Ancient dog mitochondrial lineages indigenous to North America recovered from Jamestown Colony

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Key findings

- This is the earliest known example of maternal indigenous dog lineages recovered from dog remains at a European colonial site.
- Phylogenetic analysis places the dogs from Jamestown with other ancient North American dog lineages of the Midwest.

Introduction

Dogs of the Americas share a unique genetic history with humans. Humans introduced domesticated dogs to the Americas from Asia approximately 17-13 kya (Ni Leathlobhair *et al.* 2018), concurrent with the first migrations of humans to North America (Moreno-Mayar *et al.* 2018). Though subsequent introductions of dogs occurred in the far north, maternal lineages derived from the first dogs spread throughout the Americas until European contact in the late 15th century. The European colonization of the Americas drastically impacted the demography of indigenous dogs. Multiple studies have demonstrated that mitochondrial lineages of European dogs almost completely replaced indigenous ones during the ongoing process of European colonization (Castroviejo-Fisher *et al.* 2011, Leonard 2002, van Asch *et al.* 2013). However, it is unclear when and how rapidly this replacement occurred.

Other major historical events in recent centuries have also substantially altered global dog diversity and variation. Modern dog breeding, which arose near the beginnings of European colonialism, intensified artificial selection (Jansson and Laikre 2018). Large conflicts, most notably World War I and World War II, had profound effects for many dog populations, with some breeds reaching extremely low population numbers (Howell 2013), and many European breeds had to be reconstituted due to loss of mating control. Thus contemporary European dog breeds only represent a fraction of the total dog diversity that existed 500 years ago. To further explore the complicated genetic history of European and North American dogs and reveal the diversity of dogs before the effects of colonization and 20th century conflicts, we extracted mitochondrial DNA from cranial elements recovered from the earliest permanent English colony in the Americas, Jamestown.

Methods

Extractions and ancient DNA pipeline

Six premolars underwent ancient DNA extraction and library preparation protocols as outlined in Cui *et al.* (2013) at a dedicated ancient DNA facility at the Carl R. Woese Institute for Genomic Biology at the University of Illinois Urbana-Champaign. Five of six samples were sequenced using the NovaSeq$^{(TM)}$ 6000 platform. Read files were run through Schubert *et al.*'s (2014) PALEOMIX pipeline and mapped to the dog mitochondrial genome (NC_002008.4) using BWA-MEM. Reads with Phred quality scores less than 30 were filtered out. Consensus sequences were generated using a Perl script (Bergey 2018).

Phylogenies

Jamestown sequences were combined with publicly available modern and ancient canid sequences including coyotes and wolves. Sequences (n = 1380) were aligned using MUSCLE (Edgar 2004) and manually curated. Neighbor-joining trees were created using PAUP* (Swofford 2003) with red fox (*Vulpes vulpes*) as the outgroup. A Bayesian phylogeny was constructed using BEAST 1.10.4 (Suchard *et al.* 2018) with a strict clock informed by tip dates, a Bayesian skyline plot demographic model, and a GTR substitution model. Multiple MCMC chains were run for 250 million generations and inspected for convergence in Tracer v1.7 (Rambaut *et al.* 2018).

Results

Of the five samples submitted for sequencing, only two [JR118236 (1607-1610) and JR68100 (1617-1624)] had enough quality reads to reconstruct the mitochondrial genome. The Jamestown sequences clustered with other ancient North American dog sequences from previously published works (Ameen *et al.* 2019 and Ni Leathlobhair *et al.* 2018) in both the neighbor-joining tree and the Bayesian phylogeny. In these analyses, they form a clade with dogs from the Janey B. Goode, Angel Mounds, and Scioto Cavern sites in the Midwest.

Zooarchaeological analysis identified cutmarks on the cranial elements of all dogs except one. Most of the cutmarks were located on the lateral surface of the maxilla, posterior to the infraorbital foramen, and superior to fourth premolar. Cutmarks were also found along the medial surface of the mandible inferior to the first molar. Cutmarks were relatively shallow and narrow.

