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BRIEF COMMUNICATION

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Ancient mitochondrial DNA and ancestry of Paquimé inhabitants, Casas Grandes (A.D. 1200–1450)

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Abstract

Objectives: The Casas Grandes (Paquimé) culture, located in the Northwest of Chihuahua, Mexico reached its apogee during the Medio Period (A.D. 1200–1450). Paquimé was abandoned by the end of the Medio Period (A.D. 1450), and the ancestry of its inhabitants remains unsolved. Some authors suggest that waves of Mesoamerican immigrants, possibly merchants, stimulated Paquimé's development during the Medio Period. Archaeological evidence suggests possible ties to groups that inhabited the Southwestern US cultures. This study uses ancient DNA analysis from fourteen samples to estimate genetic affinities of ancient Paquimé inhabitants.

Materials and methods: DNA was extracted from 14 dental ancient samples from Paquimé. PCR and Sanger sequencing were used to obtain mitochondrial control region sequences. Networks, PCoA, and Nei genetic distances were estimated to compare Paquimé haplotypes against available past haplotypes data from Southwestern and Mesoamerican groups.

Results: Haplogroups were characterized for 11 of the samples, and the results revealed the presence of four distinct Amerindian mitochondrial lineages: B ($n = 5$; 45%), A ($n = 3$; 27%), C ($n = 2$; 18%) and D ($n = 1$; 10%). Statistical analysis of the haplotypes, haplogroup frequencies, and Nei genetic distances showed close affinity of Paquimé with Mimbres.

Discussion: Although our results provide strong evidence of genetic affinities between Paquimé and Mimbres, with the majority of haplotypes shared or derived from ancient Southwest populations, the causes of cultural development at Paquimé still remain a question. These preliminary results provide evidence in support of other bioarchaeological studies, which have shown close biological affinities between Paquimé and Mimbres, a Puebloan culture, in the Southwestern US.

KEYWORDS
Medio Period, Mesoamerica, migration, paleogenetics, Southwest US

1 | INTRODUCTION

The Casas Grandes (Paquimé) culture, located in the Northwest of Chihuahua, Mexico, reached its apogee during the Medio Period (A.D. 1200-1450) (Di Peso, 1974; VanPool and VanPool, 2007). Notable pieces of architecture such as ceremonial platforms, ball courts, great wall houses and aqueducts, as well as other archaeological materials such as polychrome pottery, characterize Paquimé's archaeological remains. Additionally, significant amounts of marine shells, copper bells, pieces of turquoise and other faunal remains suggest that this cultural center acted as an important trading hub (Di Peso, 1974; Minnis and Whalen, 2015; VanPool and VanPool, 2007; Whalen, 2013).

Paquimé was abandoned by the end of the Medio Period (A.D. 1450), under unknown circumstances (Phillips and Gamboa, 2015). The ethnohistoric record suggests that Paquimé's inhabitants moved to the north to join the Puebloan groups after a violent conflict with Western groups, such as the Sonoran Opata, which according to Riley (1985) was the largest indigenous group that once lived in what is now Sonora, Mexico. Oral accounts collected by Bandelier indicated that Casas Grandes people were enemies with the Opatans, which had

destroyed some of the Casas Grandes towns (Deeds, 1981; Yetman, 2010). Some authors argue that rather than conflict with another group, internal factors such as social class tensions could have initiated political instability, resulting in interpersonal violence and finally the abandonment of Paquimé (Gamboa, 2008; Phillips and Gamboa, 2012, 2015; Whalen and Minnins, 2012).

1.1 | Insights into Paquimé's population ancestry

Di Peso and colleagues (1974) suggested that waves of Mesoamerican immigrants, possibly merchants, stimulated Paquimé's development during the Medio Period. Based on material culture, Di Peso (1974) suggested that immigrants possibly came from Tula. Located around 80 kilometers northwest of modern Mexico City, Tula was the capital of the Toltec civilization and probably the most influential center in central Mexico between A.D. 750-1200 (Healan and Cobean, 2012), which predates the Medio period at Paquimé. Evidence of human sacrifice, elaborate elite burials, and the ritual use of human bone at Paquimé remain some of the most convincing evidence of religious influences by Tula as Mesoamerican center (Di Peso, 1974; McGuire, 2012).

In addition, Paquimé includes evidence of scarlet macaw breeding (*Ara macao*), with presumed trade from the tropical lowlands of Mexico, demonstrating a persuasive connection with Mesoamerican cultures (Di Peso, 1974). Macaw feathers were important in rituals among Southwestern groups, and Paquimé played a central role in their production and distribution (Hargrave, 1970; Minnis et al., 1993). Furthermore, mathematical and astronomical models of construction, as well as I-shaped ball courts at Paquimé were similar to those in Mesoamerica, but infrequent further north, suggesting a Mesoamerican influence at the site (Di Peso, 1974; Naylor, 1995; Ríos and Luján, 2015; Wilcox, 1991).

