AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY 143:591–600 (2010)

Reconstructing the Population History of Nicaragua by Means of mtDNA, Y-Chromosome STRs, and Autosomal STR Markers

Carolina Nuñez,[1]* Miriam Baeta,[1] Cecilia Sosa,[1] Yolanda Casalod,[1] Jianye Ge,[2,3] Bruce Budowle,[2,3] and Begoña Martínez-Jarreta[1]

[1]*Laboratory of Forensic Genetics, Faculty of Medicine, University of Zaragoza, 50009 Zaragoza, Spain*
[2]*Institute of Investigative Genetics, Health Science Center, University of North Texas, Ft Worth, TX 76107*
[3]*Department of Forensic and Investigative Genetics, Health Science Center, University of North Texas, Ft Worth, TX 76107*

KEY WORDS Central America; genetic admixture; Mestizos; Native Americans

ABSTRACT Before the arrival of the Spaniards in Nicaragua, diverse Native American groups inhabited the territory. In colonial times, Native Nicaraguan populations interacted with Europeans and slaves from Africa. To ascertain the extent of this genetic admixture and provide genetic evidence about the origin of the Nicaraguan ancestors, we analyzed the mitochondrial control region (HVSI and HVSII), 17 Y chromosome STRs, and 15 autosomal STRs in 165 Mestizo individuals from Nicaragua. To carry out interpopulation comparisons, HVSI sequences from 29 American populations were compiled from the literature. The results reveal a close relationship between Oto-manguean, Uto-Aztecan, Mayan groups from Mexico, and a Chibchan group to Nicaraguan lineages. The Native American contribution to present-day Nicaraguan Mestizos accounts for most of the maternal lineages, whereas the majority of Nicaraguan Y chromosome haplogroups can be traced back to a West Eurasian origin. Pairwise *F*st distances based on Y-STRs between Nicaragua and European, African and Native American populations show that Nicaragua is much closer to Europeans than the other populations. Additionally, admixture proportions based on autosomal STRs indicate a predominantly Spanish contribution. Our study reveals that the Nicaraguan Mestizo population harbors a high proportion of European male and Native American female substrate. Finally, the amount of African ancestry is also interesting, probably because of the contribution of Spanish conquerors with North African genetic traces or that of West African slaves. Am J Phys Anthropol 143:591–600, 2010.

Nicaragua is the largest country of Central America, bounded on the North by Honduras, the East by the Caribbean Sea, the South by Costa Rica, and the West by the Pacific Ocean. The origin of the Nicaraguan population, as most populations of Latin American countries, is composed of a substantial ethnic admixture. The colonization of Nicaragua in 1524 (Séjourné, 1972) introduced diverse populations that interacted and mixed with the resident Native Americans. These populations were the European (mainly Spanish) and the sub-Saharan African (who were brought as slaves). Nowadays, the Nicaraguan population is composed of Mestizos (69%), Caucasoids (17%), Africans (9%), and Amerindians (5%; CIA World Factbook, 2007). This panorama makes the study of the Nicaraguan population very interesting in terms of population genetic diversity and admixture.

The first migratory waves from Mesoamerica that established in Nicaragua were the Chorotega (or Mangue). Later, this group was forced to move from their territories to the central region of Nicaragua because of the arrival of the Nicarao (Tous, 2003). Another Nicaraguan group was the Subtiaba (or Maribio), which may be related to the Chorotega. These groups mainly spoke languages of the Uto-Aztec and Oto-Manguean families. The Chontales (or Matagalpa) also occupied the central mountain region of Nicaragua. Their language has been classified into the Chibcha family (Paul, 2009).

The Caribbean coast of Nicaragua, where the Spanish did not settle until the 19th century, has a different history. Tribes with various dialects and languages related to Chibcha inhabited most of this area (Constenla, 2002). The Bawihka group, who lived in Northeast Nicaragua, mixed with African slaves from the British Caribbean territories. They expanded and began to control other indigenous groups such as the Sumus, Ramas, and Garifundas (Helms, 1996). This Afro-indigenous group became known as Miskito and today is the most numerous indigenous group in Nicaragua (VIII Censo de Población y IV de Vivienda, 2005).

