



ORIGINAL ARTICLE

Ancient DNA analysis reveals temporal and geographical patterns of mitochondrial diversity in pre-Hispanic populations from Central Argentina

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Abstract

Objectives: The study of the ancient populations of Central Argentina has a crucial importance for our understanding of the evolutionary processes in the Southern Cone of South America, given its geographic position as a crossroads. Therefore, the aim of this study is to evaluate the temporal and geographical patterns of genetic variation among the groups that inhabited the current territory of Córdoba Province during the Middle and Late Holocene.

Methods: We analyzed the mitochondrial haplogroups of 74 individuals and 46 Hypervariable Region I (HVR-I) sequences, both novel and previously reported, from archeological populations of the eastern *Plains* and western *Sierras* regions of the province of Córdoba. The HVR-I sequences were also compared with other ancient groups from Argentina and with present-day populations from Central Argentina by pairwise distance analysis and identification of shared haplotypes.

Results: Significant differences in haplogroup and haplotype distributions between the two geographical regions were found. *Sierras* showed genetic affinities with certain ancient populations of Northwestern Argentina, while *Plains* resembled its neighbors from Santiago del Estero Province and the Pampas region. We did not observe genetic differences among the *pre 1200* and *post 1200 yBP* temporal subsets of individuals defined by the emergence of horticulture, considering both geographical samples jointly.

Conclusions: The observed patterns of geographical heterogeneity could indicate the existence of biologically distinct populations inhabiting the mountainous region and the eastern plains of Córdoba Province in pre-Hispanic times. Maternal lineages analyses support a scenario of local evolution with great temporal depth in Central Argentina, with continuity until the present.

1 | INTRODUCTION

The analysis of genetic diversity is useful for studying the evolutionary history of human populations. However, the patterns of variation observed in present-day populations do not necessarily reflect those that existed in the past.

This is especially the case for Native American populations, whose genetic diversity has been shaped by processes related to the European Conquest, such as population reduction due to epidemics, warfare and slavery (Livi-Bacci, 2006; Llamas et al., 2016). In addition, massive input of European and African genes in subsequent

centuries has further masked the patterns of genetic diversity existing in pre Columbian times (Homburger et al., 2015; Montinaro et al., 2015; Ongaro et al., 2019).

The analysis of ancient DNA (aDNA), that is, the study of genetic material of archeological origin, has helped to overcome these limitations, proving to be a valuable tool for understanding human population dispersal, settlement patterns, interactions between prehistoric populations, and development of regional population histories (Raff et al., 2011). There is a growing body of research on the genetic profile of ancient American populations, which provides a temporal context for the study of the peopling of the continent (de la Fuente et al., 2015; Lindo et al., 2018; Llamas et al., 2016; Moraga et al., 2010; Moreno-Mayar et al., 2018; Nakatsuka, Lazaridis, et al., 2020; Posth et al., 2018). Besides, several aDNA studies of pre-Hispanic populations from Argentina have been published, covering most of the territory (Arencibia et al., 2019; Carnese et al., 2010; Crespo et al., 2016, 2018, 2020; García et al., 2021; Mendisco et al., 2014; Motti et al., 2015; Nakatsuka, Luisi, et al., 2020; Nores & Demarchi, 2011; Nores et al., 2011, 2017; Parolin et al., 2020; Postillone et al., 2017, 2020; Roca-Rada et al., 2021; Russo, Dejean, et al., 2018; Russo et al., 2017; Russo, Mendisco, et al., 2018; Tavella et al., 2020).

The focus of this work are the ancient populations that occupied the current territory of the province of Córdoba in Central Argentina during the Holocene, which have been studied since late nineteenth century (Ameghino, 1885). The archeological evidence indicates that this region has been inhabited by humans since the Pleistocene–Holocene transition, about 11 000 years ago (Cattáneo et al., 2016; Rivero, 2010; Rivero et al., 2019). It was proposed that the colonization of this territory was made along the least-cost routes, through plain rivers with an east–west direction (Fabra et al., 2007). These populations occupied different environments with a subsistence strategy based mainly on the hunting of deer (*Ozotoceros bezoarticus*) and guanaco (*Lama guanicoe*), and the gathering of wild fruits (Laguens et al., 2009). About 1500–1000 years BP, they incorporated small-scale cultivation of maize (*Zea mays*), squash (*Cucurbita* sp.), and beans (*Phaseolus* sp.), but maintained a mixed subsistence strategy (Fabra & González, 2019), supplemented with the collection of *algarrobo* (*Prosopis* sp.), *chañar* (*Geoffroea decorticans*) and *caranday* palm (*Trithrinax campestris*) fruits (Medina et al., 2014; Tavarone et al., 2019).

For the last two millennia, some features of the material culture (Serrano, 1945), diet, physical activity (Fabra et al., 2012), and mortuary practices (Fabra et al., 2009), suggest that distinctive regional developments emerged between the groups that inhabited the western mountains (*Sierras*) and those from the eastern plains (*Plains*) of the province of Córdoba. The populations from the

Sierras region lived in semi-subterranean dwellings, forming scattered villages (Berberían, 2020) close to water courses and nearby farms (Pastor & Berberían, 2007). Regarding the burial of the dead, simple primary burials in a lateral decubitus position with hyperflexed legs, without grave goods, are most frequent (Fabra et al., 2009). In the *Plains*, archeological information is scarce, although recent investigations confirm occupation in the area since at least 4500 BP (Laguens et al., 2009), in close connection with the lake environment of the Mar Chiquita lagoon (Bonofiglio, 2011; Fabra, 2020). In comparison with what was reported in *Sierras*, populations from the *Plains* buried their dead in a dorsal decubitus position with arms and legs extended, generally without grave goods (Fabra et al., 2009).

The study of the ancient populations of the *Sierras* and *Plains* regions of Córdoba Province is of crucial importance for our understanding of the population dynamics of the Southern Cone of South America, given the province's geographic position as a crossroads. In a recent study by our group (García et al., 2021), we reported 92 modern mitogenomes from Central Argentina (provinces of Córdoba, San Luis, and Santiago del Estero) and 22 ancient mitogenomes from archeological sites of Córdoba Province. The analysis of these data revealed a large number of new clades within the mitochondrial DNA tree, including several local clades of great temporal depth with continuity until the present, suggesting that they originated in Central Argentina and expanded from there to neighboring regions. Besides, we detected the presence of characteristic lineages of other regions (Gran Chaco, Western, Northeastern and North-western Argentina), revealing the existence of gene flow to Central Argentina.

Although that work provided substantial information about the past populations that inhabited Central Argentina, many questions arising from the archeological and the genetic evidence remain unsolved: Was there biological variability across this wide and ecologically diverse region, consistent with the archeological evidence, or was its human population homogeneous? Did the populations of *Sierras* and *Plains* of the current territory of Córdoba Province have different genetic affinities with surrounding populations? Did the horticultural groups described in the early colonizers' chronicles replace the ancient hunter-gatherers that inhabited the region since at least 11 000 years ago, or was there a population continuity over time, accompanied by local cultural development? How do the ancient groups relate to present-day populations of the region?

Here, we report new mitochondrial DNA data of human remains from different archeological contexts and analyze them together with previously reported data

(García et al., 2012, 2021; Nores et al., 2011, 2017; Nores & Demarchi, 2011). The aim of this study is to investigate the evolutionary history of the late Middle and Late Holocene populations from Córdoba Province, focusing on the temporal and geographical patterns of genetic variation.

2 | MATERIALS AND METHODS

2.1 | The area of study

The samples come from an area located approximately between 30° and 33° S and 62° and 65° W, covering part of the territory of Córdoba Province (Figure 1). The *Sierras* region occupies the west of the province, spanning the mountain chains known as Sierras Grandes, Sierras Chicas, and Sierras Occidentales (Capitanelli, 1979). The latter continues to the southwest with the Sierras de Comehingones, extending into San Luis Province, altogether constituting the archeological region of Sierras Centrales (González, 1952). The *Plains* region, towards the Northeast of the province of Córdoba, comprises Mar Chiquita, the largest salty lagoon in South America and the fifth in the world (Fabra, 2020). This body of water is characterized by its periodic variation in extension (ranging between 2000 and 8000 km²), depth, and salinity over time (Piovano et al., 2009).

2.2 | The sample

Eighty-three individuals from 40 archeological sites were included in this study (Supplementary Table 1), both novel ($N = 26$) and previously reported (García et al., 2012, 2021; Nores et al., 2011, 2017; Nores & Demarchi, 2011). Figure 1 shows the approximate location of the archeological sites from which the human remains were unearthed.

The skeletal remains are housed in the collections of the Museo de Antropología (Facultad de Filosofía y Humanidades, Universidad Nacional de Córdoba) and other public and private museums of Córdoba Province. Sixty samples were dated by AMS (Accelerator Mass Spectrometry), corresponding to 63 individuals (three double inhumations were included in this study). Twenty-nine of them (one newly reported) were processed in the Graduate School of Frontier Sciences, University of Tokyo (MTC); 15 in the W. M. Keck Carbon Cycle Accelerator Mass Spectrometry Laboratory, University of California, Irvine (UCI AMS); 14 in the NSF-Arizona AMS Laboratory, University of Arizona (AA); and two (one newly reported) in the Laboratorio de Tritio

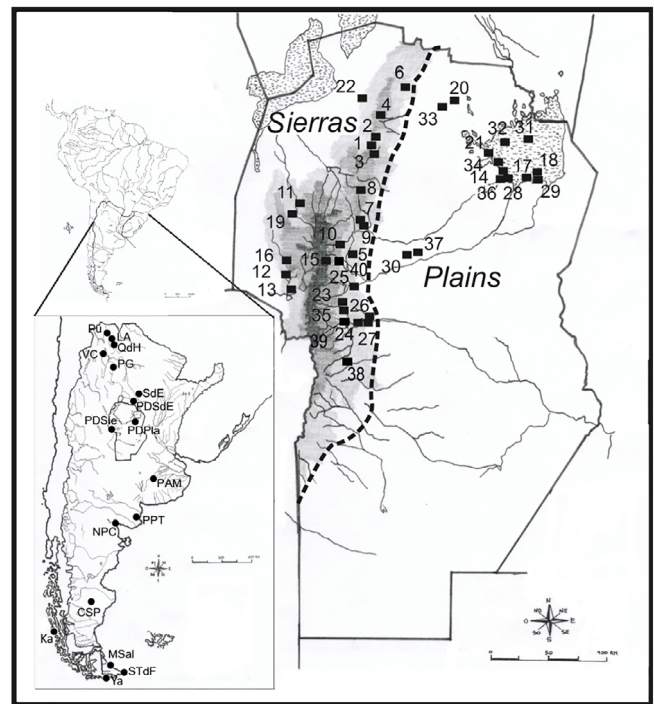


FIGURE 1 Geographical location of the archeological sites from which the human remains analyzed in this work originate. *Sierras*: 1. San Esteban, 2. Dique El Cajón, 3. Ayampitín, 4. Ischilín, 5. Constantinopla, 6. Cerro Colorado, 7. La Granja, 8. El Vado, 9. Agua de Oro, 10. Cuesta Blanca, 11. Rosca Yaco, 12. Guasmara, 13. Loma Bola, 15. Copina, 16. Cañada Larga, 19. Charquina, 22. Nunsacat, 23. Loteo 5, 24. Banda Meridional del Lago, 25. Potrero de Garay, 26. Embalse Río Tercero, 26. Central Nuclear, 27. Quillinzo, 35. Campo Potrero del Monte. *Plains*: 14. El Diquecito, 14. Laguna del Plata, 14. Los Surgentes, 17. Marull, 18. Isla Orihuella Costa Sur, 20. Pozo de las Ollas, 21. Estancia La Elisa, 28. Miramar, 28. Colonia Muller, 28. Mar Chiquita, 29. Orihuella, 30. Costasacate, 30. Rincón, 31. Isla Tigre, 32. El Mistolar, 33. Laguna de la Sal, 34. La Rueda, 34. Costa La Fortuna, 36. La Para, 37. Cosme, 38. Alpa Corral, 39. Club UBSISA - Amboy, 40. Potrerillo de Larreta. Inset: Location of Córdoba Province within Argentina, and Argentina within South America, along with the locations of ancient groups and current populations included in distance analysis. Abbreviations of ancient groups: CSP: Central-southern Patagonia, Ka: Kawéskar, LA: Los Amarillos, MSaI: Salesian Mission in Tierra del Fuego, NPC: Northern Patagonia coast, PAM: Pampas region, PG: Pampa Grande, PPT: Pampa-Patagonia transition, PLA: Plains of Córdoba, Pu: Puna, QdH: Quebrada de Humahuaca, SdE: Santiago del Estero, SIE: Sierras of Córdoba, STdF: Southern Tierra del Fuego, VC: Valles Calchaquíes, Ya: Yámana. Modern groups: PDPla: Present-day populations of the plains of Córdoba Province, PDSdE: Present-day populations of Santiago del Estero Province, PDSie: Present-day populations of Sierras Centrales (Córdoba and San Luis Provinces)

y Radiocarbono-LATYR, Universidad Nacional de La Plata (LP). Direct radiocarbon dates ranging from 4525 ± 20 to 345 ± 20 yBP were calibrated with OxCal 4.4

(Bronk Ramsey, 2009), using the SHCal20 Southern Hemisphere calibration curve (Hogg et al., 2020) (Supplementary Table 1).

