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# Ancient bears provide insights into Pleistocene ice age refugia in Southeast Alaska

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## Abstract

During the Late Pleistocene, major parts of North America were periodically covered by ice sheets. However, there are still questions about whether ice-free refugia were present in the Alexander Archipelago along the Southeast (SE) Alaska coast during the last glacial maximum (LGM). Numerous subfossils have been recovered from caves in SE Alaska, including American black (*Ursus americanus*) and brown (*U. arctos*) bears, which today are found in the Alexander Archipelago but are genetically distinct from mainland bear populations. Hence, these bear species offer an ideal system to investigate long-term occupation, potential refugial survival and lineage turnover. Here, we present genetic analyses based on 99 new complete mitochondrial genomes from ancient and modern brown and black bears spanning the last ~45,000 years. Black bears form two SE Alaskan subclades, one preglacial and another postglacial, that diverged >100,000 years ago. All postglacial ancient brown bears are closely related to modern brown bears in the archipelago, while a single preglacial brown bear is found in a distantly related clade. A hiatus in the bear subfossil record around the LGM and the deep split of their pre- and postglacial subclades fail to support a hypothesis of continuous occupancy in SE Alaska throughout the LGM for either species. Our results are consistent with an absence of refugia along the SE Alaska coast, but indicate that vegetation quickly expanded after deglaciation, allowing bears to recolonize the area after a short-lived LGM peak.

## KEYWORDS

last glacial maximum, paleogenetics, refugia, Southeast Alaska, *Ursus americanus*, *Ursus arctos*

## 1 | INTRODUCTION

During the Late Pleistocene, the Laurentide and Cordilleran Ice Sheets periodically covered much of North America, including portions of, or perhaps the entire, Alexander Archipelago located in

Southeast (SE) Alaska. While the Laurentide Ice Sheet was at its maximum extent during the global last glacial maximum (LGM), a period that spanned ~26–19 thousand years ago (ka; Clark et al., 2009), the Pacific margin of the Cordilleran Ice Sheet only reached its greatest extent ~20–17 ka (local LGM; Booth et al., 2003; Darvill et al., 2018;

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Lesnek et al., 2018; Lesnek et al., 2020; Porter & Swanson, 1998; Praetorius & Mix, 2014; Walcott et al., 2022). However, gaps in knowledge concerning the Cordilleran Ice Sheet chronology and westernmost extent and a complex sea-level history have left open questions about the presence of ice-free land (refugia) along the North Pacific Coast (NPC) during the local LGM, and the potential effects of such refugia on the biogeography of North American fauna and flora, and a viable migration route for the initial peopling of the Americas (Moreno-Mayar et al., 2018; Potter et al., 2018; Raghavan et al., 2015).

Based on the high level of endemism in the Alexander Archipelago today (MacDonald & Cook, 2007), the presence of pine pollen as early as 15.4–15.2 ka (Ager et al., 2010), and geologic mapping of ice limits (Barrie & Conway, 1999; Carrara et al., 2007; Hetherington et al., 2003; Lacourse et al., 2005), areas along the coastal Cordilleran Ice Sheet margin are hypothesized to have been ice-free during the LGM. Recent studies based on cosmogenic exposure dating, however, have demonstrated that the outer coast of the southern Alexander Archipelago became ice-free only by  $16.3 \pm 0.8$  ka, whereas ice retreated from the coastal northern Alexander Archipelago at  $15.1 \pm 0.9$  ka (Lesnek et al., 2018, 2020; Walcott et al., 2022). Hence, sites above modern-day sea level that were previously mapped as glacial refugia in the archipelago were most likely covered by ice during the local LGM until between  $\sim 16.3$  and 15.1 ka, and with the inner fjords and sounds of the southern archipelago ice-free by  $\sim 15$  ka (Clague, 1989; Dyke, 2004; Lesnek et al., 2018, 2020; Walcott et al., 2022). Nevertheless, deglaciation occurred earlier and possibly faster along the North Pacific Coast than the continental ice-free corridor between the Cordilleran and Laurentide Ice Sheets east of the Coast Range that became ecologically viable  $\sim 13.5$  ka (Clark et al., 2022; Dyke, 2004; Heintzman et al., 2016; Stalker, 1977). This regional difference in timing in deglaciation potentially created a migration route thousands of years earlier along the coast. Furthermore, sea levels during the last glaciation were considerably lower, exposing parts of the continental shelves, now submerged, that could have acted as ice age refugia (Baichtal et al., 2021; Walcott et al., 2022).

In SE Alaska today, 82 mammal species representing 116 taxa (subspecies and monotypic species) have been documented. Of these taxa, 30 are endemic to the region, including three nominal subspecies of brown bears and two subspecies of black bear. Although the validity of some of these endemics has been questioned (MacDonald & Cook, 2007), several island or coastal endemics belong to distinct genetic lineages when compared to continental populations found farther east (Cook et al., 2006; Sawyer et al., 2019). Examples include American black bear (*Ursus americanus*), brown bear (*U. arctos*), Pacific marten (*Martes caurina*), Haida ermine (*Mustela haidarum*) and several small mammals (Byun et al., 1997; Colella et al., 2018, 2021; Cook et al., 2006; Dawson et al., 2014; Hope et al., 2016; Jackson & Cook, 2019; Puckett et al., 2015; Sawyer et al., 2019; Talbot & Shields, 1996; Wooding & Ward, 1997). Extensive paleontological work in the Alexander Archipelago recovered thousands of mammalian remains from multiple caves, including human remains and

artefacts (Aqil et al., 2023; Dixon, 2015; Lindo et al., 2017), and some of the oldest New World dog remains (da Silva Coelho et al., 2021). The Shuká Káa (On Your Knees) Cave on Prince of Wales Island is so far the only cave with a preglacial record with specimens radiocarbon dated to more than 45 ka (Heaton & Grady, 2003), while radiocarbon-dated subfossils from all other caves have been of postglacial age. A gap in the subfossil record in this cave around 19.8–17.2 calibrated thousands of years before present (cal kyr B.P.) likely represents a period when the cave entrance was covered in ice, coinciding with the local LGM (Heaton & Grady, 2003; Lesnek et al., 2018; Walcott et al., 2022). However, some species that were present in the archipelago before the LGM are still found there, indicating a long residence in the region, possibly including survival in ice age refugia that are now submerged (Heaton et al., 1996; Heaton & Grady, 2003).

The fossil record indicates a clear transition in the environment and climate in the region (Heaton & Grady, 2003). Before the local LGM, SE Alaska was a forested environment inhabited by American black bears, brown bears, caribou (*Rangifer tarandus*), red fox (*Vulpes vulpes*) and hoary marmot (*Marmota caligata*). This was followed by the peak of the Wisconsin glaciation that allowed Arctic species to occupy the region, such as ringed seal (*Pusa hispida*) and arctic fox (*V. lagopus*). Returning to a forest environment after the local LGM, however, some species had disappeared from the region, including caribou and marmot, while others migrated to the region, like deer (*Odocoileus hemionus*). Genetic data from ancient samples allow us to explore mammal diversity and response to climate change during the Late Pleistocene (Cooper et al., 2015). Furthermore, insights into the biogeographic history of native fauna provide a better understanding of the ice age history in the region. For example, differentiating whether animals remained in the region during the local LGM, or if they disappeared during the LGM and recolonized the islands when the ice retreated, would contribute a crucial proxy to our knowledge of the existence of ice age refugia.

Bears represent the most abundant mammal species in the subfossil record from caves in the Alexander Archipelago. Their history in the region spans more than 45 cal kyr B.P., and they were in the archipelago both before and after the local LGM (Heaton & Grady, 2003). Therefore, bears offer an opportune system to investigate whether they survived in SE Alaska ice age refugia. American black bears and brown bears are today both found in the Alexander Archipelago; however, black bears only inhabit the southern islands (south of Frederick Sound), whereas brown bears are mainly found on the northern islands of Admiralty, Baranof and Chichagof and also referred to as 'ABC brown bears' (Talbot & Shields, 1996). Nevertheless, the subfossil record indicates that the distribution of the two bear species overlapped until at least  $\sim 8$  cal kyr B.P. in the southern part of the archipelago (Heaton & Grady, 2003). American black bears are endemic to North America, and based on mitochondrial DNA, the species shares a last maternal common ancestor around  $\sim 3.57$  million years ago with its closest relative, the Asiatic black bear (*U. thibetanus*; Puckett et al., 2015). Two maternal lineages have been identified, clades A (continental) and B

