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Author: Laena Lindahl

Advisor: Dr. Maria Nieves-Colón

The Significance of Ancient Mitochondrial DNA in Establishing Genetic Ancestry

Abstract

Within this study, the mitochondrial genomes of ancient Homo sapiens were analyzed to mark ancestral connections among the ancient people of the Caribbean and Puerto Rico; samples examined were found across Southern and Central America. Literature references house large amounts of genomic data which can be compared to each other. These papers show that mitochondrial DNA can help make connections between groups through maternal haplogroup identification. This project not only compiled and organized this data but gave insight to how mitochondrial DNA can be used to find genetic links among a group of ancient people which can later be compared to modern.

Introduction

The spread of colonialism and imperialistic practices has damaged the cultural and ancestral history of groups within Central and Southern America. Current scientists work to re-establish much of this information through the preparation and analysis of ancient and modern genomic samples that can be put into a comparative database for the continuation of the fields' study[27].

Ancient samples tend to be better preserved in colder environments, so DNA does not deteriorate. Certain areas within the South and Central American regions have conditions that are more likely to house samples that can be analyzed. However, the majority of these areas, including the Caribbean, have a more tropical climate[27]. Due to this, most samples conserved are from less-decomposable structures such as teeth, bones, and, in a few cases, hair. However, remaining DNA can be analyzed. Mitochondrial DNA (mtDNA) is more commonly analyzed than nuclear DNA (nDNA) due to the higher number of copies found in mtDNA, as opposed to the single copy of nDNA. mtDNA offers a representation of the maternal lineage alone; therefore, individuals with the same genomic patterns share a maternal link in ancestry.

Unlike the linear double-helix strands of nDNA, mtDNA is found in a double stranded circular configuration[28]. This circular structure contains multiple regions which are analyzed either separately or as a whole genome. Within this study, whole genome sequencing (WGS) and D-loop regions of the mtDNA were assessed. The D-loop houses the HVRI and HVRII hypervariable regions which, as the name implies, have many different base pairings (bp)

inclusive only to those with similarities in haplogroup: within the 16,569 bp of the mt genome, HVRI is found in positions 16400-16569 and HVRII in 0-400[1,2]. Similarities in the mtDNA genome and D-loop can identify a sample's maternal haplogroup. Southern America usually houses haplogroups A, B, C, and D with diversity in the sequences to further divide them into sub-haplogroups[25].

Carbon dating (C^{14}) dating is also an important concept in these references. C^{14} dating uses the presence of isotope C^{14} to identify when the organism may have died. These ancient beings exchange carbon with the environment while living. Following their death, the amount of carbon begins to decrease according to the half-life of carbon as it is experiences to radioactive decay. Therefore, the smaller the amount of C^{14} detected, the older it is due to the radioactive decay of that isotope in the sample[26]. In terms of this study, though the mtDNA is not an active participant in this calculation, the age of a sample relates to how far the genetic lineage can be traced back in these individuals.

Methods

The data compiled was primarily from studies involving ancient individuals within the Southern and Central American regions. Initially, this study was meant to compare modern samples to that of ancient; however, the majority of time was spent analyzing papers and data from ancient-focused sources.

Literature references were originally found through PubMed, Wiley Online Library, PNAS, Science Advances, Bio One and Science Direct. Some papers were in Spanish and were reviewed by Dr. Nieves-Colón to determine whether they applied to the study. Papers were noted and organized within a google sheet document that labeled each reference by the author/date, region, country in which it was found, the population examined, the date ranges of the sample (found in BC, CE, AD, AP, cal BP, BP and converted to CE/BC format for this study) the kind of data (mtDNA, whole genome, HVRI, HVRII, Y-Chromosome) and finally the accession numbers. Papers that indicated the site where samples were found were used to create a map of ancient samples- the map was constructed in Google's My Maps application. Latitude and longitude numbers were inputted if present in papers; for papers that gave only city/site locations, they were searched and selected based only on name.

