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Range-wide evolutionary relationships and historical demography of brown bears (*Ursus arctos*) revealed by whole-genome sequencing of isolated central Asian populations

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Abstract

Phylogeographic studies uncover hidden pathways of divergence and inform conservation. Brown bears (*Ursus arctos*) have one of the broadest distributions of all land mammals, ranging from Eurasia to North America, and are an important model for evolutionary studies. Although several whole genomes were available for individuals from North America, Europe and Asia, limited whole-genome data were available from Central Asia, including the highly imperilled brown bears in the Gobi Desert. To fill this knowledge gap, we sequenced whole genomes from nine Asian brown bears from the Gobi Desert of Mongolia, Northern Mongolia and the Himalayas of Pakistan. We combined these data with published brown bear sequences from Europe, Asia and North America, as well as other bear species. Our goals were to determine the evolutionary relationships among brown bear populations worldwide, their genetic diversity and their historical demography. Our analyses revealed five major lineages of brown bears based on a filtered set of 684,081 single nucleotide polymorphisms. We found distinct evolutionary lineages of brown bears in the Gobi, Himalayas, northern Mongolia, Europe and North America. The lowest level of genetic diversity and the highest level of inbreeding were found in Pakistan, the Gobi Desert and Central Italy. Furthermore, the effective population size (N_e) for all brown bears decreased over the last 70,000 years. Our results confirm the genetic distinctiveness and ancient lineage of brown bear subspecies in the Gobi Desert of Mongolia and the Himalayas of Pakistan and highlight their importance for conservation.

KEYWORDS
brown bear, evolutionary relationship, historical demography, whole-genome sequencing

1 | INTRODUCTION

Globally, the current rate of species extinction and declines has exceeded historic baselines (Cowie et al., 2022) and is largely linked to anthropogenic causes, including habitat destruction and fragmentation, environmental changes and persecution (Ceballos et al., 2015). Species with limited geographic ranges are especially vulnerable and are often considered the greatest conservation challenge (Lester et al., 2007). However, widely distributed species also present many challenges (Allendorf & Luikart, 2009), including how and where to focus on conservation efforts. Increasingly, genomic data are helping to address this issue by providing detailed descriptions of historical and contemporary connectivity, genetic diversity, demography and, perhaps most importantly, robust assessment of taxonomic units (Funk et al., 2012). Designation of species, subspecies or evolutionary unique populations leads to the recognition by the International Union for Conservation of Nature (IUCN), the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) and other national and international regulatory authorities (Haig et al., 2006). Thus, this information is critical to conservation managers and decision-makers to help guide conservation (Allendorf & Luikart, 2009; Funk et al., 2012).

The brown bear (*Ursus arctos)* is among the most widely distributed terrestrial carnivores (Nowak, 1999) and is often considered a generalist (Bojarska & Selva, 2012). However, distinct populations exhibit unique and specialized adaptations. There are 14–16 identified subspecies that inhabit temperate rainforests to harsh desert environments (McLellan et al., 2017). Their genetic relationships, demographics and basic life history have received substantial attention, making them model species for large mammals (Davison et al., 2011). Fossil data indicate that brown bears originated in Asia, and their historic distribution extended throughout Europe and North America and reached northern Africa during the Pleistocene (Kurtén, 1968; McLellan & Reiner, 1994). Although brown bears have a Holarctic distribution, their range has decreased mainly due to direct persecution over the past two centuries, leaving many fragmented, small populations at risk, especially in Asia and Europe (McLellan et al., 2017). A recent study based on 95 new brown bear genomes revealed the range-wide evolutionary history of the species (de Jong et al., 2023); however, included only one sample (Himalayan brown bear) from Central Asia and no samples from Mongolia. Thus, our study remains essential for understanding phylogeographic history and conserving brown bears, especially in Central Asia.

For the last two decades, maternally inherited mitochondrial DNA (mtDNA) sequences of brown bears have been used extensively to understand the phylogeography of this species with 9–12 distinct intraspecific clades identified across their current range (Anijalg et al., 2018; Hirata et al., 2013; Miller et al., 2006; Waits et al., 1998). However, recent studies based on the Y chromosome show a different pattern that suggests more connectivity, less variation and fewer (4–5) haplogroups (Bidon et al., 2014; Hirata et al., 2017). Brown bears exhibit male-biased dispersal, which can explain the differences in demography and evolutionary patterns found between these sex-linked markers (McLellan & Hovey, 2001; Proctor et al., 2004). The use of multiple biparentally inherited nuclear microsatellite loci has been effective for evaluating contemporary population structure, connectivity and demography, but such data provide limited information about global evolutionary relationships, genetic diversity and historical demography of this species (Paetkau et al., 1998; Waits & Paetkau, 2005). Genomic data have been extremely useful for delineating taxonomic units among brown bears (Hailer et al., 2012; Miller et al., 2012), despite earlier confounding factors caused by hybridization and introgression between brown and polar bears (Cahill et al., 2015; Lan et al., 2022; Miller et al., 2012).

While genomic data are available throughout much of the species' range, the isolated populations in Central Asia, specifically the Gobi Desert of Mongolia and the Himalayas of Pakistan, have limited data, rendering evolutionary relationships among brown bears in these areas uncertain. Mitochondrial DNA studies have revealed five maternal lineages for brown bears in Asia (Galbreath et al., 2007; Lan et al., 2017; Masuda et al., 1998; Matsuhashi et al., 2001; Tumendemberel et al., 2019). Only a few studies, using biparentally inherited DNA, have focused on brown bears from the Asian continent. They have shown that brown bears in the Gobi Desert of Mongolia are isolated from other bear populations in Central Asia (Tumendemberel et al., 2019), and brown bears on Hokkaido Island, Japan, are genetically distinct from continental brown bears (de Jong et al., 2023; Endo et al., 2021; Hirata et al., 2017).

To improve our understanding of the evolutionary history of brown bears with a focus on Central Asia, we generated whole-genome data from nine brown bears, including samples from the Gobi Desert in south-western Mongolia, the Himalaya Mountain Range in Pakistan and northern Mongolia. We combined these data with previously published whole-genome data for brown and polar bears across their global ranges. Our objectives were to (1) identify global evolutionary relationships of brown bears, (2) evaluate the genomic support for the taxonomic subspecies status of brown bears in Central Asia, (3) estimate the level of genetic diversity of the identified lineages and (4) reconstruct historical trends of effective population size for brown bears. Finally, we discuss the conservation implications of our findings.

2 | MATERIALS AND METHODS

We collected seven tissue and two hair samples from six geographic regions in Asia, including the Gobi Desert ($n=3$), Altai Mountains ($n=1$), Sayan ($n=1$), Khentii ($n=1$), and Ikh Khyangan ($n=1$) in Mongolia and the Himalaya Mountains in Pakistan ($n=2$; Table S1). The Mongolian Ministry of Environment and Tourism and the Institute of General and Experimental Biology, Mongolian Academy of Sciences, approved the collection of samples and methods. Sample export and import were permitted by the CITES appendix I (export: MN1000500, 18/1148; import 12US807212/9 and 8US59229C/9).

Total DNA was extracted using DNeasy Blood and Tissue kit (Qiagen Inc.) and checked using a Qubit fluorometer (Invitrogen) and 1% agarose gel electrophoresis. We prepared ~300 ng of pure DNA in ≤50 μL of EDTA-free buffer for the 150-bp library preparation. Whole-genome sequencing was conducted using the Illumina HiSeq4000 (Illumina, Inc.). We retrieved raw whole-genome sequencing data for an additional 25 bears from GenBank (https://www.ncbi.nlm.nih.gov/; Table S1) to include in our analyses. These samples included 15 brown bears (Barlow et al., 2018; Benazzo et al., 2017; Cahill et al., 2015; Taylor et al., 2018), 4 polar bears (*Ursus maritimus*; Miller et al., 2012), 1 Asiatic black bear (*Ursus thibetanus*), 1 spectacled bear (*Tremarctos ornatus*), 1 sun bear (*Ursus malayanus*), 1 sloth bear (*Melursus ursinus*; Kumar et al., 2017), and the extinct 2 cave bear species; 1 Caucasian cave bear (*Ursus kudarensis*) and 1 Gamssulzen cave bear (*Ursus ingressus*; Barlow et al., 2018). We also downloaded the complete mtDNA genome for 64 brown bears from GenBank.

2.1 | Checking raw data, mapping, and filtering

We checked the sequencing quality of raw fastq files using fastqc v0.11.3 (Andrews et al., 2011). We trimmed raw reads using Sickle (Joshi & Fass, 2011), removed index and adapter sequences and merged paired reads using FastqToSam, MarkIlluminaAdapters and BuildBamIndex in the Picard tool (Board Institute, 2018). The clean BAM files were mapped to the grizzly bear (*U. a. horribilis*) reference genome (Taylor et al., 2018) using BWA v0.7.17 (Li & Durbin, 2010). After mapping, duplicates were marked and removed using rmdup commands in SAMtools (Li et al., 2009) and Picard version 1.106 (Board Institute, 2018).