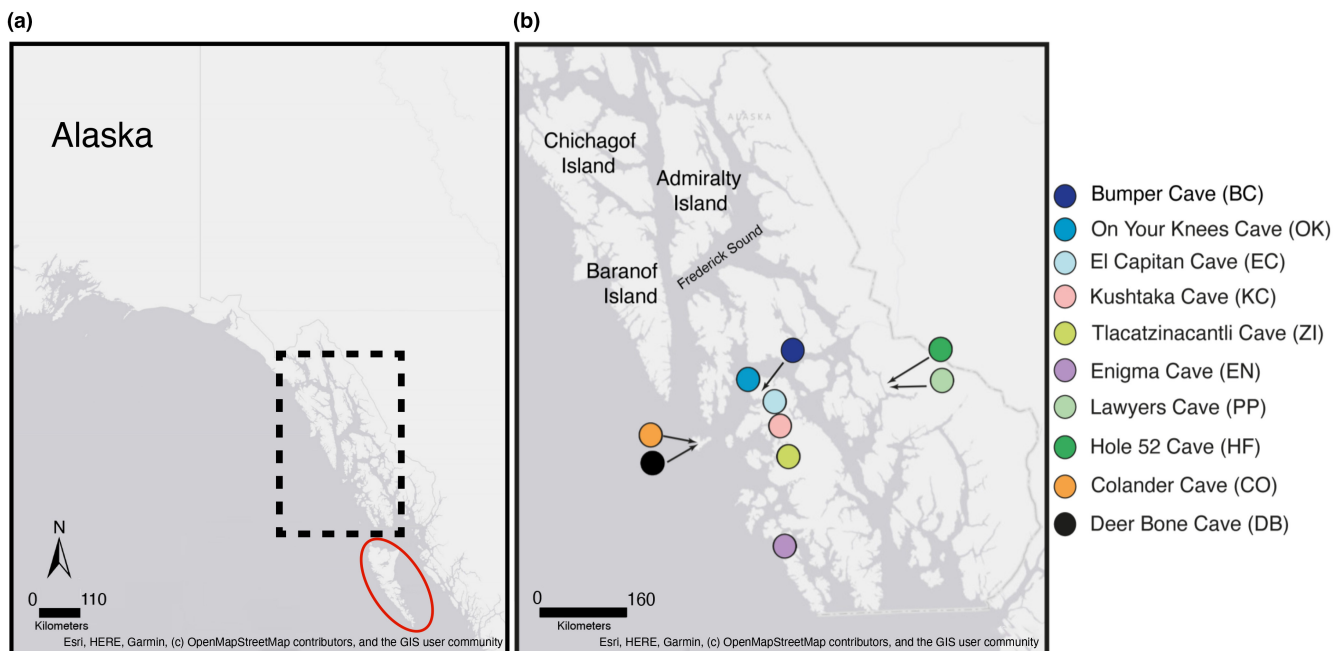
(coastal). Continental bears are found in mainland North America from Canada to Mexico and are separated into two subclades, East and West. Black bears with the coastal haplotype are only found along the Pacific Coast from southeastern Alaska to California (Byun et al., 1997; Stone & Cook, 2000; Wooding & Ward, 1997). The brown bear, on the contrary, is a Holarctic species that comprises considerable mitochondrial DNA haplotype diversity, divided into at least six distinct clades that largely correspond to geographic distribution (Davison et al., 2011). In addition, several extinct genetic subclades have been identified, including two from North America (Barnes et al., 2002; Davison et al., 2011; Salis et al., 2021). Brown bears' mitochondrial phylogeny is paraphyletic, as polar bears (*Ursus maritimus*) are nested inside brown bear mitochondrial diversity and related most closely to the ABC brown bears. Hence, the mitochondrial haplotype of ABC brown bears is more similar to the haplotype of polar bears than it is to most other brown bear populations (Barnes et al., 2002; Davison et al., 2011; Miller et al., 2012; Salis et al., 2021; Talbot & Shields, 1996), leaving the origin of ABC brown bears an enigma.

Here, we present results from genetic analyses of 72 ancient bear subfossils excavated from caves in Alexander Archipelago spanning the last ~45 cal kyr B.P., with a gap in the fossil record from about 30 to 14 cal kyr B.P. We aimed to investigate the evolutionary history of American black bears and brown bears along the North Pacific Coast to better understand the origin of these distinct lineages. For example, if all bears in the region, predating and following the gap around the LGM, belong to the same lineage, it may suggest that they

survived the local LGM in habitable ice age refugia in the region. On the contrary, if they belong to separate lineages, they may have become extirpated in SE Alaska during the LGM and a different population of bears recolonized the region, possibly from refugia elsewhere, once deglaciation began. Thus, comparing genetic data of ancient and modern samples can be used as a proxy to test whether the region provided ice age refugia along the North Pacific Coast, constraining the timing and extent of deglaciation along the westernmost edge of the Cordilleran Ice sheet, as well as assess the impacts of climate change on large mammals in SE Alaska during the Late Pleistocene.

## 2 | MATERIALS AND METHODS

Seventy-two ancient bear samples from the southern part of Alexander Archipelago were analysed (Table S1). The subfossils were excavated from 10 different caves: Bumper Cave (BC), Deer Bone Cave (DB), Colander Cave (CO), El Capitan Cave (EC), Enigma Cave (EN), Hole 52 Cave (HF), Kushtaka Cave (KC), Lawyers Cave (PP), On Your Knees Cave (OK) and Tlacatzinacantli Cave (ZI; Figure 1; Table S1). The taxonomic identity of almost all the samples had been previously predicted based on their morphology, most of them to species level due to their size (Heaton & Grady, 2003). We also included new mitogenome data from 16 modern black bears representing the main mitochondrial clades (Coastal and Continental East and West), including black bears from Southeast Alaska, in addition to 11 new brown bear mitogenomes from Alaska (Table S2).



**FIGURE 1** Maps of study area and location of caves. (a) Map of southern Alaska and northern British Columbia, dashed black box indicates Southeast (SE) Alaska, and the red box indicates Haida Gwaii, an archipelago off the northern Pacific coast of Canada and just south of the Alexander Archipelago. (b) Map of SE Alaska showing the location of caves where bears' remains were found and the Admiralty, Baranof and Chichagof (ABC) islands.

## 2.1 | Radiocarbon dating and stable isotopes

Fifty-eight subfossils were initially  $^{14}\text{C}$  radiocarbon-dated, and stable isotope  $\delta^{13}\text{C}$  values based on bone collagen were provided from previous work (Heaton & Grady, 2003). An additional 19 bear samples were dated by the University of Arizona AMS Laboratory in 2019, including two samples, UAMES 53102 and UAMES 53089, that had been dated previously. The latter two samples were redated to assess both the robustness of the previous radiocarbon dating and the potential impact of using an ultrafiltration protocol. Because no significant difference between the two methods was observed (see below), the other 17 samples were radiocarbon dated without using an ultrafiltration protocol (see Table S1 for all radiocarbon dates). The  $^{14}\text{C}$  dates were calibrated using the following scheme depending on the contribution of marine carbon based on the  $\delta^{13}\text{C}$  values: For samples with  $\delta^{13}\text{C}$  lower than  $-21\text{‰}$ , the IntCal20 (Ramsey & Lee, 2013) calibration curve was applied. A curve based on 100% of marine carbon contribution was used for samples with  $\delta^{13}\text{C}$  above  $-14.5\text{‰}$ . For samples between  $-21\text{‰}$  and  $-14.5\text{‰}$ , a mixed marine and NH atmospheric calibration curve was used (Ramsey & Lee, 2013), with the % marine carbon contribution calculated based on the cut-off values for a fully terrestrial and marine diet (Fedje et al., 2021). Calibrations were performed in OXCAL v4.3 (Ramsey & Lee, 2013), with corrections for the local marine reservoir effect (Schmuck et al., 2021), and 2-sigma dates were reported (Table S1). The median probability date was used in downstream analyses. Stable isotope  $\delta^{13}\text{C}$  values from modern bears were obtained from previously published studies (Barnes et al., 2002; Hilderbrand et al., 1996; Hobson et al., 2000; Kosintsev et al., 2022; Mangipane et al., 2020; Stern et al., 2021).

## 2.2 | DNA extraction, PCR amplification, mitochondrial enrichment and mitogenome mapping

DNA from subfossils was extracted in a dedicated cleanroom facility appropriate for ancient DNA research and separated from any processing of modern samples. Ancient DNA extractions followed the protocol described in Dabney et al. (2013), with some modifications as described in da Silva Coelho et al. (2021). As part of an initial screening to confirm the taxonomic identity of the samples, PCR reactions were prepared in the dedicated cleanroom, adding  $21\ \mu\text{L}$   $\text{H}_2\text{O}$ ,  $0.4\ \mu\text{M}$  of each forward and reverse primer, and  $2\ \mu\text{L}$  of extracted genomic DNA to each GE illustra PuReTaq Ready-To-Go PCR bead (GE Healthcare). For samples previously identified as *Ursus*, amplifications were performed using two primer pairs that amplify the *Ursus* control region and *cytb* (primers 161F/162R & 164R/165F, respectively; Lan et al., 2017). PCR products were purified using a MinElute PCR Purification kit (Qiagen) and Sanger sequenced directly using the same primers as in the PCR. DNA from modern samples was extracted using either a salt extraction procedure (Medrano et al., 1990; Sonsthagen et al., 2004) or a DNeasy Blood & Tissue kit (Qiagen).

