Received: 4 October 2023 | Revised: 24 November 2023 | Accepted: 11 December 2023

DOI: 10.1111/mec.17251

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Genomic variation in montane bumblebees in Scandinavia: High levels of intraspecific diversity despite population vulnerability

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Funding information
Vetenskapsrådet, Grant/Award Number: 2018-05973; Naturvårdsverket, Grant/Award Number: 225-21-003

Handling Editor: Sean D. Schoville

Abstract

Populations of many bumblebee species are declining, with distributions shifting northwards to track suitable climates. Climate change is considered a major contributing factor. Arctic species are particularly vulnerable as they cannot shift further north, making assessment of their population viability important. Analysis of levels of whole-genome variation is a powerful way to analyse population declines and fragmentation. Here, we use genome sequencing to analyse genetic variation in seven species of bumblebee from the Scandinavian mountains, including two classified as vulnerable. We sequenced 333 samples from across the ranges of these species in Sweden. Estimates of effective population size (N_E) vary from ~55,000 for species with restricted high alpine distributions to 220,000 for more widespread species. Population fragmentation is generally very low or undetectable over large distances in the mountains, suggesting an absence of barriers to gene flow. The relatively high N_E and low population structure indicate that none of the species are at immediate risk of negative genetic effects caused by high levels of genetic drift. However, reconstruction of historical fluctuations in N_E indicates that the arctic specialist species *Bombus hyperboreus* has experienced population declines since the last ice age and we detected one highly inbred diploid male of this species close to the southern limit of its range, potentially indicating elevated genetic load. Although the levels of genetic variation in montane bumblebee populations are currently relatively high, their ranges are predicted to shrink drastically due to the effects of climate change and monitoring is essential to detect future population declines.

KEYWORDS
bumblebees, climate change, conservation genetics, genomics/proteomics, population dynamics, population genetics – empirical

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1 | INTRODUCTION

Bumblebees are important pollinators, particularly in temperate regions of the northern hemisphere. However, declines in species abundance and distribution have been reported globally in recent decades (Cameron et al., 2011; Cameron & Sadd, 2020; Williams et al., 2009). According to the IUCN Red List, nearly one-quarter of the bumblebee species that have been assessed are declining (De Meulemeester et al., 2016). As bumblebees are mainly cold adapted, they are particularly vulnerable to climate change, which is predicted to have a major negative impact on their populations (Cameron & Sadd, 2020). Climatic niche modelling of future suitable habitats on the basis of multiple future climate change scenarios has shown that the majority of species are predicted to lose a significant proportion of their current ranges within this century. Up to 36% of species are predicted to lose more than 80% of their current range (Ghisbain et al., 2023; Rasmont et al., 2015).

The distributions of many North American and European bumblebee species have shifted northwards in recent decades (Kerr et al., 2015; Martinet et al., 2015; Soroye et al., 2020). However, although the southern edges of their distributions have retreated rapidly, expansions of the northern edges are reported to be occurring much more slowly. Climate change is the most likely explanation for these changes, and in particular the increased occurrence of extreme weather events that limit survival. Bumblebee populations in the arctic and alpine regions are particularly threatened by climate change because it is impossible for them to shift their distributions further north (Kerr et al., 2015; Rasmont et al., 2015; Soroye et al., 2020; Williams & Osborne, 2009). The assessment of the viability of arctic and mountain specialist bumblebee species is, therefore, high priority.

Analysis of genetic diversity is a powerful way to assess population viability and inform conservation strategies (Allendorf et al., 2010; Hohenlohe et al., 2021). Rarer species of bumblebees in Europe and North America have been found to exhibit the lower levels of genetic diversity and the elevated levels of genetic differentiation (Cameron et al., 2011; Charman et al., 2010; Darvill et al., 2006; Ellis et al., 2006; Kent et al., 2018; Lozier et al., 2011; Lozier & Cameron, 2009), which suggests that an analysis of genetic variation could be an important way to assess conservation status of bumblebees. Analysis of genetic diversity on a whole-genome scale enables a range of more powerful analyses than traditional conservation genetic analyses based on limited numbers of loci (Webster et al., 2023). These include reconstructing population history and quantifying levels of inbreeding and genetic load in natural populations (Allendorf et al., 2010; Hohenlohe et al., 2021; Supple & Shapiro, 2018; Webster et al., 2023).

The main focus of this study is to provide an assessment of population history and vulnerability based on genome-wide variation in bumblebee species found in mountain habitats in Sweden, which are among the most threatened by climate change (Rasmont et al., 2015). We focus on six species that are restricted to arctic and mountain environments, and one species (*Bombus pascuorum*) that is present in the Swedish mountains but is also widespread in multiple habitats throughout Europe (Table 1; Figure 1). Of the montane species, *Bombus balteatus*, *Bombus hyperboreus*, *Bombus pyrrhopygus* and *Bombus alpinus* belong to the subgenus Alpinobombus (Cameron et al., 2007; Williams et al., 2008). *B. balteatus* is relatively common in the Swedish mountains, whereas the other three have a more restricted range, inhabiting higher latitudes and elevations (Rasmont et al., 2015; Williams et al., 2019). *Bombus lapponicus* and *Bombus monticola* belong to the subgenus Pyrobombus. Both of these species are found throughout the Swedish mountains (Rasmont et al., 2015). In addition to their range in Scandinavia, two of the species are also present in mountain environments further south in Europe. *B. monticola* is found in high elevations at many locations across Europe including the British Isles, Pyrenees, Alps and Balkans. *B. alpinus* occurs at the highest elevations in the Alps in addition to the Scandinavian mountains. The other montane species *B. lapponicus*, *B. pyrrhopygus*, *B. balteatus* and *B. hyperboreus* are not present south of Scandinavia. All of these species apart from *B. alpinus* and *B. monticola* are also found further east in Siberia (Rasmont et al., 2015; Williams et al., 2019). In contrast to the other species, *B. hyperboreus* is a parasitic species, which lays eggs in the nests of other bumblebee species (Ødegaard et al., 2015).

TABLE 1 Species of bumblebees analysed in this study.

Bombus sp.	Subgenus	English	Swedish	IUCN red list (3.1)	Climate risk[a]	Distribution in Europe
alpinus	Alpinobombus	Alpine bumblebee	Alphumla	Vulnerable	HHR	European highlands and Arctic
pyrrhopygus	Alpinobombus	Polar bumblebee	Polarhumla	Least concern	HHHR	Arctic
balteatus	Alpinobombus	Golden-belted bumblebee	Fjällhumla	Least concern	HHR	Scandinavian highlands and Arctic
hyperboreus	Alpinobombus	High-Arctic bumblebee	Tundrahumla	Vulnerable	HHHR	Arctic
lapponicus	Pyrobombus	Lapland bumblebee	Lapphumla	Least concern	HHR	Scandinavian highlands and Arctic
monticola	Pyrobombus	Mountain bumblebee	Berghumla	Least concern	HHR	European highlands and Arctic
pascuorum	Thoracobombus	Common carder bee	Åkerhumla	Least concern	R	Common

[a] Climate risk categories: HHHR, extremely high climate change risk, loss of >95% of range by 2100; HHR, very high climate change risk, loss of >85% of range by 2100, HR, high climate change risk, loss of >70% of range by 2100, R, climate change risk, loss of >50% of range by 2100, LR, lower climate change risk, <50% loss of range by 2100 (Rasmont et al., 2015).

All of these montane bumblebee species are threatened by climate change. Those that are judged to be most at risk are the ones restricted to the highest elevations and latitudes: *B. pyrrhopygus*, *B. alpinus* and *B. hyperboreus*. Of these, *B. alpinus* and *B. hyperboreus* are classified as vulnerable in the species red list whereas all the other species studied here are considered least concern (Table 1) (IUCN, 2022). However, these risk assessments have been shown to be unreflective of projected population trends that model future scenarios of climate change (Ghisbain et al., 2023). Species have been classified into climate risk categories based on species distribution modelling (Rasmont et al., 2015). Two of the species under study here, *B. pyrrhopygus* and *B. hyperboreus*, are assigned the most extreme risk class, HHHR, indicating that >95% of their inhabitable range is predicted to be lost by 2100 under a realistic future climate change scenario. The other four montane species, *B. alpinus*, *B. balteatus*, *B. lapponicus* and *B. monticola*, are assigned the category HHR, indicating a loss of >85%.

We also included the samples of *Bombus pascuorum* in the study, which is a member of the subgenus Thoracobombus. In contrast to the other species, it is common all over Europe, present in a range of habitats from the Arctic to the Mediterranean. It is the most abundant bumblebee species in Europe and is relatively unthreatened by climate change compared to other species (Rasmont et al., 2015). It therefore serves as a useful comparison to other species as it is expected to have relatively high levels of genetic variation. *Bombus pascuorum* is a highly polytypic species with 24 described subspecies found throughout Europe (Lecocq et al., 2015). Four of these subspecies are found in Sweden: *B. p. smithianus* north of the Arctic circle, *B. p. sparreanus* in northern Sweden south of the Arctic circle, *B. p. pallidofacies* in central and southern Sweden, *B. p. gotlandicus* on Gotland. *B. pascuorum* is the most widespread and abundant bumblebee species in Europe and can survive in urban and suburban areas where other bumblebee species are scarce (Rasmont et al., 2015). *B. pascuorum* is assigned the lower climate risk category R, indicating >50% predicted loss in a similar time.