Despite these cultural corollaries, it is possible that goods circulated in a trade route with Mesoamerican groups rather than a significant migration before the Medio Period. The presence of foreign objects is not a conclusive indicator of population movements and even less convincing for posterior Paquimé cultural development (Punzo and Villalpando, 2015).

Shaffer (1999) suggests that Southwestern groups in the present day U.S. shared common mortuary practices, architecture, ceramics, and general symbolic and ceremonial behaviors. Lekson (1999b) argues that Paquimé's archaeological remains, such as settlement patterns and massive terraced architecture, are typical of the Pueblo groups; which refers to settled groups inhabiting what is today New Mexico, that are historically linked to the Hopi, Zuni, Acoma, Laguna, or several Rio Grande tribes (Lekson, 1999b). Lekson's Chaco Meridian hypothesis suggests possible ties to groups that inhabited the Southwest (Lekson, 1999a; Skibo et al., 2002). Also, Di Peso (1974) reported black-on-white pottery, typical of the Mimbres culture, at the Paquimé site before the Medio period (Di Peso, 1974; Whalen and Minnis, 2003). Additionally, the presence of imported macaws from Mexican lowlands at Mimbres between A.D. 1000-1200 suggests that ties to the south were well established before Paquime's apogee (Creel and McKusick, 1994).

LeBlanc et al.'s (2008) re-evaluation of the alleged connection of the Mimbres with the Chihuahuan Casas Grandes (Paquimé) population weakened the potential biological relationship between the regions. However, the relationship between the Mimbreños and Casas Grandes population continues to be relevant, particularly as the Mimbres population may have moved, at least in part, to the Casas Grandes region after the Mimbres valley was abandoned around A.D. 1130. The succeeding Black Mountain phase in the Mimbres region bears many similarities to the cultural remains of the Chihuahuan population, and Shafer (1999) has suggested that the Black Mountain phase was part of the Chihuahuan interaction sphere.

Archaeologists have studied Paquimé from many perspectives to understand the cause of its collapse and the fate of its people. This study uses ancient DNA (aDNA) analysis to add another window through which to view ancient Paquimé. The study of past human population movements through aDNA has recently become possible. Specifically with mitochondrial DNA haplotypes, questions about ancestry can be addressed and enrich the archaeological discussion.

1.2 | The ancient genetic landscape of the southwest and mesoamerica

The genetic history of native populations in the Americas has focused on mitochondrial DNA studies in modern groups. In recent decades, such studies have been enriched by the possibilities of conducting ancient DNA studies, which have led to changes in previous hypotheses about the genetic structures of prehistoric populations (Handt et al., 1994; Pääbo et al., 2004; Rasmussen et al., 2014). Most of the mitochondrial DNA of native groups in the Americas is divided among five haplogroups: A2, B2, C1, D1 and X2, and their derived variants (Fagundes et al., 2008; Horai et al., 1993; Torroni and Wallace, 1995).

For the Southwest, ancient DNA studies based on 139 individuals from twelve different archaeological sites have revealed that haplogroups B, C, and D, were most prevalent in the region (Carlyle et al., 2000; Raff et al., 2011; Snow et al., 2011). One of the populations studied with aDNA is that which inhabited the Mimbres Valley: NAN Ranch Ruin (Shafer, 2003), Cameron Creek (Bradfield, 1931), Swarts Ruin (Cosgrove and Cosgrove, 1932), the Harris Site (Haury, 1936), and Treasure Hill (Cogrove, 1923). This area is located in what is now southwestern New Mexico, during A.D. 200–1130, and its inhabitants produced the distinctive pottery referred to as black-on-white. Unfortunately, at the end of this period, the Mimbres population vanished, and its fate remains unknown (Shaffer, 1999). However, aDNA from skeletal material has shown that Mimbres' population was closely related to ancient Puebloan groups, such as those from the Tommy Site, a Chacoan outlier outside of Farmington, New Mexico (Snow et al., 2011). The Mimbres valley is geographically close to Paquimé (Figure 1), and could have been genetically similar. Of particular interest, the Mimbres' decline seems to correlates with the rise of Paquimé.

In general, past Mesoamerican haplogroup frequencies for the Postclassic period show a higher frequency of haplogroups A, followed by B, although they vary from 47 to 65% and 10 to 37% respectively (Kemp et al., 2005; Solórzano, 2006). Based on ancient DNA analyses

FIGURE 1 Geographic distribution of aDNA samples discussed in the text. Map drawn by Adam K. Benfer

in Mesoamerica, on average haplogroup A shows the highest frequency (53%), followed by C (18%). These average estimates may be biased by the findings in Copan, which showed a frequency of 89% for haplogroup C (Merriwether et al., 1997). This is not the case for Central Mexico where haplogroup C fluctuates between 4% and a maximum of 8% (Kemp et al., 2005; Merriwether et al., 1997).