In order to assess geographic variability in the distribution of mtDNA haplogroups, we separated the total sample in two regional sets. *Sierras* ($N = 37$) includes individuals from sites located in the mountainous region (average age of 1245 cal yBP from 34 dated samples). *Plains* ($N = 46$) is composed by samples from sites of the eastern plains: 43 from the shores, islands or nearby the Mar Chiquita lagoon, and 3 from the area of Río Segundo city (average age of 1459 cal yBP from 29 dated samples). To investigate possible temporal trends in the genetic variation, we also defined two temporal subsamples based on the subset of 63 individuals dated by AMS, separated according to the average calibrated date (Supplementary Table 1). The subsample *pre 1200 yBP* ($N = 17$) comprises individuals dated from 4525 ± 20 to 1585 ± 15 yBP, spanning a temporal range of 3699 calibrated years, while the *post 1200 yBP* ($N = 46$) includes samples dating from 1280 ± 20 to 345 ± 20 yBP, covering a period of 791 calibrated years (Supplementary Table 1). The dividing line between the two temporal subsamples was defined based on the timing of the incorporation of horticulture and pottery production (Fabra & González, 2019; Laguens et al., 2009), a marked increase in the demographic density in the region (Fabra, 2020) and the differences observed in the treatment of the dead, among other indicators (Fabra et al., 2009; González & Fabra, 2019).

2.3 | Ethical statement

This study was performed following ethical guidelines for working with human remains (Aranda et al., 2014), treating these deceased individuals respectfully. Skeletal samples were analyzed with the proper permits from the Province of Córdoba Government, which includes the express consent and authorization of the Council of Communities of Indigenous Peoples of the Province of Córdoba. Our research program on ancient human remains was approved by the Ethics Committee of the CEMIC (Comité de Ética en Investigación, Centro de Educación Médica e Investigaciones Clínicas “Norberto Quirno”).

2.4 | DNA extraction

Several precautions were taken to avoid contamination of the ancient DNA with modern DNA templates (Pääbo et al., 2004). To rule out recent contamination, we analyzed the mtDNA from the co-authors that handled the

remains. Sample collection was done by MF, whose mitochondrial lineage is J (16069 16126 16311 16519), while all the laboratory analyses were performed by RN, whose maternal lineage is T2e (16126 16153 16294 16519), both of European origin.

We followed an extraction protocol based on decalcification and digestion. Bone and tooth samples were decontaminated with 6% bleach for 5–15 min, rinsed multiple times with sterile water, and then allowed to dry overnight. Samples were powdered using a dental drill. About 0.25 g of pulverized sample was decalcified and digested in 1 ml of 0.5 M EDTA and 1 mg of proteinase K at 56°C, incubating for 24–48 h with mild rocking. The DNA was subsequently extracted from the supernatant, obtained after centrifugation at 5000g for 5 min, using the Wizard SV Gel and PCR Clean-Up System (Promega). An extraction blank was included for every set of six archeological samples processed. The extraction procedure was carried out at least twice with different samples of each skeletal individual, and the aDNA obtained was used for independent determinations.

2.5 | APLP analysis

Mitochondrial haplogroups were determined for each ancient sample by amplified product-length polymorphisms analysis (APLP) (Umetsu et al., 2005). The determination of the four main Native American haplogroups was performed using two PCR reactions in a final volume of 20 μ L, one of which contained the primers to determine the haplogroups A and D, and the other for B and C. PCR reactions included 1X GoTaq Buffer (Promega), 0.20 mM dNTPs (Promega), 0.28 μ M of each primer, 0.05 U/ μ l GoTaq DNA Polymerase (Promega), 0.1 mg/ml BSA, and 2 μ L of DNA extract. To characterize haplogroups A, C, and D we used the set of three primers described in Umetsu et al. (2005), and those published by Wrischnik et al. (1987) for direct genotyping of haplogroup B. The PCR program included an initial cycle of 6 min DNA denaturation at 94°C, 40 cycles of denaturation for 30 s, annealing at 53°C for 45 s and elongation at 72°C for 30 s, and a final elongation step of 5 min. Each PCR reaction included a negative control and an extraction blank to identify false-positive amplification and exogenous DNA contamination during extraction of aDNA, respectively. The product of both PCRs was seeded with a molecular size marker in native gels of 8% acrylamide:bisacrylamide (19:1), subsequently stained for 30 min with SYBR Safe 1:10000 (Invitrogen) and visualized in UV light transilluminator.

2.6 | Sequencing of hypervariable region I of mtDNA

Once individual Native American haplogroups were determined by APLP, a portion of the HVR-I mtDNA of up to 400 base pairs (bp) long was sequenced. This was done by using two or three pairs of overlapping primers, whose sequences and coordinates are listed in Supplementary Table 2. Although widely used in aDNA HVR-I studies, these amplification strategies leave small (8–23 bp) interspersed gaps of endogenous aDNA without coverage in overlapping primer positions (Supplementary Table 2). PCR reactions were carried out with the same reagents and cycling parameters as in APLP, but using 5–6 μ L of aDNA template in a final volume of 55 μ L, and 50 cycles of amplification. PCR products were sent for purification and sequencing to Macrogen (Seoul, Korea). To detect the presence of sequencing errors, both DNA strands were sequenced and any ambiguities were solved by resequencing.

The obtained sequences were manually edited and then aligned with the revised Cambridge Reference Sequence (Andrews et al., 1999) using Sequencher version 5.3 (Gene Codes Corporation). Sub-haplogroups were determined with reference to Phylotree mtDNA tree Build 17 (van Oven & Kayser, 2009) and published data.

2.7 | Statistical analysis at intra-population level

Intra-population molecular diversity indices of the temporal and geographical samples were calculated over the HVR-I sequences from np 16024 to np 16400 (regardless of the coverage gaps generated by the sequencing strategy), after removing mutational hotspots following van Oven and Kayser (2009). Genetic differences between geographical regions and temporal subsamples were studied employing the Exact test (Raymond & Rousset, 1995) to compare haplogroup frequencies, and through the analysis of molecular variance (AMOVA) on the haplotype distribution. All the above-mentioned analyses were performed with Arlequin software version 3.5.2.2 (Excoffier & Lischer, 2010).

2.8 | Comparison with ancient and contemporary populations from Argentina

The HVR-I sequences from the *Plains* and *Sierras* samples were compared with other ancient groups from Argentina to investigate their affinities (references in Supplementary Table 3). Sequences from different archeological sites and

chronologies were grouped together based on their geographical proximity (Figure 1), except for the Los Amarillos site, located in Quebrada de Humahuaca, which was considered as a separate population given its relatively large sample size and its unusually high incidence of haplogroup (Hg) A2 (Mendisco et al., 2011; Russo, Mendisco, et al., 2018). The ancient data were also compared with those of contemporary individuals with Native American lineages from small, semi-rural populations of Central Argentina (García et al., 2018; Pauro et al., 2013) to check for population continuity up to the present (Supplementary Table 3). Localities from Córdoba and San Luis Provinces that roughly overlapped with the archeological sites were grouped into the two geographical regions defined in this study for the ancient individuals (Present-day Sierras Centrales and Present-day Plains). Finally, two populations from the southern plains of Santiago del Estero Province, located to the northwest of the Mar Chiquita lagoon, were included in the Present-day Santiago del Estero group. The sample from the capital city of Córdoba (García et al., 2018) was not included to reduce the possibilities of misrepresenting the local genetic diversity, since it is a cosmopolitan city with many recent migrants from other regions of Argentina and neighboring countries.

Sequences were trimmed to a common region between positions 16024–16410. Five sequences with major reading gaps, two from *Plains* (#85 and #118) and three from Misión Salesiana “La Candelaria” (Motti et al., 2019), were removed. Phylogenetically uninformative sites such as indels or poly-C stretches at positions 16182–16183 and 16193 were excluded from the analyses, as recommended (van Oven & Kayser, 2009). The Φ ST distance calculation was performed with Arlequin 3.5.2.2 (Excoffier & Lischer, 2010), using the substitution model with the lowest BIC in MEGA 6 (Tamura et al., 2013). Significant comparisons after 10 000 permutations ($p \leq 0.05$) reject the null hypothesis of no difference between populations. The pairwise Φ ST distances were linearized with Slatkin's method (Slatkin, 1995) and used as input for both a hierarchical clustering, performed with the UPGMA algorithm of the `hclust` function from R stats, and a nonmetric multidimensional scaling (MDS), calculated with the `metaMDS` function in the `vegan` R package (Oksanen et al., 2019). The dendrogram and MDS were plotted with `factoextra` (Kassambara & Mundt, 2017) and `ggpubr` (Kassambara, 2018) R packages, respectively.

To further investigate the geographic distribution of the haplotypes identified in the pre-Hispanic populations of Córdoba Province, we constructed separate median-joining networks (Bandelt et al., 1999) for haplogroups A2, B2 and D1 using Network 10.2.0.0 (<http://www.fluxus-engineering.com/sharenet.htm>). The ancient and

contemporary sequences used for the distance calculations (Supplementary Table 3) were trimmed to a fragment spanning from np 16101 to np 16365, prioritizing the reading frame obtained in this study. Two sequences from Quebrada de Humahuaca (Postillone et al., 2017) and a sequence from *Sierras* (#117) were removed due to their short length after trimming. The C1 haplotype network was not generated because the length of the selected fragment prevents the differentiation of its main sub-haplogroups (C1b, C1c and C1d), which in turn makes it impossible to establish any robust phylogenetic hypothesis. The haplotype networks were processed using the maximum parsimony (MP) calculation (Polzin & Daneshmand, 2003) and visualizing the minimum number of trees necessary for explaining the original network. Both the weighting of homoplastic sites and the geographic coherence of the haplotypes' distribution were considered in order to avoid and/or resolve non-phylogenetic reticulations.

3 | RESULTS

Throughout the study, both the extraction and PCR controls consistently showed negative results. We obtained 17 successful haplogroup determinations by APLP out of the 26 newly analyzed individuals. These results were added to previously published data from our group (García et al., 2012, 2021; Nores & Demarchi, 2011; Nores et al., 2011, 2017) to generate a database of 74 genotyped individuals in total (Table 1) for further statistical analyses of the ancient genetic variation in the province of Córdoba.

