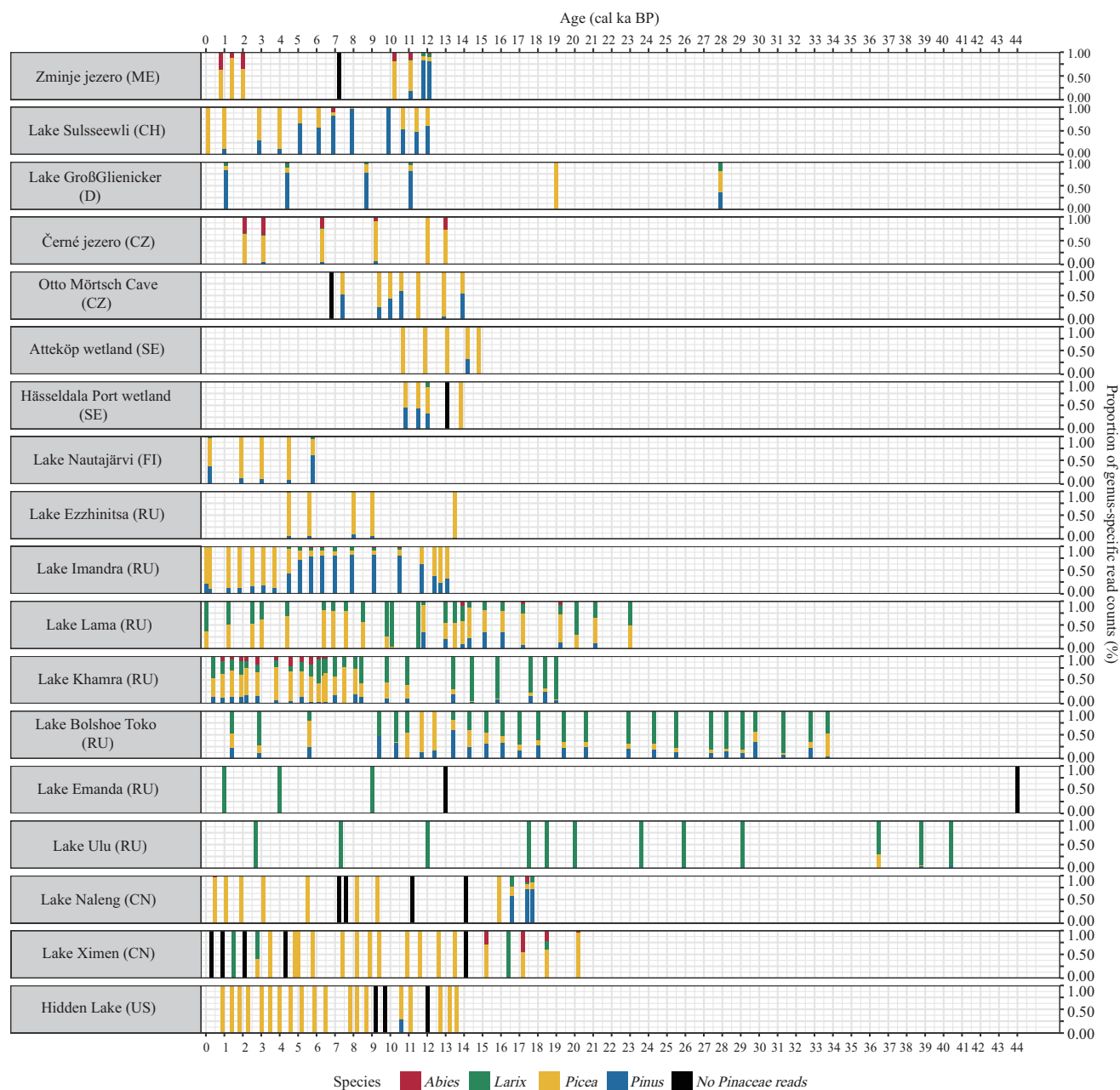


FIGURE 2 Proportion of genus-specific high-quality read counts in all samples and sites resulting from competitive mapping to the concatenated reference genome composed of the four complete genus-specific chloroplast (cp) genomes used for hybridization bait design (Meucci et al., 2026: tab. 3D) with the exception of the Batagay megaslump samples, which were excluded due to low resolution and out-of-range sample dating. Each bar represents the relative abundance of mapped reads for the four Pinaceae genera (*Abies*, *Larix*, *Picea*, and *Pinus*), using the threshold of six reads as the minimum expected read count and ordered by calibrated age (in calibrated 1000 years before present). Absolute counts are provided in Appendix S1: Figures S1–S19 and Meucci et al. (2026: tab. 6).

pinus (Väliiranta et al., 2011). This is consistent with previous genetic and morphological studies supporting widespread hybridization between *Pic. abies* and *Pic. obovata* across northern Eurasia during the Late Glacial and Early Holocene, leading to the formation of hybrid populations that expanded into Fennoscandia

(Chen et al., 2019; Tollefsrud et al., 2015; Tsuda et al., 2016; Volkova et al., 2014).

In contrast, the major *Picea* expansion during the Late Holocene likely involved different hybrid populations, predominantly related to *Pic. abies* lineages from western and southern Europe (Figure 3A; see

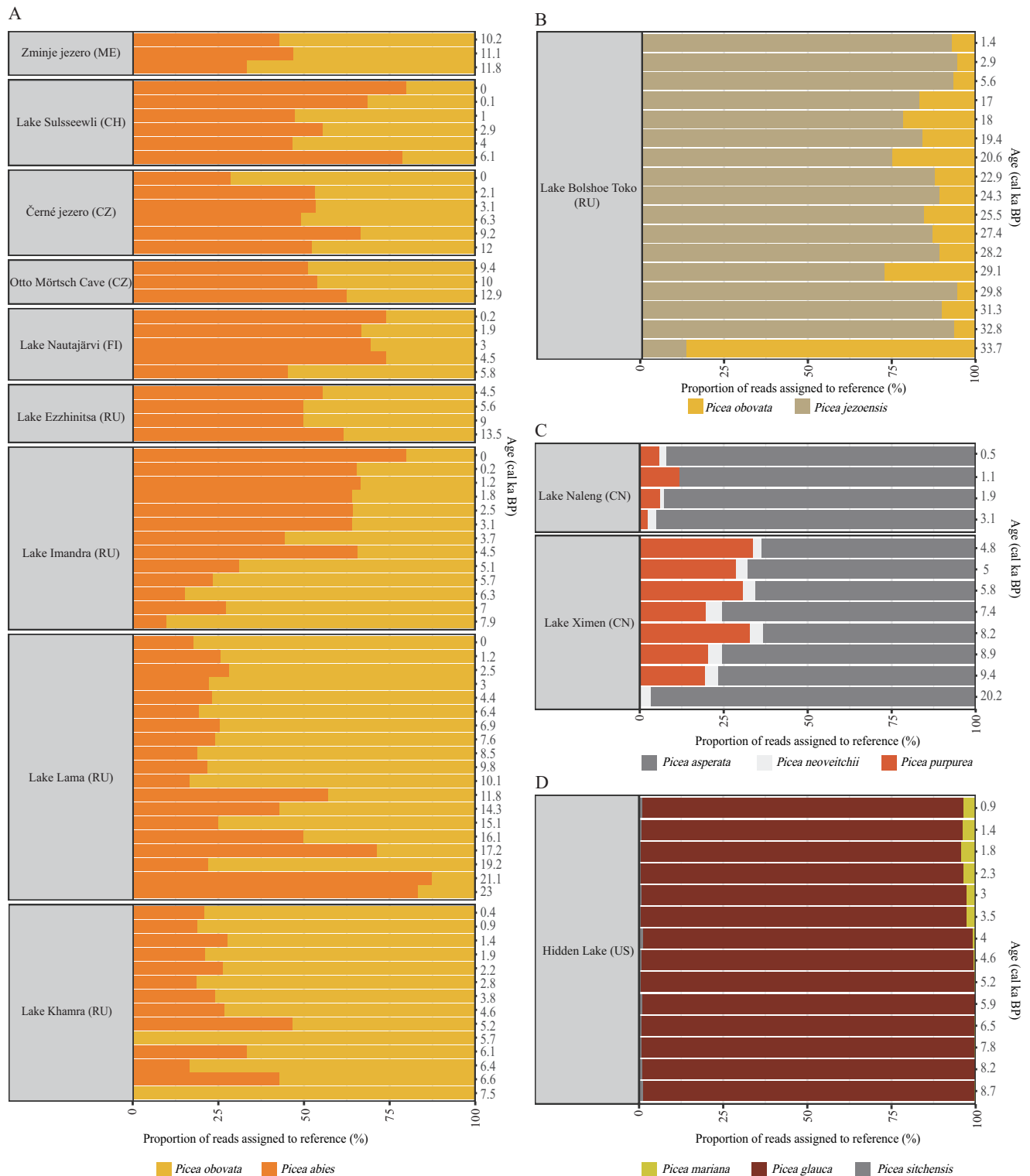


FIGURE 3 Proportions of read counts assigned to reference by single-nucleotide-polymorphism (SNP)-based species identification of *Picea*-specific reads mapped to the chloroplast (cp) reference genome of the phylogenetically and geographically distant species *Picea pungens* (NC_067714.1). Reads were assigned based on single-species variants ascertained from the alignment of species-specific cp reference genomes to *Pic. pungens* (Meucci et al., 2026: tab. 3D). Each bar represents a sample, ordered by calibrated age (in calibrated 1000 years before present) within each site.

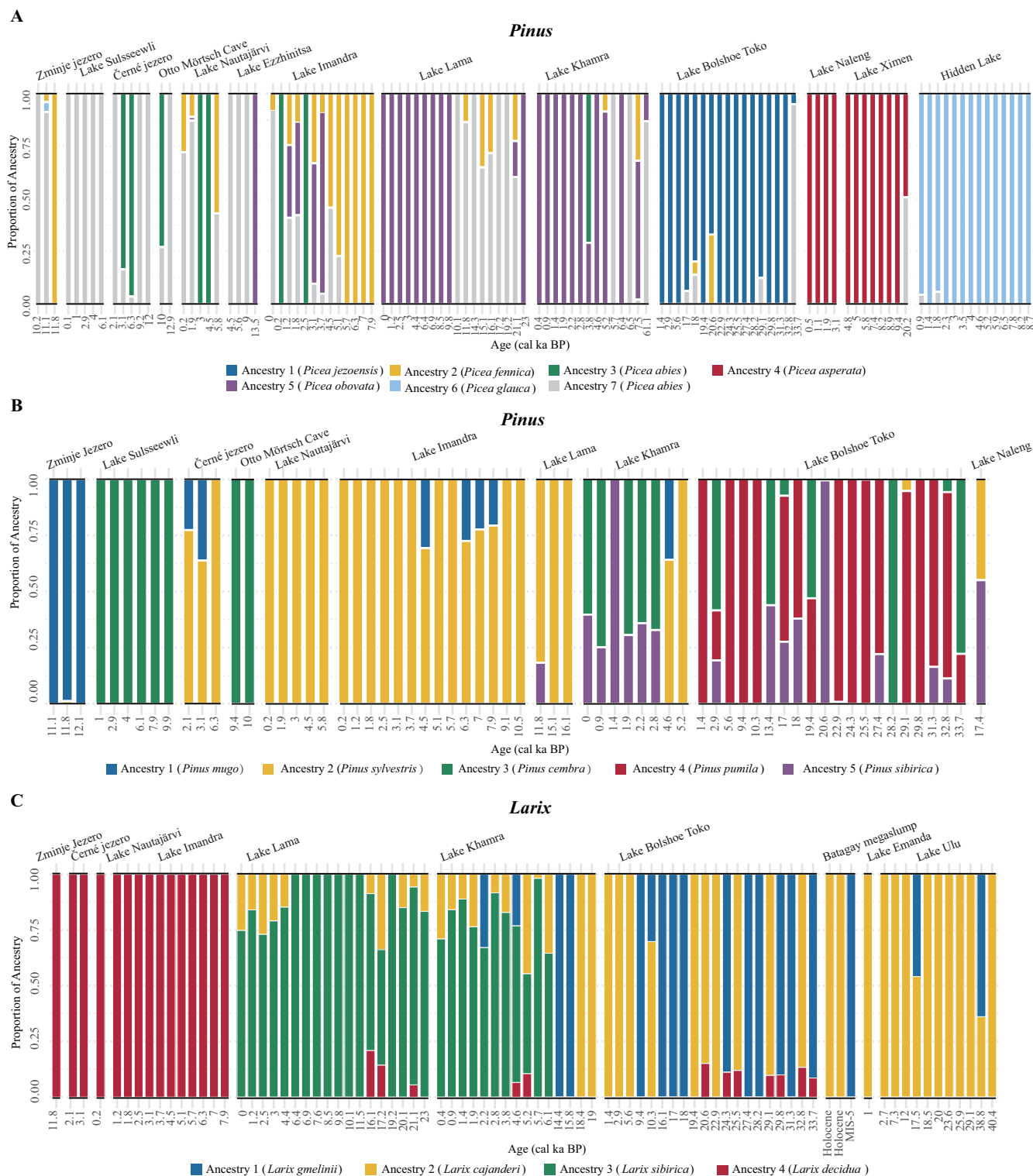
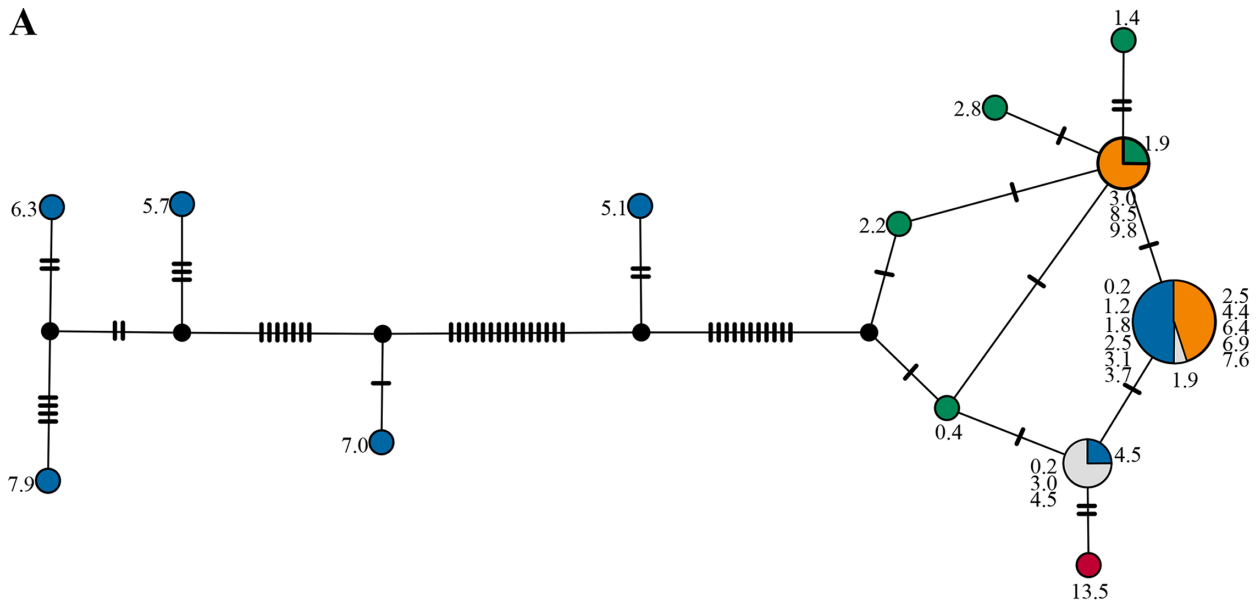


FIGURE 4 Admixture plots showing the proportions of genetic ancestries based on single-nucleotide polymorphism (SNP) variants for: (A) *Picea* (seven ancestries), (B) *Pinus* (five ancestries), and (C) *Larix* (four ancestries). Each vertical bar represents one sample ordered chronologically within each site. Sites are separated by empty bars and arranged from west to east by longitude. Colored segments indicate the proportional contribution of each inferred genetic ancestry. Species names assigned to each ancestry in the legend represent the most likely taxonomic interpretation based on the overlap of SNP-based species identification results, admixture patterns, and historical geographic distributions inferred from previous paleoecological proxies.