Within the papers, figures and supplementary information often held the accession numbers or documents of the samples. Accession numbers could be traced to GenBank, The European Nucleotide Archive (ENA), and EMPOP. These databases hold FASTA and FASTq files which were downloaded and organized within Google drive. However, many papers had a lack of provided accession numbers, accessions which did not work, drive folders without data, or mis-referenced a source. Pre-analyzed haplogroups from 3 sources were compared to find overlap between samples and demonstrate further analysis[3,5,19]. FASTA and FASTq files' base pairings were not analyzed in this study. However, data within papers was acknowledged.

Results

Fig. 1 Anceint mtDNA Sample Map
Map detailing the locations in which ancient samples were found within the literature collected. (Some did not have locations stated in the literature.)
([3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24])

Country	*n*	Reference
Argentina	13	[3, 11, 13]
Peru	195	[3, 11, 13, 18, 19, 24]
Chile	17	[3, 11, 13, 23,]
Bolivia	20	[3, 13]
Mexico	59	[3, 4, 7, 9, 17, 20,]
Ecuador	2	[3]
Costa Rica	10	[4]
Colombia	63	[4, 5, 8,]
Belize	20	[6, 11]
Brazil	49	[10, 11]
Guatemala	41	[12]
Bahamas	44	[14, 15, 16, 22]
Dominican Republic	12	[15]
Cuba	60	[15,16]
Puerto Rico	19	[15,16]
St. Lucia	13	[16]
Guadeloupe	5	[16]
Panama	21	[21]
Total	663	

Table 1: Ancient mtDNA samples by Country
Number of ancient samples (*n*) found within each South/Central American country with corresponding references.

Haplogroup	*n*	Reference
A2	14	[3,5]
B2	26	[3,5,19]
B2b	11	[3]
C1b	27	[3,5,19]
C1c	7	[3,19]
D1	7	[3,19]

Table 2: Haplogroups of Ancient Samples from References
Shows the number of specimens (*n*) that are found within each haplogroup, and which references contribute. Haplogroup list in Appendix 2, Table 2.