3.1 | Haplogroup distribution between temporal and geographical samples

The relative frequencies of each haplogroup in the total sample and in each geographical and temporal sample are presented in Table 2. The total sample from Córdoba Province showed a high incidence of haplogroup C, while the other three haplogroups were present at intermediate frequencies. However, the haplogroup distribution was not homogeneous across the geographic space, with statistically significant differences between *Sierras* and *Plains* (Exact test p value = 0.0005 ± 0.0003). The most conspicuous difference was the high incidence of haplogroup B in *Sierras* (39.4%), whereas in *Plains* only one individual (2.4%) carried this haplogroup. Conversely, haplogroups A, C, and D were more frequent in *Plains* than in *Sierras*. In contrast, temporal subsamples showed nonsignificant differences (Exact test p value = 0.2177 ± 0.0041).

3.2 | Sequence analysis

Reliable mtDNA HVR-I sequences are reported from 46 of the 74 individuals with an assigned Hg (Table 1), 16 of which are newly obtained and 30 were previously published (García et al., 2012, 2021; Nores et al., 2017). mtDNA sequencing of 62% of the total sample was an expected result in degraded aDNA samples. The better performance of APLP to determine mtDNA haplogroups (74 out of 83 samples, 89%) was due to the smaller fragment sizes amplified with APLP primers. In all cases the HVR-I sequences were consistent with the haplogroup determination (Table 1).

From the 46 analyzed sequences, 20 different haplotypes were identified. Nucleotide diversity for the whole Córdoba Province sample was $\pi = 0.0131$. Relatively higher diversity was found in the *Sierras* sample compared to *Plains*, while there was no difference between *post 1200 yBP* and *pre 1200 yBP* nucleotide diversity levels (Supplementary Table 4).

The AMOVA results revealed significant differentiation between *Sierras* and *Plains* samples (FST: 0.1821, $p = 0.000$), but there were no differences between the *pre 1200 yBP* and *post 1200 yBP* temporal subsamples (FST: -0.0033 , $p = 0.441$). Therefore, *Sierras* and *Plains* were considered separately for genetic distance calculations. It was not possible to test the temporal differentiation within each ancient geographical sample given the small N obtained after both partitions.

3.3 | Population comparisons

Genetic distances between the *Plains* and *Sierras* samples and 14 ancient groups from Argentina, as well as three modern populations from Central Argentina, are presented in a UPGMA tree and a MDS plot (Figure 2A, B). Distinctive clusters, highlighted in different colors in both graphics, showed a coherent geographic pattern. *Plains* (PLA) clustered together with the ancient and contemporary samples from the southern plains of Santiago del Estero Province (SdE and PDSdE) and with the ancient population of Pampas region (PAM). *Sierras* (SIE) was close to the population from the Pampa Grande archeological site in Northwestern Argentina. It is interesting to note that, while *Sierras* differed significantly from its modern counterpart (PDSie), *Plains* had a non-significant genetic distance not only with the current populations living in the area (PDPla) but also with the present-day population of Sierras Centrales (PDSie) (Supplementary Table 5). Finally, the ancient populations from Northern and Central-Southern Patagonia, and Tierra del Fuego (STdF and MSal) all clustered together. It is important to take into account that some of these

TABLE 1 Genetic information of the samples analyzed in this work. Samples with new genetic data are highlighted in bold

Region	Period	Sample ID	Archeological site/individual	Average calibrated date (yBP)	Map code	HVR-I (+ 16000)	Reading frame	Haplotype	Haplogroup (AFLP)	
PLAINS	pre 1200 yBP	56†	Mar Chiquita I5	5141	28	223T 242T 311C 325C 362C‡	16023–16410	D1j	D	
		140	Estancia La Elisa I2	4535	21	223T 298C 325C 327T+	16023–16422	C1	C	
		123†	El Diquecito CR II	2563	14	223T 298C 325C 327T*	16023–16422	C1	C	
		122†	El Diquecito CO II	2521	14	051G 223T 259T 271C 298C 311C 325C 327T+	16023–16422	C1d	C	
		121†	El Diquecito CQ II	2426	14	092C 223T 298C 325C 327T 390A*+	16023–16422	C1	C	
		126	El Diquecito CS II	2273	14	092C 223T 298C 325C 327T 390A+	16023–16422	C1	C	
			118	El Mistolar 06 II	2239	32	223T 298C 325C 327T	16023–16422 (unread gap: 16184–16220)	C1	C
			103#	El Diquecito CG II	1805	14	223T 298C 325C 327T*	16023–16422	C1	C
			94#	Colonia Muller II	1442	28	093C 182C 183C 189C 217C+	16023–16422	B2	B
		post 1200 yBP	47	Laguna del Plata II	1115	14	223T 298C 325C 327T	16101–16410	C1	C
			102#	El Diquecito CE II	1069	14	093C 223T 242T 311C 325C 362C+	16023–16422	D1j	D
			112#	Marull 52/2	1094	17	na			A
			87#	Orihuea II	887	29	na			A
			111#	Isla Tigre II	830	31	na			D
		96#	El Diquecito CL II	867	14	223T 298C 325C 327T*	16101–16410	C1	C	
		99†	Isla Orihuea II	646	18	223T 298C 325C 327T	16023–16422	C1	C	
		101#	El Diquecito CB II	665	14	223T 298C 325C 327T*	16023–16422	C1	C	

(Continues)

TABLE 1 (Continued)

Region	Period	Sample ID	Archeological site/individual	Average calibrated date (yBP)	Map code	HVR-I (+ 16000)	Reading frame	Haplotype	Haplogroup (APLP)
		130†	El Diquecito CB I2	665	14	223T 325C 362C*	16023-16422	D1	D
		124†	El Diquecito CA II	612	14	187T 189C 209C 223T 325C 362C*+	16023-16422	D1g5	D
		55#	El Mistolar MirI	623	32	189C 223T 325C 362C	16023-16422	D1	D
		143	Orihuela 08 II	603	29	223T 298C 325C 327T	16101-16410	C1	C
		32#	Laguna de la Sal	587	33	223T 290T 319A 362C	16101-16410	A2	A
		114†	Costasacate 45	583	30	na			A
		125†	El Diquecito CP II	572	14	187T 189C 209C 223T 325C 362C*	16101-16410	D1g5	D
		97#	El Diquecito CM II	486	14	223T 298C 325C 327T*	16023-16422	C1	C
		1#	Rincón 2607	516	30	192T 223T 290T 319A 362C	16101-16410	A2	A
		82#	La Para 4	388	36	na			C
		27	Miramar II		28	na			C
		48	La Para 3		36	223T 242T 311C 325C 362C	16023-16422	D1j	D
		51	Los Surgentes 6		14	223T 248T 298C 325C 327T	16101-16410	C1	C
		52	Costa La Fortuna 7		34	na			A
		53	Mar Chiquita 3		28	na			D
		54	Laguna del Plata 2		14	na			A
		57	Mar Chiquita 12		28	111T 223T 263C 290T 319A 362C+	16101-16410	A2	A
		58	Miramar 4		28	223T 298C 325C 327T 390A	16101-16410	C1	C
		60	Miramar 11		28	223T 325C 362C	16101-16410	D1	D

ND

TABLE 1 (Continued)

Region	Period	Sample ID	Archeological site/individual	Average calibrated date (yBP)	Map code	HVR-I (+ 16000)	Reading frame	Haplotype	Haplogroup (APLP)
		85	Laguna del Plata 1981		14	223T 298C 325C 327T	16023-16422 (unread gap: 16184-16220)	C1	C
		86	La Rueda II		34	na			C
		145	Pozo de las Ollas 44-1563		20	na			C
		146*	El Diquecito 10 CAI		14	223T 298C 325C 327T	16175-16410	C1	C
		147*	El Diquecito 10 CBII		14	na			C
SIERRAS	<i>pre 1200 yBP</i>	199	Alpa Corral	5072	38	142T 183C 189C 217C+	16023-16422	B2	B
		16	Club UBSISA - Amboy	4127	39	na			A
		61#	Agua de Oro CB I2	3545	9	na			C
		120#	Agua de Oro CB II	3545	9	na			C
		115†	Copina II	2750	15	na			C
		104	El Vado II	2113	8	142T 183C 189C 217C+	16023-16422	B2	B
	<i>post 1200 yBP</i>	42#	La Granja II	1168	7	223T 298C 325C 327T+	16023-16422	C1	C
		40#	Cuesta Blanca II	930	10	na			B
		152	Potrerillo de Larreta	958	40	223T 298C 325C 327T+	16023-16422	C1	C
		34#	Potro de Garay E8/53	914	25	142T 183C 189C 217C+	16023-16422	B2	B
		110#	Potro de Garay E341	836	25	na			B
		108†	Quillinzo 29	836	27	na			C
		95	San Esteban II	839	1	na			C

(Continues)

TABLE 1 (Continued)

Region	Period	Sample ID	Archeological site/individual	Average calibrated date (yBP)	Map code	HVR-I (+ 16000)	Reading frame	Haplotype	Haplogroup (APLP)
		105#	Loma Bola I1A	818	13	182C 183C 189C 217C+	16101–16410	B2	B
		35#	Guasmara I2	814	12	111T 172C 223T 290T 319A 362C+	16023–16422	A2	A
		116#	Potrero de Garay E4/57	788	25	187T 189C 209C 223T 325C 362C+	16023–16422	D1g5	D
		36†	Potrero de Garay E6/56	801	25	na			B
		14†	Campo Potrero del Monte I2	703	35	na			C
		98#	Rosca Yaco II	689	11	142T 183C 189C 217C+	16023–16422	B2	B
		70#	Banda Meridional del Lago II	611	24	223T 298C 325C 327T	16023–16422	C1	C
		41	Copina I2	608	15	111T 178C 223T 290T 319A 362C+	16023–16422	A2	A
		39#	Cerro Colorado A106	614	6	na			D
		17#	Ayampitín I1	578	3	183C 189C 223T 294T 298C 325C 327T+	16023–16422	C1	C
		151	Loteo 5 I1	557	23	142T 182C 183C 189C 217C 390A+	16023–16422	B2	B
		153	Loteo 5 I2	557	23	142T 182C 183C 189C 217C 390A+	16023–16422	B2	B
		30#	Cañada Larga I2	439	16	na			A
		109#	Ischilin I1	430	4	223T 298C 325C 327T+	16023–16422	C1	C
		37#	Charquina A113	423	19	na			B
		117#	Potrero de Garay E9	413	25	183C 189C 217C	16150–16410	B2	B

TABLE 1 (Continued)

Region	Period	Sample ID	Archeological site/individual	Average calibrated date (yBP)	Map code	HVR-I (+ 16000)	Reading frame	Haplotype	Haplogroup (APLP)
		107#	Nunsacat I2	406	22	187T 189C 209C 223T 325C 362C	16023-16410	D1g5	D
		62#	Agua de Oro CC II	377	9	na			C
	ND	106#	Central Nuclear		26	na			A
		144	Dique El Cajón		2	na			D

Note: #Sample included in haplogroup frequency analysis in Nores & Demarchi, 2011; Nores et al., 2011. †Samples included in haplogroup frequency analysis in Nores et al., 2011. ‡HVR-I sequence reported in García et al., 2012. +Mitogenome reported in García et al., 2021. *HVR-I sequence and/or haplogroup reported in Nores et al., 2017. Abbreviations: na, no amplification observed; ND, not dated.

TABLE 2 Distribution of Native American mitochondrial haplogroups (percentage values in parentheses) in the total sample and in the temporal and geographical subsamples

Sample group	Date range (yBP)	Average date (yBP)	Coverage (years)	N	Hg A	Hg B	Hg C	Hg D
Total ^a	4525–345	1178	4180	74 (of 83)	13 (17.6)	14 (18.9)	33 (44.6)	14 (18.9)
<i>Plains</i>	4525–370	1274	4155	41 (of 46)	8 (19.5)	1 (2.4)	22 (53.7)	10 (24.4)
<i>Sierras</i>	4450–345	1085	3015	33 (of 37)	5 (15.2)	13 (39.4)	11 (33.3)	4 (12.1)
<i>pre 1200 yBP</i>	4525–1585	2447	3695	15 (of 17)	1 (6.7)	3 (20.0)	10 (66.6)	1 (6.7)
<i>post 1200 yBP</i>	1280–345	743	791	43 (of 46)	8 (18.6)	11 (25.6)	15 (34.9)	9 (20.9)
<i>Plains pre 1200 yBP</i>	4525–1585	2368	3284	9 (of 10)	0 (0)	1 (11.1)	7 (77.8)	1 (11.1)
<i>Plains post 1200 yBP</i>	1241–370	759	822	18 (of 19)	5 (27.8)	0 (0)	7 (38.9)	6 (33.3)
<i>Sierras pre 1200 yBP</i>	4450–2156	2573	2080	6 (of 7)	1 (16.7)	2 (33.3)	3 (50.0)	0 (0)
<i>Sierras post 1200 yBP</i>	1280–345	731	735	25 (of 27)	3 (12.0)	11 (44.0)	8 (32.0)	3 (12.0)

^aSixteen of the positive samples in this study have not been dated yet, so the total N of 74 does not coincide with the sum of the number of individuals in the temporal subsamples.