A



B

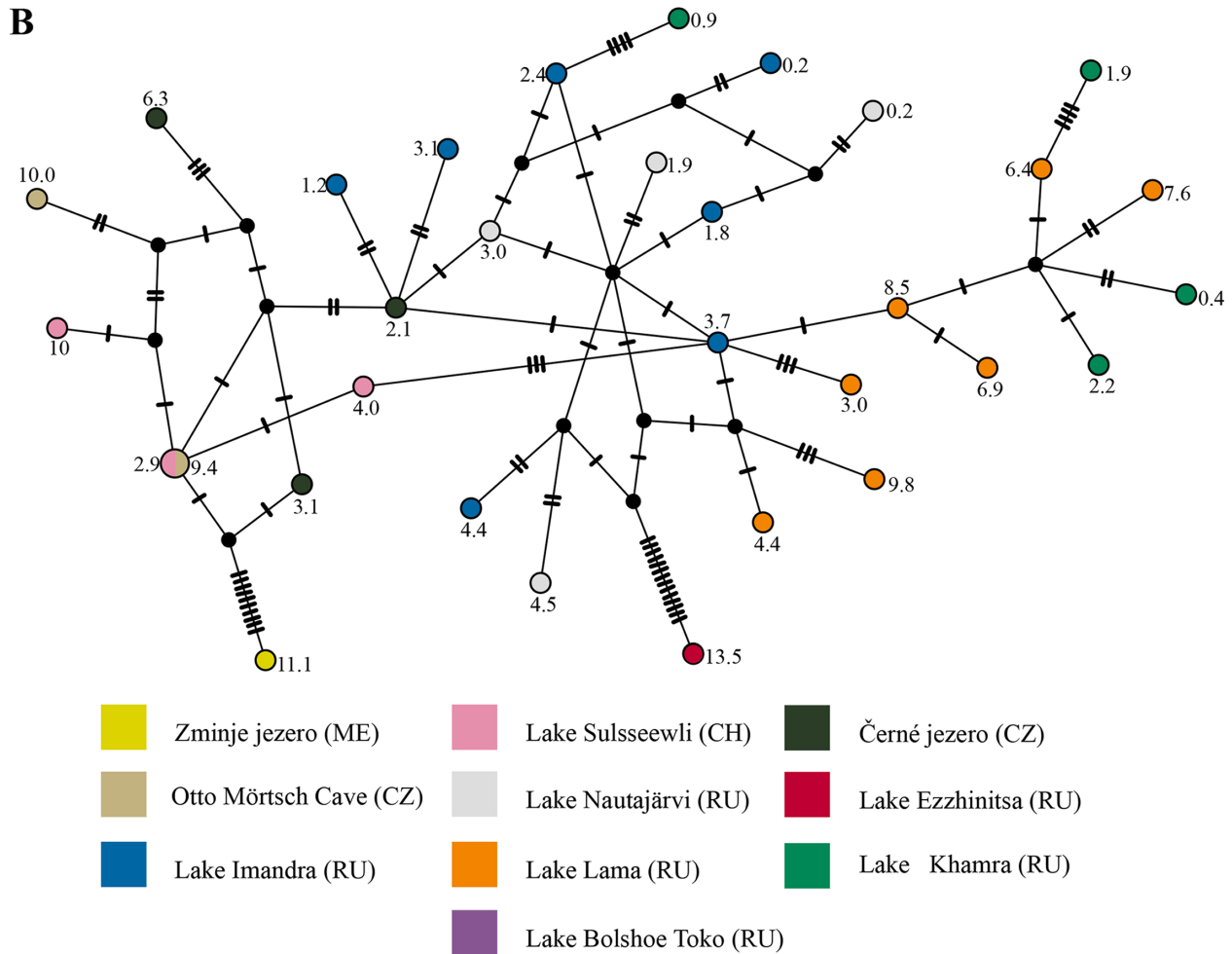


FIGURE 5 Templeton, Crandall, and Sing (TCS) haplotype networks based on *Picea* variants: (A) 46 segregating sites and (B) 56 segregating sites. Each circle represents a haplotype; larger circles indicate multiple samples sharing the same haplotype; size of the circle reflects the number of samples with this haplotype. Sample ages (in calibrated 1000 years before present) are shown next to circles. Hatch marks on edges indicate inferred mutations (bp differences). Circle colors correspond to sampling sites (see legend); black internal nodes represent inferred haplotypes.

Mid-to-Late Holocene expansion of Pinaceae across central and northern Europe and potential evidence of population contact). Despite the 99% identity (BLAST, NCBI) between the cp genomes of *Pic. abies* (GenBank: HF937082.1, NC_021456.1) and *Pic. obovata* (this study, submitted to NCBI under GenBank accession number PV631374), admixture analysis using seven ancestral populations ($K = 7$) successfully distinguished the genetic patterns of the two species. This was achieved despite a slightly higher cross-validation error for $K = 7$ compared to the optimal $K = 3$ (0.33 vs. 0.28) (Figure 4A; Appendix S1: Figures S31 and S32).

Eastern spruce populations expanded to southwestern Russia in the Late Glacial, central European populations during the Holocene

In summary, our data suggest a potential Late Glacial migration route of *Pic. obovata* or hybrids with *Pic. abies* genetically closer to eastern lineages, extending from central Siberia through northern Europe to southwestern Russia. Additionally, we identified a northward recolonization of *Pic. abies* from central Europe via southwestern Russia starting from the Early Holocene.

We detected a high number of *Picea* sequences in samples from Lake Ezzhinitza (Tver region, Russia) dating to the Late Glacial (13.5 cal ka BP; 1302 reads) (Appendix S1: Figure S2; Meucci et al., 2026: tab. 6). The 13.5 cal ka BP sample exhibited an admixed variant pattern of *Pic. abies* and *Pic. obovata* (Figure 3A; Appendix S1: Figure S20), with admixture analysis revealing an ancestry interpreted as *Pic. obovata*—shared with eastern populations from Lake Lama (north-central Siberia, Russia) and Lake Khamra (southwestern Yakutia, Russia) (Figure 4A). This pattern is further supported by the TCS haplotype network, which shows a closer genetic relationship to samples from northern and eastern sites rather than to those from central or southern Europe (Figure 5B). These results likely reflect a Late Glacial migration route of *Pic. obovata* or hybrids with *Pic. abies* genetically closer to eastern lineages, extending from central Siberia through northern Europe and reaching as far as southwestern Russia. Our findings are also in agreement with previous phylogeographic studies, which reported genetic differences between northeastern and central-southern European *Picea* populations (Tollefsrud et al., 2008). These differences were attributed to migration barriers such as the Carpathian Mountains and the spruceless area called Polish lowland disjunction (Chen et al., 2019; Lockwood et al., 2013; Tsuda et al., 2016). Niche differentiation might also play a role

in our signal as genetic analyses of modern *Picea* populations in the Scandes Mountains indicate that genotypes closer to *Pic. obovata* dominate under harsh conditions at high elevations (Korecký et al., 2021).

Holocene samples yielded a much lower number of captured sequences (68–248 reads) than the Late Glacial sample, possibly due to increased DNA degradation associated with warmer temperatures and related elevated microbial activity, and changes in lake physicochemical conditions (Capo et al., 2021; Jia et al., 2021). Holocene samples shared ancestry with central European populations, likely indicating *Pic. abies* (Figure 4A; see *Mid-to-Late Holocene expansion of Pinaceae across central and northern Europe and potential evidence of population contact*). This suggests a northward recolonization of *Pic. abies* from central Europe during the Early Holocene, potentially reaching as far as Lake Imandra on the Kola Peninsula from the Middle Holocene.

Spruce presence near the Laurentide ice margin in southern Sweden during the Late Glacial

In summary, our data support previous findings that *Pic. abies* was already present in southern Sweden, near the ice sheet margin, during the Late Glacial (Nota et al., 2022).

We captured a low number of *Picea* sequences (4–34 reads) in samples from two sites in southern Sweden—Atteköping and Hässeldala Port wetlands—dated between 14.8 and 10.7 cal ka BP (Appendix S1: Figures S3 and S4; Meucci et al., 2026: tab. 6). Although this sequence number was insufficient for SNP-based species identification, only one sample fell below the established threshold of six reads, which was based on the average read count observed in negative controls. Nevertheless, because long-distance pollen transport cannot be excluded, these sparse *Picea* reads may partly reflect extra-local input rather than strictly local presence. Despite the challenges of performing mapDamage analysis due to the low sequence count, the C > T substitution frequency displayed patterns characteristic of ancient DNA (Appendix S1: Figure S30). These results confirm earlier findings using the same sediment material, showing that *Pic. abies* was already present in southern Sweden near the ice sheet margin during the Late Glacial (Nota et al., 2022). Our findings are also in agreement with evidence from low-abundance pollen signals indicating the Early Holocene presence of *Picea* in the Scandes Mountains (Giesecke, 2005). However, our data do not allow us to speculate on whether this population originated from local survival near the southern Sweden ice margin or from early immigration from the Baltic region

(Giesecke, 2013; Nota et al., 2022). At both sites, the lowest number of Pinaceae reads was recorded at 13.1 cal ka BP (9 and 10 reads), coinciding with an early cooling phase in northern Europe preceding the onset of the Younger Dryas (Muschitiello et al., 2015), which might have caused a population decline in southern Sweden.

Spruce and fir presence in Czechia in the Late Glacial and Early Holocene

In summary, we detected an earlier-than-expected presence of *Pic. abies* and *Abies alba* during the Late Glacial and Early Holocene in Bohemia (Otto Mörtisch Cave and Černé jezero).

At the Otto Mörtisch Cave (northern Bohemia), we detected a low-abundance presence of *Picea* (15–280 reads) and *Pinus* (2–47 reads) in samples dated between 13.9 and 10.6 cal ka BP (Appendix S1: Figure S6; Meucci et al., 2026: tab. 6). Sample 12.9 cal ka BP had sufficient data (280 reads) to detect a SNP pattern identified as *Pic.*

abies via admixture analysis (Figure 4A), that is, significantly earlier than previous pollen records indicating its appearance after 9 cal ka BP (Pokorný et al., 2023).

A high number of captured Pinaceae sequences was detected in two samples dated to 10 and 9.4 cal ka BP, predominantly from *Picea* (5604–14,008 reads) and *Pinus* (4361–4989 reads), with *Abies* (142 reads) and *Larix* (14–29 reads) present in low abundance. In particular, SNP-based species identification and admixture analysis detected mainly *Pic. abies*, *Pinus cembra*, and *A. alba* (Figures 3A, 4, 6A, and 7). The presence of *Pin. cembra* is in agreement with previous evidence of its seeds being a staple food source in Mesolithic diets of hunter-gatherers (10.2–9.9 cal ka BP), supported by the discovery of charred seed shells at the Malý Faraon site in northern Bohemia (Pokorný et al., 2022). *Pin. cembra* likely had a much larger distribution area before the last glacial period and survived the LGM in refugia south of the Central European Alps and near the Carpathians, from where it expanded during the Late Glacial (Gugerli et al., 2023; Lendvay et al., 2014). However, our data do

