Received: 4 November 2022 | Revised: 13 February 2023 | Accepted: 22 February 2023

DOI: 10.1002/ajpa.24727

RESEARCH ARTICLE

AMERICAN JOURNAL OF BIOLOGICAL ANTHROPOLOGY
The Official Journal of the American Association of Biological Anthropologists
WILEY

Ancient mitogenomes from the Southern Pampas of Argentina reflect local differentiation and limited extra-regional linkages after rapid initial colonization

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Funding information
Agencia Nacional de Promoción Científica y Tecnológica, Grant/Award Numbers: PICT 0381, PICT 2015-1405, PICT 2020-3937; Consejo Nacional de Investigaciones Científicas y Técnicas, Grant/Award Numbers: 2021-11220200103037CO, BecExt 2017, PIP 2015-11220150100953CO, PUE 2016 IDACOR; Howard Hughes Medical Institute; National Geographic Society; Universidad Nacional del Sur, Grant/Award Number: CECYT-UNS 24/I266

Abstract

Objective: This study aims to contribute to the recovery of Indigenous evolutionary history in the Southern Pampas region of Argentina through an analysis of ancient complete mitochondrial genomes.

Materials and Methods: We generated DNA data for nine complete mitogenomes from the Southern Pampas, dated to between 2531 and 723 cal BP. In combination with previously published ancient mitogenomes from the region and from throughout South America, we documented instances of extra-regional lineage-sharing, and estimated coalescent ages for local lineages using a Bayesian method with tip calibrations in a phylogenetic analysis.

Results: We identified a novel mitochondrial haplogroup, B2b16, and two recently defined haplogroups, A2ay and B2ak1, as well as three local haplotypes within founder haplogroups C1b and C1d. We detected lineage-sharing with ancient and contemporary individuals from Central Argentina, but not with ancient or contemporary

samples from North Patagonian or Littoral regions of Argentina, despite archeological evidence of cultural interactions with the latter regions. The estimated coalescent age of these shared lineages is ~10,000 years BP.

Discussion: The history of the human populations in the Southern Pampas is temporally deep, exhibiting long-term continuity of mitogenome lineages. Additionally, the identification of highly localized mtDNA clades accords with a model of relatively rapid initial colonization of South America by Indigenous communities, followed by more local patterns of limited gene flow and genetic drift in various South American regions, including the Pampas.

KEYWORDS

ancient DNA, mitochondrial DNA, native Americans, Pampas region, Southern Cone

1 | INTRODUCTION

The first studies of complete mitochondrial genomes (mitogenomes) from the Americas carried out in the late 2000s greatly contributed to our understanding of peopling processes of the two continents, North and South America (e.g., Fagundes et al., 2008; Perego et al., 2010; Tamm et al., 2007). Today, a little more than a decade later, we are better positioned to address finer-scale questions of regional and temporal population dynamics given the higher number of available mitogenomes (e.g., Barbieri et al., 2017; Bodner et al., 2012; Delgado Rodríguez, Kassadjikova, & Fehren-Schmitz, 2021; de Saint Pierre et al., 2012a; Figueiro et al., 2022; García et al., 2021; Gómez-Carballa et al., 2018; Roca-Rada et al., 2021; Simão et al., 2019), most of which have been sampled from contemporary populations.

Unfortunately, there are geographic regions in the Americas where it is less possible to evaluate pre-European contact population genetics using contemporary data due to genocidal practices and heavy-handed relocations of Indigenous peoples by European colonizers (Livi-Bacci, 2006). Moreover, the massive inflow of peoples from the Eurasian and African continents over the past five centuries has blurred pre-contact patterns of genetic diversity in the Americas generally (Crawford, 2001). For example, the Pampas region of Argentina, our area of study, was preferentially settled by West Eurasian immigrants from the mid-19th century until the first decades of the 20th century (Lattes, 1973), and continues to draw people from all over Argentina and from border nations (Busso, 2007; Calvelo, 2012; Rosso, 2018). Therefore, to best explore pre-European contact genetic diversity in the populations of the Pampas region, it is necessary to analyze ancient DNA (aDNA) from bioarchaeological samples (e.g., Llamas et al., 2016; Nakatsuka et al. 2020; Posth et al. 2018; Roca-Rada et al., 2021).

Here we present complete mitochondrial genomes of nine ancient individuals from the southern portion of the Pampas dating to the Late Holocene (~3500–500 BP). We contextualize our results within extant archeological and ethnohistorical records to shed light on the human occupational dynamics of the region. Moreover, because the Southern Pampas region of Argentina harbors some of the earliest archeological sites in the Americas, this study simultaneously seeks to further our understanding of initial human colonization and subsequent occupation dynamics of the South American continent more broadly.

2 | BACKGROUND

The Pampas region of Central-Eastern Argentina forms part of South America's "Southern Cone," the latter comprising the modern-day territories of Uruguay, Chile, Argentina, and Southern Brazil. The Pampas region is divided into two ecoregions, the Dry Pampas and the Humid Pampas (Figure 1). This study's focus is on the southern portion of the Humid Pampas, known as the Southern Pampas.

The Southern Pampas shows some of the earliest evidence of human occupation in South America, starting at ~14,000 cal BP at the site of Arroyo Seco 2 (Politis, Gutiérrez, Rafuse, & Blasi, 2016; Prates, Politis, & Steele, 2013). However, human presence in the region during the first millennium of occupation seems to have been minimal given the scarcity of archeological sites thus far found (Prates, Politis, & Perez, 2020). Although taphonomic processes could have affected preservation of earlier sites, it is notable that during the subsequent Pleistocene/Holocene transition (~13,000–11,000 BP), the number of reported archeological sites is much higher, suggesting that human presence in terms of sheer numbers of people was correspondingly higher. The archeological sites during this transition period are characterized by the appearance of fishtail projectile points, and by lithic raw material sourced both locally and from the territory now comprising Uruguay, with the latter phenomenon suggestive of extensive exchange networks (Flegenheimer, Bayón, Valente, Baeza, & Femenías, 2003). In addition, cultural bonds with North Patagonia were established by this point, as evidenced by similarities in lithic technology and landscape use patterns across these two regions (Flegenheimer, Miotti, & Mazzia, 2014).