Note: Date range, average date, and coverage refer to samples with positive results for Hg determination, as well as N, with the total value between parentheses.

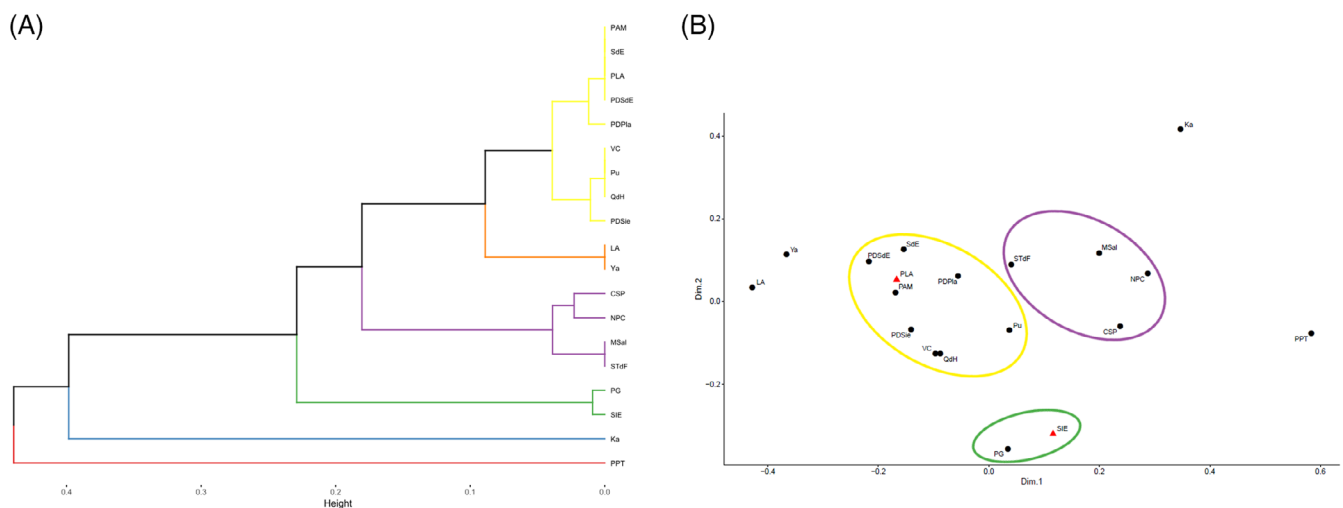


FIGURE 2 (A) UPGMA dendrogram of pairwise Φ_{ST} distances, cut at $k = 6$ to enhance clusters visualization. (B) MDS plot of the pairwise Φ_{ST} genetic distances between *Plains* and *Sierras* subsamples (red triangles) and 17 ancient and modern populations (references in Supplementary Table 3). Stress = 0.1269. Color ellipses highlight main population clusters. Abbreviations of ancient and modern groups are detailed in the legend of Figure 1

configurations in the UPGMA and the MDS plots could have been affected by the small sample sizes of the ancient groups, and therefore may not reflect actual genetic affinities.

A number of ancient populations appeared as outliers for several possible reasons. Geographically isolated groups such as the Kawéskar and Yámana from Tierra del Fuego had significant genetic distances with all other populations, while the Pampa-Patagonia transition sample (PPT), with a high frequency of D1g, as well as Los Amarillos sample (LA), mostly constituted by A2

haplotypes, were also genetically differentiated from the remaining samples (Figure 2 and Supplementary Table 5).

3.4 | Ancient maternal lineages of Central Argentina in the regional context

We report sequences of the mtDNA HVR-I for five individuals identified as Hg A, all of which have different haplotypes (Table 1 and Figure 3). Two samples exhibited a reversion at np 16111, which has been described in

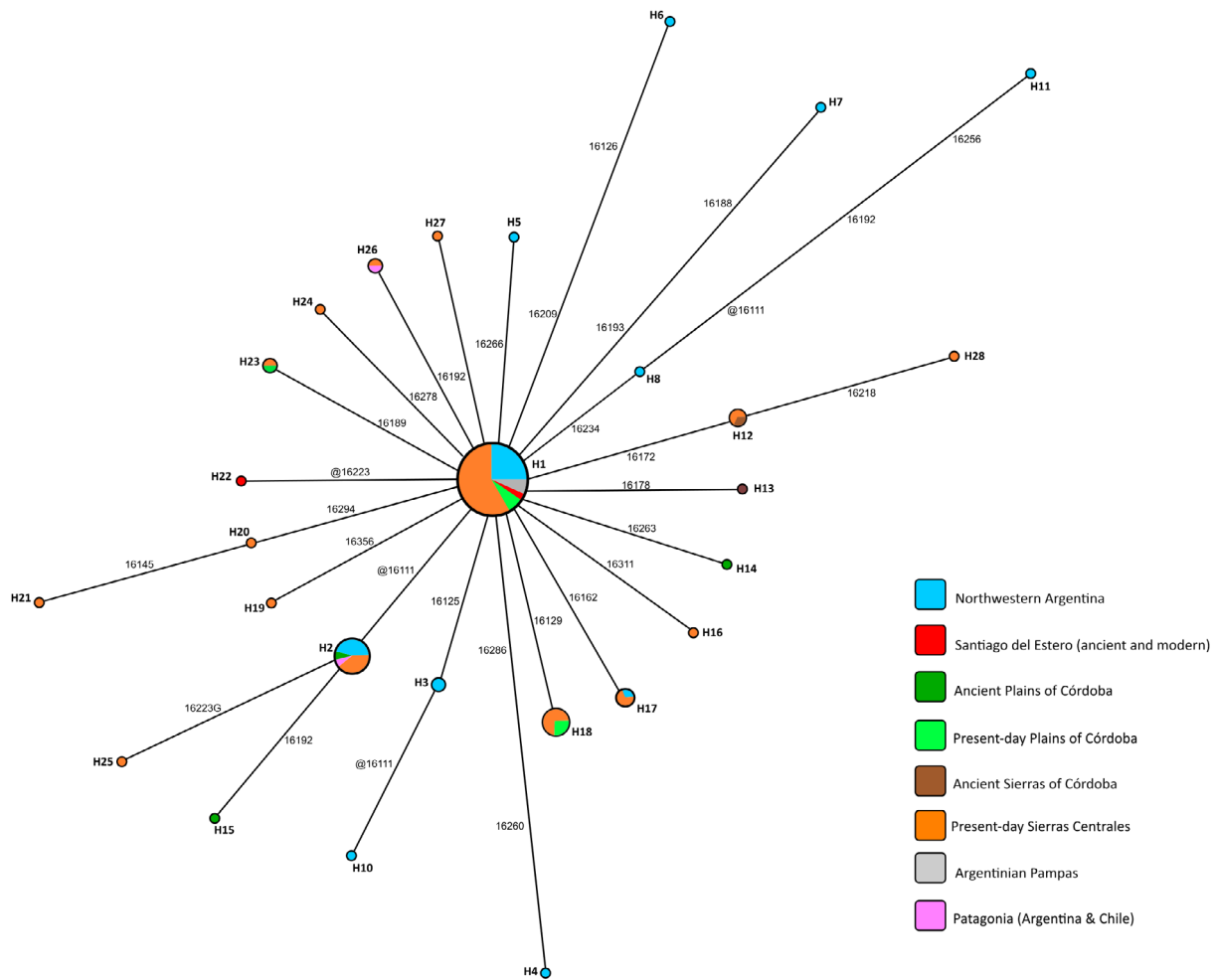


FIGURE 3 Median-joining network of haplogroup A2 HVR-I (16101–16365) haplotypes from ancient and present-day individuals of the Southern Cone of South America (Supplementary Table 3). Colors reflect the geographic sources of the included sequences. Areas of circles are proportional to haplotype frequencies

several South American populations, likely as a result of the high-mutation rate at this position (Soares et al., 2009). The transition at np 16192, another highly homoplastic site observed in an ancient sample from *Plains* (H15 in the network), has been previously reported in both ancient and contemporary South American populations, but without a consistent geographical pattern that would allow any phylogenetic hypothesis to be established. Sample #35 from *Sierras*, which shared haplotype A2₊₁₆₁₇₂ (H12) with present-day individuals from the Sierras Centrales region, has been recently included into a new local clade (A2bb1) within the A2₊₁₅₀ lineage (García et al., 2021), characteristic of the populations of Central and Western Argentina (García et al., 2021; Motti, 2012; Motti et al., 2017). The haplotypes A2₊₁₆₂₆₃ (H14), observed in sample #57 from the Mar Chiquita lagoon, and A2₊₁₆₁₇₈ (H13) of sample #41 from *Sierras*, were described as derived haplotypes of another new local clade, A2bc, also within the A2₊₁₅₀

lineage (García et al., 2021). Neither A2_{+150–16263} nor A2_{+150–16178} have been identified in any other modern or ancient populations from South America.

We also obtained nine B2 HVR-I sequences (most of them from previously published mitogenomes), eight from *Sierras* and one from *Plains* subsamples, ranging from 4450 ± 80 to 420 ± 41 yBP (Table 1 and Figure 4). Six samples from *Sierras* presented the B2₊₁₆₁₄₂ motif (H2 in the network), which was shared with individuals currently inhabiting this region and also found in an ancient individual from Pampa Grande site, in Northwestern Argentina (Carnese et al., 2010). It is worth mentioning that none of the ancient *Plains* sequences belonged to this clade, and only one individual from present-day *Plains* carried a derived haplotype with two additional mutations (H15). Two individuals (#105 and #94) displayed the B2 nodal haplotype (H1) within the reading frame of this study, although it should be noted that #105 had a mutation at np 11590 that is diagnostic