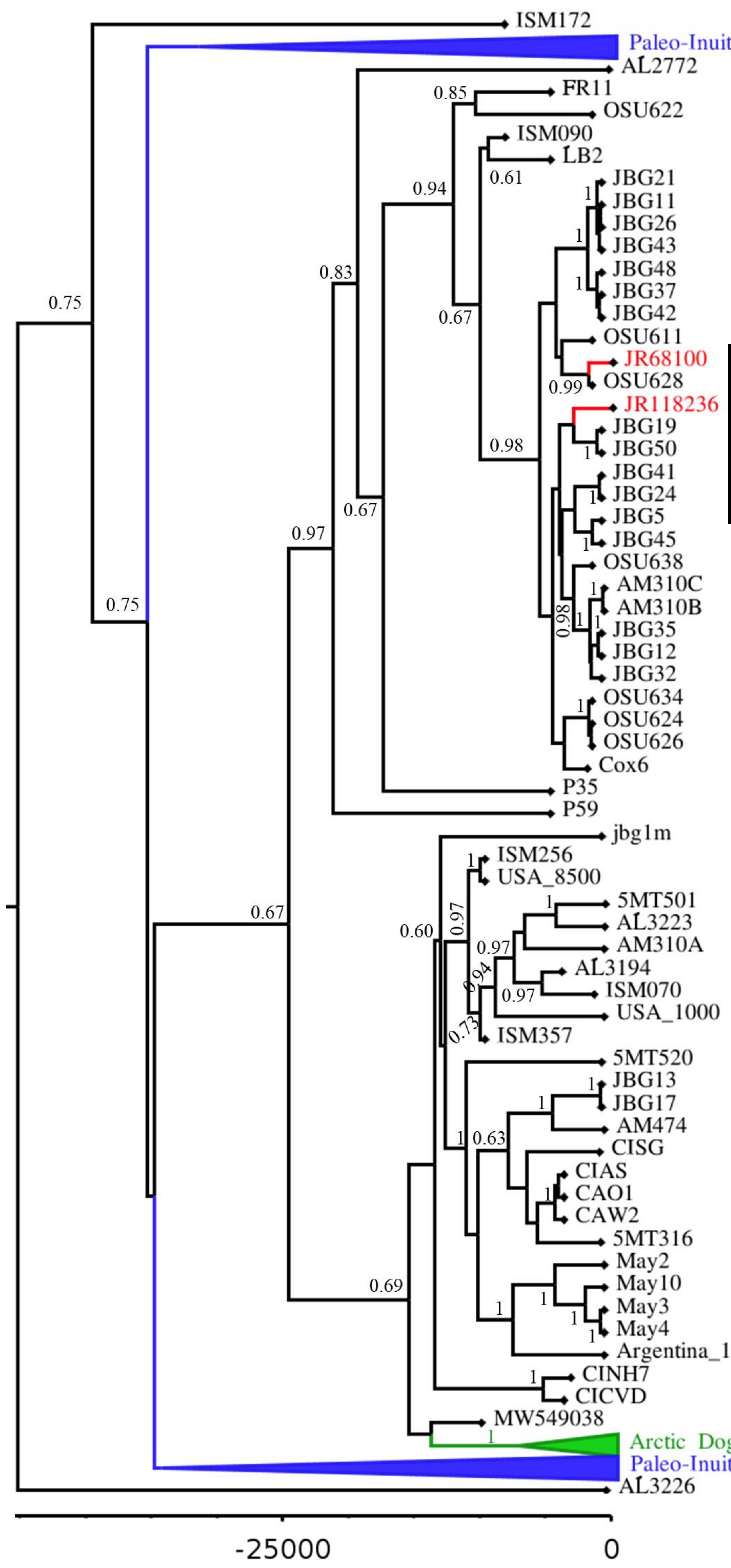

Figure 1: Bayesian phylogeny of North American dogs. Tree was generated using a sample of ancient dog mitochondrial sequences (n = 173). Jamestown dogs are labelled in red, Inuit (Thule) dogs are colored blue, and dogs from sites in Greenland, Canada, and Alaska without a known cultural context are shown in green. Posterior probabilities greater than 60% are shown.