Aligned reads that were properly paired, mapped uniquely and had high quality (Phred score ≥ 30) were used as input for base quality score recalibration (Genome Analysis Toolkit v3.7 (GATK); Danecek et al., 2011; McKenna et al., 2010). To obtain a set of "known variants" for recalibration, raw variant genotypes were called using default parameters and a minimum base quality Phred score of 20 with GATK UnifiedGenotyper, which was followed by GATK BaseRecalibrator and GATK PrintReads. We checked recalibration results using Qualimap (Okonechnikov et al., 2016). After recalibration, we obtained genetic variants, including SNPs and InDels in each scaffold. Joint genotyping of all 34 samples was implemented in GATK following the authors recommended best practices (DePristo et al., 2011; McKenna et al., 2010) using HaplotypeCaller (Poplin et al., 2017; Van der Auwera et al., 2013). SelectVariants tool in GATK was run to trim unused alternative alleles. We used VariantAnnotator in GATK v4.1.4.0 (McKenna et al., 2010) to add VariantType and AlleleBalance annotations to the variant call file. We filtered the raw SNPs with the following criteria using the Variant Filtration tool in GATK: quality by depth (QD) < 2.0, Phred-scaled *p* value (FS) > 60.0, root mean square of the mapping quality (MQ) < 40.0, variants with mapping quality rank-sum test approximation (MQRankSum) < −12.5 and a read position rank-sum test approximation (ReadPosRankSum) < −8.0. During this initial filtering step, we retained 63,788,272 variable sites of 64,369,585 SNPs and 10,113,052 InDels. We selected variants and excluded non-variants using SelectVariants in GATK. We used VCFtools v0.1.14 (Danecek et al., 2011) to run the next filtering steps with the following thresholds: genotype quality (minQ) ≥ 30, the minimum mean depth of coverage (min-meanDP) ≥ 10, the maximum mean depth of coverage (max-meanDP) ≤ 50, no missing data (max-missing 1.0) and no singletons. Before the genetic structure and phylogenetic analysis, we further filtered SNPs with linkage disequilibrium pruning (LD-pruning) with the threshold of window size = 50 kb, step size = 5 kb and r^2 threshold of .2 in Plink v1.90 (Chang et al., 2015).

2.2 | Population structure and phylogeny

We evaluated population structure using the LEA R package (Frichot & François, 2015) admixture analysis based on sparse nonnegative matrix factorization (snmf; Frichot et al., 2014) with 50 repeats for the full dataset and 10 repeats for the brown bears only dataset and principal component analysis (PCA) using Plink v1.90 (Chang et al., 2015). Results were visualized using the ggplot2 package (Wickham, 2016) in program R v.4.0 (Team R. C., 2017). We used spectacled bear as an outgroup to conduct phylogenetic analysis. We converted the SNPs vcf file to sequence format using vcf2pylip.py v2.0 (Ortiz, 2019) and genoToSequency.py (Martin et al., 2015). We used PAUP (Swofford, 2002) to calculate Akaike information criterion (AIC) and Bayesian information criterion (BIC) to identify an appropriate substitution model, which we used in further analyses to infer the phylogeny of brown bears. We used: (1) maximum likelihood (ML) with FastTree (Price et al., 2010) and RAxML (Stamatakis, 2014), and (2) coalescent-based SVDquartets method (Chifman & Kubatko, 2014) in the PAUP v.4 (Swofford, 2002). We also produced a Neighbour-Net network (Bryant & Moulton, 2004) based on a generalized time-reversible model (GTR) (Tavaré, 1986; Yang, 1994) with ML distance and considered empirical substitution rates using SplitsTree v.4.15 (Huson, 1998).

To evaluate brown bear maternal lineage relationships, we obtained complete mtDNA, which we filtered through base recalibration stages (Genome Analysis Toolkit v3.7 (GATK); Danecek et al., 2011; McKenna et al., 2010) from the filtered mapping files of each of the nine brown bears in Central Asia using mtDNA-Server protocols (Weissensteiner et al., 2016). Each individual's filtered contigs were aligned using the command 'Map to the Reference' with the same brown bear reference mtDNA genome (NC_003427.1, Taylor et al., 2018) using Geneious v20.0.5 (https://www.geneious.com). We removed all sites with ambiguities from the complete mtDNA genome alignment, resulting in an alignment of 12,990 base pairs (bp) length. We constructed a phylogenetic tree based on the best-fitting nucleotide substitution model, HKY + G (Hasegawa et al., 1985), using PAUP v.4 (Swofford, 2002) and MEGA v.X (Kumar et al., 2018).

2.3 | Genetic diversity and differentiation

We calculated observed heterozygosity (H_O), inbreeding coefficient (F) and overall pairwise F_{ST} (fixation index; Weir & Cockerham, 1984) using VCFtools v0.1.14 (Danecek et al., 2011) and plink v1.9 (Chang et al., 2015). We included 30 individuals: 24 brown bears, 4 polar bears, 1 Caucasian cave bear and 1 Gamssulzen cave bear. To focus on the population structure and genetic distance between brown bears and the most closely related paraphyletic species, including the extinct cave bears and polar bears, we excluded samples from long-diverged Asiatic black bear, spectacled bear, sun bear and sloth bear (Kumar et al., 2017). The *Dxy* and π estimates for these 30 individuals were also calculated across scaffolds using a sliding window approach in Python (popgenWindows.py, Martin et al., 2015). We specified all sliding window runs with nonoverlapping 20 kb sliding windows by calculating the number of differences in a minimum of 100 bases.

2.4 | Historical effective population size

We estimated changes in effective population size over time for 10 populations of brown bears (≥8× coverage) using pairwise sequentially Markovian coalescent model analysis, PSMC (Li & Durbin, 2009). We used the samtools mpileup and bcftools call functions to remove sites with mean map and base qualities <20 (−q 20 -Q 20 -C50 -u) from the post-score calibrated data (BSQR) without GATK filtering and linkage disequilibrium (LD) pruning to obtain the filtered fasta-like sequence dataset. We defined a generation time of 10 years based on brown bear lifespan (Miller & Waits, 2003; Paetkau et al., 1998; Tallmon et al., 2004) and a mutation rate of 1×10^{-8} year/site based on previous studies (Kumar et al., 2017; Miller et al., 2006). When running, we used default parameters and 100 bootstrap replicates.

3 | RESULTS

3.1 | Sequencing, mapping, filtering and variant calling

We collected tissue and hair samples and sequenced DNA from nine samples from Central Asia across six geographic regions, including the Gobi Desert ($n=3$; mean depths: 11.5–17) in southern Mongolia, Altai Mountains ($n=1$; mean depth: 8.03), Sayan ($n=1$; mean depth: 6.44), Khentii ($n=1$; mean depth: 10.1) and Ikh Khyangan ($n=1$; mean depth: 14.2) in northern Mongolia and the Himalaya Mountains in Pakistan ($n=2$; mean depth: 8.55 and 12.5) at an average of 11× coverage. After mapping and filtering, we generated 34 individuals' datasets of 684,081 variable nucleotides from genomes of ~2.3 billion nucleotides for further phylogenetic analysis. Genetic diversity and population structure were calculated using the final filtered SNPs from 30 bears of four species, including 24 brown bears, 4 polar bears and 2 species of extinct cave bears.

3.2 | Population structure and phylogeny

The admixture snmf analysis with LEA separated 30 individuals into two groups at $K=2$: polar bears and all other samples (Figure 1). The lowest cross-entropy values were obtained at $K=3$ (Figure S1), and this placed brown bears of the Gobi Desert and the Himalayas of Pakistan into their own genetic group separate from polar bears and all other bears. The PCA results also supported this grouping (Figure 2). Following the recommendations from previous authors of admixture analyses (Alexander & Lange, 2011; Evanno et al., 2005, Frichot & François, 2015; Pritchard et al., 2000), we also consider results of values of K greater than 3, which provide biologically meaningful information about relationships among brown bear populations, particularly in light of the recent publication (de Jong et al., 2023). At $K=4$, the North American brown bears show distinct ancestry, and at $K=5$, Mongolian and European brown bears split off. At $K=6$, the Himalayan bears split from the Gobi bears, and at $K=7$, the ancient brown bear and cave bears separate (Figure 1). As K values increased, bears showed more mixed-ancestry coefficients, but the splits remained biologically meaningful. We present summary results for seven clusters: polar bears, cave bears and five subgroups of modern brown bears and ancient brown bears (Figure 2). Similar structuring results were obtained when the admixture (Figure S2) and PCA analyses (Figure S3) were conducted on only brown bears.

The estimates of mean pairwise F_{ST} and absolute divergence (D_{XY}) showed that polar bears had the greatest pairwise genetic differentiation and divergence from other groups ($F_{ST\ =}$ 0.26–0.56; $D_{XY\ =}$ 0.091–0.113; Table S2 and S3). Gobi and Himalayan bears had greater differentiation ($F_{ST}=0.25$–0.61; $D_{XY}=0.097$–0.115) from the other brown populations in northern Mongolia, Europe and North America than pairwise F_{ST} between brown bears in northern Mongolia, Europe and North America ($F_{ST}=0.02$–0.04; $D_{XY}=0.103$–0.104). The genetic differentiation between Gobi/Himalaya and northern Mongolia and Europe was less ($F_{ST}=0.247$–0.321) than between Gobi and Himalaya ($F_{ST}=0.506$). In contrast, D_{XY} estimates showed that Gobi and Himalayan bears had slightly less divergence ($D_{XY}=0.09$) than between Gobi/Himalayan and northern Mongolia and Europe ($D_{XY}=0.113$–0.115).