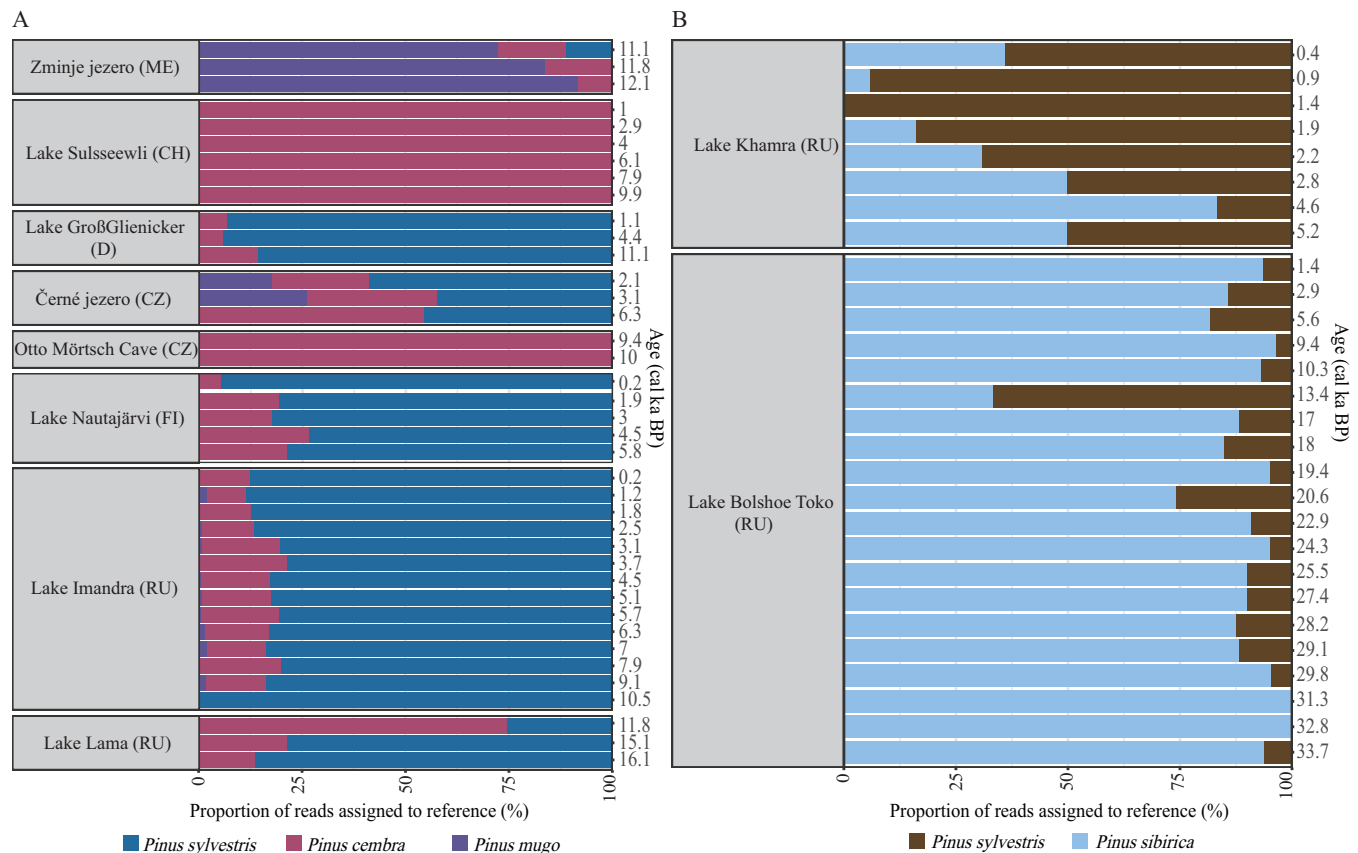


FIGURE 6 Proportions of read counts assigned to reference by single-nucleotide-polymorphism (SNP)-based species identification of *Pinus*-specific reads mapped to the chloroplast (cp) reference genome of the phylogenetically and geographically distant species *Pinus taeda* (NC_021440.1). Reads were assigned based on single-species variants ascertained from the alignment of species-specific cp reference genomes to *P. taeda* (Meucci et al., 2026: tab. 3D). Each bar represents a sample, ordered by calibrated age (in calibrated 1000 years before present) within each site.

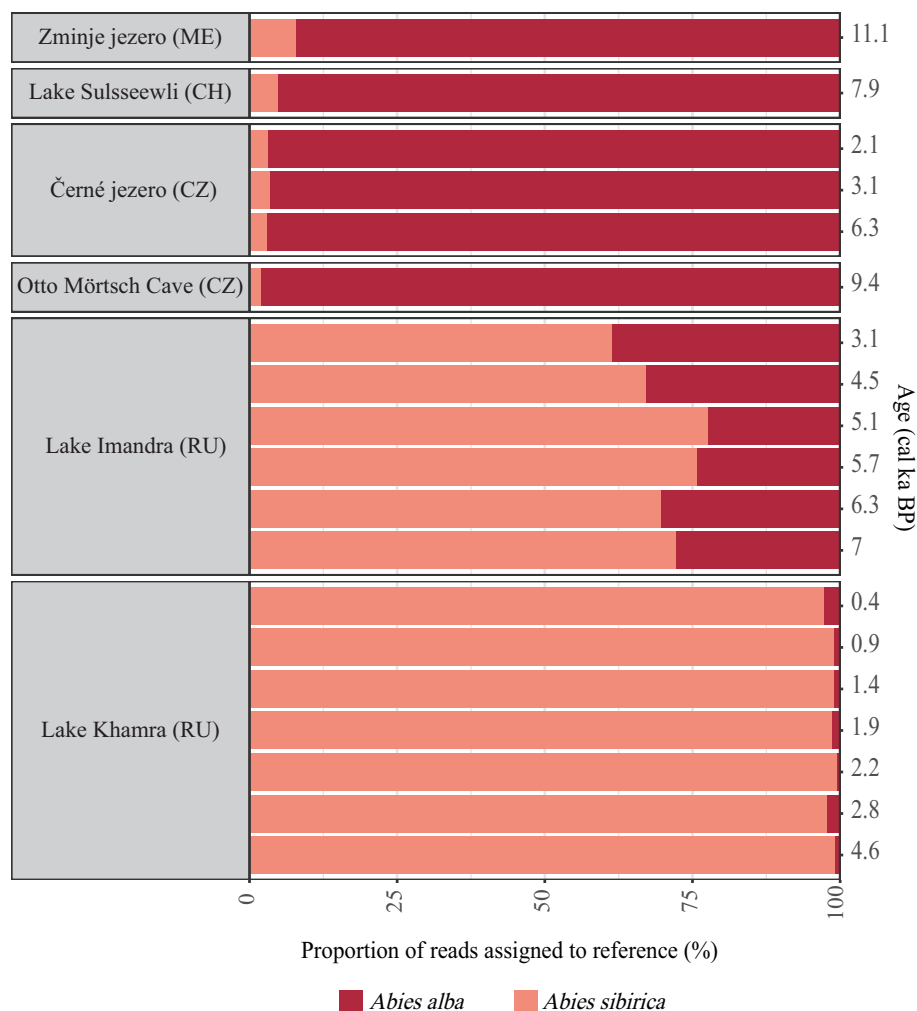


FIGURE 7 Proportions of read counts assigned to reference by single-nucleotide-polymorphism (SNP)-based species identification of *Abies*-specific reads mapped to the chloroplast (cp) reference genome of the phylogenetically and geographically distant species *Abies balsamea* (NC_042778.1). Reads were assigned based on single-species variants ascertained from the alignment of species-specific cp reference genomes to *A. balsamea* (Meucci et al., 2026: tab. 3D). Each bar represents a sample, ordered by calibrated age (in calibrated 1000 years before present) within each site.

not provide sufficient information to conclude the source of recolonization in northern Bohemia. We recorded *A. alba* at 9.4 cal ka BP, approximately 3000 years earlier than indicated by pollen records from the site (Pokorný et al., 2023) and the wider region (Liepelt et al., 2009).

A low number of Pinaceae sequences were identified in subsequent Middle Holocene samples (7.4 and 6.8 cal ka BP), likely due to poor DNA preservation. Indeed, pollen records indicate an abundant presence of *Pinus* throughout the entire Holocene (Pokorný et al., 2023). This degradation can be attributed to the cave's environmental conditions, including relatively high temperatures (7.8°C) and moisture levels, and a sedimentary environment characterized by high pH, a low sedimentation rate, and temporarily oxic conditions (Kukla et al., 2025).

At Černé jezero (southwestern Bohemia), in agreement with results from Otto Mörtzsch Cave, we observed an earlier-than-expected presence of *Picea* (40–689 reads) and *Abies* (15–27 reads) sequences in Late Glacial and Early Holocene samples (13–9.2 cal ka BP), together with *Pinus* (2–22 reads) at lower-than-expected abundance (Appendix S1: Figure S7; Meucci et al., 2026: tab. 6). This contrasts with the higher abundance of *Pinus* pollen during the Late Glacial reported in previous studies (Mateo-Beneito et al., 2024). This discrepancy might be explained by a combination of higher pollen production and dispersal capacity of *Pinus* and limited DNA preservation affecting the Late Glacial to Middle Holocene samples. Regarding *Abies*, as Otto Mörtzsch Cave confirms a signal at 9.4 cal ka BP, the earlier occurrence at Černé jezero may reflect very sparse local individuals, although

long-distance pollen transport or minor contamination cannot be excluded.

Mid-to-Late Holocene expansion of Pinaceae across central and northern Europe and potential evidence of population contact

In summary, we found paleogenomic evidence of a coordinated expansion of *Picea* during the Late Holocene across central and northern European sites, with closely related haplotypes likely reflecting contact between central-southern and northeastern European populations.

At Černé jezero (southwestern Bohemia), from 6.3 cal ka BP onward, we observed a notable increase in captured DNA sequences, indicating a mixed forest primarily dominated by *Picea* (3489–39,466 reads) and *Abies* (1213–22,600 reads), with *Pinus* (251–1105 reads) and *Larix* (47–229 reads) present in lower abundance (Appendix S1: Figure S7; Meucci et al., 2026: tab. 6). These patterns are consistent with pollen data from the site (Kuneš et al., unpublished) as well as with evidence from the broader region (Abraham et al., 2016; Kaiser et al., 2023).

SNP-based species identification showed the presence of *A. alba* (Figure 7), *Pinus* as predominantly represented by *Pin. sylvestris*, with *Pin. cembra* and *Pinus mugo* occurring in lower abundance (Figure 6A; see *Holocene Pinus dynamics in Eurasia*), and an admixed variant pattern in *Picea* and *Larix* identified as *Pic. abies* and *Larix decidua* by admixture analysis (Figures 3A, 4A, and 8A; see *Early migration of pine and spruce in northwestern Russia* and *Widespread low-abundance larch populations and evidence of hybridization across Europe during the Holocene*).

The TCS network (Figure 5B) further supports a close genetic relationship among *Picea* populations from central and southern European sites, consistent with the presence of *Pic. abies*. These samples were more closely related to Late Holocene populations from Lake Imandra (Kola Peninsula) and Lake Nautajärvi (central-southern Finland) than to the geographically nearer but much older sample 13.5 cal ka BP from Lake Ezzhinitza (southwestern Russia). Indeed, the latter was more closely related to northern European and Siberian sites, suggesting a Late Glacial connection between southwestern Russian and northeastern populations that was not present with central European populations. In contrast, *Picea* likely underwent a coordinated expansion during the Late Holocene across Europe, as indicated by parallel increases observed from 6.3 cal ka BP in Bohemia (Černé jezero; Appendix S1: Figure S7), around 4 cal ka BP at

high altitudes in the Swiss Alps (Lake Sulsseewli; Appendix S1: Figure S8), a minor increase in northern Germany (Lake Groß Glienicker; Appendix S1: Figure S9), and from approximately 4.5 cal ka BP in northern Europe (Lake Imandra and Lake Nautajärvi; Appendix S1: Figures S1 and S5), with peaks between 3.7 and 1.8 cal ka BP. These results support the hypothesis of a northward expansion of *Picea* in Europe, gradually replacing *Pinus* at higher latitudes, as reflected in a visible genetic shift toward *Pic. abies* variants at Lake Imandra (Figures 3A and 4A). This expansion was likely driven by climatic warming and wetting during the HTM (Giesecke, 2013; Giesecke et al., 2017; Lenz et al., 2021; Tollefsrud et al., 2008). Therefore, a contact between northeastern and central-southern European *Picea* populations was likely established, potentially facilitated by continuous or patchy forest corridors. Subsequently, cooling after approximately 1.9 cal ka BP led to reduced spruce abundance at both northern sites, with sharp declines possibly exacerbated by logging impacts in recent centuries at Lake Nautajärvi.

Potential low-abundance *A. sibirica* populations may have reached the Kola Peninsula during the Middle Holocene expansion

We detected low-abundance *Abies* sequences at Lake Imandra (Kola Peninsula; 34–221 reads) and Lake Nautajärvi (central-southern Finland; 14–75 reads), appearing from the Early (9.1 cal ka BP) and Middle Holocene (5.8 cal ka BP), respectively (Appendix S1: Figures S1 and S5; Meucci et al., 2026: tab. 6). This contrasts with previous pollen and metabarcoding studies, which did not detect *Abies* at these sites (Lenz et al., 2021; Ojala & Alenius, 2005; Poliakova et al., 2025).

SNP-based species identification was possible for *Abies* sequences from Lake Imandra, revealing an admixed pattern dominated by *A. sibirica* variants across all samples (Figure 7). These results suggest that low-density *A. sibirica* populations migrated westward from Siberia at the Early Holocene. During this expansion, they may have encountered *A. alba* populations migrating northward from southern refugia such as the Carpathians (Liepelt et al., 2009) and potentially resulting in hybridization. Our data indicate that these hybrid populations expanded as far north as the Kola Peninsula. This is consistent with palynological records of *A. sibirica* in the Russian Plain during the Early and Middle Holocene (Smirnova et al., 2018), which have been linked to westward migration via lowland corridors in the Mid-Urals (Hiebenga et al., 2024). Although