A number of forensic and genetic population studies have focused on the American continent. Nevertheless,

Additional Supporting Information may be found in the online version of this article.

*Correspondence to: Carolina Nuñez Domingo, Laboratory of Forensic Genetics, Faculty of Medicine, University of Zaragoza, c/Domingo Miral s/n, 50009 Zaragoza, Spain.
E-mail: cnunezdo@gmail.com

Received 16 February 2010; accepted 15 May 2010

DOI 10.1002/ajpa.21355
Published online 18 August 2010 in Wiley Online Library (wileyonlinelibrary.com).

some populations are still genetically undefined, particularly populations from Central America such as those residing in Nicaragua.

Mitochondrial DNA and Y chromosome markers are very useful tools for investigating the genetic history of populations (Wallace et al., 1999; Jobling and Tyler-Smith, 2003). Lineage-based genetic marker variation has shown a strong phylogeographical structure among continental areas to the point that the majority of haplogroups are confined to a single continent and can be used to trace past and recent migrations (Roewer et al., 1996, 2005; Sampietro et al., 2005; Tamm et al., 2007). Several studies of different populations of Latin America, using these polymorphisms, observed strong sexual genetic asymmetries between male and female gene pools (Green et al., 2000; Mesa et al., 2000; Carvalho-Silva et al., 2001; González-Andrade et al., 2007). Thus, the mitochondrial DNA shows a predominantly Native American contribution whereas the Y chromosome indicates a substantial European contribution. These findings are in agreement with the historical records, which document that the majority of immigrants who entered America during colonial times were males (Sánchez-Albornoz, 1977). In addition, this asymmetry was reinforced by the social attitude of accepting marriage between European males and Native American females, whereas relationships between Native American men and European females were persecuted (Rodríguez- Delfin et al., 2001).

In the present study, we analyzed the mitochondrial DNA control region, 17 Y chromosome STRs, and 15 autosomal STRs of a population from Nicaragua, with the following aims: a) the genetic characterization of the Nicaraguan population, b) to survey the geographic origin of Nicaraguan ancestors, c) to determine the extent of the admixture present among the current Mestizo population, and d) to evaluate the differential sexual contribution to the gene pool of Nicaragua.

MATERIALS AND METHODS

Sample collection and DNA extraction

A total of 165 bloodstains were collected in FTA® paper from healthy unrelated Mestizo individuals who were born and reside in different departments of Nicaragua (Chinandega, Leon, Managua, Carazo, Chontales, Matagalpa, Esteli, Madriz, Nueva Segovia, Jinotega, Atlántico Norte, and Atlántico Sur). Informed consent was obtained from all individuals participating in the study. DNA was extracted using the Chelex-100 method (Walsh et al., 1991).

PCR and mitochondrial DNA sequencing

The hypervariable regions HVSI (positions 15996–16401) and HVSII (positions 29–408) of the mtDNA genome were analyzed. PCR was performed using a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA) in 20 μl reactions using the HotStar Taq Plus Master Mix kit (Qiagen, Chatsworth, CA), including 1 μl of the extracted DNA (0.5–3 ng of DNA), and following the manufacturer's conditions with little modifications. Primers for HVSI were L15996-H16401 and for HVSII L29-H408 (Vigilant et al., 1991), both including the M13 sequence. DNA products were then purified using the MinElute PCR Purification kit (Qiagen, Chatsworth, CA) and sequenced in both directions using M13 primers. Sequencing reactions were performed using the BigDye® Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) following the manufacturer's conditions with slight modifications. Purification of sequencing products was performed with DyEx 2.0 Spin kit (Qiagen, Chatsworth, CA). Sequence reaction products were then analyzed on the ABI Prism™ 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences obtained were analyzed using the Sequencing Analysis software (Applied Biosystems, Foster City, CA) and compared with the rCRS using the SeqScape software (Applied Biosystems, Foster City, CA).

Haplogroups were assigned by means of mtDNAmanager (Lee et al., 2008) and checked following the most updated versions of the mitochondrial DNA phylogeny (Achilli et al., 2008; van Oven et al., 2009).