Maximum-likelihood (ML) and coalescence (based on SVDquartets) trees were not consistent in placement of the divergent lineages but consistent in identifying at least five distinct divergent groups of modern brown bears (Figure 3a; Figure S4). For brown bears, the major lineage splits were the same five genetic groups of modern brown bears as detected in admixture analyses (i.e. northern Mongolia and Siberian Russia, Europe, Gobi, Himalaya and North America). The three primary differences between the two phylogenetic results were (1) the placement of brown bears in North America, which has shared ancestry to the Gobi/Himalayas in coalescence with lower statistical support for this lineage, whereas ML

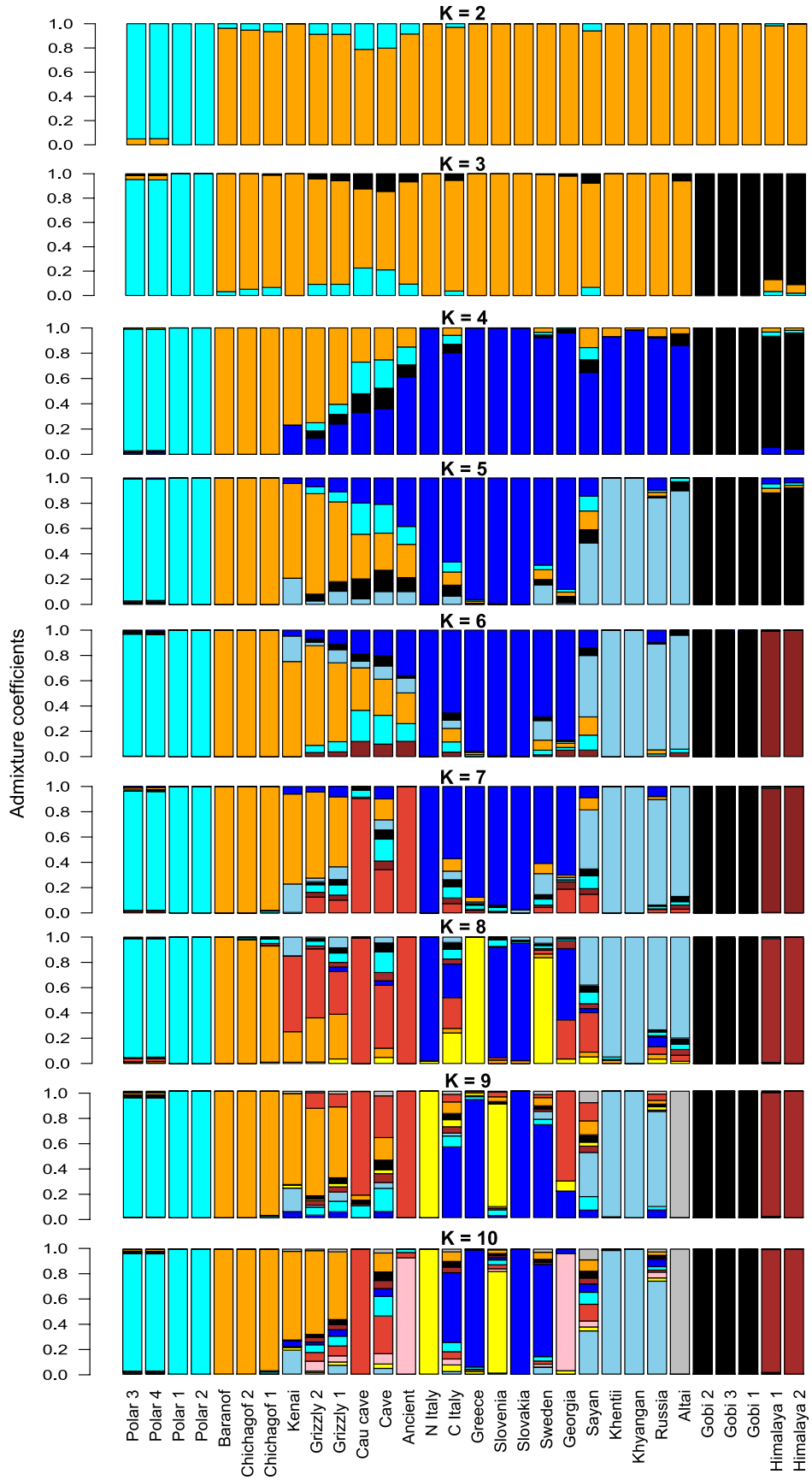

FIGURE 1 Population structure results ($K = 2$–10) from snmf analysis with the LEA R package from 24 brown bears (*Ursus arctos*), two species of cave bears (*U. ingressus* and *U. kudarensis*) and four polar bears (*U. maritimus*) based on 684,081 nuclear SNPs.

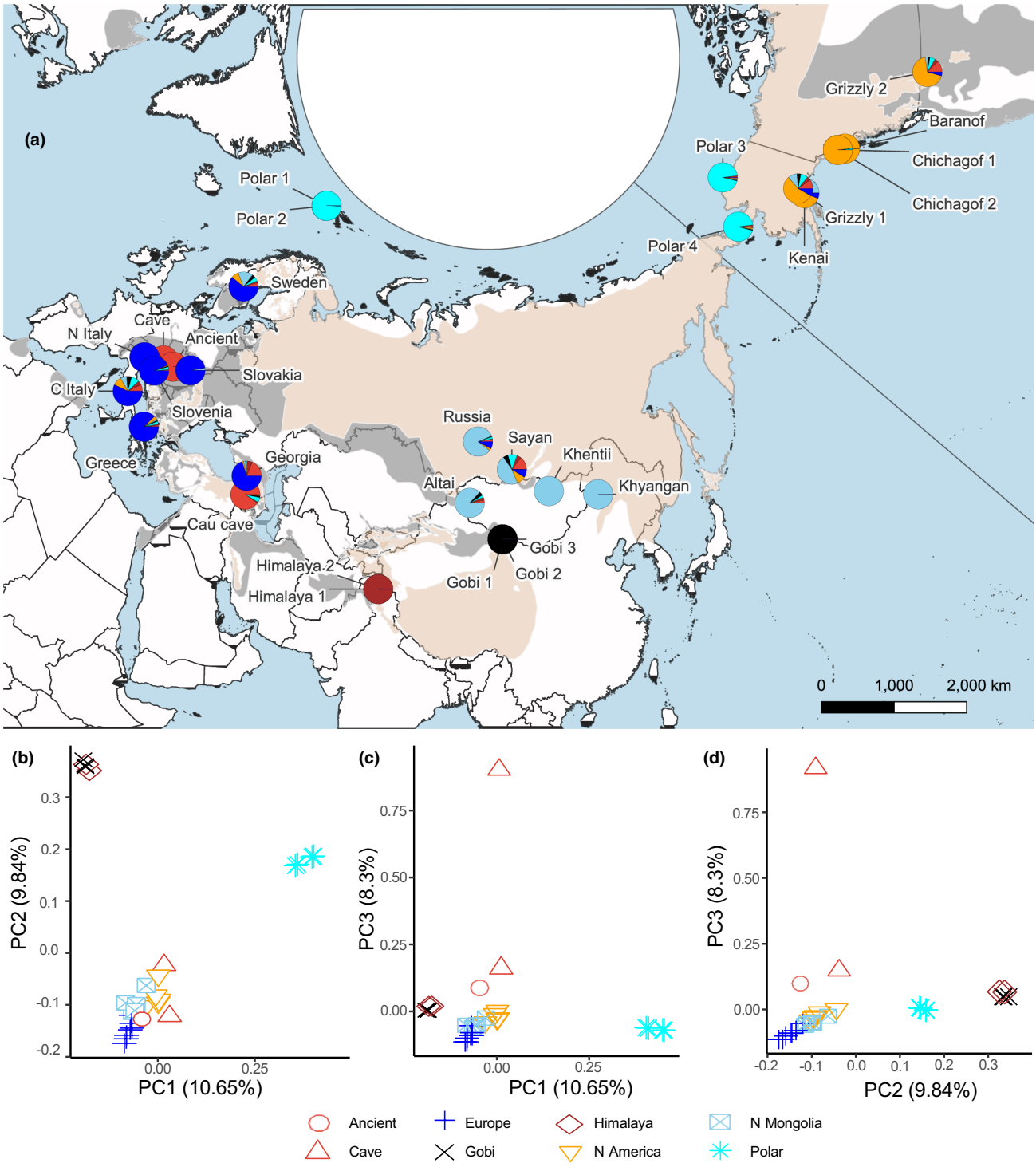

FIGURE 2 (a) Sampling locations with pie charts indicating per cent ancestry assigned to each individual at $K = 7$ from LEA R Package. Results from principal component analyses: (b) PC1 vs. PC2, (c) PC1 vs. PC3, (d) PC2 vs. PC3. These analyses are based on 684,081 nuclear SNPs from 24 brown bears (*Ursus arctos*), 2 species of cave bears (*U. ingressus* and *U. kudarensis*) and 4 polar bears (*U. maritimus*).

tree showed that the North American bears have shared ancestry with northern Mongolia bears; (2) the sample from Kenai, AK was the ancestral lineage to the Europe and northern Mongolia bears based on coalescence, while the ML tree supported it to be the same lineage as the other brown bears in North America; (3) the Ancient brown bear (the Pleistocene brown bear from Winden Cave, Austria; C^{14} dated ~41,201 ± 895 years ago) placed as the ancient lineage of Gobi/Himalaya and North America based on coalescence, whereas ML showed Ancient and Georgia brown bears were ancestral to the bears in Europe. Given that SVDquartets is generally more reliable than ML for inferring species tree estimation from the multilocus SNPs dataset (Vachaspati & Warnow, 2018) and the support for the