The archeological record of the Early Holocene (~11,700–8200 BP) is sparser than that of the earlier Pleistocene/Holocene transition, though the oldest ancient human remains in the Southern Pampas come from this period (Flegenheimer et al., 2010; Politis, Gutiérrez, &

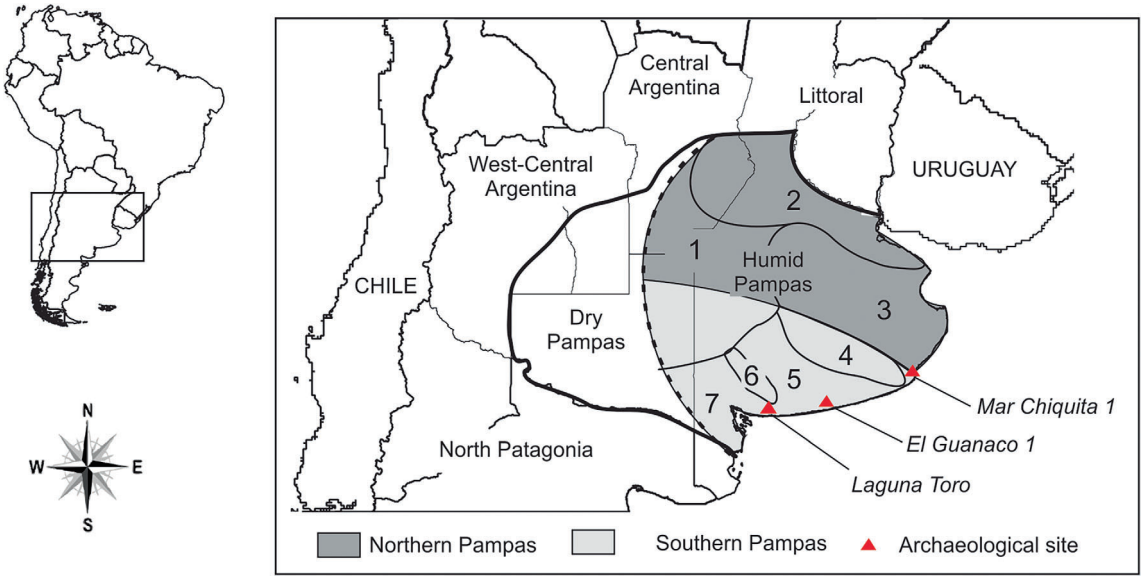

FIGURE 1 Map showing the featured archeological sites with human remains, the eco-regional subdivisions of the Pampas (modified from Politis and Madrid, 2001), and surrounding regions and territories. 1—Northwest Pampas. 2—Northern Pampas. 3—Depressed Pampas. 4—Tandilia. 5—Interserrana. 6—Ventania. 7—Southwest Pampas.

Scabuzzo, 2014). During the Middle Holocene (~8200–3500 BP), a new type of triangular, stemless lithic point appeared in this region (Escola, 2014), as well as throughout other regions in the Southern Cone. This period is also characterized by a decrease in occupation intensity as evidenced by the low number of archeological sites (Martínez et al. 2015). Barrientos and Pérez (2005) initially proposed that the Southern Pampas experienced population discontinuity—even population replacement—during the Middle Holocene, noting both the relative absence of archeological sites and significant morphological change in recovered crania. This proposition has since been countered by Martínez et al. (2015) who demonstrate that there are, in fact, several more archeological sites than were previously known. Still, the Middle Holocene harbors fewer sites than the previous time period, a phenomenon they argue can best be explained by changes in settlement patterns and taphonomic biases. Also, Politis (2008), based on the continuity in technology, burial places, and use of space, supports a model of population continuity.

During the Late Holocene (~3,500–500 BP), the population density and complexity of Pampas groups increased, as inferred from the introduction and development of new technologies (Politis, 2008; Politis & Madrid, 2001). The archeological record shows the presence of lithic raw materials originating in Tandilia, an area within the Southern Pampas (see Figure 1), in several archeological sites from the surrounding regions of North Patagonia (Martínez, 2008), the Dry Pampas (Berón, 2007), and the northern portion of the Humid Pampas (Acosta, Escudero, Terzaghi, Loponte, & Jimeno, 2010; Gonzalez, Frère, & Fiore, 2007; Heider, 2016), suggesting continual interactions among neighboring populations. In particular, Southern Pampas and North Patagonian populations shared common cultural practices such as secondary burials (Mazzia et al., 2004; Martínez, Flensborg, Bayala, & López, 2007), morphological patterns of cranial deformation (Serna et al., 2019), etching patterns on ceramic plates (Crivelli Montero, Eugenio, Pardiñas, & Silveira, 1997; Oliva & Algrain, 2004), and rock art designs (Madrid, Politis, & Poiré, 2000; Mazzanti, 2006). Other archeological evidence indicates that people from the Southern Pampas interacted with those in the Littoral region, as evidenced by the presence of corn scraps (Mazzanti, 2006) and similar decoration motifs in ceramic assemblages (Mazzanti, 2006). Also during this period, macro-regional social links and long-range exchange networks expanded, as evidenced by the novel presence of exotic elements. These include necklace beads made of "roca verde," or copper oxidation minerals from the South-Central Andes (Vecchi, 2021).

Initial Spanish contact and colonial era ethnographic information on Pampas groups is scarce. A few written documents from the 16th and 17th centuries note the presence of Indigenous ethnic communities in the region and give them the geographic ascription of "Pampas" (Nacuzzi, 1998). According to Casamiquela (1965), those groups were northern Tehuelche, an ethnic group belonging to the Chon linguistic family that also occupied the Patagonian region (discussed in Nacuzzi, 1998). Even though European settlers did not consolidate control over the Southern Pampas until the 19th century, changes triggered by Spanish colonization in the 16th century, such as the introduction of horses and cattle, had already generated consequential changes in mobility and in demographic conformation of the region's Indigenous groups.

Later ethnohistoric records from the 18th and 19th centuries recount the presence of highly mobile groups who were ethnically Mapuche; European colonizers named them "Araucanos" after the Araucanía region in Chile. Araucano groups occupied a vast area, ranging from the trans-Andean region in what is now Chile, to the southern Sierras Pampeanas in Central Argentina and North Patagonia. Some ethnohistorians and archeologists have hypothesized that

the Araucanian presence in the Pampas, a phenomenon referred to as the "Araucanization of the Pampas," is relatively recent, and that their initial migration and subsequent large-scale movements effected a replacement of the region's prior residents. Ethnohistorians have further debated about whether this so-called Araucanization process began prior to, or as a consequence of, Spanish conquest, and if it involved a replacement of a prior group, whether that group was ethnically Tehuelche (Nacuzzi, 1998; Mandrini & Ortelli, 2002; Villar & Otero, 2012).

In sum, the early known archeological record of up to the Late Holocene—covering more than 10,000 years—tells a long-term story of relatively sparse human occupation of the Southern Pampas, with somewhat fluctuating population sizes that some have interpreted as evidence of discontinuity during the Middle Holocene. The later archeological record of the Late Holocene of the Southern Pampas and its regional surroundings is much more intensive, indicating higher numbers of individuals who are interacting locally and extra-regionally, at least at the level of cultural exchange.