Quality checking. To minimize the effects of laboratory and documentation errors, two independent members of the laboratory read the sequences separately. In some cases where the sequences were not sufficiently clear or possible phylogenetic inconsistencies were observed, DNA extraction and sequencing were repeated.

Y chromosome genotyping

Y chromosome short tandem repeat (STR) markers included in the AmpFlSTR® Yfiler® PCR Amplification kit (Applied Biosystems, Foster City, CA) were analyzed in the ABI Prism™ 310 Genetic Analyzer according to the manufacturer's recommendations. This set of markers was selected because it includes the "European minimal haplotype" (DYS19, DYS385a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, and DYS393; Kayser et al., 1997), the Scientific Working Group on DNA Analysis Methods recommended Y-STR loci (DYS438 and DYS439), and the highly polymorphic loci DYS437, DYS448, DYS456, DYS458, Y GATA H4, and DYS635 (formerly known as Y GATA C4) to further increase the power of discrimination (Mulero et al., 2006). Allele identification was performed by GeneMapper™ ID software, version 3.1 (Applied Biosystems, Foster City, CA).

As an approximate approach, although not 100% accurate, haplogroups were determined with 23-Haplogroup Beta Program of Haplogroup Predictor (Athey, 2006). This tool is based on a Bayesian approach based upon allele frequencies, which were calculated from collections of haplotypes extracted from published articles and databases (Athey, 2005). Such an approach does result in the probability that a Y-STR haplotype is in a haplogroup. The haplogroup assigned was the one that scored the highest probability. Additionally, we performed haplogroup determination by comparing with data sets from the literature in which both Y chromosome single nucleotide polymorphism (SNP) markers and Y-STRs had been typed (Bosch et al., 1999; Bortolini et al., 2003; Zegura et al., 2004; Adams et al., 2008; Coelho, 2009; Hammer et al., 2009). Haplogroup nomenclature is provided according to the most recent update (Karafet et al., 2008). Haplotypes consisting of nine Y-STR loci were searched in the largest Y chromosome haplotype database, YHRD, (Roewer et al., 2001) to observe the concordance between haplogroups and the frequency of each haplotype in worldwide metapopulations.

Abbreviations

AMOVA	analysis of molecular variance
HVS	hypervariable regions
MDS	multidimensional scaling
YHRD	Y chromosome haplotype database.

Autosomal STRs

Fifteen autosomal STRs included in the AmpFlSTR® Identifiler® PCR Amplification kit (D8S1179, D7S820, D3S1358, D13S317, D16S539, D2S1338, D19S433, D5S818, D21S11, CSF1PO, vWA, THO1, TPOX, D18S51, and FGA; Applied Biosystems, Foster City, CA) were analyzed following the manufacturer's recommendations. The amplified products and reference ladders provided with the kit were analyzed in the ABI Prism™ 310 Genetic Analyzer. Electrophoresis results were analyzed using GeneMapper™ ID software, version 3.1.

Statistical analysis

Mitochondrial DNA. Diversity indices of HVSI sequences (haplotype and nucleotide diversity, and mean number of pairwise differences) were calculated using Arlequin 3.1 software (Excoffier et al., 2005). Nucleotide and sequence diversity were computed as proposed by Nei (1987). A median-joining network of Nicaraguan HVSI sequences was constructed using the Network 4.5.1.0 package (Bandelt et al., 1999). Positions 16,029–16,362 were considered, and nucleotide position was given a weight as in Brandstätter et al. (2008). Values of ρ and σ were converted to age estimates using the most recent mutation rate available for synonymous mutations of the HVSI segment of one transition per 16,677 years (in the nucleotide position range 16,051–16,400) (Soares et al., 2009).