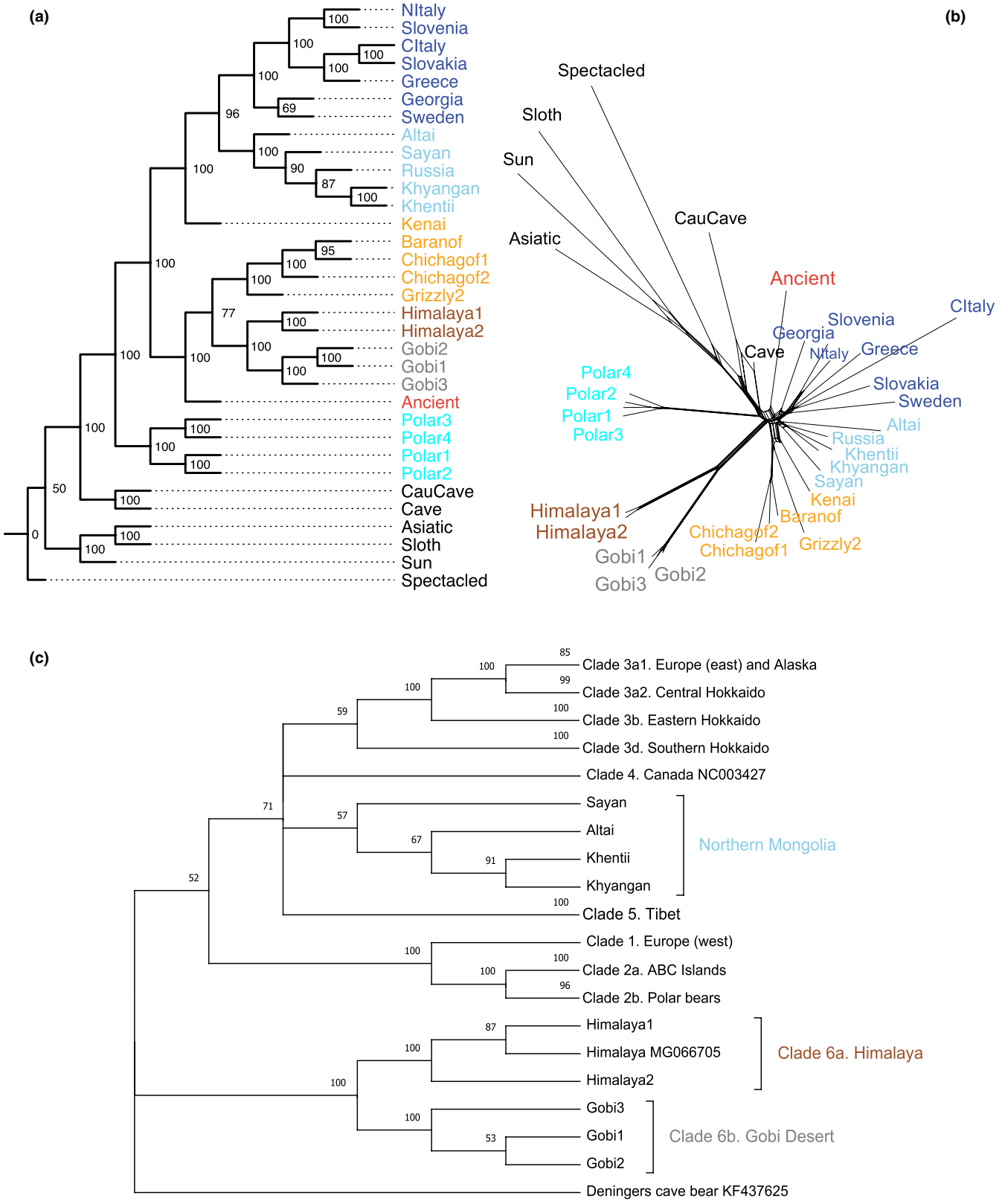

FIGURE 3 (a) Phylogenetic tree based on SVDquartets of brown bears (*Ursus arctos*) and their relationship with polar bears (*U. maritimus*), two species of cave bears (*U. ingressus* and *U. kudarensis*), sloth bear (*Melursus ursinus*), sun bear (*Helarctos malayanus*), Asiatic black bear (*U. thibetanus*) and Andean bear (*Tremarctos ornatus*) based on nuclear 684,081 nuclear SNPs from the whole-genome SNPs dataset. (b) Neighbour-net tree from the whole-genome SNPs dataset using SplitsTree4. (c) Maximum-likelihood phylogenetic tree of brown bears based on mtDNA data (11,261 base pairs). *Ursus spelaeus* and *U. deningeri* were used as outgroups. The numbers at nodes represent bootstrapping support values.

SVDquartets patterns across our other analysis methods, we conclude those results are best supported.

The neighbour-net analysis showed that polar bears have an early divergence from a shared ancestor with brown and cave bear lineages (Figure 2b). Samples from similar geographic origins were clustered on the tree, and most samples showed early divergence with splits occurring close to the centre/origin. Only the Gobi and Himalayan brown bears had a noticeable amount of shared ancestry before bifurcating into two distinct lineages.

The ML consensus tree using mtDNA from 54 brown bears in Asia, Europe, Hokkaido and North America showed 9–10 divergent clades/subclades. Similar to whole-genome results, the mtDNA tree clustered Gobi and Himalaya as a divergent monophyletic group with two geographically distinct subclades (Figure 2c). However, this tree placed the Gobi and Himalaya as the sister lineages of all other brown bears. This tree also showed divergent lineages on Hokkaido Island, Japan, but we did not have a whole genome from this region to compare.

3.3 | Genetic diversity

Among extant brown bear populations, observed heterozygosity (H_O) and nucleotide diversity (π) were lowest for Central Italy, Gobi and Himalayan brown bears (H_O=0.037–0.055, π=0.047–0.050) and polar bears (H_O=0.045–0.056, π=0.057) (Table S4). Diversity estimates for the extinct Pleistocene brown and cave bears were even lower (H_O=0.001–0.065, π=0.041–0.069). However, the brown bears in most of Europe, northern Mongolia and Siberian Russia had relatively higher genetic diversity (H_O=0.051–0.108, π=0.101–0.108, Figure 4, Table S4).

3.4 | Historical demography

The trajectory of historical effective population sizes (N_e) for all brown bears with >8× coverage genomes was inferred using pairwise sequential Markovian coalescent (PSMC) analysis (Li & Durbin, 2011). Brown bears across all regions showed an overall decrease in N_e, with highs ~1 mya and lows most recently ~10 ka. Across time, we observed two bottlenecks. However, three different patterns of N_e were identified among the individuals; (1) the Gobi, Central Italy in Apennine and Alps Mountain Ranges; (2) Slovenia, Khentii and Khyangan in Mongolia and Himalaya; and (3) Sweden and North America (Figure 5, Figure S5). The N_e of group 1 showed a relatively steep decrease between ~1 mya and ~200 ka. Group 2 showed a modest decrease starting from ~800 to 200 ka. Group 3 decreased slightly, starting ~300–400 ka until ~100 ka. Between ~80 and 120 ka, N_e estimates of group 1, including the Gobi and most European brown bears, increased to >40,000 individuals, while the other brown bears had lower N_e (~10,000–25,000 individuals). Throughout time, brown bears (n=2) in both ABC and mainland North America had lower N_e than brown bears in Eurasia.

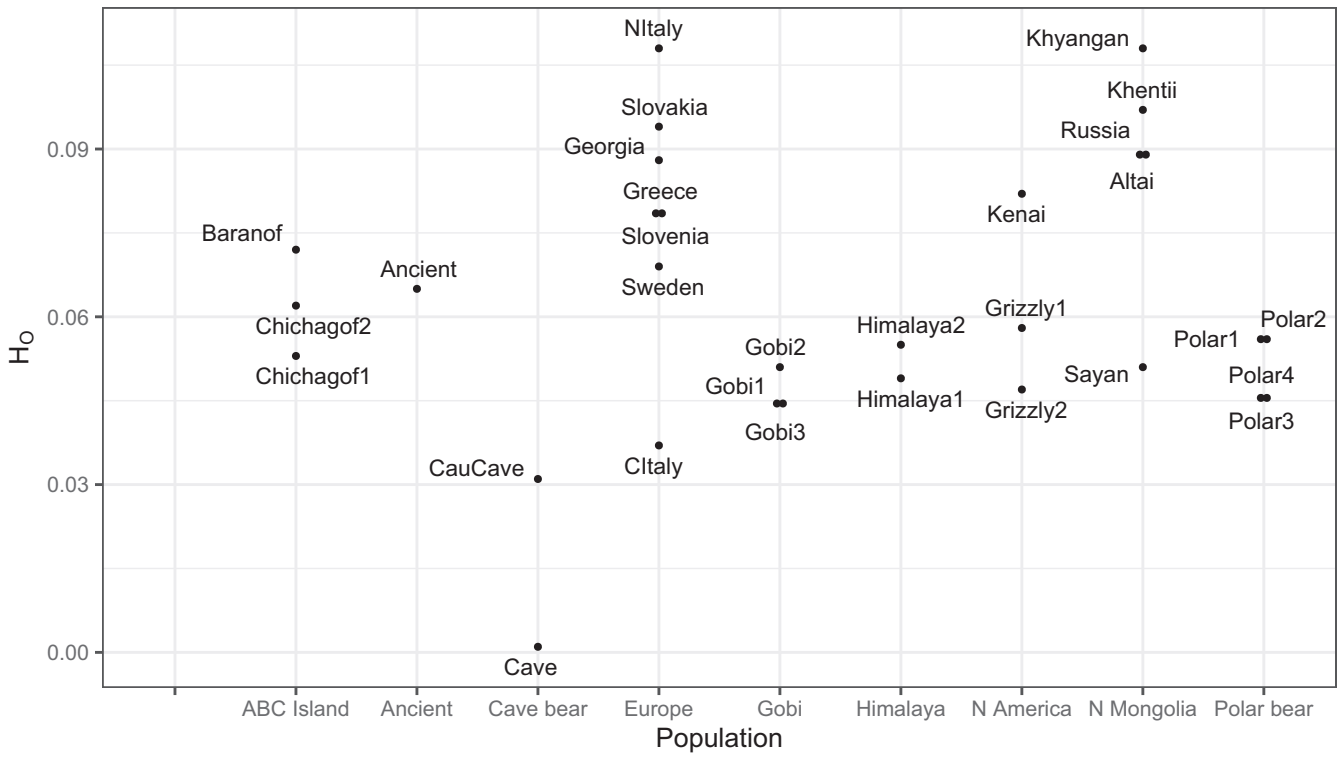

FIGURE 4 Observed heterozygosity (H_O) estimates for the 6 populations from 24 brown bears (*Ursus arctos*), ancient brown bears, 2 species of cave bears (*U. ingressus* and *U. kudarensis*) and 4 polar bears (*U. maritimus*) based on 684,081 nuclear SNPs. Dots show the estimates for each corresponding individual.

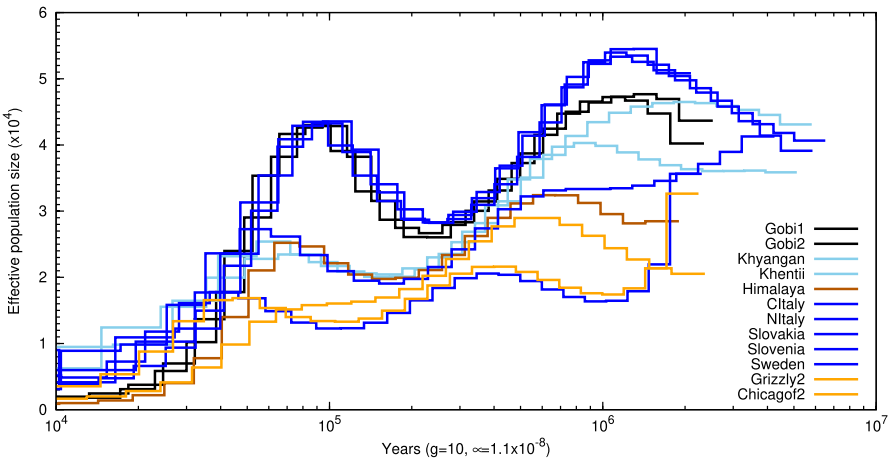

FIGURE 5 Historical effective population sizes (N_e) using the pairwise Markovian coalescence (PSMC) analyses for all brown bear genomes with 8×–18× coverage.

4 | DISCUSSION

Our work provides a comprehensive whole-genome assessment of brown bear evolutionary history, genetic diversity and genetic structure and offers new insights for isolated populations of conservation concern in Central Asia. All analyses based on our whole-genome dataset of 684,081 unlinked nuclear SNPs divided modern brown bears into five distinct groups, including populations in (1) the Gobi Desert, Mongolia, (2) the Himalayas of Pakistan, (3) northern Mongolia and Russia, (4) Europe and (5) North America (Figure 1 and 2, Table S2 and S3, Figure S2). These whole-genome results differ from mtDNA or Y chromosome studies on placement and number of groups and divergent lineages. Mitochondrial DNA studies tend to identify more haplogroups, which are specific for regional levels such as clade 3a2, 3b2 and 3d for Hokkaido brown bears (e.g. Anijalg et al., 2018; Hirata et al., 2013; Keis et al., 2013; Lan et al., 2017; Miller et al., 2006; Tumendemberel et al., 2019; Waits et al., 1998). Y chromosomal studies show male-mediated gene flow of brown bears, less variation and more complicated phylogeography with less clear regional differences (Bidon et al., 2014; Hirata et al., 2017). These differences reflect differing movement patterns between sexes; female brown bears are highly philopatric, while males tend to disperse long distances (Proctor et al., 2004; Stoen et al., 2006; Swenson et al., 1998; Zedrosser et al., 2007).

Our nuclear SNPs phylogenetic results indicated that all North American brown bears shared the same ancestry, which is consistent with recent studies (de Jong et al., 2023; Lan et al., 2022). MtDNA phylogeography studies suggest that brown bears in North America arrived in three different temporal migration waves from Asia ~34–279 kya, representing clades 2a, 3 (subclades 3a and 3b) and 4 (Anijalg et al., 2018; Miller et al., 2006; Waits et al., 1998). Y chromosome studies also indicate multiple migrations and have found two haplotypes (BR1 and BR4) in mainland North America and one haplotype (BR5) found only in ABC island bears (Hirata et al., 2017). Although we found all North American brown bears share the same lineage based on nuclear SNPs, we do not believe this refutes the hypothesis of multiple migrations but rather supports substantial admixture following migrations. Pairwise genetic differentiation between ABC and mainland North America was lower than others, which may be the result of more recent splits and continued low levels of male-mediated gene flow between these groups, despite being separated by the ocean. Interestingly, according to the SVDquartets tree, the brown bear in Kenai was placed as a sister lineage to the lineages of Europe and northern Mongolia, which may be a remnant of one of the multiple migrations from Asia. Our admixture and phylogenetic results suggest that some modern brown bears in Eurasia, at least those in the Sayan region in Mongolia and Caucasus, Georgia, retain higher levels of ancestral alleles compared to the bears in Altai, Russia, Central Italy and Sweden. All results are consistent with the hypothesis that brown bears migrated to North America from Asia (Anijalg et al., 2018; Keis et al., 2013; Kurtén, 1968, 1976; Tumendemberel et al., 2019).

We identified three primary brown bear lineages/groups in Eurasia using whole-genome data: Europe, northern Mongolia and Siberian Russia, and the Gobi and Himalaya. Within Europe and northern Mongolia, we identified a secondary split. This distinction between Europe and northern Mongolia (including Siberian Russia) was supported by all our results, including phylogenetic trees, admixture and genetic distance/divergence estimates. Previous mtDNA studies suggested brown bears in Europe (eastern lineage) and northern Mongolia and Siberian Russia were a continuous large population that shared similar matrilineal mtDNA haplotypes belonging to clade 3a1, which is also the most widely distributed subclade throughout Eurasia, Russian Far East, Sakhalin Island in Russia and western Alaska (Anijalg et al., 2018; Hirata et al., 2013; Keis et al., 2013; Miller et al., 2006). However, a recent mtDNA study that used additional samples from Central Asia showed a split between northern Mongolia and Siberian Russia and eastern Europe based on 3b and 3a1 haplogroups, respectively (Salomashkina et al., 2014; Tumendemberel et al., 2019), which is consistent with our genomic study. The recently published whole-genome study (de Jong et al., 2023) has also shown the same result. Shared historical ancestry and connectivity throughout the larger brown bear populations in Siberian Russia were also supported by the estimates of lower genetic divergence between Europe and northern Mongolia than in North America and Europe. Further studies with more continuous

sampling of this region may help identify the geographic area coinciding with this split.

Mainland Asia is a hotspot of phylogenetic diversity having three distinct lineages corresponding to three geographic regions: northern Mongolia and Siberian Russia, Gobi and Himalaya. Most northern Mongolian and Siberian Russian bears were a sister clade to the European bears. Interestingly, the samples from Altai and Sayan showed similar ancestry to other northern Mongolian brown bears but had admixture with the ancient Pleistocene brown and cave bear samples. Contemporary gene flow seems to occur throughout northern Mongolia, except for Gobi and Himalayan populations (Tumendemberel et al., 2019). In the southern range, the Gobi and Himalayan brown bears are monophyletic sister groups that share a common lineage. This evolutionary pattern has also been found in the grey wolf (*Canus lupus*), another Holarctic-distributed carnivore, which showed early divergent clades in the southern range, including Tibetan and Indian wolves (Pilot, 2021). Similarly, snow leopards (*Uncia uncia*) also showed genetic differentiation among northern (Mongolia), central (Himalaya and Tibetan plateau), and western (Tian Shan and Pamir) populations, each of which is considered subspecies (Janecka et al., 2017).

Both whole-genome SNPs and the larger mtDNA dataset supported the monophyletic lineages of Gobi (clade 6a) and Himalaya (clade 6b) samples. Previously, mtDNA studies of the partial control region, cytochrome B and COII did not detect distinct phylogenetic lineages between Gobi and Himalayas of Pakistan despite having different haplogroups (Galbreath et al., 2007; Lan et al., 2017; Tumendemberel et al., 2019). Matrilineal lineage clade 6 was placed as the sister lineage to all other currently existing brown bear lineages in previous studies (Lan et al., 2017; Tumendemberel et al., 2019). However, this study suggests that Gobi and Himalayan bears are sisters to the North American lineage and are the most genetically divergent groups. This likely indicates their early divergence and long-term isolation.

Despite the Gobi and Himalayan bears being sister taxa, the current gene flow does not appear to exist between these groups. In fact, we found greater genetic differentiation based on F_{ST} between the brown bears in Gobi and the Himalayas than between Gobi and others in northern Mongolia and Siberia or Europe. Geographic isolation, possibly exacerbated by anthropogenic influences and adaptations to the different environmental conditions in the Gobi and Himalayas, may have played an important role in separating these lineages. The combination of restricted gene flow and small population sizes (Tumendemberel et al., 2021) likely increased genetic drift leading to low genetic diversity and further genetic differentiation. Previous research using microsatellites demonstrated that Gobi bears were the most divergent of all groups sampled in Central Asia (Tumendemberel et al., 2019). Our results suggest more connectivity between the bears in the Gobi Desert and the Altai Mountains of Mongolia, which are geographically close (~300 km). Interestingly, previous work found an admixed individual (~100-year-old sample) between the Altai Mountains and the Gobi Desert (Tumendemberel et al., 2019).

Around the Pleistocene epoch ~100–120 ka, the historical N_e of brown bears in Gobi increased in a manner consistent with European bears (Apennines and Alps) while N_e of Himalayan and northern Mongolian bears remained lower. Frequently, individuals with similar patterns of N_e indicate shared evolutionary histories, including demography and genetic connectivity (Nadachowska-Brzyska et al., 2016). However, the phylogeny suggests earlier divergence between Gobi and European than between Gobi and Himalayan bears. This may reflect more suitable climatic conditions (Janz et al., 2021) in regions at lower elevations inhabited by Gobi and European bears which allowed for population increase, while the colder climate at higher elevations (Altai-Sayan, Khentii, Ikh Khyangan Mountains in northern Mongolia as well as the Himalayas) restricted the Himalayan and northern Mongolian bear populations.

We found extremely low genetic diversity and high inbreeding in smaller geographically isolated brown bear populations in the Gobi Desert in Mongolia, the Himalayas in Pakistan and the Apennines in Central Italy, and relatively high genetic diversity in larger, more connected populations in northern Mongolia and Siberian Russia, Europe and North America. Another small and isolated brown bear population, which also has concerningly low genetic diversity based on a previous whole-genome study is in the Pyrenees Mountain range in Spain ($F=0.57$; Benazzo et al., 2017). Hokkaido brown bears are also isolated but have a large population size and were previously found to have an intermediate level of genetic diversity ($H_E=0.10$–0.11) compared to estimates for other brown bears ($H_E=0.05$–0.18; Endo et al., 2021). Our low diversity and high inbreeding estimates for polar bears, ancient Pleistocene brown bears and cave bears were consistent with previous studies (Cahill et al., 2015; Hailer et al., 2012; Miller et al., 2012).

Two additional geographically isolated Asian brown bear populations exist in Hokkaido and Tibet that were not represented in this study. Based on a recent whole-genome sequencing study, Hokkaido brown bears are a separate lineage from the continental brown bears (Endo et al., 2021), and brown bears in Tibet are in a separate maternal lineage (clade 5, Figure 2) but have shared ancestry with brown bears in southern Hokkaido (Hirata et al., 2013). Including whole-genome samples from across species distributions will continue to increase our understanding of brown bear evolutionary history in Asia and worldwide.

Conservation of a species as a whole can benefit from identifying and understanding differences among intraspecies taxa, such as subspecies (Mace, 2004). Characteristics leading to subspecies designation include (1) unique evolutionary lineages (Lidicker, 1962; Smith & Patton, 1980), (2) divergence between the monophyletic lineages (Orr, 2005; Wright, 1969), (3) genetic differentiation considering both adaptive and neutral loci (Hey & Pinho, 2012; Hohenlohe et al., 2010), (4) phenotypic differences (Haig et al., 2006) and (5) geographic separation (Lande, 1988). Previous work based on partial mtDNA (927 base pairs) illustrated that Gobi and Himalayan brown bears were genetically unique from other brown bear populations (Lan et al., 2017; Tumendemberel et al., 2019). Subsequently, this study confirmed these results, but Gobi and Himalayan brown bears

are unique monophyletic groups in both mtDNA and nDNA. In combination with morphological and ecological differences (Bold, 1967), and geographic separation (McLellan et al., 2017; Sokolov & Orlov, 1992), our work confirms the subspecies status of brown bears in the Gobi Desert (*U.a.gobiensis*; Shiirevdamba et al., 2013) and those in the Himalayas (*U.a.isabellinus*; Galbreath et al., 2007; Lan et al., 2017). We also found brown bears in Europe and northern Mongolia and Siberian Russia represent divergent monophyletic lineages in mtDNA and nDNA, lending some credence to European brown bears (*U.a.arctos*) and East Siberian brown bears (*U.a.collaris*; Heptner, 1998), but these bears exist in populations without distinctly defined geographic limits, possibly overlapping (McLellan et al., 2017). Further studies based on genomic, demographic and movement data are needed to clarify the connectivity.

Our study provides new insights into the evolutionary history of brown bears through the use of whole-genome data and the inclusion of previously unsampled populations in Central Asia. Conserving each of the distinct lineages is important for the long-term evolution of the species since each contains unique evolutionary potential (Waples & Gaggiotti, 2006). However, many of these unique lineages persist in small, isolated populations at risk of extinction (O'Brien & Mayr, 1991). Additionally, several small populations in the species' southern range were found to have low genetic diversity at the genome level, potentially reducing their adaptive and functional capacity. Historically, all brown bear lineages had large fluctuations in N_e, but current N_e is lower than at any time in the past. The question remains if populations can be sustained at this low N_e. Maintaining connectivity that currently exists and possibly restoring historical connectivity may help prevent further declines and provide opportunities to transfer genetic variation and adaptive traits. This work has increased our understanding of the phylogeography of brown bear populations globally and provides a valuable genomics dataset for future studies in specific conservation and evolutionary-related questions, such as local adaptations to diverse environments.

AUTHOR CONTRIBUTIONS

OT, LPW, SAH, PAH, MP and AZ designed the research; OT and SAH conducted the data analyses; SAH, PAH, JS, AZ, MS, MP, JLK and LPW conducted review and editing; OT, AZ, JLK and LPW provided funding acquisition, project administration and resources; and OT wrote the paper.

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CONFLICT OF INTEREST STATEMENT

Authors declare no competing interest.

DATA AVAILABILITY STATEMENT

Raw sequence reads are deposited into GenBank under the project ID: PRJNA997569, and individual genotype data are submitted to Fig Share (10.6084/m9.figshare.21385455). We published our analytical scripts used for this manuscript in the GitHub repository (https://github.com/odko2008/Analyses/tree/main).

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REFERENCES

Alexander, D. H., & Lange, K. (2011). Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. *BMC Bioinformatics*, *12*(1), 246.

Allendorf, F. W., & Luikart, G. (2009). *Conservation and the genetics of populations*. John Wiley & Sons.

Andrews, S., Lindenbaum, P., Howard, B., & Ewels, P. (2011). *FastQC: High throughput sequence QC report v 0.10. 1*. Cambridge, UK, Babraham Bioinformatics.

Anijalg, P., Ho, S. Y. W., Davison, J., Keis, M., Tammeleht, E., Bobowik, K., Tumanov, I. L., Saveljev, A. P., Lyapunova, E. A., Vorobiev, A. A., Markov, N. I., Kryukov, A. P., Kojola, I., Swenson, J. E., Hagen, S. B., Eiken, H. G., Paule, L., & Saarma, U. (2018). Large-scale migrations of brown bears in Eurasia and to North America during the late Pleistocene. *Journal of Biogeography*, *45*(2), 394–405. https://doi.org/10.1111/jbi.13126

Barlow, A., Cahill, J. A., Hartmann, S., Theunert, C., Xenikoudakis, G., Fortes, G. G., Paijmans, J. L., Rabeder, G., Frischauf, C., & Grandal-d'Anglade, A. (2018). Partial genomic survival of cave bears in living brown bears. *Nature Ecology & Evolution*, *2*(10), 1563–1570.

Benazzo, A., Trucchi, E., Cahill, J. A., Delser, P. M., Mona, S., Fumagalli, M., Bunnefeld, L., Cornetti, L., Ghirotto, S., Girardi, M., Ometto, L., Panziera, A., Rota-Stabelli, O., Zanetti, E., Karamanlidis, A., Groff, C., Paule, L., Gentile, L., Vila, C., … Bertorelle, G. (2017). Survival and divergence in a small group: The extraordinary genomic history of the endangered Apennine brown bear stragglers. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(45), E9589–E9597. https://doi.org/10.1073/pnas.1707279114

Bidon, T., Janke, A., Fain, S. R., Eiken, H. G., Hagen, S. B., Saarma, U., Hallstrom, B. M., Lecomte, N., & Hailer, F. (2014). Brown and polar bear Y chromosomes reveal extensive male-biased gene flow within brother lineages. *Molecular Biology and Evolution*, *31*(6), 1353–1363. https://doi.org/10.1093/molbev/msu109

Board Institute. (2018). *Picard toolkit*. Broad Institute. http://broadinstitute.github.io/picard/

Bojarska, K., & Selva, N. (2012). Spatial patterns in brown bear *Ursus arctos* diet: The role of geographical and environmental factors. *Mammal Review*, *42*(2), 120–143.

Bold, A. (1967). Mongolian brown bear and mazaalai. *Scientific Proceedings of the Institute of Biology of the Mongolian Academy of Sciences*, *2*, 5–50.

Bryant, D., & Moulton, V. (2004). Neighbor-net: An agglomerative method for the construction of phylogenetic networks. *Molecular Biology and Evolution*, *21*(2), 255–265.

Cahill, J. A., Stirling, I., Kistler, L., Salamzade, R., Ersmark, E., Fulton, T. L., Stiller, M., Green, R. E., & Shapiro, B. (2015). Genomic evidence of geographically widespread effect of gene flow from polar bears into brown bears. *Molecular Ecology*, *24*(6), 1205–1217. https://doi.org/10.1111/mec.13038

Ceballos, G., Ehrlich, P. R., Barnosky, A. D., García, A., Pringle, R. M., & Palmer, T. M. (2015). Accelerated modern human-induced species losses: Entering the sixth mass extinction. *Science Advances*, *1*(5), e1400253. https://doi.org/10.1126/sciadv.1400253

Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S., Purcell, S. M., & Lee, J. J. (2015). Second-generation PLINK: Rising to the challenge of larger and richer datasets. *Gigascience*, *4*(1), 7.

Chifman, J., & Kubatko, L. (2014). Quartet inference from SNP data under the coalescent model. *Bioinformatics*, *30*(23), 3317–3324.

Cowie, R. H., Bouchet, P., & Fontaine, B. (2022). The sixth mass extinction: Fact, fiction or speculation? *Biological Reviews*, *97*(2), 640–663. https://doi.org/10.1111/brv.12816

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., & Sherry, S. T. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158.