Although Mesoamerican population dynamics have changed dramatically over the last 500 years, the remaining groups reflect high diversity and differentiation; these populations are still showing high levels of ancestral isolation and genetic drift (Moreno-Estrada et al., 2014). The haplogroup frequencies obtained by ancient DNA studies in Mesoamerica reflect mitochondrial haplogroup differences between prehistoric populations' genetic makeup, but could also be affected by sampling bias. The distribution of variants by temporal period in earlier Central Mexico is also unclear, and this is another limitation and may lead to partial temporal sampling.

It should be kept in mind that ancient Mesoamerican genetic structure should be addressed cautiously, since its definition and distribution could have changed respective to modern times. Nowadays, 291 Mesoamerican ethnic groups remain in a vast geographic region that ranges from northern Mexico to southern Central America (Braswell et al., 2002; Carmack and Salgado-González, 2006; Pailes and Whitecotton, 1995). The exact prehistoric Mesoamerican population distribution and movements remain unclear, especially at border regions (McCafferty et al., 2012; Snow et al., 2011).

Based on modern genetic data there are two major differences between Southwest and Mesoamerican groups: the frequency of haplogroup A tends to decrease in the Southwest and to increase in Mesoamerica, while haplogroup B tends to present an opposite distribution (Snow et al., 2011, Gorostiza et al., 2012). It seems that a clinal distribution of these two haplogroup frequencies is evident in modern groups such as Cora, Huichol and Tarahumara, and the Akimel and Tohono O'odham (Kemp, 2006; Malhi, 2003). It would be expected that this is the tendency for ancient groups inhabiting the region. There are exceptions, however, as seen at the Mine Canyon site in the Southwest, which shows a high frequency of haplogroup A (Snow et al., 2011).

Despite limitations with contamination issues or sample bias due to variation in skeletal material preservation, ancient DNA studies become even more relevant in revealing insights of the genetic structure of populations. Populations with a shared ancestry and/or living in geographical proximity are expected to display similar haplotypes that are grounded by relatedness into haplogroups (Emery et al., 2015). The

haplotypes and haplogroups facilitate studies of population origins, genetic structure, and gene flow (Underhill and Kivisild, 2007).

Given that the ancestral origin of the people that inhabited Paquimé city persists as an object of study (Phillips and Gamboa, 2015), the aim of this study is to test genetic similarities from Paquimé skeletal remains to observe maternal lineages in relation to ancient Mesoamerican or ancient Southwestern groups. Inasmuch as the geographical location of Paquimé is closer to the Southwest cultural area, we hypothesize that the inhabitants of this center would carry similar mitochondrial haplogroup frequencies to ancient southwestern groups. Ancient mitochondrial DNA could add valuable information to understanding ancestral links of the people living in Paquimé.

2 | MATERIALS AND METHODS

DNA was extracted from 14 dental samples from Paquimé to explore possible biological affiliations of Paquimé inhabitants during the Medio Period. Extraction was conducted in a dedicated aDNA laboratory at the University of Calgary. The tooth samples were collected from Museo de las Culturas del Norte, INAH Chihuahua, as part of a larger project on Casas Grandes bioarchaeological research at the University of Calgary. Preparation and DNA extraction of samples followed strict protocols for contamination control and detection, including positive air pressure, the use of protective clothing, UV sources for workspace decontamination, and laminar flow hoods for extraction and PCR set-up, and separated pre- and post-PCR (Polymerase Chain reaction) areas.

For surface decontamination, samples were submerged in 6% sodium hypochlorite for 10 minutes, rinsed twice with ultrapure water, and irradiated with 254-nm ultraviolet light for 30 minutes per side before being crushed into powder using a vice. The powder was transferred to a 15 ml tube and incubated at 50°C while shaking gently overnight in 5 ml of extraction solution (0.5M EDTA pH8, 0.25% SDS, 0.5 mg/ml proteinase K). Following this, DNA was extracted using the silica spin column protocol (Yang et al., 1998), and finally was eluted with a 50 ul TET buffer to continue with the PCR reaction. At the end of the process, two DNA extracts were obtained, from only one tooth, which were used as templates to conduct PCR reactions.

We examined the resulting ancient control region mitochondrial DNA, specifically at the hypervariable regions I and II, covering approximately 300 bp per segment, targeting haplogroup-determining variants. For the PCR amplifications, eight overlapping primer pairs were used: F15989 - R16158, F16112 - R16251, F16190 - R16322, F16268 - R16410, F34 - R159, F109 - R240, F151 - R292, and L220 - R377. All primers design followed Gabriel et al. (2001) except for R16251 [5′-GGA GTT GCA GTT GAT GT-3′]. The PCR reaction volume was 30 μl containing 50 mM KCL and 10 mM Tris-HCL, 2.5 mM MgCl2, 0.2 mM dNTP, 1.5 mg/mL BSA, 0.3 μM each primer, 4-6 μl DNA sample and 2.25-3.75U AmpliTaq Gold™. Each PCR reaction utilized a minimum of 4 μl; samples that failed to amplify with that amount of DNA were reamplified using an additional 1 μl of template; the maximum of DNA extract utilized was 6 μl. Reactions were amplified for 40 cycles and amplified fragments of ~140 bp. The conditions of PCR amplification were as follows: the initial denaturing took place at 95°C for 12 min, followed by 40 cycles at 95°C for 30 sec, 50°C for 30 sec, 72°C for 60 sec followed by a final extension at 72°C.