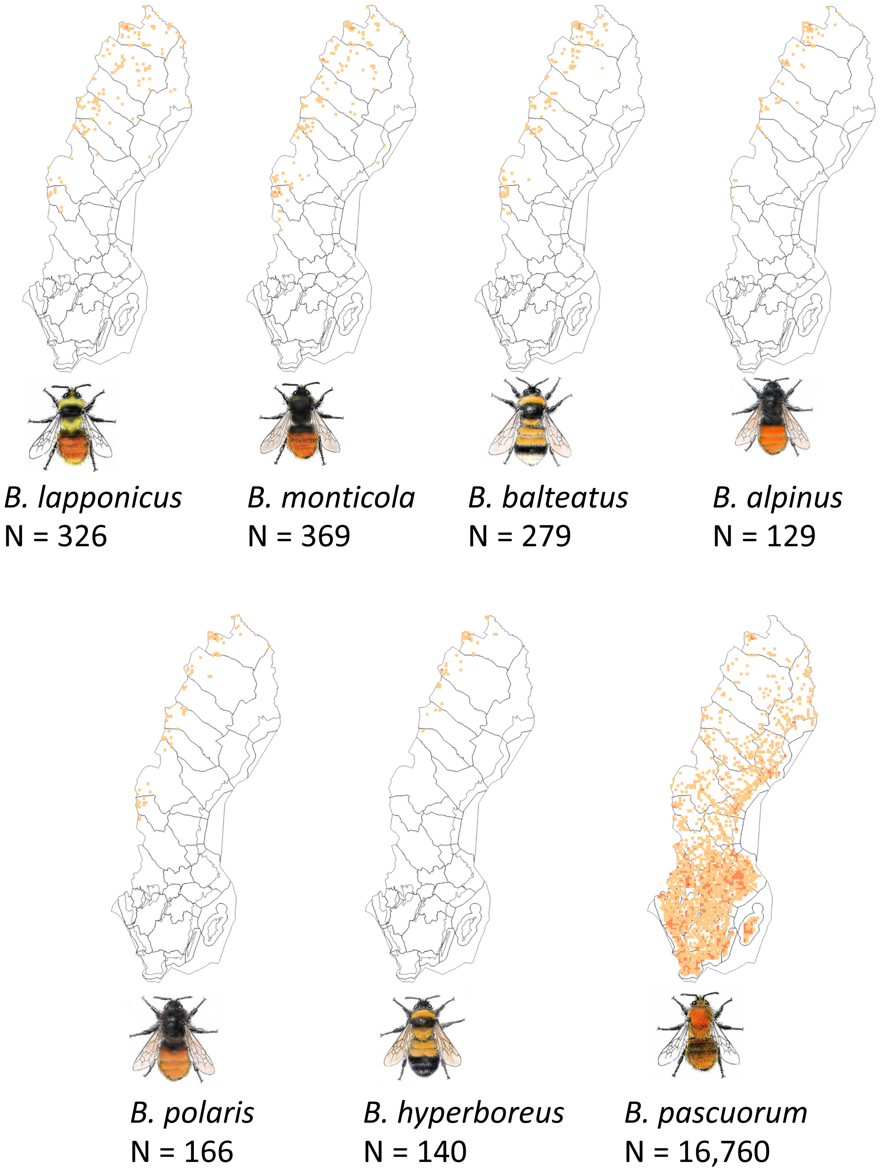

FIGURE 1 Distribution of study species in Sweden. Points on the map represent observations since 1997 reported in Swedish Species Observation System (*N* is the number of observations). *B. lapponicus*, *B. monticola* and *B. balteatus* are found widely in the Swedish mountains and in the Arctic. *B. alpinus*, *B. pyrrhopygus* and *B. hyperboreus* are restricted to higher elevations and latitudes in these areas. *B. pascuorum* is a common bumblebee in the whole of Europe, distributed from the Arctic to the Mediterranean. Maps from Swedish Species Observation System (artportalen.se).

Here, we performed whole-genome sequencing on 333 samples of the seven bumblebee species collected throughout their ranges in Sweden. The data were used to estimate a range of population genetic parameters for each species to inform about their conservation status and population viability. We first generated phylogenetic trees of all the samples in order to confirm evolutionary relationship between species and search for the presence of any previously unknown cryptic species. We next performed an analysis of population structure in each species to detect the presence of subpopulations and barriers to gene flow across their ranges. We estimated the levels of genetic variation across the genome and used these values to estimate effective population size (N_E) for each species. We also performed a reconstruction of historical variation in N_E using the programme SMC++, which analyses variation in coalescent time and the site frequency spectrum of genetic variation across the genome (Terhorst et al., 2017). Finally, we analysed the length distribution of runs of homozygosity (ROH) in samples of each species to infer the prevalence of inbreeding (Ceballos et al., 2018).

2 | METHODS

2.1 | Sampling and DNA extraction

Specimens of seven bumblebee species were collected during the summer of 2021 from several locations in Sweden. This involved field trips to high elevation locations in Lapland, Härjedalen and Jämtland to collect montane species and additional collections of the widespread species *B. pascuorum* in locations throughout Sweden. Sampling locations are displayed in Figure 2, a summary of collections is found in Table 2 and a full list of samples and associated data is presented in Table S1. Samples were captured with sweeping hand nets and kept temporarily in Falcon tubes accompanied with ice packs. Species and sex/caste of each sample were identified using a guide (Ødegaard et al., 2015). The identity of each sample was confirmed by the genetic analysis, which also enabled a subset of samples for which field identification was ambiguous or incorrect to be correctly identified (see below). Samples were stored in 95% ethanol at −20°C. The DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit from a middle leg and thorax muscle taken from the coxal cavity of the middle leg.

2.2 | Sequencing and variant calling

All species from the Pyrobombus and Alpinobombus subgenera studied here are closely related to a single species from their subgenus for which a high-quality genome assembly is available. It is, therefore, possible to use one genome assembly from each subgenus to map reads from all of the other species of that subgenus. We used a genome assembly of *B. lapponicus sylvicola* (ASM1967717v1; PRJNA646847) to map Pyrobombus species and a genome assembly of *B. kirbiellus* (ASM1920181v1; PRJNA704506) to map Alpinobombus species. Both of these genome assemblies

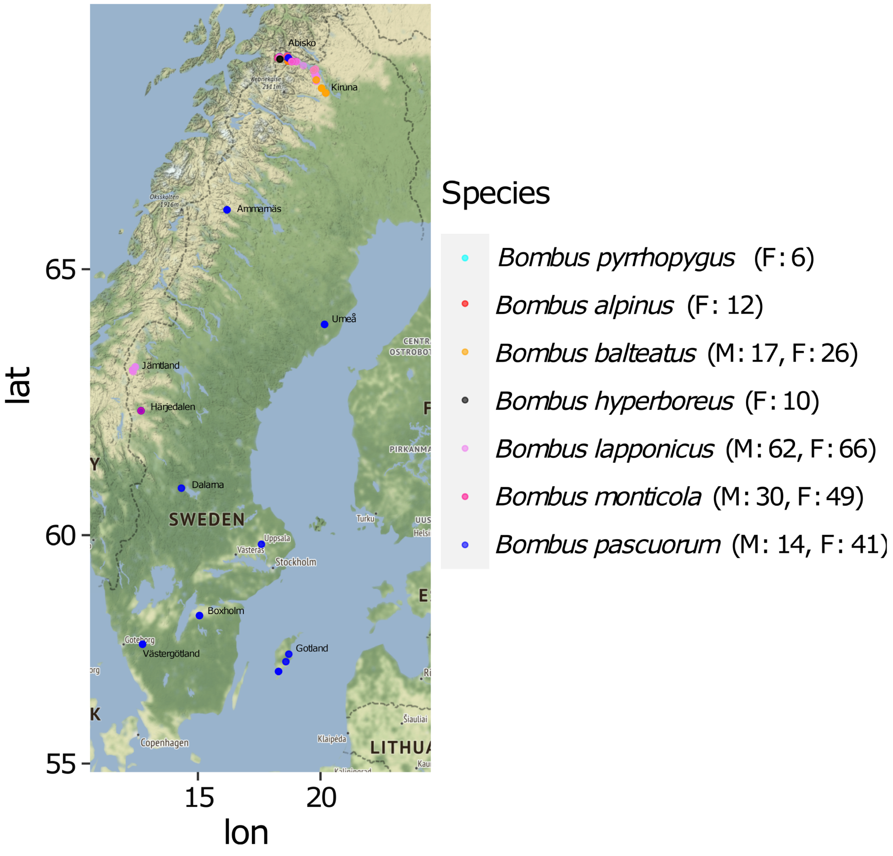

FIGURE 2 Map of bumblebee sample collection for this study. The solid dots in different colours denote the sampling sites for each species across Sweden. Number of males (M) and females (F; workers and queens) collected are given. Map from Stamen design (CC BY 4.0).

TABLE 2 Number and location of bumblebee samples used in this study.