Davison, J., Ho, S. Y. W., Bray, S. C., Korsten, M., Tammeleht, E., Hindrikson, M., Ostbye, K., Ostbye, E., Lauritzen, S. E., Austin, J., Cooper, A., & Saarma, U. (2011). Late-quaternary biogeographic scenarios for the brown bear (*Ursus arctos*), a wild mammal model species. *Quaternary Science Reviews*, *30*(3–4), 418–430. https://doi.org/10.1016/j.quascirev.2010.11.023

de Jong, M. J., Niamir, A., Wolf, M., Kitchener, A. C., Lecomte, N., Seryodkin, I. V., Fain, S. R., Hagen, S. B., Saarma, U., & Janke, A. (2023). Range-wide whole-genome resequencing of the brown bear reveals drivers of intraspecies divergence. *Communications Biology*, *6*(1), 153.

DePristo, M. A., Banks, E., Poplin, R., Garimella, K. V., Maguire, J. R., Hartl, C., Philippakis, A. A., del Angel, G., Rivas, M. A., Hanna, M., McKenna, A., Fennell, T. J., Kernytsky, A. M., Sivachenko, A. Y., Cibulskis, K., Gabriel, S. B., Altshuler, D., & Daly, M. J. (2011). A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature Genetics*, *43*(5), 491–498. https://doi.org/10.1038/ng.806

Endo, Y., Osada, N., Mano, T., & Masuda, R. (2021). Demographic history of the brown bear (*Ursus arctos*) on Hokkaido Island, Japan, based on whole-genomic sequence analysis. *Genome Biology and Evolution*, *13*(9), evab195.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Frichot, E., & François, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution*, *6*(8), 925–929.

Frichot, E., Mathieu, F., Trouillon, T., Bouchard, G., & François, O. (2014). Fast and efficient estimation of individual ancestry coefficients. *Genetics*, *196*(4), 973–983.

Funk, W. C., McKay, J. K., Hohenlohe, P. A., & Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends in Ecology & Evolution*, *27*(9), 489–496.

Galbreath, G. J., Groves, C. P., & Waits, L. P. (2007). Genetic resolution of composition and phylogenetic placement of the Isabelline bear. *Ursus*, *18*(1), 129–131. https://doi.org/10.2192/1537-6176(2007)18[129:Grocap]2.0.Co;2

Haig, S. M., Beever, E. A., Chambers, S. M., Draheim, H. M., Dugger, B. D., Dunham, S., Elliott-Smith, E., Fontaine, J. B., Kesler, D. C., & Knaus, B. J. (2006). Taxonomic considerations in listing subspecies under the US endangered species act. *Conservation Biology*, *20*(6), 1584–1594.

Hailer, F., Kutschera, V. E., Hallstrom, B. M., Klassert, D., Fain, S. R., Leonard, J. A., Arnason, U., & Janke, A. (2012). Nuclear genomic sequences reveal that polar bears are an old and distinct bear lineage. *Science*, *336*(6079), 344–347. https://doi.org/10.1126/science.1216424

Hasegawa, M., Kishino, H., & Yano, T.-A. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, *22*(2), 160–174.

Heptner, V. G. (1998). *Mammals of the Soviet Union: Sirenia and Carnivora (Sea Cows; Wolves and Bears)*. Smithsonian Institution Libraries and the National Science Foundation.

Hey, J., & Pinho, C. (2012). Population genetics and objectivity in species diagnosis. *Evolution*, *66*(5), 1413–1429. https://doi.org/10.1111/j.1558-5646.2011.01542.x

Hirata, D., Mano, T., Abramov, A. V., Baryshnikov, G. F., Kosintsev, P. A., Murata, K., & Masuda, R. (2017). Paternal phylogeographic structure of the brown bear (*Ursus arctos*) in northeastern Asia and the effect of male-mediated gene flow to insular populations. *Zoological Letters*, *3*, 21. https://doi.org/10.1186/s40851-017-0084-5

Hirata, D., Mano, T., Abramov, A. V., Baryshnikov, G. F., Kosintsev, P. A., Vorobiev, A. A., Raichev, E. G., Tsunoda, H., Kaneko, Y., Murata, K., Fukui, D., & Masuda, R. (2013). Molecular phylogeography of the brown bear (*Ursus arctos*) in Northeastern Asia based on analyses of complete mitochondrial DNA sequences. *Molecular Biology and Evolution*, *30*(7), 1644–1652. https://doi.org/10.1093/molbev/mst077

Hohenlohe, P. A., Bassham, S., Etter, P. D., Stiffler, N., Johnson, E. A., & Cresko, W. A. (2010). Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. *PLoS Genetics*, *6*(2), e1000862.

Huson, D. H. (1998). SplitsTree: Analyzing and visualizing evolutionary data. *Bioinformatics*, *14*(1), 68–73.

Janecka, J. E., Zhang, Y., Li, D., Munkhtsog, B., Bayaraa, M., Galsandorj, N., Wangchuk, T. R., Karmacharya, D., Li, J., Lu, Z., Uulu, K. Z., Gaur, A., Kumar, S., Kumar, K., Hussain, S., Muhammad, G., Jevit, M., Hacker, C., Burger, P., … Jackson, R. (2017). Range-wide snow leopard phylogeography supports three subspecies. *Journal of Heredity*, *108*(6), 597–607. https://doi.org/10.1093/jhered/esx044

Janz, L., Rosen, A. M., Bukhchuluun, D., & Odsuren, D. (2021). Zaraa Uul: An archaeological record of Pleistocene-Holocene palaeoecology in the Gobi Desert. *PLoS One*, *16*(4), e0249848.

Joshi, N., & Fass, J. (2011). *Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ files*. In (Version 1.33) GitHub repository. https://github.com/najoshi/sickle

Keis, M., Remm, J., Ho, S. Y. W., Davison, J., Tammeleht, E., Tumanov, I. L., Saveljev, A. P., Mannil, P., Kojola, I., Abramov, A. V., Margus, T., & Saarma, U. (2013). Complete mitochondrial genomes and a novel spatial genetic method reveal cryptic phylogeographical structure and migration patterns among brown bears in North-Western Eurasia. *Journal of Biogeography*, *40*(5), 915–927. https://doi.org/10.1111/jbi.12043

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549.

Kumar, V., Lammers, F., Bidon, T., Pfenninger, M., Kolter, L., Nilsson, M. A., & Janke, A. (2017). The evolutionary history of bears is characterized by gene flow across species. *Scientific Reports*, *7*, 46487. https://doi.org/10.1038/srep46487

Kurtén, B. (1968). *Pleistocene mammals of Europe*. Addine Publishing Co.

Kurtén, B. (1976). *The cave bear story: Life and death of a vanished animal*. Columbia University Press.

Lan, T., Leppälä, K., Tomlin, C., Talbot, S. L., Sage, G. K., Farley, S. D., Shideler, R. T., Bachmann, L., Wiig, Ø., & Albert, V. A. (2022). Insights into bear evolution from a Pleistocene polar bear genome. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(24), e2200016119.

Lan, T. Y., Gill, S., Bellemain, E., Bischof, R., Nawaz, M. A., & Lindqvist, C. (2017). Evolutionary history of enigmatic bears in the Tibetan plateau–Himalaya region and the identity of the yeti. *Proceedings of the Royal Society B-Biological Sciences*, *284*(1868), 20171804. https://doi.org/10.1098/rspb.2017.1804

Lande, R. (1988). Genetics and demography in biological conservation. *Science*, *241*(4872), 1455–1460. https://doi.org/10.1126/science.3420403

Lester, S. E., Ruttenberg, B. I., Gaines, S. D., & Kinlan, B. P. (2007). The relationship between dispersal ability and geographic range size. *Ecology Letters*, *10*(8), 745–758.

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with burrows-wheeler transform. *Bioinformatics*, *25*(14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li, H., & Durbin, R. (2010). Fast and accurate long-read alignment with burrows-wheeler transform. *Bioinformatics*, *26*(5), 589–595. https://doi.org/10.1093/bioinformatics/btp698

Li, H., & Durbin, R. (2011). Inference of human population history from individual whole-genome sequences. *Nature*, *475*(7357), 493–496.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Lidicker, W. Z. (1962). The nature of subspecies boundaries in a desert rodent and its implications for subspecies taxonomy. *Systematic Zoology*, *11*(4), 160–171.

Mace, G. M. (2004). The role of taxonomy in species conservation. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, *359*(1444), 711–719. https://doi.org/10.1098/rstb.2003.1454

Martin, S. H., Davey, J. W., & Jiggins, C. D. (2015). Evaluating the use of ABBA–BABA statistics to locate introgressed loci. *Molecular Biology and Evolution*, *32*(1), 244–257.

Masuda, R., Murata, K., Aiurzaniin, A., & Yoshida, M. C. (1998). Phylogenetic status of brown bears *Ursus arctos* of Asia: A preliminary result inferred from mitochondrial DNA control region sequences. *Hereditas*, *128*(3), 277–280. https://doi.org/10.1111/j.1601-5223.1998.00277.x

Matsuhashi, T., Masuda, R., Mano, T., Murata, K., & Aiurzaniin, A. (2001). Phylogenetic relationships among worldwide populations of the brown bear *Ursus arctos*. *Zoological Science*, *18*(8), 1137–1143. https://doi.org/10.2108/zsj.18.1137

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., & DePristo, M. A. (2010). The genome analysis toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research*, *20*(9), 1297–1303. https://doi.org/10.1101/gr.107524.110

McLellan, B., Proctor, M., Huber, D., & Michel, S. (2017). *Ursus arctos (amended version of 2017 assessment). The IUCN Red List of Threatened Species: E. T41688A121229971*.