What complementary information does genomic evidence provide to help us better understand the nature and extent of group interactions, both within and outside of the Southern Pampas? Genomic analyses of the broader Pampas region, whether autosomal or mitochondrial, are in their infancy: a 2018 analysis based on whole genome data proposed an overall degree of genetic continuity in the Southern Cone since about 8,600 cal BP, which is the date of the most ancient Pampas sample thus far analyzed (Posth et al., 2018). And, a more recent analysis by Roca-Rada et al. (2021) based on mitogenomes from the Early and Middle Holocene in the Pampas region reveals the continuous presence of lineages D1j and D1g, which are widespread in both ancient and present-day populations throughout the Southern Cone, as well as the presence of other novel mitogenomes that could not be related to any modern or ancient subclades. These analyses have certainly contributed broad spatio-temporal information on the Southern Cone, but could not resolve local genetic relationships given the lack of recent mitogenomes from the region.

We undertook a mitogenomic analysis of nine ancient individuals from the Late Holocene Southern Pampas to gain additional insights into the region's human occupation, with the goal of also informing larger questions on the early peopling of the continent. Though we cast a wide net by exploring mitogenomic relationships with both proximal and distant groups within and outside of the territory of Argentina, we also specifically ask: (1) Is there evidence of genetic temporal continuity in the Southern Pampas? (2) Is there evidence of gene flow with proximate or distant regions, regardless of whether complementary archeological evidence for such interactions exists?

3 | SUBJECTS AND METHODS

3.1 | Archeological sites and samples

A total of nine individuals from three Late Holocene archeological sites of the Southern Pampas, in Buenos Aires province, Argentina, were analyzed in this work. The dates of these sites range from 2531 to 793 cal BP (Table 1 and Figure 1).

The Mar Chiquita 1 site (37°42′ S 57°23′ W) is in the southern limit of the Depressed Pampas, located in the county of Mar Chiquita. The site is adjacent to the Atlantic coast in a sector that was formed about 2200 to 3000 years ago by sedimentary accumulation produced by littoral drift (Violante, Parker, & Cavallotto, 2001). An increase in coastal erosion in recent years (Merlotto & Bértola, 2009) resulted in the exposure of archeological remains, including the human remains studied here. The site yielded human remains of three individuals: one adult male (66JM) dated to 793 years cal BP, one sub-adult female (67JM), and another indeterminate sub-adult that was not analyzed in this work (Aldazabal, Eugenio, & García Laborde, 2020). Along with these individuals, three artifacts were found, two produced from coastal pebbles and another from guanaco (*Lama guanicoe*) bone.

The El Guanaco 1 site (38°41′ S 59°39′ W) is in the Interserrana sub-region, 13 km away from the Atlantic coast. The site is at a low elevation mound and about 100 m from the northeastern margin of El Lucero lake. The lithic artifacts found on the site were made from a wide variety of raw materials with diverse origins (i.e., from the nearby coast, and the areas of Tandilia and Ventania). Based on this diversity and the amount of the lithic material transported, Bayón and Flegenheimer (1998) characterized the site as a preferred landscape that was repeatedly occupied. The first human remains found at the site correspond to two individuals who were recovered in a salvage operation, which consist of a primary burial of an adult individual (68JM) and an infant of about 4 years of age (69JM) (Flegenheimer et al., 2002). In subsequent excavations, another primary burial of an adult individual (70JM) and a secondary burial consisting of at least three individuals, dated to between 2240 and 2531 years cal BP, were recovered (Mazzia, Scabuzzo, & Guichón, 2004). The former two burials correspond to juvenile individuals (71JM and 72JM); the latter adult burial remains unanalyzed. Stable carbon isotope analyses indicated a preponderant consumption of terrestrial resources (Flegenheimer et al., 2002).

The Laguna Toro site (38°50′ S 61°25′ W) is located in the Southwest area of the Humid Pampas, about 12 km from the coast and near the shallow lake of the same name. Two adult individuals from this site were analyzed. One of them was found as scattered and poorly preserved skeletal remains associated with a few lithic artifacts (74JM), while a second individual (75JM), dated to 2414 years cal BP, was found as a primary burial in a north–south orientation. For this individual, both genomic (discussed later) and isotopic data are published, with the latter indicating a diet preponderant with terrestrial resources (Nakatsuka, et al., 2020a; Scabuzzo, Frontini, Vecchi, & Bayón, 2016). Ceramic fragments, malacological remains, and a substantial collection of lithic artifacts, composed of projectile points, scrapers, pecking/grinding tools (mills, mortars, pestles), as well as cores and flakes, were also found at the site.

This work with ancient human remains has been carried out following the guidelines and recommendations of the Argentine Association of Biological Anthropology (Asociación de Antropología Biológica Argentina, 2007). Indigenous communities have not claimed descendancy nor reclaimed remains as of yet. Teeth were exported

TABLE 1 List of ancient samples from the Southern Pampas analyzed in this study, including associated metadata, radiocarbon date, and mitochondrial haplogroup.

Sample ID	Archeological site	Archeological ID	Morphological sex	Tooth type	Calibrated radiocarbon age with a 95.4% probability in years BP (conventional radiocarbon age, lab code)	Average of 95.4% CI date range in cal BP (present defined as 1950 CE)	Haplogroup	GenBank ID
66JM	Mar Chiquita 1	MCh_ind 1	Male	Molar	906–680 cal BP (910 ± 50 BP, LP-3256)	793	C1b	OP605616
67JM	Mar Chiquita 1	MCh_ind 2	Female	Molar	906–680 cal BP (associated with 66JM)	793	B2b16	OP605617
68JM	El Guanaco 1	EG_ind 1	Male	Molar	2712–2349 cal BP (2470 ± 60 BP, BETA-128180)	2531	C1b	OP605618
69JM	El Guanaco 1	EG_ind 2	Indeterminate	Premolar	2340–2139 cal BP (2280 ± 30 BP, BETA-137745)	2240	B2ak1	OP605619
70JM	El Guanaco 1	EG_ind 3 (Skull 154)	Male	Canine	2712–2345 cal BP (2460 ± 60 BP, PTA-8520)	2529	B2b16	OP605620
71JM	El Guanaco 1	EG_ind 6 (Skull 294)	Female	Molar	2712–2139 cal BP (associated with 68JM, 69JM, and 70JM)	2426	B2b16	OP605621
72JM	El Guanaco 1	EG_ind 5 (Skull 195)	Male	Molar	2712–2139 cal BP (associated with 68JM, 69JM, and 70JM)	2426	B2ak1	OP605622
74JM	Laguna Toro	LT_ind 1	Male	Molar	2685–2143 cal BP (associated with 75JM)	2414	C1d + 194	OP605623
75JM	Laguna Toro	LT_ind 2	Female	Molar	2685–2143 cal BP (2369 ± 52, UGAMS-16601)	2414	A2ay	OP605624

for DNA analysis with corresponding permissions from the Dirección Provincial de Museos y Preservación Provincial de la Provincia de Buenos Aires, and the Instituto Nacional de Antropología y Pensamiento Latinoamericano of Argentina.