Bombus sp.	Province									Total
	Lappland	Jämtland	Härjedalen	Västerbotten	Uppland	Dalarna	Östergötland	Västergötland	Gotland	
alpinus	12	0	0	0	0	0	0	0	0	12
pyrrhopygus	5	1	0	0	0	0	0	0	0	6
balteatus	42	0	1	0	0	0	0	0	0	43
hyperboreus	9	1	0	0	0	0	0	0	0	10
lapponicus	109	16	3	0	0	0	0	0	0	128
monticola	63	1	15	0	0	0	0	0	0	79
pascuorum	23	0	4	8	2	2	4	4	8	55
Total	263	19	23	8	2	2	4	4	8	333

are derived from samples from the Rocky Mountains, United States (Christmas et al., 2021, 2022). *B. lapponicus sylvicola* in United States is an extremely close relative of *B. lapponicus* in Sweden. The most recent taxonomic revision considers them to be the same species (*B. lapponicus*) although they were previously considered to be two species, *B. sylvicola* (North America) and *B. lapponicus* (Eurasia) (Martinet et al., 2019). A similar situation exists for *B. kirbiellus* in North America and *B. balteatus* in Eurasia. In this case, these two species were previously both considered to be *B. balteatus*, but the most recent taxonomic revision has split *B. balteatus* into two species *B. kirbiellus* in North America and *B. balteatus* in Eurasia (Williams et al., 2019). The genome assemblies that are available are therefore from species currently known as *B. lapponicus sylvicola* (previously *B. sylvicola*) and *B. kirbiellus* (previously *B. balteatus*), which are closely related to the study species *B. lapponicus* and *B. balteatus* found in Sweden. *Bombus pascuorum* is distantly related to the other study species and we used a publicly available genome assembly (iyBomPasc1.1; PRJEB43481) to map reads from this species.

Paired-end sequencing libraries were prepared with the Nextera DNA Flex Library Preparation Kit and samples were sequenced on an Illumina NovaSeq6000 with 2×150bp reads. The read data for each species were mapped to their corresponding reference genomes. *B. monticola* and *B. lapponicus* were mapped to *B. lapponicus sylvicola* (Pyrobombus). *B. balteatus*, *B. alpinus*, *B. hyperboreas* and *B. pyrrhopygus* were mapped to *B. kirbiellus* (previously *B. balteatus*, Alpinobombus), and *B. pascuorum* was mapped to *B. pascuorum* (*Thoracobombus*). Mapping was performed using the BWA-MEM algorithm (Li, 2013). Mappings were sorted and written to bam files using samtools. Picard was used to add read groups and mark duplicate reads in the bam files that were then indexed in samtools. The variants were called following the GATK best practices for non-model species (Poplin et al., 2018). HaplotypeCaller was run with each bam files with flag '-ploidy' to generate individual-specific gVCF files. All gVCF files were merged on a per contig basis by GenomicsDBImport and called variants by GenotypeGVCFs. The resulting variant data sets were hard-filtered following the GATK recommended thresholds.

Samples were initially all called as diploids, which allowed us to confidently confirm the sex of each sample by the absence of heterozygous sites in haploid males. Samples for which the sex assigned from identification in the field was incongruent with ploidy were examined morphologically in more detail to confirm sex. Heterozygous variants that were detected when HaplotypeCaller was run with '-ploidy 2' for haploid samples were considered unreliable and filtered out for each cohort data set. All samples were assigned their correct ploidy to infer genotypes for the final data set.

Additional SNP filters were applied with bcftools (Li, 2011): (1) only including biallelic SNPs with minimum minor allele count of 2 or greater and excluding SNPs with read depth and genotype quality per site in each sample under 3 and 20, respectively (-v snps -m 2 -M 2 --min-ac 2:minor -e 'FMT/DP <3 | FMT/GQ <20'); (2) excluding sites with missing data exceeding 0.5, that is, 'F_MISSING >0.5'; (3) excluding sites with high mean depth across all samples and excessive heterozygosity (INFO/DP>X | INFO/ExcessHet>25), where X=1000 for both *B. pascuorum* and Alpinobombus data sets and X=3134 for Pyrobombus to exclude sites with depth in the top 5% of the distribution. The additional filters INFO/DP and INFO/ExcessHet allowed us to remove sites due to alignments to multiple regions and variant calling errors. Three SNP data sets were generated, corresponding to sets of samples mapping to each of the three reference genomes, ready for the downstream analyses.

In order to directly compare the levels of genetic variation in *B. lapponicus* and *B. balteatus* with their sister species in North America, the gVCF files generated by HaplotypeCaller were combined with a similar data set of samples collected previously in the United States Rocky Mountains (Christmas et al., 2021, 2022) (NCBI PRJNA646847 and PRJNA704506). The variants were then genotyped and filtered for the two combined sets of gVCF files following the same steps and filters as above. For the combined data sets, 'INFO/DP >25000' was used. This cut-off was chosen by examining the INFO/DP distributions in each data set.

2.3 | Population structure

Reconstruction of neighbour-joining (NJ) trees (Saitou & Nei, 1987), principal component analyses (PCA) and admixture (Alexander et al., 2009) analyses were performed to reveal the genetic diversity of each geographical population using the SNP data sets. For these analyses, only

SNPs with minor allele frequency greater than 5% were included. Scripts provided by Simon Martin were used to calculate distance matrix for NJ trees taking into account mixed ploidy of samples (https://github.com/simonhmartin/genomics_general). The trees were made and visualized with FastMe (Guindon & Gascuel, 2003) and FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/), respectively.

PCA analyses for each species were implemented in PLINK v1.9 (Chang et al., 2015). To better present the geographical clustering, one sample (DA_2) of *B. pascuorum* overlapped with sample DA_03 was removed due to their close relatedness, probably from the same nest. The Admixture program was used for eight runs of population structure by specifying the level of ploidy for each sample (Alexander et al., 2009). The best-supported values of K were inferred by selecting the one with the lowest cross-validation error by adding '--cv' flag in this program.

2.4 | Confirmation of species identification

The NJ trees were used in conjunction with detailed morphological analysis to correctly identify samples where species identification in the field was incongruent with genetic analysis. This was particularly important for species with similar colouration patterns, which is the case for *B. lapponicus* and *B. monticola*, and for *B. alpinus* and *B. pyrrhopygus*. A WILD M5-47147 stereo microscope for preparation was used for this purpose.

All specimens of *B. lapponicus* in this study had yellow hairs on the last two tergites in contrast to samples of *B. monticola* which had orange red hairs on the last tergites. Detailed analysis of this character was particular important for populations of *B. lapponicus* in the southern part of the mountain chain, which are darker than northern populations and difficult to distinguish from *B. monticola* in the same area (Ødegaard et al., 2015).

A good diagnostic field character to separate *B. alpinus* and *B. pyrrhopygus* proved to be the colour of hairs on the two first metasomal segments. In *B. alpinus*, there is a distinct limit between the black hairs on the first tergite and the red hairs on the second tergite. In *B. pyrrhopygus*, the colour transition is much more diffuse and does not necessarily follow the tergite limitation. In addition, the cuticle in *B. pyrrhopygus* is densely shagreened and dull while in *B. alpinus*, it has a smoother, shinier surface. This can preferably be seen on the outer surface of the hind tibiae. After detailed morphological analysis of all samples for which genetic analysis was incongruent with field identification, we were able to confidently determine the species for each sample.

2.5 | Estimation of effective population size

Effective population size (N_E) for each species was estimated using an estimate of population mutation rate, Watterson's estimator (θ_W), involving two equations: $\theta_W = \frac{K}{a_n}$, where K is the number of segregating sites in the species and a_n is the (n−1)th harmonic number, n denoting the number of chromosome sets. N_E can be obtained using an estimate of θ_W per base and an estimate of the mutation rate per base μ based on $\theta = 2pN_E\mu$, where p is the ploidy of the species ($p = 1.5$ for haplodiploid bumblebees) (Ferretti & Ramos-Onsins, 2015; Fu, 1994). For these analyses, we used a direct estimate of mutation rate from *B. terrestris* ($\mu = 3.6 \times 10^{-9}$) (Liu et al., 2017).

2.6 | Detection of runs of homozygosity (ROH)

The segments of ROHs for females from each species were assayed using the '--homozyg' flag in PLINK v1.9 (Chang et al., 2015), which uses a sliding window approach to scan each individual's genotype at each marker position across the genome. The following criteria were defined to present the length of ROHs: (1) a minimum ROH length of 10 kb containing at least 50 SNPs; (2) a maximum gap between consecutive SNPs of 1 Mb; (3) a minimum density of one SNP in 50 kb; (4) a sliding window of 50 SNPs across the genome with one SNP step; (5) no tolerance of heterozygous sites and a maximum three missing genotypes; (6) a default window threshold of 0.05. The lengths of ROH were divided into three classes: 0.01–0.5 Mb, 0.5–1 Mb, 1–1.5 (detected maximum length) Mb.

2.7 | Inference of population demographic history

SMC++ (Terhorst et al., 2017) was used to infer past fluctuations in N_E. This method uses the inferred distribution of coalescence times at loci across the genome and the site frequency distribution of SNPs to infer temporal variation in N_E (Terhorst et al., 2017).To ensure the reliability of SNP calling, only female samples with read coverage greater than 13× were selected for running SMC++, in line with other studies (Wang et al., 2021). None of the samples included in this analysis showed high relatedness according to the PCA analysis. The mean depth per individual was estimated using the '--depth' flag in VCFtools (Danecek et al., 2011). The previously filtered VCF files were used as inputs by keeping only these samples. Eventually, 49 samples were used for the demographic inference, including seven *B. pascuorum*, 10 *B. monticola* and 11 *B. lapponicus*, as well as 12 *B. balteatus* and three for *B. alpinus*, *B. hyperboreus* and *B. pyrrhopygus*, respectively. We used the '-d' option to specify between 3 and 12 different samples per species as 'distinguished individuals' across runs in order to generate a composite likelihood, which should result in improved estimates of population size history (Terhorst et al., 2017). Estimations from SMC++ were scaled using a generation time (g) of 1 year and a mutation rate (μ) 3.6×10^{-9} substitutions per site per year (see above).