McLellan, B., & Reiner, D. C. (1994). A review of bear evolution. *Bears: Their Biology and Management*, *9*, 85–96. https://doi.org/10.2307/3872687

McLellan, B. N., & Hovey, F. W. (2001). Natal dispersal of grizzly bears. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, *79*(5), 838–844. https://doi.org/10.1139/cjz-79-5-838

Miller, C. R., & Waits, L. P. (2003). The history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): Implications for conservation. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(7), 4334–4339.

Miller, C. R., Waits, L. P., & Joyce, P. (2006). Phylogeography and mitochondrial diversity of extirpated brown bear (*Ursus arctos*) populations in the contiguous United States and Mexico. *Molecular Ecology*, *15*(14), 4477–4485. https://doi.org/10.1111/j.1365-294X.2006.03097.x

Miller, W., Schuster, S. C., Welch, A. J., Ratan, A., Bedoya-Reina, O. C., Zhao, F. Q., Kim, H. L., Burhans, R. C., Drautz, D. I., Wittekindt, N. E., Tomsho, L. P., Ibarra-Laclette, E., Herrera-Estrella, L., Peacock, E., Farley, S., Sage, G. K., Rode, K., Obbard, M., Montiel, R., … Lindqvist, C. (2012). Polar and brown bear genomes reveal ancient admixture and demographic footprints of past climate change. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(36), E2382–E2390. https://doi.org/10.1073/pnas.1210506109

Nadachowska-Brzyska, K., Burri, R., Smeds, L., & Ellegren, H. (2016). PSMC analysis of effective population sizes in molecular ecology and its application to black-and-white Ficedula flycatchers. *Molecular Ecology*, *25*(5), 1058–1072.

Nowak, R. M. (1999). *Walker's mammals of the world* (6th ed.. Vol. 1). John Hopkins University Press.

O'Brien, S. J., & Mayr, E. (1991). Bureaucratic mischief: Recognizing endangered species and subspecies. *Science*, *251*(4998), 1187–1189.

Okonechnikov, K., Conesa, A., & Garcia-Alcalde, F. (2016). Qualimap 2: Advanced multi-sample quality control for high-throughput sequencing data. *Bioinformatics*, *32*(2), 292–294. https://doi.org/10.1093/bioinformatics/btv566

Orr, H. A. (2005). The genetic basis of reproductive isolation: Insights from drosophila. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(1), 6522–6526. https://doi.org/10.1073/pnas.0501893102

Ortiz, E. (2019). *vcf2phylip v2. 0: convert a VCF matrix into several matrix formats for phylogenetic analysis. Version v2. 0. Zenodo*.

Paetkau, D., Waits, L. P., Clarkson, P. L., Craighead, L., Vyse, E., Ward, R., & Strobeck, C. (1998). Variation in genetic diversity across the range of north American brown bears. *Conservation Biology*, *12*(2), 418–429. https://doi.org/10.1046/j.1523-1739.1998.96457.x

Pilot, M. (2021). Disentangling the admixed trails of grey wolf evolution. *Molecular Ecology*, *30*(24), 6509–6512. https://doi.org/10.1111/mec.16261

Poplin, R., Ruano-Rubio, V., DePristo, M. A., Fennell, T. J., Carneiro, M. O., Van der Auwera, G. A., Kling, D. E., Gauthier, L. D., Levy-Moonshine, A., Roazen, D., Shakir, K., Thibault, J., Chandran, S., Whelan, C., Lek, M., Gabriel, S., Daly, M. J., Neale, B., MacArthur, D. G., & Banks, E. (2017). *Scaling accurate genetic variant discovery to tens of thousands of samples*. (pp. 201178).

Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2–approximately maximum-likelihood trees for large alignments. *PLoS One*, *5*(3), e9490.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959. ://WOS:000087475100039.

Proctor, M. F., McLellan, B. N., Strobeck, C., & Barclay, R. M. R. (2004). Gender-specific dispersal distances of grizzly bears estimated by genetic analysis. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, *82*(7), 1108–1118. https://doi.org/10.1139/z04-077

Salomashkina, V. V., Kholodova, M. V., Tuten'kov, O. Y., Moskvitina, N. S., & Erokhin, N. G. (2014). New data on the phylogeography and genetic diversity of the brown bear *Ursus arctos* Linnaeus, 1758 of Northeastern Eurasia (mtDNA control region polymorphism analysis). *Biology Bulletin*, *41*(1), 38–46. https://doi.org/10.1134/S1062359014010087

Shiirevdamba, T., Shardarsuren, O., Erdenejav, G., Amgalan, T., & Tsetsegmaa, T. (2013). *Mongolian red book*. Ministry for Nature and the Environment of Mongolia.

Smith, M. F., & Patton, J. L. (1980). Relationships of pocket gopher (Thomomys bottae) populations of the lower Colorado River. *Journal of Mammalogy*, *61*(4), 681–696.

Sokolov, V., & Orlov, V. (1992). *A new species of present bears–Ursus gobiensis sp. n.–mazaalai or Gobi bear*. Internationales Symposium: Erforschung der biologischen Ressourcen der Mongolei, Halle/Saale, Germany.

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312–1313.

Stoen, O. G., Zedrosser, A., Saebo, S., & Swenson, J. E. (2006). Inversely density-dependent natal dispersal in brown bears *Ursus arctos*. *Oecologia*, *148*(2), 356–364. https://doi.org/10.1007/s00442-006-0384-5

Swenson, J. E., Sandegren, F., & Soderberg, A. (1998). Geographic expansion of an increasing brown bear population: Evidence for presaturation dispersal. *Journal of Animal Ecology*, *67*(5), 819–826. https://doi.org/10.1046/j.1365-2656.1998.00248.x

Swofford, D. (2002). *PAUP*: Phylogenetic analysis using parsimony (* and other methods)*. Sinauer Associates.

Tallmon, D. A., Bellemain, E., Swenson, J. E., & Taberlet, P. (2004). Genetic monitoring of Scandinavian brown bear effective population size and immigration. *The Journal of Wildlife Management*, *68*(4), 960–965.

Tavaré, S. (1986). Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences*, *17*(2), 57–86.

Taylor, G. A., Kirk, H., Coombe, L., Jackman, S. D., Chu, J., Tse, K., Cheng, D., Chuah, E., Pandoh, P., & Carlsen, R. (2018). The genome of the north American brown bear or grizzly: *Ursus arctos* ssp. horribilis. *Genes (Basel)*, *9*(12), 598. https://doi.org/10.3390/genes9120598

Team R. C. (2017). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Tumendemberel, O., Tebbenkamp, J. M., Zedrosser, A., Proctor, M. F., Blomberg, E. J., Morin, D. J., Rosell, F., Reynolds, H. V., Adams, J. R., & Waits, L. P. (2021). Long-term monitoring using DNA sampling reveals the dire demographic status of the critically endangered Gobi bear. *Ecosphere*, *12*(8), e03696.

Tumendemberel, O., Zedrosser, A., Proctor, M. F., Reynolds, H. V., Adams, J. R., Sullivan, J. M., Jacobs, S. J., Khorloojav, T., Tserenbataa, T., Batmunkh, M., & Waits, L. P. (2019). Phylogeography, genetic diversity, and connectivity of brown bear populations in Central Asia. *PLoS One*, *14*(8), e0220746.

Vachaspati, P., & Warnow, T. (2018). SVDquest: Improving SVDquartets species tree estimation using exact optimization within a constrained search space. *Molecular Phylogenetics and Evolution*, *124*, 122–136.

Van der Auwera, G. A., Carneiro, M. O., Hartl, C., Poplin, R., Del Angel, G., Levy-Moonshine, A., Jordan, T., Shakir, K., Roazen, D., & Thibault, J. (2013). From FastQ data to high-confidence variant calls: The genome analysis toolkit best practices pipeline. *Current Protocols in Bioinformatics*, *43*(1), 11.10.11–11.10.33.

Waits, L. P., & Paetkau, D. (2005). Noninvasive genetic sampling tools for wildlife biologists: A review of applications and recommendations for accurate data collection. *The Journal of Wildlife Management*, *69*(4), 1419–1433.

Waits, L. P., Talbot, S. L., Ward, R. H., & Shields, G. F. (1998). Mitochondrial DNA phylogeography of the north American brown bear and implications for conservation. *Conservation Biology*, *12*(2), 408–417. https://doi.org/10.1046/j.1523-1739.1998.96351.x

Waples, R. S., & Gaggiotti, O. (2006). INVITED REVIEW: What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, *15*(6), 1419–1439.

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population-structure. *Evolution*, *38*(6), 1358–1370. https://doi.org/10.2307/2408641

Weissensteiner, H., Forer, L., Fuchsberger, C., Schöpf, B., Kloss-Brandstätter, A., Specht, G., Kronenberg, F., & Schönherr, S. (2016). mtDNA-server: Next-generation sequencing data analysis of human mitochondrial DNA in the cloud. *Nucleic Acids Research*, *44*(W1), W64–W69. https://doi.org/10.1093/nar/gkw247

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer.

Wright, S. (1969). *Evolution and the genetics of populations: Vol. 2. The theory of gene frequencies*. University of Chicago Press.

Yang, Z. (1994). Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: Approximate methods. *Journal of Molecular Evolution*, *39*(3), 306–314.

Zedrosser, A., Stoen, O. G., Saebo, S., & Swenson, J. E. (2007). Should I stay or should I go? Natal dispersal in the brown bear. *Animal Behaviour*, *74*, 369–376. https://doi.org/10.1016/j.anbehav.2006.09.015

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