To compare the present Nicaraguan results with other American populations, HVSI sequences from 1,649 American individuals were collected from the literature. American populations used for this purpose were: a) North Americans: Triqui, Tarahumara, Purepecha, Otomí, Mixtec, Xochimilco, Zitlala, Ixhuatlancillo, Necoxtla, Yucatec Maya, and Pima from Mexico (Sandoval et al., 2009), Nuu-Chah-Nulth, Haida, and Bella Coola from Canada (Ward et al., 1991, 1993), Navajo and Apache from Arizona (Budowle et al., 2002); b) Central Americans: Embera, Wounan, and Ngöbé from Panamá (Kolman et al., 1995; Kolman and Bermingham, 1997), Cuba (Mendizabal et al., 2008), El Salvador (Salas et al., 2009); c) South Americans: Arequipa, Tayacaja, San Martin, and Quechua from Peru (Fuselli et al., 2003; Sandoval et al., 2009), Araucarian from Chile (Horai et al., 1993), Cayapa and Waorani from Ecuador (Rickards et al., 1999; Baeta et al., 2009), Yanomami from Brasil (Easton et al., 1996). Population genetic structure was tested by analysis of molecular variance (AMOVA) (Excoffier et al., 1992), and pairwise Fst distances were calculated as proposed by Tajima and Nei (1984) using the Arlequin 3.1 software. To estimate the most likely putative origin (North, Central, or South America) of the Nicaraguan sequences, the probability of origin for each subcontinental region was calculated by a Bayesian approach (Mendizabal et al., 2008).

Y chromosome. The haplotype diversity and population pairwise Fst values were calculated with the Arlequin 3.1 software. Population pairwise Fst values were estimated for Nicaragua, Spain (n = 148; Martin et al., 2004), Italy (n = 79; Capelli et al., 2006), Germany (n = 89; Rodig et al., 2007), United Kingdom (n = 249; Ballard et al., 2005), France (n = 100; Keyser-Tracqui et al., 2003), Arabic and Berber-speaking populations from Morocco (n = 109; Quintana-Murci et al., 2004), Guiné-Bissau (n = 161; Rosa et al., 2006), Angola (n = 236; Coelho et al., 2009), and Kichwa Native American (n = 101; González-Andrade et al., 2007) populations. To maximize the amount of overlapping loci and samples nine Y-STR loci were considered (DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393, and DYS385a/b) for this purpose.

Autosomal STRs. Molecular diversity parameters were determined with the Arlequin 3.1 software. Admixture proportions based on 15 autosomal STRs were computed with Admix 2.0 (Dupanloup and Bertorelle, 2001) using Spanish (Camacho et al., 2007), Mexican Amerinds (Barrot et al., 2005), and Equatorial Guinea (Alves et al., 2005) together with Angola (Beleza et al., 2004) as ancestral populations. Admixture proportions and their standard deviations were estimated from 100,000 bootstrap iterations. This program can compute the estimator mY, initially described in Bertorelle and Excoffier (1998) and extended to any number of parental populations by Dupanloup and Bertorelle (2001). This estimator takes into consideration the frequencies of different alleles as well as the degree of molecular divergence between them. In the case of multilocus data, this approach is possible only when all loci have approximately the same mutation rate (Bertorelle and Excoffier, 1998). Since all 15 autosomal STRs analyzed herein have not similar mutation rates (Short Tandem Repeat DNA Internet DataBase), we have computed admixture coefficients separately for three classes of loci with similar mutation rates: a) THO1 and TPOX; b) D8S1179, D7S820, D3S1358, D13S317, D16S539, D2S1338, D19S433, D5S818, D21S11, CSF1PO, and vWA; c) D18S51 and FGA. We obtained a final estimate from the average over classes of loci, weighted by the number of loci involved in each class.

RESULTS

Mitochondrial DNA

Haplotype and sequence diversity. A total of 68 different HVSI, 55 different HVSII, and 95 different HVSI/II haplotypes were found in the Nicaraguan population sample (ignoring the common insertions at positions 310 and 315 in the HVSII polyC tract). Mitochondrial DNA haplotypes and their corresponding haplogroups are provided in Supplementary file 1. Two samples were removed from the total because of inconclusive profiles. Some HVSI haplotypes are common in the sample, such as 16111T, 16223T, 16290T, 16319A, and 16362C, which are present in 16 individuals. Similar profiles were observed differing with transitions 16187 C→T and 16299 A→G, in 30 and 12 individuals, respectively.