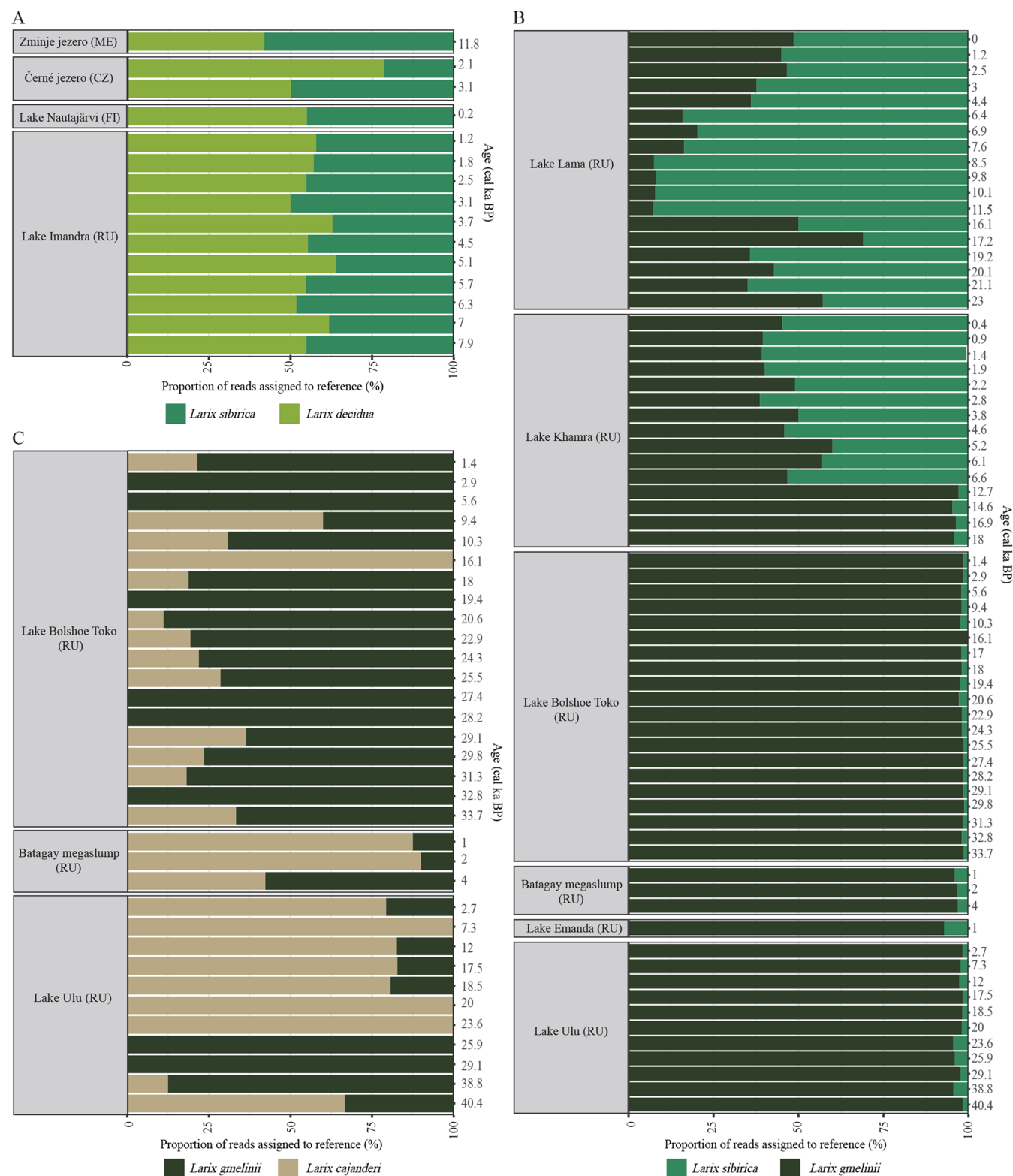


FIGURE 8 Proportions of read counts assigned to reference by single-nucleotide-polymorphism (SNP)-based species identification of *Larix*-specific reads mapped to the chloroplast (cp) reference genome of the phylogenetically and geographically distant species *Larix potaninii* (KX880508.1). Reads were assigned based on single-species variants ascertained from the alignment of species-specific cp reference genomes to *L. potaninii* (Meucci et al., 2026; tab. 3D). Each bar represents a sample, ordered by calibrated age (in calibrated 1000 years before present) within each site.

the presence of *Abies* at such northern latitudes had not been previously reported, *A. sibirica* pollen grains were found as far north as Lake Lyadhej-To, on the northwestern rim of the Polar Ural Mountains, during the Early Holocene (Andreev et al., 2005). However, its migration route remains unknown, whether via the Mid-Urals or through a northern Ural corridor.

Holocene *Pinus* dynamics in Eurasia

In summary, we found *Pin. sylvestris* dominating throughout the Holocene in northern Germany, and during the Early and Middle Holocene in northern Europe, while *Pin. cembra* was confined to higher elevations in the Alps and Sudetes. In Siberia, *Pin. sylvestris* reached its northernmost known range at Lake Lama on the Taymyr Peninsula during the LGM and Late Glacial. *Pinus sibirica* was found in southwestern Yakutia and *Pin. pumila* in the southeast, with potential contact and hybridization between the two taxa.

At Lake Groß Glienicker (northern Germany), Holocene samples revealed increasing abundances of all Pinaceae taxa, with *Pinus* consistently dominant (86–730 reads) over *Picea* (18–75 reads), *Larix* (6–54 reads), and *Abies* (4–21 reads) (Figure 2; Appendix S1: Figure S9; Meucci et al., 2026: tab. 6). This is largely consistent with pollen records (Andreev, unpublished), though the stronger *Pinus* signal in pollen likely reflects a combination of its high pollen production and dispersal capacity, along with potential poor *sedaDNA* preservation in Early to Middle Holocene sediments. Notably, *L. decidua* was absent from the pollen record (see [Widespread low-abundance larch populations and evidence of hybridization across Europe during the Holocene](#)).

At Lake Nautajärvi (central-southern Finland), only the oldest sample from the Middle Holocene showed higher *Pinus* than *Picea* abundance (Figure 2; Appendix S1: Figure S5; Meucci et al., 2026: tab. 6), reflecting an Early–Middle Holocene *Pinus* dominance that was gradually replaced by *Picea* from the Middle to Late Holocene. This shift is well visible at Lake Imandra (Figure 2; Appendix S1: Figure S1), where *Pinus* dominated over *Picea* from 11.7 to 5.1 cal ka BP. In contrast, pollen records from Lake Nautajärvi consistently show a stronger *Pinus* signal throughout the Holocene (Ojala & Alenius, 2005), likely due to its high pollen production and dispersal efficiency.

Low-abundance *Pinus* sequences (9–27 reads; Figure 2; Appendix S1: Figure S4; Meucci et al., 2026: tab. 6) were also detected at the Hässeldala Port wetland site

(southern Sweden) from 12 cal ka BP, although not in sufficient quantity for SNP-based species identification.

SNP data from Lake Imandra, Lake Groß Glienicker, and Lake Nautajärvi confirm *Pin. sylvestris* as the dominant species, with minor representation of *Pin. mugo* (Figure 6), as supported by admixture analysis (Figure 4B). In contrast, the low proportion of variants assigned to *Pin. cembra* across all sites—based on SNP-based species identification—likely reflects taxonomic misassignments of *Pin. sylvestris* variants due to their high sequence similarity (~96% identity; BLAST, NCBI), stemming from shared variants with underrepresented subpopulations in the available reference genomes.

In central Europe, *Pinus* was also found in low abundance at Černé jezero (southwestern Bohemia), within a forest dominated by *Picea* and *Abies*, and exhibited a variant pattern mainly assigned to *Pin. sylvestris*, with lower contributions from *Pin. mugo* (Figures 4B and 6A; Appendix S1: Figure S7; Meucci et al., 2026: tab. 6). At the Otto Mörtch Cave (northern Bohemia), *Pin. cembra* was detected from the Late Glacial (13.9 cal ka BP), and with particularly high abundance in the Early Holocene samples (10 and 9.4 cal ka BP; 4361 and 4989 reads), at times reaching levels comparable to *Picea* (Figure 2; Appendix S1: Figure S6; Meucci et al., 2026: tab. 6). At Lake Sulsseewli in the northern Swiss Alps, *Pin. cembra* dominated over other Pinaceae during the Early and Middle Holocene (12–5.1 cal ka BP; 7–7203 reads), but occurred in lower abundance in the Late Holocene within a *Picea*-dominated forest.

TCS network analysis (Figure 9A) revealed two major genetic clusters: one dominated by *Pin. cembra* (Lake Sulsseewli, Otto Mörtch Cave), and another by *Pin. sylvestris* (Černé jezero, Lake Gross Glienicker, Lake Imandra, Lake Nautajärvi). The haplotype from Zminje Jezero clustered with the latter group but showed greater divergence, possibly indicating the presence of *Pin. mugo* or *Pin. mugo*–*Pin. sylvestris* hybrids (Sikora & Celiński, 2024). Admixture analysis with five ancestral populations supports these genetic patterns (Figure 4B).

In Siberia, *Pinus* was present at low abundance between the LGM and the Early Holocene at Lake Lama (Figure 2; Appendix S1: Figure S11; Meucci et al., 2026: tab. 6), with read peaks at 18.4, 16.1, and 13.4 cal ka BP (8–278 reads). At Lake Khamra (Figure 2; Appendix S1: Figure S12; Meucci et al., 2026: tab. 6), *Pinus* reads remained low (8–50 reads) from 19 cal ka BP till the Middle Holocene, then increased from 2 cal ka BP (64–3838 reads), which coincides with known *Pinus* expansion in Yakutia recorded in pollen sequences from this site (Baisheva et al., 2024) and the

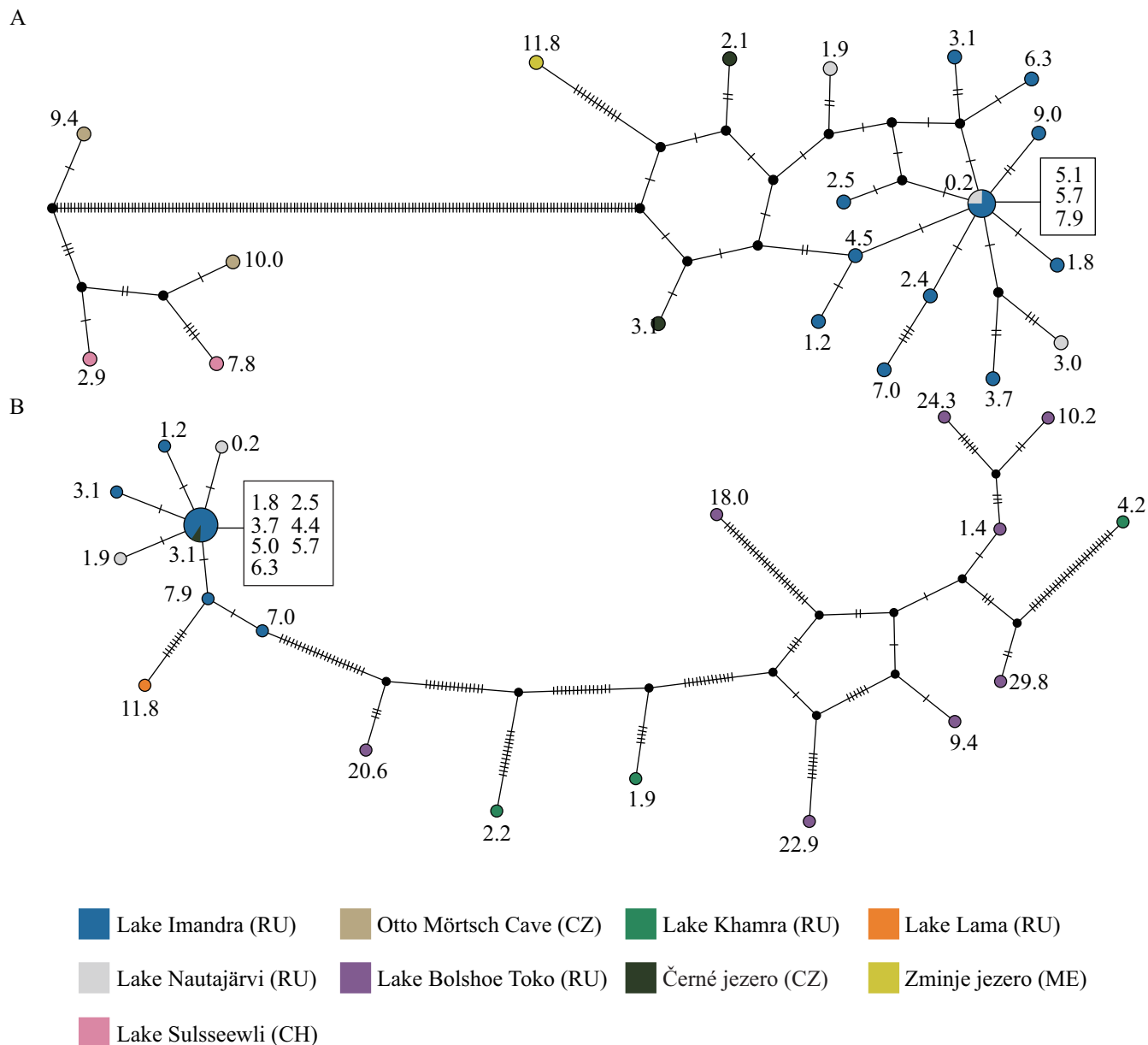


FIGURE 9 Templeton, Crandall, and Sing (TCS) haplotype networks based on *Pinus* variants: (A) 208 segregating sites and (B) 91 segregating sites. Each circle represents a haplotype; larger circles indicate multiple samples sharing the same haplotype; size of the circle reflects the number of samples with this haplotype. Sample ages (in calibrated 1000 years before present) are shown next to the circles. Hatch marks on edges indicate inferred mutations (bp differences). Circle colors correspond to sampling sites (see legend); black internal nodes represent inferred ancestral haplotypes.

wider region (Cao et al., 2019). At Lake Bolshoe Toko, *Larix*, *Pinus*, and *Picea* were relatively high abundant throughout the record (33.7–1.4 cal ka BP), with *Pinus* generally less abundant than *Larix* but more so than *Picea*. A notable decline at the Late Glacial–Holocene transition is potentially reflecting the impact of the Younger Dryas (Figure 2; Appendix S1: Figure S13; Meucci et al., 2026: tab. 6).

SNP-based species identification (Figure 6A) and admixture analysis (Figure 4B) indicate the presence of

Pin. sylvestris at Lake Lama, consistent with the TCS haplotype network (Figure 9B), which places Lake Lama closely related to the European sites characterized by *Pin. sylvestris*. At Lake Khamra, *Pin. sylvestris* was detected in low abundance during the Middle Holocene, while the dominant signal reflected admixture between *Pin. cembra* and *Pin. sibirica* (Figure 4B), likely representing *Pin. sibirica*, which could be considered a subspecies of *Pin. cembra* (*Pin. cembra* subsp. *sibirica*). At Lake Bolshoe Toko, *Pin. pumila* predominated (Figures 4B and

6B), with temporary incursions of *Pin. sibirica*, suggesting episodic expansions into southeastern Siberia. Modern genetic studies in the contact zone of these taxa have identified cryptic hybrids with *Pin. sibirica* morphology but *Pin. pumila* mitochondrial DNA, indicating interspecific hybridization (Petrova et al., 2018). Pollen data from Lake Bolshoe Toko (Courtin et al., 2021) detected *Pinus* subgenus *Diploxylon*-type pollen, indicating the presence of *Pin. sylvestris* in the lake vicinity from approximately 7 cal ka BP to the present; however, this was not supported by our results. The TCS haplotype network (Figure 9B) supports these findings, showing greater genetic diversity in samples from Lake Khamra and Lake Bolshoe Toko—characterized by multiple *Pinus* species and likely hybrids—compared to the low genetic structure observed in European sites dominated by *Pin. sylvestris*, consistent with previous evidence (Żukowska et al., 2023).