Electrophoresis on 2% agarose gels was used to visualize positive amplifications of targeted fragments. PCR products were sequenced using forward and/or reverse primers at Eurofins Genomics, Louisville, KY. The obtained sequences were visually edited using ChromasPro software (www.technelysium.com.au), and truncated to remove primer sequences. Edited sequences were compared by alignments against the Cambridge reference (rCRS NC_012920), through BioEdit 7.2.5 software (www.mbio.ncsu.edu/BioEdit).

Haplogroups determination and phantom mutations were estimated with the software HaploGrep (Kloss-Brandstaetter et al., 2010). Phantom mutations are systematic artifacts generated in the course of the sequencing process. The number of artifacts depends on the sort of automated sequencer and sequencing chemistry employed, as well as other lab-specific factors (Brandstätter et al., 2005). Confirmation of haplogroups was based on the presence/absence of determining haplotype variants following O'Rourke and Raff (2010).

For further contamination control, extraction blanks were included during all processes and PCR reactions, which always tested negative for contamination. To assure that the results were replicable, sequenced samples underwent repeat extraction and amplification. Cloning was not undertaken in this study as repeat amplification and sequencing were capable of identifying and resolving damage-induced ambiguities. Further analysis at the sequence level was necessary to exclude any modern DNA that survived the decontamination treatments. During sequence analysis, authentic ancient DNA showed cytosine deamination, since changing variants did not correspond to any expected mutation in Native American groups. Repeat amplifications and sequencing solved the most common form of miscodings, C-T and G-A transitions, and did not interfere with haplotype assignment. In addition, mitochondrial control region was sequenced for all individuals working in the laboratory space to allow for identifying potential contaminants. Finally, the samples two, three, four, and six (Table 1) were replicated at the ancient DNA laboratory of the University of Montana, Missoula. For the extraction process the same protocol described here was used, however amplification, sequencing, and haplogroups assigment followed Snow et al. (2010).

Analysis of the results included, first, the genetic affinity estimated between Paquimé and other past contemporaneous populations from the surrounding regions. For such a comparison, populations were expected to share appropriate data such as base pair range in HVR-I (16112-16410 bp), and time period encompassed (Mata-Míguez et al., 2012; Snow et al., 2010, 2011). The use of more recent DNA data was avoided to minimize interference from factors such as genetic drift and other colonial migration processes, thus ensuring a consistency of results with other studies of ancient DNA from the same time period (Gonzáles-Martín et al., 2015).

The ancient samples from the Southwest came from different sites in the Mimbres Valley: Swartz Ruin (nine samples), NAN Ranch Ruin

TABLE 1 Mitochondrial haplotypes and haplogroups (HG) retrieved for the Paquimé samples analyzed

	ID#	HVR-I (minus 16000)	HVR-I reading range (minus 16000)	HVR-II	HVR-II reading range	HG
1	8-11A	223T 290T 319A 362C	112-410	221R 235G 263G 315+C	109-389	A
2	CH159-2A	298C 362C	024-410	263G	109-389	A
3	7-14A	223T 362C	112-410	199C 204C 263G	109-292	A
4	9-16B	106A 111T 183C 189C 217C 244A	000-410	152C 263G 315+C	109-389	B
5	19-CPB	183C 189C	024-410	263G 309+C 315+C	109-389	B
6	1-4B	183C 189C 261T 378T	024-410	73G 263G 309+C 315+C	34-389	B
7	17-6B	183C 189C 217C 325C	112-410	228A 263G 309+C 315+C	109-389	B
8	5-4	189C 217C	112-410	73G 263G 309+C 315+C	109-389	B
9	14-1A	183C 189C 223T 298C 325C 327T	024-410	73G 98T 120T	34-240	C
10	44-13H	223T 298C 325C 327T	112-410	215G 263G 290-291del 309+C 315+C	109-389	C
11	2-14A	265G 296T 362C	000-410	263G 309+C 315+C	109-389	D
12	44-13 HL2[a]	362C	268-410	—	—	D?
13	44I-13[a]	223T 290T	112-315	170Y 235G 251A 260A 263G	104-292	A?
14	44F[a]	319A	190-410	315+C	220-389	A?

IUPAC code is used for base ambiguities.
[a]Denotes a sample not included in subsequent analysis due to lack of sufficient coverage.