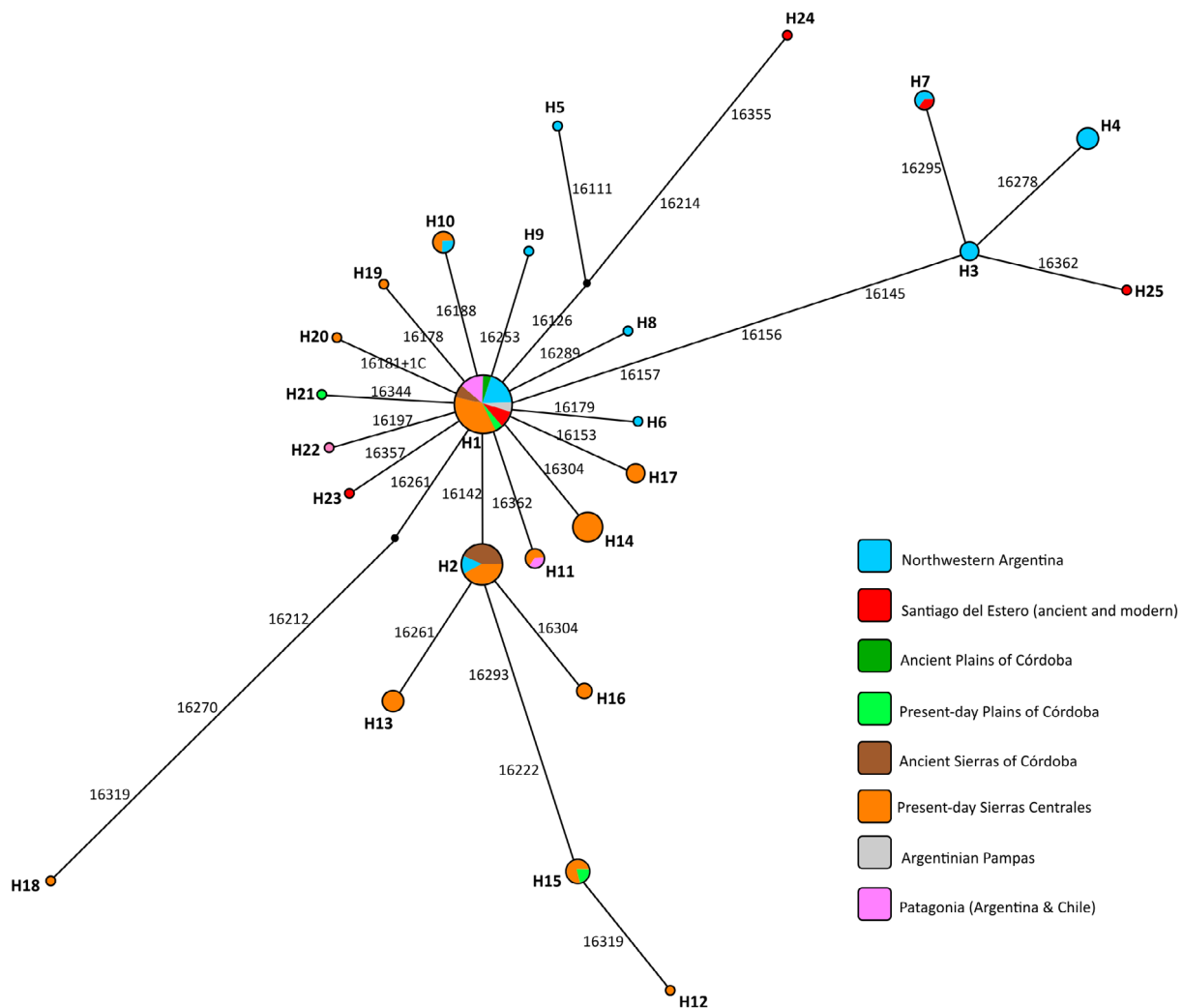


FIGURE 4 Median-joining network of haplogroup B2 HVR-I (16101–16365) haplotypes from ancient and present-day individuals of the Southern Cone of South America (Supplementary Table 3). Colors reflect the geographic sources of the included sequences. Inferred missing haplotypes are presented as black dots. Areas of circles are proportional to haplotype frequencies

of sub-haplogroup B2ak (García et al., 2021); while #94 had an additional transition at position 16093, a fast-evolving site (Hasegawa et al., 1993; Meyer et al., 1999; Wakeley, 1993) that is present in many branches of the mitochondrial phylogenetic tree (Soares et al., 2009).

Haplogroup C is the most represented lineage in our pre-Hispanic sample, as well as among the contemporary inhabitants of the province of Córdoba (García et al., 2018). We report 22 C1 HVR-I sequences, 17 from *Plains* and 5 from *Sierras* (Table 1). Seven of the *Plains* sequences were drawn from El Diquecito site and already published by Nores et al. (2017), while eight Hg C1 sequences were trimmed from complete mitogenomes (García et al., 2021). Given the extent of the considered fragment, it was only possible to distinguish the sub-haplogroup C1d from other C1 sequences, while C1b and C1c diagnostic sites fell outside of range.

For this reason, most of the samples without mitogenome information ($N = 12$) were assigned to the C1 nodal haplotype, and only one was identified as C1d by the presence of a transition at np 16051. This sequence from El Diquecito site, dated in 2438 yBP, displayed additional polymorphisms at np 16259, 16271 and 16311, which represent a motif that was originally reported in a modern mitochondrial genome from Salta Province, Argentina, assigned to C1d1b (Perego et al., 2010), and recently redefined as C1d1b2 (García et al., 2021). Haplotype C1d₊₁₆₂₅₉₋₁₆₂₇₁₋₁₆₃₁₁ was detected in present-day populations of Gran Chaco, Northwestern and Central Argentina (Cabana et al., 2006; García et al., 2018, 2021; Motti, 2012; Pauro et al., 2013; Perego et al., 2010; Sevini et al., 2013), Uruguay (Figueiro et al., 2011), and in an ancient individual from Santiago del Estero Province (Tavella et al., 2020).

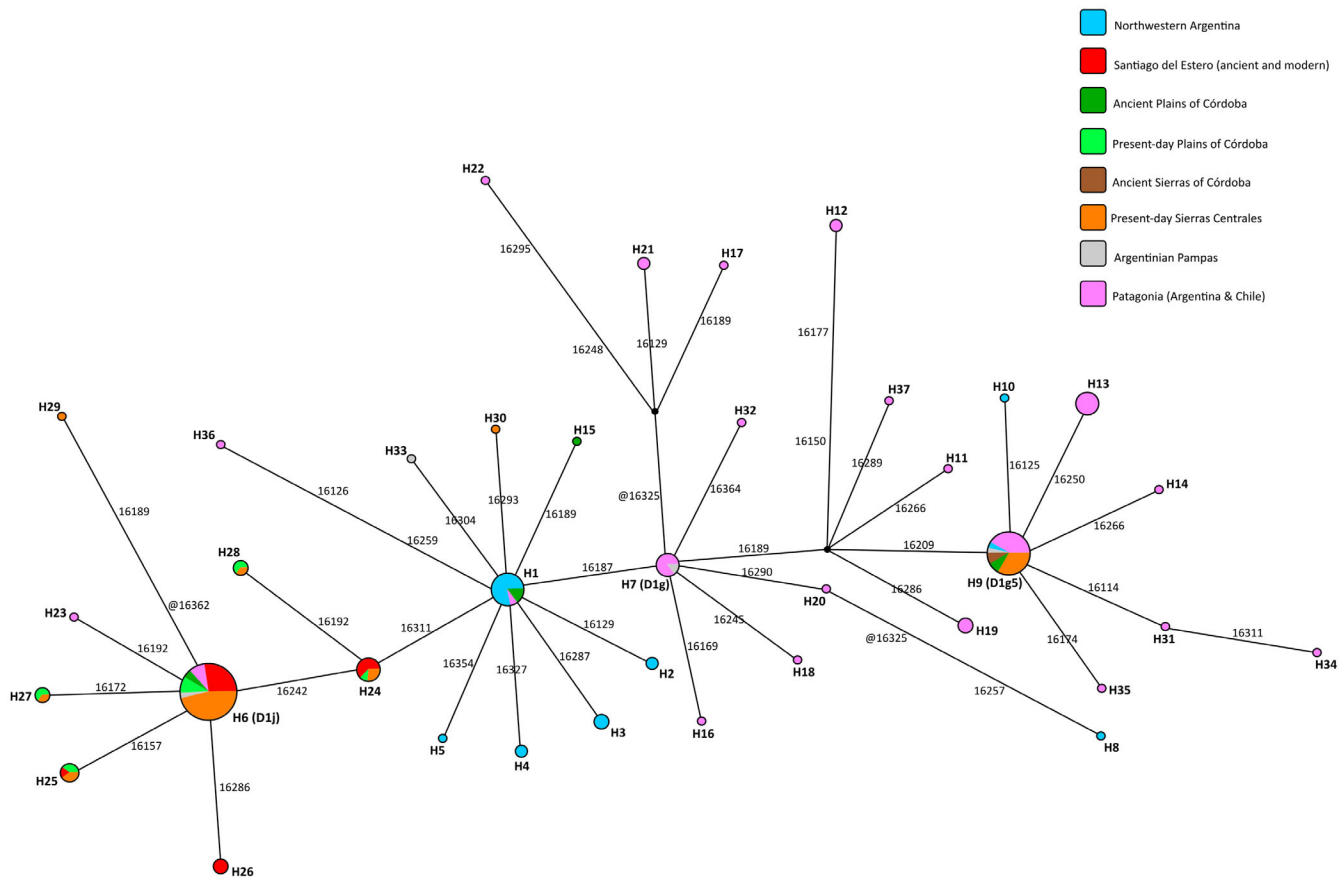


FIGURE 5 Median-joining network of haplogroup D1 HVR-I (16101–16365) haplotypes from ancient and present-day individuals of the Southern Cone of South America (Supplementary Table 3). Major sub-haplogroups are labeled. Colors reflect the geographic sources of the included sequences. Inferred missing haplotypes are presented as black dots. Areas of circles are proportional to haplotype frequencies

Three C1 samples from *Plains* differed from the nodal sequence by a mutation at np 16390, while two of them had an additional polymorphism at np 16092 (note that the third sequence starts at np 16101). In Argentina, the C1₊₁₆₃₉₀ motif is found in sequences belonging to haplogroups C1b and C1c. As whole mitogenomes are available for both samples from El Diquecito site, it is possible to determine that they belong to C1b by the presence of the diagnostic mutation at np 493. The co-occurrence of transitions 16092 and 16390 observed in the mitochondrial control region of these ancient C1b samples was not detected in any other individual from South America (García et al., 2021). The motif C1₊₁₆₂₄₈, observed in an undated sample from the *Plains*, was reported in six individuals from Brazil (Cardena et al., 2013; Poletto et al., 2019). Finally, a sample from Ayampitín site, in *Sierras* (600 yBP), presents the motif C1_{+16189–16294}, so far not reported in any South American population.

Ten sequences assigned to haplogroup D1 are reported in Table 1 and Figure 5. Two sequences carried the nodal D1 haplotype (H1 in the network). Four

individuals (two from *Plains* and two from *Sierras*) belonged to the sub-haplogroup D1g (D1₊₁₆₁₈₇) with additional mutations at positions 16189 and 16209 (H9), which are diagnostic of lineage D1g5 (Bodner et al., 2012). D1g5 is mainly distributed in populations from Patagonia, both modern (de Saint Pierre et al., 2012; Gómez-Carballa et al., 2016), and ancient (Arencibia et al., 2019; Crespo et al., 2017, 2020; Motti et al., 2019; Parolin et al., 2019). It was also found in modern populations of Western Argentina (García et al., 2018; Motti, 2012), Central Chile (Gómez-Carballa et al., 2016), and in ancient samples from the Puna in Northwestern Argentina (Postillone et al., 2017) and from the Pampas region (Roca-Rada et al., 2021).

Three ancient individuals from *Plains* belonged to D1j lineage (D1_{+16242–16311}, H6), and one of them exhibited an additional mutation at np 16093 (not displayed on the D1 network since it falls outside the selected range). D1j is very frequent in Central and Western Argentina (Bodner et al., 2012; García et al., 2012, 2018, 2021; Motti, 2012; Pauro, 2015) and was present in ancient samples from Santiago del Estero (Tavella et al., 2020), Patagonia



(Crespo et al., 2017), Paraná Delta (Nores et al., 2019), the eastern Pampa-Patagonia transition (Postillone et al., 2020), and the Pampas region (Roca-Rada et al., 2021). Finally, one individual had the D1 nodal motif plus T16189C (H15), an haplotype only detected before in the current population of Córdoba city (Salas et al., 2008) and in Gran Chaco (Sevini et al., 2014).

4 | DISCUSSION

One of the questions this study aimed to address was whether the cultural differences observed between the groups that inhabited the two main ecological regions of Córdoba Province in the Holocene were correlative to the genetic divergence among them. Our results show significant differences in mtDNA haplogroup frequency and haplotypes distribution between the ancient samples from *Sierras* and *Plains*, mainly explained by the high frequency of Hg B in *Sierras*, where it reaches 39.4% (N = 13), against 2.4% (N = 1) in the *Plains* (Table 2). These genetic differences are clearly reflected in the pairwise distances, calculated from the HVR-I sequences.

