1-1-2018

Mitochondrial DNA Analysis of Mazahua and Otomi Indigenous Populations From Estado de Mexico Suggests a Distant Common Ancestry

Angelica González-Oliver
Facultad de Ciencias, Departamento de Biología Celular, Universidad Nacional Autónoma de México, Ciudad de México, México, goliver@unam.mx

Ernesto Garfias-Morales
Facultad de Ciencias, Departamento de Biología Celular, Universidad Nacional Autónoma de México, Ciudad de México, México, egarfias@comunidad.unam.mx

D G. Smith
Department of Anthropology, University of California, Davis, Davis, California, USA, dgsmith@ucdavis.edu

Mirsha Quinto-Sánchez
Facultad de Medicina, Departamento de Biología Celular, Universidad Nacional Autónoma de México, Ciudad de México, México, elmirsha@gmail.com

Mitochondrial DNA Analysis of Mazahua and Otomi Indigenous Populations from Estado de Mexico Suggests a Distant Common Ancestry

Angélica González-Oliver,[1]* Ernesto Garfias-Morales,[1] D. G. Smith,[2] Mirsha Quinto-Sánchez[3]

[1]Facultad de Ciencias, Departamento de Biología Celular, Universidad Nacional Autónoma de México, Ciudad de México, México.

[2]Department of Anthropology, University of California, Davis, Davis, California, USA.

[3]Facultad de Medicina, Departamento de Biología Celular, Universidad Nacional Autónoma de México, Ciudad de México, México.

Correspondence to: Angélica González-Oliver, Departamento de Biología Celular, Universidad Nacional Autónoma de México, Circuito Exterior S/N. Coyoacán. Ciudad de México. 04510. México. E-mail: goliver@unam.mx.

KEY WORDS: MITOCHONDRIAL DNA, OTOMANGUEAN, MEXICAN POPULATIONS, CENTRAL MEXICO

Abstract The indigenous Mazahua and Otomi have inhabited the same localities in Estado de Mexico since pre-Columbian times. Their languages, Mazahua and Otomi, belong to the Otomanguean linguistic family, and, while they share cultural traditions and a regional history that suggest close genetic relationships and common ancestry, the historical records concerning

their origin are confusing. To understand the biological relationships between Mazahua and Otomi we analyzed the mitochondrial DNA (mtDNA) genetic variation. We identified the mtDNA haplogroups by restriction fragment length polymorphism typing and sequenced the hypervariable region I of the mtDNA control region in 141 Mazahua and 100 Otomi. These results showed that Otomi exhibit a higher frequency of haplogroup A than B, whereas Mazahua exhibit the opposite pattern. The most frequent subhaplogroups are A2, B2 and C1, in order of their frequency, among the Otomi EM. Meanwhile the subhaplogroups B2, D1, and A2, in that order, are most common among the Mazahua 1. The most frequent haplotypes (Ht) of haplogroups A and B are Ht2 (A) and Ht58 (B2g1) in the Mazahua 1 and Ht8 (A2), Ht22 (A2ao1) and Ht53 (B2c2b) in Otomi EM. The genetic differences between the Mazahua and Otomi EM suggest a distant shared ancestry and a moderate degree of maternal admixture that has not obscured the difference of their mitochondrial DNA patterns. These unexpected results suggest the Mazahua and Otomi probably descend from the same group but separated very early and admixed with other Mesoamerican populations before their arrival in Central Mexico. The historical evidence of conflicting relations between the Mazahua and Otomi and the almost nonexistence of marriage between them could be responsible for maintaining only a moderate degree of maternal admixture.

Approximately 85.2% of the 228,566 Mazahua people and 42.4% of the 209,714 Otomi speaking people inhabit the Estado de Mexico (INEGI 2005), (Figure 1). Both have subsisted primarily on corn and agave agriculture but also make elaborate textile handicrafts and maintain a commercial interchange of their products to supplement their income (Scheffler 1992). The Mazahua and Otomi have their own cultural traditions and system of governance (e.g. the Mazahua Supreme Board) that represents them in the Mexican government.

The Otomi have lived at high altitudes, up to 3000 m above sea level, in Central Mexico since pre-Columbian times and presently inhabit the cold lands East and West from Toluca in Estado de Mexico (Torquemada 1723; de Alva 1891; Soustelle 1993). The Mazahua inhabit several localities North of Toluca, to the North of Nahua groups. The Mazahua and Otomi have lived nearby in the same localities, but their borderline has fluctuated due to frequent conflicts between them, the intrusion of mestizos (urban Spanish speaking people) and the influence of economic and political factors, particularly after the Spanish conquest (Soustelle 1993).

The Mazahua (*hñáthó*) and Otomi (*hñahñu*) languages both belong to the Otomanguean language family (Campbell 1979; Ortíz-Álvarez 2005) which, together with the Ocuiltec, Pame and Chichimec languages, comprise the Otomi-Pame group, one of the most important in Mexico due to its wide distribution in the highlands, its number of speakers and its antiquity (Soustelle 1993). Glottochronologyical estimates based on seven Otopame languages without written documentation prior to the 16th century, which included the *hñáthó* and *hñahñu* languages, indicated that all languages represent a single micro-phylum (Otomanguean) with an initial subdivision around 2700 YBP (Cazes 1976). However, other glottochronologyical studies suggested 6000 YBP (Renfrew et al. 2000) and 6400 YBP (Campbell 2000). The proto-Otomanguean homeland is controversial but was proposed in the Tehuacán Valle, Puebla, and

probably also in sites outside this region. Conversely, the internal diversification of the micro-phylum began somewhere beyond the northern border of Mesoamerica, in San Luis Potosi, or further north; according to this hypothesis the two lineages (ñña-maklasinka and meko) of this micro-phylum presuppose the existence of a population that moved southward to settle in Mesoamerica (Cazes 1976).

According to Clavijero (2003), the Mazahua and Otomi languages were very similar because the Mazahua people were once Otomi that later separated from them. On the other hand, Benavente-Motolinía (1989), reported that all people speaking languages of the Otomanguean linguistic family ("Otomiano groups") descend ultimately from a Chichimec group. Many historical records from XVI Century report the arrival in pre-Columbian times of nomadic people, collectively named Chichimec, from North to Central Mexico. The name Chichimec sometimes refers not to specific ethnic groups but rather more generally to nomadic people from the North. This is consistent with the fact that several Chichimec groups spoke Nahuatl but others spoke Otomi and Tarascan languages (Carmack et al. 1996).

Historians do not agree on the origin of the Mazahuas and Otomies nor when they arrived in Central Mexico. According to de Alva (1891), five tribes under the leadership of Ehécatl, Cohuatzin, Mazacóhuatl (the Mazahua leader), Tlapánhuitz and Hurrz arrived in Central Mexico in a single migration approximately AD 538 (González and Gutierrez 1999). The Otomi people, named after their leader Oton (Sahagún 1999), also called Otomitl, settled where Tula would later be founded, but sometime later abandoned Tula due to frequent invasions of Chichimec groups and migrated to Central Mexico (Soustelle 1993). The *Anales de Cuauhtitlán* (1885) also mention the arrival of several groups in Central Mexico at this time.

After the Mazahua and Otomi arrived at Estado de Mexico, they inhabited and dominated this region until it was conquered by the Nahua speaking Mexica of Tenochtitlán (Nolasco 1999). Nguemore, in the locality of Jocotitlán, part of the Otomi kingdom of Xaltocan, was the central government site of the Mazahua population where tributes were collected by the Aztecs. The Mexica dominated the Otomi and Mazahua groups until the Spanish conquest (Garduño 1999).

There is little archaeological evidence of Otomi-Pame sites. The Huamango site from Acambay, Estado de Mexico, was inhabited by Otomies at least since AD 850. Later, Huamango was dominated by Toltecs between AD 900 and 1300. This site was an important ceremonial and commercial center (Lagunas 1997).

Historical records and both cultural and linguistic affiliations suggest evident connections between the Mazahua and Otomi populations. However, the historical information concerning their origin and genetic relationships is confusing. One possibility is that they were migrant people of the same group that arrived in Central Mexico during the settlement of this region and, therefore, share a close common ancestry and should not show genetic differences. On the other hand, they might have descended from the same group but separated before they migrated to Central Mexico and, therefore, share a more distant ancestry and, consequently, exhibit genetic differences.

Native American mtDNAs descend from five founding haplogroups, A, B, C, D and X, that can be identified by restriction fragment length polymorphisms (RFLPs) or, in the case of haplogroup B, by a 9 base pair (bp) deletion. Additional mutations in the mtDNA control region differentiate the main haplotypes from the same haplogroup (Torroni et al. 1992, 1993; Peñaloza-Espinosa et al. 2007; Tamm et al. 2007; Sandoval et al. 2009; Kemp et al. 2010; Perego

et al. 2010; Gorostiza et al. 2012; Mata-Míguez et al. 2012; Álvarez-Sandoval et al. 2015). The frequencies of these haplogroups differ significantly among contemporary Native American populations, and these differences are often correlated with geography, linguistic and/or cultural affiliation (Lorenz and Smith 1996; Smith et al. 1999; Bolnick et al. 2003). Studies of mtDNA diversity in Mexican Native populations demonstrate that their differences are correlated with geographical location but not language affiliation (Sandoval et al. 2009; Kemp et al. 2010). Studies in the Northeast and Southwest regions of North America also reveal similar haplogroup frequencies in populations living in the same geographic region, even when the populations belong to different language families (Malhi et al. 2001). While such populations usually share the founding mtDNA haplotypes of the mitochondrial haplogroups, only closely related populations share derived haplotypes that are identical or that differ by only a few mutations (Torroni et al. 1993; Lorenz and Smith 1997; Bolnick et al. 2003). Thus, the analysis of the mtDNA haplogroups and haplotypes have the potential to provide a better understanding of genetic relationships among ancient and contemporary indigenous populations of Mesoamerican origin.

It has been observed that Mesoamerican populations are characterized by high frequencies of haplogroup A, lower frequencies of haplogroups B, C and D and absence of haplogroup X (Peñaloza-Espinosa et al. 2007; Sandoval et al. 2009; Kemp et al. 2010; Mata-Míguez et al. 2012), a genetic pattern that is also observed in pre-Columbian Mesoamerican populations (Gonzalez-Oliver et al. 2001; Kemp et al. 2005; Raff et al. 2011). However, our previous mtDNA studies revealed that Mazahua exhibit a higher frequency of haplogroup B than A, a circumstance rarely reported in the literature for Mexican populations (Kemp et al. 2010), while Otomi presented the opposite pattern (Romero-García 2010; González Oliver et al. 2013).

The difference in haplogroup frequencies suggests a distant genetic relationship between the two populations, an unexpected conclusion due to the close historical and linguistic connections between them, as detailed above.

In the present study we analyzed mtDNA haplotypes and haplogroups of Mazahua and Otomi individuals from several additional communities in the Estado de Mexico that have inhabited the same geographical region from pre-Columbian times to examine finer scale genetic relationships between the two populations. Our aim was to assess whether the Mazahua and Otomi populations show genetic differences consistent with a common maternal ancestry and a very early separation of the two groups. We compared our results with those reported in the literature for Nahua populations from Central Mexico and other Otomanguean speaking indigenous populations and characterized the mtDNA genetic diversity within and between the populations belonging to the Otomanguean language family.

Materials and Methods

Sample Collection. We collected buccal swab samples from 149 Mazahua and 101 Otomi individuals that inhabit localities of Estado de Mexico, Mexico. The individuals selected for study were not related, lived in the same localities where their parents and grandparents were born and recognized themselves as Mazahua or Otomi natives practicing their cultural traditions and speaking their own languages. We collected the biological samples according to the ethical guidelines of the Helsinki Declaration. The local tribal and municipal government authorities gave their permission for sample collection, and all individuals sampled were informed of the objectives of the study and voluntarily signed a letter of consent to use their samples in this study.

mtDNA Characterization. DNA was extracted from the buccal swabs using the Qiagen QIAamp DNA Blood kit as previously described (Malhi et al. 2008; Kemp et al. 2010) and screened for the five main Native American mitochondrial haplogroups. For haplogroups A, B, C and D we used the primers and PCR conditions described by Gonzalez-Oliver et al. (2001), with the following modifications: 1.5 mM $MgCl_2$ and 0.02 units/25 μl Platinum Taq (Invitrogen). Haplogroup X was screened as described in Kemp et al. (2010). The PCR amplifications were conducted in an Eppendorf Master Cycler thermocycler. A portion of the amplicons were separated on 12% (for haplogroups A, C and D) or 14% (for haplogroup B) polyacrylamide gels. The sizes of the amplicons were determined by comparison with ϕX174 DNA/HaeIII fragments size standard after staining with ethidium bromide. Haplogroups A, C, and D were typed by restriction analysis as reported in González Oliver et al. (2001, 2013).

The nucleotide positions (nps) 16,021-16,373 of the hypervariable region I (HVRI) of the mitochondrial genome of 141 Mazahua and 100 Otomi individuals were amplified using the primers L15,975 and H16,401 proposed by Vigilant et al. (1989). Eight Mazahua and one Otomi sample were of insufficient quantity after the haplogroup analysis and, therefore, could not be sequenced. The PCR reactions were carried out in 25 μl volumes containing 100 μM of each dNTP, 0.1 μM each primer, 1.5 mM $MgCl_2$, 0.02 units of Platinum Taq (Invitrogen), 1 X buffer supplied by the manufacturer and 1-2 μl of DNA template. A program of 40 cycles of 1 min melting at 94ºC, 1 min annealing at 55ºC and 1 min extension at 72ºC was used for amplification. Negative PCR controls were included in every set of PCR reactions. To confirm amplifications of the appropriate fragment, 3 μl of PCR products together with ϕX174 DNA/HaeIII fragments size standard were visualized on 12% polyacrylamide gels with ethidium bromide. The HVRI amplicons were purified using EXO-SAP enzymes following the supplier's

recommendations and then further purified using Centri-Sep spin columns from Princeton Separations. Both forward and reverse sequencing was conducted with the Big Dye Terminator v3.1 Cycle Sequencing kit of Applied Biosystems on an automatic ABI Prism 310 (Applied Biosystem) at Instituto de Biología, UNAM, C.U. campus.