Holocene Pinaceae dynamics in the Swiss Alps

In summary, the results from Lake Sulsseewli (northern Swiss Alps) reveal an Early Holocene dominance of *Pin. cembra* and a subsequent Late Holocene expansion of *Pic. abies*, reflecting climate-driven succession and increasing anthropogenic influence on high-altitude forest composition.

We identified *Pic. abies*, *Pin. cembra*, and *A. alba* at Lake Sulsseewli by SNP-based species identification (Figures 3A, 6A, and 7). In particular, during the Early Holocene, we detected low read numbers of *Picea* (36 reads) in the 10.7 cal ka BP sample and *Pinus* (7–39 reads) in samples dated between 12 and 10.7 cal ka BP (Appendix S1: Figure S8; Meucci et al., 2026: tab. 6). Given the low read numbers, these signals should be interpreted with caution and may reflect very sparse local individuals occupying sheltered microsites or DNA input from lower elevation populations via long-distance transport (e.g., pollen). Nevertheless, their occurrence is consistent with postglacial recolonization from glacial refugia south of the Central European Alps (Gugerli et al., 2023; Latalowa & van der Knaap, 2006; Ravazzi, 2002), potentially persisting at very low abundance during the Younger Dryas (Appendix S1: Figure S8; Meucci et al., 2026: tab. 6). This was followed by an expansion of *Pinus* during the Early Holocene, identified as *Pin. cembra* through SNP-based species identification (Figure 6A), peaking at 7.9 cal ka BP. Early Holocene treeline rise with pine dominance is also reported from other sites in the Swiss Alps (Thöle et al., 2016). This coincides with rising temperatures and

increased precipitation during the HTM (Garcés-Pastor et al., 2022). In contrast, spruces remained in very low abundance (7–39 reads). Limited *A. alba* sequences (235 reads) were also detected, particularly at 7.9 cal ka BP (Figure 7) confirming pollen records from other sites in the Swiss Alps (Huonder et al., 2024). These findings suggest sparse spruces and firs within a pine-dominated forest, with pines likely serving as the pioneer conifer species in this region due to their superior adaptation to Alpine montane environments (Neale & Wheeler, 2019). Fluctuations in the abundance of both *Pinus* and *Picea* were observed during the subsequent period, with a decline at 6.9 cal ka BP, a slight increase at 6.1 cal ka BP, and another decline at 5.1 cal ka BP. These changes coincided with the onset of increased agricultural and grazing activities in the vicinity of Lake Sulsseewli (Garcés-Pastor et al., 2022).

A second expansion of *Pin. cembra*, though in lower abundance, occurred alongside a major expansion of *Pic. abies* (Figures 2 and 3A; see *Early migration of pine and spruce in northwestern Russia*) around 4 cal ka BP (3591 reads), peaking at 3 cal ka BP (11,980 reads) and becoming dominant over pines. This expansion was likely driven by a combination of Late Holocene climatic conditions and human activity that may have favored the growth of *Pic. abies*. These findings are consistent with previous pollen and metabarcoding data (Garcés-Pastor et al., 2022). The decline in *Pic. abies* abundance at 1 cal ka BP coincides with a sharp increase in microcharcoal concentrations reported by Garcés-Pastor et al. (2022), suggesting intensified fire activity likely linked to forest clearance. During the Late Holocene (2.9–1.0 cal ka BP), we also detected a very low-density presence of firs (34–35 reads) and evidence of rare occurrence of larches (15–33 reads), within a forest primarily dominated by spruces and pines.

Widespread low-abundance larch populations and evidence of hybridization across Europe during the Holocene

In summary, our data provide the first paleogenomic evidence of sparse *Larix* populations across northern and central Europe during the Early and Middle Holocene, indicating a more extensive Holocene range than previously recognized. The high levels of admixture between *L. decidua* and *L. sibirica* cp genomes suggest historical hybridization between the two species.

At Lake Imandra (Kola Peninsula), *Larix* showed a low signal starting at 10.5 cal ka BP (13 reads), increasing from 7.9 cal ka BP (185 reads), peaking at 5.7 cal ka BP (466 reads), and then gradually declined toward the Late

Holocene, resembling the dynamics of *Pinus* and *Abies* (Appendix S1: Figure S1; Meucci et al., 2026: tab. 6). However, *Larix* presence was significantly lower than *Pinus* but higher than *Abies*, suggesting that the Early and Middle Holocene featured a sparse presence of larches and firs within a pine-dominated forest, until the expansion of spruces, which outcompeted other Pinaceae genera in the Late Holocene. These findings may represent the first *sedDNA* record of larch populations at such a northern range on the Kola Peninsula, although the results should be interpreted with caution due to a relatively low number of captured sequences and the lack of *Larix* detection in pollen (Lenz et al., 2021) and metabarcoding record (Poliakova et al., 2025) from the same sediment core.

Previous evidence has documented a larch population along the southeastern coast of the Kola Peninsula, identified as a western population of *L. sibirica* and referred to as *Larix archangelica*, *L. sibirica* ssp. *rossica*, or *Larix sukaczewii* due to distinct morphological traits (Kozhin & Sennikov, 2016). This population is considered a relic of a formerly broader Middle Holocene distribution. Additionally, Holocene macroremains of *L. sibirica* (8.7–7.5 cal ka BP) were discovered in the Scandes Mountains, Sweden (Kullman, 1998), and recent pollen findings at Lake Kuutsjärvi, northeast Finland (Salonen et al., 2024), further extending the species' past range westward beyond its previously known Early Holocene limit in Northwest Russia (Kuosmanen et al., 2015). These findings contrast with our genetic data, which likely indicate the presence of *L. decidua* in these regions; however, the presence of hybrid populations offers a plausible alternative explanation.

We detected *Larix* populations also in northern Germany (Lake Gross Glienicker; 12–54 reads), northern Bohemia (Otto Mörtzsch Cave; 14–29 reads), and the Dinaric Alps (Zminje Jezero; 22–167 reads) from the Early Holocene; in central-southern Finland (Lake Nautajärvi; 47–146 reads) and southwestern Czechia (Černé Jezero; 47–229 reads) from the Middle Holocene; and in the northern Swiss Alps (Lake Sulsseewli; 15–33 reads) during the Late Holocene. These widespread occurrences challenge most of the previous pollen and macrofossil records from the same sediment cores or nearby regions, which did not detect *Larix*. This is likely due to its low pollen productivity and limited dispersal capacity, resulting in highly localized signals (Wagner et al., 2015).

Previous evidence suggests that *L. decidua* expanded from glacial refugia in southern Europe, such as the southern Alps and Carpathians, shortly after the LGM, reaching the central European lowlands and occasionally extending to areas near the Baltic Sea (Wagner et al., 2015). Further studies showed that during the

Holocene, its distribution became largely confined to mid- and high-elevation disjunct populations in the Alps, Sudeten, and Carpathian mountain ranges (Dudová & Szabó, 2022; Jankovská & Pokorný, 2015; Schulte, Li, et al., 2022), corresponding to its modern range. Additional records indicate isolated occurrences in the Polish lowlands (Lewandowski, 1997), in southwestern Czechia since the Late Glacial (Dudová & Szabó, 2022; Vondrák et al., 2019), and in northwestern Czechia during the Late Holocene (Pokorný et al., 2023). However, our *sedDNA* results reveal a wider historical distribution of larches, albeit at low densities, across Europe throughout the Holocene—significantly expanding their known ecological range.

SNP-based species identification revealed a highly admixed pattern of variants between *L. decidua* and *L. sibirica* in all European sites (Figure 8A). The available reference genomes of *L. decidua* (NCBI GenBank: AB501189.1, NC_016058.1) and *L. sibirica* (NCBI GenBank: MF795085.1, NC_036811.1) exhibit 99% identity (BLAST, NCBI), suggesting that the admixture observed in our data likely reflects historical introgressive hybridization. Although this admixture has not been extensively studied, it is consistent with the known capacity for hybridization among *Larix* species, which contributes to taxonomic ambiguities between species and subspecies (Abaimov, 2010; Polezhaeva et al., 2010). In Europe, *L. decidua* remains the only formally recognized species, although several subspecies or varieties have been described in its disjunct populations (Pâques et al., 2013). Although the potential historical hybridization, admixture analysis could separate a shared ancestry among *Larix* sequences from European sites indicating the presence of *L. decidua*, in contrast to the patterns observed at Lake Lama (north-central Siberia) and Lake Khamra, which likely indicate *L. sibirica* (Figure 4C). The TCS network including only the one sample with higher *Larix* sequences content per site in order to increase shared variant positions for the construction of the haplotype also revealed two distinct haplotype clusters separating the European and Siberian samples (Figure 10A).

Overall, we hypothesize that *L. sibirica* may have expanded westward at low densities during the Early Holocene, potentially reaching the Kola Peninsula and southern Finland. Upon contact with *L. decidua*, which migrated northward from glacial refugia in the Sudeten Mountains, central Poland, or the Carpathians, hybridization likely occurred. Small, previously undetected populations established during postglacial migration may have served as local zones for hybridization and further expansion in the Early Holocene.

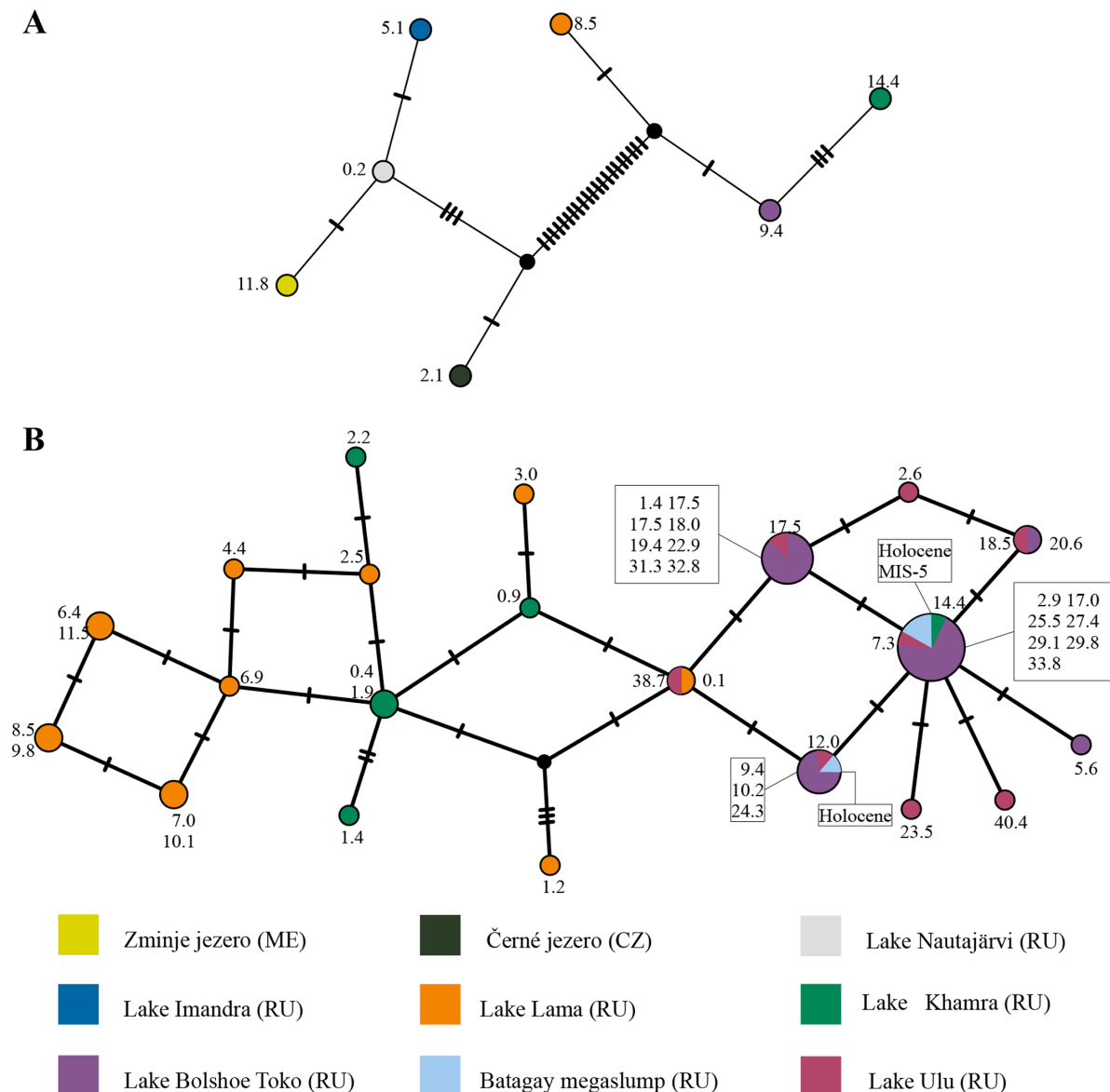


FIGURE 10 Templeton, Crandall, and Sing (TCS) haplotype networks based on *Larix* variants: (A) 30 segregating sites, (B) 14 segregating sites. Each circle represents a haplotype; larger circles indicate multiple samples sharing the same haplotype; size of the circle reflects the number of samples with this haplotype. Sample ages (in calibrated 1000 years before present) are shown next to the circles. Hatch marks on edges indicate inferred mutations (bp differences). Circle colors correspond to sampling sites (see legend); black internal nodes represent inferred ancestral haplotypes.

Early presence and lineage shift of *Pic. abies*, with sparse *L. decidua* in Late Glacial pine-dominated woodlands of the Dinaric Alps

In summary, our *sedaDNA* data reveal an unexpectedly early presence of *Pic. abies* in the Late Glacial, followed by a shift from eastern to western lineages at the Younger Dryas–Holocene transition and subsequent expansion to dominance over pine during the Early Holocene. We confirm the presence of *Pin. mugo* and provide the first evidence of low-abundance *L. decidua*

within Late Glacial, pine-dominated woodlands of the Dinaric Alps.

At Zminje jezero (Dinaric Alps, Montenegro), Pinaceae sequences were predominantly detected in sediments from the Younger Dryas/Holocene transition (Figure 2; Appendix S1: Figure S10; Meucci et al., 2026: tab. 6), while sediments from warm Holocene conditions showed very low signals, likely due to poor DNA preservation. Specifically, we found *Pinus* at higher abundance (285–2381 reads) than *Picea* (38–308 reads) in the two Younger Dryas samples (12.1–11.8 cal ka BP). Then, we observed a decrease of *Pinus* (356 reads) and the

expansion of *Picea* (1231 reads) at 11.1 cal ka BP. This is in agreement with paleoecological evidence indicating an altitudinal shift of *Picea* at the onset of the Holocene in response to rising summer temperatures of approximately 2–4°C (Cagliero et al., 2023; Heiri et al., 2015; Schmidhauser et al., 2024). The detection of *Picea* reads as early as 12.1 cal ka BP likely reflects a local, low-density spruce population during the Younger Dryas, but an influence from long-distance pollen transport cannot be excluded. This occurrence predates the first stomata evidence by 600 years and the first pollen records by 400 years.

SNP-based species identification of *Pinus* sequences predominantly identified dwarf mountain pine (*Pin. mugo*) variants (Figure 6A; Appendix S1: Figure S24), confirming previous pollen and stomata-based assumptions, which were limited to the subgenus or genus level (Beug, 2004; Cagliero et al., 2023; Finsinger & Tinner, 2020). Only a few *Pin. sylvestris* variants were detected, contrasting with pollen evidence. The signal attributed to *Pin. cembra* is based on a low number of reads and may reflect taxonomic misassignments due to the high genetic similarity (~96% identity; BLAST, NCBI) with *Pin. sylvestris* and *Pin. mugo*, likely resulting from shared variants with underrepresented subpopulations in the available reference genomes. However, admixture analysis using four or five ancestral populations (Figure 4B; Appendix S1: Figure S33) identified a distinct ancestry at Zminje Jezero, likely confirming the presence of *Pin. mugo*. The TCS network (Figure 9A) further supports this, revealing that the haplotype from the 11.8 cal ka BP sample was distinct yet related to the cluster of samples assigned to *Pin. sylvestris*, with its closest connection to the 2.1 cal ka BP sample from Černé jezero, which also showed signals of *Pin. mugo*. Given the documented natural hybridization and gene flow between *Pin. mugo* and *Pin. sylvestris* (Heuertz et al., 2010; Kormutak et al., 2017; Wachowiak & Prus-Głowacki, 2008), caution is suggested when interpreting cpDNA-based identifications without supporting macrofossil evidence.

SNP-based species identification of *Picea* sequences at Zminje Jezero revealed an admixed pattern between *Pic. abies* and *Pic. obovata* (Figure 3A), likely reflecting historical hybridization. However, admixture analysis further resolved distinct patterns (Figure 4A), showing that the 11.8 cal ka BP sample shared ancestry with a hybrid population genetically closer to eastern populations, which we attributed to *Pic. fennica*—a lineage likely formed during the Early Holocene in the Russian Plain (see discussion of *Early migration of pine and spruce in north-western Russia*). In contrast, the 11.1 and 10.2 cal ka BP samples shared ancestry with *Pic. abies* populations from central European sites. This is supported by the TCS haplotype network analysis, which shows the 11.1 cal ka BP

sample closely related to central European haplotypes from Lake Sulsseewli, Černé jezero, Otto Mörtzsch Cave, as well as Late Holocene samples from Lake Imandra (Figure 5B; Appendix S1: Figure S36B). Whereas, the 11.8 cal ka BP sample was more closely related to Middle Holocene samples from Lake Imandra, although with a significant genetic distance, likely reflecting different historical hybridization patterns. We therefore hypothesize that the Younger Dryas triggered a population shift at Zminje Jezero—from a population likely originating from northeastern lineages during the Younger Dryas to one more closely related to central European populations expanding at the onset of the Holocene.

A. alba dynamics resembled those of *Picea*, with low sequence abundance during the Younger Dryas (12.1–11.8 cal ka BP; 12–75 reads) and a peak at 11.1 cal ka BP (302 reads), in agreement with paleoecological findings (Cagliero et al., 2023). As previously discussed (see *Widespread low-abundance larch populations and evidence of hybridization across Europe during the Holocene*), we also identified *L. decidua* for the first time in a Late Glacial pine-dominated forest of the Dinaric Alps, several hundred kilometers south of its modern southeastern range. However, this larch population was likely very sparse, as indicated by the absence of pollen, stomata, or needles (Cagliero et al., 2023).

Glacial persistence and Holocene genetic shift of Pinaceae in northern Siberia

In summary, our results provide the first paleogenomic evidence that spruces, pines, and firs, alongside previously known larch populations, persisted through the LGM near Lake Lama (western Taymyr Peninsula), representing their northernmost refugium. During the Holocene, *L. sibirica* and *Pic. obovata* expanded, with both taxa exhibiting clear genetic shifts after the Younger Dryas, underscoring the significant ecological impact of this climatic event.

At Lake Lama, we observed a stable presence of Pinaceae sequences during the LGM (23–19.2 cal ka BP), with *Picea* being the most abundant (77–326 reads), followed by *Larix* (110–203 reads), *Pinus* (1–69 reads), and *Abies* (3–45 reads) (Figure 2; Appendix S1: Figure S11; Meucci et al., 2026: tab. 6). These results provide the first paleogenetic evidence of continuous spruce, pine, and fir presence through the LGM in the Lake Lama region and confirm earlier findings of *Larix* survival by Schulte, Meucci, et al. (2022). Our observations are further supported by shotgun sequencing data from the same sediment core (von Hippel et al., 2025). In contrast, the pollen study reported only low and sporadic

Picea signals, first appearing at the onset of the Late Glacial (Andreev et al., 2004). Despite its northern location, the Lake Lama region likely experienced milder glacial conditions, functioning as a refugium for all four Pinaceae genera. The lake's position, at 53 m above sea level in a west–east valley along the western Putorana Plateau, combined with hydrothermal activity, likely hosted a warm and humid microclimate (Andreev et al., 2004).

SNP-based species identification revealed a highly admixed pattern between *Pic. abies* and *Pic. obovata*, with alternating dominance between the two species during the LGM and Late Glacial (Figure 3A). However, admixture analysis revealed an ancestry shared with European samples, indicating the presence of populations genetically closer to western *Pic. abies* than those that inhabited Lake Lama during the Holocene (Figure 4A). This suggests that the range of western *Pic. abies* may have reached central Siberia during the LGM and Late Glacial, potentially placing Lake Lama within the hybridization zone with *Pic. obovata*. Whereas after the Younger Dryas–Holocene transition, beginning with the sample dated to 10.1 cal ka BP, a marked shift occurred toward a variant pattern dominated by *Pic. obovata*, which remained stable throughout the Holocene.

SNP-based species identification of *Larix* sequences revealed extensive admixture between *L. gmelinii* and *L. sibirica* in the LGM and Late Glacial samples (23–16.1 cal ka BP; Figure 8B), consistent with Lake Lama's location within their known hybrid zone (Schulte, Meucci, et al., 2022). At 11.5 cal ka BP, we observed a major expansion of *Larix* (20,863 reads), predominantly characterized by *L. sibirica* variants reflecting Early Holocene warming (Epp et al., 2018). Interestingly, an evident genetic shift in the variant patterns of both larches and spruces occurred after the Younger Dryas cooling event, reflecting its significant environmental impact. Indeed, the overall Pinaceae abundance showed the lowest abundance between 13.9 and 11.8 cal ka BP.

Forest densification went along with the shift in the generic variants in *Larix* and *Picea* populations. A significant decline in *Larix* occurred around 7.6 cal ka BP, coinciding with a major spruce expansion confirmed by pollen and *sedaDNA* metabarcoding and shotgun record (Andreev et al., 2004; von Hippel et al., 2022, 2025) pointing to *Pic. obovata* as indicated by our record. This shift likely reflects warmer conditions and soil development (von Hippel et al., 2025) favoring spruce growth. A more favorable climate is also indicated by the mid-Holocene *Larix* range extension over 500 km beyond the modern treeline in Yamal and Taymyr (Payette et al., 2002). Both *Larix* and *Picea* declined in the Late

Holocene in accordance with paleoecological evidence from this and further regional sites (Klemm et al., 2016).

SNP-based species identification and admixture analysis of *Pinus* sequences showed the presence of *Pin. sylvestris* population during the Late Glacial as discussed in *Holocene Pinus dynamics in Eurasia* (Figures 4B and 6A). Although the current northern limit of *Abies* is several hundred kilometers south of Lake Lama, we detected low-abundance *Abies* sequences during the LGM, Late Glacial (23–13.9 cal ka BP), and Early Holocene (11.8–8.5 cal ka BP; 16–27 reads), suggesting transient, low-density *Abies* populations within spruce- and larch-dominated forests before retreating in the Middle to Late Holocene. However, SNP-based analysis was not possible due to the limited number of sequences. While *A. sibirica* pollen grains are large and heavy, and thus often underrepresented in pollen records, no prior pollen data have indicated *Abies* presence during the Late Glacial in nearby southern regions. Only sporadic fir pollen has been reported as far north as the northwestern rim of the Polar Ural Mountains during the Early Holocene (Andreev et al., 2005). Therefore, further studies are needed to confirm these findings.

Southwestern Yakutia as a glacial refugium for Pinaceae and Holocene expansion patterns

In summary, our analyses from Lake Khamra (southwest Yakutia) revealed potential glacial refugial populations for *L. gmelinii*, *Picea*, and *Pinus*. During the Holocene, *Pic. obovata*, *L. sibirica*, *Pin. sibirica*, and *A. sibirica* expanded into the region.

Sediment samples from the LGM (19 cal ka BP) and Late Glacial (18.4–14.4 cal ka BP) showed higher numbers of *Larix* sequences (74–1281 reads) compared to *Pinus* (15–50 reads) and *Picea* (6–34 reads) (Figure 2; Appendix S1: Figure S12; Meucci et al., 2026: tab. 6). These results suggest the presence of glacial refugial populations dominated by *Larix*, with only sparse *Picea* and *Pinus*. Given Lake Khamra's relatively southern location in a transitional permafrost zone and increased LGM continentality due to the extended shelves, it likely experienced mild LGM conditions. A marked decline in all Pinaceae, especially *Larix* (0–40 reads), occurred at 13.4 cal ka BP, possibly due to climatic disturbance prior to the Younger Dryas. Between 10.9 and 5.7 cal ka BP, overall Pinaceae sequence abundance remained low (0–143 reads), which we attribute to poor DNA preservation as we have pollen evidence of forest cover (Baisheva et al., 2024). Beginning around 5.2 cal ka BP, Pinaceae sequences gradually increased, with *Picea* becoming

dominant. This trend is in agreement with metabarcoding results but contrasts with pollen data indicating *Pinus* as dominant (Baisheva et al., 2024), likely due to its higher pollen productivity and dispersal capacity. From 1.9 cal ka BP onward, all *Pinaceae* genera declined until 1.4 cal ka BP, followed by renewed expansion during the last 1000 years, primarily dominated by *Larix* and *Picea*, with *Pinus* and *Abies* in lower abundance.

SNP-based species identification revealed a shift in the variant pattern of *Larix* populations, from a predominantly *L. gmelinii* signal during the Late Glacial (19–14.4 cal ka BP) to a highly admixed pattern dominated by *L. sibirica* from 6.5 cal ka BP onward (Figure 8B). The only Late Glacial sample included by the TCS network (14.4 cal ka BP) showed a closely related haplotype with samples from Lake Bolshoe Toko and Lake Ulu (Figure 10B), which are associated with *L. gmelinii* and *L. cajanderi*, rather than with the Holocene samples from Lake Khamra. These findings suggest Lake Khamra was a refugium for *L. gmelinii*, while *L. sibirica* expanded eastward during the Holocene, extending the hybridization zone between the two species to the northern treeline on the Taymyr Peninsula (Epp et al., 2018; Schulte, Meucci, et al., 2022), the eastern Putorana Plateau and western Yakutia.

SNP-based species identification of *Pinus* sequences could be performed only on the Late Holocene samples (Figure 6B). As discussed in *Holocene Pinus dynamics in Eurasia*, admixture analysis primarily indicates the presence of *Pin. sibirica* during the Late Holocene (from 2.8 cal ka BP), with a signal of *Pin. sylvestris* detected at 5.5 and 4.6 cal ka BP (Figure 4B).

SNP-based species identification of *Picea* sequences showed a relatively stable population of *Pic. obovata* (Figure 3A) with haplotypes closely related to Lake Lama samples (Figure 5B), highlighting its genetic structure along the west–east geographical gradient. *Abies* sequences indicated *A. sibirica* throughout the Late Holocene (Figure 7).

Pinaceae dynamics in the southeastern Siberian Mountains during the last 34 ka

In summary, our paleogenetic record from the southeastern Siberian mountains indicates larch-dominated forests since the late Marine Isotope Stage (MIS) 3, with stable pine and spruce populations persisting through the LGM. In particular, we identified potential hybrids of *L. gmelinii* and *L. cajanderi*, along with the presence of *Pin. pumila* and *Picea jezoensis*.

At Lake Bolshoe Toko (southeastern Yakutia), our results revealed *Larix* exhibiting the highest overall

abundance, followed sequentially by *Picea* and *Pinus* (Figure 2; Appendix S1: Figure S13; Meucci et al., 2026: tab. 6). Between the late MIS 3 and the Late Glacial (33.7–17 cal ka BP), all three *Pinaceae* taxa maintained a relatively high and continuous presence, with peaks at 33.7, 29.8, and 22.9 cal ka BP, followed by a gradual decline due to the harsher LGM conditions. However, a substantial number of *Pinaceae* sequences (386–6656 reads) persisted between 20.6 and 17 cal ka BP. These findings suggest a larch-dominated forest from the late MIS 3, with a stable presence of pine and spruce that survived the LGM. Our results differ from the pollen record, which suggests a pine-dominated forest during the LGM—likely influenced by its higher pollen production and dispersal capacity—while showing only sparse *Larix* and *Picea* signals (Courtin et al., 2021).

Between 16.1 and 10.9 cal ka BP, we observed a low range of *Pinaceae* sequences (0–267 reads). However, we would have expected a more substantial *Pinaceae* presence during the Late Glacial and Early Holocene due to the relative warmer climate, as indicated by pollen data (Courtin et al., 2021). This discrepancy may be attributed to poor DNA preservation, possibly due to increased freshwater input from glacial retreat between 15 and 10 cal ka BP, which could have decreased water conductivity, reducing Mg^{2+} availability needed for DNA stabilization (Capo et al., 2021; Jia et al., 2021). *Larix* and *Pinus* reoccurred in the Early Holocene samples (10.3 cal ka BP) and continued with consistently high abundances up to the most recent sample at 1.4 cal ka BP (643–4821 reads). The main Holocene expansion of *Picea*, likely originating from warmer and more humid southeastern regions (Aizawa et al., 2007; Lockwood et al., 2013), was detected during the HTM (5.6 cal ka BP), temporarily becoming dominant over the other *Pinaceae* genera.