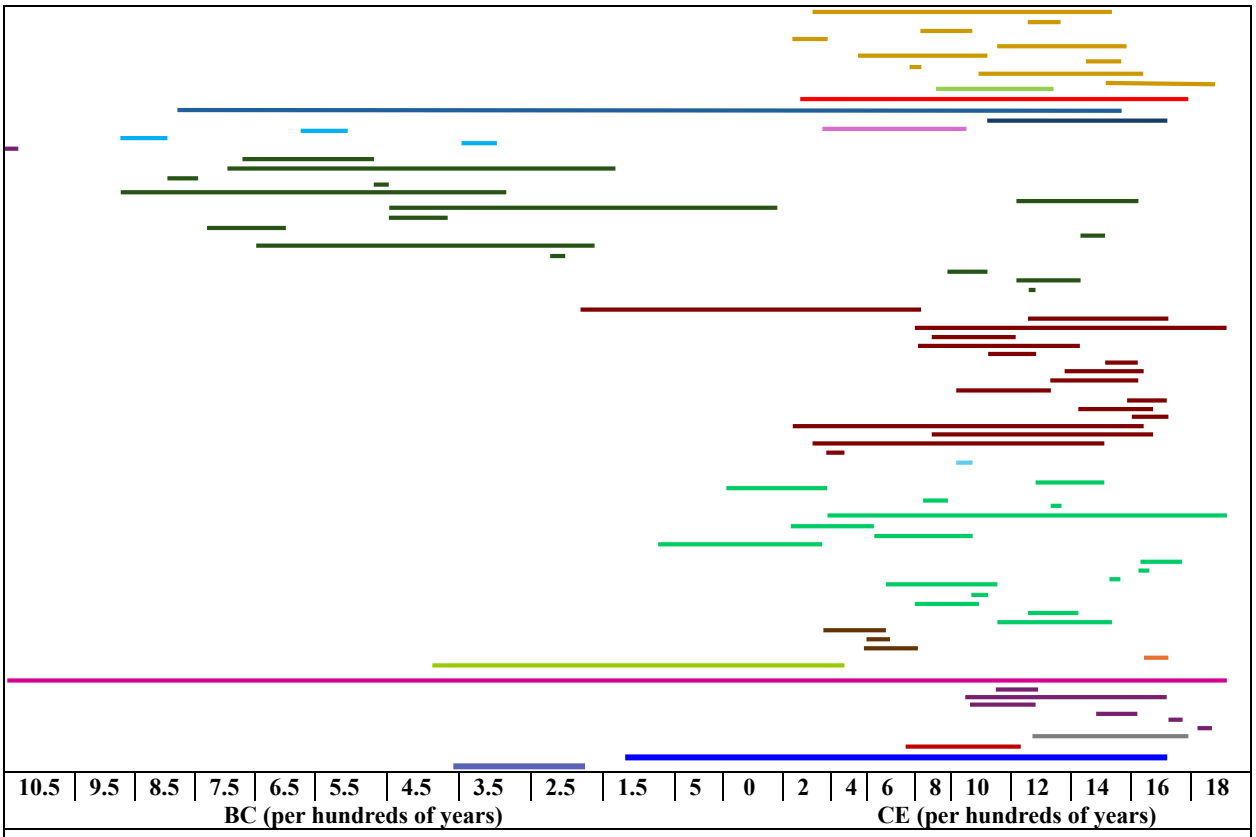

Fig. 2 Calculated Date Range of Ancient Samples from References
Graph showing the calculated age range of ancient samples from reference papers, found by C^{14} dating, in hundreds of years. (BC/CE) [3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24]. Graph color ID in Appendix 1 Table 1a, C^{14} dates in Table 1b.