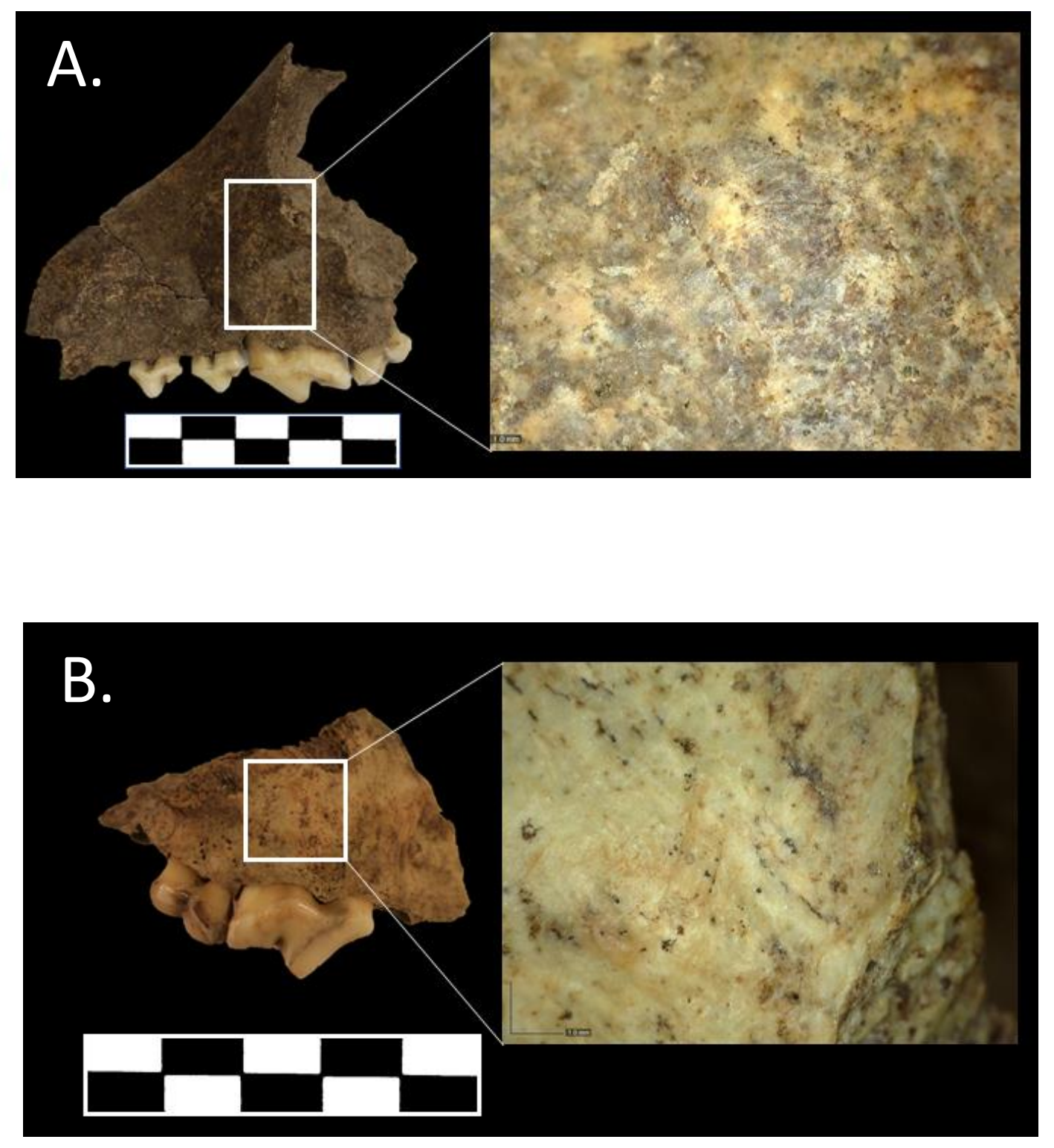

Figure 2: Cutmarks on dog elements.
A. Image of JR68100 cranial fragment and magnified image of two parallel cutmarks.
B. Image of JR118236 and magnified image of cutmarks on the lateral portion of maxilla bone.

Conclusions

Our analysis suggests that Jamestown dogs carried mitochondrial lineages derived from the first population of dogs introduced during the first peopling of the Americas. The Jamestown sequences cluster with sequences from Scioto Cavern dogs that date to 200 BC - 500 AD, dogs from Angel Mounds (12VG1) dated between 1050 – 1450 AD, and dogs from the Janey B. Goode site (11S1232), which was occupied between 650 and 1400 AD. Interestingly, the large clade containing the Jamestown dogs and pre-contact mid-Atlantic and Midwest dogs is a sister to a small clade that includes a mitogenome of an ancient Alaskan dog. We included dogs from Thule archaeological sites in our analysis, and it is clear that the indigenous mitochondrial lineages of the Jamestown dogs were distantly related to those of Thule dogs, which likely arrived in the Americas around 1000 AD (Tackney *et al.* 2019; Ni Leathlobhair *et al.* 2018). The Jamestown dogs thus belong to a very successful maternal lineage of dogs with a geographic distribution extending from Alaska through the mid-Atlantic until the time of European contact and colonization.