3 | RESULTS

3.1 | Field sampling of seven bumblebee species

The samples of the study species were collected during the summer of 2021. Sample collection in Abisko and the vicinity yielded samples of all seven species (263 samples in total). Additional collections were also made in Jämtland (19 samples) and Härjedalen (23 samples). Samples of the widespread species *B. pascuorum* were collected from throughout its range in Sweden, including the island of Gotland. This incorporated six main geographic areas in which four different subspecies and hybrids between them were located.

The final data sets consisted of 333 samples (Webster, 2022). Three of the species—*B. alpinus*, *B. pyrrhopygus* and *B. hyperboreus*—have a more restricted distribution at higher elevations and latitudes. These species were found in lower numbers than the other, more widespread, montane species and were only present in a subset of localities. The sampling locations for all seven species are displayed in Figure 2. A summary of samples collected in each location is presented in Table 2. A full list of samples that were sequenced and associated metadata is presented in Table S1.

3.2 | Whole-genome sequencing and bioinformatic analysis

All samples were subjected to Illumina whole-genome sequencing. Raw sequence reads from each sample were aligned to the appropriate reference genome to generate three separate multi-species alignments: (1) The 207 Pyrobombus samples (*B. lapponicus* and *B. monticola*) were aligned to *B. lapponicus sylvicola* (Christmas et al., 2021). (2) The 71 Alpinobombus samples (*B. alpinus*, *B. pyrrhopygus*, *B. balteatus*, *B. hyperboreus*) were aligned to the *B. kirbiellus* reference assembly (Christmas et al., 2022). (3) The 55 *B. pascuorum* samples aligned to the *B. pascuorum* reference genome assembly. Mean sequencing depth was 10.5 x per individual and did not vary substantially between species (Table S1).

We first determined ploidy of each sample by detecting the presence of heterozygous sites. This analysis confirmed whether samples were male (haploid) or female (diploid). One sample of *B. hyperboreus* was identified morphologically as a male, but was found to be diploid on the basis of the genetic analysis, indicating that it is a diploid male (see below for additional analysis). We updated sample classifications in cases of incorrect classification in the field. Species identity was then confirmed by constructing NJ trees for each of the three multispecies alignments. The seven different species were clearly distinguishable from NJ trees. A small number of samples that had been incorrectly classified were identified from NJ trees and correctly classified. In addition, the identity of samples of the two species *B. alpinus* and *B. pyrrhopygus*, which are difficult to identify due to their convergence in morphology, was confirmed by a combination of genetic clustering in NJ trees and detailed morphological analysis. The numbers in Table 2 refer to correctly classified samples.

3.3 | Levels of genetic variation

We estimated the levels of genetic variation in all species using Watterson's theta (θ_W) and nucleotide diversity (average number of pairwise differences, π) (Watterson, 1975) (Table 3). Estimates of θ_W

TABLE 3 Genetic variation in seven Swedish bumblebee species.

Bombus sp.	Male	Female	Haploid genomes sampled[a]	No. of SNPs	Watterson's theta per base (θ_W) (%)	Nucleotide diversity (π) (%)	Effective population size (N_E)	Observed heterozygosity (H_{OBS})
alpinus		12	24	570,149	0.061	0.08	56,534	0.333
pyrrhopygus		6	12	1,390,927	0.184	0.236	170,545	0.371
balteatus	17	26	69	2,370,054	0.197	0.24	182,673	0.283
hyperboreus		10	20	660,899	0.074	0.099	68,978	0.306
lapponicus	62	66	194	3,095,046	0.21	0.227	194,583	0.280
monticola	28	48	124	1,085,476	0.08	0.091	73,814	0.298
pascuorum	14	41	96	3,800,079	0.241	0.273	222,742	0.218

[a] Sum of haploid genomes found in both haploid and diploid individuals.

are 0.06%–0.25% (Wallberg et al., 2014), which we converted into estimates of long-term N_E based on a direct estimate of mutation rate in the bumblebee *Bombus terrestris* (see Section 2). Levels of N_E vary between species. The highest estimates of N_E are exhibited by *B. pascuorum* (~220,000; Table 3). This is consistent with the fact that it is a widespread species in Europe, found in many habitats, which may be expanding in numbers (Rasmont et al., 2015). Two other species that are relatively widespread in the Swedish mountains, *B. lapponicus* and *B. balteatus*, also exhibit relatively high N_E (~195,000 and ~180,000, respectively). *B. pyrrhopygus* also exhibited relatively high N_E (~170,000), which is surprising considering that it restricted to high elevations and was the species observed the fewest number of times during collection. Conversely, those species with the most restricted distributions had significantly lower levels of genetic diversity. Estimates of N_E for *B. alpinus* and *B. hyperboreus* were both in the vicinity of 60,000 (Table 3). Surprisingly, *B. monticola* also has an N_E in this range, despite being one of the more abundant montane species. In general, however, differences in levels of long-term N_E between species were consistent with expectations based on species abundance and range size.

3.4 | Population structure

We estimated population structure and species relationships of all species using three methods: Neighbour-joining (NJ) trees (Saitou & Nei, 1987), principle component analysis (PCA) and admixture analyses (Alexander et al., 2009). The NJ tree of *B. lapponicus* and *B. monticola* reveals that the samples cluster by species with no additional structuring apparent (Figure 3a). Samples of *B. lapponicus* from Lappland, Jämtland and Härjedalen showed no evidence of population substructure or clustering by geography in the PCA (Figure S1). This was further supported by the admixture analysis, in which a single population was the best supported model by cross-validation (Figure 3b). Samples of *B. monticola* from Abisko formed clusters according to PCA (Figure S1). However, the three samples that form a separate cluster were collected in exactly the same location and the most likely reason for the clustering is therefore that they are related. Admixture analysis for *B. monticola* also supports a single panmictic population (Figure 3b).

NJ trees for the species of subgenus Alpinobumbus—*B. balteatus*, *B. alpinus*, *B. pyrrhopygus* and *B. hyperboreus*—reflect the evolutionary relationships between these species (Figure 4a). Tree topologies are the same as those published based on analyses of phenotypic characters and molecular barcodes (Williams et al., 2019), which place *B. alpinus* and *B. pyrrhopygus* as sister taxa. No significant evidence of clustering either within or among geographical regions was observed for any of the four Alpinobombus species using PCA (Figure S1). A single population was the best-supported model by cross-validation using admixture for all species (Figure 4b). However, it should be noted that the sample sizes for *B. alpinus*, *B. pyrrhopygus* and *B. hyperboreus* were all relatively low (6–12 individuals per species), which results in low power to detect population structure. *B. alpinus* was only sampled in Abisko, which means it is impossible to identify genetic structuring among geographic regions for this species. For *B. hyperboreus*, a single sample collected in Jämtland did not cluster with the other samples from Abisko, which could indicate the presence of geographical structure in the species although more samples would be required to confirm or refute this.

In contrast to the species already discussed, *B. pascuorum* is a polytypic species found in a wide range of habitats across Europe (Lecocq et al., 2015). The sampling strategy for this species incorporated the four subspecies present in Sweden, in addition to samples identified as hybrids between pairs of subspecies collected in hybrid zones. The NJ tree of these samples revealed limited levels of clustering (Figure 5a). The samples collected from Gotland, from the subspecies, *B. p. gotlandicus*, were clearly separated from the other samples. A limited amount of separation was also observed for samples from Abisko, consisting of the subspecies *B. p. smithianus*. The remaining samples, consisting of *B. p. sparreanus*, *B. p. pallidofacies* and hybrids between subspecies, collected over a wide geographical area, do not show evidence of substructure. The PCA plot reveals a similar pattern, with samples from Gotland and Abisko forming separate clusters from the remaining samples from mainland Sweden. These remaining samples do not show evidence for geographic structuring (Figure S1). The admixture analysis also revealed that samples from Abisko and Gotland formed separate clusters from the remaining samples (Figure 5b). Cross-validation analysis indicated that $K=2$ had best support, implying two major populations, one on Gotland and one on mainland Sweden, although the admixture plots also show some degree of distinctness of the Abisko population.

Taken together, these analyses indicate that the six montane bumblebee species studied here do not exhibit obvious population substructure across their ranges in Sweden. By contrast, the widespread polytypic species *B. pascuorum* exhibits a degree of substructure. A population on Gotland appears to be isolated from the mainland population. Furthermore, samples collected in Abisko appear to have a degree of isolation from other mainland populations further south, which display different colouration patterns and are described as belonging to different subspecies (Lecocq et al., 2015).

3.5 | Inference of historical fluctuations in N_E using SMC++

We used the program SMC++ to infer historical fluctuations in N_E in all seven of the study species (Figure 6). We infer that populations of *B. hyperboreus* have declined since ~10^4 years ago (the end of the last ice age). The analysis suggests that current N_E could be less than 5000, although historically it has been more than 10 times higher (Table 3, ~70,000). The species *B. pyrrhopygus* also shows signs of decline in this period, which could indicate a contemporary N_E around 25,000 compared to a value of ~75,000 estimated from θ_W. The other montane species under investigation do not show the signals of major population expansions or declines and have been relatively stable during the Holocene. In contrast to the arctic and

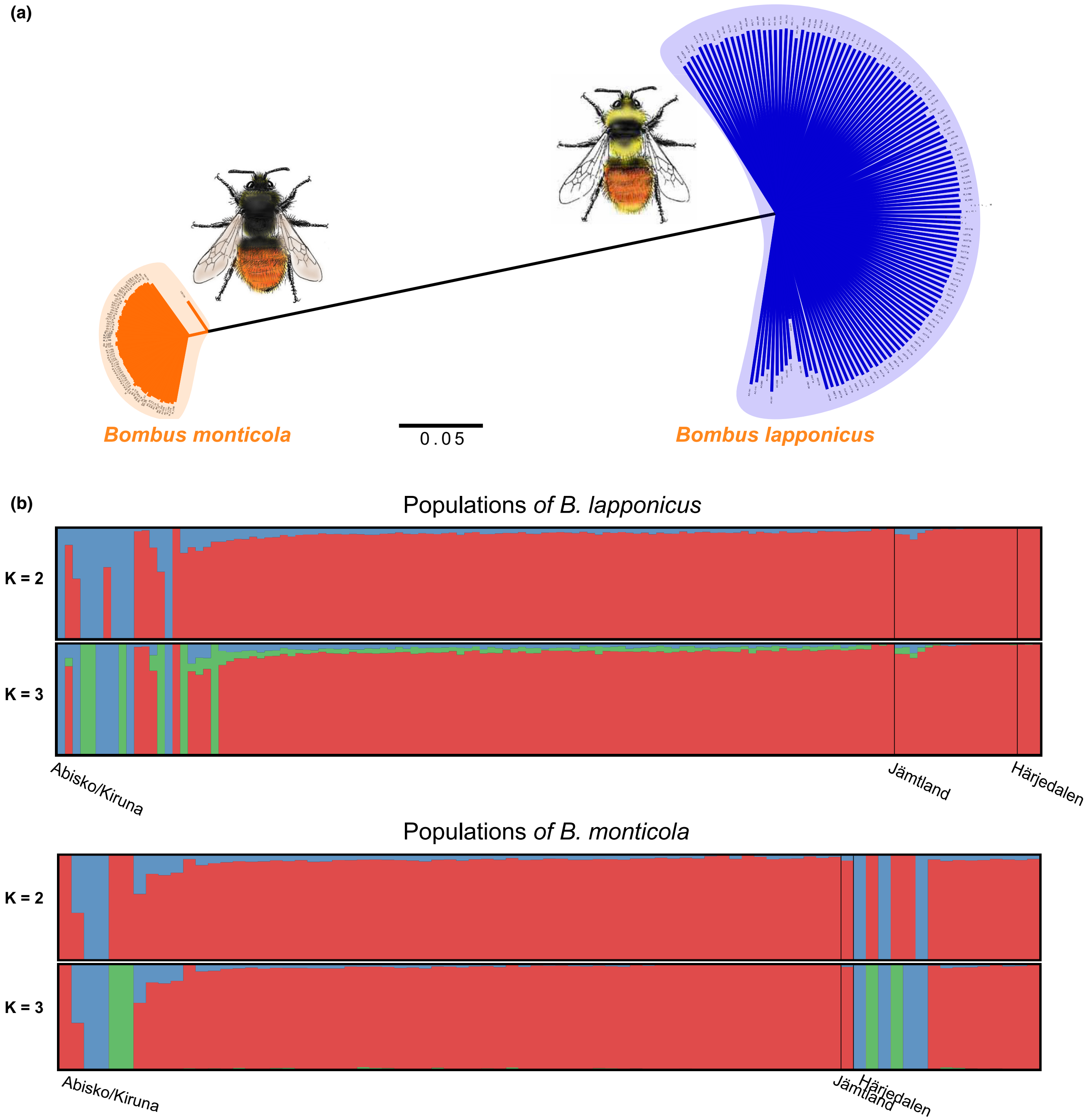

FIGURE 3 (a) Neighbour-joining tree for samples of *B. lapponicus* and *B. monticola* generated using whole-genome sequence data. Both species are in the subgenus Pyrobombus. Each external branch represents an individual sample. (b) Admixture analyses for all geographical populations of *B. lapponicus* (top) and *B. monticola* (bottom). No significant structure was detected for either species and $K=1$ was the best supported parameter value for both species.

montane species, there are signs that *B. pascuorum* has undergone a population expansion during this period.

3.6 | Runs of homozygosity

We scanned the genomes of all diploid samples (typically females) for runs of homozygosity (ROH) (Table 4; Figures S2–S5). Figure 7 shows the proportion of the genome of each sample that falls into ROH >0.1 Mb, grouped by species. The most striking finding was a single individual of *B. hyperboreus* (WO_001) with >55% of the genome lying in ROH longer than 0.1 Mb (Figure 7). This pattern is predicted to result from a sib-mating. This sample was also classified as a diploid male, which indicates that it is homozygous at the sex-determining locus. The sample is the only *B. hyperboreus* collected from Jämtland. All of the other samples of this species were found in the vicinity of Abisko.

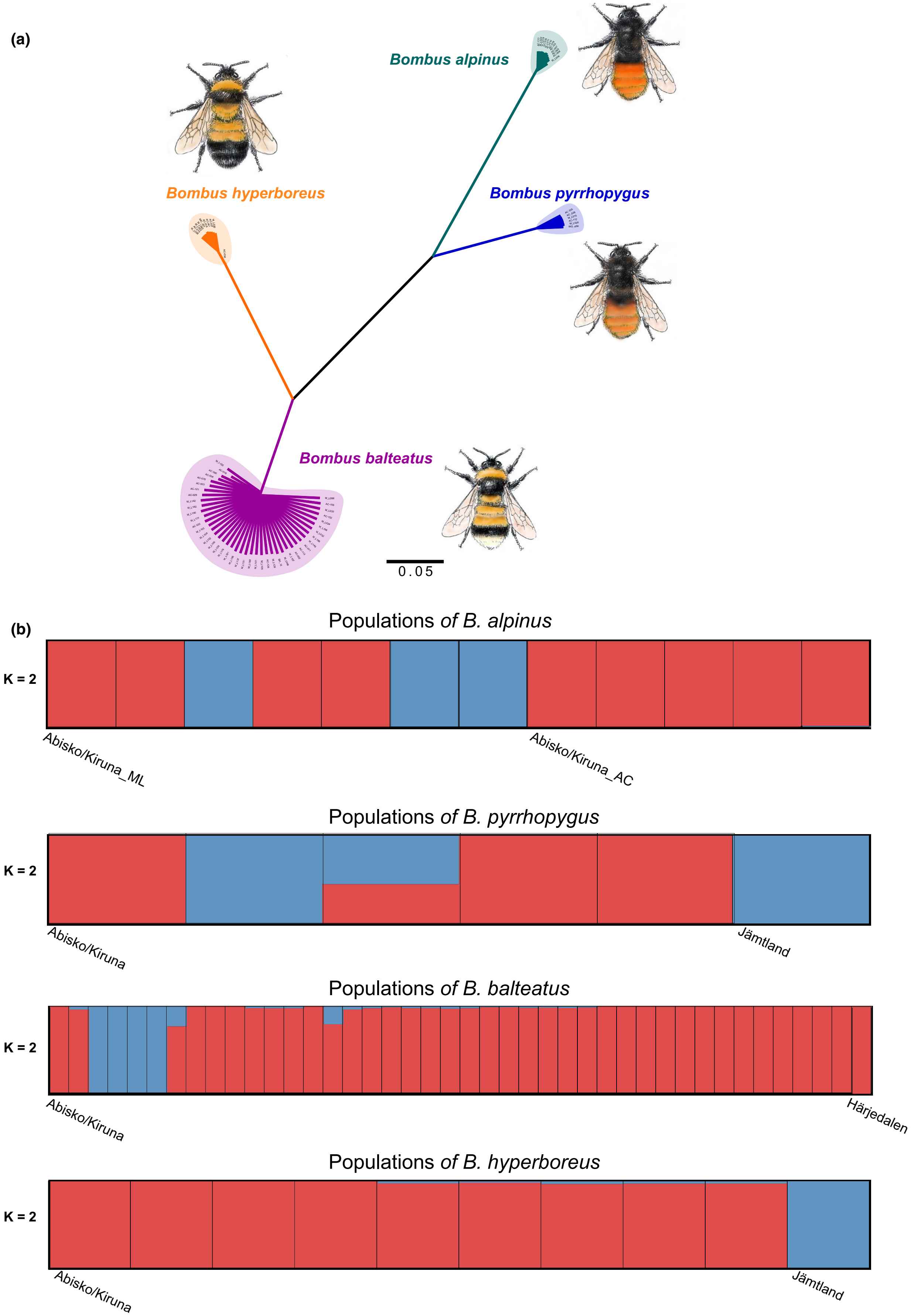
(a)
Bombus alpinus
Bombus hyperboreus
Bombus pyrrhopygus
Bombus balteatus
0.05
(b)
Populations of B. alpinus
K = 2
Abisko/Kiruna_ML
Abisko/Kiruna_AC
Populations of B. pyrrhopygus
K = 2
Abisko/Kiruna
Jämtland
Populations of B. balteatus
K = 2
Abisko/Kiruna
Härjedalen
Populations of B. hyperboreus
K = 2
Abisko/Kiruna
Jämtland

FIGURE 4 (a) Neighbour-joining tree for *B. alpinus*, *B. pyrrhopygus*, *B. balteatus* and *B. hyperboreus*. All species are in the subgenus Alpinobombus. Each external branch represents an individual sample. (b) Admixture analyses for all geographical populations of *B. alpinus*, *B. pyrrhopygus*, *B. balteatus* and *B. hyperboreus*. No significant structure was detected for any of the species and $K = 1$ was the best supported parameter value for all species.

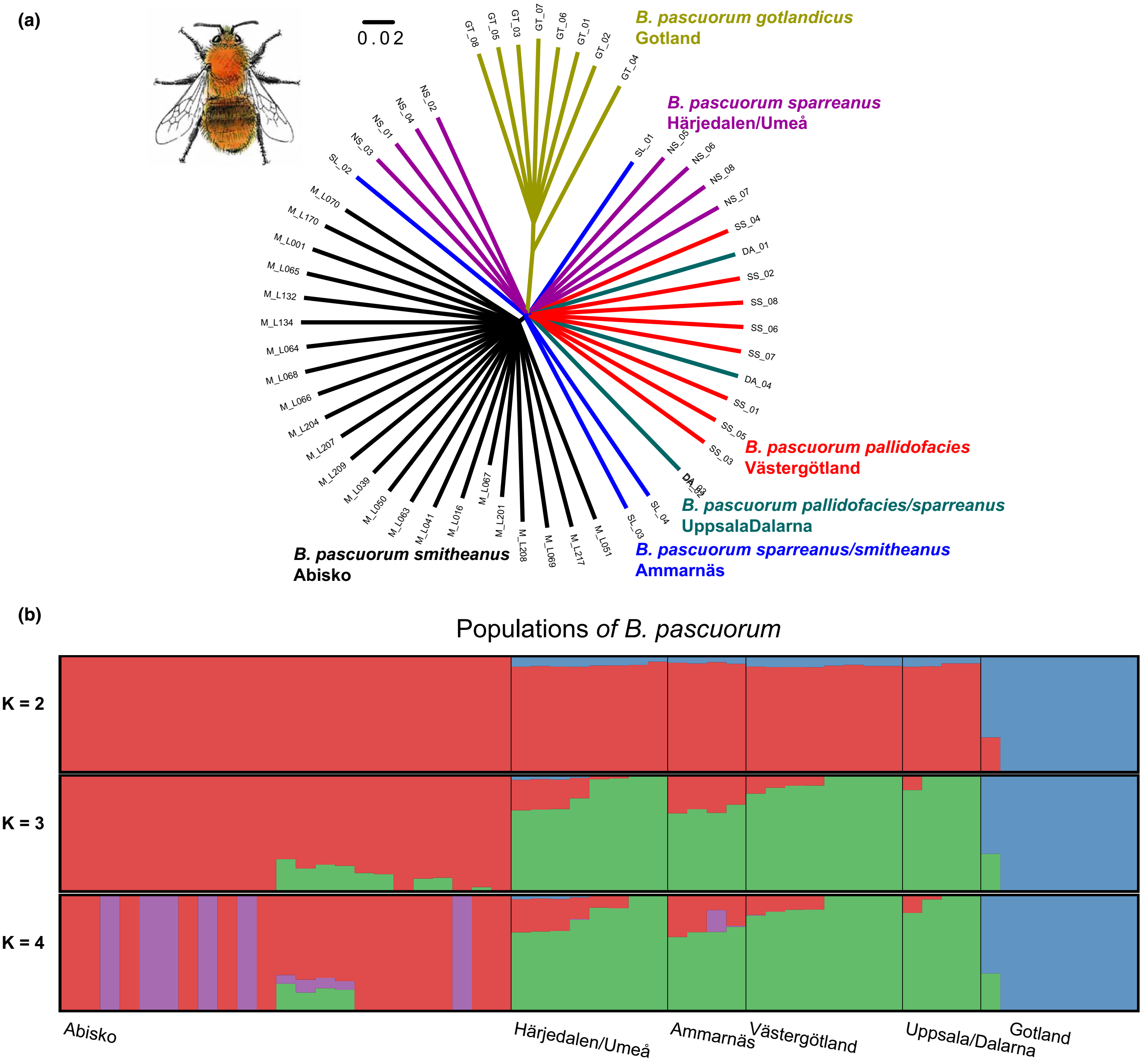

FIGURE 5 (a) Neighbour-joining tree for *B. pascuorum*. Several subspecies of *B. pascuorum* are present in Sweden. Each external branch represents an individual sample. Sampling locations and subspecies are indicated, including several from hybrid zones. (b) Admixture runs for all geographical populations of *B. pascuorum*. The best supported parameter value is $K = 2$, which indicates the presence of a distinct subpopulation on Gotland (*B. pascuorum gotlandicus*).

A small number of individuals of *B. pyrrhopygus*, *B. pascuorum* and *B. monticola* were also identified with 5%–20% of the genome covered by ROH blocks longer than 0.1 Mb indicating that inbreeding occurs sporadically in these species as well (Figure 7). Outliers with long ROH tracts were not detected in the other species, but may still occur at low frequencies. Only a small number of samples contained any ROHs longer than 1 Mb, and for the majority of species, none of samples had any such long ROHs (Table 4). The mean coverage of the genome by ROH is <5% for all species apart from *B. hyperboreus*, where the presence of the one highly inbred diploid male individual strongly influences this measure. Average levels of ROH in *B. pascuorum* are high compared

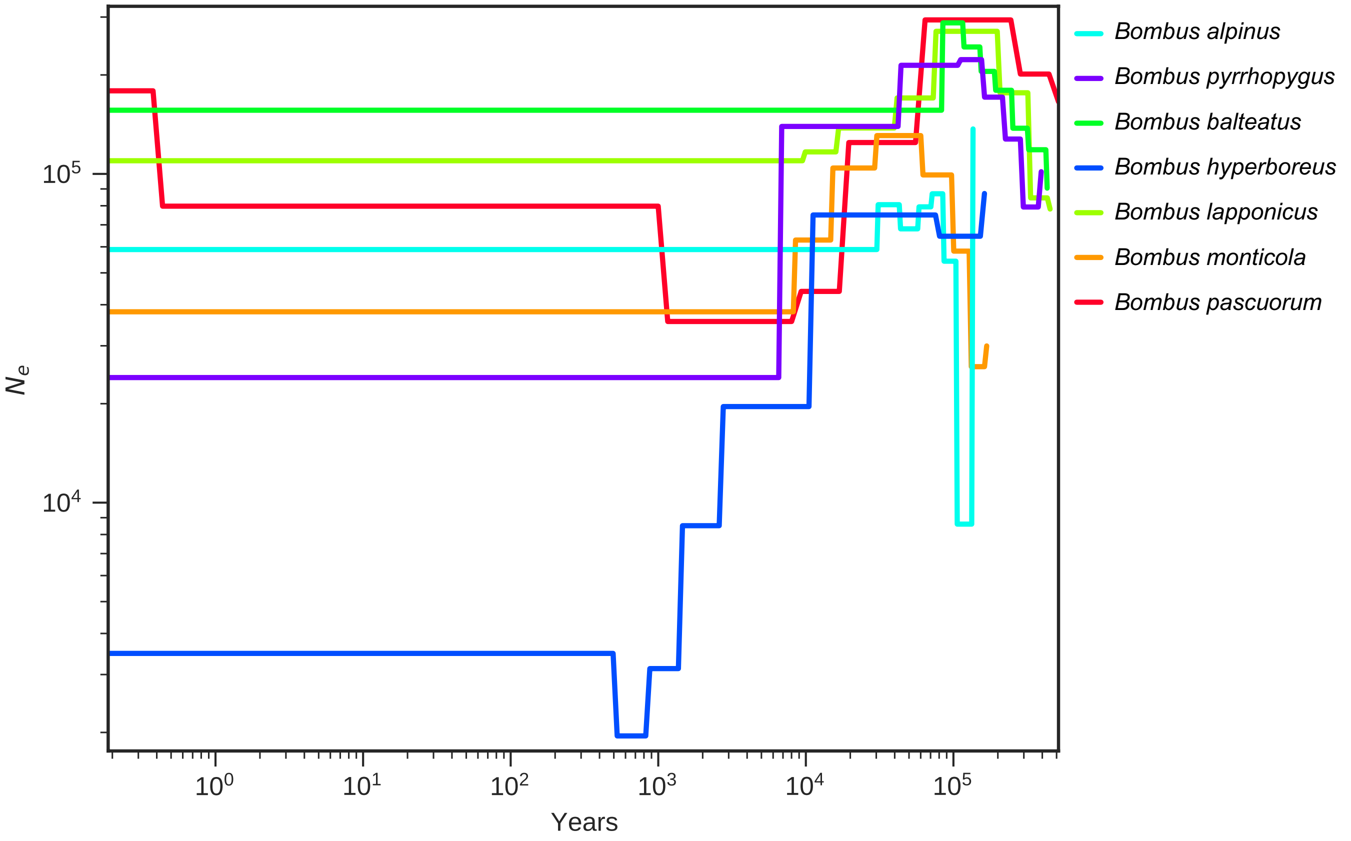

FIGURE 6 Estimation of historical variation in N_E using the SMC++ method. The *x*-axis denotes years before present. Strong signals of population decline are inferred for *B. hyperboreus*, whereas population size increases are inferred for *B. pascuorum*.

to the other species, with ROH covering ~5% of the genome on average (Table 4).

3.7 | Comparison of levels of genetic variation with North American montane bumblebee species

Sister species of *B. lapponicus* and *B. balteatus* are found in mountain and arctic locations in North America. These species are highly similar in morphology and recently evolutionarily diverged from their European counterparts. The sister species of *B. lapponicus* in North America is also known as *B. lapponicus* (subspecies *sylvicola*). The sister species of *B. balteatus* in North America is *B. kirbiellus*. We compared our results to a data set of 214 samples of *B. lapponicus* and 299 samples of *B. kirbiellus* from the Rocky Mountains, Colorado, United States (Christmas et al., 2021, 2022). The data set generated here also permitted comparisons of genetic diversity in this population (Table 5). To do this, we jointly called genetic variation in both data sets simultaneously in order to directly compare the levels of genetic variation. For the two populations of *B. lapponicus* (USA and Sweden), we find that the levels of nucleotide diversity and N_E are extremely similar (~160,000). We find that *B. kirbiellus* (USA) has significantly higher N_E compared to *B. balteatus* (Sweden) ($N_E \approx 310{,}000$ compared to 170,000).

4 | DISCUSSION

We used whole-genome sequencing to survey genetic variation and analyse population history and structure of seven bumblebee species found in Sweden: six species restricted to arctic and mountain environments and one widespread species. In general, we find a lack of population structure in all of the montane species, and comparable levels of N_E in both montane and widespread species. We identify some indications of declines and elevated genetic load in the rarer montane species. The results are useful to evaluate the conservation status of each species and to inform management decisions.

4.1 | Relatively high N_E and low population structure in montane bumblebees

For all of the species under study here, we estimate long-term N_E from levels of genetic variation to be greater than 50,000. Variation in estimates of N_E among species reflects differences in their range and abundance. *B. pyrrhopygus*, *B. balteatus* and *B. lapponicus* have N_E close to that estimated for the widespread species *B. pascuorum* (~200,000), which indicates their range and abundance are also relatively large. These levels of N_E are similar to other widespread social bees such as honeybees (Wallberg et al., 2014). By contrast,

TABLE 4 Summary statistics of runs of homozygosity (ROH).

Bombus sp.	Female no.	Total no. of ROH of each length			Mean total length of ROH per sample (Mb)	Mean genome coverage by ROH per sample (%)
		0.01–0.1 Mb	0.1–1 Mb	>1 Mb		
alpinus	12	301	48	0	1.72	0.69
pyrrhopygus	6	290	87	0	5.17	2.07
balteatus	26	4797	55	0	4.51	1.80
hyperboreus	10	1844	804	1	25.47	10.18
lapponicus	53	7511	771	0	5.45	2.16
monticola	49	3799	971	2	6.99	2.77
pascuorum	41	12,329	1325	3	14.42	4.69

B. alpinus, *B. monticola* and *B. hyperboreus* have lower N_E than the other montane bumblebee species. *B. alpinus* and *B. hyperboreus* have a more restricted distribution in at higher elevations and latitudes (Figure 1) (Ødegaard et al., 2015; Rasmont et al., 2015) and are therefore expected to have smaller population sizes. A value of N_E for a population less than 500 is commonly used to suggest it is vulnerable and likely to experience the negative effects of genetic drift and inbreeding (Allendorf et al., 2022). Levels of N_E in the species studied here are substantially higher than this.

The six montane species of bumblebees studied here did not show evidence for population structure among the montane locations where they were collected in Lapland, Jämtland and Härjedalen. This indicates that there are no substantial barriers to gene flow and that the populations have historically been continuous across the Swedish mountain range. Studies of genetic structure *B. kirbiellus* and *B. lapponicus sylvicola* from North America that are sister species of two of the species studied here (Martinet et al., 2019; Williams et al., 2019) do not show evidence for geographical substructure within the Rocky Mountains, Colorado, United States (Christmas et al., 2022). Other studies have, however, found evidence for genetic differentiation of these populations on different mountain ranges in North America (Koch et al., 2017; Whitley, 2018). We find that *B. lapponicus* samples collected from populations in Sweden and Colorado have similar levels of genetic variation, whereas populations of *B. kirbiellus* from Colorado have significantly higher levels of genetic variation compared to its sister species *B. balteatus* in Sweden. This indicates that *B. kirbiellus* may be relatively more abundant in North America, which is consistent with observations from field collections (Christmas et al., 2022).

In general, studies in both Europe and North America have found that relatively abundant bumblebee species such as *B. terrestris* do not exhibit population structure over thousands of kilometres (Colgan et al., 2022; Ghisbain et al., 2020; Heraghty et al., 2023; Lozier et al., 2011), whereas very rare and declining species (e.g. *B. muscorum*) may show structure on a scale of only 10 km (Darvill et al., 2006). Genetic differentiation of populations can occur in mountain ranges if species are restricted to high elevations, as with *B. vancouverensis* in the southern part of its range in the western United States (Jackson et al., 2018; Lozier et al., 2023). The results presented here are therefore consistent with observations from other studies indicating that bumblebees do not exhibit population structure in the absence of geographical barriers unless they have particularly low abundance.

We analysed population structure in four subspecies of *B. pascuorum*, which are geographically separated and differ in colouration (Lecocq et al., 2015). The subspecies *B. p. gotlandicus*, which is restricted to the island of Gotland, was clearly genetically distinct from the other subspecies, consistent with isolation in allopatry. In addition, the subspecies *B. p. smithianus*, present in the far north of Sweden, was genetically separable from other subspecies (Figure 5). In contrast, however, the two subspecies *B. p. sparreanus* and *B. p. pallidofacies* were indistinguishable genetically, indicating that there are unlikely to be any barriers to gene flow between these two subspecies.

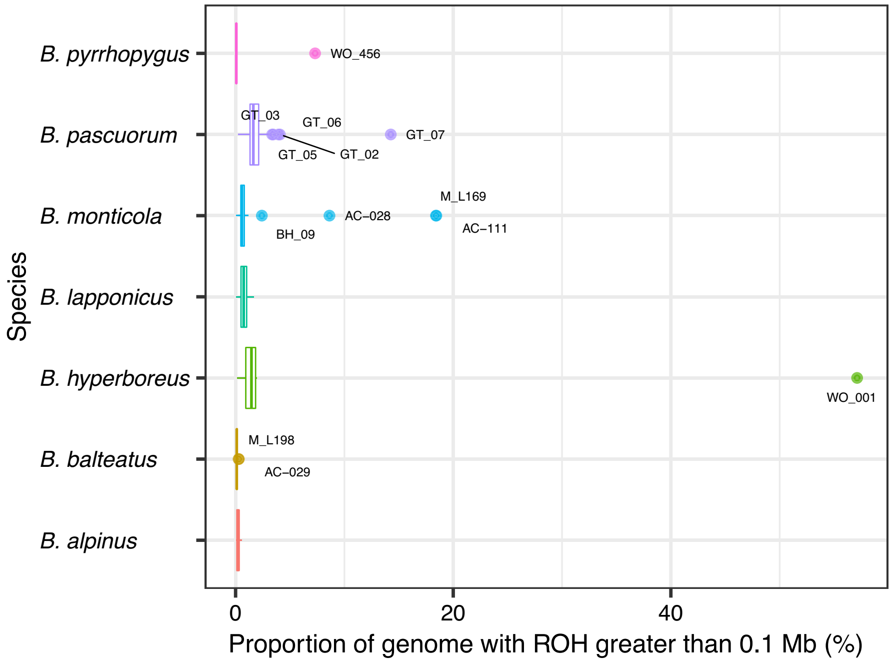

FIGURE 7 Proportion of genome of each sample comprising ROH > 0.1 Mbp. Outliers are labelled.

TABLE 5 Comparison of genetic variation in related species from Northern Europe and North America.

Species	Haploid genomes sampled[c]	No. of SNPs	Watterson's theta per base (θ_w) (%)	Nucleotide diversity (π) (%)	Effective population size (N_E)
lapponicus cluster					
B. lapponicus (SWE)	194	2,514,341	0.171	0.161	158,075
B. lapponicus[a] (USA)	428	3,010,424	0.18	0.219	166,652
balteatus cluster					
B. balteatus (SWE)	69	2,250,496	0.187	0.207	173,458
B. kirbiellus[b] (USA)	598	5,824,609	0.334	0.271	309,427

[a] The species in North America currently named *Bombus lapponicus* (subspecies *sylvicola*) was previously considered to be a separate species named *Bombus sylvicola* (Martinet et al., 2019).

[b] The species *Bombus kirbiellus* in North America was previously known as *Bombus balteatus* (Williams et al., 2019).

[c] Sum of haploid genomes found in both haploid and diploid individuals.

4.2 | Evidence of inbreeding and population declines

Haplodiploid organisms such as bumblebees and other Hymenoptera are particularly vulnerable to inbreeding because it can lead to homozygosity at the sex locus, which produces diploid males, which are unable to successfully fertilize females. The presence of diploid males can therefore severely reduce the fitness of a population. When populations become small and inbreeding is common, this effect can lead to a particularly extreme extinction vortex (Lozier & Zayed, 2017; Zayed & Packer, 2005). Diploid male production has been observed in populations of rare and declining bumblebee species and is an indicator of high vulnerability (Darvill et al., 2006; Ellis et al., 2006).