The SNP-based species identification of *Larix* sequences revealed an alternation between samples exhibiting an admixed signal of *L. gmelinii* with a lower abundance of *L. cajanderi* variants and samples displaying only *L. gmelinii* variants (Figure 8C). Lake Bolshoe Toko is located on the southwestern edge of the hybridization zone between these two species, centered in central Yakutia (Haupt et al., 2024). This suggests that different hybrid populations alternately dominated and expanded in the Lake Bolshoe Toko area over time.

As discussed in *Holocene Pinus dynamics in Eurasia*, *Pinus* sequences were predominantly assigned to *Pin. pumila* across all samples (Figures 4B and 6B), with a minor proportion of *Pin. sibirica* variants, likely reflecting incursions and potential hybridization, as previously observed (Petrova et al., 2018).

The SNP-based species identification of *Picea* sequences revealed a dominance of *Pic. jezoensis* variants

in all samples, except for the oldest MIS 3 sample at 33.7 cal ka BP, which was dominated by *Pic. obovata* variants (Figure 3B). The TCS network further confirmed that the haplotype from the 33.7 cal ka BP sample was most closely related to samples from lakes Khamra and Lama, both characterized by the presence of *Pic. obovata* or hybrids with *Pic. abies* (Appendix S1: Figure S36C). Additionally, we observed pronounced genetic divergence among Lake Bolshoe Toko, suggesting either the influx of distinct *Pic. jezoensis* subpopulations over time or a higher intraspecific genetic variability in *Pic. jezoensis* compared to *Pic. obovata*.

Larix dynamics in the hybridization zone of *L. gmelinii* and *L. cajanderi* in the East Siberian Mountains over the last 40 ka

In summary, our data from the Oymyakon Upland region (Lake Ulu) provide new paleogenomic evidence for glacial persistence of *Larix*, and a transition from *L. gmelinii*- to *L. cajanderi*-dominated hybrid populations, reflecting the region's role as a refugium and a hybridization zone.

At Lake Ulu, we found a consistent presence of *Larix* (42–1712 from the MIS 3 to the LGM samples [40.4–20 cal ka BP]). A significant expansion was observed at 18.5 cal ka BP, with consistently high abundances (7391–8905 reads) persisting until the Late Holocene (2.7 cal ka BP) (Appendix S1: Figure S14; Meucci et al., 2026: tab. 6). In contrast, *Pinus* (3–82 reads) and *Picea* (2–21 reads) remained consistently low in abundance and were not detected in earlier metabarcoding results (Jia et al., 2024).

Although distinguishing between *L. gmelinii* and *L. cajanderi* in low-coverage samples remains challenging due to their high genetic similarity (99% identity; BLAST, NCBI) of their cp genomes, SNP-based species identification of *Larix* sequences identified predominantly *L. gmelinii* with a minor signal of *L. cajanderi* variants from 40.4 to 25.9 cal ka BP (Figure 8C; Appendix S1: Figure S28). From 23.6 cal ka BP through the Holocene, *L. cajanderi* variants became dominant, indicating that Lake Ulu likely sat at the eastern boundary of a hybridization zone between the two species. Recent genetic studies suggest divergence between *L. gmelinii* and *L. cajanderi* from a common ancestor occurred around 20.4 cal ka BP (Haupt et al., 2024). Post-divergence, *L. gmelinii* likely expanded westward into Yakutia, while *L. cajanderi* spread eastward into Chukotka. An admixed signal between the two species before the LGM was found also at Lake Bolshoe Toko (see *Pinaceae dynamics in the southeastern Siberian Mountains during the last 34 ka*) and

in the very ancient sample from MIS 5 retrieved from the Batagay megaslump which may represent ancestral populations before the divergence of the two species.

Conversely, if divergence predated 40 cal ka BP, our data suggest that western *L. gmelinii* or closely related hybrids extended as far east and north as Lake Ulu. Upon contact with eastern *L. cajanderi* populations, hybridization might have occurred. However, only hybrids genetically closer to *L. cajanderi* appear to have survived the LGM at Lake Ulu, likely due to enhanced resilience to extreme cold and frost potentially acquired through adaptive introgression (Meucci et al., 2025). Recent nuclear DNA analyses of modern *Larix* individuals (Meucci et al., 2025) support a hybridization hypothesis rather than the coexistence of distinct taxa, showing that the degree of genetic admixture between the two species follows a west–east longitudinal gradient across a broad contact zone in central Yakutia. Therefore, our findings emphasize the dual role of Lake Ulu's region as both a refugium and a hybridization zone, shaping the Holocene dynamics of *Larix* in eastern Siberia.

Northeastern Tibetan Plateau (Nianbaoyuze Mountains)

In summary, we found *Picea* sedaDNA with uncertain origin during the LGM. We confirmed finding from pollen of an Early Holocene expansion of spruce forests, but identified *Picea asperata*, with new evidence of low-abundance presence of larches, pines, and firs.

At Lake Ximen, our findings revealed 177 *Picea* reads at 20.2 cal ka BP (Appendix S1: Figure S17; Meucci et al., 2026: tab. 6), which is markedly above the contamination threshold, identified as *Pic. asperata* by SNP-based species identification (Figure 3C). While between 18.5 and 10.1 cal ka BP, we found a very low number of captured sequences (1–27 reads). These findings confirm the record of Pinaceae pollen which was attributed to an extra-regional signal, suggesting that boreal forests persisted at lower elevations during the glacial period (Herzschuh et al., 2014). It remains uncertain whether or not the paleogenetic evidence also derives from pollen material or whether it indicates a local low-density population of spruces at Lake Ximen during the LGM. A postglacial expansion of *Picea* was starting at 9.4 cal ka BP, peaking at 8.9 cal ka BP, and gradually declining until 4.8 cal ka BP in accordance with evidence from pollen (Herzschuh et al., 2014) and plant metabarcoding data from the same site (Shen et al., 2024). After the Younger Dryas cooling (12.9–11.7 cal ka BP), the Early and Middle Holocene experienced a warmer and wetter

climate, which likely facilitated the expansion of spruce-dominated forests, potentially with other rare Pinaceae. This is supported by our results, which show a very low number of *Larix* (6–46 reads), *Pinus* (4–28 reads), and *Abies* (1–14 reads) sequences. An extremely low number of Pinaceae sequences (1–9 reads), mostly below the threshold of 6 reads set in our study (see *Hybridization capture*), was detected in core sediments younger than 4.3 cal ka BP in accordance with the metabarcoding record (Shen et al., 2024). This suggests that Late Holocene cooling and reduced precipitation likely caused Pinaceae loss at Lake Ximen (Herzschuh et al., 2014).

SNP-based species identification indicated a predominance of *Pic. asperata* variants in all samples, with *Picea purpurea* being less abundant but increasing during the postglacial expansion. The cp genome of *Pic. purpurea* was found to be closely related to other *Picea* species in eastern Tibet, such as *Picea crassifolia*, *Picea neveitchii*, *Picea brachytyla*, *Picea wilsonii*, and *Picea likiangensis* (Sun et al., 2014). This close genetic relationship is likely due to introgressive hybridization, as suggested by previous studies (Li et al., 2010; Lockwood et al., 2013), making species identification based on single-species variants challenging. However, according to previous evidence, only *Pic. likiangensis* overlaps with *Pic. purpurea* in the northeastern areas of the Tibetan Plateau. Since *Pic. purpurea* typically grows at higher altitudes than *Pic. likiangensis* (Du et al., 2011), we focused exclusively on *Pic. purpurea* in our analysis.

Southeastern Tibetan Plateau (Hengduan Mountains)

In summary, our results indicate a spruce-dominated open forest during the Holocene and uncertain origin of *sedaDNA* signals from the Late Glacial samples.

At Lake Naleng in the Hengduan Mountains, we detected low numbers of captured Pinaceae sequences in samples dated between 17.7 and 15.9 cal ka BP, primarily *Pinus* (4–103 reads) and *Picea* (7–15 reads), with *Abies* only present in the 17.4 cal ka BP sample (20 reads) (Figure 2; Appendix S1: Figure S18; Meucci et al., 2026: tab. 6). Previous metabarcoding, shotgun sequencing (Liu et al., 2024; Liu, Kruse, et al., 2021; Liu, Li, et al., 2021), and pollen studies (Kramer et al., 2010a, 2010b) reconstructed an alpine steppe environment for this period, without evidence of Pinaceae. As observed at Lake Ximen, this discrepancy suggests that Pinaceae may have occurred in very low abundance or that hybridization capture retrieved trace cpDNA from wind-transported pollen. Early Holocene samples (9.2 and 8.2 cal ka BP) showed

low *Picea* sequence counts (0–92 reads), likely indicating an initial low-density expansion, which did not allow for species determination. During the Middle Holocene, we observed an expansion of *Pic. asperata* (Figure 8C), with *Pic. purpurea* in lower abundance, peaking at 3.1 and 1.8 cal ka BP and declining after 1.1 cal ka BP—likely due to climate cooling—consistent with previous findings (Liu et al., 2024; Liu, Kruse, et al., 2021; Liu, Li, et al., 2021). A low signal of *Abies* was found from 3.1 cal ka BP (8–29 reads).

Lake Ximen and Lake Naleng records showed different vegetation dynamics, particularly during the Late Holocene, which Shen et al. (2024) attributed to differences in connectivity to glacial lowland refugia. The TCS network reflected geographic patterns, with haplotypes from Lake Ximen more closely related to northern sites such as Lake Bolshoe Toko than those from Lake Naleng (Appendix S1: Figure S37A).

Picea forest dynamics in south-central Alaska: Early Holocene colonization and Late Holocene high-density expansion

In summary, our single-site investigation from south-central Alaska confirms pollen-based evidence of Early Holocene colonization by *Picea* forests and reveals an exceptionally dense forest expansion during the Late Holocene.

At Hidden Lake in the Kenai Mountains (Kenai Peninsula, Alaska, USA), we detected no evidence of Pinaceae forest during the Late Glacial and Early Holocene (13.6–9.2 cal ka BP; 2–20 reads). A substantial increase in *Picea* sequences at 8.7 cal ka BP (10,922 reads) indicates a dense initial recolonization, consistent with pollen data (Ager, 1983; Broadman et al., 2022), followed by a stable *Picea* presence throughout the Middle Holocene (8.7–4.6 cal ka BP; 8932–26,126 reads) (Appendix S1: Figure S19; Meucci et al., 2026: tab. 6). The TCS haplotype network (Appendix S1: Figure S37B) revealed close genetic relationships among Middle Holocene samples, suggesting recolonization by one or a few closely related *Picea* populations. This supports earlier findings of unique regional haplotypes in central Alaska, consistent with postglacial expansion from small, scattered LGM refugia in local ice-free zones (Anderson et al., 2006; Edwards et al., 2014). This expansion was likely triggered by the Early Holocene warming and diminished ground-water influence, as the retreat of the Cordilleran Ice Sheet to the east created drier, less saturated soils favorable for spruce colonization (Broadman et al., 2022).

A notable decrease in *Picea* abundance was observed at 4 cal ka BP (3494 reads). Although this decline was not

reflected in pollen records (Ager, 1983; Broadman et al., 2022), it coincides with the onset of glacial advances in southern Alaska around 3.5–4.0 cal ka BP (Kaufman et al., 2016). Subsequently, a major expansion occurred at 3.5 cal ka BP, marked by exceptionally high sequence counts (49,012–92,326 reads) and increased haplotype diversity. These findings suggest the influence of multiple factors, including the influx of genetically distinct *Pic. glauca* subpopulations and the possible arrival of another conifer, *Tsuga mertensiana* (mountain hemlock), which was detected in pollen records around this time (Ager, 1983; Broadman et al., 2022). Due to high cpDNA similarity, *Tsuga* sequences may have been captured by our probes, potentially contributing to the elevated read counts. Unfortunately, no complete chloroplast of American species of *Tsuga* are available.

Particularly in the Late Holocene samples (3.5–0.9 cal ka BP), we found low numbers of sequences from *Larix* (153–323 reads), *Pinus* (63–160 reads), and *Abies* (27–58 reads)—previously undetected in pollen records, indicating a sparse, low-density presence, especially of larches.

SNP-based species identification revealed a consistent dominance of *Pic. glauca* variants across all samples (Figure 3D). Although organelle gene introgression from *Picea sitchensis* has been documented in western North America (Lockwood et al., 2013), no such signal was detected here.

SUMMARY AND CONCLUSIONS

We applied hybridization capture method to equimolar library pools from 238 sediment samples from 19 Late Quaternary archives from Eurasia and Alaska targeting the complete cp genomes of four Pinaceae genera (*Picea*, *Pinus*, *Larix*, and *Abies*). This study improved the methodology of Schulte, Meucci, et al. (2022) by significantly expanding the breadth of coverage and read depth within the captured bait regions. As a result, the revised approach yielded more diverse, deduplicated, and comprehensive sequencing data.

Our investigation faced both the advantages and limitations of using hybridization capture for analyzing Pinaceae dynamics at a continental scale since the LGM. The method provided reliable time series data for absolute abundances and relative compositions of Pinaceae genera at each site, thus enabling detailed tracing of Pinaceae dynamics across broad geographic areas and through time. Compared with traditional paleoecological methods such as pollen and plant macrofossil analyses, hybridization capture was highly effective in detecting low-abundance and previously undetected Pinaceae

populations. Despite these strengths, the method has several limitations. Poor DNA preservation affected several samples deposited during the warm conditions of the Early and Middle Holocene. Quantification of absolute Pinaceae reads may be affected by the high sensitivity of the method and its ability to retrieve nontarget and diverged sequences. Closely related species to those used in the bait design may be captured more efficiently than genetically distant taxa, potentially biasing abundance estimates. Moreover, conserved regions of the chloroplast genome may lead to nontarget capture across diverse plant taxa; to mitigate this, rRNA genes were excluded from bait design. Additionally, taxonomic classification and competitive mapping against a concatenated cp genome of the four Pinaceae targeted genera, with stringent read filtering, minimized potential biases from reads mapping to nonspecific conserved regions shared across multiple genera. Another potential source of bias in abundance estimates is the capture of trace amounts of paternally inherited cpDNA from long-distance wind-dispersed pollen, particularly from high pollen producers such as *Pinus*. This risk could be particularly pronounced in alpine lake sedimentary archives characterized by low local plant inputs but significant pollen deposition from nearby dense mountain forests, as possibly occurred at our eastern Tibetan Plateau sites during the LGM. However, we found no consistent proportional relationship between genus-specific pollen abundance and corresponding *sedaDNA* counts. Taxon-specific differences in DNA preservation, likely influenced by tissue composition, may also introduce biases in read quantification, though this remains unexplored in the scientific literature.