The recent report of 92 modern mitogenomes from Central Argentina and 22 ancient mitogenomes from Córdoba Province allowed the description of a large number of new mitochondrial clades, many of which have an ancient local origin and later spreading to surrounding regions. The same study also detected lineages in Central Argentina that are characteristic of other regions, revealing a complex dynamic of gene flow to and from this region (García et al., 2021). In the present work, after confirming the genetic differentiation between *Sierras* and *Plains*, we assessed the comparison of each group separately with ancient and modern populations from Argentina.

While the pre-Hispanic inhabitants of the *Sierras* show genetic affinities with ancient groups of Northwestern Argentina, those who inhabited the eastern *Plains* resemble their neighbors from Santiago del Estero Province and the Pampas region. The genetic dissimilarity between *Plains* and *Sierras*, together with the significant morphological differences between both groups (Fabra & Demarchi, 2013), could indicate the existence of biologically distinct populations inhabiting the mountainous region and the area spanning the eastern plains and the Mar Chiquita lagoon of Córdoba Province during the late Middle and Late Holocene. As mentioned before, differences in some features of their material culture (Serrano, 1945) and, particularly, mortuary practices (Fabra et al., 2009) also support this statement.

Another question we introduced was whether the horticultural groups described by the early colonizers' chronicles replaced the ancient hunter-gatherer

populations or if, instead, there was a biological continuity over time accompanied by a local cultural development. The high incidence of Hg B in *Sierras* in the *post 1200 yBP* subsample (44%, Table 2) was interpreted in a previous work (Nores et al., 2011) as a signal of gene flow into the *Sierras* region from populations with high incidence of Hg B, such as the South-Central Andean region, including modern day Perú, Northern Chile and Northwestern Argentina (Russo, Mendisco, et al., 2018), or the Gran Chaco region (Demarchi et al., 2001). However, our analysis of mtDNA haplogroups and HVR-I sequences does not support this hypothesis, since no genetic structure was observed between temporal subsets. Furthermore, seven of the eight Hg B2 samples from *Sierras* belong to B2ak, and five of them are grouped in the exclusive ancient clade B2ak1a, diagnosed by a mutation in the coding region at np 13145 (García et al., 2021). The distribution and high frequency of lineage B2ak1 (B2₊₁₆₁₄₂) in present-day populations from Central and West-Central Argentina (García et al., 2018; Motti, 2012), as well as its occurrence in the oldest *Sierras* individual (4450 ± 80 yBP), support instead a scenario of local origin with great temporal depth and a later dispersal to Northwestern Argentina. This process would explain the presence of B2ak1 in a later individual of 1300 yBP from Pampa Grande site (Carnese et al., 2010). In line with this hypothesis, the coalescent age for this clade was estimated at 8945 yBP (García et al., 2021).

Four individuals (two from *Plains* and two from *Sierras*) carry the haplotype D1g₊₁₆₁₈₉₋₁₆₂₀₉ (D1g5). This lineage is widely distributed in extant populations of Western, Northwestern and Central Argentina (Motti et al., 2019; Posth et al., 2018). Even though it is also found in Patagonia, this lineage constitutes one of the many widespread D1g haplotypes in that region. The finding of the basal D1g lineage in a Middle Holocene sample (~7800 cal yBP) and a Late Holocene derived D1g5 sample (~1600 cal yBP) was postulated as evidence of the presence of D1g5 in the Pampas before populations bearing these mtDNA lineages migrating southwards (Roca-Rada et al., 2021). Considering that the ages of the D1g5 individuals from this study range between 889 and 387 yBP, we propose that the observed distribution of D1g5 in Central and Western Argentina could be explained by a single demographic event, probably more recent than the spread of the other D1g variants across Patagonia. This scenario is coherent with the estimation of a divergence time of only 6000 years for D1g5 (Roca-Rada et al., 2021).

Three sequences from the *Plains* belong to D1j, a lineage characterized by the transitions T152C, C16242T, and T16311C, which is observed at high frequency in current populations from Central and Western Argentina (García et al., 2012; Motti et al., 2017). Most interestingly, one of

them is the oldest sample included in this study (García et al., 2012), belonging to an individual found at the shores of the Mar Chiquita lagoon who was dated in 4525 ± 20 yBP, which evidences the deep roots of this lineage in the region. This finding was considered additional support for the hypothesis of a local origin for the D1j lineage in West-Central Argentina, based on its present-day distribution (García et al., 2012). However, a recent finding of D1j in an Early Holocene mitogenome from Laguna de los Pampas site (~10 000 cal yBP) points towards an origin in the Argentinian Pampas for D1j, from where it may have spread across the Southern Cone (Roca-Rada et al., 2021), explaining more recent occurrences in Córdoba Province, Patagonia (Crespo et al., 2017), Paraná Delta (Nores et al., 2019), and the eastern transition of the Pampa-Patagonia regions (Postillone et al., 2020).

Finally, we assessed the relationship between the ancient groups and the current populations of the region. On one hand, we found three samples that exhibit the private haplotypes $A2_{+150-16178}$, $A2_{+150-16263}$, and $C1_{+16189-16294}$, so far not found in any modern population, which would suggest they represent extinct ancient lineages. Given that those haplotypes were identified in relatively recent samples (578 and 608 cal yBP), it is possible that they were lost during or closely after the Spaniard conquest. On the other hand, we detected a remarkable similarity in the mitochondrial lineages of the ancient *Plains* individuals and the contemporary populations of Central Argentina, suggesting genetic continuity until the present.

Even though further work incorporating more samples from a wider geographical and temporal range is needed to answer some of the questions raised in this study, it constitutes a step forward towards a better understanding of the maternal evolutionary history of the populations that inhabited Central Argentina during the Middle and Late Holocene.

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CONFLICT OF INTEREST

We declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Rodrigo Nores: Conceptualization-Equal, Data curation-Lead, Formal analysis-Equal, Funding acquisition-Supporting, Investigation-Lead, Methodology-Lead, Project administration-Supporting, Resources-Supporting, Software-Equal, Supervision-Supporting, Validation-Lead, Visualization-Supporting, Writing-original draft-Equal.

María Pía Tavella: Data curation-Supporting, Formal analysis-Equal, Methodology-Supporting, Software-Equal, Visualization-Equal, Writing-original draft-Equal.

Mariana Fabra: Formal analysis-Supporting, Funding acquisition-Supporting, Investigation-Supporting, Resources-Supporting, Writing-original draft-Supporting.

Darío A. Demarchi: Conceptualization-Equal, Data curation-Supporting, Formal analysis-Equal, Funding acquisition-Lead, Investigation-Equal, Methodology-Supporting, Project administration-Lead, Resources-Lead, Software-Supporting, Supervision-Lead, Writing-original draft-Equal.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the article.

REFERENCES

- Ameghino, F. (1885). Informe sobre el Museo Antropológico y Paleontológico de la Universidad Nacional de Córdoba durante el año 1885. *Boletín de La Academia Nacional de Ciencias de Córdoba (República Argentina)*, VIII, 347–360.
- Andrews, R. M., Kubacka, I., Chinnery, P. F., Lightowlers, R. N., Turnbull, D. M., & Howell, N. (1999). Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nature Genetics*, 23(2), 147. <https://doi.org/10.1038/13779>
- Aranda, C., Barrientos, G., & Del Papa, M. (2014). Código deontológico para el estudio, conservación y gestión de restos humanos de poblaciones del pasado. *Revista Argentina de Antropología Biológica*, 16(2), 111–113. <https://doi.org/10.17139/raab.2014.0016.02.05>
- Arencibia, V., Crespo, C. M., García Guraieb, S., Russo, M. G., Dejean, C. B., & Goñi, R. (2019). Análisis genético poblacional de grupos cazadores recolectores del Holoceno tardío del Lago Salitroso (Santa Cruz, Argentina). *Revista Argentina de Antropología Biológica*, 21(2), 4. <https://doi.org/10.24215/18536387e004>
- Bandelt, H. J., Forster, P., & Rohlf, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology*



- and *Evolution*, 16(1), 37–48. <https://doi.org/10.1093/oxfordjournals.molbev.a026036>
- Berberián, E. (2020). Potrero de Garay: una entidad sociocultural tardía de la región serrana de la provincia de Córdoba (República Argentina). *Comechingonia. Revista de Arqueología*, 24(1), 5–68. <https://doi.org/10.37603/2250.7728.v24.n1.28238>
- Bodner, M., Perego, U. A., Huber, G., Fendt, L., Rock, A. W., Zimmermann, B., ... Parson, W. (2012). Rapid coastal spread of first Americans: Novel insights from South America's Southern Cone mitochondrial genomes. *Genome Research*, 22(5), 811–820. <https://doi.org/10.1101/gr.131722.111>
- Bonofiglio, M. M. (2011). Las comunidades productoras de alimentos de la llanura. In E. Berberíán, B. Bixio, M. M. Bonofiglio, M. C. González Navarro, M. Medina, S. Pastor, et al. (Eds.), *Los Pueblos indígenas de Córdoba* (pp. 95–103). Córdoba, Argentina: Ediciones del Copista.
- Bronk Ramsey, C. (2009). Bayesian analysis of radiocarbon dates. *Radiocarbon*, 51(1), 337–360. <https://doi.org/10.1017/s0033822200033865>
- Cabana, G. S., Merriwether, D. A., Hunley, K., & Demarchi, D. A. (2006). Is the genetic structure of Gran Chaco populations unique? Interregional perspectives on native South American mitochondrial DNA variation. *American Journal of Physical Anthropology*, 131(1), 108–119. <https://doi.org/10.1002/ajpa.20410>
- Capitanelli, R. G. (1979). Geomorfología. In J. B. Vázquez, R. A. Miatello, & M. E. Roqué (Eds.), *Geografía Física de la provincia de Córdoba* (pp. 213–296). Editorial Boldt.
- Cardena, M. M. S. G., Ribeiro-dos-Santos, Â., Santos, S., Mansur, A. J., Pereira, A. C., & Fridman, C. (2013). Assessment of the relationship between self-declared ethnicity, mitochondrial haplogroups and genomic ancestry in Brazilian individuals. *PLoS One*, 8(4), e62005. <https://doi.org/10.1371/journal.pone.0062005>
- Carnese, F. R., Mendisco, F., Keyser, C., Dejean, C. B., Dugoujon, J. M., Bravi, C. M., ... Crubézy, E. (2010). Paleogenetical study of pre-Columbian samples from Pampa Grande (Salta, Argentina). *American Journal of Physical Anthropology*, 141(3), 452–462. <https://doi.org/10.1002/ajpa.21165>
- Cattáneo, R., Izeta, A. D., & Caminoa, J. M. (2016). A fishtail projectile point from the southern Pampean Hills, Characato, Córdoba, Argentina. *PaleoAmerica*, 2(3), 274–276. <https://doi.org/10.1080/20555563.2016.1200348>
- Crespo, C. M., Cardozo, D. G., Tessone, A., Vázquez, M., Kisielinski, C., Arencibia, V., ... Dejean, C. B. (2020). Distribution of maternal lineages in hunter-gatherer societies of the southern coast of Tierra del Fuego, Argentina. *American Journal of Physical Anthropology*, 173, 709–720. <https://doi.org/10.1002/ajpa.24107>
- Crespo, C. M., Favier Dubois, C., Russo, M. G., Lanata, J. L., & Dejean, C. B. (2017). First analysis of ancient mtDNA genetic diversity in northern coast of Argentinean Patagonia. *Journal of Archaeological Science: Reports*, 12, 91–98. <https://doi.org/10.1016/j.jasrep.2017.01.011>
- Crespo, C. M., Lanata, J. L., Cardozo, D. G., Avena, S. A., & Dejean, C. B. (2018). Ancient maternal lineages in hunter-gatherer groups of Argentinean Patagonia. Settlement, population continuity and divergence. *Journal of Archaeological Science: Reports*, 18, 689–695. <https://doi.org/10.1016/j.jasrep.2017.11.003>
- Crespo, C. M., Russo, M. G., Hajduk, A., Lanata, J. L., & Dejean, C. B. (2016). Variabilidad mitocondrial en muestras pre-colombinas de la Patagonia Argentina. Hacia una visión de su poblamiento desde el ADN antiguo. *Revista Argentina de Antropología Biológica*, 19(1), 21. <https://doi.org/10.17139/raab.19.1.21>
- de la Fuente, C., Galimany, J., Kemp, B. M., Judd, K., Reyes, O., & Moraga, M. (2015). Ancient marine hunter-gatherers from Patagonia and Tierra del Fuego: Diversity and differentiation using uniparentally inherited genetic markers. *American Journal of Physical Anthropology*, 158, 719–729. <https://doi.org/10.1002/ajpa.22815>
- de Saint Pierre, M., Bravi, C. M., Motti, J. M. B., Fuku, N., Tanaka, M., Llop, E., ... Moraga, M. (2012). An alternative model for the early peopling of southern South America revealed by analyses of three mitochondrial DNA haplogroups. *PLoS One*, 7(9), e43486. <https://doi.org/10.1371/journal.pone.0043486>
- Demarchi, D. A., Panzetta-Dutari, G. M., Motran, C. C., López de Basualdo, M. A., & Marcellino, A. J. (2001). Mitochondrial DNA haplogroups in Amerindian populations from the Gran Chaco. *American Journal of Physical Anthropology*, 115(3), 199–203. <https://doi.org/10.1002/ajpa.1074>
- Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10(3), 564–567. <https://doi.org/10.1111/j.1755-0998.2010.02847.x>
- Fabra, M. (2020). Vivir en los límites del Gran Chaco y la región pampeana: aportes al estudio de las poblaciones de la costa sur de Laguna Mar Chiquita (Córdoba) desde la Arqueología y la Bioantropología. *Revista Del Museo de La Plata*, 5(2), 657–684. <https://doi.org/10.24215/25456377e135>
- Fabra, M., & Demarchi, D. A. (2013). Análisis morfogeométrico aplicado al estudio de los patrones espaciales y temporales de variación morfológica craneofacial en poblaciones del centro de Argentina. *Cuadernos Del Instituto Nacional de Pensamiento Latinoamericano*, 1(1), 87–101.
- Fabra, M., & González, C. (2019). Oral health and diet in populations of Central Argentina during the late Holocene: Bioarchaeological and isotopic evidence. *Latin American Antiquity*, 30(4), 818–835. <https://doi.org/10.1017/laq.2019.69>
- Fabra, M., González, C., & Salega, M. S. (2012). Modos de vida e historia biológica de poblaciones de las sierras y las llanuras de Córdoba (Argentina): aproximaciones desde el registro bioarqueológico. *Revista Argentina de Antropología Biológica*, 14, 87–104.
- Fabra, M., Laguens, A. G., & Demarchi, D. A. (2007). Human colonization of the central territory of Argentina: Design matrix models and craniometric evidence. *American Journal of Physical Anthropology*, 133(4), 1060–1066. <https://doi.org/10.1002/ajpa.20634>
- Fabra, M., Salega, M., & González, C. (2009). Comportamiento mortuorio en poblaciones prehispánicas de la región austral de las Sierras Pampeanas durante el Holoceno. *Arqueología*, 15, 165–186.
- Figueiro, G., Hidalgo, P. C., & Sans, M. (2011). Control region variability of Haplogroup C1d and the tempo of the peopling of the Americas. *PLoS One*, 6(6), e20978. <https://doi.org/10.1371/journal.pone.0020978>
- García, A., Nores, R., Motti, J. M. B., Pauro, M., Luisi, P., Bravi, C. M., ... Demarchi, D. A. (2021). Ancient and modern