3.2 | Ancient DNA extraction, mitochondrial capture, and next-generation sequencing

Tooth samples underwent surface decontamination using a household bleach solution (6% sodium hypochlorite) with a subsequent rinse with ddH_2O. Whole teeth were then pulverized using a SPEX SamplePrep 6770 Freezer/Mill in dedicated clean rooms at the University of Tennessee, Knoxville (UTK). Ancient DNA extracts were obtained from the tooth powder and further processed both at UTK and at Harvard Medical School (HMS), performing different procedures at each location with the same batch of pulverized samples. At UTK, aDNA was extracted from 250 mg of powder following Dabney et al. (2013). Illumina aDNA libraries were prepared using the NEBNext Ultra II DNA Library Prep kit (New England Biolabs) according to manufacturer's instructions. Mitochondrial DNA was enriched by targeted capture through in-solution hybridization using the myBaits kit (Daicel Arbor Biosciences, Ann Arbor, USA), which is based on the methodology developed by Gnirke et al. (2009). The enriched

products were sequenced on an Illumina MiSeq sequencer at the UTK Next-Gen Sequencing Core Facility using v.2150 cycle kits for 2 × 76 cycles. At HMS, aDNA extraction from 75 mg of tooth powder and double-stranded Illumina sequencing libraries were performed with an automated liquid handler using silica-coated magnetic beads (Rohland, Glocke, Aximu-Petri, & Meyer, 2018). aDNA extracts were pretreated with the enzyme uracil-DNA glycosylase (UDG) to minimize analytical artifacts caused by cytosine-to-thymine substitutions present at the ends of aDNA molecules (Rohland, Harney, Mallick, Nordenfelt, & Reich, 2015). Libraries were enriched for fragments that overlapped mtDNA following Maricic, Whitten, & Pääbo (2010), and were sequenced on an Illumina NextSeq500 using v.2150 cycle kits for 2 × 76 cycles. Dental material from all nine ancient individuals screened in this study yielded useful mitochondrial DNA data in both laboratories.

3.3 | Sequence data processing

The raw sequence data generated at UTK was processed with the AdapterRemoval program (Lindgreen, 2012) to merge paired reads, remove adapters, and filter low quality reads. FASTQ files were aligned to the revised Cambridge Reference Sequence (rCRS; Andrews et al. 1999) using the BWA algorithm (Li & Durbin, 2009), and Samtools 1.10 (Li, 2011) was used to remove duplicates and filter Q30 quality. Damage patterns were assessed using MapDamage (Jónsson, Ginolhac, Schubert, Johnson, & Orlando, 2013).

For HMS raw sequence data, the procedures described in Nakatsuka et al. (2020a) were followed. Briefly, paired forward and reverse reads that overlapped by at least 15 nucleotides were merged using custom software (https://github.com/DReichLab/ADNA-Tools), restricting to merged sequences of at least 30 base pairs. FASTQ files were aligned to the mitochondrial reference sequence (RSRS, Behar et al., 2012) using BWA (version 0.7.15-r1140). Two nucleotides from the end of each sequence were removed, and damage patterns were assessed using MapDamage (Jónsson et al. 2013).

The same downstream analysis was conducted with BAM files obtained from both labs. The percentage of coverage of the mitochondrial sequence and the average number of times that each nucleotide was read were obtained with the Qualimap software (Konstantin et al., 2015). For mtDNA haplogroup estimation, variant call format files (VCFs) were generated using Samtools 1.10 and processed through HaploGrep 2 (Weissensteiner et al., 2016) for PhyloTree Build 17 (van Oven & Kayser, 2009) to assign haplogroups.

3.4 | Comparative mitochondrial DNA databases

To conduct our analyses, we used three comparative databases of published human mtDNA sequences, maintained in-house, that include (1) 2672 contemporary and 160 ancient mitogenomes from throughout the Americas (Table S1a), (2) 5348 complete contemporary and ancient control region (hypervariable regions [HV] I and II) sequences from South America (Table S1b), and (3) 2640 contemporary and ancient HVI sequences from South America (Tables S1c). We used the latter two databases to manually screen for diagnostic polymorphisms to supplement the currently limited nature of the extant mitogenome database from South America.

3.5 | Phylogeny estimation

Phylogenetic trees were estimated from the obtained complete mitogenomes by incorporating all closely related, published mitogenomes from South America (Table S2). We built maximum parsimony trees, one for each of the relevant subhaplogroups within A2, B2, C1b, and C1d using mtPhyl v 4.015 (2013; https://sites.google.com/site/mtphyl/home). The trees were hand edited with reference to PhyloTree Build 17 (van Oven & Kaiser, 2009) and previously published trees in García et al. (2021).

3.6 | Coalescence age estimation

Coalescence times for founding clades A2 and B2, and the three local clades B2b16, B2ak, and pre-A2ay, as well as the subclades within these, were estimated using a Bayesian approach with tip dates calibration applying BEAST v1.10.4 (Drummond & Rambaut, 2007). The analysis included the nine newly generated mitogenomes together with all previously published mitogenomes that were used for phylogenetic analysis of the branches of interest ($N = 16$), plus a representative sample of contemporary and ancient Indigenous mitogenomes from South America ($n = 1022$) (updated from García et al., 2021). This set of mitogenomes was chosen to perform computationally feasible Bayesian analyses on a genetically and geographically diverse dataset. Multiple sequence alignment of the data set was constructed with the online service of MAFFT (Katoh, Rozewicki, & Yamada, 2019) using the progressive FFT-NS-2 setting. We trimmed the alignments by excluding hypervariable position 16519 and length variations at positions 303–315, 514–525, and 16181–16193. BEAST was run on the resulting 1047 aligned mitogenomes under the HKY evolution model. We used the radiocarbon dates of ancient samples as calibration points, given that tip calibration has been shown to yield more consistent results than node dating (Rieux et al., 2014). The substitution rate reported in Soares et al. (2009) was used as a prior parameter. A strict clock model was applied taking into account the nature of human mtDNA evolution, as similar generation times and DNA repair mechanisms are expected (Ho & Duchêne, 2014). Three independent MCMC chains were run over 100 million states, each with a sampling frequency of 10,000. We then used LogCombiner v1.10 in BEAST (Drummond & Rambaut, 2007) to combine the log files from the three independent runs with a 10% burn-in fraction. Finally, Tracer v1.7.2 (Rambaut et al., 2018) was used to ensure convergence of the MCMC chains and an Estimated Sample Size (ESS).