In our analysis, we identified one example of a diploid male—a *B. hyperboreus* collected in Jämtland. This sample also had the highest degree of inbreeding detected in any sample: >50% of its genome consisted of ROH greater than 100 kbp in length. It was the only *B. hyperboreus* collected in Jämtland. It is noteworthy that this is the only observation of *B. hyperboreus* this far south reported in Swedish Species Observation System, which has records over the last 25 years, with all of the other 143 reports restricted to Lapland (the majority in the vicinity of Abisko). This indicates that this sample was found at or close to the southern limit of the species distribution in the Swedish mountains. This finding indicates that inbreeding is occurring in the population of *B. hyperboreus*, particularly on the edges of its range where it is most likely to be influenced by the effects of climate change, although it is not possible to determine the extent of inbreeding in a population based on the observation of a single diploid male. We do not find evidence for inbreeding in any other species. Relatively high average levels of ROH are found in *B. pascuorum*. As *B. pascuorum* is an extremely widespread bumblebee, it is unlikely that it is experiencing inbreeding. It is therefore unlikely that the average levels of ROH observed in any of the species here have been influenced by inbreeding as they do not differ markedly from those observed in *B. pascuorum*.

Although we estimate that N_E is relatively high in all of the study species, it is important to note that our estimate of N_E from θ_W reflects the harmonic mean over evolutionary time since the coalescence of the sequences in the sample, rather than being an estimate of contemporary N_E (Nadachowska-Brzyska et al., 2022). We also performed an SMC++ analysis to estimate fluctuations in N_E over the evolutionary history of the populations (Terhorst et al., 2017). A significant finding of this analysis was that *B. hyperboreus* and to a lesser extent *B. pyrrhopygus* showed signals of population decline during the current postglacial period in the last ~10,000 years. Both of these species have restricted arctic distributions, but may have been more widespread earlier in the current post-glacial period when conditions were more favourable in a greater part of Europe. It is therefore possible that their populations have declined in the current post-glacial period. This could suggest that the range of these species has shrunk and that current N_E is much smaller than predicted from overall levels of genetic variation. The observation of inbred individuals in these species from the ROH analysis also supports this suggestion. By contrast, *B. pascuorum* is currently the most widespread and abundant bumblebee in Europe and is able to survive in a range of modern habitats (Lecocq et al., 2015). The SMC++ analysis indicates that this species may have expanded since the last ice age, although it is possible that the weak population structure observed in this species may have influenced this result.

A contrasting pattern of inferred population history using SMC approaches is exhibited by the montane bumblebee *B. vancouverensis*, which shows greater degree of population fragmentation along a north–south gradient in western North America. In this species, more isolated southern montane populations show lower genetic variation and population declines (Lozier et al., 2023). This could reflect a greater degree of habitat fragmentation, whereby subpopulations of this species have become geographically isolated at high elevation mountain peaks. By contrast, the common North American bumblebee *B. vosnesenskii* shows low population structure and no evidence of decline (Heraghty et al., 2023), similar to our observations in *B. pascuorum*.

4.3 | Absence of cryptic species

As mentioned above, the montane bumblebee species studied here have sister species in North America and their subgenera have circumpolar distributions (Martinet et al., 2019; Williams et al., 2019). Previous studies have used whole-genome sequencing to assess genetic variation and population structure in *B. lapponicus sylvicola* (sister species of *B. lapponicus*) and *B. kirbiellus* (sister species of *B. balteatus*) in several high-elevation locations in Colorado, United States (Christmas et al., 2021, 2022). One significant finding was the detection of the presence of a previously unknown cryptic species, which was indistinguishable from *B. lapponicus sylvicola* on the basis of standard morphological characters: 67 of 281 samples originally identified as *B. l. sylvicola* formed a distinct cluster of samples, termed 'incognitus', that were highly divergent from the remaining samples (Christmas et al., 2021). However, we did not observe any distinct clusters related to *B. lapponicus* in our data set of 129 samples. This is consistent with the hypothesis that 'incognitus' is restricted to North America, and whether it occurs outside of Colorado is unknown.

4.4 | Assessment of species vulnerability

Arctic and mountainous regions of Scandinavia harbour bumblebee species that are most threatened by climate change and are also projected to become refugia for more European bumblebee species in future (Ghisbain et al., 2023). Habitat loss due to agriculture or urbanization is not currently prevalent in these regions, although could potentially increase in future, which could have negative effects on bumblebees (Rasmont et al., 2015). Three of the montane species studied here, *B. lapponicus*, *B. monticola* and *B. balteatus*, are found relatively commonly in the Swedish mountains. Of these, *B. monticola* is also found at higher elevations in the United Kingdom and in mountain ranges in mainland Europe, whereas the other species are restricted to the Scandinavian mountains. According to the IUCN Red List, all these species are categorized as 'least concern' (IUCN, 2022). However, they also fall in the climate risk category HHR (very high risk), indicating they are predicted to lose >85% of their current range by 2100 (Rasmont et al., 2015). This implies that all of these species would occupy a significantly reduced range in the Scandinavian mountains, and *B. monticola* would additionally lose much of its range elsewhere in Europe except the high Alps.

Our analysis of genome variation in these three species did not uncover any evidence for depleted levels of genetic variation that would suggest they lie in the immediate risk zone for experiencing negative genetic effects due to inbreeding or genetic drift. We find the levels of variation to be lower than expected in *B. monticola* based on its relative abundance, but no indications of population decline or inbreeding. This suggests that the populations are currently healthy, despite the prediction that they will be strongly threatened by climate change in future.

The other three montane species, *B. alpinus*, *B. pyrrhopygus* and *B. hyperboreus*, have a much more restricted distribution in the Swedish mountains, occurring at higher elevations and latitudes (Rasmont et al., 2015). Of these, *B. alpinus* is also present at higher altitudes in the Alps, whereas the others are restricted to Scandinavia. *B. alpinus* and *B. hyperboreus* are categorized as 'vulnerable' according to the red list, whereas *B. pyrrhopygus* is considered 'least concern' (IUCN, 2022). *B. hyperboreus* and *B. pyrrhopygus* are placed in the HHHR climate risk category (extremely high risk), indicating they are predicted to lose >95% of their range by 2100 whereas *B. alpinus* is placed in category HHR (very high risk) (Rasmont et al., 2015).

Among these three species, we find evidence of population declines or inbreeding over evolutionary time. *B. hyperboreus* is a less abundant species with a very restricted range and, in contrast to the other species studied here, it has a specialized parasitic lifestyle. Our analysis indicated that this species has become much less abundant in the current postglacial period, and we uncovered one highly inbred diploid male individual among the 10 samples we collected. This species is considered to be one of the most vulnerable to extinction in Europe (Rasmont et al., 2015) and our analysis suggests it may be suffering negative genetic effects of population declines, particularly in the southern limit of its range. We also find evidence that *B. alpinus* and *B. pyrrhopygus* exhibit lower or declining N_E relative to the other species, consistent with their relatively restricted abundance and distribution.

Climate change is the main threat to the six montane bumblebee species studied here (Ghisbain et al., 2023). Our analysis suggests their current ranges in the Swedish mountains would be sufficient to support healthy populations in future. However, these ranges are predicted to shrink drastically under future scenarios of climate change (Rasmont et al., 2015), which could lead to population bottlenecks and fragmentation and eventually loss of all suitable habitat. Monitoring of these populations using both genetic and conventional methods would be advisable in order to understand how their populations are affected by changing climate in future.

AUTHOR CONTRIBUTIONS

Matthew T. Webster designed the research. Aoife Cantwell-Jones, Richard J. Gill, Björn Cederberg and Matthew Webster collected samples and performed species identification. Yuanzhen Liu, Anna Olsson and Tuuli Larva performed the DNA extractions. Yuanzhen Liu analysed the data. Matthew T. Webster wrote the manuscript with feedback from all co-authors.

ACKNOWLEDGEMENTS

This project was mainly funded by the Swedish Environmental Protection Agency (agreement nr. 225-21-003). The project made use of facilities at the Abisko Scientific Research Station, which is connected to SITES (Swedish Infrastructure for Ecosystem Science), INTERACT (International Network for Terrestrial Research and Monitoring in the Arctic) and SLU (Swedish University of Agricultural Sciences). Aoife Cantwell-Jones is funded by the NERC Science and Solutions for a Changing Planet Doctoral Training Programme, Imperial College London (NE/S007415/1). Collections were also supported by grants from INTERACT (H2020—agreement no. 730938) and Quekett Microscopical Club awarded to Richard Gill. The computational analysis was enabled by resources provided by the Swedish National Infrastructure for Computing (SNIC) at UPPMAX partially funded by the Swedish Research Council through grant agreement no. 2018-05973. We also acknowledge support from the National Genomics Infrastructure in Stockholm funded by Science for Life Laboratory, the Knut and Alice Wallenberg Foundation and the Swedish Research Council. We thank the following people for help with sample collection: Johan Lindell (Lappland), Willhelm Osterman (Jämtland), Peter Nolbrant (Västergötland), Niklas Johansson (Östergötland), Sven Hellqvist (Västerbotten). Turid Everitt and Matthew Christmas assisted with data analysis.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

DATA AVAILABILITY STATEMENT

All sequence data generated by the study have been deposited at the National Center for Biotechnology Information (NCBI) together with associated metadata under BioProject PRJNA890771. Scripts for bioinformatic analysis are available at GitHub: https://github.com/yzliu01/Swedish_mountain_bumblebee.

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SUPPORTING INFORMATION

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How to cite this article: Liu, Y., Olsson, A., Larva, T., Cantwell-Jones, A., Gill, R. J., Cederberg, B., & Webster, M. T. (2024). Genomic variation in montane bumblebees in Scandinavia: High levels of intraspecific diversity despite population vulnerability. *Molecular Ecology*, *33*, e17251. https://doi.org/10.1111/mec.17251