- mitogenomes from Central Argentina: New insights into population continuity, temporal depth and migration in South America. *Human Molecular Genetics*, 30(13), 1200–1217. <https://doi.org/10.1093/hmg/ddab105>
- García, A., Pauro, M., Bailliet, G., Bravi, C. M., & Demarchi, D. A. (2018). Genetic variation in populations from Central Argentina based on mitochondrial and Y chromosome DNA evidence. *Journal of Human Genetics*, 63(4), 493–507. <https://doi.org/10.1038/s10038-017-0406-7>
- García, A., Pauro, M., Nores, R., Bravi, C. M., & Demarchi, D. A. (2012). Phylogeography of mitochondrial haplogroup D1: An early spread of subhaplogroup D1j from Central Argentina. *American Journal of Physical Anthropology*, 149(4), 583–590. <https://doi.org/10.1002/ajpa.22174>
- Gómez-Carballa, A., Moreno, F., Álvarez-Iglesias, V., Martín-Torres, F., García-Magariños, M., Pantoja-Astudillo, J. A., ... Salas, A. (2016). Revealing latitudinal patterns of mitochondrial DNA diversity in Chileans. *Forensic Science International: Genetics*, 20, 81–88. <https://doi.org/10.1016/j.fsigen.2015.10.002>
- González, A. R. (1952). Antiguo horizonte precerámico en las Sierras Centrales de la Argentina. *RUNA. Archivo Para Las Ciencias Del Hombre*, 5, 110–133.
- González, C., & Fabra, M. (2019). La muerte, los ritos, lo sagrado: prácticas funerarias entre los habitantes de la costa sur de la Laguna Mar Chiquita y llanuras adyacentes en el holoceno tardío (Córdoba, Argentina). *Jangwa Pana*, 18(3), 445–468. <https://doi.org/10.21676/16574923.3191>
- Hasegawa, M., Di Rienzo, A., Kocher, T. D., & Wilson, A. C. (1993). Toward a more accurate time scale for the human mitochondrial DNA tree. *Journal of Molecular Evolution*, 37(4), 347–354. <https://doi.org/10.1007/BF00178865>
- Hogg, A. G., Heaton, T. J., Hua, Q., Palmer, J. G., Turney, C. S. M., Southon, J., ... Wacker, L. (2020). SHCal20 southern hemisphere calibration, 0–55,000 years cal BP. *Radiocarbon*, 62(4), 759–778. <https://doi.org/10.1017/RDC.2020.59>
- Homburger, J. R., Moreno-Estrada, A., Gignoux, C. R., Nelson, D., Sanchez, E., Ortiz-Tello, P., ... Bustamante, C. D. (2015). Genomic insights into the ancestry and demographic history of South America. *PLoS Genetics*, 11(12), e1005602. <https://doi.org/10.1371/journal.pgen.1005602>
- Kassambara, A. (2018). ggpubr: “ggplot2” Based Publication Ready Plots. R package version 0.1.7.
- Kassambara, A., & Mundt, F. (2017). factoextra: Extract and visualize the results of multivariate data analyses. R package version 1.0.5.
- Laguens, A. G., Fabra, M., Santos, G. M., & Demarchi, D. A. (2009). Palaeodietary inferences based on isotopic data for pre-Hispanic populations of the Central Mountains of Argentina. *International Journal of Osteoarchaeology*, 19(2), 237–249. <https://doi.org/10.1002/oa.1064>
- Lindo, J., Haas, R., Hofman, C., Apata, M., Moraga, M., Verdugo, R. A., ... Di Rienzo, A. (2018). The genetic prehistory of the Andean highlands 7000 years BP through European contact. *Science Advances*, 4(11), eaau4921. <https://doi.org/10.1126/sciadv.aau4921>
- Livi-Bacci, M. (2006). The depopulation of Hispanic America after the conquest. *Population and Development Review*, 32(2), 199–232. <https://doi.org/10.1111/j.1728-4457.2006.00116.x>
- Llamas, B., Fehren-Schmitz, L., Valverde, G., Soubrier, J., Mallick, S., Rohland, N., ... Haak, W. (2016). Ancient mitochondrial DNA provides high-resolution time scale of the peopling of the Americas. *Science Advances*, 2(4), e1501385. <https://doi.org/10.1126/sciadv.1501385>
- Medina, M., Pastor, S., & Berberían, E. E. (2014). “Es Gente Fazil de Moverse de una Parte a Otra”. Diversidad en las Estrategias de Subsistencia y Movilidad Prehispanicas Tardías (Sierras de Córdoba, Argentina). *Complutum*, 25(1), 73–88.
- Mendisco, F., Keyser, C., Hollard, C., Seldes, V., Nielsen, A. E., Crubézy, E., & Ludes, B. (2011). Application of the iPLEXTM gold SNP genotyping method for the analysis of Amerindian ancient DNA samples: Benefits for ancient population studies. *Electrophoresis*, 32(3–4), 386–393. <https://doi.org/10.1002/elps.201000483>
- Mendisco, F., Keyser, C., Seldes, V., Rivolta, C., Mercolli, P., Cruz, P., ... Ludes, B. (2014). Genetic diversity of a late Prehispanic Group of the Quebrada de Humahuaca, northwestern Argentina. *Annals of Human Genetics*, 78(5), 367–380. <https://doi.org/10.1111/ahg.12075>
- Meyer, S., Weiss, G., & Von Haeseler, A. (1999). Pattern of nucleotide substitution and rate heterogeneity in the hypervariable regions I and II of human mtDNA. *Genetics*, 152, 1103–1110.
- Montinaro, F., Busby, G. B. J., Pascali, V. L., Myers, S., Hellenthal, G., & Capelli, C. (2015). Unravelling the hidden ancestry of American admixed populations. *Nature Communications*, 6(1), 6596. <https://doi.org/10.1038/ncomms7596>
- Moraga, M., de Saint Pierre, M., Torres, F., & Ríos, J. (2010). Vínculos de parentesco por vía materna entre los últimos descendientes de la etnia Kawéskar y algunos entierros en los canales patagónicos: evidencia desde el estudio de linajes mitocondriales. *Magallania (Punta Arenas)*, 38(2), 103–114. <https://doi.org/10.4067/S0718-22442010000200006>
- Moreno-Mayar, J. V., Vinner, L., de Barros Damgaard, P., de la Fuente, C., Chan, J., Spence, J. P., ... Willerslev, E. (2018). Early human dispersals within the Americas. *Science*, 362(6419), eaav2621. <https://doi.org/10.1126/science.aav2621>
- Motti, J. M. B. (2012). Caracterización de linajes maternos en la población actual del noroeste y centro-oeste argentinos. Universidad Nacional de La Plata.
- Motti, J. M. B., Hagelberg, E., Lindo, J., Malhi, R. S., Bravi, C. M., & Guichón, R. A. (2015). Primer genoma mitocondrial en restos humanos de la Costa de Santa Cruz, Argentina. *Magallania (Punta Arenas)*, 43(2), 119–131. <https://doi.org/10.4067/S0718-22442015000200006>
- Motti, J. M. B., Muñoz, A. S., Cruz, I., D'Angelo del Campo, M. D., Borrero, L. A., Bravi, C. M., & Guichón, R. A. (2019). Análisis de ADN mitocondrial en restos humanos del Holoceno tardío del sur de Santa Cruz. In A. Gómez Otero, A. Sandoval, & A. Benegas (Eds.), *Arqueología de la Patagonia: El pasado en las arenas* (pp. 493–503). Editorial IDEAUS, CONICET-CENPAT.
- Motti, J. M. B., Schwab, M. E., Beltramo, J., Jurado-Medina, L. S., Muzzio, M., Ramallo, V., ... Bravi, C. M. (2017). Diferenciación regional de poblaciones nativas de América a partir del análisis de los linajes maternos. *Intersecciones en Antropología*, 18(3), 271–282.
- Nakatsuka, N., Lazaridis, I., Barbieri, C., Skoglund, P., Rohland, N., Mallick, S., ... Fehren-Schmitz, L. (2020). A Paleogenomic