Statistical Analysis: Mitochondrial Haplogroups and Haplotypes. The haplogroup frequencies of the 149 Mazahua and 101 Otomi from the Estado de Mexico studied here were compared with those of 1,191 individuals from 21 other populations listed in Table 1, whose geographic locations are shown in Figure 1. Abbreviations of populations depicted in Figure 1 and the citations of studies reporting these frequencies are given in Table 1. Several Nahua samples cited in Sandoval et al. (2009) and Kemp et al. (2010) were excluded from this comparison because they represent the same populations (Peñaloza-Espinosa 2015, personal communication). Chi-square tests were used to compare the haplogroup counts between Mazahua and Otomi and other Native populations belonging to the Otomanguean or Uto-Aztecan language families. Differences at the 0.05 level of probability were regarded as statistically significant. We used the mtDNA haplogroup frequencies to conduct the principal component analysis (PCA) performed in the XLSTAT version 2017. Haplogroup diversity (h) was estimated using Arlequin version 3.5.1.2 based on the Tajima and Nei model (Tajima et al. 1984).

Mitochondrial DNA sequences were edited with the CodonCode 3.0.1 software and compared to the revised Cambridge Reference Sequence (Anderson et al. 1981; Andrews et al. 1999) using DNA Alignment version 1.3.3.1. Alignment and base calling were handchecked independently by E.G.M. and A.G.O. The assignment of mtDNA haplotypes to haplogroups was according to Achilli et al. (2008) and Kumar et al. (2011) and elaborated with haplogrep

software version 2 (http://haplogrep.uibk.ac.at) (Kloss-Brandstätter 2010; van Oven 2015; Weissensteiner et al. 2016). All the polymorphic sites of the haplotypes including the RFLP markers were considered to classify the haplotypes into haplogroups/ subhaplogroups.

Shared haplotypes within and between populations were determined in MacClade version 4.08 using the available HVRI data base of the populations reported in Table 1. We did not use the HVRI data reported by Alvarez-Sandoval et al. (2015) because the available sequences in Gene Bank are very small (111 bp).

Estimates of pair-wise population Fst values based on mtDNA haplotypes were calculated in Arlequin 3.5.1.2 (Tajima and Nei 1984; Excoffier et al. 2005). A Neighbor-Joining dendrogram based on the pair-wise Fst distances was generated using PAST Software 3.16 (Hammer et al. 2001). Haplotype diversity (h), nucleotide diversity (π) and number of segregating sites, based on Tajima and Nei model, were calculated both for all four haplogroups combined and for each of the four haplogroups individually using Arlequin 3.5.1.2. The distribution of mtDNA diversity, subdivided across geographic boundaries and the Otomanguean and Uto-Aztecan linguistic families, was evaluated using an analysis of molecular variance (AMOVA) (Excoffier et al. 2005). For geographic comparisons the populations were classified as members of the Eastern Sierra Madre, the Western Sierra Madre, or the Eje Neovolcánico (Center and Southern) groups. The statistical significance of this test was evaluated using 1,000 random permutations. Tajima's D index (Tajima 1993) was calculated to determine if the populations have evolved under the neutral theory model. Mantel tests were performed in R Statistical Software to test correlations between genetic and geographic distances among populations with at least 60 individuals sampled (R Core Team 2013). The genetic distances were estimated as pair-wise Fst values in Arlequin. Geographic distances were calculated in R

Statistical Software using decimal coordinates in cases where these were reported in the literature; some coordinates or samples localities not mentioned in Sandoval et al. (2009), and Mizuno et al. (2014) were inferred from Scheffler (1992).

Median-joining haplotype networks including all the Otomanguean and Uto-Aztecan populations were constructed in Network version 4.6.1.0 (www.fluxus-engineering.com) separately for haplogroups A, B, C and D to determine the genetic relationships among the haplotypes within each haplogroup. To avoid excessive complexity of the haplogroup A network we excluded some Otomanguean populations (Zapotec, Mixtec and Triqui) with a high number of individuals that are not located in Central Mexico. No network was constructed for haplogroup X, because this haplogroup was not detected in this study. The reticulation was resolved according to Kemp et al. (2010), initially applying a default weight of 10 to all sites, then down-weighting sites in proportion to their relative mutation rates as estimated by Meyer et al. (1999). All sequence positions with fourfold higher rates were down-weighted to four, those sites with threefold higher rates were down-weighted to five, and those sites with twofold higher rates were down-weighted to six as was proposed in Mata-Míguez et al. (2012). In the haplogroup B network, the 16,182 and 16,183 positions were disregarded and position 16,186 was down-weighted to 4 to minimize reticulation as in Kemp et al. (2010).

Results

Mitochondrial Haplogroup Frequencies. The mitochondrial haplogroup frequencies of the populations studied here and other populations with which they are compared are given in Table 1. The Mazahua and Otomi from Estado de Mexico are named Mazahua 1 and Otomi EM respectively. Haplogroups A, B, C and D are all present in the Mazahua 1 and Otomi EM, but

haplogroup X was not identified in any individual. The Otomi EM population exhibits a higher frequency of haplogroup A (57.4%) than B (20.8%), followed by haplogroup C (14.8%), and haplogroup D (7.0%) exhibits the lowest frequency. The Mazahua 1 population presents a higher frequency of haplogroup B (35%) than haplogroup A (31.5%), followed by haplogroups D (20.1%) and C (13.4%). Mazahua 1 presents higher haplogroup diversity than Otomi EM (0.7240 and 0.6061, respectively).

Sequence Diversity Analysis. The results of the diversity estimates for each mitochondrial haplogroup are shown in Supplementary Table S1. Approximately similar diversity values were obtained for all populations analyzed, with a few exceptions such as the Triqui and Zapotec 2. The values of nucleotide diversity (π) and Theta pi have very high standard deviations and are not shown in Supplementary Table S1.

Haplogroup Structure. The results of the analysis of the fine haplogroup structure with all the haplotypes from Mazahua 1 and Otomi EM and other native populations are shown in Supplementary Table S2. Not all of these haplotypes belong to the subhaplogroups A2, B2, C1 and D1, as has been previously reported in the literature for some of the populations compared (Sandoval et al. 2009; Gorostiza et al. 2012). As determined with haplogrep software, subhaplogroups A2 (50.0%), B2 (15.0%), C1 (13.0 %), A (7%), B4 (6%) and D1 (6.0%) are the most frequent among the Otomi EM, while the subhaplogroups B2 (32.6%), D1 (21.3%), A2 (16.3%), and C1 (12.0%) A (9.9%) are the most frequent among the Mazahua 1. Subhaplogroups B2 (40.0%), D1 (20%) and A2 (16%) are also the most frequent among Mazahua 2. Subhaplogroup A2 is the most frequent in all contemporary Otomi populations followed by C1 in Otomi H, Otomi S and Otomi V or B2 in Otomi EM (Table 2). Subhaplogroup A2 is the most

frequent when all individuals (1039) in the 19 populations are combined followed of B2, C1 and D1, a result that differs from the report of Sandoval et al. (2009) (A2, C1, B2 and D1). Haplotypes 8 (A2) and 22 (A2ao1) in haplogroup A and 53 (B2C2b) in haplogroup B are the most frequent among the Otomi EM, whereas haplotypes 87 (D1i2), 2 (A) and 58 (B2g1) were most common among the Mazahua 1 (Supplementary Table S2). We identified the subhaplogroup D4h3a in one Otomi individual.

Principal Component Analysis (PCA) and Neighbor-Joining Dendrogram. The PCA plot based on mtDNA haplogroup frequencies (Figure 2) and the Neighbor-Joining dendrogram based on haplotype Fst values (Figure 3) show genetic differences between the Mazahua 1 and the Otomi EM. The Mazahua 2 reported in the literature also show genetic difference with the Otomi EM analyzed in our study. The Nahua Zi are the most different from other populations.

mtDNA Genetic Diversity. The gene diversity estimates (h) based on the haplotypes of all four haplogroups show no difference between Mazahua 1 and the modern Otomi populations (Table 3). The Triqui exhibit the lowest gene diversity, and that of Zapotec 2 is lower than that of all the populations with at least 40 individuals analyzed. It is interesting that Zapotec 1 and Zapotec 2, with similar numbers of individuals analyzed, exhibit different diversity values based on the Theta S values. The Mazahua 1 and Otomi EM have the greatest number of polymorphic sites of all populations (Table 3) and exhibit similar values of nucleotide diversity (π) and Theta pi.

Unique and Shared Haplotypes. Four hundred and forty two mitochondrial haplotypes were identified among the 1039 sequences belonging to the four main mitochondrial haplogroups included in Table 4, with Mazahua 1 and Otomi EM accounting for 54 and 48 of these haplotypes, respectively. While the majority of all mtDNA haplotypes (53.2%) were shared between populations, Mazahua 1 shares a slightly higher percentage of haplotypes with other

populations (46.3%) than Otomi EM (43.8%), while Otomi EM exhibits a higher percentage of unique haplotypes (50.0%) than Mazahua 1 (38.9%). If we consider only the populations with more than 40 individual analyzed, the Otomi H, Otomi S and Otomi V share the highest percentage of haplotypes with other populations (78.1%, 64.8% and 73.5%, respectively). This contrasts sharply with the Otomi EM (43.8%), Triqui (40.0%) and Zapotec 2 (39.3%) which exhibit the lowest proportions of shared haplotypes (Supplementary Table S2). Both Mazahua 1 Otomi EM share haplotyes with other Otomi poplations. Mazahua 1 shares the largest number of haplotypes with Otomi H (15), followed by Otomi EM (14), Otomi V (13) and Otomi S (12), while Otomi EM shares the largest number of haplotypes with Otomi H (10), Otomi S (10), and Otomi V (9). While the Otomi EM share haplotypes with all the populations, Mazahua 1 does not share any haplotypes with ancient Nahua Xt2. (Supplementary Table S3). The Mazahua 1 and Otomi EM share the largest number of haplotypes belonging to the haplogroup B (5), followed by haplotypes belonging to the haplogroup A (4), haplogroup C (3) and haplogroup D (1). Most haplotypes unique to Mazahua 1 are members of haplogroups B and D (Supplementary Table S2).

Analysis of Molecular Variance and Mantel Test. The results of the AMOVA, with populations subdivided into units based on geography or linguistic affiliation (Table 5), indicate that most of the mtDNA diversity (92.95% and 93.5%, respectively) is found within populations as is expected in human populations. The genetic variation due to differences among populations within groups (5.25% and 6.64%, respectively) and among groups (less than 2%) is not significant. Tajima's D and Fu's FS values in the neutrality test were negative as has been observed for most modern populations, suggesting a relatively recent and rapid population growth (data not shown).

The Mantel test yielded a non significant correlation between genetic and geographic distances in this region (r = 0.044, P = 0.315), even when populations with fewer than 60 individuals were not considered (r = 0.193, P = 0.107107). Similar results are obtained when the mitochondrial haplotypes were considered separately (A: r = -0.053, P = 0.654; B: r = 0.156, P = 0.072; C: r = -0.078, P = 0.733; D: r = -0.130, P = 0.854).

mtDNA Haplogroups Network Analysis. The haplotype median-joining networks contain several haplotypes shared between individuals from the Otomanguean and Uto-Aztecan language families (Figures 4-7). No network contained subclades specific to language families or geographic regions. All networks have a star-like shape characteristic of populations with rapid demographic growth, and all contain reticulation caused by mutational hotspots.

The haplotypes of Mazahua 1 and Otomi EM individuals comprise central nodes of haplogroup A, B and C networks. Only the haplogroup D network lacks any Mazahua haplotype in the central node, and this network has a secondary node derived from the central node that includes Mazahua 1 and all modern Otomi populations except Otomi H (Figure 7).

Discussion

The variation in the frequency distribution of the four mitochondrial haplogroups allows testing hypotheses of relatedness between populations. Populations that are closely genetically related should exhibit similar frequencies of these haplogroups, while those with very different frequencies are probably not closely related to each other. This inference is based on the assumption that the frequencies of haplogroups in the contemporary populations are similar to those of their ancestors, although genetic bottlenecks and drift in small populations can significantly change mitochondrial haplogroup frequencies (Kaestle et al. 2001).

The general regional pattern observed for the main mtDNA haplogroups in Mesoamerican populations is a high frequency of haplogroup A, and lower frequencies of haplogroups B, C and D. This pattern is present in both ancient and contemporary populations that belong to different language families and inhabit different geographic regions of Mesoamerica; therefore it has been proposed as a very ancient regional pattern (Kemp et al. 2005). All of the populations compared in this study exhibit haplogroup A at a highest frequency, except the Mazahua 1 and Mazahua 2. Although the small number of individuals analyzed from Mazahua 2 could contribute to the difference in the frequencies between haplogroups B and A, we analyzed a larger number of individuals from Mazahua 1 than in any other population in the present study. We carefully selected the localities sampled in Estado de Mexico, including the locations of Acambay, Ixtlahuaca, Jocotitlán and Temascalcingo, all of which are towns inhabited by Mazahua and Otomi people since the pre-Columbian period. Unfortunately, the authors that analyzed Mazahua 2 did not report the localities where their samples were collected (Mizuno et al. 2014).

The distribution pattern of mitochondrial subhaplogroups, the PCA plot based on haplogroup frequencies and the Neighbor-Joining dendrogram based on genetic distance of mtDNA haplotypes indicate a genetic difference between the Mazahua 1 and the Otomi EM. Moreover, the pattern of mitochondrial subhaplogroups identified for Mazahua 1 is similar to that of Mazahua 2, and, while contemporary Otomi populations do not show similar patterns, subhaplogroup A2 predominates in all of them. These Otomi patterns are not likely to have been affected by sample size, because the numbers of individuals compared among the populations were similar.

The indigenous from Mexico typically exhibit high frequencies of haplogroup A, with, as in both Mazahua and Otomi populations, the largest number of haplotypes shared in this haplogroup. However, the largest number of haplotypes shared between Mazahua 1 and Otomi EM are those within haplogroup B. Therefore, the result found here between the Mazahua 1 and the Otomi EM is unusual.

The networks for haplogroups A, B and C exhibit similar derived and ancestral haplotypes between Otomi and Mazahua populations, suggesting common ancestry and some maternal gene flow between them; however, the central node in the network for haplogroup D includes Otomi haplotypes from contemporary populations but no Mazahua haplotypes suggesting that their common ancestry is distant. The most frequent haplotypes of haplogroups A and B differ between the Mazahua 1 (Ht2 and Ht58) and the Otomi EM (Ht8, Ht22 and Ht53) suggesting a low level of gene flow between them. It is interesting that haplogroup D has a relatively high frequency in Mazahua because this haplogroup is the less frequent in Mexican populations (Kemp et al. 2010; González-Oliver et al. 2013). While the indigenous populations from Mexico usually share ancestral haplotypes in all 4 mitochondrial haplogroups (Kemp et al. 2010; Ochoa-Lugo 2017), Mazahua 1 and Otomi EM represent an exception for haplogroup D. Although Mazahua 1 individuals share a derived node with Otomi EM, Otomi S and Otomi V, no Mazahua 1 individuals occupy the ancestral node that includes members of the Otomi populations.

These genetic differences of mtDNA observed between the Mazahua 1 and the Otomi EM suggest a distant, rather than a closely, shared ancestry dating to 6000 or more YBP. They probably separated very early and admixed with other Mesoamerican populations before their arrival in Central Mexico. This could explain that after their separation the frequency of

haplogroup A decreased and the frequency of haplogroup B increased in the Mazahuas. Other studies have suggested that population movements and gene flow were not negligible forces in North American prehistory (Malhi et al. 2002).

The higher frequency of haplogroup B than of haplogroup A found in the Mazahua population is similar to that present in other populations like the Cora and Huichol from Nayarit State, which also show a higher frequency of haplogroup B than A (approximately 53% B and 31% A) (Kemp et al. 2010). The Cora and Huichol samples were collected in localities that are in the geographic border between the American Southwest and Mesoamerica. The populations from the U.S. Southwest, with the exception of the Athapaskans, are characterized by even higher frequencies of haplogroup B, ranging from 21.9% to 85.9%, and very low frequencies or absence of haplogroup A (Malhi et al. 2003; Kemp et al. 2005; Monroe et al. 2013). A decreasing gradient in the frequency of haplogroup B occurs from American Southwest to Central Mexico (Kemp et al. 2010).

In a previous mtDNA study we compared the Mazahua and Otomi with Cora and Huichol populations; in that study the principal component analysis (PCoA plot) based on mitochondrial haplogroup frequencies showed the Mazahua were closer to Otomi populations than to the Cora and Huichol (González Oliver et al. 2013). The results of the present study are consistent with those of the earlier study in suggesting a distant common ancestry between Mazahua 1 and Otomi EM. The similarity in the B and A haplogroup patterns of Mazahua 1 and the Cora and Huichol populations could suggest some degree of genetic admixture with populations from the North of Mexico after the Mazahua and Otomi populations separated but before the Mazahua migrated to settle in Central Mexico. Although it has also been proposed that the speakers of the Otopame group have occupied the same territory since the internal diversification of the

branches of their languages (Wright 1982), members of Proto-Otopame languages, which derived the Mazahua and Otomi language, probably migrated from North to Mesoamerica (Bernal 1974). The Otomi site of Huamango is considered to have been an important site for trade between Eastern and Western Central Mexico, including that between Tula and the populations than inhabited the state of Queretaro and the Estado de Mexico (Piña 1981; Serrano 1999), during the Postclassic. Therefore some degree of genetic admixture between the Otomi and other surrounded Mesoamerican populations such as Nahuas cannot be ruled out.

The Mazahua and Otomi populations speak different, but closely related, languages of the same linguistic family; these two languages probably began to differentiate when the Mazahua and Otomi separated before their arrival to Central Mexico as two distinct groups. Historical and cultural factors, such as war and marriage customs, might have maintained these genetic differences after their settlement in Central Mexico. The Mazahua inhabited the East of Ixtlahuaca during the start of the Colonial period but were later evicted by the Otomi people. Historical records from the sixteenth through the eighteenth centuries indicate that both Mazahua and Otomi inhabited the regions of Jiquipilco, San Bartolomé (today San Bartolo Morelos) and Chapa de Mota (Soustelle 1993). However, in the eighteenth century the Otomi people conquered the regions where they reside today. Thus, the original border between the Mazahua and Otomi regions was different from that observed at present. For instance, Temascalcingo, initially inhabited only by the Mazahua people, is presently inhabited by both Otomi and Mazahua people. Soustelle (1993) reports that Otomi people of the Meseta Ixtlahuaca from Estado de Mexico do not have good relations with the Mazahua people inhabiting the same locality. The Otomi are looked down upon by the Mazahua people, reflecting the longstanding conflicts between the two populations, and marriage between the Mazahua and Otomi people are

reported to be almost nonexistent (Soustelle 1993). However, in the present study we have detected that this situation is changing in the Acambay locality.

The Neighbor-Joining dendrogram shows that the Otomi EM are closer to Otomi S than to Otomi V. The Otomi S were sampled from San Bartolo Tutotepec, adjacent to Tenango de Doria. Historical records indicate that Otomi people, including the Otomi EM, arrived in San Bartolo Tutotepec since pre-Columbian times. In contrast, the Otomi V were sampled from Cardonal, a region inhabited by Otomi people relocated by the Spanish to develop the mining industry during the Colonial period (Secretaría de Gobernación y Gobierno del Estado de Hidalgo, 1988). These relocations created a region formed by different populations, including Spanish and mestizo regions (Moreno-Alcántara et al. 2006), and might explain the genetic difference between the Otomi EM and the Otomi V. Unfortunately the authors do not report the localities from where the Otomi H were sampled (Sandoval et al. 2009).

In a previous comparative study of mitochondrial haplogroups, we identified genetic differences among the Nahua populations from Central Mexico, although these differences were based on small numbers of individuals (González Oliver et al. 2013). Gorostiza et al. (2012) reported that Nahua populations exhibit greater genetic diversity than other populations. In the present study, we confirm that Nahua populations show no close genetic relationship with each other. Nahuátl was the main language spoken during the Aztec Empire and was used as the lingua franca in Central Mexico during the European contact (Fagan 1984); therefore, the fact that different indigenous groups speak Nahuátl does not necessarily imply a recent common origin. Ancient mtDNA evidence from Xaltocan, Mexico, indicates that the Aztec expansion may have been associated with significant demographic and genetic changes in this area (Mata-Míguez et al. 2012). It is noteworthy that Nahua At and Nahua Xo show genetic differences

despite their close geographical proximity and a shared history of alliance against the Mexica since pre-Columbian times. However, at the time of Spanish arrival Atocpan was part of Xochimilco and both were included in the Triple Alliance (Tlacopan, Tenochtitlan and Texcoco) that reduced the influence of the Tepanec and promoted the founding of the Aztec empire, which also controlled the Mazahua and Otomi (Wacher 2006).

Because the mtDNA is a uniparentally inherited marker it only reflects the female population history. Therefore, these results, showing only the maternal history of Mazahuas and Otomies, could differ from that based on the male lineage, as has been reported in other studies (Malhi et al. 2008; Kemp et al. 2010). Although the mtDNA is a maternally inherited marker, the analyses of the restriction fragment length polymorphisms (RFLPs) and the single nucleotide polymorphisms (SNPs) as population markers are useful indicators of common history, of isolation or interpopulation relationships (Molnar 2002). On the other hand the mitochondrial DNA allow the description of female demographic patterns, which may be affected by different behaviors such as marriage practice, language, cultural differences, and geographic distance (Murdock 1981).

None of the haplogroup A haplotypes in the populations sampled here exhibited either the mutations 16233G and 16331G characteristic of the Athapaskan from Southwest nor the 16257T and 16263A mutations found in Tohono O'odham, Zuni and Chumash of Southern California. This supports the previous interpretation that haplogroup A in the American Southwest was not introduced by farmers from Mesoamerica (Kemp et al. 2010).

All haplogroup B haplotypes identified in the present study exhibited the 16111T mutation which has been found in Cora, Huichol and Tarahumara from North Mexico. We did not detect the subhaplogroup B2a which evolved in the Americas approximately 2,105 YBP

(Kemp et al. 2010). Haplotypes of the subhaplogroup C1 exhibited the mutational positions characteristic of these haplotypes, and the 16298C mutation is present only in the majority of the Oto-manguean populations. The 16249C and 16271C D1 mutations in members of haplogroup, are of interest, because they are found only in the Mazahua 1.

We identified subhaplogroup D4h3a8, previously reported in a Mazahua 2 individual (Mizuno et al. 2014), in an Otomi EM individual. This subhaplogroup has been reported along the Pacific coast of South America as well as in ancient individuals from North America but is not common in Mesoamerica (Kumar et al. 2011; Raff *et al.* 2011). The Native Americans haplotype database from Central Mexico (Supplementary Table S1) is a useful comparative tool that can be used to gain insight into the processes of regional origin of the indigenous populations.

Conclusions

The genetic differences of mtDNA observed between the Mazahua and the Otomi here analyzed suggest a distant shared ancestry. The two probably descend from the same group but separated very early and admixed with other Mesoamerican populations before their arrival in Central Mexico. The highest frequency of subhaplogroup B2 present in the Mazahua is unique in the populations from Central Mexico, suggesting maternal gene flow with populations from the North of Mexico. Historical and cultural factors such as war and marriage customs have probably contributed to the maintenance of a moderate level of gene flow between them after their settlement that did not masked their genetic differences.

In addition to language and ethnicity, it is very important to know the localities or communities of the individuals sampled; this information is necessary to relate the genetic data

with historical and cultural information as we have shown in the analysis of Otomi populations. In contrast with the Native populations from North America (Malhi et al. 2002), our results do not show a clear correlation between the genetic distance, the language affiliation and geographic proximity of the populations analyzed in this study.

Acknowledgements We thank all the Mazahua and Otomi individuals who provided the samples used in this study and all the persons that participated in the collect of the samples. The authors also thank Brian M. Kemp for comments on an earlier version of this manuscript. This work was supported by the Consejo Nacional de Ciencia y Tecnología (Proyecto Ciencia Básica 2008. No. 101791; 2015. No. 252130); and UC-Mexus 2008. (Small Grant Program. Number 08-0037787).

Received 10 July 2017; revision accepted for publication 12 October 2017.

Literature Cited

Achilli, A., U. A. Perego, C. M. Bravi et al. 2008. The phylogeny of the four pan-American mtDNA haplogroups: Implications for evolutionary and disease studies. *PLoS One* 3:e1764.

Alva I., F. de. 1891. *Relaciones*, Vol. 1 of *Obras históricas de don Fernando de Alva Ixtlilxochitl*, A. Chavero, ed. México, DF: Secretaría de Fomento.

Álvarez-Sandoval, B. A., L. R. Manzanilla, M. González-Ruiz et al. 2015. Genetic evidence supports the multiethnic character of Teopancazco, a neighborhood center of Teotihuacan, Mexico (AD 200–600). *PLoS One* 10:e0132371.

Anales de Cuauhtitlán. 1885. In *Anales del Museo Nacional de México*, Vol. 3, J. F. Ramírez, ed., F. Galicia-Chimalpopoca, G. Mendoza, and F. Sánchez-Solís, trans. México, DF: Ignacio Escalante.

Anderson, S., A. T. Bankier, B. G. Barrell et al. 1981. Sequence and organization of the human mitochondrial genome. *Nature* 290:457–465.

Andrews, R. M., I. Kubacka, P. F. Chinnery et al. 1999. Reanalysis and revision of the Cambridge Reference Sequence for human mitochondrial DNA. *Nat. Genet.* 23:147.

Bellwood, P. 2000. The time depth of major language families: An archaeologist's perspective. In *Time Depth in Historical Linguistics*, C. Renfrew, A. McMahon, and L. Trask, eds. Cambridge: McDonald Institute for Archaeological Research, 109–140.

Benavente, Fr. T. de. 1989. *Historia de los indios de la Nueva España*. México, DF: Porrúa.

Bernal, I. 1974. Teotihuacan. In *Historia de México*, Vol. 1, J. Lorenzo and I. Bernal, eds. Barcelona, España: Salvat Editores, 221–240.

Bolnick, D. A., and D. G. Smith. 2003. Unexpected patterns of mitochondrial DNA variation among Native Americans from the southeastern United States. *Am. J. Phys. Anthropol*. 122:336–354.

Campbell, L. 2000. *American Indian Languages: The Historical Linguistics of Native America*. New York: Oxford University Press.

Campbell, L. R., and M. Mithun. 1979. *The Languages of Native America: A Historical and Comparative Assessment*. Austin: University of Texas Press.

Carmack, R., J. Gasco, and G. Gossen, eds. 1996. *The Legacy of Mesoamerica: History and Culture of a Native American Civilization*. Upper Saddle River, NJ: Prentice Hall.

Cazes, D. 1976. Glotocronología Hña-Maclasinca-Meco (Otopame). *Amerindia* 1:65–115.

Clavijero, F. J. 2003. *Historia Antigua de México*. 10th ed. México: Porrúa.

De La Cruz, I., A. González-Oliver, B. M. Kemp et al. 2008. Sex identification of children sacrificed to the ancient Aztec rain gods in Tlatelolco. *Curr. Anthropol*. 49:520–526.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.5: An integrated software package for population genetics data analysis. *Evol. Bioinform.* 1:47–50.

Fagan, B. M. 1984. *The Aztecs*. New York: W. H. Freeman.

Garduño, C. J. 1999. *Temascalcingo: Monografía municipal.* Toluca: Instituto Mexiquense de Cronistas Municipales, Gobierno del Estado de México.

González, G. J. D., and A. P. Gutiérrez. 1999. *Villa Victoria: Monografía municipal.* Toluca: Instituto Mexiquense de Cronistas Municipales, Gobierno del Estado de México.

González-Oliver, A., E. Garfias-Morales, E. Romero-García et al. 2013. Análisis del DNA mitocondrial antiguo y contemporáneo: Un acercamiento a las relaciones genéticas en las poblaciones indígenas de Mesoamérica. *Cuicuilco Nueva Época* 20:153–171.

González-Oliver, A., L. Márquez-Morfín, J. C. Jiménez et al. 2001. Founding Amerindian mitochondrial DNA lineages in ancient Maya from Xcaret, Quintana Roo. *Am. J. Phys. Anthropol.* 116:230–235.

Gorostiza, A., V. Acunha-Alonzo, L. Regalado-Liu et al. 2012. Reconstructing the history of Mesoamerican populations through the study of the mitochondrial DNA control region. *PLoS One* 7:9:e44666.

Hammer, Ø., D. A. T. Harper, and P. D. Ryan. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* 4:1–9.

INEGI (Instituto Nacional de Estadística, Geografía e Informática). 2005. El conteo de población y vivienda 2005. http://www.beta.inegi.org.mx/proyectos/ccpv/2005/.

Kaestle, F. A., and D. Glenn-Smith. 2001. Ancient mitochondrial DNA evidence for prehistoric population movement: The Numic expansion. *Am. J. Phys. Anthropo*l. 115:1–12.

Kemp, B. M., A. González-Oliver, R. S. Malhi et al. 2010. Evaluating the farming/language dispersal hypothesis with genetic variation exhibited by populations in the Southwest and Mesoamerica. *Proc. Natl. Acad. Sci. U. S. A.* 107:6:759–764.

Kemp, B. M., A. Resendez, J. A. Roman et al. 2005. An analysis of ancient Aztec mtDNA from Tlatelolco: Pre-Columbian relations and the spread of Uto-Aztecan. In *Biomolecular Archaeology: Genetic Approaches to the Past*, D. M. Reed, ed. Carbondale: Center for Archaeological Investigations, Southern Illinois University, 22–46.

Kloss-Brandstätter, A., D. Pacher, S. Schönherr et al. 2010. HaploGrep: A fast and reliable algorithm for automatic classification of mitochondrial DNA haplogroups. *Hum. Mutat.* 32:25–32.

Kumar, S., C. Bellis, M. Zlojutro et al. 2011. Large scale mitochondrial sequencing in Mexican Americans suggests a reappraisal of Native American origins. *BMC Evol. Biol.* 11:293.

Lagunas, Z. 1997. Costumbres funerarias y características bioculturales de la población prehispánica de Huamango. *Expresión Antropol.* 6:7–28.

Lorenz, J. G., and D. G. Smith. 1996. Distribution of four founding mtDNA haplogroups among native North Americans. *Am. J. Phys. Anthropol*. 101:307–323.

Lorenz, J. G., and D. G. Smith. 1997. Distribution of sequence variation in the mtDNA control region of native North Americans. *Hum. Biol*. 69:749–776.

Malhi, R. S., J. A. Eshleman, J. A. Greenberg et al. 2002. The structure of diversity within New World mitochondrial DNA haplogroups: Implications for the prehistory of North America. *Am. J. Hum. Genet.* 70:905–919.

Malhi, R. S., A. González-Oliver, K. B. Schroedes et al. 2008. Distribution of Y chromosomes among native North Americans: A study of Athapaskan population history. *Am. J. Phys. Anthropol.* 137:412–424.

Malhi, R. S., H. M. Mortensen, J. A. Eshleman et al. 2003. Native American mtDNA prehistory in the American Southwest. *Am. J. Phys. Anthropol*. 120:108–124.

Malhi, R. S., B. A. Schultz, and D. G. Smith. 2001. Distribution of mitochondrial DNA lineages among Native American tribes of northeastern North America. *Hum. Biol*. 73:17–55.

Mata-Míguez, J., L. Overholtzer, E. Rodríguez-Alegría et al. 2012. The genetic impact of Aztec imperialism: Ancient mitochondrial DNA evidence from Xaltocan, Mexico. *Am. J. Phys. Anthropol.* 149:504–516.

Meyer, S., G. Weiss, and A. von Haeser. 1999. Pattern of nucleotide substitution and rate heterogeneity in the hypervariable regions I and II of human mtDNA. *Genetics* 152:1,103–1,110.

Minchin, P. R. 1987. An evaluation of relative robustness of techniques for ecological ordinations. *Vegetatio* 69:89–107.

Mizuno, F., J. Gojobori, L. Wang et al. 2014. Complete mitogenome analysis of indigenous populations in Mexico: Its relevance for the origin of Mesoamericans. *J. Hum. Genet.* 59:359–367.

Molnar, S. 2002. *Human Variation: Races, Types and Ethnic Groups.* Upper Saddle River, NJ: Prentice Hall.

Monroe, C., B. M. Kemp., and D. G. Smith. 2013. Exploring prehistory in the North American Southwest with mitochondrial DNA diversity exhibited by Yumans and Athapaskans. *Am. J. Phys. Anthropol.* 150:618–631.

Moreno-Alcántara, B., M. G. Garret Ríos, and U. J. Fierro-Alonso. 2006. *Otomíes del Valle del Mezquital*. Mexico, DF: Comisión Nacional para el Desarrollo de los Pueblos Indígenas.

Murdock, G. P. 1981. *Atlas of World Cultures.* Pittsburgh, PA: Pittsburgh University Press.

Nolasco, A. M. A. 1999. *Aculco: Monografía municipal.* Toluca: Instituto Mexiquense de Cronistas Municipales, Gobierno del Estado de México.

Ochoa-Lugo, M., M. Muñoz, G. Pérez-Ramírez et al. 2016. Genetic affiliation of pre-Hispanic and contemporary Mayas through maternal linage. *Hum. Biol.* 88:136–167.

Ortíz-Álvarez, M. I. 2005. *La población hablante de lenguas indígenas en México.* Temas selectos de geografía de México no. 1.3.3. México, DF: Universidad Nacional Autónoma de México.

Peñaloza-Espinosa, R. I., D. Arenas-Aranda, R. M. Cerda-Flores et al. 2007. Characterization of mtDNA haplogroups in 14 Mexican indigenous populations. *Hum. Biol*. 79:313–320.

Perego, U. A., N. Angerhofer, M. Pala et al. 2010. The initial peopling of the Americas: A growing number of founding mitochondrial genomes from Beringia. *Genome Res.* 20:1,174–1,179.

Piña-Chán, R. 1981. Investigaciones sobre Huamango y región vecina. México, DF: Gobierno del Estado de México.

Raff, J. A., D. A. Bolnick, J. Tackney et al. 2011. Ancient DNA perspectives on American colonization and population history. *Am. J. Phys. Anthropol*. 146:503–514.

R Core Team. 2013. *R: A Language and Environment for Statistical Computing.* Vienna, Austria: R Foundation for Statistical Computing.

Romero-García, E. 2010. Análisis de los linajes fundadores del DNA mitocondrial en las poblaciones contemporáneas: Mazahua y Otomí del Estado de México. Bachelor's thesis, Escuela Nacional de Antropología e Historia.

Sahagún, Fr. B. de. 1999. *Historia general de las cosas de Nueva España*. 10th ed. Mexico, DF: Porrúa.

Sandoval, K., L. Buentello-Malo, R. Peñaloza-Espinosa et al. 2009. Linguistic and maternal genetic diversity are not correlated in Native Mexicans. *Hum. Genet*. 126:521–531.

Scheffler, L. 1992. *Los indígenas mexicanos*. México, DF: Panorama Editorial.

Secretaría de Gobernación y Gobierno del Estado de Hidalgo. 1988. Los municipios de Hidalgo. In *Colección enciclopedia de los municipios de México*, Vol. 13. http://siglo.inafed.gob.mx/enciclopedia/EMM13hidalgo/index.html.

Serrano, E. 1999. *Acambay: Monografía municipal.* Toluca: Instituto Mexiquense de Cultura, Asociación Mexiquense de Cronistas Municipales, Gobierno del Estado de México.

Smith, D. G., R. S. Malhi, J. Eshleman et al. 1999. Distribution of mtDNA haplogroup X among native North Americans. *Am. J. Phys. Anthropol*. 110:271–284.

Soustelle, J. 1993. *La familia otomí-pame del México central*. México, DF: Fondo de Cultura Económica.

Tajima, F. 1993. Simple methods for testing the molecular evolutionary clock hypothesis. *Genetics* 135:599–607.

Tajima, F., and M. Nei. 1984. Estimation of evolutionary distance between nucleotide sequences. *Mol. Biol. Evol.* 1:269–285.

Tamm, E., T. Kivisild, M. Reidla et al. 2007. Beringian standstill and spread of Native American founders. *PLoS One* 2:e829.

Torquemada, Fr. J. de. 1723. *Veinte i un libros rituales i monarchia indiana*. 3 vols. Madrid: Nicolás Rodriguez Franco.

Torroni, A., Y. S. Chen, O. Semino et al. 1994. mtDNA and Y-chromosome polymorphisms in four Native American populations from southern Mexico. *Am. J. Hum. Genet.* 54:303–318.

Torroni, A., T. G. Schurr, M. F. Cabell et al. 1993. Asian affinities and continental radiation of the four founding Native American mtDNAs. *Am. J. Hum. Genet.* 53:563–590.

Torroni, A., T. G. Schurr, C. Yang et al. 1992. Native American mitochondrial DNA analysis indicates that the Amerind and Nadene populations were founded by two independent migrations. *Genetics* 130:153–162.

van Oven, M. 2015. PhyloTree Build 17: Growing the human mitochondrial DNA tree. *Forensic Sci. Int. Genet. Suppl. Ser.* 5:e392–e394.

Vigilant, L., R. Pennington, H. Harpending et al. 1989. Mitochondrial DNA sequences in single hairs from a southern African population. *Proc. Natl. Acad. Sci. U. S. A.* 86:9,350–9,354.

Wacher, M. 2006. *Nahuas de Milpa Alta*. Mexico, DF: Comisión Nacional para el Desarrollo de los Pueblos Indígenas.

Weissensteiner, H., D. Pacher, A. Kloss-Brandstätter et al. 2016. HaploGrep 2: Mitochondrial haplogroup classification in the era of high-throughput sequencing. *Nucleic Acids Res.* 44:W58–W63.

Wright, D. 1982. The sixteenth century murals of the Augustinian monastery at Ixmiquilpan. Master's thesis, Instituto Allende, Guanajuato, Mexico.

Table 1. Haplogroup Frequencies

Population	Abreviature[1]	Language Family	n	A (%)	B (%)	C (%)	D (%)	X (%)	J[2] (%)	L[2] (%)	(h)[3]	Geographic Location	Reference
Aztec[4]	Azt	Uto-Aztecan	37	62.2	16.2	5.4	16.2	0.0	0.0	0.0	0.5736	Tlatelolco, Mexico City	Kemp et al. 2005, 2010; De la Cruz et al. 2008
Mazahua 1	Mz1	Otomanguean	149	31.5	35.0	13.4	20.1	0.0	0.0	0.0	0.725	Estado de Mexico	This study
Mazahua 2	Mz2	Otomanguean	25	16.0	52.0	8.0	24.0	0.0	0.0	0.0	0.6667	Estado de Mexico	Mizuno et al. 2014
Mixtec 1	Mx1	Otomanguean	123	66.7	22.8	7.3	3.3	0.0	0.0	0.0	0.5014	Oaxaca	Torroni et al. 1994; Peñaloza et al. 2007; Malhi et al. 2008; Kemp et al. 2010
Mixtec 2	Mx2	Otomanguean	19	78.9	10.5	5.3	5.3	0.0	0.0	0.0	0.3801	Oaxaca	Sandoval et al. 2009
Nahua At	NAt	Uto-Aztecan	59	47.5	35.6	11.9	5.1	0.0	0.0	0.0	0.6423	Atocpan, Mexico City	Peñaloza et al. 2007
Nahua Ch	NCh	Uto-Aztecan	41	46.3	34.1	7.3	12.2	0.0	0.0	0.0	0.6646	Chilacachapa, Guerrero	Peñaloza et al. 2007
Nahua Cu	NCu	Uto-Aztecan	46	63.0	19.6	15.2	2.2	0.0	0.0	0.0	0.5527	Cuetzalan, Puebla	Malhi et al. 2003, 2008; Kemp et al. 2010
Nahua Co	NCo	Uto-Aztecan	38	68.4	7.9	0.0	15.8	0.0	0.0	7.9	0.4235	Coyolillo, Veracruz	Peñaloza et al. 2007
Nahua Ix	NIx	Uto-Aztecan	47	55.3	27.7	0.0	17.0	0.0	0.0	0.0	0.6013	Ixhuatlancillo, Veracruz	Peñaloza et al. 2007
Nahua Ne	NNe	Uto-Aztecan	37	51.4	40.5	8.1	0.0	0.0	0.0	0.0	0.5811	Necoxtla, Veracruz	Peñaloza et al. 2007
Nahua Xo	NXo	Uto-Aztecan	43	72.1	18.6	9.3	0.0	0.0	0.0	0.0	0.4474	Xochimilco, Mexico City	Peñaloza et al. 2007
Nahua Xt2[4]	NXt2	Uto-Aztecan[4]	15	60.0	20.0	6.7	13.3	0.0	0.0	0.0	0.619	Xaltocan, Tlaxcala	Mata-Míguez et al. 2012
Nahua Zi	NZi	Uto-Aztecan	46	65.2	30.4	2.2	2.2	0.0	0.0	0.0	0.4918	Zitlala, Guerrero	Peñaloza et al. 2007
Otomi EM	OtEM	Otomanguean	101	57.4	20.8	14.8	7.0	0.0	0.0	0.0	0.6061	Estado de Mexico	This study
Otomi H	OtH	Otomanguean	103	46.6	23.3	23.3	6.8	0.0	0.0	0.0	0.6762	Hidalgo	Peñaloza et al. 2007; Sandoval et al. 2009
Otomi S	OtS	Otomanguean	91	53.8	11.0	24.2	11.0	0.0	0.0	0.0	0.6344	Hidalgo	Gorostiza et al. 2012
Otomi V	OtV	Otomanguean	81	49.3	14.8	27.1	8.6	0.0	0.0	0.0	0.6562	Hidalgo	Gorostiza et al. 2012
Otomi Xt1[4]	OtXt1	Otomanguean[5]	10	30.0	30.0	0.0	40.0	0.0	0.0	0.0	0.7333	Xaltocan, Tlaxcala	Mata-Míguez et al. 2012
Teopancazco[4]	Teo	NA[6]	29	55.0	21.0	17.0	7.0	0.0	0.0	0.0	0.6404	Estado de Mexico	Álvarez-Sandoval et al. 2015
Triqui	Tri	Otomanguean	107	72.0	28.0	0.0	0.0	0.0	0.0	0.0	0.4073	Oaxaca	Sandoval et al. 2009
Zapotec 1	Zp1	Otomanguean	103	39.8	23.3	29.1	4.9	0.0	1.0	1.9	0.6887	Oaxaca	Malhi et al. 2008; Kemp et al. 2010
Zapotec 2	Zp2	Otomanguean	88	67.0	18.2	11.4	3.4	0.0	0.0	0.0	0.5091	Oaxaca	Mizuno et al. 2014

[1] These abbreviations are used in the Figures and some Tables.
[2] Non-Native American admixture.
[3] Haplogroup diversity based in A, B, C and D.
[4] Ancient populations.
[5] Language affiliations and biological affinity for the Xaltocan samples according to ethnohistorical documents.
[6] Not available.

Table 2. Main Mitochondrial Haplogroups/Subhaplogroups

H/SH[1]	Mz1 (n=141)	Mz2 (n=25)	Mx1 (n=65)	Mx2 (n=19)	NAt (n=44)	NCu (n=29)	NIx (n=10)	NNe (n=25)	NXo (n=35)	NXt2 (n=15)	NZi (n=14)	OtEM (n=100)	OtH (n=68)	OtS (n=91)	OtV (n=81)	OtXt1 (n=10)	Tri (n=107)	Zp1 (n=72)	Zp2 (n=88)	Total
A	14	-	5	2	-	1	-	-	3	-	-	7	4	2	11	-	4	-	-	53
A2	23	4	40	13	17	19	4	12	23	9	14	50	23	46	29	3	72	31	59	491
A19	5	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	1	-	-	8
B2	46	10	8	1	15	1	-	7	2	2	-	15	10	8	7	1	18	8	9	168
B4	5	3	4	-	3	2	1	6	3	1	-	6	7	2	5	2	12	9	7	78
B6	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
C1	17	2	5	1	8	5	3	-	3	1	.	13	20	22	22	-	-	21	10	153
C4a2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
C5a	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
C7a1a1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	2
D1	30	5	3	1	-	-	1	-	-	2	-	6	4	10	5	4	-	2	3	75
D2a’b	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
D4	-	1	-	-	1	-	1	-	-	-	-	1	-	-	1	-	-	1	-	6

[1]H/SH = Haplogroups/Subhaplogroups.

Table 3. Diversity Estimates for mtDNA Haplotypes

Population	n	No. of haplotypes	Polymorfic sites	Gene diversity (h)	Nucleotide diversity (p)	Theta (S)	SD Theta (S)	Theta (pi)	SD Theta (pi)
Mazahua 1	141	54	57	0.9582 +/- 0.0073	0.0203 +/- 0.0106	10.3216	2.6839	7.2074	3.7613
Mazahua 2	25	13	25	0.9367 +/- 0.0253	0.0184 +/- 0.0100	6.6208	2.4514	6.5273	3.5583
Mixtec 1	65	26	35	0.9563 +/- 0.0092	0.0180 +/- 0.0095	7.3779	2.2639	6.3753	3.3930
Mixtec 2	19	10	18	0.8246 +/- 0.0836	0.0103 +/- 0.0061	5.1501	2.0880	3.6622	2.1666
Nahua At	44	28	44	0.9556 +/- 0.0192	0.0228 +/- 0.0120	10.1150	3.1967	8.0955	4.2530
Nahua Cu	29	21	32	0.9729 +/- 0.0173	0.0183 +/- 0.0099	8.1484	2.8556	6.4945	3.5209
Nahua Ix	10	8	23	0.9556 +/- 0.0594	0.0215 +/- 0.0124	8.1302	3.6160	7.6292	4.3986
Nahua Ne	25	14	24	0.9100 +/- 0.0433	0.0180 +/- 0.0098	6.3560	2.3661	6.4025	3.4966
Nahua Xo	35	22	35	0.9294 +/- 0.0341	0.0148 +/- 0.0081	8.4988	2.8579	5.2558	2.8931
Nahua Xt2	15	8	21	0.8381 +/- 0.0852	0.0198 +/- 0.0110	6.4584	2.6720	7.0329	3.9240
Nahua Zi	14	5	9	0.5934 +/- 0.1438	0.0040 +/- 0.0029	2.8301	1.3591	1.4230	1.0363
Otomi EM	100	48	58	0.9671 +/- 0.0079	0.0192 +/- 0.0101	11.2026	3.0420	6.8104	3.5825
Otomi H	68	32	39	0.9666 +/- 0.0082	0.0200 +/- 0.0105	8.1431	2.4469	7.0901	3.7344
Otomi S	91	37	48	0.9560 +/- 0.0090	0.0218 +/- 0.0113	9.4440	2.6558	7.7200	4.0217
Otomi V	81	34	38	0.9642 +/- 0.0075	0.0181 +/- 0.0096	7.6339	2.2453	6.4202	3.4034
Otomi Xt1	10	7	18	0.8667 +/- 0.1072	0.0189 +/- 0.0110	6.3627	2.9015	6.7191	3.9158
Triqui	107	15	26	0.5470 +/- 0.0560	0.0129 +/- 0.0071	4.9568	1.5068	4.5948	2.5183
Zapotec 1	72	32	50	0.9581 +/- 0.0100	0.0215 +/- 0.0112	10.3158	2.9830	7.6393	3.9950
Zapotec 2	88	28	29	0.9203 +/- 0.0145	0.0142 +/- 0.0077	5.7439	1.7481	5.0300	2.7329

Theta (S) = Theta S Watterson values.
Theta (pi) = Mean number of pairwise differences.
SD = Standard deviation.

Table 4. Number of mtDNA Haplotypes

Population	n	No. Haplotypes	Singleton % (n)	Shared within a population % (n)	Shared between populations % (n)
Mazahua 1	141	54	38.9 (21)	14.8 (8)	46.3 (25)
Mazahua 2	25	13	30.7 (4)	30.7 (4)	38.4 (5)
Mixtec 1	65	26	23.0 (6)	30.8 (8)	46.2 (12)
Mixtec 2	19	10	30.0 (3)	10.0 (1)	60.0 (6)
Nahua At	44	28	35.7 (10)	14.3 (4)	50.0 (14)
Nahua Cu	29	21	42.9 (9)	19.0 (4)	38.1 (8)
Nahua Ix	10	8	37.5 (3)	0.0 (0)	62.5 (5)
Nahua Ne	25	14	21.4 (3)	14.2 (2)	64.2 (9)
Nahua Xo	35	22	40.9 (9)	4.5 (1)	54.5 (12)
Nahua Xt2	15	8	50.0 (4)	0.0 (0)	50.0 (4)
Nahua Zi	14	5	20.0 (1)	0.0 (0)	80.0 (4)
Otomi EM	100	48	50.0 (24)	6.2 (3)	43.8 (21)
Otomi H	68	32	12.5 (4)	9.3 (3)	78.1 (25)
Otomi S	91	37	27.0 (10)	8.1 (3)	64.8 (24)
Otomi V	81	34	23.5 (8)	3.0 (1)	73.5 (25)
Otomi Xt1	10	7	42.8 (3)	14.2 (1)	42.8 (3)
Triqui	107	15	33.3 (5)	26.7 (4)	40.0 (6)
Zapotec 1	72	32	34.4 (11)	21.9 (7)	43.7 (14)
Zapotec 2	88	28	46.4 (13)	14.3 (4)	39.3 (11)
Total	1039	442	34.2 (152)	12.3 (54)	53.2 (234)

Table 5. AMOVA Results

Grouping criteria	Groups	Populations	Source of variation	Percentage of variation	Fixation indices	*P*
Geography	Eastern	Nahua, Otomi (NCu, NIx, NNe, OtH, OtS, OtV)	Within populations	92.95	FSC = 0.0534	0.0000
	Center	Mazahua, Nahua, Otomi (Mz1, Mz2, NAt, NXo, NXt2, OtEM, OtXt1)	Among populations Within groups	5.25	FST = 0.0704	0.0000
	Western	Nahua (NZi)	Among groups	1.8	FCT = 0.0179	0.0508
	Southern	Mixtec, Triqui, Zapotec (Mx1, Mx2, Tri, Zp1, Zp2)				
Linguistic family	Otomanguean	Mazahua, Mixtec, Otomi, Triqui, Zapotec (Mz1, Mz2, Mx1, Mx2, OtEM, OtH, OtS, OtV, OtXt1, Tri, Zp1, Zp2)	Within populations	93.5	FSC = 0.06631	0.0000
	Uto-Aztecan	Nahua (NAt, NCu, NXo, NIx, NXt2, NZi)	Among populations within groups	6.64	FST = 0.0650	0.0000
			Among groups	-0.14	FCT = -0.0013	0.4233

Significance level, p=0.0500

Supplementary Table S1. Diversity Estimates for Each Mitochondrial Haplogroup

Population	n	Haplotype	%	Sample size	No. of haplotypes	No. polymorphic sites	Gene diversity (h)	Theta (S)	SD Theta (S)
Mz1	141	A	29.8	42	21	26	0.9082 +/- 0.0353	6.0423	2.0567
		B	36.2	51	17	18	0.8910 +/- 0.0249	4.0007	1.4187
		C	12.8	18	8	9	0.7974 +/- 0.0885	2.6166	1.2169
		D	21.3	30	8	10	0.6230 +/- 0.0930	2.5242	1.0821
Mz2	25	A	16.0	4	3	2	0.8333 +/- 0.2224	1.0909	0.87553
		B	52.0	13	6	11	0.8590 +/- 0.0633	3.5447	1.65502
		C	8.0	2	1	0	0.0000 +/- 0.0000	0.0000	0.0000
		D	24.0	6	3	6	0.6000 +/- 0.2152	2.6277	1.5531
Mx1	65	A	69.2	45	14	18	0.9192 +/- 0.0157	4.1164	1.4809
		B	18.5	12	8	10	0.9091 +/- 0.0649	3.3113	1.5926
		C	7.7	5	2	1	0.6000 +/- 0.1753	0.4800	0.4800
		D	4.6	3	2	2	0.6667 +/- 0.3143	1.3333	1.0983
Mx2	19	A	78.9	15	6	8	0.7143 +/- 0.1165	2.4603	1.2015
		B	10.5	2	2	4	1.0000 +/- 0.5000	4.0000	3.1622
		C	5.3	1	1	0	1.0000 +/- 0.0000	0.0000	0.0000
		D	5.3	1	1	0	1.0000 +/- 0.0000	0.0000	0.0000
NAt	44	A	38.6	17	10	18	0.8456 +/- 0.0886	5.3242	2.1987
		B	40.9	18	12	15	0.9216 +/- 0.0510	4.3610	1.8353
		C	18.2	8	5	4	0.8571 +/- 0.1083	1.5427	0.9605
		D	2.3	1	1	0	1.0000 +/- 0.0000	0.0000	0.0000
NCu	29	A	69.0	20	14	19	0.9526 +/- 0.0326	5.3555	2.1362
		B	10.3	3	3	4	1.0000 +/- 0.2722	2.6666	1.9189
		C	17.2	5	3	7	0.8000 +/- 0.1640	3.3600	2.0007
		D	3.4	1	1	0	1.0000 +/- 0.0000	0.0000	0.0000
NIx	10	A	40.0	4	3	2	0.8333 +/- 0.2224	1.0909	0.8755
		B	10.0	1	1	0	1.0000 +/- 0.0000	0.0000	0.0000
		C	30.0	3	2	3	0.6667 +/- 0.3143	2.0000	1.5118
		D	20.0	2	2	8	1.0000 +/- 0.5000	8.0000	6.0000
NNe	25	A	48.0	12	4	6	0.6515 +/- 0.1327	1.9868	1.0667
		B	52.0	13	10	13	0.9487 +/- 0.0506	4.1892	1.9011
		C	--	--	--	--	--	--	--
		D	--	--	--	--	--	--	--
NXo	35	A	77.1	27	16	17	0.8860 +/- 0.0540	4.4105	1.7121
		B	14.3	5	4	6	0.9000 +/- 0.1610	2.8800	1.7577
		C	8.6	3	3	7	1.0000 +/- 0.2722	4.6666	3.1269
		D	--	--	--	--	--	--	--
NXt2	15	A	60.0	9	4	9	0.5833 +/- 0.1833	3.3114	1.6995
		B	20.0	3	2	4	0.6667 +/- 0.3143	2.6666	1.9189
		C	6.7	1	1	0	1.0000 +/- 0.0000	0.0000	0.0000
		D	13.3	2	1	0	0.0000 +/- 0.0000	0.0000	0.0000
NZi	14	A	100.0	14	5	10	0.5934 +/- 0.1438	3.1445	1.4782
		B	--	--	--	--	--	--	--
		C	--	--	--	--	--	--	--
		D	--	--	--	--	--	--	--

Theta (S) = Theta S Watterson values.
SD = Standard deviation.

Supplementary Table S1. Continued

OtEM	100	A	57.0	57	27	35	0.9386 +/- 0.0185	7.5897	2.3714
		B	21.0	21	12	12	0.9048 +/- 0.0482	3.3354	1.4306
		C	15.0	15	8	14	0.8667 +/- 0.0673	4.3056	1.8861
		D	7.0	7	3	7	0.6667 +/- 0.1598	2.8571	1.5996
OtH	68	A	39.7	27	16	22	0.9174 +/- 0.0388	5.7077	2.1252
		B	25.0	17	8	9	0.8897 +/- 0.0461	2.6621	1.2467
		C	29.4	20	6	10	0.8474 +/- 0.0437	2.8187	1.2641
		D	5.9	4	2	1	0.5000 +/- 0.2652	0.5454	0.5454
OtS	91	A	72.1	49	24	32	0.9243 +/- 0.0264	7.1768	2.3191
		B	14.7	10	6	9	0.7778 +/- 0.1374	3.1813	1.6050
		C	32.4	22	5	8	0.7359 +/- 0.0549	2.1945	1.0268
		D	14.7	10	3	2	0.6444 +/- 0.1012	0.7069	0.5387
OtV	81	A	49.4	40	17	20	0.9205 +/- 0.0252	4.7019	1.6825
		B	14.8	12	4	5	0.6818 +/- 0.1019	1.6556	0.9318
		C	28.4	23	10	12	0.8913 +/- 0.0368	3.2134	1.3550
		D	7.4	6	3	3	0.6000 +/- 0.2152	1.3138	0.9097
OtXt1	10	A	30.0	3	3	5	1.0000 +/- 0.2722	3.3333	2.3231
		B	30.0	3	3	5	1.0000 +/- 0.2722	3.3333	2.3231
		C	--	--	--	--	--	--	--
		D	40.0	4	1	0	0.0000 +/- 0.0000	0.0000	0.0000
Tri	107	A	72.0	77	5	10	0.1497 +/- 0.0548	2.0347	0.8061
		B	28.0	30	10	12	0.8299 +/- 0.0462	3.0290	1.2440
		C	--	--	--	--	--	--	--
		D	--	--	--	--	--	--	--
Zp1	72	A	43.1	31	16	24	0.9075 +/- 0.0350	6.0075	2.1626
		B	23.6	17	6	6	0.7574 +/- 0.0912	1.7747	0.9189
		C	29.2	21	7	11	0.8905 +/- 0.0289	3.0574	1.3352
		D	4.2	3	3	10	1.0000 +/- 0.2722	6.6666	4.3278
Zp2	88	A	67.0	59	16	15	0.8627 +/- 0.0269	3.2284	1.1734
		B	18.2	16	9	10	0.8833 +/- 0.0612	3.0136	1.3907
		C	11.4	10	2	1	0.2000 +/- 0.1541	0.3534	0.3534
		D	3.4	3	1	0	0.0000 +/- 0.0000	0.0000	0.0000

Theta (S) = Theta S Watterson values.
SD = Standard deviation.

Supplementary Table S2. Mitochondrial DNA Haplotypes and Their Assignment into Haplogroups and Subhaplogroups in 19 Populations

Ht[a]	H/SH[b]	Polymorphisms	Mz1[c] n=141	Mz2 n=25	Mx1 n=65	Mx2 n=19	NAt n=44	NCu n=29	NIx n=10	NNe n=25	NXo n=35	NXt2 n=15	NZi n=14	OtEM[c] n=100	OtH n=68	OtS n=91	OtV n=81	OtXt1 n=10	Tri n=107	Zp1 n=72	Zp2 n=88
Ht1	A	663G,16223T,16290T,16319A	1	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
Ht2	A	663G,16223T,16290T,16319A,16362C	12	-	5	2	-	-	-	-	2	-	-	5	4	2	9	-	-	4	-
Ht3	A	663G,16186T,16223T,16290T,16319A	-	-	-	-	8	4	2	-	9	-	9	1	-	-	-	-	71	8	16
Ht4	A	663G,16223T,16290T,16294T,16319A,16362C	1	-	-	-	-	1	-	-	3	-	-	-	-	-	-	-	-	-	-
Ht5	A	663G,16104T,16223T,16290T,16319A,16362C	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	3	1
Ht6	A2	663G,16111T,16223T,16319A,16362C	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht7	A2	663G,16111T,16290T,16319A,16362C	-	-	-	-	-	-	-	-	-	-	-	2	1	-	2	-	-	-	-
Ht8	A2	663G,16111T,16223T,16290T,16319A,16362C	3	1	-	8	-	-	-	-	-	-	-	12	7	3	3	-	-	-	-
Ht9	A2	663G,16111T,16290T,16319A,16333G,16362C	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht10	A2	663G,16111T,16131C,16134T,16223T,16319A,16362C	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Ht11	A2	663G,16111T,16223T,16290T,16311C,16319A,16362C	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht12	A2	663G,16111T,16189C,16223T,16290T,16319A,16324C	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-
Ht13	A2	663G,16051G,16111T,16223T,16290T,16319A,16362C	1	-	4	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Ht14	A2	663G,16111T,16223T,16290T,16319A,16356C,16362C	1	2	-	-	-	-	-	-	-	-	-	-	1	-	5	-	-	-	-
Ht15	A2	663G,16046T,16111T,16223T,16290T,16319A,16362C	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht16	A2	663G,16111T,16114T,16223T,16290T,16319A,16356C,16362C	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht17	A2	663G,16111T,16129A,16223T,16234T,16290T,16319A,16362C	3	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-
Ht18	A2	663G,16111T,16177G,16223T,16241G,16290T,16319A,16362C	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht19	A2	663G,16093C,16111T,16114T,16223T,16290T,16319A,16356C,16362C	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht20	A2	663G,16092C,16111T,16129A,16223T,16234T,16290T,16319A,16362C	-	-	-	-	-	1	-	-	1	-	-	1	-	-	-	-	-	1	14
Ht21	A2	663G,16111T,16129A,16189C,16223T,16241C,16242A,16245A,16246G,16290T,16319A	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht22	A2ao1	663G,16111T,16223T,16264T,16290T,16319A	-	-	-	-	-	-	-	-	1	-	-	10	-	-	-	-	-	-	-
Ht23	A2ae	663G,16111T,16223T,16284G,16290T,16319A,16362C	1	-	-	-	-	-	-	-	-	-	-	2	-	1	-	-	-	-	-
Ht24	A2ae	663G,16111T,16129A,16223T,16284G,16290T,16319A,16362C	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-
Ht25	A2af	663G,16111T,16223T,16290T,16319A,16360T,16362C	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht26	A2h1	663G,16111T,16223T,16290T,16319A,16335G	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	1	-	-	-
Ht27	A2h1	663G,16111T,16223T,16290T,16319A,16335G,16362C	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-
Ht28	A2i	663G,16111T,16223T,16290T,16319A,16325C,16362C	1	-	-	-	-	-	-	-	-	-	2	-	-	-	4	-	1	-	-
Ht29	A2m	663G,16223T,16240G,16290T,16319A,16362C	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Ht30	A2q	663G,16111T,16209C,16223T,16274A,16290T, 16319A,16362C	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Ht31	A2u	663G,16111T,16136C,16189C, 16290T,16311C,16319A,16362C	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Ht32	A2u	663G,16093C,16111T,16136C,16189C,16290T,16311C,16319A,16362C	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Ht33	A2u	663G,16111T,16136C,16223T,16290T,16292T,16311C,16319A,16362C	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht34	A2u	663G,16111T,16136C,16223T,16290T,16299G,16311C,16319A,16362C	1	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-
Ht35	A2u	663G,16093C,16111T,16136C,16223T,16290T,16292T,16311C,16319A,16362C	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht36	A2u1	663G,16111T,16136C,16223T,16247G,16257T,16274A,16290T,16319A,16344T,16362C	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-
Ht37	A2v	663G,16111T,16223T,16239T,16290T,16319A,16362C	1	-	-	-	-	-	-	-	-	-	-	-	-	3	2	-	-	-	-
Ht38	A2v	663G,16051G,16111T,16223T,16239T,16290T,16319A,16362C	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht39	A2v1b	663G,16111T,16223T,16234T,16239T,16290T,16319A,16362C	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Ht40	A2w1	663G,16111T,16187T,16223T,16290T,16319A,16362C	2	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht41	A19	663G,16223T,16290T,16311C,16319A,16362C	2	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Ht42	A19	663G,16092C,16167T,16223T,16290T,16311C,16319A,16362C	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht43	B4	8281-8289d,16189C,16217C	-	2	-	-	1	-	-	-	1	-	-	1	-	-	-	-	-	-	-
Ht44	B4	8281-8289d,16183C,16189C,16217C	1	-	-	-	1	1	1	3	1	-	-	1	1	-	-	-	7	8	-
Ht45	B4	8281-8289d,16182C,16183C,16189C,16217C	4	-	3	-	1	-	-	1	-	-	-	3	4	1	4	1	10	2	-
Ht46	B4	8281-8289d,16183C,16189C,16217C,16390A	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht47	B2	8281-8289d,16182C,16183C,16189C,16217C,16278T	4	-	-	-	-	-	-	-	-	-	-	2	2	-	1	-	-	-	-

Supplementary Table S2. Continued

Ht[a]	H/SH[b]	Polymorphisms	Mz1[c] n=141	Mz2 n=25	Mx1 n=65	Mx2 n=19	NAt n=44	NCu n=29	NIx n=10	NNe n=25	NXo n=35	NXt2 n=15	NZi n=14	OtEM[c] n=100	OtH n=68	OtS n=91	OtV n=81	OtXt1 n=10	Tri n=107	Zp1 n=72	Zp2 n=88
Ht48	B2	8281-8289d,16183C,16189C,16217C,16270A,16278T	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht49	B2c2a	8281-8289d,16189C,16217C,16319A	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht50	B2c2a	8281-8289d,16182C,16183C,16189C,16217C ,16319A	3	-	-	-	-	-	-	-	-	-	-	-	2	1	6	-	-	-	-
Ht51	B2c2a	8281-8289d,16182C,16183C,16189C,16217C,16258C,16319A	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht52	B2c2b	8281-8289d,16189C,16217C,16295T	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht53	B2c2b	8281-8289d,16182C,16183C,16189C,16217C,16295T	3	-	-	-	-	-	-	-	-	-	-	6	4	5	-	1	-	-	-
Ht54	B2c2b	8281-8289d,16182C,16183C,16189C,16217C,16274A,16295T	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Ht55	B2c2b	8281-8289d,16182C,16183C,16189C,16209C,16217C,16295T	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht56	B2c2b	8281-8289d,16037G,16178C,16182C,16183C,16189C,16217C,16295T	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht57	B2g1	8281-8289d,16183C,16217C,16298C	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht58	B2g1	8281-8289d,16183C,16189C,16217C,16298C	12	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Ht59	B2g1	8281-8289d,16092C,16183C,16189C,16217C,16298C	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht60	B2g1	8281-8289d,16183C,16189C,16217C,16297C,16298C	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Ht61	B2i1	8281-8289d,16182C,16183C,16189C,16217C,16311C	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht62	B2o	8281-8289d,16092C,16182C,16183C,16189C,16217C	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht63	B2o	8281-8289d,16092C,16182C,16183C,16189C,16214T,16217C	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht64	B2t	8281-8289d,16169T,16183C,16189C,16217C,16259T,16357C	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht65	B2t	8281-8289d,16182C,16183C,16189C,16217C,16259T,16357C,16362C	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht66	B2x	8281-8289d,16129A,16145A,16183C,16217C,16319A,16323C	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht67	C1	13263G,16298C,16325C,16327T	2	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	4	9
Ht68	C1	13263G,16223T,16298C,16325C,16327T	8	2	3	-	-	-	-	-	-	-	-	5	4	5	6	-	-	-	-
Ht69	C1	13263G,16223T,16298C,16325C,16327T,16365T	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht70	C1b	13263G,16223T,16298C,16311C,16325C,16327T	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht71	C1b8	13263G,16298C,16325C,16327T,16362C	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht72	C1b13d	13263G,16051G,16223T,16298C,16325C,16327T	1	-	-	1	1	-	-	-	-	-	-	2	2	9	2	-	-	3	1
Ht73	C1b13d	13263G,16051G,16094C,16223T,16298C,16311C,16325C,16327T	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht74	C1b14	13263G,16172C,16181G,16223T,16298C,16311C,16325C,16327T	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht75	C1b14	13263G,16086C,16172C,16181G,16223T,16298C,16325C,16327T	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht76	C1c6	13263G,16153A,16223T,16298C,16325C,16327T	2	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-
Ht77	C4a2	13263G,16223T,16298C,16301T,16325C,16327T,16357C,	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht78	C5a	13263G,16184T,16223T,16261T,16298C,16325C,16343C,16348T	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht79	C7a1a1	13263G,16194G,16223T,16325C,16327T	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ht80	D1	5178A,16223T,16325C,16362C	-	-	1	-	-	-	-	-	-	-	-	2	3	5	1	-	-	-	-
Ht81	D1	5178A,16223T,16271C,16325C,16362C	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht82	D1	5178A,16223T,16249C,16325C,16362C	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht83	D1	5178A,16223T,16305G,16325C,16362C	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht84	D1	5178A,16223T,16228T,16249C,16325C,16362C	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht85	D1h2	5178A,16223T,16239T,16260T,16325C,16362C	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht86	D1h2	5178A,16093C,16223T,16239T,16260T,16274A,16325C,16362C	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ht87	D1i2	5178A,16223T,16274A,16325C ,16362C,16368C	18	-	-	-	-	-	-	-	-	-	-	4	-	1	4	-	-	-	-
Ht88	D1i2	5178A,16223T,16243C,16274A,16325C,16362C,16368C	1	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	3
Ht89	D4h3a8	5178A,16223T,16234G,16241G,16301T,16342C,16362C	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-

[a]Ht = Haplotype.

[b]H/SH = Haplogroups/Subhaplogroups.

[c]All the mtDNA hapotypes of the Mazahua 1 and Otomi EM are shown; for the rest of the populations only the haplotypes shared with Mazahua 1 and Otomi EM are included.

Supplementary Table S3. Haplotypes Shared between the Populations Compared

	Mz1	Mz2	Mx1	Mx2	NAt	NCu	NIx	NNe	NXo	NXt2	NZi	OtH	OtEM	OtS	OtV	OtXt1	Tri	Zp1	Zp2
Mz1	-																		
Mz2	3	-																	
Mx1	4	1	-																
Mx2	4	1	1	-															
NAt	7	2	3	1	-														
NCu	4	1	2	2	3	-													
NIx	3	2	2	1	3	2	-												
NNe	3	-	4	-	4	1	1	-											
NXo	7	2	2	2	4	4	3	2	-										
NXt2	-	-	2	-	1	1	-	-	-	-									
NZi	2	1	1	1	1	1	2	-	1	-	-								
OtH	15	3	6	3	9	4	4	4	6	1	1	-							
OtEM	14	4	4	3	6	3	3	3	7	1	2	10	-						
OtS	12	2	5	3	6	6	5	3	6	2	3	12	10	-					
OtV	13	3	4	4	6	5	4	2	5	1	1	16	9	15	-				
OtXt1	2	-	1	-	2	1	1	2	1	-	-	2	3	2	1	-			
Tri	3	1	2	2	3	2	2	3	2	-	2	3	4	4	2	3	-		
Zp1	9	3	7	1	5	3	4	3	5	2	2	5	5	5	2	3	4	-	
Zp2	5	3	4	1	4	3	3	-	2	1	2	5	4	5	-	-	3	-	-

Figure 1. Map of Mexico showing approximate geographic locations of the populations considered in this study.

Figure 2. Principal component analysis based on mtDNA haplogroup frequencies.

Figure 3. Neighbor-Joining dendrogram based on *Fst* values of all the mitochondrial DNA haplotypes.

Figure 4. Haplotypes neighbor-joining network of haplogroup A from Otomanguean and Yuto-Aztecan populations. The mutations identified are based on the Cambridge Reference Sequence (Anderson et al. 1981; Andrews et al. 1999). The node size is proportional to the number of individuals. The black nodes represent haplotypes not found in the populations analyzed. The white nodes represent haplotypes found in a single population. The gray nodes represent haplotypes found in different populations.

Figure 5. Haplotypes neighbor-joining network of haplogroup B from Otomanguean and Yuto-Aztecan populations. The mutations identified are based on the Cambridge Reference Sequence (Anderson et al. 1981; Andrews et al. 1999). The node size is proportional to the number of individuals. The black nodes represent haplotypes not found in the populations analyzed. The white nodes represent haplotypes found in a single population. The gray nodes represent haplotypes found in different populations.

Figure 6. Haplotypes neighbor-joining network of haplogroup C from Otomanguean and Yuto-Aztecan populations. The mutations identified are based on the Cambridge Reference Sequence (Anderson et al. 1981; Andrews et al. 1999). The node size is proportional to the number of individuals. The black nodes represent haplotypes not found in the populations analyzed. The white nodes represent haplotypes found in a single population. The gray nodes represent haplotypes found in different populations.

Figure 7. Haplotypes neighbor-joining network of haplogroup D from Otomanguean and Yuto-Aztecan populations. The mutations identified are based on the Cambridge Reference Sequence (Anderson et al. 1981; Andrews et al. 1999). The node size is proportional to the number of individuals. The black nodes represent haplotypes not found in the populations analyzed. The white nodes represent haplotypes found in a single population. The gray nodes represent haplotypes found in different populations.

Figure 1.

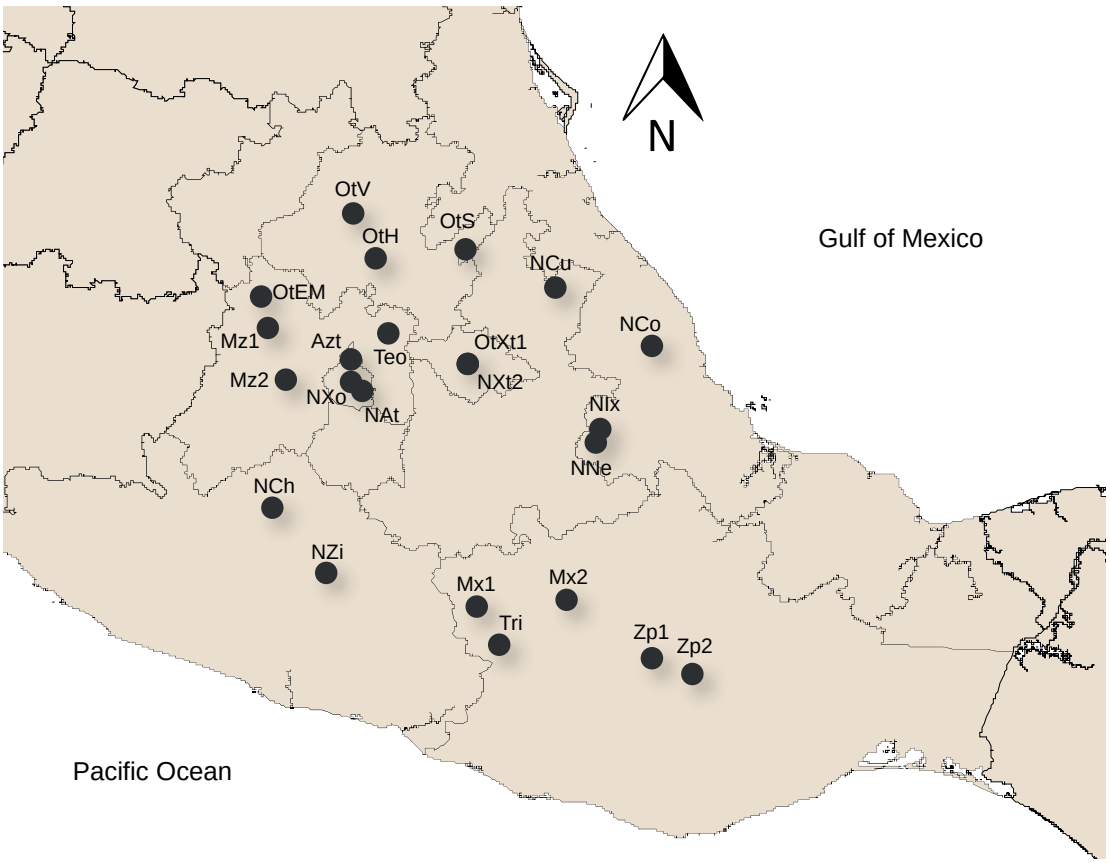


Figure 2.

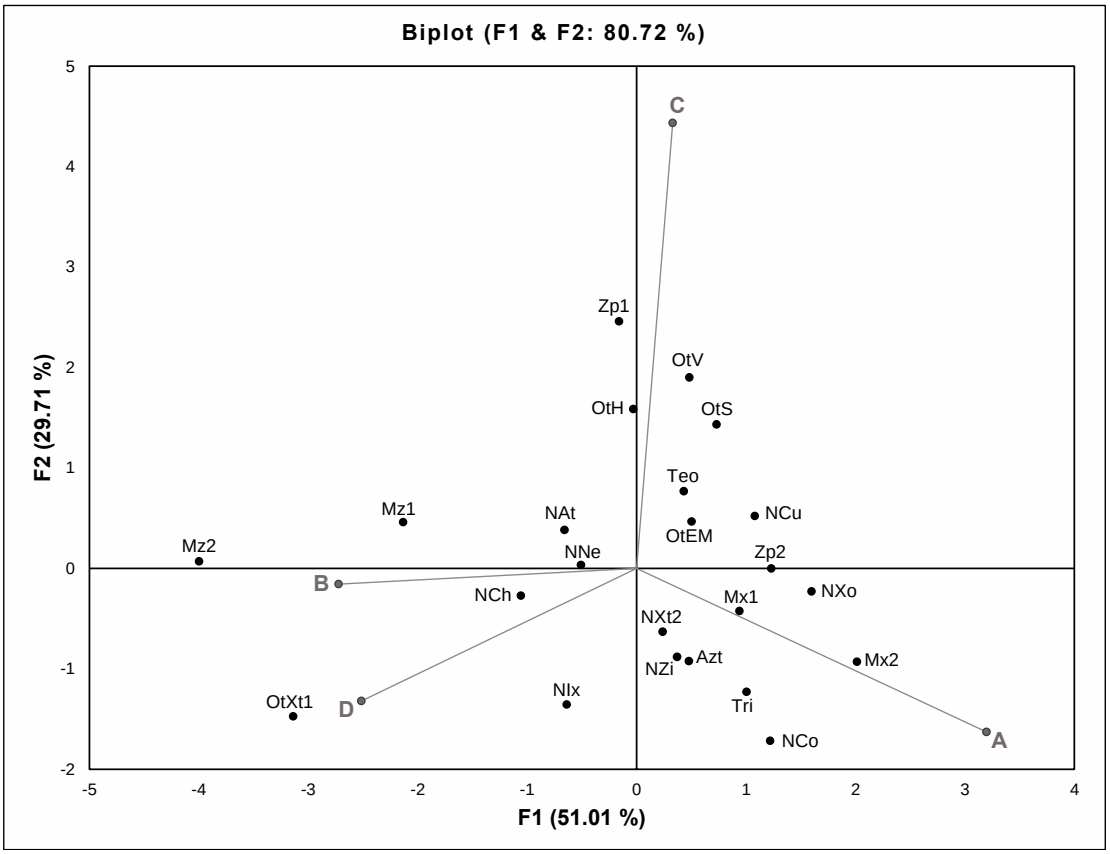


Figure 3.

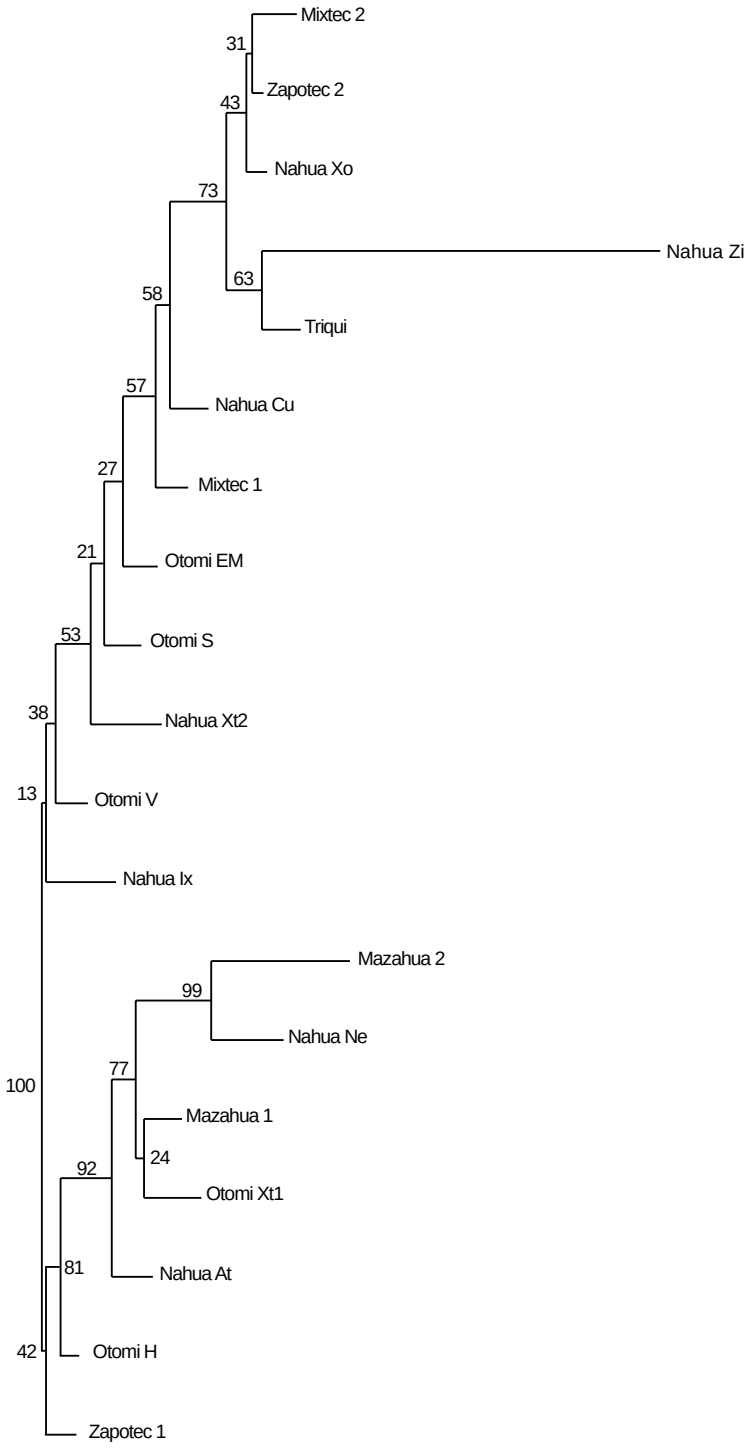

Mixtec 2
31
Zapotec 2
43
Nahua Xo
73
Nahua Zi
63
58
Triqui
57
Nahua Cu
27
Mixtec 1
21
Otomi EM
53
Otomi S
38
Nahua Xt2
13
Otomi V
Nahua Ix
Mazahua 2
99
Nahua Ne
100
77
Mazahua 1
24
92
Otomi Xt1
81
Nahua At
42
Otomi H
Zapotec 1

Figure 4.

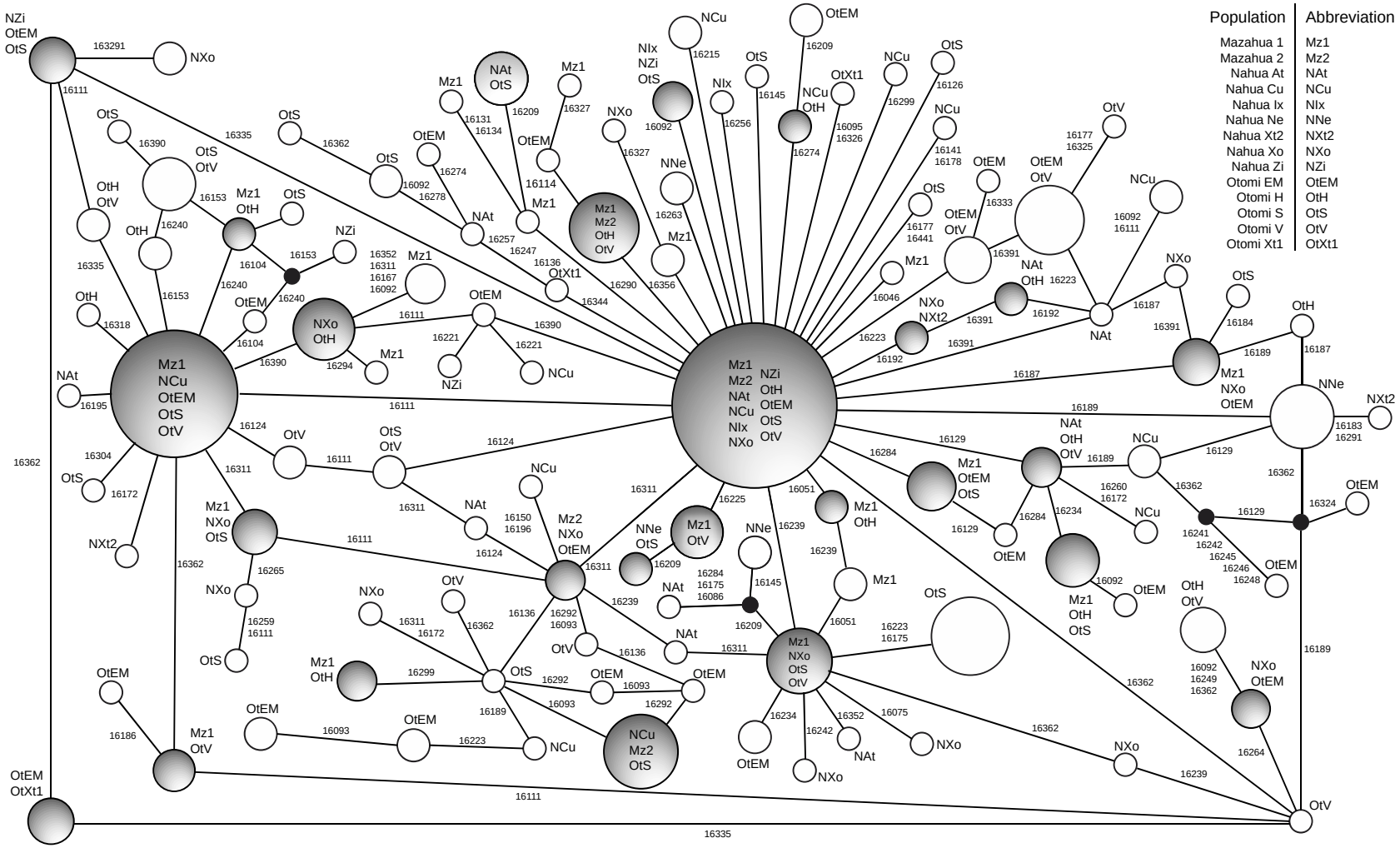

Population
Abbreviation
Mazahua 1
Mz1
Mazahua 2
Mz2
Nahua At
NAt
Nahua Cu
NCu
Nahua Ix
NIx
Nahua Ne
NNe
Nahua Xt2
NXt2
Nahua Xo
NXo
Nahua Zi
NZi
Otomi EM
OtEM
Otomi H
OtH
Otomi S
OtS
Otomi V
OtV
Otomi Xt1
OtXt1

Figure 5.

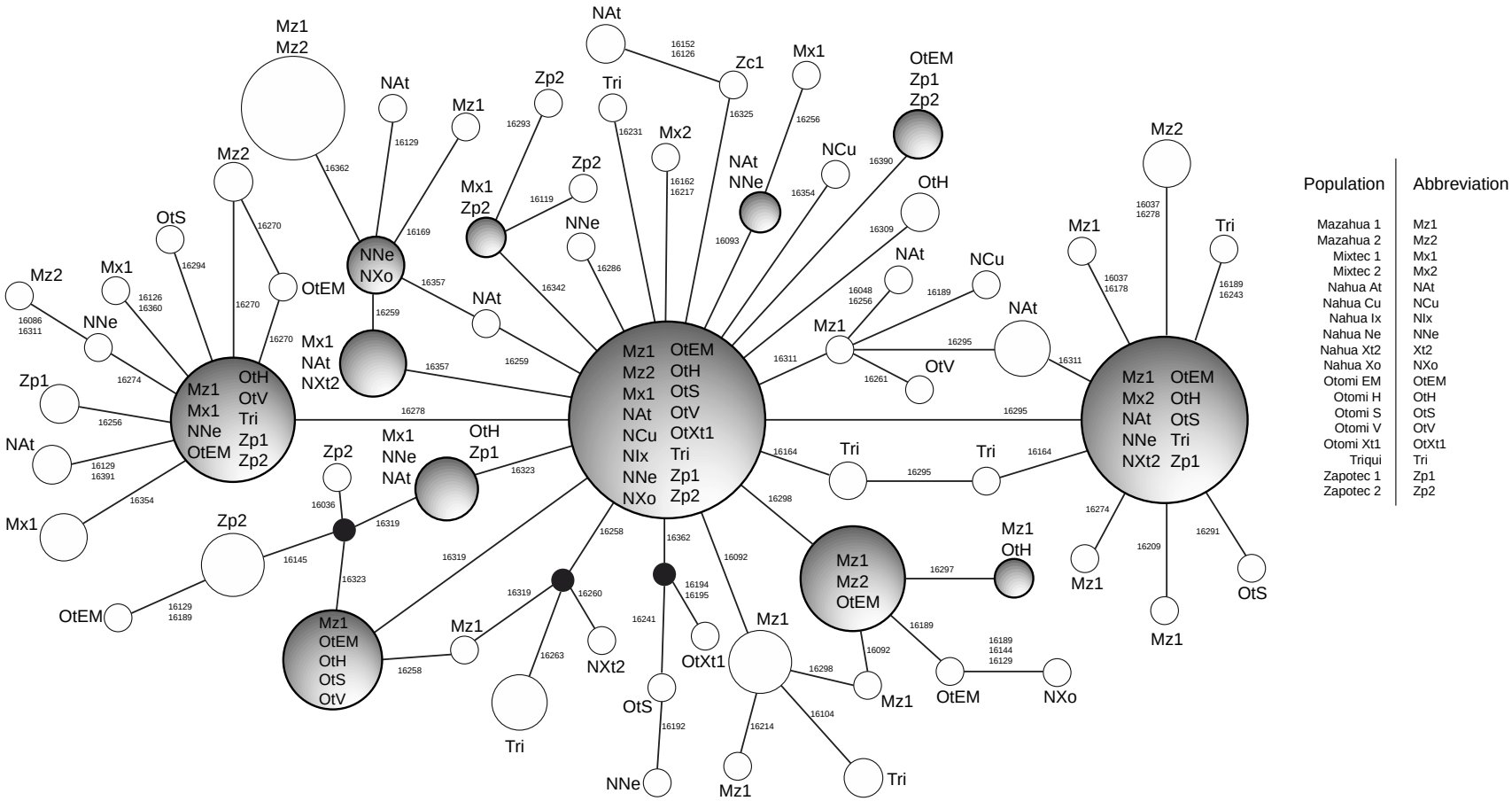

Mz1 Mz2
NAt
Zp2
Tri
Zc1
Mx1
OtEM Zp1 Zp2
Mz2
NCu
OtH
OtS
Mx1
NNe
Mz1
Tri
Mz2
OtEM
NAt
NCu
Mz1 OtEM Mz2 OtH Mx1 OtS NAt OtV Zp1 OtXt1 NIx Tri NNe Zp1 NXo Zp2
Mz1 OtH Mx1 OtV NNe Tri OtEM Zp1 Zp2
NNe NXo
Mx1 NAt NXt2
Mx1 NNe NAt
OtH Zp1
Zp2
Mz1 Mz2 OtEM
Mz1 OtEM OtH OtS OtV
Mz1 OtH
Mz1 Mx2 OtH NAt OtS NNe Tri NXt2 Zp1
Mx1
Zp2
Mz1
NXt2
OtXt1
OtS
NNe
Tri
Mz1
Mz1
Tri
OtEM
NXo
Mz1
Mz1
OtS
Population
Abbreviation
Mazahua 1 Mz1
Mazahua 2 Mz2
Mixtec 1 Mx1
Mixtec 2 Mx2
Nahua At NAt
Nahua Cu NCu
Nahua Ix NIx
Nahua Ne NNe
Nahua Xt2 Xt2
Nahua Xo NXo
Otomi EM OtEM
Otomi H OtH
Otomi S OtS
Otomi V OtV
Otomi Xt1 OtXt1
Triqui Tri
Zapotec 1 Zp1
Zapotec 2 Zp2

Figure 6.

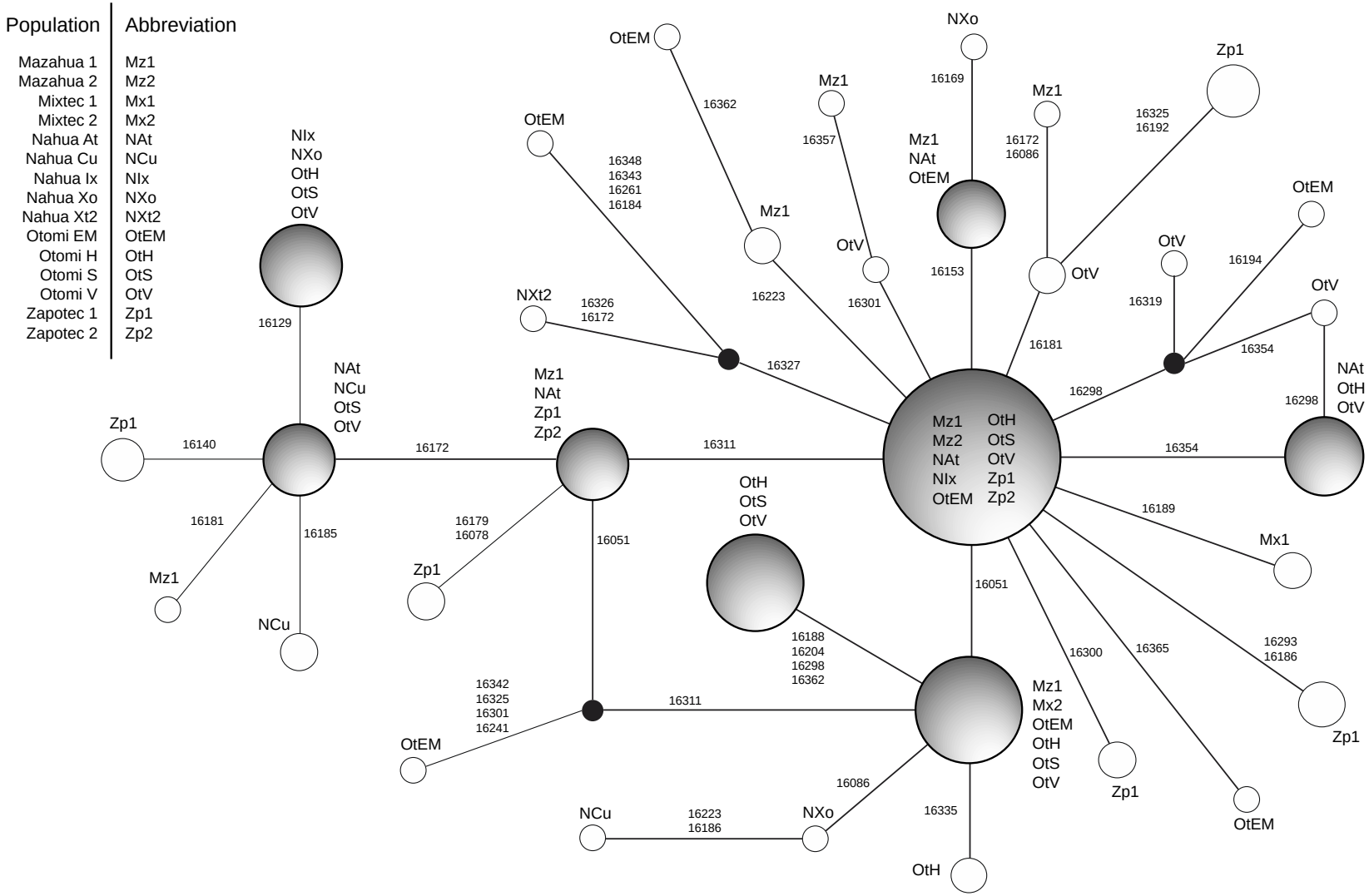
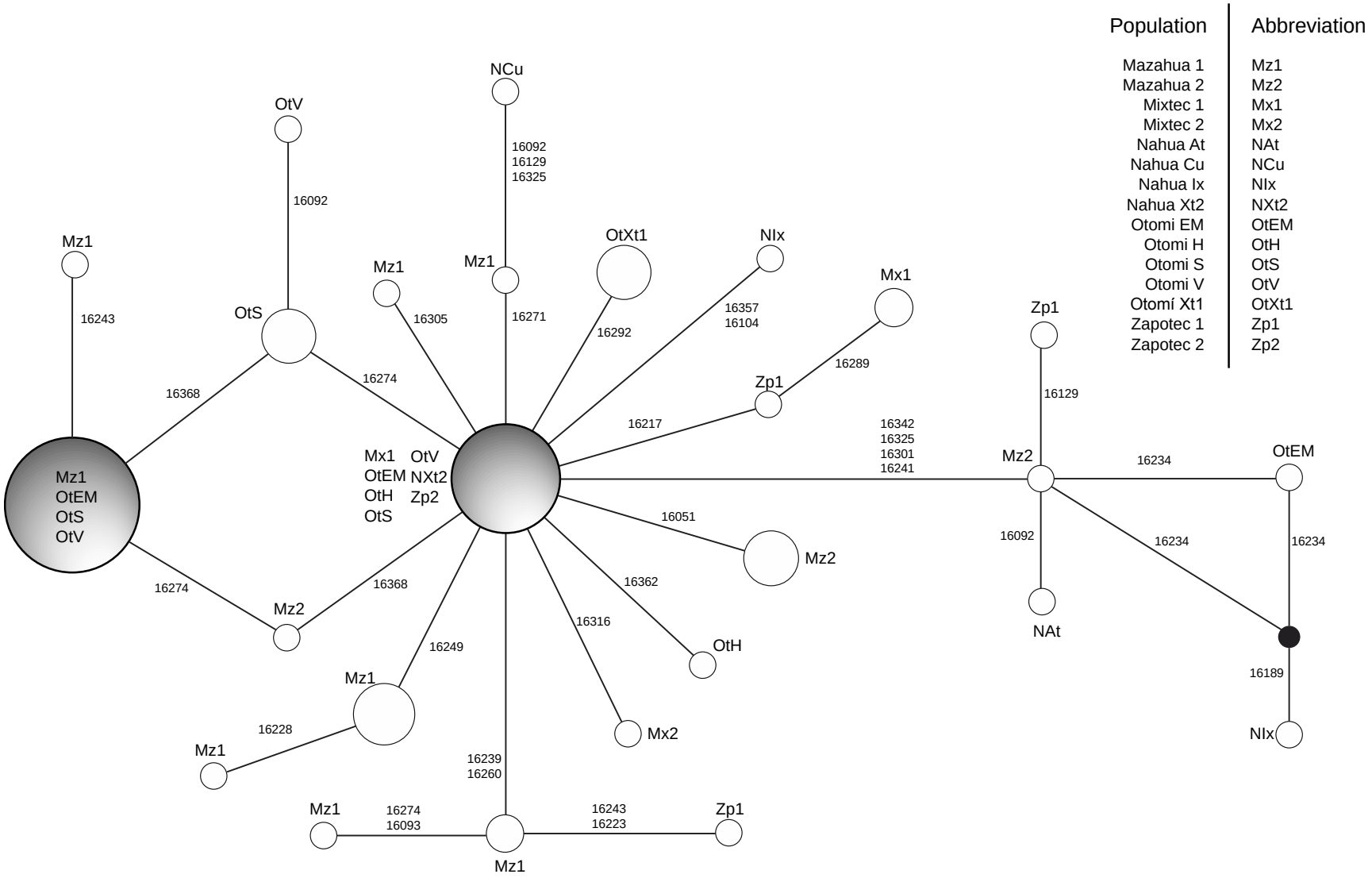

Population
Abbreviation
Mazahua 1
Mz1
Mazahua 2
Mz2
Mixtec 1
Mx1
Mixtec 2
Mx2
Nahua At
NAt
Nahua Cu
NCu
Nahua Ix
NIx
Nahua Xo
NXo
Nahua Xt2
NXt2
Otomi EM
OtEM
Otomi H
OtH
Otomi S
OtS
Otomi V
OtV
Zapotec 1
Zp1
Zapotec 2
Zp2
NIx NXo OtH OtS OtV
16129
NAt NCu OtS OtV
Zp1
16140
16172
Mz1 NAt Zp1 Zp2
16311
Mz1 Mz2 NAt NIx OtEM OtH OtS OtV Zp1 Zp2
16354
NAt OtH OtV
16298
16181
16185
Mz1
NCu
16179
16078
Zp1
16051
OtH OtS OtV
16188
16204
16298
16362
16342
16325
16301
16241
OtEM
16311
Mz1 Mx2 OtEM OtH OtS OtV
16086
NCu
16223
16186
NXo
16335
OtH
16300
16365
Zp1
OtEM
16189
Mx1
16293
16186
Zp1
16051
16327
16223
16326
16172
NXt2
16348
16343
16261
16184
OtEM
16362
OtEM
Mz1
16357
Mz1
OtV
16301
16153
Mz1 NAt OtEM
16169
NXo
16172
16086
Mz1
OtV
16325
16192
Zp1
16181
16319
OtV
16194
OtEM
16354
OtV
16298

Figure 7.


Population
Abbreviation
Mazahua 1
Mz1
Mazahua 2
Mz2
Mixtec 1
Mx1
Mixtec 2
Mx2
Nahua At
NAt
Nahua Cu
NCu
Nahua Ix
NIx
Nahua Xt2
NXt2
Otomi EM
OtEM
Otomi H
OtH
Otomi S
OtS
Otomi V
OtV
Otomí Xt1
OtXt1
Zapotec 1
Zp1
Zapotec 2
Zp2
NCu
OtV
16092
16129
16325
16092
Mz1
16243
Mz1
16305
Mz1
16271
OtXt1
16292
NIx
16357
16104
Mx1
16289
Zp1
16217
OtS
16368
16274
Mz1
OtEM
OtS
OtV
Mx1
OtEM
OtH
OtS
OtV
NXt2
Zp2
16342
16325
16301
16241
Zp1
16129
Mz2
16234
OtEM
16051
Mz2
16092
16234
16234
16274
16368
Mz2
16362
16316
OtH
16249
Mz1
16189
16228
Mz1
Mx2
NIx
16239
16260
Mz1
16274
16093
16243
16223
Zp1
Mz1
NAt