(19 samples), Cameron Creek (six samples), Harris Site (eight samples), Treasure Hill (one sample), and Unprovenanced Mimbres (three samples). Due to the small sample size of each site, these were clustered together and referred to as Mimbres in the text. Similarly, two sites from the south of Farmington in New Mexico known as the Tommy Site (13 samples) and Mine Canyon (9 samples) were considered. For descriptions of the Mimbres, Tommy and Mine Canyon sites see Snow et al. (2010, 2011). Additionally, one site was used from Mesoamerica: Basin of Mexico (25 samples; Mata-Míguez et al., 2012). A total of 86 HVR-I sequences and 36 genetic variants were compiled from published literature (Mata-Míguez et al., 2012; Snow et al., 2010, 2011). Nei genetic distances between the populations and subsequent PCoA plotting were calculated using the software GenAlex 6.5.

The second approach to biological affinities was based on haplogroup frequencies available for Mesoamerica and Southwest prehistoric populations. Nei genetic distances for haplogroup frequencies were calculated with Phylip 3.696. Fisher exact tests, in Genpop (Raymond and Rousset, 1995; Rousset, 2008), were used to evaluate the statistical significance of differences in haplogroup frequencies between pair of populations. Further, a PCoA was conducted using the haplogroup frequencies under standard covariance assumptions.

Finally, for haplotypes, a median joining network was built separately for haplogroups A, B, C, and D according to Bandelt et al. (1999) with default settings, notably without changing positions weight, using the software PopArt (Leigh and Bryant, 2015). The network grouped haplotypes available for the populations dated to between A.D. 500 and 1400, for nucleotide positions 16106–16410.

TABLE 2 Pairwise population matrix of Nei genetic distances based on sequence data (haplotypes)

Paquimé	Mimbres	Basin Mexico	Tommy Site	Mine Canyon	
0.000					Paquimé
0.012	0.000				Mimbres
0.019	0.008	0.000			Tommy Site
0.079	0.082	0.082	0.000		Mine Canyon
0.038	0.044	0.044	0.043	0.000	Basin of Mexico

3 | RESULTS

Mitochondrial haplogroups were characterized for 11 of the fourteen samples attempted (78.5%), and the results revealed the presence of four distinct Amerindian mitochondrial lineages: B ($n = 5$; 45%), A ($n = 3$; 27%), C ($n = 2$; 18%); and D ($n = 3$; 10%). The sequences were deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/; accession numbers KY400149- KY400159).

The HVR-I and HVR-II sequencing results and corresponding haplotype variants and haplogroups are shown in Table 1.

Three out of the 14 samples were excluded from the analysis because of a lack of complete information, specifically they did not produce DNA in all PCR targets: 44I-13 and 44-13 HL2, and 44F.

The 11 samples with complete results presented different combinations of variants (haplotypes) within the particular haplogroups. Most of the variants present were transitions. For samples 4 and 7 the haplogroup B4 was detected due to the presence of transition np16217 T-C, which is one of the most common in Native American groups (Achilli et al., 2008). HVR-I transversion np16183 A-C was observed in five of

FIGURE 2 Principal coordinates (PCoA) based on Nei's genetic distances for the HVR-I (16112-16410 bp) haplotypes

the individual samples, while in HVR-II np263 A-G was present in 10 samples.

A deletion of base pairs 290 and 291 was only observed once (sample 44-13 HLI) and it helped to define the haplogroup lineage C1. Phantom mutations were detected in samples 2 and 11, for position 16306 of the HVR-I, which were excluded from the analysis.

Nei's genetic distances based on sequences of the HVR-I (16112-16410 bp) showed closer genetic affinity of Paquimé with Mimbres than any other population (Table 2; Figure 2). The Tommy Site was also closely related to the Paquimé results, with both populations demonstrating a strong Southwestern haplotype pattern.

The second approach based on available data for haplogroup frequencies was also analyzed for Paquimé's contemporaneous groups from the prehistoric Southwest and Mesoamerica (Table 3). Statistical analysis of the haplogroup frequencies, or Nei genetic distances, also showed close affinity of Paquimé with Mimbres, and both Paquimé

TABLE 3 Ancient mitochondrial haplogroup frequencies of Paquimé and selected comparative populations

		Haplogroups					
Population/Site	***n***	**A**	**B**	**C**	**D**	**Age**	**Citation**
Paquimé	*11*	*0.27*	*0.45*	*0.18*	*0.10*	*A.D. 1200–1450*	This study
Mimbres	46	0.11	0.48	0.17	0.24	A.D. 550–1130	Snow et al., 2011
Tommy Site	36	0.03	0.69	0.14	0.14	A.D. 800–1100	Snow et al., 2010
Mine Canyon	12	0.58	0.33	0.08	0.00	A.D. 1100–1300	Snow et al., 2010
Ancestral Puebloan	38	0.11	0.71	0.18	0.00	A.D. 300–1000	Carlyle, 2003
Fremont	36	0.00	0.75	0.13	0.06	A.D. 400–1350	Parr et al., 1996
Western Basketmaker II	23	0.13	0.78	0.04	0.04	A.D. 50–500	Carlyle, 2003; LeBlanc et al., 2007
Basin of Mexico	25	0.48	0.24	0.04	0.24	A.D. 1240–1521	Mata-Míguez et al., 2012
Tlatelolco	38	0.57	0.21	0.07	0.14	A.D. 1454	De la Cruz et al., 2008
Tetetzontlilco	36	0.70	0.10	0.16	0.03	A.D. 1531–1600	Solórzano, 2006
Yucundaa	41	0.54	0.24	0.17	0.05	A.D. 1544	Warinner et al., 2012
Xcaret	24	0.88	0.04	0.08	0.00	A.D. 600–1521	González-Oliver et al., 2001
Teopancazco	29	0.55	0.21	0.17	0.07	A.D. 200–550	Alvarez-Sandoval et al., 2015
Total	**395**						

TABLE 4 Pairwise population matrix of Nei genetic distances based on haplogroup frequencies of populations in Table 3

0.0000												Paquimé
0.2144	0.0000											Teopancazco
0.074	0.5359	0.0000										**Mimbres**
0.1684	0.0305	0.4881	0.0000									Mine Canyon
0.1240	0.8209	0.0503	0.6296	0.0000								Tommy Site
0.2117	0.0725	0.3745	0.0934	0.6582	0.0000							Basin of Mexico
0.2515	0.0217	0.5355	0.0429	0.8528	0.0223	0.0000						Taltelolco
0.6135	0.0753	1.3648	0.1158	2.2770	0.1803	0.0771	0.0000					Xcaret
0.0900	0.6428	0.1024	0.4623	0.0262	0.6388	0.7315	1.5461	0.0000				Ancestral Puebloan
0.1838	0.0018	0.4959	0.0205	0.7347	0.0799	0.0283	0.0907	0.5623	0.0000			Yucundaa
0.1527	0.9274	0.0730	0.6932	0.0021	0.7522	0.9663	2.8256	0.0264	0.8261	0.0000		Fremont
0.4280	0.0268	0.9455	0.0773	1.4864	0.1292	0.0446	0.0148	1.1125	0.0381	1.7159	0.0000	Tetetzontlilco
0.1159	0.6893	0.1104	0.4597	0.0272	0.5744	0.6994	1.5510	0.0212	0.6071	0.0251	1.1778	0.0000 Western Basketmaker

and Mimbres were closer to other Southwestern samples than to ancient Mesoamericans (Table 4, Figure 3). The haplogroup frequency distribution of the Paquimé group was not statistically significant different from that of most other at the 0.05 level of probability ($P < 0.0038$ with Bonferroni's Correction (Abdi, 2007)). The only populations whose haplogroup frequencies distributions were significantly different from that of Paquimé at the 0.05 level of probability were those of Xcaret (0.000000), Tetetzontlilco (0.000120), and Fremont (0.000150).

The haplotype networks constructed with Paquimé and comparable sequence data from other groups (Table 2; Figure 4) provided a clear indication of the grouping between Paquimé and Southwest groups' haplotypes. The haplotype A_16223T_16290T_16319A_16362C (8-11A) was the only one shared between Paquimé and Mine Canyon, while the other haplotypes were unique to Paquimé.

Moreover, the haplotype B_16183C_16189C_16217C_16325C that characterizes the individual 17-6B had previously been described in Aztec groups from Central Mexico (Mata-Míguez et al., 2012). Finally, Paquimé haplotypes belonging to haplogroups C and D derived from Southwestern groups, although they were only found in Paquimé (Figure 4).

Finally, the aDNA data reported here are thought to be authentic because all consensus haplotypes and haplogroups were confirmed

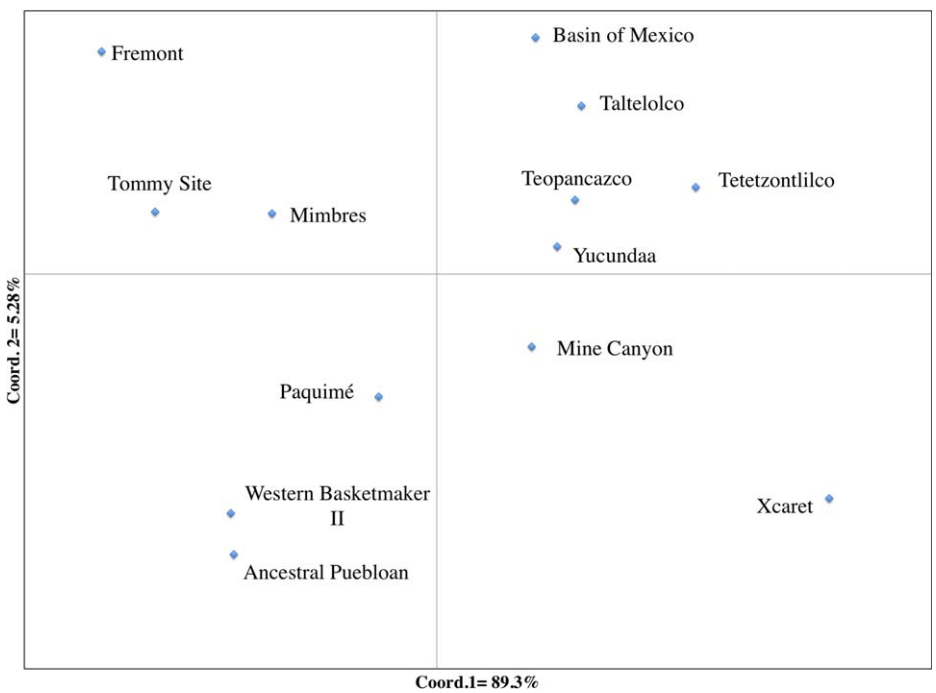

FIGURE 3 Principal coordinates (PCoA) based Nei genetic distances for ancient mitochondrial haplogroup frequencies

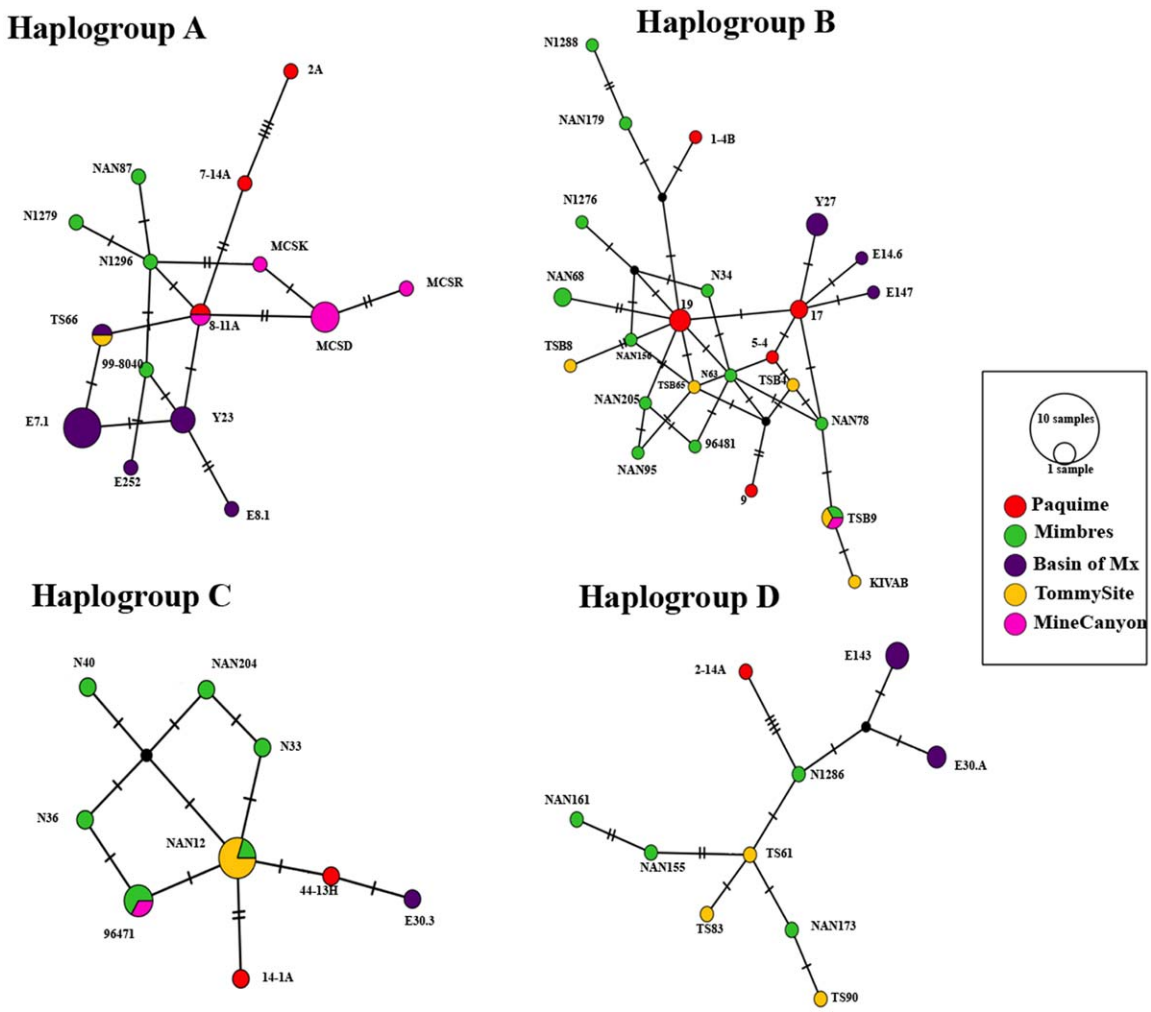

FIGURE 4 Median-joining network for haplogroups A, B, C, and D obtained using the HVR-I haplotypes available for Paquimé and contemporary sites. Sequences nodes corresponding to samples collected from Paquimé are in red color. Black circles represent median vectors, haplotypes that existed at one time but are now extinct, or haplotypes that were simply not sampled in this study

through repeated extractions and amplifications for all samples. Frequencies and variants were present according to and in line with other data published for the same region. Rigorous procedures were conducted in all stages of analysis to prevent and detect contamination, and the subset of samples sent to the aDNA laboratory in Missoula, Montana, confirmed our results. Furthermore, mitochondrial haplogroups of the laboratory workers at Calgary and Montana corresponded to the H1a and the X haplogroups respectively, and did not match any of the haplogroups found in the study. Additionally, no human DNA had been worked on previously in the clean lab, and all of the reagents tested negative for human DNA.

4 | DISCUSSION AND CONCLUSIONS

This study provides the first paleogenetic data from Paquimé, Casas Grandes associated with the Medio Period (A.D. 1200–1450). While the sample size is small, it provides initial insights into Paquimé's ancient genetic structure, consisting of similar haplotype patterns to those observed in other prehistoric U.S. Southwest groups, such as the Tommy Site (0.019), but in particular to Mimbres with a genetic distance of only 0.012 (Table 2). These results are consistent with other bio-affinities, such as crown morphology and dental metrics, which previously supported similarities between the Paquimé population and Mimbres sites (LeBlanc, 2008; Turner, 1999). Furthermore, LeBlanc et al. (2008) have suggested not only possible gene flow between these two populations but also the possibility of genetically related founders. The disappearance of Mimbres culture has been partially attributed to the development of Paquimé as a new political, economic and religious center, in the area (Adams et al., 1987). According to Shaffer (1999), Mimbres was one of the most populated and influential cultures in the Southwest and should be considered when studying Paquimé, especially due to the geographical proximity of the regions (Figure 1). Also, Lekson (1999a) proposed that these interactions between Paquimé and Mimbres influenced Paquimé's cultural apogee. Although our results provide strong evidence of genetic affinities between Paquimé and Mimbres, with the majority of haplotypes shared or derived from ancient Southwest populations, the causes of cultural development at Paquimé still remain a question.

Haplogroup frequency analysis is not a sensitive indicator of genetic change (Emery et al., 2015); however it still consistently shows Mimbres as the closest population to Paquimé (Table 3; Figure 3). Further genetic testing of complete mitochondrial genomes from more individuals is necessary to estimate the amount of past gene flow (Lawson et al., 2012). In this study, distinct genetic variants in the HVR-I also showed similarity with Mimbres, but such similarity was not found in relation to the geographically contiguous Tommy and Mine Canyon sites, possibly due to the presence of different maternal lineages or sample size. Unfortunately, there are no reported variants for HVR-II that would allow for a comparison of the control region's haplotypes to other past populations. Whole mitogenome haplotypes information could be useful in future larger sample sizes to contrast against modern populations, and also to better understand the effects of genetic drift (Carlyle et al., 2000; Gonzáles-Martín et al., 2015; Gorostiza et al., 2012; Kemp, 2006; Kemp et al., 2010; Malhi, 2003; Raff et al., 2011; Tamm et al., 2007). Currently, this analysis is underway on a larger number of aDNA samples from the Casas Grandes site, which might shed more light on the Southwest versus Mesoamerican ancestry in Paquimé as well as to evaluate lineages continuity.

Other archaeological data such as funerary contexts, grave offerings, and stable isotopes in each of these individuals could also enrich the interpretations. For instance, preliminary strontium isotope results show that at least one of the five individuals belonging to haplogroup B was distinctly non-local, while two other haplogroup B individuals had strontium isotope signatures very close to being non-local. Two samples belonging to haplogroup A, more commonly found in Mesoamerican groups, presented a local strontium isotope signature (Offenbeker et al., 2015).

Finally, this study does not support a significant relation between Mesoamerican groups and Paquimé. These novel, albeit preliminary, results provide interesting evidence that is consistent with previous archaeological and bioarchaeological studies that consider Paquimé's interactions as more significant with other Southwestern and Northern Mexico groups (LeBlanc et al., 2008; Lekson, 1999a,b; Shaffer, 1999; Turner, 1999). It is also possible that Paquimé and Mimbres shared similar population genetic events, including genetically related founder families. A further genetic study, expanding this study sample size, is necessary to explore whether significant gene flow between these two populations occurred. If patterns in a larger sample size from Paquimé tend in the same direction, it would be possible to discard significant migration movements from and to Mesoamerica, although it would be also important to contrast this data with Y chromosome, and potentially nuclear, genetic patterns.

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