4 | RESULTS

Complete mitochondrial genomes were successfully recovered from the nine sets of individual remains. Because we typed these mitogenomes using independent aDNA extractions with differing protocols at different laboratories, we were able to obtain complete genomes with ~100% coverage, even for those with low coverage sequence reads (e.g., 71JM, 72JM, and 74JM). We were also able to independently corroborate all polymorphic site assignments. Average coverage depth was 59.3× (9.8×–125.5×). Diagnostic damage patterns indicate recovery of endogenous aDNA from the nine archeological samples. Table S3 reports coverage/depth statistics and damage scores for each sample. All newly generated mitogenomes are available in GenBank (accession numbers OP605616-OP605624).

4.1 | Haplogroups, haplotypes, and estimated phylogenies

The nine novel Southern Pampas mitogenomes comprise eight distinct haplotypes, five of which correspond to haplogroup B2, two to haplogroup C1b, and one each to haplogroups A2 and C1d. From the continental dataset of 2832 mitogenomes, we considered the sequences most closely related to our newly genotyped samples, and used them to reconstruct the phylogenetic tree shown in Figure 2; near matches from HVI and HVII sequences are not included.

Three samples (66JM, 68JM, and 74JM; #s 24, 23, and 25, respectively, in Figure 2) represent unique mitogenome sequences without meaningful matches to our comparative databases, although near-matches for 66JM were found via manual screening. The derived mitochondrial control region (CR) motif C1b$_{+16192-16291-16362}$ was found in two contemporary sequences from West-Central Argentina, both carrying an extra transition at 16144 (Motti, 2012), as well as CR motif C1b$_{+16192-16291}$ was found in one contemporary Uruguayan HVI sequence (Sans et al., 2021).

Sample 75JM (# 1) belongs to the recently described A2ay clade, characterized by diagnostic variants 64-152-7049-16192 (García et al., 2021). The motif A2$_{+64-152-16192}$ is shared with a mid-Holocene Pampean mitogenome (# 6, B2S19; Llamas et al., 2016), although the absence of the polymorphism at nucleotide position 7049 in B2S19 indicates that the latter is derived from a pre-A2ay clade. The A2ay diagnostic CR sites are found in other publications of partial sequences: the A2$_{+152-16192}$ motif has been described in seven modern samples from the Argentine cities of Córdoba and Buenos Aires (Catelli et al., 2011), in five samples from the Chilean central-southern regions of Biobío, Araucanía, and Los Lagos (Gómez-Carballa et al., 2016), and in one Argentine Mapuche (de Saint Pierre et al., 2012b), while the A2$_{+16192}$ motif is present in HVI sequences of ancient samples from North Patagonia (Crespo, Russo, Hajduk, Lanata, & Dejean, 2017) and Córdoba province (Fabra et al., 2022; Nores et al., 2022).

We identified a new clade within B2b, named B2b16, that is characterized by the presence of a transition at position 3316. This clade comprises four ancient samples: two from the El Guanaco 1 site (70JM and 71JM; #s 20 and 21, respectively), another from the Mar Chiquita 1 site (67JM; # 19), and one mitogenome from the Northwest Pampas in Buenos Aires province (# 22, LCH.E2-I2.1; Roca-Rada et al. 2021). The B2$_{+16173}$ CR motif is also found in contemporary individuals from Córdoba province in Central Argentina (Salas, Jaime, Álvarez-Iglesias, & Carracedo, 2008), Peru (Cabana et al., 2014), Bolivia (Bert, Corella, Gené, Pérez-Pérez, & Turbón, 2004; Gayà-Vidal et al., 2011), and Chile

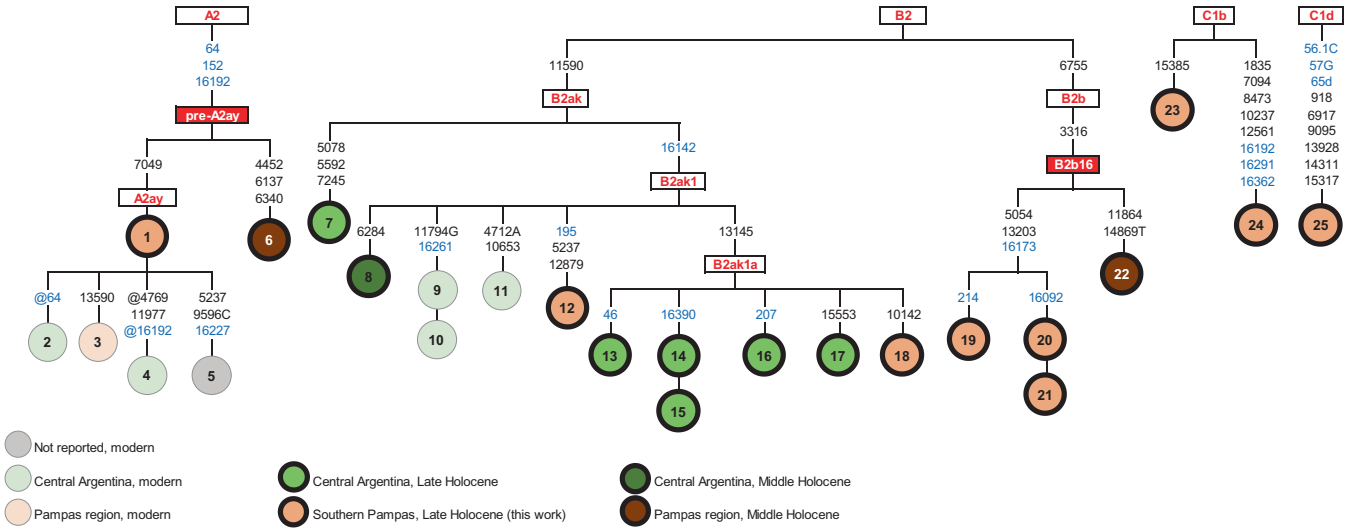

FIGURE 2 Estimated phylogenies, including the nine ancient Southern Pampas mitogenomes together with their most closely related mitogenomes from our South American dataset. Numbers correspond to single sequences discussed in the text and to references in Table S2. Different colors correspond to different geographic regions at different time periods. Circles with bold outlines represent ancient samples. Clades B2b16 and pre-A2ay, indicated by red-filled rectangles, are defined for the first time in this study, whereas white-filled rectangles represent previously described clades. Polymorphisms are shown on each branch; they are transitions unless a specific polymorphism is otherwise indicated. The @ prefix indicates a back mutation. CR mutations are in blue font.