We identified several refugia sites in non-glaciated Siberia, including some previously unrecognized, as potential habitats for Pinaceae species over the past 45 ka. This identification was facilitated by the high sensitivity of hybridization capture techniques. Multi-taxa refugia acted as hybridization hotspots, likely fostering hybrids with enhanced resilience to the harsh environmental conditions of the LGM. These refugia thus appear critical in shaping postglacial recolonization patterns across northern Eurasia.

At Lake Lama (western Taymyr Peninsula), we confirmed the presence of hybrid refugial populations of *L. gmelinii*–*L. sibirica* (Schulte, Meucci, et al., 2022) and documented, for the first time, potential hybrid populations of *Pic. abies*–*Pic. obovata*, along with refugial populations of *Pin. sylvestris* and potentially *Abies*. This discovery extends the known northernmost boundary of these taxa's glacial refugia, possibly supported by relatively mild climatic conditions linked to local hydrothermal activity.

At Lake Khamra (southwestern Yakutia), *L. gmelinii* dominated, accompanied by minor populations of *Picea* and *Pinus* dating back to approximately 19 cal ka BP, suggesting a nearby refugium. Given its more southerly and continental position, Lake Khamra likely experienced moderate LGM conditions, including relatively warm summers.

Further east, Lake Bolshoe Toko (southeastern Yakutia) emerged as a more extensive refugium, hosting multiple Pinaceae species such as *Pic. jezoensis*, *Pin. pumila*, *Pin. sibirica*, and *L. gmelinii*. Previously, only scattered occurrences of *Pinus* had been recorded at this site.

At Lake Ulu (Oymyakon Upland), our findings support previous evidence of *Larix* refugial populations (Jia et al., 2024). In addition, our findings indicate that the Lake Ulu region likely functioned as a hybridization zone between *L. gmelinii* and *L. cajanderi* during the MIS 3, potentially contributing to the emergence of hybrids genetically closer to *L. cajanderi* with improved tolerance to extreme cold and frost that enabled their survival through the LGM (Meucci et al., 2025). Additionally, we documented the first evidence of sparse, intermittent occurrences of *Pinus* and *Picea* populations from the late MIS 3 through the Holocene. However, further research is necessary to clarify whether these rare populations also represent refugial groups. Overall, our results confirm the presence of extensive *Larix* refugia in eastern Siberia and suggest that hybridization may have enhanced adaptability, reducing the effects of genetic bottlenecks. This likely facilitated rapid postglacial expansion and contributed to *Larix* dominance during the Holocene, supporting the hypothesis proposed by Herzschuh (2019).

Our approach provides insights into the postglacial Pinaceae dynamics in Eurasia. While our results largely confirm earlier findings, they add detailed species-level resolution and serve as an additional proxy for quantitative analyses. Following deglaciation during the Late Glacial and Early Holocene, Pinaceae species underwent complex range expansions driven by climate and competitive interactions.

Data from high-latitude European site, Lake Imandra (western Kola Peninsula), and the high-altitude Lake Sulsseewli (northern Swiss Alps) confirm that postglacial succession began with open pine forests (*Pin. sylvestris* and *Pin. cembra*, respectively), subsequently replaced by spruce forests (*Pic. abies*) due to warmer, wetter climates and improved soil conditions (Birks & Birks, 2004; Giesecke et al., 2017; Kuneš et al., 2011).

Similarly, we observed rapid replacement of *Pin. mugo* by *Pic. abies* and *A. alba* in the Dinaric Alps (Zminje jezero) at the Holocene onset, approximately 600 years earlier than previous paleoecological evidence (Cagliero et al., 2023).

Findings from Lake Nautajärvi (central-southern Finland), southern Sweden (Atteköping and Hässeldala Port wetlands), Lake Ezzhinitza (southwestern Russia), and central European sites from Bohemia (Černé jezero and Otto Mörtzsch Cave) revealed *Pic. abies*-dominated forests with co-occurring, lower abundance pine populations (*Pin. sylvestris* and *Pin. cembra*) throughout the Holocene. In contrast, *Pin. sylvestris* was the dominant taxon in northern Germany (Lake Groß Glienicker).

Additionally, we present the first paleogenetic evidence of sparse *Larix* populations across Europe during the Early and Middle Holocene, suggesting a wider Holocene distribution than previously recognized. At the Siberian sites, *Larix* functioned as an early-successional species during the Late Glacial (Figure 2), benefiting from traits such as seasonal needle shedding to avoid frost damage and shallow root systems adapted to permafrost conditions (Herzschuh, 2019).

Evergreen taxa expanded primarily during warmer periods, showing regionally distinct dynamics. At the north-central Siberian Lake Lama, *Pic. obovata*—or hybrids with *Pic. abies*—expanded from the Early Holocene. In southwestern Yakutia (Lake Khamra), *Pic. obovata* became dominant during the Middle Holocene, while *Pin. sibirica* expanded during the Late Holocene in lower abundance, resulting in a relatively balanced representation of all four Pinaceae genera. In southeastern Yakutia (Lake Bolshoe Toko), although *L. gmelinii* remained dominant throughout the MIS 3 and the Holocene, *Pin. pumila* and *Pic. jezoensis* exhibited coordinated, parallel patterns of expansion. In contrast, *Larix* remained consistently dominant in Far East Siberia (Batagay megaslump, Lake Ulu, Lake Emanda), with *L. cajanderi* dominating throughout the Holocene, while other Pinaceae taxa were likely absent or present only as rare, scattered populations.

Range shifts driven by glacial cycles facilitated contact between previously isolated populations or species, either in shared refugia or along postglacial expansion routes, likely promoting hybridization between different species. Targeting full-length, paternally inherited cpDNA in Pinaceae offers a valuable marker for detecting hybridization, as its increased sensitivity to introgressive events increases intraspecific variation. However, inferring admixture from environmental DNA remains challenging, since co-occurring sequences in sediment samples may not necessarily originate from the same population. With respect to *Picea*, our results revealed an admixed pattern of *Pic. abies* and *Pic. obovata* variants throughout the Holocene across all European sites, likely reflecting historical hybridization—patterns that we were able to disentangle through admixture analysis (Figure 4A). At Lake Imandra, we detected the influx of distinct spruce

populations along the Holocene. During the Late Glacial and Early Holocene, spruce populations originating east of the Urals or from Central Siberia likely expanded westward into the Russian Plain, where they encountered northward-migrating *Pic. abies*. This contact may have facilitated hybridization, potentially resulting in hybrids referred to as *Pic. fennica*, which subsequently spread at low density toward the Kola Peninsula in the Early Holocene. From the Middle Holocene onward, populations dominated by *Pic. abies* variants migrated to the Kola Peninsula likely from southern or western regions. These patterns suggest that Lake Imandra lies within the *Pic. abies*–*Pic. obovata* hybridization zone or was colonized by hybrids formed at lower latitudes, as the Russian Plain, potentially carrying traits advantageous for northward migration. At Lake Lama, we identified an admixed variant pattern between *Pic. abies* and *Pic. obovata* during the LGM and Late Glacial, with alternating dominance suggesting the presence of distinct hybrid populations. These shared a common ancestry (Figure 4A) with Holocene European populations, indicating Lake Lama as both a glacial refugia and a hybridization zone during the LGM from where *Pic. abies*–*Pic. obovata* hybrids might have formed and migrated westward during the Late Glacial. This also shows that the hybridization zone between the two species extended much farther east than previously documented during the LGM and Late Glacial. During the Holocene, samples from Lake Lama showed shared ancestry with those from Lake Khamra, Lake Ezzhinita, and to a lesser extent Lake Imandra. Variant patterns became predominantly assigned to *Pic. obovata*, suggesting a local population shift increasingly influenced by eastern populations. This also indicates a westward displacement of the hybridization zone, placing Lake Lama outside its extent during the Holocene.

With respect to the newly detected sparse *Larix* populations across Europe, we identified a highly admixed variant pattern between *L. decidua* and *L. sibirica* across all European sites, likely indicating historical introgressive hybridization. However, admixture analysis was able to separate the species, suggesting that these European *Larix* populations correspond to *L. decidua*. In Siberia, at Lake Lama, we detected an admixed signal of *L. sibirica* and *L. gmelinii* during the LGM and Late Glacial (23–16.1 cal ka BP), indicating that this site was already located in the hybridization zone between the two species during its role as a glacial refugium. In contrast, the region around Lake Khamra, which also likely served as a glacial refugium, was dominated by *L. gmelinii* until the Late Glacial (14.4 cal ka BP), including the potential influence of *L. cajanderi* around 17–18 cal ka BP. Therefore, southwestern

Yakutia was probably near the *L. gmelinii*–*Larix*–*L. cajanderi* contact zone during the LGM and Late Glacial. Later, we detected a predominant *L. sibirica* signal from the Early Holocene (11.5 cal ka BP) at Lake Lama indicating a westward expansion of *L. sibirica* likely facilitated by HTM-induced warming and a corresponding decrease in permafrost. As *L. sibirica* is sensitive to permafrost (Abaimov, 2010), its spread may have been enabled by a thinner active permafrost layer, extending the hybridization zone into southwestern Yakutia. Indeed, from the Middle Holocene both Lake Lama and Lake Khamra were likely into the hybridization zone between *L. sibirica* and *L. gmelinii*. Further east, at Lake Bolshoe Toko, we found evidence of hybridization between *L. gmelinii* and *L. cajanderi* from the late MIS 3 through the LGM, when the area likely served as a refugium, and in the Late Holocene. Lake Bolshoe Toko is located near the southern limit of the continuous permafrost zone and at the southwestern edge of the hybridization zone between the two species (Haupt et al., 2024). This indicates that different hybrid populations alternately expanded in the Lake Bolshoe Toko region. At the Batagay megaslump (northern Yakutia), and at Lake Ulu (Oymyakon Upland), we detected admixed signals between *L. gmelinii* and *L. cajanderi* in the oldest samples: MIS 5 (130–80 cal ka BP) at Batagay and MIS 3 (40.4–38.8 cal ka BP) at Lake Ulu. These signals may represent an ancestral population predating the separation of the two species, or evidence that central Yakutia served as a hybridization zone much earlier than previously documented. At Lake Ulu, only hybrids genetically closer to *L. cajanderi* survived the LGM, likely due to enhanced resilience to withstand extreme cold and frost. This highlights the dual role of the Lake Ulu region as both refugium and hybridization zone, shaping Holocene *Larix* dynamics in eastern Siberia.

With respect to *Abies*, we found a potential low-abundance population at Lake Imandra on the Kola Peninsula, showing an admixed variant pattern dominated by *A. sibirica* with minor *A. alba* contribution. These results suggest that *A. sibirica* populations potentially migrated westward from Siberia during the Early Holocene. During this migration, they may have encountered *A. alba* expanding northward from southern refugia such as the Carpathians, possibly leading to hybridization in northern regions. These inferred migration routes appear to align with those observed for *Pic. obovata* migrating from the east in the Early Holocene, and *Pic. abies* and *L. decidua* expanding from southern or western regions in the Middle Holocene. This points to a potential broader Pinaceae migration pattern in response to postglacial climate change, likely reflecting shared climatic niches and joint refugia.

The continued development and expansion of hybridization capture for *sedaDNA* hold great potential for advancing paleogenomic research on how glacial cycles and past environmental changes have shaped the modern distribution of plant species. Future advancements could include integrating mitochondrial and nuclear DNA target sequences to complement chloroplast-based phylogeographic studies, further refining our understanding of species distributions in space and time. Expanding the application of this method across broader geographical and temporal scales and to further taxa could uncover new evidence of glacial refugia and hybridization hotspots. Ultimately, this knowledge may help inform conservation and climate adaptation research by improving predictions of future vegetation responses to environmental change.

AUTHOR CONTRIBUTIONS

Stefano Meucci: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); project administration (equal); software (lead); validation (lead); visualization (lead); writing—original draft (lead); writing—review and editing (equal). **Kathleen R. Stoof-Leichsenring:** Conceptualization (supporting); formal analysis (supporting); investigation (supporting); methodology (equal); project administration (supporting); resources (lead); supervision (supporting); writing—original draft (supporting); writing—review and editing (supporting). **Yanrong Zhang:** Investigation (supporting); writing—review and editing (supporting). **Andrei A. Andreev:** Writing—original draft (supporting); writing—review and editing (supporting). **Konstantin V. Krutovsky:** Methodology (supporting); writing—review and editing (supporting). **Boris K. Biskaborn:** Investigation (supporting); writing—review and editing (supporting). **Inger G. Alsos:** Investigation (supporting); writing—review and editing (supporting). **Laura Parducci:** Investigation (supporting); writing—review and editing (supporting). **Kevin Nota:** Writing—review and editing (supporting). **Sandra Garcés-Pastor:** Writing—review and editing (supporting). **Petr Kuneš:** Investigation (supporting); writing—review and editing (supporting). **Walter Finsinger:** Investigation (supporting); writing—review and editing (supporting). **Eleonora Cagliero:** Investigation (supporting); writing—review and editing (supporting). **Daniel Vondrák:** Investigation (supporting); writing—review and editing (supporting). **Jaroslav Kukla:** Investigation (supporting); writing—review and editing (supporting). **Darrell Kaufman:** Investigation (supporting); writing—review and editing (supporting). **Barbara Wohlfarth:** Investigation (supporting); writing—review and editing

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Supporting tables and the bioinformatic pipeline (Meucci et al., 2026) are available from Zenodo: <https://doi.org/10.5281/zenodo.18698942>. The age-depth models for the sediment records were adopted from the studies cited in Meucci et al. (2026: tab. 1). The raw shotgun DNA sequencing data are available in the European Nucleotide Archive (ENA) under BioProject accession number PRJEB98989. The chloroplast genome assembly of *Pic. obovata* is available in NCBI GenBank under accession number PV631374.

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SUPPORTING INFORMATION

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

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