Notably, the mitochondrial lineages recovered from the Jamestown dogs were not European. This is not entirely unexpected based on historical accounts (Haile 1998) and archaeological data (Kelso and Straube 2012) that indicate a degree of exchange and interaction between indigenous Algonquian-speakers with European colonists at Jamestown. However, the nature of the interactions between indigenous and European populations is unknown, as is the role of dogs in any interactions or exchange. The skeletal elements analyzed here were found to have cutmarks on them, and the archaeological context is consistent with these dogs having been eaten for food by humans. One of the dogs (JR118236) is associated with the Starving Time (the winter of 1609-1610), which was a period of resource scarcity that forced residents to eat other animals not typically consumed as food and, in some cases, humans (Kelso and Straube 2012). Dog remains from layer JR2361C, however, likely represent continued reliance on Indigenous dogs for a decade or more after the starving time. Notably, the colony suffered many periods of potential collapse after its initial establishment because of failure to generate reliable food sources. Though our analysis cannot determine if these dogs were a common food source for either indigenous or European individuals at Jamestown, it was not unusual for indigenous North Americans (Tito *et al.* 2011) or European colonists (Schwartz 1997) to use dog meat as a protein source during periods of stress.

Further analysis is required to determine the full ancestry of the Jamestown dogs, as these dogs may either represent an early admixed population or be of entirely indigenous dog ancestry. Sequencing of the nuclear genome is necessary to accurately infer the full ancestry of these dogs and provide a window into dog diversity in North America (and possibly Europe) circa 1600 AD. Genomic analysis of these unique 17th century samples would shed light on the ways in which dogs were involved in the interactions between indigenous populations and early Europeans.

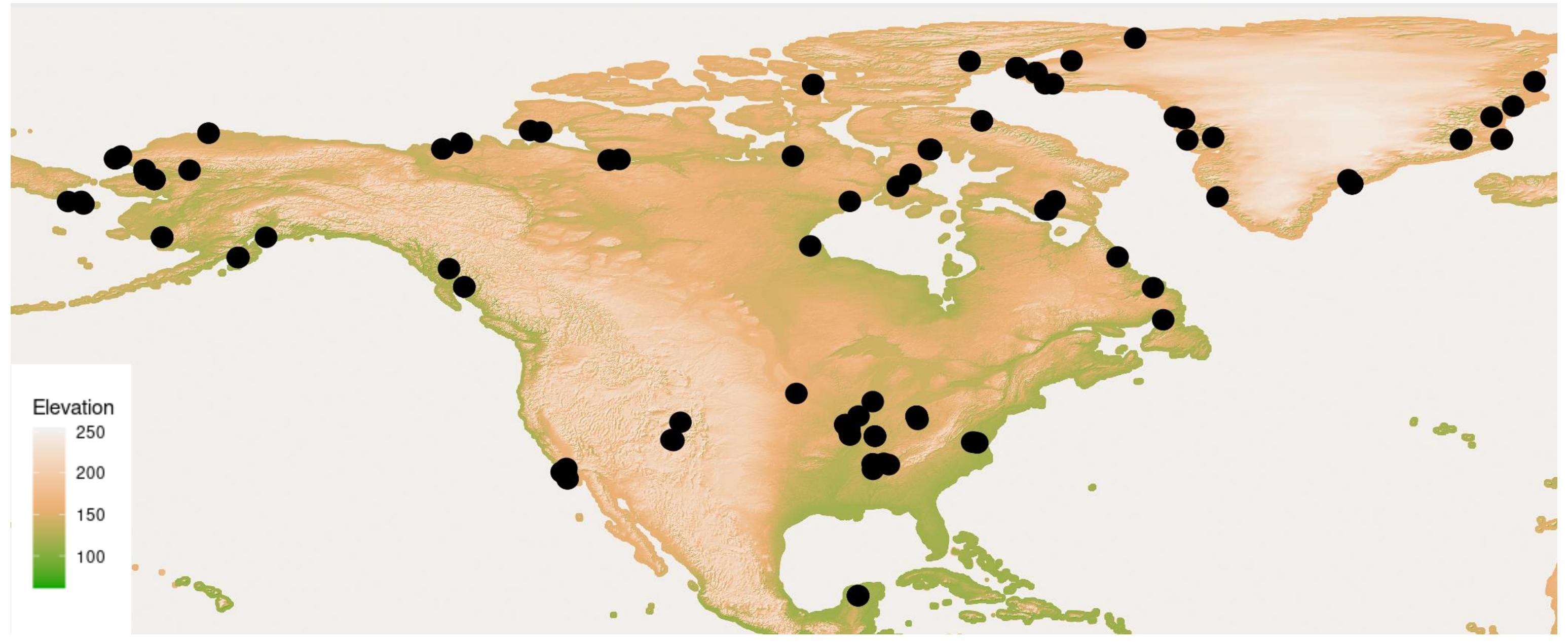

Figure 3: Locations of ancient North American dog sequences used in the phylogeny. Samples were collected from previously published studies. Black dots represent the geographic locations of 159 ancient dog samples for which latitude and longitude data were available.

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References: