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ORIGINAL PAPER

Ancient mitochondrial DNA and population dynamics in postclassic Central Mexico: Tlatelolco (AD 1325–1520) and Cholula (AD 900–1350)

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Abstract

The past composition and genetic diversity of populations from Central Mexico during the Postclassic period (AD 900–1520) are still little understood. Two of the largest centres of ancient groups, Tlatelolco and Cholula, declined after European conquest and questions about their relationships with other Central Mexican cities and ritual activities have been debated. Tlatelolco was a Mexica group that practiced the Quetzalcoatl cult and human sacrifice, including the sacrifice of children, while Cholula was considered the main pilgrimage centre and multiethnic city during the Postclassic. This study analysed the mitochondrial DNA control region of 28 human skeletal samples to estimate the genetic affinities of individuals buried at Tlatelolco and Cholula. Amelogenin analysis and whole genome sequencing (WGS) were also applied to determine the sex of the 15 Tlatelolco subadults from sacrificial contexts. Networks, PCoA and Nei genetic distances were calculated to compare Tlatelolco and Cholula haplotypes with available ancient haplotype data from Mesoamerican groups and the two borderland areas, Paquimé and Greater Nicoya. Mitochondrial haplogroups were characterized for 11 of the 15 samples from Tlatelolco (73%) and 12 samples out of 13 from Cholula (92%), revealing the presence of four distinct Amerindian mitochondrial lineages at Tlateloloco, A ($n = 6$; 55%), B ($n = 2$; 18%), C ($n = 1$; 9%) and D ($n = 2$; 10%); and three lineages in Cholula, A ($n = 5$; 42%), B ($n = 5$; 42%) and C ($n = 2$; 16%). Statistical analysis of the haplotypes, haplogroup frequencies and Nei genetic distances showed close affinity of Tlatelolco's subadults with ancient Mexica (Aztecs) and closer affinities between Cholula and the Xaltocan of the Basin of Mexico. Sex determination of Tlatelolco subadult sacrifice victims revealed that 83% were females, in contrast to previous studies of subadult sacrificial patterns at the site. Together, these results demonstrate the multi-ethnic nature of religious and economic centres in Postclassic Central Mexico.

Keywords Mesoamerica · Human sacrifice · Mitochondrial DNA · Genetic distance · Haplogroups · Sex determination

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Introduction

Scholars have widely studied the ancient Mexican cities. However, as technological advances progress, the genetic composition of individuals living in these centres and the biological sex of infant ritual victims have been explored only recently. During the Postclassic Period (AD 900–1520), Tlatelolco and Cholula (located about 100 km apart) represented two important communities in Central Mexico, which were contemporaneous to the Toltec and Aztec empires (Nichols and Pool 2012; Carmack et al. 2007) (Fig. 1). The most distinctive aspects of Tlatelolco and Cholula as religious and economic centres were their ritual spaces (e.g., pyramids) and large market plazas (Guillien Arroyo 1999; McCafferty 2007). Ethnohistorical accounts suggest cultural similarities between the two cities (Carballo 2016), while other accounts highlight their differences (Hirth 1996; Smith 2008a, b;

Fig. 1 Site locations discussed in the text and noted in Table 1. ArcGIS 10.4 software (http://www.esri.com/software/arcgis) was used to generate the figure. Service layer credits Natural Earth. Free vector and raster map data @ naturalearthdata.com. We acknowledge the use of imagery from the NASA Worldview application (https://worldview.earthdata.nasa.gov/) operated by the NASA/Goddard Space Flight Center Earth Science Data and Information System (ESDIS) project (ESDIS 2017; Patterson and Vaughn Kelso 2017)

Tsukamoto et al. 2014). However, information on the genetic diversity of the inhabitants and their relation to other contemporaneous groups in Central Mexico is scarce.

Tlatelolco

Formed around AD 1337, Tlatelolco was a sister city of Aztec Tenochtitlan, both now buried beneath present-day Mexico City. The historical events associated with the origin of Tlatelolco and its development have been a subject of controversy in the historical and archaeological literature (Orozco y Berra 1960; Gonzales Rul 1996). Ethnohistoric sources suggest that the *Tlatelolcas* descended from Tula, mixed with other non-Toltec groups that repopulated the Basin of Mexico after the collapse of the Tula Empire (Davies 1977; Smith 1984; van Zantwijk 1985). Lake Texcoco gave them advantages for the transportation of goods and people and transformed into a large marketplace, which later became a commercial city (Lopez Austin 2001). Ethnohistoric sources identify Tlatelolco as the biggest and most diversified marketplace in Mesoamerica during the Late Postclassic. It may have served up to 50,000 people daily, providing the opportunity for multicultural trade as merchants, called *pochteca*, moved goods and captives over long distances, and also contributed new slaves for sacrificial ceremonies (Gonzales Torres 2001; Lopez Austin 2001). Tlatelolco experienced a demographic explosion during the transition from the Middle Postclassic (AD

1150–1350) to the Late Postclassic (AD 1350–1520) (Smith and Berdan 2003), and the pre-Conquest population represented one of largest groups of Mexica origin, declining rapidly after the Spanish conquest (Livi-Bacci 2006; Vågene et al. 2017).

Tlatelolco was a neighbouring city to Tenochtitlan and was populated by the same group of people, who were of Nahua affiliation. In spite of their geographic proximity, Tenochtitlan and Tlatelolco demonstrated differences in their religious practices. On the one hand, Tenochtitlan followed the Aztec religion that featured a polytheistic pantheon, with multiple gods worshiped in each city but with particular patron deities, while Tlatelolco followed the Quetzalcoatl cult, which included human sacrifice, among other traditions. This human sacrifice coincided with battle celebrations, the end or beginning of new seasons, and offerings to the deities of rain, fertility and agriculture (de Sahagún et al. 1975; Gonzales Torres 2001). Sacrificed victims were usually of Mexica origin and included men and women, but young children were preferred for certain ceremonies dedicated to the rain god, Tlaloc (Roman Berrelleza 1991; Gonzales Torres 2001). However, according to Gonzalez Torres (2001), the *pochteca* also contributed to the movement of slaves from potentially different groups in Mesoamerica for sacrifices, ceremonies and other purposes. In this study, we applied ancient mtDNA analysis to explore the genetic affinity and sex of subadults found in a ritual context in Tlatelolco; we hypothesized that subadults from the Tlatelolco ceremonial context would display a genetic affinity with other Mexica (Aztec) ancient samples.

Cholula

Cholula, in the Puebla-Tlaxcala Valley, was a sacred city in central Mexico that had been continuously occupied since about 1000 BC (Kubler 1968; McCafferty 2001). During the Postclassic, Cholula represented a pilgrimage and religious hub dedicated to the cult of Quetzalcoatl (McCafferty 1996). The ethnic origins of the Cholula inhabitants has been debated and apparently changed through time (McCafferty 2007). Architectural, symbolic and sourcing materials have demonstrated similarities between Cholula and Teotihuacan during the Classic Period (AD 250–600), suggesting that the inhabitants of these two cities shared an ethnic background (McCafferty 2001). However, the arrival of Olmeca-Xicallanca groups from the Gulf Coast also suggests an admixture of populations during the Epiclassic and Postclassic Periods (AD 600–1520) (McCafferty 2007). According to Carrasco (1971), Cholula was a multicultural city inhabited by groups such as the Toltec, Chichimec and Mixtec during the Postclassic Period. It is not clear how much of the Valley was under the control of Cholula and other cities, but Cholula became prominent during the Postclassic Period as a pilgrimage core for people throughout Mesoamerica (Smith and Berdan 2003; Lind 2012).

McCafferty (2007) suggests that Cholula's ethnic composition changed through time in an unbroken sequence, but supporting bioarchaeological evidence is not conclusive. In contrast, Muller (1973) suggests that around AD 700, Cholula suffered depopulation, and some inhabitants fled as far as southern Central America. She suggests that Cholula was later repopulated, and its core grew exponentially during the Postclassic to become the Tlaxcala/Cholula region during the Middle Postclassic (Smith and Berdan 2003). According to ethnohistoric sources, the original populations in the Puebla Valley were the Otomangue-speaking groups, which would have had multiethnic components (Paddock 1987). A component of this population was the Olmeca-Xicallanca, a Nahua-affiliated group that ruled in Cholula from AD 800 to 1300, believed by some to be a combination of Mixtecas and Chocho-Popolocas (Muller 1973). However, other ethnohistoric sources argue that Nahuatl-speaking people arrived in Tlaxcala and the north of the Puebla Valley between AD 1210 and 1230 (Smith 1984). Additional interpretations suggest the influence of Gulf coast ethnic groups in Cholula during the Epiclassic and Early Postclassic period (McCafferty 2007).

Cholula also functioned as an important economic heart; it had a major international market, was a central point of production and distributed elaborate polychrome ceramics (McCafferty 2007). Its market stocked jewels, precious stones and delicate featherwork (Duran 1971). Cholula's cultural material and religious expressions (which often featured the feathered serpent iconography of the god Quetzalcoatl) can be found in ceramics from the Basin of Mexico to Yucatan and down to southern Central America, and it is worth noting that Cholula was also the centre where Mixteca-Puebla style goods were produced and traded (Duran 1971; McCafferty and Steinbrenner 2005).

Ancient mitochondrial DNA could add valuable information to understand the genetic links of the people living in Cholula during the Postclassic. Given that Cholula has been considered a multi-ethnic cultural hub, we hypothesized that ancient Cholula inhabitants would have distinct mitochondrial haplogroup profiles in comparison with the Nahua of Central Mexico.

Ancient mitochondrial haplogroups and the genetic landscape in ancient Central Mexico: implications for Tlatelolco and Cholula

Previous studies of populations from Central Mexico have contributed to an understanding of demographic changes and sociocultural events. Mitochondrial ancient DNA studies have revealed a higher frequency of haplogroup A over haplogroup B, and low frequencies for haplogroups C and D

(Table 1). The most studied Mesoamerican site using ancient DNA (aDNA) is Tlatelolco, with mitochondrial haplogroup information recovered in three separate studies conducted by Kemp et al. (2005), Solórzano Navarro (2006), and De La Cruz et al. (2008). Only one of these studies sequenced the Hypervariable Region-I (HVR-I) of the mitochondria (Solórzano Navarro 2006), usually studied for establishing maternal lineages, while the other two explored general diagnostic mitochondrial DNA (mtDNA) haplogroup variants (Kemp et al. 2005) and determined the sex of the children sacrificed to Tlaloc (De La Cruz et al. 2008). These previous studies provide haplogroup distributions for Tlatelolco ranging from 46 to 65% for haplogroup A, 13–37% for haplogroup B, 10–18% for haplogroup D, and finally, 4–7% for haplogroup C. Similar results were obtained by Mata-Míguez and colleagues (2012) using samples from Xaltocan, an Early and Middle Postclassic site in the Basin of Mexico. We expected to find a similar haplogroup distribution amongst the Tlatelolco subadults in this study if, as suggested by ethnohistoric records, the sacrificial victims mostly belonged to the local Mexica population.

Despite the predominance of haplogroup A in Central Mexico (Solórzano Navarro 2006; Álvarez-Sandoval et al. 2015), a small group of samples ($n = 10$) from the Xaltocan group has shown a different pattern, with equal frequencies for both haplogroups A and B (Mata-Míguez et al. 2012), leading the investigators to suggest that this population was related to a different ethnic group, perhaps the Otomí. Although these results could be strongly affected by sample size, it could also indicate that the populations in Mesoamerica may carry different haplogroup patterns than expected. For example, Mayan aDNA samples from the Yucatan Peninsula have shown higher levels of haplogroup A than other haplogroups, with the second most frequent being haplogroup C (González-Oliver et al. 2001). In contrast, Mayan aDNA samples from Copán reported by Merriwether and colleagues (2006) found an absence of haplogroup A and B for a small group of samples ($n = 9$), with the highest frequency being haplogroup C (80%). Similar results were observed in 25 Mayan samples from an early colonial site in Belize by Elwess and colleagues (2015) that reported a

Table 1 Mitochondrial haplogroup frequencies of Cholula and Tlatelolco sites and selected Mesoamerican ancient populations

Population/site	*N*	Haplogroup frequency (%)					References[a]
		A	B	C	D	Century AD	
Tlatelolco subadult[a]	*11*	55	18	9	18	1350–1519	This study
Cholula	*12*	42	42	16	0	250–1400	This study
Tlatelolco							
1) Tlatelolco[b]	*23*	65	13	4	18	1325	(Kemp et al. 2005)
2) Tlatelolco[b]	*30*	46	37	7	10	1350–1400	(Solórzano Navarro 2006)
3) Tlatelolco[c]	*14*	57	21	7	14	1454–1457	(De La Cruz et al. 2008)
Xaltocan							
Pre-Aztec conquest (PrAz)[d]	*10*	30	30	0	40	1240–1541	(Mata-Míguez et al. 2012)
Post-Aztec conquest (PoAz)[d]	*15*	60	20	6	13		
Tetetzontlilco	*30*	70	10	17	3	1531–1600	(Solórzano Navarro 2006)
Teotihuacán	*36*	58	25	14	3	300–700	(Aguirre Samudio et al. 2016)
Teopancazco	*29*	55	21	17	7	200–550	(Álvarez-Sandoval et al. 2015)
Yucundaa	*41*	54	24	17	5	1544	(Warinner et al. 2012)
Mayas[e]	*38*	60	0	34	5	250–1500	(Ochoa-Lugo et al. 2016)
Xcaret	*24*	88	4	8	0	600–1521	(González-Oliver et al. 2001)
Copán	*9*	0	0	88	12	700–1300	(Merriwether et al. 2006)
Paquimé	*15*	20	47	27	7	1200–1450	(Morales-Arce et al. 2017a, b)

[a] These samples belong to individuals from a sacrificial context

[b] These samples belong to adults of the general population burial excavated in 1965 and 1966 from the ceremonial center of Tlatelolco

[c] These samples were identified as male subadults, by molecular analysis, from a sacrificial context dedicated to the God of the Rain

[d] Hypothesized language and ethnic affiliations correspond to Otomí before the conquest of Xaltocan by Aztecs (Mata-Míguez et al. 2012)

[e] These Mayan samples are from Mexico, mostly from archaeological sites in Tabasco and Chiapas and a few from Yucatán and Quintana Roo (Ochoa-Lugo et al. 2016)

haplogroup C frequency of 64% followed by D (28%) and B (8%), highlighting also the absence of haplogroup A. Overall, however, the amount of ancient genetic data on Mayan groups is limited, and haplogroup distributions may be significantly influenced by poor preservation and DNA amplification bias. Nevertheless, these Mayan group observations are relevant to the study of Cholula's populations, as this city could have had Gulf Coast Maya groups among its inhabitants during the Epiclassic period (AD 600–900). Finally, only one group of ancient samples ($n = 9$) had been previously analysed for Cholula (AD 1100–1500) that reported a frequency of 100% for haplogroup A (Juárez Martín 2002). If Cholula's population was primarily composed of people of Central Mexican origin, we expected that its inhabitants would present higher frequencies of haplogroup A compared to other haplogroups. Alternatively, higher frequencies of haplogroups B or C would be observed if its population was indeed composed of a more diverse, multi-ethnic population.

Analyses of ancient DNA should be addressed cautiously. Population dynamics models analysing genetic diversity of remaining Mesoamerican groups from Central Mexico, such as Otomí, Nahua and Tepehua, suggest a demographic expansion during the Archaic and Formative periods, while their populations started to decline after the Classic period, and abruptly in colonial times (González-Martín et al. 2015). For this purpose, it is necessary to increase the database of ancient samples and DNA sequence coverage to better approach questions on past Mesoamerican group compositions and movements. Through ancient mtDNA analysis of 28 samples from Central Mexico, 15 from Tlatelolco and 13 from Cholula, our study aims to assess the genetic affinities of these two sites with other ancient groups from Central Mexico to augment the understanding of the past genetic structure in correspondence with ritual practices and culture history in Mesoamerica. Moreover, as the sex of subadult skeletons is difficult to assess based on morphological features alone, ancient DNA techniques are also applied to accurately identify the sex ratio of the subadult sacrificial victims from Tlatelolco.

Materials and methods

Archaeological sites and samples

The two archaeological sites analysed in this study date to AD 250–1520 (Table 2). The Tlatelolco samples correspond to the Late Postclassic Period (AD 1350–1520). Excavations at Tlatelolco were conducted between 1961 and 1962 in a ceremonial context associated to the Templo Mayor (Guilliem Arroyo 2016). Samples analysed from Cholula, Puebla, date to the Postclassic Period (AD 900–1350), except for one sample that dates to the Classic Period (AD 250–600); these samples were excavated between 2009 and 2013 in San Andres, Cholula, Puebla (Saenz Serdio and Cedillo Ortega 2016). A graphical summary of the methods is provided in Fig. S3 (Supplementary Material 2).

A total of 28 individuals were selected for this analysis (Table 2), with one skeletal element analysed per individual, except for Cholula samples Cho#9 and Cho#10 which belong to the same individual (Table 3). Fifteen individuals from Tlatelolco were sampled from the Dirección de Antropología Física, INAH-Mexico D.F. by Jose Antonio Pompa y Padilla, donated to the University of Calgary in the early 1990s (Guilliem Arroyo 2016). Thirteen individuals were sampled from excavations conducted by the INAH-Puebla, donated for aDNA analysis to the University of Calgary in 2015 (Saenz Serdio and Cedillo Ortega 2016). A map with the approximate locations of the sites noted in the text was created using Esri's ArcMap 10 (Fig. 1). Permission to sample the Cholula material and for conducting destructive analysis was provided by the INAH Puebla and Coordinación Nacional de Arqueología, Mexico (#401-3-1922). For the Tlatelolco material, permission for sampling and analysis was granted by Dirección de Antropología Física, INAH, Mexico.

DNA extraction

All 29 bone samples were first extracted within the dedicated clean laboratory at the University of Calgary, Canada. Subsequently, the 15 Tlatelolco samples were independently re-extracted and analysed within the dedicated ancient DNA

Table 2 Archaeological sites, dates and number of samples processed

Site	Period	Date	N	Sample IDs
Tlatelolco	Late Postclassic	AD 1350–1519	15	IF #1–IF #15
Cholula	Classic	AD 250–900	1	Cho #5
	Early Postclassic	AD 900–1150	4	Cho #1–Cho #4
	Middle Postclassic	AD 1150–1350	8	Cho #6–Cho#13, Cho #24
Total			28	

Table 3 Archaeological samples processed

Sample ID (laboratory)	Collection name	Burial	Individual	Age at death[d]	Sample type
Tlatelolco[a]					
IF #1	1	55		7–12	Rib
IF #2	2	103		4–6	Rib
IF #3	3	14		7–12	Metatarsal
IF #4	4	37		7–12	Rib
IF #5	5	51		0–3	Rib
IF #6	6	24		4–6	Rib
IF #7	7	13		4–6	Rib
IF #8	8	50		0–3	Rib
IF #9	9	12		4–6	Metatarsal
IF #10	10	93		0–3	Rib
IF #11	11	16		4–6	Rib
IF #12	12	1		0–3	Rib
IF #13	13	25		7–12	Rib
IF #14	14	35		4–6	Rib
IF #15	15	14D		0–3	Rib
Cholula[b,c]				Sex[d]	
Cho#1	Col-1	7		Male	Tooth RP4
Cho#2	Col-2	16		Female	Tooth LM3
Cho#3	Col-3	3	3	Female	Tooth LM3
Cho#4	Col-4	11		Female	Tooth RM3
Cho#5	R3-1	1		Male	Metacarpal
Cho#6	S13-1	2B		–	Tooth I
Cho #7	S13-2	33G		–	Phalange
Cho#8	S13-3	105		–	Phalange
Cho#9	S11-1		13	Female	Metatarsal
Cho#10	S11-2				Metatarsal
Cho#11	S11-3	7		Female	Metatarsal
Cho#12	S11-4	9		Male	Tooth LM3
Cho#13	S11-5		14	–	Tooth M
Cho#24	0–21K-13W	6 IV		Female	Metatarsal

[a] Samples from Tlatelolco were excavated between 1961 and 1962

[b] Cholula's teeth identification followed the Standard's book (Buikstra and Ubelaker 1994)

[c] Samples excavated between 2009 and 2013, except for CHO#24 which was excavated in 1967 (Guilliem Arroyo 2016; Saenz Serdio and Cedillo Ortega 2016)

[d] Age at death and sex estimations are described in the site reports of Tlatelolco and Cholula, respectively (Guilliem Arroyo 2016; Saenz Serdio and Cedillo Ortega 2016)

laboratory in BioArCh, University of York, UK. Both laboratories follow comprehensive controls for the prevention and detection of contamination including the use of UV filtered ventilation and positive airflow, UV sources for workspace decontamination; protective clothing such as Tyvex suits, masks and disposable gloves; and separation of pre- and post-PCR workspaces, equipment, and consumables. Non-template controls (including blank extractions and negative PCR controls) were processed alongside the experimental samples during all the analytical steps to monitor for the presence of contamination. In the case of ribs, metacarpals and tarsals, a ~1-cm^2 piece of bone was cut for the extraction process, while the entire tooth was used for dental samples.

In both laboratories, the bone samples were chemically decontaminated through submersion in 6% sodium hypochlorite for 10 min, rinsed twice with ultrapure water and irradiated with 254-nm ultraviolet light for 30 min on two sides. Subsequently, samples were reduced to powder and incubated at 50 °C overnight in 5 ml of lysis buffer (0.5 M EDTA, pH 8.0, 0.5 mg/ml proteinase K and 0.12% SDS). After demineralization, DNA extraction and purification followed the MiniElute silica spin column protocol by Yang and colleagues (1998, 2008) and the DNA was eluted twice with 50 μl aliquots of TET buffer (in Calgary) or Qiagen EB buffer (in York).

mtDNA amplification and sequence analysis

Mitochondrial DNA was analysed by amplification of the Hypervariable Regions I and II (HVR-I and HVR-II) covering ~300 bp per region. Eight overlapping fragments of the HVR-I and HVR-II were amplified and analysed following the procedures described in Morales-Arce and colleagues (2017b) to determine maternal haplotypes of the individuals.

In addition, three overlapping fragments were used to replicate amplifications and target the HVR-II and partial HVR-I region in the Cholula samples (mt1–1 5′-TAA CTC CAC CAT TAG CAC CC-3′, mt3–1 5′-CAC CCT ATT AAC CAC TCA CG-3′, mt4–2 5′-ATT ATT TAT GGC ACC TAC GTT C-3′) as described in Katzenberg and colleagues (2005). PCR was run for 40 cycles at 95 °C for 30 s (denaturing), 55 °C for 30 s (annealing) and 72 °C for 45 s (extension), with an initial 12 min denaturing period at 95 °C. Extraction blanks and PCR negative controls were run along with each PCR set. 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 alignment against the Cambridge reference (rCRS NC_012920), through BioEdit 7.2.5 software (Hall 1999).

Sequences were edited using the sequence coordinates of the revised Cambridge Reference Sequence (rCRS) (Andrews et al. 1999). Most of the sequences targeted the mtDNA control region from 16,024 to 16,410 (encompassing HVR-I) and 34 to 389 (encompassing HVR-II) (Stoneking 2000). Haplotypes were assigned based on the observation of

variable positions for each sequence in each sample in comparison with updated databases of the mitochondrial human phylogeny in PhyloTree (van Oven and Kayser 2009; van Oven 2015) and additional Web-based searches for near-matches. Confirmation of haplogroups followed diagnostic presence/absence of variants according to O'Rourke and Raff (2010). To authenticate the mtDNA analysis results, samples from Cholula underwent repeat extraction and amplification within the University of Calgary laboratory, while for the Tlatelolco samples, mtDNA haplogroups were confirmed through analysis of full and partial mtDNA genomes obtained through whole genome sequencing (WGS) (see "Whole genome library preparation and sequencing"). Rather than undertaking cloning, we confirmed haplotypes using those mtDNA genomes with greater than 5× depth of coverage for the Tlatelolco samples (Supplementary Material 1), and through repeat sequencing for Cholula samples.

Statistical analysis and population comparison

First, genetic affinities of the Tlatelolco and Cholula samples were compared with other populations from Central Mexico. The comparative populations were selected based on available coverage of HVR-I (16,106–16,378 bp), and contemporaneous time periods (AD 250–1519). The sequences from the published aDNA data corresponding to the Mesoamerican Classic and Postclassic periods were compiled from the literature (Solórzano Navarro 2006; Mata-Míguez et al. 2012; Álvarez-Sandoval et al. 2015; Morales-Arce et al. 2017b) corresponding in total to 105 HVR-I sequences and 62 variants. The comparative ancient samples came from the sites of Tlatelolco (27 samples), Basin of Mexico-Xaltocan (25 samples), Teopancazco (15 samples), Mayas from Mexico (38) samples, and finally an external non-Mesoamerican group, Paquimé (15 samples). Nei genetic distances between the populations and a haplotype PCoA were calculated using the software GenAlex 6.5 (Peakall and Smouse 2006, 2012). Nei genetic distance is a measure of differentiation between populations (Nei 1972). The effect of geographic distance on the genetic affinities was also considered (S4 Table). The statistical significance of the correlation between geographic and genetic distance matrices was evaluated by the Mantel test with 1000 permutations (Mantel 1967). GenAlex 6.5 was used to make a geographic distance matrix and to calculate the Mantel test.

The second approach to biological affinities/differentiation was based on haplogroup frequencies available for Mesoamerican prehistoric populations (Table 1). Nei genetic distances for haplogroup frequencies were calculated with Phyllip 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 each pair of populations. Furthermore, 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 following Bandelt and colleagues (1999) with default settings, notably without changing position weight, using the software NETWORK 5.0. The network includes haplotypes belonging to populations who lived between AD 200–1519; for nucleotide positions from 16,106 to 16,378 of the HVR-I. The same populations were used for genetic haplotype affinities, with the exception of samples from Jícaro, Greater Nicoya, that were added in the construction of the haplogroup B network. These are the only extant haplotypes for ancient Central America, a Mesoamerican borderland (Morales-Arce et al. 2017a). The Jícaro, Greater Nicoya, samples were omitted from the other population analyses due to small sample size ($n = 3$).

Sex determination and whole genome sequencing

Amelogenin analysis

Sex determination for the Tlatelolco subadults was conducted through targeted PCR amplification of the amelogenin locus, as well as through WGS sex identification methods (Skoglund et al. 2013). At the University of Calgary, PCR amplifications targeted the amelogenin locus as described in Dudar and colleagues (2003) (Common primer 5′-TCA TGA ACC ACT ACT CAG GRA GG-3′, XA 5′-TAG AGT GTG ACT ATC TTA GAA TCA GG-3′, YA 5′-AAA GAG AGG AAA TTA TAT GCC CAA AGT T-3′). Reactions were amplified for 60 cycles and co-amplified fragments of 120 bp for the Y-chromosome, and 155 bp for the X-chromosome. The PCR conditions consisted of 95 °C for 30 s (denaturing), 50.2 °C for 30 s (annealing) and 70 °C for 45 s (extension), with an initial 12 min denaturing period at 95 °C. The visualization of X- and Y-chromosome PCR products was conducted on MetaPhor agarose 3% stained with ethidium bromide. The PCR blank reactions did not show spot contamination during the collection of the data.

Whole genome library preparation and sequencing

At the University of York, the DNA extracts from all samples were converted into double-stranded Illumina sequencing libraries for shotgun sequencing following the protocol by Meyer and Kircher (2010) and modified according to Fortes and Paijmans (2015). Libraries were prepared using 25 μl of DNA, and individual P5 and P7 barcodes were included for each sample. Libraries were constructed from blank extracts, and negative controls were processed along with all samples and monitored for contamination. Three microlitres of the resulting libraries were amplified and indexed in a 25-μl

reaction containing 1× AmpliTaq Buffer, 2 mM MgCl2, 0.1 mg/ml BSA, 0.25 mM dNTPs, 1.25 U AmpliTaq Gold 360, 0.2 μM IS4 Forward Primer and 0.2 μM each of individually barcoded P7 Indexing Primer. Amplification thermal cycling conditions were as follows: 10 min at 94 °C; between 14 and 19 cycles of 30 s at 94 °C (denaturing), 45 s at 60 °C (annealing) and 45 s at 72 °C (extension); and a final extension step of 5 min at 72 °C. Amplified libraries were purified using Qiagen MinElute PCR purification columns, quantified using a Qubit 2.0 Fluorometer, and quality assessed on an Agilent 2100 Bioanalyzer, using a High Sensitivity Chip. The indexed library from one sample (IF#4) was paired-end sequenced (read length 75 bp) on a NextSeq platform at the University of Potsdam to determine whether the endogenous DNA content would be sufficient for sex identifications. The remaining successfully indexed libraries (12 of 15 samples) were pooled in equimolar concentrations (along with blanks and negative controls) and single-end sequenced (with read length 80 bp) on a HiSeq2000 Illumina platform at the National Highthroughput DNA Sequencing Centre, University of Copenhagen, Denmark. Raw sequence data were deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/; BioProject PRJNA423230; SRA accession: SRP127309); the WGS results are presented in the Supplementary Material 1.

Whole genome data analysis

The raw reads obtained from the sequenced libraries were trimmed for adapter and P5 index sequences using cutadapt v1.11 (Martin 2011). During P5 index trimming, one error in the index sequence was allowed (parameter −e 0.125). The reads were filtered to a minimum phred-scaled quality score of 20 (−q 20), and any sequence less than 20 bp in length (−m 20) or that did not match the correct P5 index was discarded from analysis. The individual reads from each sample were then mapped to the human reference genome (hg38) using the Burrows-Wheeler Alignment 0.7.5a (Li and Durbin 2009) with the following parameters (bwa aln −q 20 −n 0.01 −l 1024) to increase the maximum number of mismatches and disabled the seed length (Schubert et al. 2012). Reads were sorted and PCR duplicates removed using Samtools v0.1.19 (Li and Durbin 2009). Mean depth of coverage for the mtDNA genomes was calculated using Samtools, taking into account depth at all positions (including zero depth). Mean fragment lengths were estimated using BAMStats (http://bamstats.sourceforge.net/). Sequence length distributions and damage patterns were assessed through the mapDamage2.0 package (Jonsson et al. 2013).

Sex identification was undertaken on the human genomic sequences by calculating the ratio of reads aligning to the Y chromosome to reads aligning to both sex chromosomes (Ry) based on the method proposed by Skoglund and colleagues (Skoglund et al. 2013). The 95% confidence interval (CI) was computed as $Ry \pm 1.96 \times Ry \times (1 - Ry)/(Nx + Ny)$, where Nx and Ny are the total number of reads aligning to the X chromosome and Y chromosome, respectively. Only reads with a mapping quality above 30 were considered.

To confirm the mitochondrial haplogroups of Tlatelolco samples produced through HVR analyses, the obtained genomic sequences were aligned to the rCRS using the same mapping parameters, as above and consensus mitochondrial genome sequences were called using Samtools mpileup. Haplogroups were assigned based on defining mutations identified through mthap (http://dna.jameslick.com/mthap/).

Results

Ancient DNA preservation

Overall, the Tlatelolco and Cholula samples demonstrated good preservation, considering their antiquity and provenience. The success rate for PCR amplifications was higher in Cholula (92%) than in Tlatelolco (73%), and in total for this study, we amplified mitochondrial DNA by PCR for 82% of the samples.

Indicators of DNA preservation from WGS of the Tlatelolco samples were consistent with the results obtained through PCR amplifications. The endogenous human DNA content of the bones was quite variable, ranging from 0.04 to 86% with a mean of 25% (Supplementary Material 1). At Tlatelolco, the 10 samples with > 1% endogenous human DNA were all successful for HVR amplifications. Of the four samples that failed mtDNA amplification, two contained < 0.5% human DNA (IF#10 and IF#12) and the other two contained insufficient DNA for Illumina library preparation (IF#8 and IF#14). Authentication of WGS results was undertaken through the assessment of post-mortem degradation including short sequence length and characteristic misincorporation patterns, particularly the deamination of cytosine (C-T) at the 5′ ends of molecules (Jonsson et al. 2013; Ginolhac et al. 2017). An increase in C-T mutations (3–20%, mean = 13%) was observed towards the 5′ and 3′ ends of molecules in all libraries, except for IF#12, which was discarded from subsequent WGS analysis (Supplementary Material 1; S2 Fig.).

Mitochondrial DNA analysis

Based on HVR sequences, the mitochondrial haplogroups were characterized for 11 of the 15 samples from Tlatelolco (73%) and 12 samples out of 13 from Cholula (92%). The results revealed the presence of four distinct Amerindian mitochondrial lineages in Tlatelolco: A (n = 6; 55%), B (n = 2; 18%), C (n = 1; 9%) and D (n = 2; 10%). In contrast, only three Amerindian mitochondrial

lineages are present in Cholula: A ($n = 5$; 42%), B ($n = 5$; 42%), and C ($n = 2$; 16%) (Table 4).

The HVR-I and HVR-II haplotype variants are shown in Table 4. In eight samples, the sequence reading ranges were fragmented and did not cover the complete targeted sequence range, which caused difficulties in the use of software such as HaploGrep to define haplogroups (Bandelt et al. 2012; Röck et al. 2013). The sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/; accession numbers MG550891–MG550915).

Distinctive transitions of the Native American haplogroups were observed in both hypervariable regions. Samples_IF#1, 3, 4, 5, 7, 9, and Cho#3, 6, 7, 12, 24 assigned to haplogroup A presented diagnostic transitions T146C, A153G, C16111T, C16290T, G16319A, T16362C, except for sample_Cho#3 that instead showed transitions A235G and C16290T.

Samples_IF#2, 13, Cho#2, 5, 9, 9/10, 13 assigned to haplogroup B showed the common Native American transition T16217C, except for sample_Cho#8 that presented transitions T16189C, T16298C and T16311C. HVR-I transversion A16183C was observed in samples_IF#2, 13, Cho#8, 9/10, and 13 for haplogroup B. Haplogroup C samples_IF#11, Cho#4 and 5, presented transitions T16325C, C16327T, and only sample_Cho#11 displayed a deletion of base pairs 290–291 characteristic of haplogroup C1. Finally, in haplogroup D, samples_IF#6 and 15 presented transitions C16223T and T16362C. Haplogroup D was absent in the Cholula samples (Table 4).

Table 4 Mitochondrial haplotypes and haplogroups (H) of the HVR-I and HVR-II for each sample from Tlatelolco and Cholula

		HVR-I																																HVR-II																		
ID	H	16026C	16111C	16136T	16145G	16147C	16153G	16172T	16182A	16183A	16189T	16201C	16217T	16209T	16223C	16234C	16235A	16240A	16241A	16290C	16298T	16300A	16301C	16311T	16319G	16325T	16327C	16342T	16345A	16348C	16344C	16362T	16377C	41C	48C	64C	73A	130G	142T	146T	152T	153A	195T	217T	235A	263A	310T	320C	325C	327C	340C	377C
IF# 1	A2		T		A										N	N	N	N	N	T					A							C				T				C		G		N	N			Y		Y	Y	
IF# 3	A2		T											C	T										A							C				T	G			C		C			G	G						
IF# 4	A2		T					N	N	N	N				T					T		G			A							C					G						N	N	N	N	N	N	N	N	N	N
IF# 5	A2		T												T					T					A							C					G			C		C			G	G						
IF# 7	A2		T			Y									T		G			T					A						T	C					G			C		G			G	G						
IF# 9	A2		T	C											T					T				C	A							C				T	G			C		G			G	G						Y
IF# 13	B									C	C		C																							G			Y		Y		N	N	N	N	N	N	N	N	N	N
IF# 2	B								M	C			C																								G									G	C					
IF# 11	C							C							T						C					C	T		R	Y			Y				G				N	N	C	C		N						
IF# 6	D	Y	N	N			A					T			T	T		G			N	N	N	N	N	N	N	N	N			C			Y	T	G	R		N	N	N	N	N	G	G	N	N	N	N	N	N
IF# 15	D4 h3														T	A			G				T					C				C		Y			G									G						

		HVR-I																				HVR-II																	
ID	H	16111C	16129G	16181A	16183A	16189T	16213G	16217T	16223C	16239C	16240A	16278C	16290C	16298T	16311T	16319G	16325T	16327C	16354C	16362T	16391G	44.1C	64C	73A	114C	146T	150C	152T	153A	199T	204T	225G	235A	250T	263A	267T	290-291 del	309.1	315.1
Cho #3	A2								T		G		T				N	N	N	N	N		T	G		C		C	G		C	A	G						
Cho #6	A2	T	A			C			T				T			A				C	N			G		C							G		G			C	C
Cho #7	A2	T							T	T			T			A				C	N		T	G		C		C	G				G					C	C
Cho #12	A2	T							T	T			T			A				C		C	T	G	C			C	G				G		G			C	C
Cho #24	A2	T														A				C	N		T	G		C			G				G		G			C	C
Cho #2	B							C														N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Cho #5	B			N	N	C		C									N	N	N	N	N			G											G			C	
Cho #8	B				C	C	N	N	N	N	N			C	C							N	N	N	G										G	T		C	C
Cho #9	B4				C	C	A	C				T												G			T								G			C	C
Cho #10					C	Y	A	C				T												G			T								G			C	C
Cho #13	B4				C	C		C				Y												G											G			C	C
Cho #4	C	T					N	N	N	N	N			C	C		C	T	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Cho #11	C1			G					T					C			C							G											G		✓		C
Cho #1	?	N	N						T										T		A	N	N	N	N	N	N	N	N	C	C			C	G			C	C

Nei genetic distances based on sequences of the HVR-I (16106–16,378 bp) showed closer genetic affinity of the Tlatelolco subadults with sequences from Tlatelolco and those identified as Aztecs in Xaltocan (PoAz_Xaltocan), than to any other group (0.007) (Table 5).

Sequences from Cholula displayed the closest relationship with PrAz_Xaltocan (0.023) although the Tlatelolco subadults were even more closely related to this group (0.014) (Table 5; Fig. 2). Both Tlatelolco subadults and Cholula presented the highest genetic distances with samples from Teopancazco (Teotihuacan) which represent a multiethnic neighbourhood in Teotihuacan combining locals and migrants (Álvarez-Sandoval et al. 2015) (Table 5; Fig. 2). Genetic distances with Paquimé and Mayas showed similar results in relation to Tlatelolco and Cholula (Table 5). To further investigate the patterns of genetic variation in geographic space, the Mantel test was used to measure the correlation between geographic and Pairwise Nei genetic distance matrices. The results showed a non-significant correlation between the genetic and geographical distance matrices ($r = 0.01589$, $P < 0.683$), indicating that the levels of genetic differences between these populations do not depend on geographic distances (S4 Table).

The analyses of haplogroup frequencies consistently showed a closer genetic affinity of Tlateololco subadults to the sacrificial victims from the same site reported by De la Cruz and colleagues (2008) (0.003004) and also to the Po_Az (0.00499) (Table 6; Fig. 3).

However, the haplogroup frequency distribution of the Tlatelolco subadults was not statistically different from that of most of the others at the 0.05 level of probability ($P < 0.0038$ with Bonferroni's correction (Abdi 2007)), except for the Maya sites of Copán and Xcaret (Table 7).

Based on haplogroup frequencies, Cholula shows closer genetic affinity to Tlatelolco (0.03018) (Table 6; Fig. 3) than to most others. However, Cholula's haplogroup distributions were only significantly different from those of PrAz_Xaltocan (0.0013), Mayas (0.0001), Xcaret (< 0.0001) and Copán (< 0.0001) (Table 7).

The haplotype networks constructed for haplogroup A (Table 4; Fig. 4) showed that Tlatelolco-subadult haplotypes derive from other Central Mexican populations; however, these subadults carried unique haplotypes, not related between them, which is observable in the dispersion from the basal node in the samples (Fig. 4). At the same time, Cholula haplotypes derived from Mesoamerican haplotypes, and only two individuals, sample_Cho#7 and Cho#12, carry identical haplotypes which indicate most possibly maternally related individuals (Fig. 4). The same individuals share this haplotype with two ancient samples from the Mexican Maya area described by Ochoa and colleagues (2016), named T3E9 and E7P65, from the sites of Comalco and Tenosique, respectively, in Tabasco (AD 500–1000). Haplogroup B, C and D networks show that Tlatelolco and Cholula samples carried mostly unique haplotypes, and Tlatelolco samples are associated with the Aztecs rather than other groups (Fig. 4). None of the Cholula samples show an association to Teopancazco (Teotihuacan) haplotypes in the networks (Fig. 4).

Finally, a larger sample size could increase the ability to understand the association of the haplogroup D haplotypes in Mesoamerica, especially from the Mayan area, where this haplogroup has been described for past populations (Merriwether et al. 2006). Maternal lineages from Cholula did not derive from currently available sequences from the Maya area (Ochoa-Lugo et al. 2016). The two haplogroup D samples from the Tlatelolco subadults not only exhibit mutations C16223T and T16362C but also carry unique haplotypes (Fig. 4). Finally, there were no Cholula samples assigned to this haplogroup. Unfortunately, the lack of reported HVR-II sequences also limits the comparisons of more control region variants with other populations from ancient Central Mexico.

Sex identification of Tlatelolco subadults

Targeted amplification of the amelogenin locus was successful for eight of the 15 samples from Tlatelolco, indicating a

Table 5 Pairwise population matrix of Nei genetic distances based on sequence data

Tlatelolco subadults	Cholula	Tlatelolco	PrAz_Xaltocan	PoAz_Xaltocan	Teopancazco	Paquimé	Mayas	
0.000								Tlatelolco subadults
0.023	0.000							Cholula
0.015	0.027	0.000						Tlatelolco
0.014	0.023	0.015	0.000					PrAz_Xaltocan
0.007	0.025	0.015	0.014	0.000				PoAz_Xaltocan
0.250	0.197	0.209	0.263	0.256	0.000			Teopancazco
0.028	0.022	0.024	0.015	0.030	0.270	0.000		Paquimé
0.022	0.030	0.022	0.021	0.021	0.281	0.030	0.000	Mayas

Fig. 2 Principal coordinates (PCoA) based on Nei's genetic distances for the HVR-I haplotypes (16,106–16,378 bp)

female sex for all successful samples (S1 Fig.). Subsequent WGS confirmed the female sex for these eight samples and identified two additional females and two additional males within the assemblage (10 females, two males total) (Supplementary Material 1). Together, the sex identification results indicate a sex ratio of 5:1, and a dominance of female subadults within the sacrificial assemblage.

Authenticity of the obtained results

The authenticity of the obtained results can be demonstrated through a number of criteria, including (1) good overall preservation of DNA within the Tlatelolco assemblage, as evidenced by a mean endogenous DNA > 20%; (2) repeat amplifications of the samples within the same and different laboratories produced consistent mtDNA haplotypes and haplogroups; (3) where one individual was represented by two bone elements (Cho#9 and Cho#10), consistent haplotypes were obtained from both elements (Tables 2, 3); (4) the sex determination results obtained through PCR amplification of the amelogenin locus were consistent with those obtained through WGS; (5) misincorporation patterns in the WGS data was consistent with degraded templates (S2 Fig); (6) all of the samples produced haplotypes consistent with Amerindian lineages, while none of the laboratory workers in Calgary or York exhibit Native American haplogroups; and (7) blank extractions and/or negative controls failed to produced PCR amplifications in the mtDNA or amelogenin analyses.

Discussion and conclusions

The analysis of Tlatelolco's and Cholula's genetic affinities shows that their cultural history of rituals and ethnic compositions is also reflected in their genetic structure. As expected, a higher frequency of haplogroup A was observed for the Tlatelolco subadults. The observance of close genetic similarities of the Tlatelolco subadults to Tlatelolcas and Aztecs supports the ethnohistoric accounts (Broda de Casas 1971) that suggest that Tlatelolcas offered children from within their immediate social group for ceremonial purposes. However, sex determination of the Tlatelolco subadults also revealed that ten of the victims (83%) corresponded to females, a pattern different from the one found by De La Cruz et al. (2008), who molecularly identified the sex of other sacrificed children as exclusively male. This discrepancy could reflect the variety of rituals in Tlatelolco, as depending on the Aztec calendar, in which subadult females could have been preferred for certain sacrificial practices (Motolinía 1950; Broda de Casas 1971). For example, Broda de Casas (1971) suggests that young female ceremonial victims would impersonate the lake. The study of additional skeletons associated with diverse ceremonial contexts in Tlatelolco and Tenochtitlan is necessary to understand the patterns of this Mexica practice further.

Secondly, this study aimed to evaluate Cholula's Postclassic population and its genetic affinity to other Mesoamerican groups. We found in Cholula an equal representation of haplogroups A and B (42%) and a complete lack of haplogroup D; our finding contrasts with the rest of Central Mexico where haplogroup A is more common, and

Table 6 Pairwise population matrix of Nei genetic distances based on haplogroup frequencies listed in Table 4

Cholula	Tlatelolco-infants	1)Tlatelolco	2)Tlatelolco	3)Tlatelolco	PrAz_Xaltocan	PoAz_xaltocan	Tetetzontlilco	Teotihuacan	Teopancazco	Yucundaa	Mayas	Xcaret	Copán	Paquimé	
0.0000															Cholula
0.160531	0.0000														Tlatelolco-infants
0.233998	0.011131	0.0000													1) Tlatelolco
0.03018	0.071397	0.119338	0.0000												2) Tlatelolco
0.133778	0.003004	0.012729	0.053416	0.0000											3) Tlatelolco
0.35302	0.205146	0.276581	0.195798	0.222105	0.0000										PrAz_Xaltocan
0.146616	0.00499	0.008575	0.063048	0.00093	0.248036	0.0000									PoAz_Xaltocan
0.209272	0.05122	0.039496	0.160013	0.048902	0.521294	0.041194	0.0000								Tetetzontlilco
0.071186	0.037988	0.055681	0.048002	0.025681	0.369365	0.025715	0.033828	0.0000							Teotihuacan
0.090993	0.025981	0.046697	0.061574	0.021652	0.339907	0.023272	0.026928	0.005081	0.0000						Teopancazco
0.067296	0.036344	0.062169	0.049047	0.028251	0.348013	0.030704	0.037773	0.00258	0.001841	0.0000					Yucundaa
0.328055	0.135254	0.137558	0.312102	0.15264	0.696388	0.148333	0.048609	0.119881	0.085646	0.104071	0.0000				Mayas
0.309696	0.083776	0.045133	0.226375	0.077064	0.625255	0.062056	0.016015	0.074704	0.075258	0.090655	0.096486	0.0000			Xcaret
1.355892	1.686114	2.341542	1.984363	1.949454	2.378528	2.116414	1.439762	1.512058	1.243273	1.258842	0.700534	2.412246	0.0000		Copán
0.045965	0.269895	0.401984	0.081302	0.251522	0.250006	0.281338	0.427468	0.205382	0.214393	0.183776	0.53476	0.613472	1.077819	0.0000	Paquime

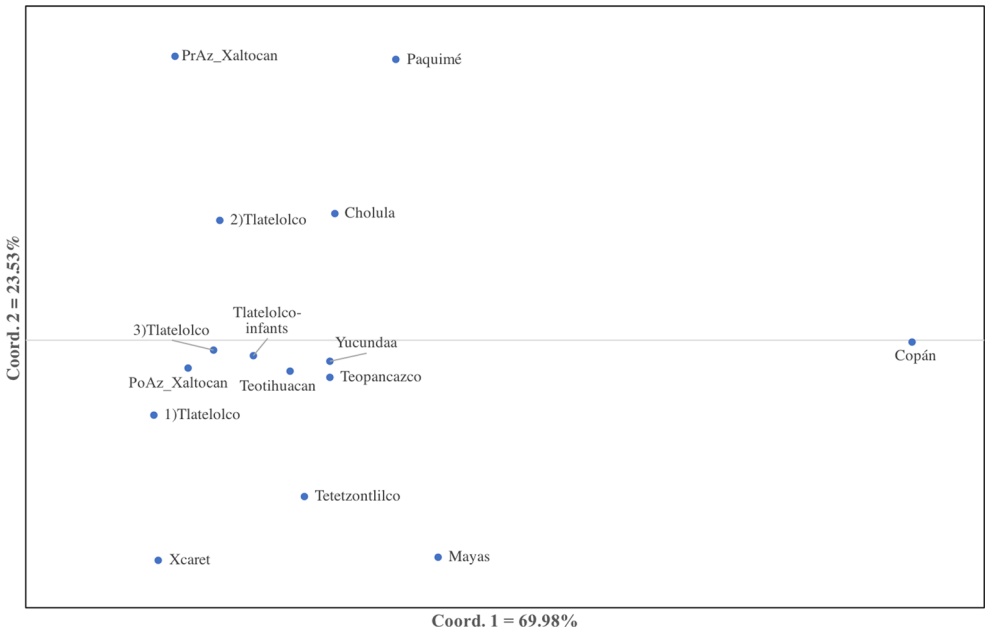

Fig. 3 Principal coordinates (PCoA) based on Nei's genetic distances for ancient mitochondrial haplogroup frequencies (Table 1) in Central Mexico

haplogroup D is commonly observed in lower frequencies (Table 3). The reason for Cholula's different haplogroup distribution from others in Central Mexico is unknown but might relate to ethnohistorical and archaeological evidence of ethnic migration to the city in the Epiclassic period (AD 600–900) (McCafferty 2007). Although Cholula sequence-based genetic distances show closer affinity to PrAz_Xaltocan (Table 4), the haplogroup distribution between them is significantly different (Tables 6 and 7) probably due to the absence of haplogroup D in Cholula, versus a 40% presence in the PrAz_Xaltocan group (Table 5). However, haplogroup frequencies do not evaluate directly the lineage relationships. The use of only haplogroup frequencies limits the evaluation of other relevant aspects of the demographic history such as the extent of admixture, drift and natural selection (Kivisild 2015). A closer examination of haplotypes shows strong similarities between Cholula inhabitants and the PrAz_Xaltocan populations (0.023), with several derived variants, but it would be necessary to increase the dataset from Xaltocan past groups to clarify this relationship. With respect to mtDNA haplotypes, a higher genetic distance of Cholula samples to Teopancazco (0.250) than to any other Central Mexican population questions the hypothesized population's movements from Teotihuacan to Cholula after the collapse of the Classic period. This point deserves much further investigation by adding more samples for each period in Cholula. Lastly, Cholula also presented a significantly different pattern from the Mayan populations here analysed (Table 7), which would not currently support a significant influx of Maya origin people to Cholula. Furthermore, the Maya themselves were culturally diverse (Sharer and Traxler 2006), and additional genetic evidence is required to more fully characterize their genetic makeup.

Network analysis also revealed that most of the Cholula individuals carry unique haplotypes. However, two samples, _Cho#7 and _Cho#12, share identical haplotypes (Fig. 4), which could suggest a maternal relation. The two individuals belong to different archaeological excavations, both corresponding to the Middle Postclassic. This maternal similarity could be the product of a haplotype that was highly frequent in the population at that time. Nevertheless, it is intriguing that

Table 7 *P* value comparisons between Cholula, Tlatelolco and populations listed in Table 2. *P* values were calculated using Genepop (Raymond and Rousset 1995; Rousset 2008). Results marked with a (*) are statistically significant from one another at the 0.5 level, when corrected with Bonferroni's correction ($\alpha = 0.0038$)

	Tlatelolco subadults	Cholula	Region
1) Tlatelolco	0.7330	0.0019	Central Mexico
2) Tlatelolco	0.3337	0.2431	Central Mexico
3) Tlatelolco	1.0000	0.0848	Central Mexico
PrAz_Xaltocan	0.1585	0.0013*	Central Mexico
PoAz_Xaltocan	0.9689	0.0574	Central Mexico
Tetetzontlilco	0.0863	0.0076	Central Mexico
Teopancazco	0.4790	0.2025	Central Mexico
Teotihuacán	0.1089	0.2821	Central Mexico
Yucundaa	0.2087	0.3407	Mixtec
Mayas	0.0008*	0.0001*	Maya
Xcaret	0.0019*	< 0.0001*	Maya
Copán	< 0.0001*	< 0.0001*	Maya
Paquimé	0.1156	0.4667	Greater Southwest USA

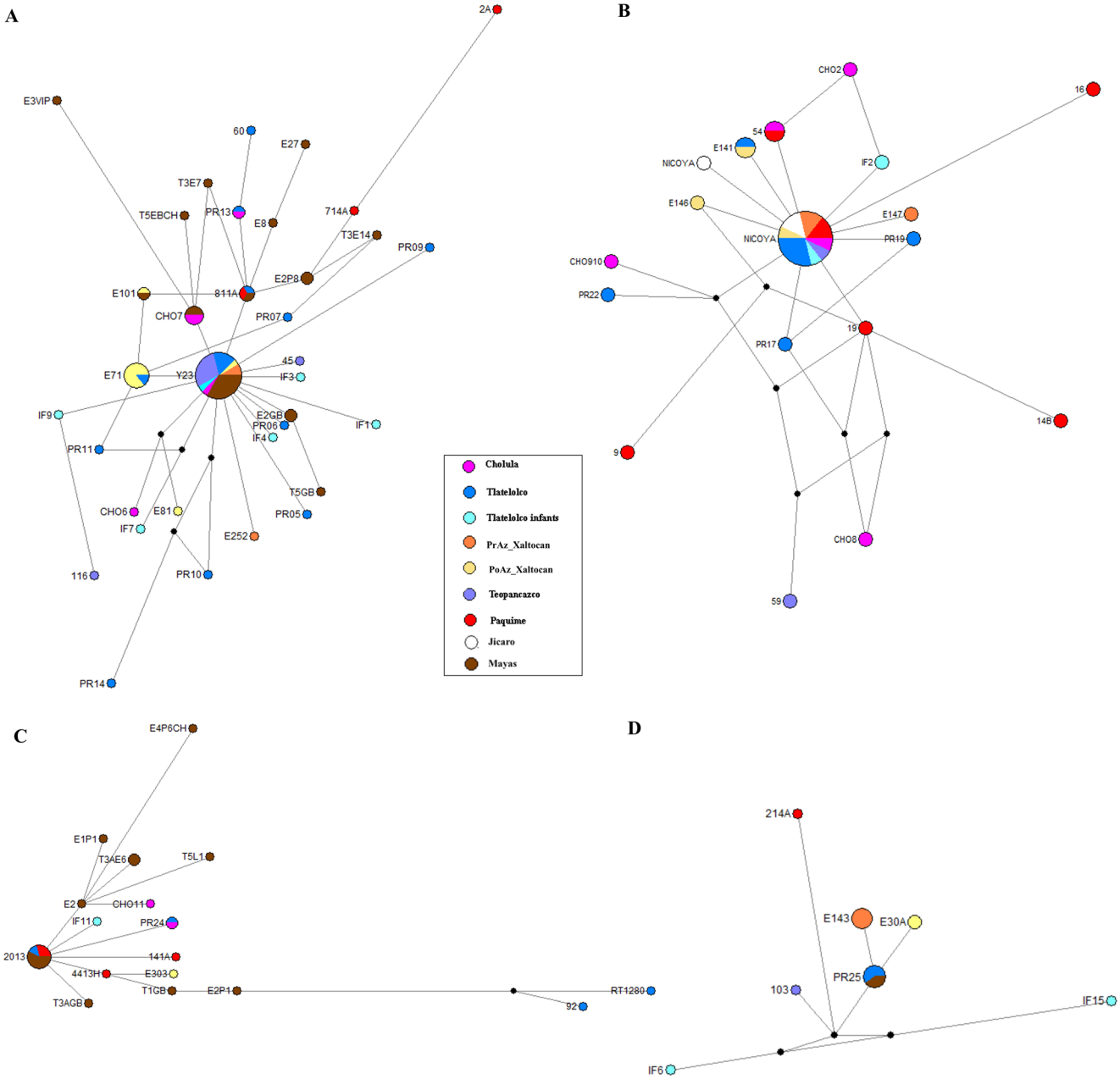

Fig. 4 Haplogroups A, B, C and D haplotype networks. Node size is dependent on the number of samples sharing that haplotype. Black circles represent median vectors, haplotypes that were not sample in this study

the same haplotypes have been described for ancient Maya in Tabasco. The multiculturality suggested for Cholula is perhaps reflected by the genetic analyses, as they are separate from other Nahua-affiliated groups. However, it is necessary to increase the sample size from Cholula to evaluate if its genetic diversity reflects a multi-origin component of the population by period.

Other samples such as those from Jícaro, Greater Nicoya, did not group in the haplogroup B network analysis with any other derived haplotype from Central Mexico, which supports the theory that Greater Nicoya could have a different ancestry than suggested from Central Mexico, and in contrast to traditional interpretations of migration out of Mexico (Fowler 1989; McCafferty and Dennett 2013; Mccafferty 2015). However, the analysis of whole mitochondrial genomes and a larger number of samples from both areas would be required to confirm this pattern.

Additionally, this study found that genetic distances are not dependent on the geographic distances, which may support a previous dental traits study by Willermet et al. (2013), who argue that cultural differences, more than geographic distances, explain the patterns of biological differentiation in Central Mexico.

Furthermore, this study demonstrates the feasibility of conducting molecular sex determination and ancestry analysis in the diverse world of ancient Central Mexico. Both sites, Tlatelolco and Cholula, showed a high percentage of mitochondrial DNA recovery considering their excavation from geographic areas considered unfavourable for ancient DNA preservation (Hofreiter et al. 2015). The Cholula samples (represented mostly by teeth) presented a higher mtDNA amplification rate (92%) than Tlatelolco (73%) (consisting principally of ribs and metatarsals). Nevertheless, the successfully amplified samples from the latter site demonstrated an average endogenous human DNA content > 20% (and as high as 85%), which is exceptional for skeletal elements other than the petrous portion of the skulls and tooth cementum (Hansen et al. 2017).

Although several aDNA studies have been carried out on Mesoamerican populations, the data continues to be limited, predominantly concentrating on haplogroup frequencies rather than sequencing HVR-I. As the availability of technology increases, it is necessary to obtain higher resolution genetic information to characterize Central Mexican groups. Future paleogenetic research in Mesoamerica could benefit from whole mitogenome sequencing and nuclear recovery techniques to expand population structure data and understand the geographic and temporal dynamics. In this study, the HVR-II was not used for comparison due to the lack of available data; however, it was useful to clarify haplogroup determination, which is recommended for conducting comparisons at the population level (Parson et al. 2014).

Finally, this study represents important progress in the understanding of ancient Mexican populations. We have demonstrated the possibility of conducting ancient DNA analysis on highly degraded skeletal samples from Mexico, which could be enhanced by the future application of other methods such as Y-STR profiles to understand paternal lineages and next-generation sequencing to obtain whole mitogenomes and nuclear information.

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Author contributions were as follows: AYMA, NS and CS designed the research. AYMA, JH and KM performed the experiments. AYMA, JH and CS analysed the data. GM, CS and NS provided materials and resources. AYMA wrote the paper, with input from the other co-authors.

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Data availability statement HVRI and HVRII sequences are available in the NCBI GenBank accession numbers MG550891–MG550915. Whole genome sequence data for Tlatelolco samples are available in the GenBank SRA under accession SRP127309.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

Abdi HH (2007) The Bonferonni and Šidák corrections for multiple comparisons. Encycl Meas Stat 1:1–9. https://doi.org/10.4135/9781412952644

Aguirre Samudio AJ, Gonzales Sobrino BZ, Alvarez-Sandoval BA et al (2016) Genetic history of the classic period of teotihuacan's burials in Central Mexico. Rev Argentina Antropol Biológica 19. https://doi.org/10.17139/raab.2017.0019.01.02

Álvarez-Sandoval BA, Manzanilla LR, González-Ruiz M, Malgosa A, Montiel R (2015) Genetic evidence supports the multiethnic character of Teopancazco, a neighborhood Center of Teotihuacan, Mexico (AD 200-600). PLoS One 10:e0132371. https://doi.org/10.1371/journal.pone.0132371

Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N (1999) Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23:147

Bandelt HJ, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48

Broda de Casas J (1971) Las fiestas aztecas de los dioses de la lluvia una reconstruccion segun las fuentes del siglo XVI. Rev Esp Antropol Am 6:245–327. https://doi.org/10.5209/rev_REAA.1971.v6.26650

Buikstra JE, Ubelaker DH (1994) Standards for data collection from human skeletal remains: proceedings of a seminar at the Field Museum of Natural History. Arkansas Archeological Survey Research Series, Fayetteville

Carballo DM (2016) Urbanization and religion in ancient Central Mexico. Oxford University Press, New York

Carmack RM, Gasco J, Gossen GH (2007) The legacy of Mesoamerica : history and culture of a native American civilization, second. Pearson Prentice Hall, New Jersey

Carrasco P (1971) Los barrios antiguos de Cholula. In: Castro Morales E (ed) Estudios y Documentos de la Region de Puebla-Tlaxcala Vol.III. Instituto Poblano de Antropologi e Historia, Mexico , pp 9–88

Davies N (1977) The Toltecs until the fall of Tula. University of Oklahoma Press, Norman

De La Cruz I, Gonzalez-Oliver A, Kemp BMM et al (2008) Sex identification of children sacrificed to the ancient Aztec rain gods in Tlatelolco. Curr Anthropol 49:519–526. https://doi.org/10.1086/587642

de Sahagún B, Garibay KAM, de Bustamante CM (1975) Historia general de las cosas de Nueva España. x, 1093

Dudar JC, Waye JS, Saunders SR (2003) Determination of a kinship system using ancient DNA, mortuary practice, and historic records

in a upper Canadian pioneer cemetery. Int J Osteoarchaeol 13:232–246. https://doi.org/10.1002/oa.680
Duran FD (1971) Book of the gods and rites and the ancient calendar. University of Oklahoma Press, Norman
Elwess NL, Kopp M, Lavioe E et al (2015) Analysis of ancient mitochondrial DNA within the Tipu Maya collection. Internet J Biol Anthropol 8:1–7. https://doi.org/10.5580/IJBA.30723
ESDIS N (2017) Moderate resolution imaging spectroradiometer (MODIS) terra corrected reflectance (true color), 30 m resolution, 3rd December 2017. In: NASA Worldview. Greenbelt, Maryl. Earth Obs. Syst. Data Inf. Syst. (EOSDIS), Earth Sci. Data Inf. Syst. Proj. Flight Proj. Dir. Goddard Sp. Flight Cent. (GSFC), Natl. Aeronaut. Sp. Ad. https://worldview.earthdata.nasa.gov/ . Accessed 18 Dec 2017
Fortes GG, Paijmans JLA (2015) Analysis of whole mitogenomes from ancient samples. Methods Mol Biol 1347:179–195. https://doi.org/10.1007/978-1-4939-2990-0_13
Fowler WR (1989) The cultural evolution of ancient Nahua civilizations: the Pipil-Nicarao of Central America. University of Oklahoma Press, Oklahoma
Ginolhac A, Rasmussen M, Gilbert MTP, Willerslev E, Orlando L (2017) mapDamage : testing for damage patterns in ancient DNA sequences. Bioinformatics 27(15):2153–2155. https://doi.org/10.1093/bioinformatics/btr347
Gonzales Rul F (1996) Tlatelolco a traves de los tiempos 50 anos despues (1944–1994) Tomo II Etnohistoria. Instituto Nacional de Antropologia e Historia, Mexico
Gonzales Torres Y (2001) Sacrifice and ritual violence. In: Carrasco D (ed) The Oxford encyclopedia of mesoamerican cultures. Oxford University Press, Mexico
González-Martín A, Gorostiza A, Regalado-Liu L, Arroyo-Peña S, Tirado S, Nuño-Arana I, Rubi-Castellanos R, Sandoval K, Coble MD, Rangel-Villalobos H (2015) Demographic history of indigenous populations in Mesoamerica based on mtDNA sequence data. PLoS One 10(8):e0131791. https://doi.org/10.1371/journal.pone.0131791
González-Oliver A, Márquez-Morfín L, Jiménez JC, Torre-Blanco A (2001) Founding Amerindian mitochondrial DNA lineages in ancient Maya from Xcaret, Quintana Roo. Am J Phys Anthropol 116:230–235
Guilliem Arroyo S (2016) Reporte de muestras de Tlatelolco. Mexico D.F
Guillien Arroyo S (1999) Ofrendas a Ehecatl-Quetzalcoatl en Mexico-Tlatelolco. Proyecto Tlatelolco, 1987–1996. Instituto Nacional de Antropologia e Historia, Mexico
Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98
Hansen HB, Damgaard PB, Margaryan A, Stenderup J, Lynnerup N, Willerslev E, Allentoft ME (2017) Comparing ancient DNA preservation in petrous bone and tooth cementum. PLoS One 12(1):e0170940. https://doi.org/10.1371/journal.pone.0170940
Hirth KG (1996) Political economy and archaeology: perspectives on exchange and production. J Archaeol Res 4(3):203–239
Hofreiter M, Paijmans JLA, Goodchild H, Speller CF, Barlow A, Fortes GG, Thomas JA, Ludwig A, Collins MJ (2015) The future of ancient DNA: technical advances and conceptual shifts. BioEssays 37:284–293. https://doi.org/10.1002/bies.201400160
Jonsson H, Ginolhac A, Schubert M et al (2013) mapDamage2.0: fast approximate Bayesian estimates of ancient DNA damage parameters. Bioinformatics 29:1682–1684. https://doi.org/10.1093/bioinformatics/btt193
Juárez Martín A (2002) Parentesco Biológico entre los pobladores prehispánicos de Cholula, mediante el análisis molecular de sus restos óseos. Escuela Nacional de Antropología e Historia, Mexico
Katzenberg MA, Oetelaar G, Oetelaar J, Fitzgerald C, Yang D, Saunders SR (2005) Identification of historical human skeletal remains : a case study using skeletal and dental age , history and DNA. Int J Osteoarchaeol 15:61–72. https://doi.org/10.1002/oa.748
Kemp BM, Reséndez A, Román Berrelleza JA et al (2005) An analysis of ancient Aztec mtDNA from Tlatelolco: pre-Colombian relations and the spread of Uto-Aztecan. In: Reed DM (ed) Genetic approaches to the past. Center for archaeological investigations. Southern Illinois University, Illinois, pp 22–47
Kubler G (1968) La traza colonial de Cholula. Estud Hist Novahispana: 111–127
Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760. https://doi.org/10.1093/bioinformatics/btp324
Lind M (2012) The kingdom and pilgrimage center of Cholula. In: Fields VM, Pohl JMD, Lyall VI, de Avila Blomberg A (eds) Children of the Plumed Serpent: Art and ritual in Mesoamerica's Late Antiquity. Los Angeles County Museum of Art, Los Angeles, p 256
Livi-Bacci M (2006) The depopulation of hispanic America after the conquest. Popul Dev Rev 32:199–232. https://doi.org/10.1111/j.1728-4457.2006.00116.x
Lopez Austin A (2001) Aztec. In: The Oxford encyclopedia of Mesoamerican cultures. Oxford University Press, New York, pp 68–72
Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220
Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnetjournal 17:10–12
Mata-Míguez J, Overholtzer L, Rodríguez-Alegría E, Kemp BM, Bolnick DA (2012) The genetic impact of Aztec imperialism: ancient mitochondrial DNA evidence from Xaltocan, Mexico. Am J Phys Anthropol 149:504–516. https://doi.org/10.1002/ajpa.22152
McCafferty G (1996) Reinterpreting the great pyramid of Cholula, Mexico. Anc Mesoamerica 7:1–17. https://doi.org/10.1017/S0956536100001255
McCafferty G (2001) Cholula. In: The Oxford encyclopedia of Mesoamerican cultures. Oxford University Press, New York, pp 202–205
McCafferty G (2007) So what else is new? A Cholula centric perspective on lowland/Highland interaction during the classic/Postclassic transition. In: Kowalski JK, Kristan-Graham C (eds) Twin Tollans: Chichen Itza, Tula, and the Epiclassic to early Postclassic Mesoamerican world. Dumbarton Oaks, Washington, DC, pp 449–481
Mccafferty G (2015) The Mexican legacy in Nicaragua , or problems when data behave badly. 25:110–118. https://doi.org/10.1111/apaa.12053
McCafferty GG, Dennett CL (2013) Ethnogenesis and hybridity in protohistoric Nicaragua. Archaeol Cult Mix 191
McCafferty G, Steinbrenner L (2005) The meaning of the Mixteca-Puebla style stylistic tradition on the southern periphery of Mesoamerica: the view from Nicaragua. In: Waters-Rist A, Cluny C, Mcnamee C, Steinbrenner L (eds) 33rd annual Chacmool conference. The Arqueological Association of the University of Calgary, Calgary, pp 282–292
Merriwether DA, Reed DM, Ferrell RE, Ferrel RE (2006) Ancient and contemporary mitochondrial DNA variation in the Maya. In: Whittington SL, Reed DM (eds) Bones of the Maya: Studies of Ancient Skeletons. University of Alabama Press, Tuscaloosa, pp 208–217
Meyer M, Kircher M (2010) Illumina sequencing library preparation for highly multiplexed target capture and sequencing. Cold Spring Harb Protoc 5:1–11. https://doi.org/10.1101/pdb.prot5448
Morales-Arce AY, Hofman CA, Duggan AT, Benfer AK, Katzenberg MA, McCafferty G, Warinner C (2017a) Successful reconstruction of whole mitochondrial genomes from ancient Central America and Mexico. Sci Rep 7:18100. https://doi.org/10.1038/s41598-017-18356-0

Morales-Arce AY, Snow MH, Kelley JH, Anne Katzenberg M (2017b) Ancient mitochondrial DNA and ancestry of Paquime inhabitants, Casas Grandes (A.D. 1200-1450). Am J Phys Anthropol 163:616–626. https://doi.org/10.1002/ajpa.23223

Motolinía T (1950) History of the Indians of New Spain. The Cortés Society, Berkeley

Muller F (1973) La extensionk arqueologica de Cholula a traves del tiempo. Comunicaciones:19–22

Nei M (1972) Genetic distance between populations. Am Nat 106:283–292

Nichols DL, Pool CA (2012) The Oxford handbook of Mesoamerican archaeology. Oxford University Press, Oxford

O'Rourke DH, Raff JA (2010) The human genetic history of the Americas: review the final frontier. Curr Biol 20:R202–R207

Ochoa-Lugo MI, Muñoz M de L, Pérez-Ramírez G et al (2016) Genetic affiliation of pre-Hispanic and contemporary Mayas through maternal linage. Hum Biol 88:136–167. https://doi.org/10.13110/humanbiology.88.2.0136

Orozco y Berra M (1960) Historia antigua y de la conquista de Mexico, vol. 4. Editorial Porrúa, Mexico

Paddock J (1987) Cholula en Mesoamerica. Notas Mesoamericanas 10: 21–68

Parson W, Gusmão L, Hares DR, Irwin JA, Mayr WR, Morling N, Pokorak E, Prinz M, Salas A, Schneider PM, Parsons TJ, DNA Commission of the International Society for Forensic Genetics (2014) DNA Commission of the International Society for forensic genetics: revised and extended guidelines for mitochondrial DNA typing. Forensic Sci Int Genet 13:134–142. https://doi.org/10.1016/j.fsigen.2014.07.010

Patterson T, Vaughn Kelso N (2017) Cross blended hypso with relief, water, drains, and ocean bottom, 1:10 raster. Version 3.2.0. In: Nat. Earth. Free Vectors Raster Map Data. http://www.naturalearthdata.com/downloads/10m-cross-blend-hypso/cross-blended-hypso-with-relief-water-drains-and-ocean-bottom/. Accessed 18 Dec 2017

Peakall R, Smouse PE (2006) GENALEX 8: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research–an update. Bioinformatics 28:2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetic software for exact tests and ecumenicism. J Hered 86:248–249

Roman Berrelleza JA (1991) A study of skeletal material from Tlatelolco. In: Carrasco D (ed) To change place: Aztec ceremonial landscapes. University Press of Colorado, Niwot, pp 9–19

Rousset F (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol Ecol Resour 8: 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Saenz Serdio MA, Cedillo Ortega C (2016) Informe parcial para el procesamiento de muestras de ADN mitocondrial antiguo en el Laboratorio de la Universidad de Calgary. Cholula, Puebla

Schubert M, Ginolhac A, Lindgreen S, Thompson JF, Al-Rasheid KAS, Willerslev E, Krogh A, Orlando L (2012) Improving ancient DNA read mapping against modern reference genomes. BMC Genomics 13:178

Sharer RJ, Traxler LP (2006) The ancient Maya, 6th edn. Stanford University Press, Stanford

Skoglund P, Storå J, Götherström A, Jakobsson M (2013) Accurate sex identification of ancient human remains using DNA shotgun sequencing. J Archaeol Sci 40:4477–4482. https://doi.org/10.1016/j.jas.2013.07.004

Smith ME (1984) The Aztlan migrations of the Nahuatl chronicles: myth or history? Ethnohistory 31:153–186

Smith ME (2008a) The Aztec empire. In: Brumfield EM, Feinman GM (eds) The Aztec world. Abrams, New York, pp 121–136

Smith ME (2008b) Aztec city-state capitals. University Press of Florida, Gainesville

Smith ME, Berdan FF (2003) Postclassic Mesoamerica. In: Smith ME, Berdan FF (eds) The Postclassic Mesoamerican world. The University of Utah Press, pp 3–13

Solórzano Navarro E (2006) De La Mesoamérica Prehispanica a la Colonial: La huella del DNA antiguo. Universidad Autonoma de Barcelona

Stoneking M (2000) Hypervariable sites in the mtDNA control region are mutational hotspots. Am J Hum Genet 67:1029–1032

Tsukamoto K, Inomata T, Campiani A et al (2014) Mesoamerican plazas : arenas of community and power. University of Arizona Press, Tucson

Vågene ÅJ, Campana MG, Robles García NM, et al (2017) Salmonella enterica genomes recovered from victims of a major 16th century epidemic in Mexico. bioRxiv

van Oven M (2015) PhyloTree build 17: growing the human mitochondrial DNA tree. Forensic Sci Int Genet Suppl Ser 5:e392–e394

van Oven M, Kayser M (2009) Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat 30: E386–E394. https://doi.org/10.1002/humu.20921

van Zantwijk R (1985) The aztec arrangement: the social history of pre-Spanish Mexico. University of Oklahoma Press, Norman, Oklahoma

Warinner C, García NR, Spores R, Tuross N (2012) Disease, demography, and diet in early colonial New Spain: investigation of a sixteenth-century Mixtec cemetery at Teposcolula Yucundaa. Lat Am Antiq 23:467–489

Willermet C, Edgar HJH, Ragsdale C, Aubry BS (2013) Biodistances among Mexica, Maya, Toltec, and Totonac groups of central and coastal Mexico. Chungara, Rev Antropol Chil 45:447–460

Yang DY, Eng B, Waye JS, Dudar JC, Saunders SR (1998) Technical note: improved DNA extraction from ancient bones using silica-based spin columns. Am J Phys Anthr 105:539–543. https://doi.org/10.1002/(SICI)1096-8644(199804)105:4<539::AID-AJPA10>3.0.CO;2-1

Yang DY, Liu L, Chen X, Speller CF (2008) Wild or domesticated: DNA analysis of ancient water buffalo remains from North China. J Archeol Sci 35:2778–2785. https://doi.org/10.1016/j.jas.2008.05.010