To assemble complete mitochondrial genomes of ancient samples, library preparation, mtDNA target enrichment, whole genome enrichment and shotgun sequencing were performed by Arbor Daicel Biosciences (<http://www.arborbiosci.com>; see Table S1). DNA from all samples was target-enriched using a custom-designed ursid mitogenome bait panel (Lan et al., 2017). In addition, to test the application of whole genome enrichment (WGE) on these specimens, WGE was performed for three samples (UAMES 53085, UAMES 53102 and UAMES 53123) following the protocol described in Lan et al. (2022). Both methods followed the standard MYBAITS v. 3.0 protocol, with equal masses of libraries pooled, bead-templated and sequenced using the Ion PI Chip Kit v2 chemistry and sequenced on the Ion Proton platform. Following sequencing, reads were demultiplexed, quality-trimmed and filtered using the default settings on the Ion TORRENT SUITE v. 4.4.3. Additionally, low-depth Illumina shotgun sequencing was performed for 10 samples (Table S1). Truseq dual-barcoded libraries were prepared without sonication, using the blunt-end ligation module from the NEBNext Fast DNA library preparation kit (New England BioLabs) with an extended double-time treatment and blunt-end adapters synthesized and paired-end sequenced on an Illumina HiSeqX platform.

Mitogenome data of modern samples were generated via shotgun sequencing protocols on a 454 Life Sciences GS-Jr FLX, (Roche) using library preparation and sequencing protocols similar to those described in Sonsthagen et al. (2013), or on an Illumina MiSeq following the manufacturer's recommended protocols, both conducted at the U.S. Geological Survey's Alaska Science Center, Anchorage, Alaska. In addition, Illumina library preparation and shotgun sequencing of some modern samples were carried out at Novogene AIT, Singapore.

To assemble mitogenome consensus sequences from ancient samples, Illumina reads were first trimmed using Trimmomatic (Bolger et al., 2014). Based on initial identification of samples to species performed using BLAST (Morgulis et al., 2008), all Ion Torrent and Illumina reads were then aligned separately and mapped using BWA v. 0.7.13 (Li & Durbin, 2009), with the *aln* algorithm and seed set to 1024, against available mitochondrial genomes (*Ursus americanus* NC-003426.1, *Ursus arctos* JX-196368.1 and *Ursus arctos* NC-003427). Unmapped reads were extracted with samtools (Li et al., 2009) and mapped with BWA-mem (Li, 2013) using the default settings. PCR duplicates were removed using the MarkDuplicates tool in Picard using the lenient validation stringency. If a sample was sequenced multiple times, the BAM files generated from different mappings were merged using Picard MergeSamFiles (<http://broadinstitute.github.io/picard/>). The consensus was called using samtools mpileup (Li, 2011) and the default settings. Mapping statistics were calculated with bedtools (Quinlan, 2014). Low-coverage samples were mapped to all three references to assess potential reference bias. Modern samples were mapped using BWA-mem using default settings. PCR duplicate removal, consensus calling and calculation of mapping statistics were performed using the same pipeline mentioned above.



## 2.3 | DNA degradation assessment

The well-known degradation patterns of ancient DNA, and the expected high rate of nucleotide misincorporation that is mainly caused by deamination, can be used to confirm ancient DNA authenticity. We used MAPDAMAGE 2.0 (Jónsson et al., 2013), which uses an approximate Bayesian estimation of the damage patterns to assess DNA degradation patterns in the reference mapped assembly of each ancient bear individual.

## 2.4 | Mitogenome sequence data analysis

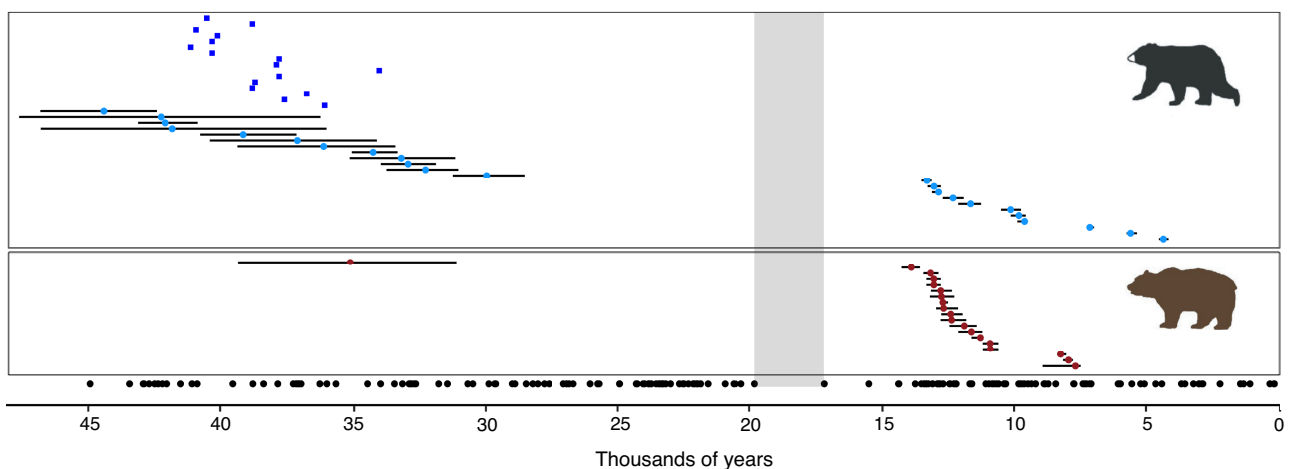
In addition to the 99 ancient and modern mitogenomes generated for this study, complete mitogenomes of *Ursus americanus* (3), *U. arctos* (370), *U. maritimus* (32), *U. thibetanus* (11), *U. spelaeus* (14), *U. ingressus* (3) and *U. deningeri* (2) were downloaded from the National Center for Biotechnology Information (NCBI) GenBank database. Reads from an additional 19 samples were downloaded from the NCBI Sequence Reads Archive (SRA) and their mitogenomes assembled using the same pipeline as for the modern mitogenomes generated for this study (see Table S2).

Three separate data sets were created, all including modern and ancient bears from Eurasia and North America. The first data set contained 553 bear sequences (data set 553) that included sequence data from all samples. The second data set contained data from 513 bear samples (data set 513), comprising sequences with a minimum average mitogenome coverage of 5× of dated and calibrated bears only, including bears from Salis et al. (2021) that had their date indirectly estimated. The third data set comprised 485 bears (data set 485), composed of similar mitogenomes as the second data set, but only excluding samples that were indirectly dated by Salis et al. (2021). The sequences were aligned in MAFFT (Katoh

et al., 2019), with manual adjustment in GENEIOUS PRIME 2022.0.1 (<https://geneious.com>) to exclude tandem repeats from the control region. To make all sequences within the alignments of similar lengths, 24 base pairs from the beginning and 45 at the end of the alignment were trimmed. Maximum-likelihood analyses of all three data sets were performed using RAXML-HPC Black Box 8.2.12 (Stamatakis, 2014), with 1000 bootstraps using the GTR substitution model that was chosen as the best model by IQ-TREE (Nguyen et al., 2015). A Bayesian tree was generated from the data set 553, using BEAST 2.7.1 (Bouckaert et al., 2019). The GTR+G+I substitution model and a constant-size coalescent model were used. Trees were sampled every 1000 states from a total of 75 million states. To obtain estimated divergence dates, BEAST 2.7.1 (Bouckaert et al., 2019) analysis was performed on the data sets containing mitogenomes with a minimum depth of 5× and calibrated radiocarbon dates (data sets 513 and 485). Age calibration of divergence time estimation was performed by adding tip dates using calibrated radiocarbon dates and estimated dates of all the bear samples included in the analyses. Trees were sampled every 1000 states from a total of 100 million states. Two additional data sets, one only comprised of *U. americanus* and the second only of *U. arctos* with an ABC haplotype, were created and used to generate statistical parsimony networks using the TCS algorithm (Clement et al., 2000) as implemented in POPART (Leigh & Bryant, 2015).

## 3 | RESULTS

We generated mitogenome data for 72 ancient bear subfossil samples, 59 of which were radiocarbon dated with ages ranging from >41 to ~4 <sup>14</sup>C kyr and calibrated dates ranging from ~44.5 to ~4.3 cal kyr B.P. with a notable hiatus in the fossil record between ~30 and ~14 cal kyr B.P. (Figure 2). Two samples (UAMES



**FIGURE 2** Timeline of *U. americanus* (top) and *U. arctos* (bottom) in Southeast Alaska. Each dot represents a dated sample. Blue squares represent noncalibrated samples; hence, the x-axis for those samples is <sup>14</sup>C kyr. For all other samples, the x-axis is in calibrated kyr before present (B.P.). Horizontal lines represent the range of 2-sigma calibrated dates. Black dots represent subfossils from other species found in Alexander Archipelago caves (Heaton & Grady, 2003; Lesnek et al., 2018). The shaded grey area indicates the timing of the local last glacial maximum.

53102 and UAMES 53089) were related to assess a possible impact of ultrafiltration pretreatment of the collagen and robustness of the older  $^{14}\text{C}$  radiocarbon dating methods on the subfossil ages. For UAMES 53102, when collagen was ultrafiltered, it returned a date of  $12,090 \pm 70$   $^{14}\text{C}$  years, while it was  $12,080 \pm 60$   $^{14}\text{C}$  years without ultrafiltration. For the older sample, UAMES 53089, the date was  $>36,400$   $^{14}\text{C}$  years with ultrafiltration and  $>35,800$   $^{14}\text{C}$  years without. Their ages previously obtained by Heaton and Grady (2003) were 12,300  $^{14}\text{C}$  years and 38,400  $^{14}\text{C}$  years, respectively. Because there was no marked difference between the methods applied, the dates provided by Heaton and Grady (2003) for those two samples were used on downstream analyses and newly generated radiocarbon dates were estimated without using an ultrafiltration protocol.

The ancient mitogenomes had an average depth ranging from 0.01x to 300x and a breadth ranging from 1% to 100%, with 49 near-complete mitogenomes ( $>80\%$  coverage; Table S1). The newly assembled mitogenomes exhibited an increased rate of cytosine deamination at the 5'-end of the reads and an increased rate of guanine to adenine substitution close to the 3'-end of the reads (Figure S1 shows misincorporation plots from samples with a mitogenome coverage  $>5\times$ ), as expected for damage patterns of ancient DNA and confirming the authenticity of the DNA. In addition, we assembled 27 new modern bear mitogenomes (Table S2). Phylogenetic analyses based on maximum-likelihood RAXML and Bayesian inference BEAST analyses were performed on all three data sets, and the results are shown in the following figures: data set 553 (Figure 3;

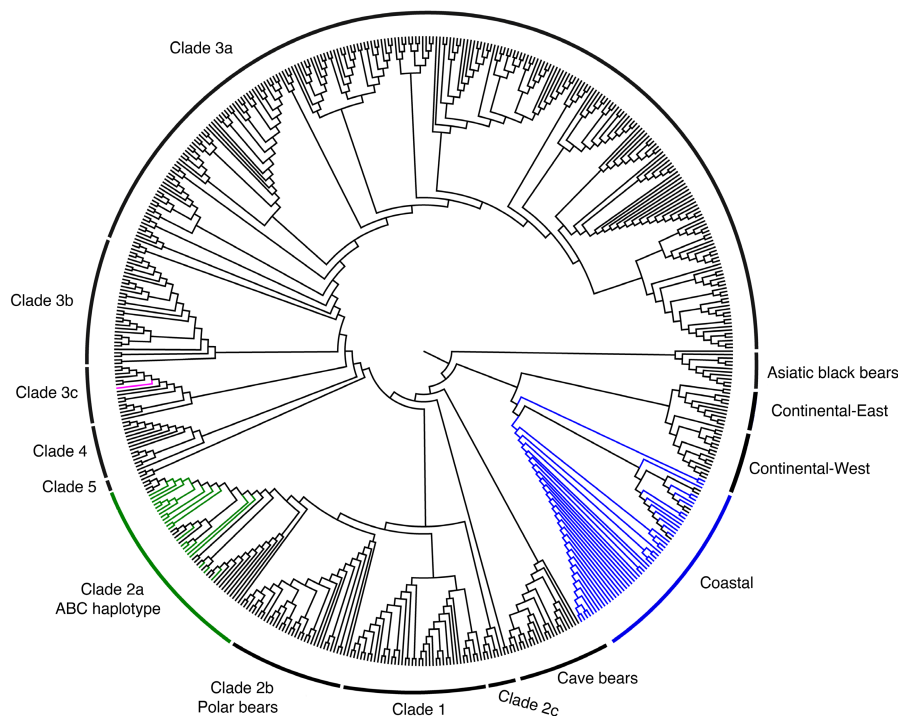
Figures S2 and S3), data set 513 (Figures 4 and 5; Figures S4 and S5), and data set 485 (Figures S6–S9), respectively.

### 3.1 | Preglacial black bears were as large as modern brown bears

Initial taxonomic identifications of almost all subfossil samples had been predicted based on morphology, and among the 72 samples, 35 samples had been identified as brown bears and 25 as black bears, nine as bears of unknown species affinity, one as a seal (*Phoca* sp.), one as an unidentified marine mammal, and one as a wolverine (*Gulo gulo*; Heaton & Grady, 2003). Based on our genetic analyses, of the 72 subfossils, 22 were genetically identified as brown bear and 50 were identified as American black bear (Table S1). Of note, all the bears that were initially identified as brown bears based on their morphological size measurements and later confirmed genetically as black bear were preglacial and dated to  $\sim 44.5$  to  $\sim 30$  cal kyr B.P., suggesting that preglacial American black bears could have reached the larger size of modern brown bears.

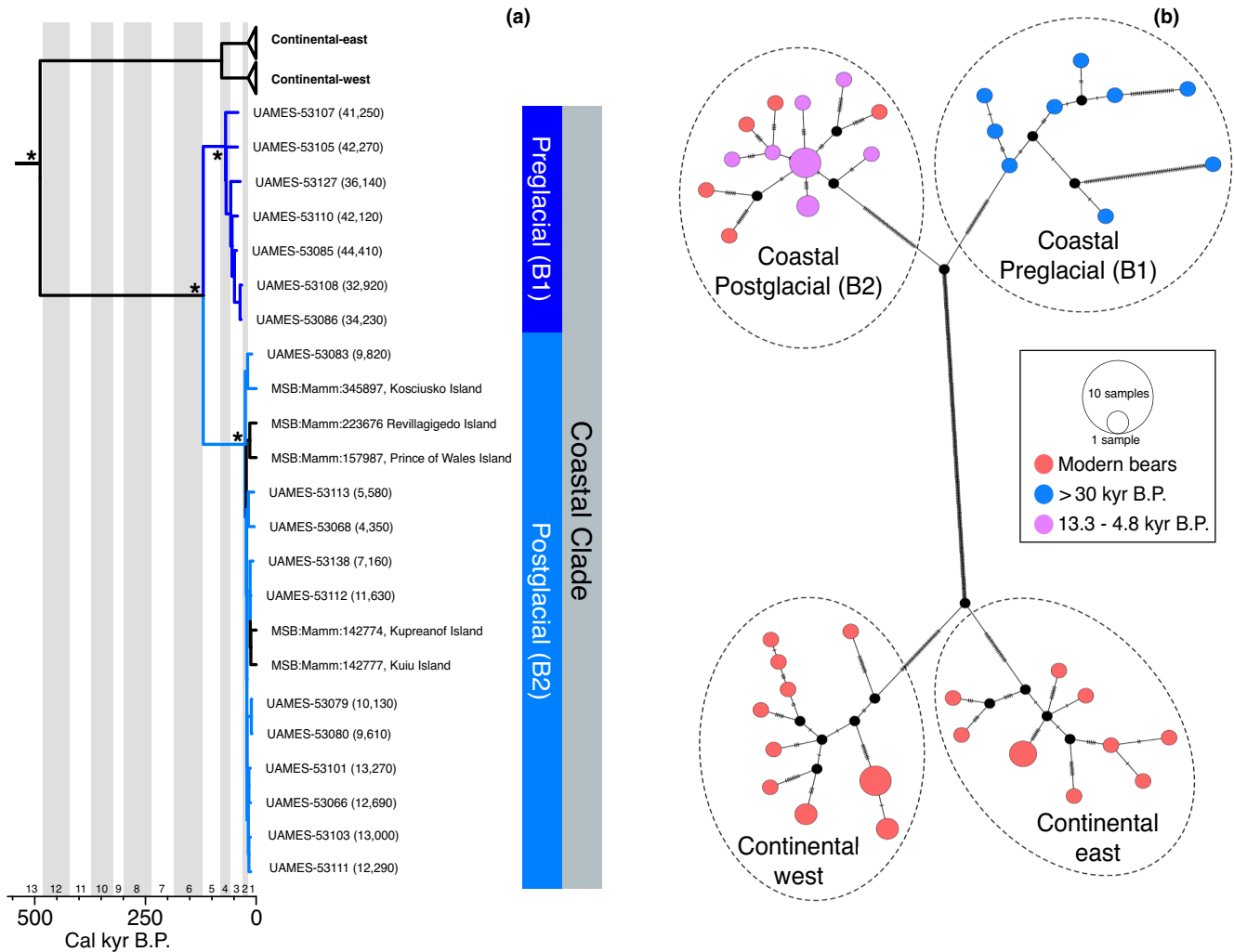
### 3.2 | Ancient pre- and postglacial American black bears comprise distinct coastal clades

The phylogenetic analyses of all data sets grouped the American black bear mitogenomes into the main coastal and continental



**FIGURE 3** Phylogenetic cladogram based on maximum-likelihood analysis of the complete data set (553 mitogenome sequences). Coloured branches indicate ancient bears from Southeast (SE) Alaska used in this study. American black bears are indicated by the subclades 'Continental-East', 'Continental-West', and 'Coastal'. Brown bear subclades are indicated as clades 2 to 5, except clade 2b that represents polar bears. ABC, Admiralty, Baranof and Chichagof, also referred to as 'ABC brown bears'.

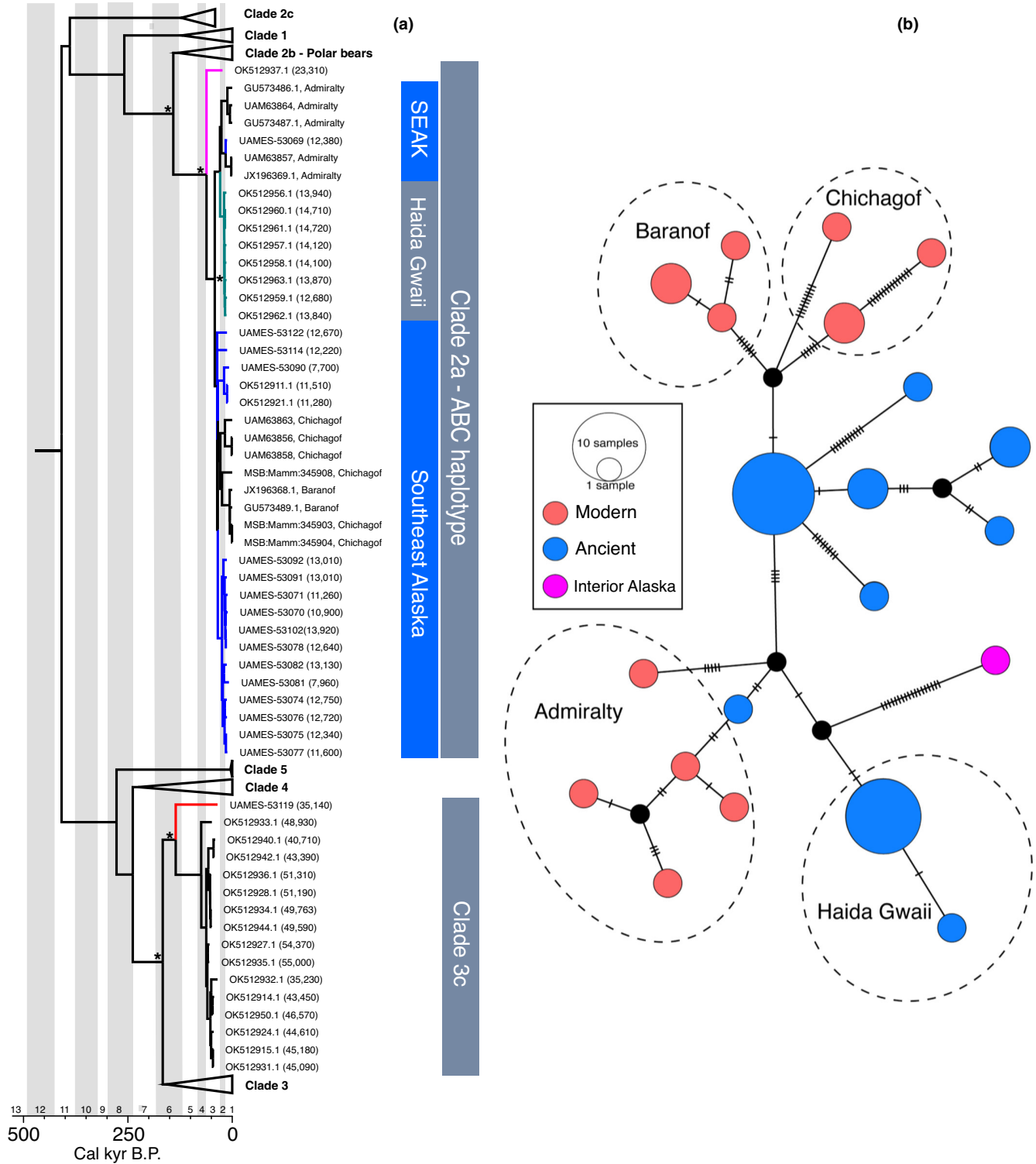




**FIGURE 4** Phylogenetic relationships of black bears (*Ursus americanus*). (a) A tip-calibrated Bayesian phylogenetic tree of *U. americanus* based on 513 sequences (data set 513) showing two subclades of coastal black bears. Stars indicate that the node is supported in maximum-likelihood (bootstrap support over 95%) and Bayesian analyses (posterior probability over 0.95). Grey and white bars represent the different marine isotope stages (MIS; numbers indicate the MIS), grey bars indicate glacial periods and white bars interglacial. Coloured branches represent ancient bears, and black shows modern bears. See Figure S5 for the full phylogenetic tree. (b) TCS haplotype network based on 60 *Ursus americanus* mitochondrial genome sequences showing the two main mitochondrial clades: Continental (a) and Coastal (b). The Coastal clade is split into preglacial (B1) and postglacial (B2) subclades. Circle sizes are proportional to the number of individuals with each haplotype and coloured according to their calibrated age group. B.P., before present. Cal kyr B.P. refers to calibrated thousands of years before present.

clades. The continental clade was further subdivided into two subclades, with bears from Maine and Pennsylvania grouped together in the East clade and bears from New Mexico to Alaska grouped in the West clade. In the coastal clade, all preglacial black bears that inhabited SE Alaska form a monophyletic group (B1), which is sister to a clade that encompasses postglacial ancient black bears and modern black bears that belong to the coastal clade (B2; Figure 4a). Maximum-likelihood and Bayesian analyses of the data set including all 553 mitogenome sequences displayed overall similar relationship. However, in the Bayesian tree (Figure S3), eight undated, low-coverage samples (coverage < 5x) grouped as sister to the whole coastal clade or the postglacial subclade, while in the maximum-likelihood tree (Figure S2), only two of the low-coverage samples grouped as sister to the coastal clade, while the remaining samples

placed within either the post- or the preglacial subclades. The split between the preglacial subclade (presumably an extinct clade of bears dated ~44.5 to ~30 cal kyr B.P.) and the postglacial clade (ancient and modern bears younger than ~13.2 cal kyr B.P.) was statistically well-supported in all phylogenetic analyses (bootstrap value of 100% and posterior probability of 1.0). The divergence time estimates derived from data sets 485 and 513 were similar. Because it is the most inclusive data set of the two, only estimated divergence times from data set 513 are reported and presented in Figure 4a (see Figure S8 for the tree from data set 485, and Figures S4–S7 for the complete tree from data sets 513 and 485). The American black bears' maternal lineage diverged from Asiatic black bears 1.5 million cal years B.P. (95% HPD 1378–1646 cal kyr B.P.). The coastal clade shared a last common ancestor with the continental clade ~487 cal



**FIGURE 5** Phylogenetic relationships of brown bears (*Ursus arctos*). (a) A tip-calibrated Bayesian phylogenetic tree of *U. arctos* and *U. maritimus* based on 513 sequences (data set 513). The magenta branch represents the bear with the ABC haplotype from Interior Alaska. Black branches represent modern bears. Blue represents ancient ABC bears from Southeast (SE) Alaska, while dark green represents ancient bears with ABC haplotype from Haida Gwaii. The red branch shows the single preglacial brown bear found in SE Alaska. Stars indicate that the node is supported in maximum-likelihood and Bayesian analyses (bootstrap support >95 and posterior probability >0.95). Grey and white bars represent marine isotope stages (MIS) 13 to 1, with grey bars indicating glacial periods, and white bars interglacial. See Figure S5 for the full phylogenetic tree. (b) TCS haplotype network based on 40 *Ursus arctos* mitochondrial genome sequences from clade 2a. Circle sizes are proportional to the number of individuals with each haplotype indicating whether it is modern or ancient, except for the bear from interior Alaska with the ABC haplotype. ABC, Admiralty, Baranof and Chichagof, also referred to as ‘ABC brown bears’. Cal kyr B.P. refers to calibrated thousands of years before present.

kyr B.P. (95% HPD 432–545 cal kyr B.P.) (Figure 4a). Subclades B1 and B2 shared a last common ancestor ~119 cal kyr years B.P. (95% HPD 100–140 cal kyr B.P.). The preglacial clade coalesced ~65 cal kyr B.P. (95% HPD 57–77 cal kyr B.P.) and the postglacial ~24 cal kyr B.P. (95% HPD 18–31 cal kyr B.P.). The haplotype network (Figure 4b) showed similar groupings as in the phylogenetic trees for coastal and continental black bears. The coastal and continental clades are separated by at least 365 mutations, which represents 2.2% genetic differentiation, and the two pre- and postglacial subclades inside the coastal lineage differ from each other by at least 62 substitutions.

### 3.3 | Ancient pre- and postglacial brown bears are distant relatives

#### 3.3.1 | A preglacial brown bear from the Alexander Archipelago belongs to an extinct mainland clade

Among the 72 subfossil bear samples analysed in this study, only a single preglacial brown bear (UAMES 53119) was identified, aged 31.7 <sup>14</sup>C kyr (median date of 35.1 cal kyr B.P.). The sample grouped as sister to clade 3c, an extinct brown bear clade that is distantly related to the ABC brown bear haplotype and instead more closely related to bears that today are found throughout Russia, western Europe and mainland Alaska (Barnes et al., 2002; Salis et al., 2021; Figure 5a; see Figure S9 for the tree from data set 485, and Figures S4–S7 for the complete tree from data sets 513 and 485). UAMES 53119 is the first and so far only record of a 3c bear from the coast; all other bears that also belong to clade 3c were found in interior Alaska and Yukon and dated from ~55–35 cal kyr B.P. (Barnes et al., 2002; Salis et al., 2021). The clade 3c is sister to clade 3, receiving high bootstrap support and posterior probability values (Figure 5a). In the maximum-likelihood analyses of data set 485, clade 3c is nested inside clade 3 being sister to clade 3a, although it receives low bootstrap support (Figure S6). Although UAMES 53119 was placed as sister to the whole 3c clade based on Bayesian analyses, with a high posterior probability (Figure 5a), it was nested inside this clade in the maximum-likelihood analyses (Figures S2, S4, S6). Clade 3c diverged from clade 3 ~165.5 cal kyr B.P. (95% HPD 150–184 cal kyr B.P.). UAMES 53119 shared a last common ancestor with clade 3c ~134.5 cal kyr B.P. (95% HPD 120–152 cal kyr B.P.).

#### 3.3.2 | Ancient Alexander Archipelago postglacial brown bears are close relatives of modern ABC brown bears

All analysed brown bears excavated from caves in SE Alaska, with radiocarbon dates ranging from ~12.3 to ~7.0 <sup>14</sup>C kyr (calibrated dates ranging from ~14 to ~7.7 cal kyr B.P.), grouped in a strongly supported clade with modern brown bears from the Alexander Archipelago (Figure 5a; see Figures S4–S7 for the complete tree from data sets 513 and 485). Furthermore, eight samples, dated

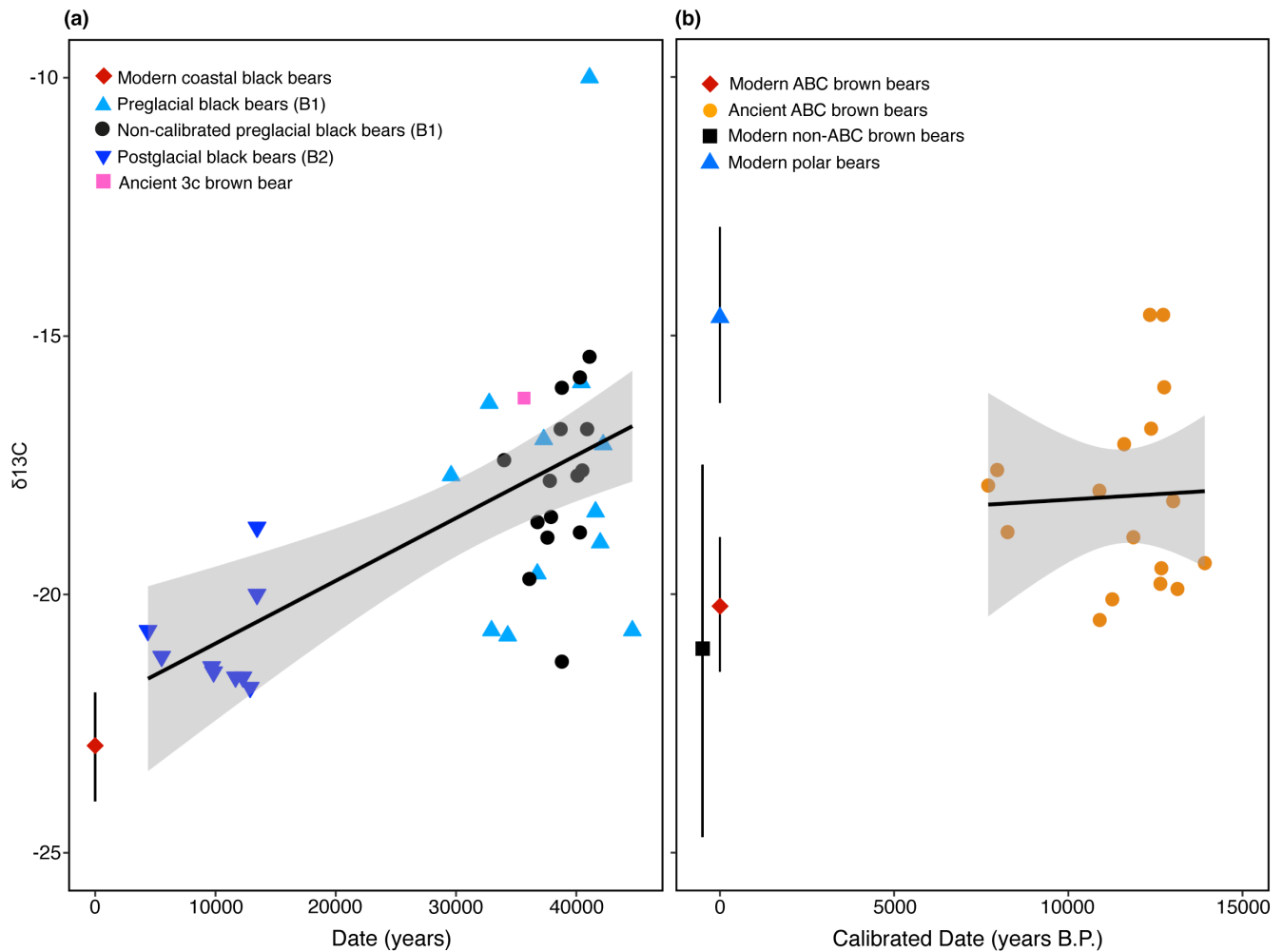
14.7–12.7 kyr B.P., of brown bears from Haida Gwaii, an archipelago off the northern Pacific coast of Canada just south of the Alexander Archipelago (Figure 1a), also grouped in this clade. Hence, all brown bears with an ABC haplotype are postglacial and the last record of an ABC haplotype bear in the southern islands of the archipelago is BC-00351, dated ~7.7 cal kyr B.P. (Table S1). A bear, purportedly recovered from Engineer Creek Mine near Fairbanks, Alaska, dated to ~23 cal kyr B.P. (Salis et al., 2021), was confirmed as sister to all other brown bears with the ABC haplotype, from both SE Alaska and Haida Gwaii. Based on Bayesian phylogenetic analysis of data set 513 (Figure 5a), brown bears with an ABC haplotype form two supported subclades, the first encompassing modern bears from Admiralty Island, ancient bears from Haida Gwaii, and one ancient bear from SE Alaska (UAMES 53069). The second clade encompasses modern bears (Baranof and Chichagof) and ancient bears from SE Alaska. Divergence date estimates based on data set 513 (see Figure S9 for the complete tree from data set 485) place a last common ancestor of the bear sample from Interior Alaska and the ABC haplotype clade before the LGM at ~61 cal kyr B.P. (95% HPD 50–72 cal kyr B.P.), during the MIS 4. The two subclades (Haida Gwaii/Admiralty bears and Baranof/Chichagof/ancient bears) shared a last common ancestor ~41.5 cal kyr B.P. (95% HPD 35–49 cal kyr B.P.), during MIS 3. The split between those groups is not recovered in any maximum-likelihood analyses. The haplotype network shows that ancient SE Alaska brown bear samples are placed between modern bears from Admiralty and Baranof/Chichagof, respectively (Figure 5b). Modern bears from Baranof and Chichagof islands, although only separated from the ancient samples by a single mutation, branched out of a cluster formed by ancient bears of different ages, 14–11.1 cal kyr B.P.

### 3.4 | Stable isotopes ( $\delta^{13}\text{C}$ ) show a shift in diet after the last glacial maximum

The preglacial American black bears from the extinct clade B1 display a range of stable isotope  $\delta^{13}\text{C}$  values from -10‰ to -20.8‰ (Figure 6a), with a mean  $\delta^{13}\text{C}$  equal to -17.35‰ and an average of 49% of marine carbon contribution. On the contrary, the postglacial black bears exhibit a range from -18.7‰ to -23.2‰ with a mean  $\delta^{13}\text{C}$  equal to -21.2‰ (Figure 6a). The preglacial brown bear had a  $\delta^{13}\text{C}$  value equal to -16.2‰, similar to black bears that inhabited the archipelago before the LGM. Ancient postglacial brown bears with an ABC haplotype had a range of -14.6‰ to -21.5‰, with a  $\delta^{13}\text{C}$  mean of -18.18‰, which represents ~52% of marine carbon contribution on average (Figure 6b).

## 4 | DISCUSSION

Recent studies based on cosmogenic exposure dating have demonstrated that areas in SE Alaska above sea level today were covered in ice during the local LGM (20–17 ka) and deglaciation began between ~16.3 to 15.1 ka along the coast (Lesnek et al., 2018, 2020; Walcott



**FIGURE 6** Trends in bear stable isotope  $\delta^{13}\text{C}$  values over time. (a) Stable isotope values from pre- and postglacial American black bears and one preglacial brown bear (with a 3c haplotype; see Figure 5). The vertical black line indicates the range of  $\delta^{13}\text{C}$  values in modern black bears (see Methods for references). The x-axis for the 'non-calibrated preglacial black bears' group (black circles) is in  $^{14}\text{C}$  years; for all other groups, it is in calibrated years before present (B.P.). (b) Stable isotope values from postglacial ABC brown bears. Vertical black lines indicate ranges of  $\delta^{13}\text{C}$  values among modern ABC brown bears, non-ABC brown bears and polar bears (see Section 2 for references). The black regression lines and 95% confidence intervals shaded in grey indicate the trend over time based on ancient samples in both plots. ABC, Admiralty, Baranof and Chichagof, also referred to as 'ABC brown bears'.

et al., 2022). Nevertheless, due to considerable sea-level changes in the region (Baichtal et al., 2021; Bard et al., 1990; Shugar et al., 2014), areas that may now be submerged could have served as Pleistocene coastal refugia (Lesnek et al., 2020; Walcott et al., 2022), which could help explain the high level of endemism in the archipelago (MacDonald & Cook, 2007). For example, native bears in the region today appear to represent populations distinct from the mainland, suggesting long-term isolation in the archipelago (Byun et al., 1997; Davison et al., 2011; MacDonald & Cook, 2007; Puckett et al., 2015; Talbot & Shields, 1996; Wooding & Ward, 1997). Therefore, they represent an obvious system within which to investigate whether they are descendants of bears that survived in the region during the LGM or bears that recolonized the area when deglaciation began. Our results presented here from analyses of mitochondrial genomes of numerous ancient and modern bears suggest that bears did not reside in the Alexander Archipelago during the local LGM. Instead,

preglacial populations appear to have become extirpated, and the archipelago was recolonized by postglacial bears when conditions were again favourable. The earliest bear record after the LGM is an ABC brown bear dated to ~14 cal kyr B.P. The earliest black bear record comes from the same cave, dating ~700 years later than the first brown bear record. It is likely that brown bears colonized the Canadian Haida Gwaii archipelago (Figure 1a), prior to migrating to SE Alaska, perhaps as early as ~17 cal kyr B.P. (a single specimen that has only been assessed morphologically), or at least after ~14.7 until 12.7 cal kyr B.P. (Fedje et al., 2021; Salis et al., 2021). Since then, there are no records of brown bears in Haida Gwaii. The first black bear record in the Canadian archipelago dates back to ~13.3 cal kyr B.P. (Fedje et al., 2021). It is noteworthy that both bear species must have survived in the Alexander Archipelago during the Younger Dryas, a cooling period from ~12.8 to 11.5 ka where temperatures dropped close to glacial temperatures (Kaufman et al., 2016). The

presence of both bear species, about 2300 years after the onset of deglaciation (Lesnek et al., 2018, 2020; Walcott et al., 2022), suggests that deglaciation happened relatively fast. Vegetation must have quickly expanded allowing at least the southern islands of the Alexander Archipelago (south of Frederick Sound) to support two large mammal species by 14 ka.

#### 4.1 | Genetic data suggest that black bears did not survive in Southeast Alaska during the LGM

At least two distinct American black bear haplogroups inhabited the Alexander Archipelago in different time periods over the last ~45,000 years, one extinct that may have inhabited the area only before the LGM and a second one that apparently recolonized the area once the Cordilleran ice sheet retreated. The presence of a now-extinct subclade composed of ancient preglacial coastal bears that range in ages from ~44.5 to 30 cal kyr B.P. suggests that considerable local extinctions happened during black bear evolution and that the LGM may have been a strong driver of such extinctions. Considerable extinction of brown bear genetic haplotype diversity has previously been reported (Barnes et al., 2002), but until now this has not been demonstrated for American black bears. The deep split of the two coastal black bear haplogroups, ~119 cal kyr B.P., and the finding that preglacial and postglacial bears are exclusively found in one of these subclades, suggest that black bears did not reside in the Alexander Archipelago during the LGM. Instead, they recolonized the area after the LGM, likely from refugia along the Pacific coast or from refugia now submerged (Puckett et al., 2015; Walcott et al., 2022). It has been hypothesized that due to their limited geographic distribution and genetic distinctness, coastal black bears may have been isolated in the Pacific Northwest for most of the species' evolution and inhabited a coastal refugium during the LGM (Pedersen et al., 2021; Puckett et al., 2015). Even though the location of the northwest refugium is unknown, Puckett et al. (2015) suggested that it could have been located on the Northwestern Pacific Coast, south of the Cordilleran ice sheet, instead of the coastal regions of Alaska and British Columbia. Once deglaciation began, black bears could have moved northwards along the coast, reaching SE Alaska by ~13.3 cal kyr B.P. However, it is important to note that despite the deep divergence in time and 62 mutations, the two American black bear coastal subclades are sister groups. Coupled with an incomplete fossil record and lower sea levels during the LGM, we cannot ignore a less supported alternative hypothesis that preglacial black bears indeed did survive in now-submerged refugia in the archipelago, followed by range expansion once deglaciation began. The observed haplotype turnover could have been a consequence of population size reduction due to the harsh conditions during the peak of the glaciation that associated with genetic drift may have caused a loss of one of the haplotype lineages entirely. Alternatively, a small bear population could have remained in the archipelago but was quickly replaced (either by genetic drift or competition) by the postglacial mitochondrial lineage as new bear

populations colonized once the ice retreated. Nevertheless, for either scenario, the two haplogroups remain unsampled among both pre- and postglacial bears.

#### 4.2 | Brown bears inhabited the southern islands in the Alexander Archipelago before and after the LGM

While brown bears with the distinct ABC haplotype today inhabit the northern islands of the Alexander Archipelago only, modern brown bears that inhabit the SE Alaska mainland carry the distantly related 3b haplotype (Davison et al., 2011). Based on the modern distribution of brown bears in the archipelago, Klein (1965) hypothesized that the species never colonized the southern islands. This hypothesis was refuted when brown bear subfossils were recovered from the southern islands (Heaton et al., 1996), although it was not known whether those ancient brown bears were related to modern ABC bears or bears from the mainland. We recovered one brown bear from the On Your Knees cave on Prince of Wales Island that dates to ~35 cal kyr B.P. This ancient bear is sister to clade 3c, a haplotype that now appears to be extinct but used to inhabit interior Alaska and Yukon Territory between ~50 and ~33 cal kyr B.P. (Barnes et al., 2002). This is the first genetically confirmed record of a brown bear from the Alexander Archipelago that does not carry the ABC haplotype. The 3c clade is positioned as a sister clade to clades 3a and 3b, which today are present in Eurasia and northern North America. It has been reported that clade 3c bears migrated to North America during the marine isotope stage (MIS) 4 (71–57 ka; Salis et al., 2021), although our data support a hypothesis that this migration happened earlier, and perhaps as early as 150–120 ka, during the MIS 6 (Illinoian Glaciation; Dyke et al., 2004), or at the beginning of MIS 5 (Eemian interglacial period; Dyke et al., 2004). During the Pleistocene glacial periods, the Beringian land bridge that extended from the Lena River Valley in Siberia to the Yukon Territory in Canada was exposed, facilitating migrations between Eurasia and North America (Elias et al., 1996; Jakobsson et al., 2017). Hence, it is likely that migration of clade 3c brown bears to North America happened during MIS 6.

The origin of ABC brown bears is still unclear. To date, the only preglacial brown bear from the Alexander Archipelago represents a distantly related lineage, so a postglacial replacement of haplotype in the archipelago seems likely. Previous studies failed to determine when bears representing the ABC haplotype clade migrated to North America, or if this distinct haplotype evolved in the New World (Davison et al., 2011; Salis et al., 2021). One proposed hypothesis concerning their origin has been referred to as the 'population conversion theory', which places a brown bear-polar bear hybrid origin of ABC brown bears to after the LGM (Cahill et al., 2013). Recently, however, genome sequence analyses, including from an ancient polar bear, and statistical fitting of data to alternative admixture graph scenarios instead favoured an ancient introgression from brown bears into the ancestor of polar bears, possibly dating



back over 150,000 years (Lan et al., 2022; Miller et al., 2012), although the issue of gene flow, and its timing and direction, between the two species continues to be debated (de Jong et al., 2023; Wang et al., 2022). It is possible that the ABC haplotype may once have represented a geographically more widespread lineage, also occurring in the more southernly Haida Gwaii archipelago at least from around 14.7 until 12.7 cal kyr B.P. as well as interior Alaska around 23 cal kyr B.P. (Salis et al., 2021). The bear specimen dated to 23 cal kyr B.P. could be the first record of the ABC haplotype in interior Alaska, although its provenance has been questioned (Barnes et al., 2002; Salis et al., 2021). Nevertheless, our analyses suggest an origin of the Alexander Archipelago/Haida Gwaii haplotype clade ~42 cal kyr B.P., significantly prior to the local LGM, and that the last common ancestor of the broader ABC clade, including the bear remains from interior Alaska, lived ~61 cal kyr B.P. Hence, our results do not support a hypothesis that brown bears occupied the region during the LGM. Instead, they are consistent with a formerly more widespread ABC brown bear maternal lineage and that the ancestors of postglacial Alexander Archipelago brown bears recolonized the region following the LGM glacial retreat, likely from ice-free Beringia. Similar to the black bears, however, alternative scenarios cannot be ruled out, including unsampled haplotypes due to an incomplete fossil record.

### 4.3 | Other species occupied the Alexander Archipelago during the bear hiatus

During the local LGM (20–17 ka) in the Alexander Archipelago, there is a remarkable gap in subfossil ages that likely reflects complete ice cover of the On Your Knees Cave entrance (Heaton & Grady, 2003; Lesnek et al., 2018). Although the gap in ages of subfossil bears excavated from caves in the region span a longer period, from ~30 to 14 cal kyr B.P., the presence of other species in the fossil record during this time indicates that an incomplete fossil record is not the main reason for the hiatus of bear subfossils. For example, Arctic species, such as arctic fox and ringed seal, occupied the region, suggesting that ice was present in the region. Foxes only disappeared from the cave fossil record at ~22 cal kyr B.P., while seals persisted until ~20 cal kyr B.P. Near the end of the LGM, the first species in the fossil record were ringed seals at ~17 cal kyr B.P., suggesting the persistence of sea ice until then, followed by foxes that reemerged around the same time as brown bears (Heaton & Grady, 2003).

### 4.4 | Southeast Alaska was mostly ice-free when postglacial bears recolonized the area

Stable isotope signatures can provide insights into paleodiets. Because marine sources have a higher concentration of  $\delta^{13}\text{C}$ , stable isotope data will show the relative contributions of marine and terrestrial carbon sources to diet (Fry & Sherr, 1989; Tafuri et al., 2009). Using a model with cut-offs for different diets, bones with  $\delta^{13}\text{C}$  values higher than  $-14.5\text{‰}$  may reflect a diet with a contribution consisting of

largely marine carbon, whereas values between  $-21\text{‰}$  and  $-14.5\text{‰}$  indicate a mixed contribution of marine and terrestrial diet, and values lower than  $-21\text{‰}$  indicate a fully terrestrial diet (Fedje et al., 2021). With a mean  $\delta^{13}\text{C}$  value of  $-17.35\text{‰}$ , preglacial American black bears in the Alexander Archipelago likely had diets consisting of a mix of marine and terrestrial sources (~49% of marine carbon), while postglacial black bears had a mean  $\delta^{13}\text{C}$  equal to  $-21.2\text{‰}$ , indicating a largely terrestrial diet, more similar to modern American black bears, which have values below  $-20\text{‰}$  (Hobson et al., 2000). One outlier sample (UAMES 53107, a preglacial bear that was morphologically identified as brown bear due to its size) had a  $\delta^{13}\text{C}$  equal to  $-10\text{‰}$ , comparable to marine mammals, including polar bears, suggesting that some bears may have lived entirely on a marine diet. It is also possible, however, that this value is erroneous, and a new stable isotope analysis is warranted. The markedly different trends in  $\delta^{13}\text{C}$  values between the pre- and postglacial black bears indicate that black bears shifted their diet from a mixed diet to one dominated by terrestrial sources. Such shift may have been caused by changing environmental conditions, with black bears living during the Wisconsin Glaciation having less access to terrestrial sources as the climate was cooling. By the time postglacial bears recolonized SE Alaska, the region largely ice-free (Lesnek et al., 2020), they likely had higher availability of terrestrial sources than before the LGM.

A shift in diet and environment may also explain the apparent shift in size observed among the black bear subfossils. Even though modern black bears are generally smaller than brown bears, it has been reported that Late Pleistocene bears of both species were often larger when compared to modern and Holocene bears (Döppes & Pacher, 2014; Graham, 1991; Kosintsev et al., 2022; Marciszak et al., 2015, 2019; Wolverton, 2008; Wolverton & Lyman, 1998). Half of the preglacial black bears analysed for this study were initially morphologically identified as brown bears mainly due to their larger size. Nevertheless, genetic analyses confirmed their identity as black bears. Based on the subfossil record, it is challenging to properly differentiate a black bear from a brown bear as the key characteristics to differentiate the bears to species often are missing in subfossils, and size is not reliable when analysing ancient bears (Gordon, 1977; Graham, 1991).

Three ancient ABC brown bears dated ~12.7 to ~12.3 cal kyr B.P. had  $\delta^{13}\text{C}$  values as high as polar bears, suggesting a fully marine diet, while some of the oldest ABC bears have values comparable to modern ABC bears, implying a more mixed diet. In other cases, contributions of marine carbon were as low as 20%. This indicates that the earliest ABC bears had a highly variable diet with better access to or higher consumption of marine food sources, compared with today's population.

### 4.5 | Ecological and climate change may have caused extirpation of brown bears from the southern islands of the Alexander Archipelago

The last record of an ABC brown bear in the southern islands of the archipelago dates to ~7.7 cal kyr B.P. Today, brown bears in the archipelago are mainly known from the northern islands of



Admiralty, Baranof and Chichagof. Due to an incomplete fossil record, it is possible that ABC brown bears inhabited the southern part of the archipelago until more recent times. Presence of brown bears has been reported on Mitkof Island (south of Frederick Sound and close to the mainland), but there appears to be no established population (Harper & McCarthy, 2013; Klein, 1965; MacDonald & Cook, 1996). These records may represent recent migrants from the mainland, although no genetic data are available to affirm this. In any case, the reason for the extirpation of brown bears from the southern islands is unknown. The fossil record indicates that a dense forest habitat developed in the region during the Holocene, while the northern islands may have presented more open habitats, which is more favourable for brown bears (Heaton & Grady, 2003; MacDonald & Cook, 1996; Wooding & Ward, 1997). The forest appearance may also have been a reason for the extirpation of brown bears in Haida Gwaii (Fedje et al., 2021). After the LGM deglaciation, sea levels in the region varied and at time surpassed the modern sea level. During the early Holocene, sea levels on Prince of Wales Island were higher when compared to the northern islands (Baichtal et al., 2021). Furthermore, temperatures in the mid-Holocene decreased resulting in an abrupt cooling event ~8.2 ka (Rohling & Pälike, 2005). The sea-level changes associated with a more forested environment and colder temperatures could have contributed to the local extinction of brown bears on the southern islands.

## 5 | CONCLUSIONS

Our findings presented here are consistent with the absence or only limited presence of ice-free refugia along the extant SE Alaska coast during the LGM, at least for mammal species that require a large home range. We have no evidence that bears inhabiting the Alexander Archipelago today survived in the region during the LGM. However, given the rapid sea-level rise following the glacial retreat in SE Alaska, it is possible that refugia were available in areas of the now-submerged continental shelf (Baichtal et al., 2021; Walcott et al., 2022) and that smaller species with small home ranges, such as Pacific marten (Colella et al., 2021), could have survived in Alexander Archipelago refugia during the LGM. Nonetheless, evidence indicates that the peak of the local LGM was relatively short-lived along the coast, where deglaciation happened rapidly, and the vegetation quickly expanded once conditions were more favourable allowing bears to recolonize the area at least by ~2300 years after deglaciation began.

Given the limitations of analyses based solely on maternally inherited mitochondrial DNA (Ballard & Whitlock, 2004), future whole genome studies of coastal American black and brown bears subfossils from SE Alaska will provide better insights into the evolutionary history of these distinct lineages in the region. Furthermore, additional subfossil radiocarbon dating and genetic analyses, also of other and smaller mammals, may help to more fully assess the impact of climate change and refugial survival along the Pacific coast during the late Pleistocene.

## AUTHOR CONTRIBUTIONS

Charlotte Lindqvist and Sandra L. Talbot designed the study; Flavio Augusto da Silva Coelho, Stephanie Gill, Crystal M. Tomlin, Marilena Papavassiliou, Sarah A. Sonsthagen, Sean D. Farley, Joseph A. Cook, George K. Sage, Timothy H. Heaton and Sandra L. Talbot provided samples and/or generated the mitogenome sequence data; Flavio Augusto da Silva Coelho and Charlotte Lindqvist analysed the data; Timothy H. Heaton provided the cave and paleontological context; Flavio Augusto da Silva Coelho and Charlotte Lindqvist wrote the manuscript with contributions from all authors.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The mitochondrial genome sequences generated in this study are deposited in the NCBI GenBank database with Accession no. OQ318898 to OQ318974, OQ333075 to OQ333090, and OQ439773 to OQ439778. Data sets and untrimmed sequence alignment of all samples are available on Dryad Digital Repository (<https://doi.org/10.5061/dryad.brw15dvdk>).

## BENEFIT-SHARING STATEMENTS

Benefits from this research accrue from the sharing of our data and results on public databases as described above.

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