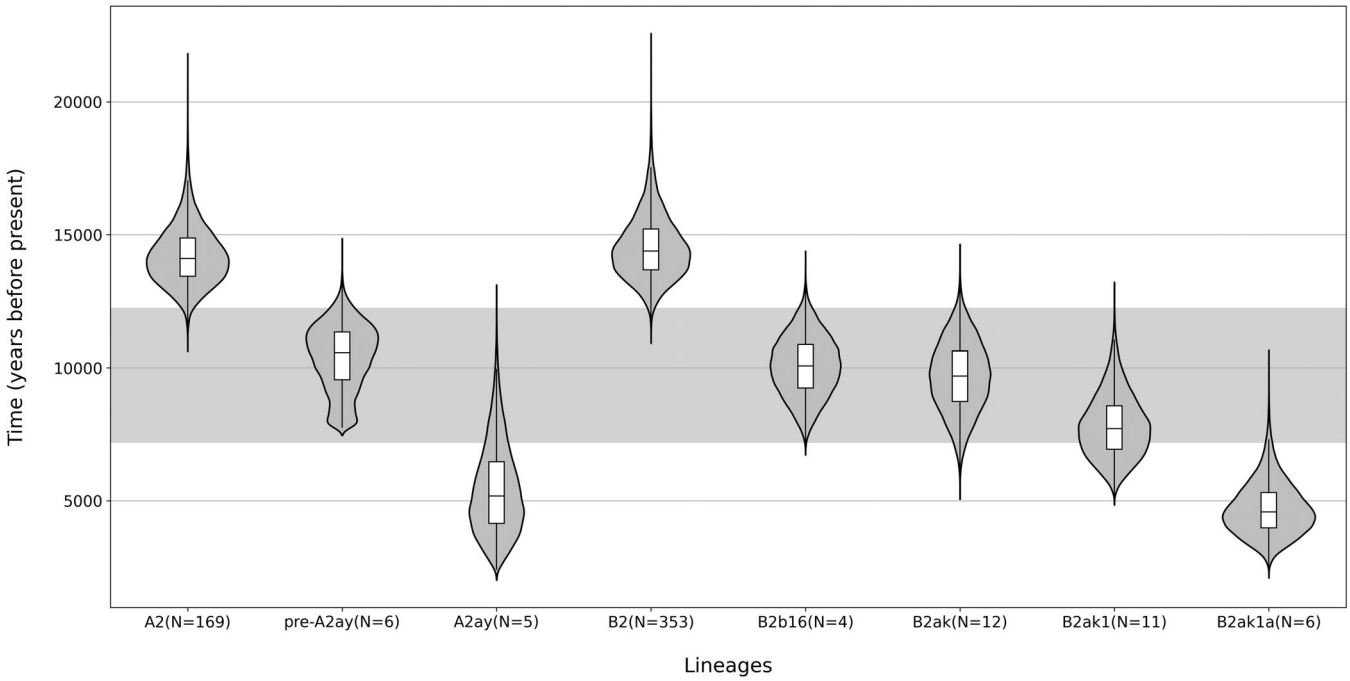

FIGURE 3 TMRCA for clades discussed in the text. The gray bar delineates the 95% Highest Posterior Density of Bayesian estimates of the three major regional clades, pre-A2y, B2b16, and B2ak.

(Gómez-Carballa et al., 2016). El Guanaco 1 samples 70JM and 71JM shared the same exact haplotype, indicating a matrilineal link, though they were not part of the same burial. Meanwhile, two other individuals from El Guanaco 1 (71JM and 72JM; # 21 and 12) that were interred together have different mitochondrial haplotypes. Future autosomal analyses may reveal extra-mitochondrial genetic kinship among these samples.

Two mitogenomes (69JM and 72JM; #s 18 and 12) belong to the newly described clade B2ak1 (García et al., 2021). All B2ak mitogenomes known so far derive from contemporary and ancient samples from Central Argentina (García et al., 2021). CR sequences assignable to the nested branch B2ak1 ($B2_{+16142}$) suggest that this lineage attains its highest frequency and internal branching diversity in Central (García, Pauro, Bailliet, Bravi, & Demarchi, 2018) and West-Central Argentina (Motti, 2012), and that it has wider distribution into Northwest and Northeast Argentina, and in Chile (Bobillo et al., 2010; de Saint Pierre et al., 2012b, Gómez-Carballa et al., 2016; Sanabria et al., 2019). Lineage $B2_{+16142}$ has also been reported in one of 19 individuals from the Northwest Argentina archeological site of Pampa Grande, dated to ~1300 BP (Carnese et al., 2010).

4.2 | Coalescent age estimates

Coalescent estimates for clades pre-A2ay, B2b16, and B2ak, and their subclades, varied between 4698 (B2ak1a) and 10,394 (pre-A2ay) years BP. In all cases, the ESS were above 200 (between 941 and 4381; Table S4). Interestingly, these three major local clades show similar coalescent ages, at around 10,000 BP (Figure 3). Our time to the most recent common ancestor (TMCRA) estimates for major haplogroups A2 and B2 is ~14,000 BP, which falls within the range of previous molecular date estimates (e.g., Llamas et al., 2016).

4.3 | Mitochondrial lineage continuity within the Pampas

We detected signals of genetic continuity between Middle and Late Holocene groups in the Pampas, reflected in the shared presence of the B2b16 clade in one individual from the site of Mar Chiquita 1 (793 cal BP), two individuals from the El Guanaco 1 site (2529 cal BP), and one individual from the Laguna Chica site in the Northwest Pampas (6880 cal BP) (Figure 2: haplotypes #s 19, 20, 21 and 22). Taken together, these linkages account for more than 6000 years of regional biological continuity. Additionally, pre-A2ay may be shared between a sample from the Laguna Toro site (2414 cal BP) and one individual from the Arroyo Seco 2 site in the Interserrana area (7435 cal BP) (Figure 2: haplotypes #s 1 and 6). Since this similarity is based only on CR polymorphisms, and not on a diagnostic polymorphism at position 7049, this assessment is provisional.

4.4 | Inter-regional mitochondrial lineage sharing

4.4.1 | Northern Patagonia, Argentina

No matrilineal lineages were definitively shared between the nine ancient mitogenomes and contemporary North Patagonian CR

haplotypes. Otherwise, we would have expected to find any of three predominant contemporary North Patagonian subhaplogroups—D1g, B2i2, and C1b13—currently found in about 80% of sampled Mapuche, Pehuenche, and Huilliche communities (de Saint Pierre et al., 2012b) to also be present in the Pampas region.

We did identify a possible match, however. A comparison of our ancient mitogenomes with ancient mtDNA CR data from North Patagonia reveals one possible shared subhaplogroup ($A2_{+16192}$) that is present in a sample from the North Patagonian Atlantic coast (site of Centro Minero-Criadero) dated to ~600 cal BP (Crespo et al., 2017).

4.4.2 | Central and South Patagonia, Argentina

We found no shared matrilineal lineages between the ancient mitogenomes analyzed here and published mitochondrial data for contemporary or ancient individuals from Central and South Patagonia (i.e., Arencibia et al., 2019; de la Fuente et al., 2015; de Saint Pierre et al. 2012a; Motti et al., 2019; Parolín et al., 2019; Tamburrini et al., 2022).

4.4.3 | Littoral region of Argentina, Uruguay, and Brazil

We failed to identify shared matrilineal lineages between the study samples and any mitogenome from eastern South America (see Table S1 for references). Only one published contemporary Uruguayan haplotype shares two CR polymorphisms with 66JM (# 24).

4.4.4 | Central region of Argentina

We identified two shared clades, B2ak1 and A2ay, between three ancient Southern Pampas individuals, on the one hand, and ancient and modern samples from Central Argentina (García et al., 2021), on the other. Also, an individual dated to ~4100 cal BP from Córdoba province shares the HVI $A2_{+16192}$ motif, although it carries an additional mutation at position 16248 (Fabra et al., 2022). Finally, the derived CR motif $C1b_{+16192-16291-16362}$ of 66JM (#24) was found in two complete CR sequences from West-Central Argentina (Motti, 2012).

4.4.5 | Northwest region of Argentina and Central Andes

Despite the high amount of published mitogenomes for western South America, we found no shared clades between the Southern Pampas and the Andean regions. Only the appearance of CR polymorphism 16173 in B2 samples from Peru and Bolivia could represent a trace presence of B2b16. Yet, it should be noted that in the Northwest Region of Argentina, there are mostly contemporary CR haplotypes that belong to B2ak1, with one ancient sample from the Pampa Grande site in Salta province (Carnese et al. 2010).

4.5 | Extra-regional mitochondrial lineage sharing

We found no additional instances of shared matrilineal lineages between the ancient mitogenomes analyzed here and published mitochondrial data for contemporary or ancient individuals outside of the above-listed results.

5 | DISCUSSION

The samples analyzed here represent Indigenous, pre-European contact populations of the Southern Pampas, from throughout different places and ages, but which we consider to have a shared evolutionary trajectory. Indeed, it is noteworthy that even with a relatively small genomic sample, we identified a shared mitochondrial clade among two individuals from the Interserrana area (El Guanaco 1) from the initial Late Holocene, and an individual from the Depressed Pampas (Mar Chiquita 1) from the final Late Holocene. These newly generated mitogenomic data allowed for the exploration of genetic links between groups from the Late Holocene Southern Pampas, and from other times and regions in South America. We begin by discussing our results in light of current archeological interpretations of the occupational and artifactual evidence of the Southern Pampas, and the Pampas region more broadly. We wrap up with broader insights into the early population dynamics of South America.

5.1 | Population continuity in the Southern Pampas, and in the Pampas more generally

The 6000-year record of shared lineages between Middle and Late Holocene groups in the Southern Pampas is consistent with the hypothesis of population continuity in the Pampas based on archeological evidence (Martínez et al., 2015; Politis, 2008), contrasting with Barrientos and Pérez's (2005) alternative hypothesis of extinction and population replacement. However, the possibility of small local population extinctions taking place during the Middle Holocene, followed by occasional repopulation events involving groups of the same metapopulation, as proposed by Barrientos and Pérez (2002), cannot be ruled out with our data.

5.2 | Inter-regional genetic linkages

Interestingly, for those regions for which there is archeological evidence of cultural interactions with groups in the Pampas, including the Southern Pampas, namely North Patagonia and Littoral regions of Argentina, and the territory of Uruguay, we find little compelling evidence of genetic interactions.

To further drive this point home in the case of North Patagonia, for the period of the Late Holocene (~3500–500 BP), the inter-regional haplogroup frequencies are quite distinct between the Pampas and North Patagonian ancient samples thus far analyzed: lineage B2 is

absent while D1 is highly represented (68%) in North Patagonia (Crespo et al., 2017; Postillone, Martínez, Flensborg, & Dejean, 2020), whereas 55% of samples are B2, and D1 is absent among Southern Pampas groups (this work).

Conversely, in the case of Central Argentina, we have a situation in which no similarities in material culture with the Southern Pampas has been described, except for the presence of fishtail lithic points, which are widespread in the Southern Cone. A previous study by our group (García et al., 2021) was unable to establish population genetic linkages between Central Argentina and the Pampas region. These newly generated data, however, did identify genetic linkages via the presence of two clades, B2ak1 and A2ay, that are shared among ancient and modern samples from these regions. Moreover, genomic evidence from Nakatsuka et al. (2020b) demonstrated genetic contributions from the Central Andes in the Pampas region. Given the archeological evidence that supports links between the Andean Northwest and Central Argentina (González, 1960; Rivero, 2012), and the Southern Pampean presence of B2ak1 and A2ay, we preliminarily suggest that this Andean signal is more likely due to gene flow from Central Argentina rather than to direct interaction with Andean groups. However, the presence of Andean ancestry in Central Argentina has to be assessed further to substantiate this hypothesis.

Lastly, we consider our results in light of a long-standing supposition that the Pampas region is said to have been overrun by "Araucanos" (members of the Mapuche ethnic group with origins in Northwest Patagonia) over the course of the 19th and 20th centuries, replacing prior occupants; some hypothesized that these prior occupants were members of the Tehuelche ethnic group (Casamiquela, 1965). We note that no subhaplogroup found among the ancient mitogenomes analyzed here is present among samples of contemporary Tehuelche individuals (de Saint Pierre et al. 2012b), nor among published data of ancient individuals from Central and South Patagonia, which was the historical geographical range of the Tehuelches (Arencibia et al., 2019; de la Fuente et al., 2015; Motti et al., 2019; Parolín et al., 2019). At the same time, however, the ancient mitogenome lineages also do not match with any contemporary Northern Patagonian individual samples, as noted earlier, some of whom belong to the Mapuche ethnic group.

On the one hand, the lack of northern Patagonian mitochondrial clades among the ancient Pampean samples supports the hypothesis of a later population replacement by Mapuche groups. However, our study does not support the hypothesis that earlier individuals were members of the Tehuelche ethnic group. On the other hand, the fact that we do not have genomic information on Mapuche individuals who were living in Buenos Aires province during the 19th century, prevents us from affirming that they were different from the Late Holocene individuals analyzed here. For these reasons, the mitogenomic evidence for a population replacement in the Pampas is, at this point, inconclusive.