- reconstruction of the deep population history of the Andes. *Cell*, 181(5), 1131–1145.e21. <https://doi.org/10.1016/j.cell.2020.04.015>
- Nakatsuka, N., Luisi, P., Motti, J. M. B., Salemme, M., Santiago, F., D'Angelo del Campo, M. D., ... Reich, D. (2020). Ancient genomes in South Patagonia reveal population movements associated with technological shifts and geography. *Nature Communications*, 11(1), 3868. <https://doi.org/10.1038/s41467-020-17656-w>
- Nores, R., Bonomo, M., Pauro, M., & Cabana, G. (2019). Aportes al origen de la entidad arqueológica Goya-Malabrigo según la evidencia paleogenética del Delta Superior del Paraná. In A. G. Laguens, M. Bonnín, B. Marconetto, & T. Costa (Eds.), *Libro de Resúmenes XX Congreso Nacional de Arqueología Argentina: 50 años de arqueologías* (pp. 1803–1804). Universidad Nacional de Córdoba. Facultad de Filosofía y Humanidades.
- Nores, R., & Demarchi, D. A. (2011). Análisis de haplogrupos mitocondriales en restos humanos de sitios arqueológicos de la provincia de Córdoba. *Revista Argentina de Antropología Biológica*, 13(1), 43–54 <https://revistas.unlp.edu.ar/raab/article/view/386>
- Nores, R., Fabra, M., & Demarchi, D. A. (2011). Variación temporal y espacial en poblaciones prehispánicas de Córdoba. Análisis de ADN antiguo. *Revista del Museo de Antropología*, 4(1), 187–194.
- Nores, R., Fabra, M., García, A., & Demarchi, D. A. (2017). Diversidad genética en restos humanos arqueológicos del sitio El Diquecito (Costa Sur, Laguna Mar Chiquita, Provincia de Córdoba). *Revista Argentina de Antropología Biológica*, 19(1), 7–18. <https://doi.org/10.17139/raab.19.1.12>
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., ... Wagner, H. (2019). *vegan: Community Ecology Package*. R package version 2.5-2. Cran R.
- Ongaro, L., Scliar, M. O., Flores, R., Raveane, A., Marnetto, D., Sarno, S., ... Montinaro, F. (2019). The genomic impact of European colonization of the Americas. *Current Biology*, 29(23), 3974–3986.e4. <https://doi.org/10.1016/j.cub.2019.09.076>
- Pääbo, S., Poinar, H., Serre, D., Jaenicke-Després, V., Hebler, J., Rohland, N., ... Hofreiter, M. (2004). Genetic analyses from ancient DNA. *Annual Review of Genetics*, 38(1), 645–679. <https://doi.org/10.1146/annurev.genet.37.110801.143214>
- Parolin, M. L., Cortés, L. I., Basso, N., & Scattolin, M. C. (2020). New evidence of ancient mitochondrial DNA of the southern Andes (Calchaquí valleys, Northwest Argentina, 3,600–1,900 years before present). *Human Biology*, 91(4), 225–247. <https://doi.org/10.13110/humanbiology.91.4.02>
- Parolin, M. L., Galimany, J., Gómez Otero, J., Dahinten, S., Millán, A. G., & Moraga, M. (2019). Primeras secuencias mitocondriales de la región control completa en muestras humanas del Holoceno tardío de la costa norte y centro de Patagonia, Argentina. In J. Gómez Otero, A. Sandoval, & A. Benegas (Eds.), *Arqueología de la Patagonia: El pasado en las arenas* (pp. 469–480). Editorial IDEAUS, CONICET-CENPAT.
- Pastor, S., & Berberían, E. E. (2007). Arqueología del sector central de las Sierras de Córdoba (Argentina). Hacia una definición de los procesos sociales del período prehispánico tardío (900-1573 DC). *Intersecciones en Antropología*, 8, 31–47.
- Pauro, M. (2015). Análisis molecular de linajes uniparentales en poblaciones humanas del centro de Argentina. Universidad Nacional de Córdoba.
- Pauro, M., García, A., Nores, R., & Demarchi, D. A. (2013). Analysis of uniparental lineages in two villages of Santiago del Estero, Argentina, seat of Pueblos de Indios in colonial times. *Human Biology*, 85(5), 699–719. <https://doi.org/10.3378/027.085.0504>
- Perego, U. A., Angerhofer, N., Pala, M., Olivieri, A., Lancioni, H., Kashani, B. H., ... Torroni, A. (2010). The initial peopling of the Americas: A growing number of founding mitochondrial genomes from Beringia. *Genome Research*, 20(9), 1174–1179. <https://doi.org/10.1101/gr.109231.110>
- Piovano, E. L., Ariztegui, D., Córdoba, F., Cioccale, M., & Sylvestre, F. (2009). Hydrological variability in South America below the tropic of Capricorn (Pampas and Patagonia, Argentina) during the last 13.0 ka. In F. Vimeux, F. Sylvestre, & M. Khodri (Eds.), *Past climate variability in South America and surrounding regions. From the Last Glacial Maximum to the Holocene* (Vol. 14, pp. 323–351). Developments in Palaeoenvironmental Research
- Poletto, M. M., Malaghini, M., Silva, J. S., Bicalho, M. G., & Braun-Prado, K. (2019). Mitochondrial DNA control region diversity in a population from Parana state—Increasing the Brazilian forensic database. *International Journal of Legal Medicine*, 133(2), 347–351. <https://doi.org/10.1007/s00414-018-1886-5>
- Polzin, T., & Daneshmand, S. V. (2003). On Steiner trees and minimum spanning trees in hypergraphs. *Operations Research Letters*, 31(1), 12–20. [https://doi.org/10.1016/S0167-6377\(02\)00185-2](https://doi.org/10.1016/S0167-6377(02)00185-2)
- Posth, C., Nakatsuka, N., Lazaridis, I., Skoglund, P., Mallick, S., Lamnidis, T. C., ... Reich, D. (2018). Reconstructing the deep population history of Central and South America. *Cell*, 175(5), 1185–1197.e22. <https://doi.org/10.1016/j.cell.2018.10.027>
- Postillone, M. B., Fuchs, M. L., Crespo, C. M., Varela, H. H., Carnese, F. R., Avena, S. A., & Dejean, C. B. (2017). Linajes maternos en muestras antiguas de la Puna Jujeña. Comparación con estudios de la región Centro-Sur Andina. *Revista Argentina de Antropología Biológica*, 19(1), 16. <https://doi.org/10.17139/raab.19.1.16>
- Postillone, M. B., Martínez, G., Flensburg, G., & Dejean, C. B. (2020). First analysis of mitochondrial lineages from the eastern Pampa–Patagonia transition during the final late Holocene. *American Journal of Physical Anthropology*, 171(4), 659–670. <https://doi.org/10.1002/ajpa.24016>
- Raff, J. A., Bolnick, D. A., Tackney, J., & O'Rourke, D. H. (2011). Ancient DNA perspectives on American colonization and population history. *American Journal of Physical Anthropology*, 146(4), 503–514. <https://doi.org/10.1002/ajpa.21594>
- Raymond, M., & Rousset, F. (1995). An exact test for population differentiation. *Evolution*, 49(6), 1280–1283. <https://doi.org/10.1111/j.1558-5646.1995.tb04456.x>
- Rivero, D. E. (2010). La transición Pleistoceno-Holoceno (11.000 – 9.000 AP) en las Sierras de Córdoba (Rep. Argentina). *Arqueología*, 16, 175–189.
- Rivero, D. E., Cornero, S., Truyol, G., & Neves, W. (2019). Human cranium of Candonga cave site and its implications for the initial peopling of South America. *Homo*, 70(4), 283–295. <https://doi.org/10.1127/homo/2019/1118>
- Roca-Rada, X., Politis, G., Messineo, P. G., Scheifler, N., Scabuzzo, C., González, M., ... Fehren-Schmitz, L. (2021). Ancient mitochondrial genomes from the Argentinian Pampas inform the early peopling of the Southern Cone of South America. *iScience*, 24(6), 102553. <https://doi.org/10.1016/j.isci.2021.102553>

- Russo, M. G., Dejean, C. B., Avena, S. A., Seldes, V., & Ramundo, P. (2018). Mitochondrial lineage A2ah found in a pre-Hispanic individual from the Andean region. *American Journal of Human Biology*, 30, e23134. <https://doi.org/10.1002/ajhb.23134>
- Russo, M. G., Gheggi, M., Avena, S., Dejean, C., & Cremonte, M. (2017). Linajes mitocondriales en muestras de esquina de Huajra (Jujuy, Argentina). Aportes al estudio de la ocupación incaica en la región y la procedencia de sus habitantes. *Revista Argentina de Antropología Biológica*, 19(1), 15. <https://doi.org/10.17139/raab.19.1.15>
- Russo, M. G., Mendisco, F., Avena, S. A., Crespo, C. M., Arencibia, V., Dejean, C. B., & Seldes, V. (2018). Ancient DNA reveals temporal population structure within the South-Central Andes area. *American Journal of Physical Anthropology*, 166(4), 851–860. <https://doi.org/10.1002/ajpa.23475>
- Salas, A., Jaime, J. C., Álvarez-Iglesias, V., & Carracedo, Á. (2008). Gender bias in the multiethnic genetic composition of Central Argentina. *Journal of Human Genetics*, 53(7), 662–674. <https://doi.org/10.1007/s10038-008-0297-8>
- Serrano, A. (1945). Los comechingones. Serie Aborígenes Argentinos Vol. I, Instituto de Arqueología, Lingüística y Folklore, Universidad Nacional de Córdoba.
- Sevini, F., Vianello, D., Barbieri, C., Iaquilano, N., De Fanti, S., Luiselli, D., ... Franceschi, Z. (2014). Human mitochondrial genomes reveal population structure and different phylogenies in Gran Chaco (Argentina). Unpublished. GenBank direct submission.
- Sevini, F., Yao, D. Y., Lomartire, L., Barbieri, A., Vianello, D., Ferri, G., ... Franceschi, Z. A. (2013). Analysis of population substructure in two sympatric populations of Gran Chaco, Argentina. *PLoS ONE*, 8(5), e64054. <https://doi.org/10.1371/journal.pone.0064054>
- Slatkin, M. (1995). A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, 139, 457–462.
- Soares, P., Ermini, L., Thomson, N., Mormina, M., Rito, T., Röhl, A., ... Richards, M. B. (2009). Correcting for purifying selection: An improved human mitochondrial molecular clock. *American Journal of Human Genetics*, 84(6), 740–759. <https://doi.org/10.1016/j.ajhg.2009.05.001>
- Tamura, K., Stecher, G., Peterson, D., Filipowski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30(12), 2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Tavarone, A., Colobig, M. M., & Fabra, M. (2019). Late Holocene plant use in lowland Central Argentina: Microfossil evidence from dental calculus. *Journal of Archaeological Science: Reports*, 26, 101895. <https://doi.org/10.1016/j.jasrep.2019.101895>
- Tavella, M. P., Demarchi, D. A., & Nores, R. (2020). Diversidad genética en restos humanos de contextos arqueológicos tardíos de la provincia de Santiago del Estero a partir del estudio de ADN mitocondrial. *Revista Argentina de Antropología Biológica*, 22(1), 13. <https://doi.org/10.24215/18536387e013>
- Umetsu, K., Tanaka, M., Yuasa, I., Adachi, N., Miyoshi, A., Kashimura, S., ... Osawa, M. (2005). Multiplex amplified product-length polymorphism analysis of 36 mitochondrial single-nucleotide polymorphisms for haplogrouping of east Asian populations. *Electrophoresis*, 26(1), 91–98. <https://doi.org/10.1002/elps.200406129>
- van Oven, M., & Kayser, M. (2009). Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Human Mutation*, 30(2), E386–E394. <https://doi.org/10.1002/humu.20921>
- Wakeley, J. (1993). Substitution rate variation among sites in hyper-variable region 1 of human mitochondrial DNA. *Journal of Molecular Evolution*, 37(6), 613–623. <https://doi.org/10.1007/BF00182747>
- Wrischnik, L. A., Higuchi, R. G., Stoneking, M., Erlich, H. A., Arnheim, N., & Wilson, A. C. (1987). Length mutations in human mitochondrial DNA: Direct sequencing of enzymatically amplified DNA. *Nucleic Acids Research*, 15(2), 529–541. <https://doi.org/10.1093/nar/15.2.529>

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