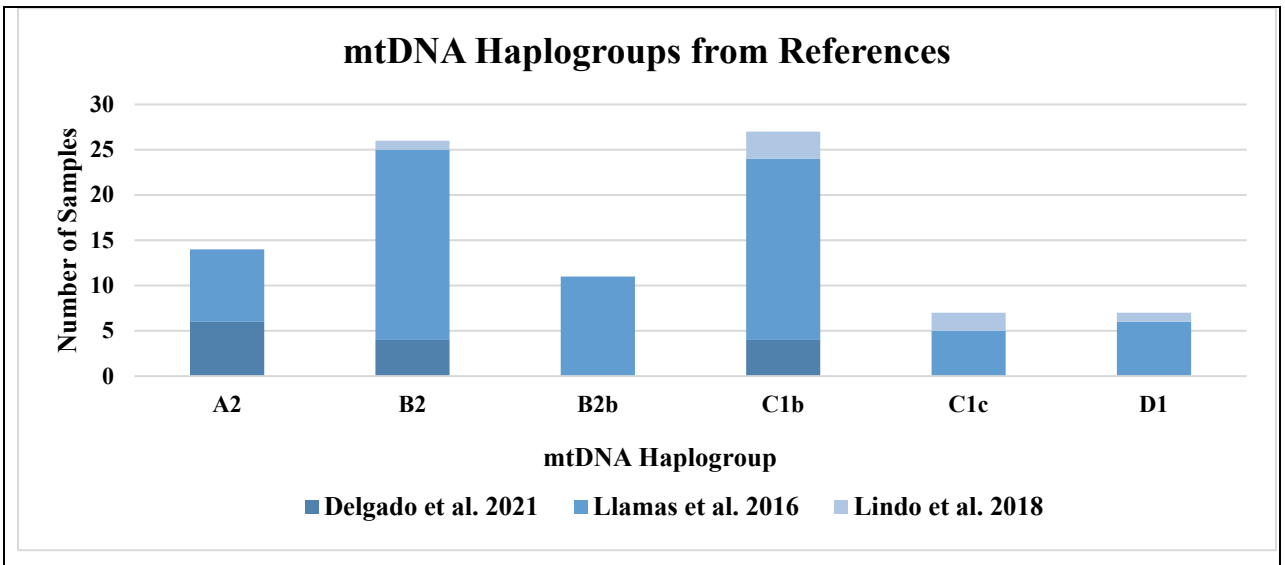

Fig. 3: mtDNA Haplogroups from References
Graph showing Haplogroups A2, B2, B2b, C1b, C1c, and D1 of ancient samples from Llamas et al. 2016, Delgado et al. 2021, and Lindo et al. 2018[3,5,19]. Haplogroup list in appendix Table 2.

Fig. 4: Map of Haplogroup C1b Samples from References
Illustrates the locations of the samples belonging to haplogroup C1b from Llamas et al. 2016, Delgado et al. 2021, and Lindo et al. 2018[3,5,19]. Areas 2 & 3 and 6 & 7 overlap due to proximity of samples' locations.

ID	Location	Year (BC/CE)	*n*	Ref.
1	Coahulia	1000-1600 CE	2	[3]
2	Aguazugue	1596-1275 BC	2	[5]
3	Vista Hermosa	1596-1275 BC	2	[5]
4	Pueblo Viejo	1428-1572 CE	3	[3]
5	Pasamayo	1000-1470 CE	2	[3]
6	Huaca Pucllana	1244-1288 CE 776-968 CE 100-650 CE	10	[3]
7	Jauranga	1000-1600 CE	2	[3]
8	Lake Titicaca	4081BC-320CE	3	[19]
9	Atacama Desert	100-650 CE	1	[3]
10	Llullaillaco	159-1430 CE	1	[3]

Table 3: C1b Haplogroup Sample Locations
ID numbers correspond to the Fig. 4 map to describe the location in which the reference specified samples were found, the C^{14} date calculated (converted to BC/CE), and the number of samples found from Llamas et al. 2016, Delgado et al. 2021, and Lindo et al. 2018[3,5,19]. Table 1b in Appendix 1 has years listed and equation for conversion.

Discussion

A total of 663 ancient samples were compiled from 21 literature references. Table 1 separates the individuals based on their country of discovery while Fig 1 places them on a map depending on the authors' description of where they were found -based on specificity, some samples may not be represented in their exact location. This map helps define how far the genetic lineages can travel and interconnect geographically separated groups. Furthermore, it implies where samples are most commonly found and could be further assessed to find what climatic conditions are most favorable in preserving samples. For instance, the largest number of samples came from Peru, 195, while the lowest from Ecuador, 2; this could be influenced by the annual climate and geographic vicinity to different landmarks. It can also be analyzed in reference to Fig. 2, which displays the possible date ranges of the individuals' deaths from C^{14} dating, to show how successful those conditions may be in terms of the longevity of preservation that climate can assure. While the mtDNA is not directly used to date a sample, this graph displays how far samples can trace genetic lineage to if compared to a modern genome. Chatters et al.

2014 had the oldest sample that was found to be from around 10500 BC while the most recent sample was from Salazar et al. 2023 falling between 1420-1532 CE.

Small-Scale Data Analysis

The use of mtDNA to determine genetic lineage is based highly on pattern recognition. When the samples are compiled, the base pairings found in the genome can be matched to another individual. From these, ancient samples alone will undergo haplotyping that will categorize them in connection to other samples within that group. To demonstrate, the haplogroupings shown in Fig 3 from Delgado et al. 2021, Lindo et al. 2018, and Llamas et al. 2016 indicate how samples can be further grouped by their genetics. Only 3 references were analyzed to show how haplotypes are necessary in the process of defining genetic lineage, this study is not meant to completely interconnect the data. Samples within each haplogroup, A2, B2b, C1b, C1c, and D1 are connected in ancestry (full list in Appendix Table 1b). Haplotyping modern individuals can indicate which ancient individuals they are related to.

In this case, many of the samples found among the references could be analyzed against each other and connections could be made. The samples shown in Fig. 4 are samples from the 3 sources which have the same haplotype: C1b. Corresponding to this fig., Table 3 defines each of these samples by their C^{14} calculated date range to show how generations of the same ancestry are widely spread geographically as well as over time. The oldest location is 8, Lake Titicaca, with 3 samples in the years 4081BC-320 CE[19]. This is a very wide range that overlaps with most of the other samples. It is possible that an ancestor traveled to begin a founder's effect of that genetic line in a new geographic area while others remained until a later time; however, based solely on the aDNA, this cannot be proved. The samples from Delgado et al. 2021, 2 and 3, are slightly younger in the range of 1596-1275 BC[5]. This could be the first settlement of the line from 8 since it is significantly closer to site 1. To further show the movement of this lineage, more samples' haplogroups would need to be identified as C1b and dated to illustrate the likely movement of them over time. Moreover, modern people within these areas could be genetically tested to see if there was a connection between them and the older samples. Comparing their genetics as well as the confirmed ages of the individuals could make a clearer map of the movement of that line and its connection with other groups. These samples show how haplogrouping can give insight to not only the familial connections but hypotheses of how those individuals spread through Southern/Central America both physically and genetically.

Availability of Reference Data

The availability of data sets was a highly contributing factor to this study as well as any comparative study. Many of the papers had data which was not available on any of the websites or databases. Others required contacting the author directly which led to awaiting a response. It's clear that in analytical research, there is a heavy reliance on other authors to publish their actual data rather than their lone analysis of it. Many of the databases were not updated to include more recent studies; as techniques regarding the replication of aDNA and a more thorough analysis are

being discovered, it's important that papers exercising them are available. There is also a lack of overlap between databases with FASTA files. ENA and GenBank were primarily used in this study and, depending on the author, they published sporadically on either or both.

Continuation of Study

While the genetic connection of the ancient individuals is important in analyzing the possible movements or interactions of certain homo sapiens within a lineage, it can be further assessed through a comparative study to modern individuals. This would be done by laying out the base pairings available and/or finding similarities in haplotypes that could distinguish one genetic line from another. Certain geographical areas could be targeted according to the hypothesized movement of ancient individuals.

Acknowledgments

I'd like to thank Dr. Maria Nieves-Colón not only for allowing me to be a part of her research, but for her incredible patience with me as I worked on this literature search and inspiring my interest in anthropology through her work. I'd also like to thank Dr. Jessica Stone and Ms. Nithya Malisetti for helping me through this process and answering my endless, if not repetitive, questions. Finally, thank you to all of those whose papers gave me the data in this study and whose analysis taught me not only about the genetic applications of it but made me appreciate how it could show ancient culture and lifestyle practices.

Appendix 1

Table 1a: Color Key for Fig. 2	
Llamas et al. 2016	
Morales-Arce et al. 2017	
Uricoechea Patino et al. 2023	
Ferraz et al. 2023	
Morales Arce et al. 2019	
Verdugo et al. 2020	
Delgado et al. 2021	
Chatters et al. 2014	
Posth et al. 2018	
Nakatsuka et al. 2020	
Schroeder et al. 2018	
Nagele et al. 2020	
Mizuno et al. 2023	
Salazar et al. 2023	
Lindo et al. 2018	
Raghavan et al. 2015	
Rosario Capodiferro et al. 2021	
Forbes-Pateman et al. 2022	
De la Fuente et al. 2018	
Fernandes et al. 2020	
Kennet et al. 2022	

Note: Most samples were separated by geographic location and dates given were generalized over all samples included (lines on the graph do not represent one individual but rather however many were found at that site). Both Raghavan et a. 2015 and Ferraz et al. 2023s' lines on the graph were composed of multiple samples which were not separated.

Table 1b: C^{14} Date Ranges for Ancient Samples (BC/CE) in Fig. 2	
Reference	C^{14} Calculated Date Ranges
De la Fuente 2018	630-1040 CE
Forbes-Pateman et al. 2022	1000-1600 CE
Rosario Capodiferro et al. 2021	870-1030 CE, 870-1519 CE, 1430-1270 CE, 1519-1540 CE, 1626-1671 CE
Raghavan et al. 2015	10651 BC-1750CE
Lindo et al. 2015	4081BC-320 CE
Salazar et al. 2023	1420-1532 CE
Nagele et al. 2020	500-710 CE, 1090-1300 CE, 181 BC- 250 CE, 660-770 CE, 380-1750 CE, 130-420 CE, 450–850 CE, 581 BC – 250 CE, 1430- 1550 CE, 1420 CE, 1330 CE, 490-930 CE, 880-1260 CE, 610-880 CE, 1020-1230 CE, 930-1330 CE

Fernandes et al.	1151 BC – 1550 CE
Schroeder et al. 2018	839-897 CE
Nakatsuka et al. 2020	600-1270 CE, 1000-1530 CE, 690-1775 CE, 700-1000 CE, 650-1200 CE, 985- 1045 CE, 1325- 1435 CE, 1175-1410 CE, 1100-1450 CE, 1400-1560 CE, 470-1030 CE, 800-1100 CE, 1285-1485 CE, 1438-1533 CE, 100-1470 CE, 750-1400 CE, 200-1390 CE, 250-385 CE
Kennet et al.	3651-1751 BC
Posth et al. 2018	8211-7521 BC, 7291- 1231 BC, 7011- 5001 BC, 5011- 4701 BC, 7481-5361 BC, 4951- 3651 BC, 411-BC – 870 CE, 1040-1410 CE, 1230-1380 CE, 6781-1501 BC, 2281- 2031 BC, 790-990 CE, 1030-1220 CE, 1040-1210 CE, 1070 CE
Ferraz et al. 2023	8051 BC – 1550 CE
Morales-Arce et al. 2019	900-1520 CE
Uricoechea Patino et al. 2023	100-1600 CE
Chatters et al. 2014	10500 BC
Verdugo et al. 2020	250-1000 CE
Delgado et al. 2021	7058-6265 BC, 4319-3573 BC, 1596-1275 BC
Morales-Arce et al. 2017	800-1250 CE
Mizuno et al. 2023	250-546 CE, 425-546 CE, 417-636 CE
Llamas et al. 2016	1430-159 CE, 1244-1288 CE, 776-968 CE, 100-650 CE, 1000-1476 CE, 1428-1572 CE, 1000-1470 CE, 850 CE, 325-440 CE, 400-1000 CE, 1000-1600 CE

Note: Some samples were found in forms other than BC/CE, cal BP or BP. To make comparisons easier, they were converted to BC/CE using the following equations:

If (cal) BP > yr. 1950 use: year (BP) – 1949

If (cal) BP < yr. 1950 use: 1950 - year (BP)

Appendix 2

Table 2: mtDNA Haplogroups from Sources		
Sample ID from reference	mtDNA haplogroup	Reference
IL2	D1	Lindo et al. 2018
IL3	B2	Lindo et al. 2018
IL4	C1b	Lindo et al. 2018
IL5	C1c	Lindo et al. 2018
IL7	C1b	Lindo et al. 2018
K1	C1b	Lindo et al. 2018
SMP5	C1c	Lindo et al. 2018