Importantly, it must be noted that the nature and distributions of genetic and cultural traits are not always correlated (Cabana & Clark, 2011), viz., the Mapuche ethnic group, so defined by shared language and cultural practices, cannot be defined by a particular set of mitochondrial lineages, because in fact no human group can be defined in such a way, given our extended human history of continuous gene flow (Moore, 1994). It should also go without saying that the absence of evidence of shared lineages between contemporary ethnic groups and ancient samples is not evidence of absence, particularly given the small numbers of ancient samples. Future genomic studies on additional samples may very well produce data establishing clear genetic linkages between past and present communities.

5.3 | Temporal depth of mitogenomic lineages in the Southern Pampas

Coalescent age estimations show three clades (pre-A2ay, B2ak, and B2b16) with similar ages of around ~10,000 years BP. These are younger than D1j and D1g (~15,600 years BP), the other Southern Cone haplogroups with broader dispersion areas (Roca-Rada et al., 2021). This age difference suggests that D1g and D1j would have already been present in the earliest populations that arrived to the Southern Cone, whose archeological signals would presumably be found in those occupations without fishtail lithic points such as the Urupez 2 and K87 sites in Uruguay (Suárez, 2017), Monte Verde in Chile (Dillehay et al., 2015), and Arroyo Seco 2 in Argentina (Prates et al., 2013).

We suggest that a process of regional differentiation might have occurred, accounting for the appearance of subclades with narrower distribution ranges such as pre-A2ay, B2ak, and B2b16.

5.4 | Role of the Southern Pampas in the peopling dynamics of South America

The Southern Pampas region was one of the first regions in South America to be populated during the Pleistocene/Holocene transition, as evidenced in the Arroyo Seco 2 site, dated to ~14,000 cal BP (Prates et al., 2013). The region also features a high density of archeological sites during the early period, between 13,000 and 11,000 cal BP, as compared with other South American regions (Prates et al., 2013).

Given the region's standing as one of the first to be populated, it is interesting that in the Pampas, as in other regions of South America (c.f., de Saint Pierre et al., 2012a; Gómez-Carballa et al., 2018; Motti et al., 2017; Taboada-Etchalar et al., 2013), experienced local differentiation and population continuity with restricted gene flow (Llamas et al., 2016; Reich et al., 2012). In this sense, we propose that the B2b16 subclade, together with the haplotypes $C1b_{+15385}$ and $C1d_{+56.1C-57G-65d-918-6917-9095-13928-14311-15317}$, could represent locally differentiated clades within the region. The results of our study show that, in fact, B2b16 has been present in the region since the Middle Holocene (6880 cal BP; Roca-Rada et al., 2021) up until at least 800 years ago.

6 | CONCLUSIONS

Here we have demonstrated genomic continuity in the Southern Pampas region of Argentina since at least 7000 years ago, indicating that communities in the Pampas did not suffer from extinction, as some have posited. Moreover, we find that rather than being peripheral to Patagonian or Littoral regions of Argentina in terms of settlement and population sizes—a condition leading to a presumption that the region must also have been genomically nondescript or unexceptional—, the Pampas region stands on its own, with a mitogenomic signature that links to regions in Central and West-Central Argentina.

AUTHOR CONTRIBUTIONS

Josefina M. B. Motti: Conceptualization (equal); data curation (supporting); formal analysis (equal); funding acquisition (supporting); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Maia Pauro:** Data curation (lead); formal analysis (equal); writing – original draft (supporting). **Clara Scabuzzo:** Resources (equal); writing – review and editing (equal). **Angelina García:** Data curation (supporting); formal analysis (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal). **Verónica Aldazábal:** Resources (equal); writing – review and editing (supporting). **Rodrigo Vecchi:** Resources (equal); writing – review and editing (supporting). **Cristina Bayón:** Resources (equal); writing – review and editing (supporting). **Nicolás Pastor:** Formal analysis (supporting). **Darío A. Demarchi:** Supervision (supporting); writing – review and editing (equal). **Claudio M. Bravi:** Data curation (supporting); supervision (supporting); writing – review and editing (equal). **David Reich:** Funding acquisition (equal); project administration (supporting); resources (equal); writing – review and editing (supporting). **Graciela S. Cabana:** Funding acquisition (equal); project administration (supporting); resources (equal); writing – original draft (lead); writing – review and editing (lead). **Rodrigo Nores:** Conceptualization (equal); funding acquisition (equal); investigation (lead); project administration (lead); supervision (lead); visualization (supporting); writing – original draft (lead); writing – review and editing (lead).

ACKNOWLEDGMENTS

The authors thank Sebastián Alioto and Juan Francisco Jiménez for their ethnohistorical insights on the region, and Facundo Cabrera Brizuela from Estancia La Arminda, Municipality of Coronel Dorrego, and the owners and staff of Estancia El Guanaco for their logistical support during archeological fieldwork. The authors are also grateful to Pierre Luisi for his valuable computational support. This work benefited from computational resources provided by the Centro de Computación de Alto Desempeño (CCAD) at the Universidad Nacional de Córdoba, which forms part of SNCAD-MinCyT, Argentina.

FUNDING INFORMATION

This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 2015-11220150100953CO and 2021-11220200103037CO) and ANPCyT (PICT 2020–3937) to Josefina M. B. Motti, Angelina García, Claudio M. Bravi, and Rodrigo Nores. Josefina M. B. Motti was supported by ANPCyT (PICT 2015–1405). Rodrigo Vecchi was supported by ANPCyT (PICT 0381) and Universidad Nacional del Sur (CECYT-UNS 24/I266). Rodrigo Nores was supported by PUE 2016 IDACOR, BecExt 2017 CONICET, and a National Geographic Society grant under the auspices of the pilot program "Ancient DNA: Peopling of the Americas, 2018." David Reich is supported by the Howard Hughes Medical Institute. Josefina M. B. Motti, Clara Scabuzzo, Verónica Aldazábal, Rodrigo Vecchi, Angelina García, Cristina Bayón, Darío A. Demarchi, Claudio M. Bravi, and Rodrigo Nores are Research Career members of CONICET. Nicolás Pastor is a member of CONICET's Research & Development support staff. Maia Pauro was a CONICET postdoctoral fellow at the time this research was conducted.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/genbank/, accession numbers OP605616-OP605624.

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How to cite this article: Motti, J. M. B., Pauro, M., Scabuzzo, C., García, A., Aldazábal, V., Vecchi, R., Bayón, C., Pastor, N., Demarchi, D. A., Bravi, C. M., Reich, D., Cabana, G. S., & Nores, R. (2023). Ancient mitogenomes from the Southern Pampas of Argentina reflect local differentiation and limited extra-regional linkages after rapid initial colonization. *American Journal of Biological Anthropology*, 1–15. https://doi.org/10.1002/ajpa.24727