LP28.2	D1	Llamas et al. 2016
LP28.3	C1b	Llamas et al. 2016
LP13.1	C1b	Llamas et al. 2016
LP13.2	B2	Llamas et al. 2016
LP13.3	C1b	Llamas et al. 2016
LP13.4	B2b	Llamas et al. 2016
LP13.5	D1	Llamas et al. 2016
LP13.6	C1b	Llamas et al. 2016
LP20.1	B2b	Llamas et al. 2016
LP13.7	B2	Llamas et al. 2016
LIB2*	B2b	Llamas et al. 2016
LP13.8	C1b	Llamas et al. 2016
LP17.1	C1b	Llamas et al. 2016
LP40.1	B2b	Llamas et al. 2016
LP40.2	B2b	Llamas et al. 2016
LP17.2	B2b	Llamas et al. 2016
LP17.5	C1b	Llamas et al. 2016
LP40.3	C1b	Llamas et al. 2016
LP17.6	C1b	Llamas et al. 2016
LP17.7	C1b	Llamas et al. 2016
LP17.8	A2	Llamas et al. 2016
LP40.4	A2	Llamas et al. 2016
LP20.3	B2b	Llamas et al. 2016
LP20.4	A2	Llamas et al. 2016
LP14.1	B2b	Llamas et al. 2016
LP20.5	B2	Llamas et al. 2016
LP14.2	D1	Llamas et al. 2016
LP20.6	C1c	Llamas et al. 2016
LP20.7	B2	Llamas et al. 2016
LP20.8	C1b	Llamas et al. 2016
LP14.5	C1b	Llamas et al. 2016
LP22.5	B2	Llamas et al. 2016
LP22.6	B2	Llamas et al. 2016
LP22.8	B2	Llamas et al. 2016
LP41.1	B2	Llamas et al. 2016
LP41.2	C1b	Llamas et al. 2016
LP41.3	C1b	Llamas et al. 2016
LP41.4	C1b	Llamas et al. 2016
LP41.5	B2	Llamas et al. 2016
LP41.6	B2	Llamas et al. 2016
LP41.7	B2	Llamas et al. 2016
LP41.8	B2b	Llamas et al. 2016
LP41.9	D1	Llamas et al. 2016
LP41.10	B2b	Llamas et al. 2016
LP41.11	C1c	Llamas et al. 2016

LP40.5	D1	Llamas et al. 2016
LP14.6	A2	Llamas et al. 2016
LP22.1	B2	Llamas et al. 2016
LP40.7	C1b	Llamas et al. 2016
LP14.7	B2b	Llamas et al. 2016
LP14.8	C1c	Llamas et al. 2016
LP22.2	C1b	Llamas et al. 2016
LP5.1	D1	Llamas et al. 2016
LP5.2	A2	Llamas et al. 2016
LP5.3	A2	Llamas et al. 2016
LP5.4	A2	Llamas et al. 2016
LP6.1	B2	Llamas et al. 2016
LP27.5	C1b	Llamas et al. 2016
LP15.2B	C1c	Llamas et al. 2016
LP27.2	B2	Llamas et al. 2016
LP15.3B	B2	Llamas et al. 2016
LP15.4B***	C1c	Llamas et al. 2016
LP15.6B	A2	Llamas et al. 2016
LP15.7B	B2	Llamas et al. 2016
LP27.3	B2	Llamas et al. 2016
LP40.10	B2	Llamas et al. 2016
LP12.1	C1b	Llamas et al. 2016
LP12.2	B2	Llamas et al. 2016
LP12.4	B2	Llamas et al. 2016
LP12.5	B2	Llamas et al. 2016
LP12.6	C1b	Llamas et al. 2016
TE03	A2	Delgado et al. 2021
TE13	B2	Delgado et al. 2021
UB3	A2	Delgado et al. 2021
AZ77	A2	Delgado et al. 2021
AZ75	B2	Delgado et al. 2021
AZ21	C1b	Delgado et al. 2021
AZ66	B2	Delgado et al. 2021
AZ68	C1b	Delgado et al. 2021
AZ46	A2	Delgado et al. 2021
AZ48	A2	Delgado et al. 2021
VIS3	B2	Delgado et al. 2021
VIS4	C1b	Delgado et al. 2021
VIS5	C1b	Delgado et al. 2021
AZ32	A2	Delgado et al. 2021
AZ33	B2d	Delgado et al. 2021

Note: Some samples/haplotypes were removed as they were not fully defined by haplogroup or sub-haplogroup[3,5,19].

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