Summary statistics for mtDNA HVSI sequence diversity are presented in Supporting Information Table S1. Haplotype and nucleotide diversity for the Nicaraguan population studied were 0.934 (±0.013) and 0.026 (±0.014), respectively. Native American populations with lower sequence diversity were Triqui (0.548 ± 0.056), Zitlala (0.593 ± 0.144), Pima (0.553 ± 0.056), and Waorani (0.222 ± 0.047).

Haplogroup composition. Figure 1A shows the frequency of each mtDNA haplogroup observed in the Nicaraguan sample. The most frequent haplogroup was the Native American A2 (73.62%), followed by haplogroup B4* (14.11%). Haplotypes classified into the B haplogroup may be considered to belong to the common B2 Native American subclade (Achilli et al., 2008), but since

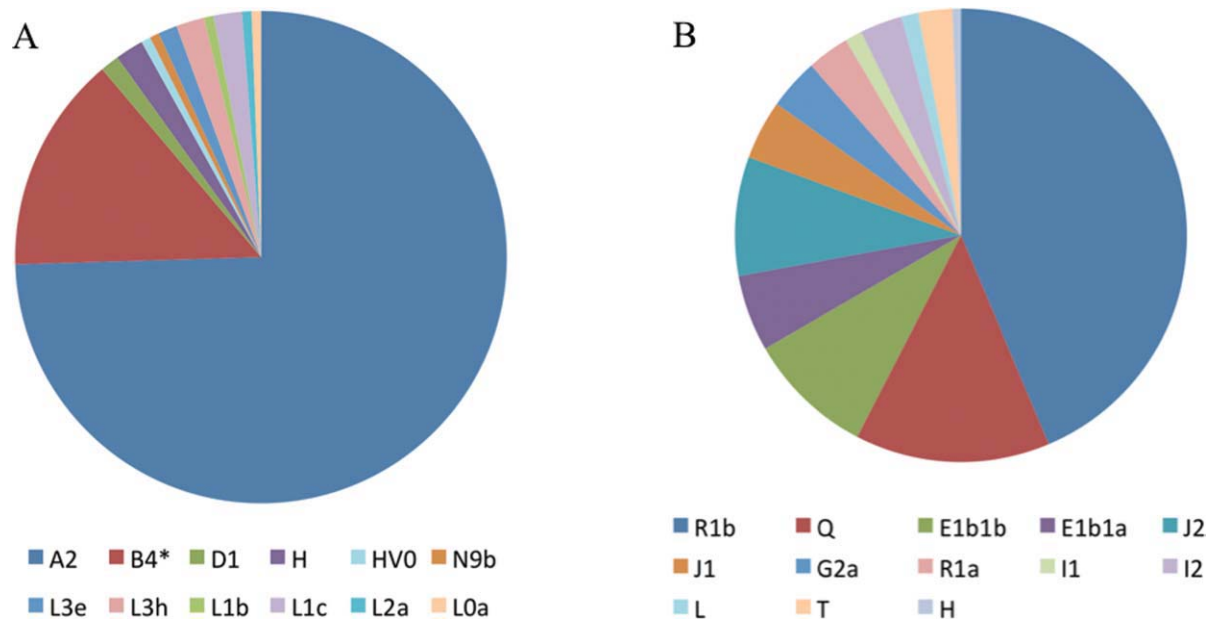

Fig. 1. Frequency of each mtDNA (**A**) and Y-chromosome (**B**) haplogroup found in Nicaragua.

the coding positions were not typed this subclade is classified as B4* haplogroup. Native American haplogroups D1 and X2a were also present, but at very low frequencies (1.22 and 0.61%, respectively). The presence of one sample belonging to the X2a haplogroup may be because of recent migration from North America, since X2a has not been observed in Central America (Perego et al., 2009). Other Native American lineages, such as C1 and D4h3 (Tamm et al., 2007; Perego et al., 2009) are absent from our Nicaraguan sample.

Other non-Native American haplogroups were observed at low frequencies, H (2.45%), V (0.61%), L0a (0.61%), L1b (0.61%), L1c (1.84%), L2a (1.22%), L3e (1.22%), and L3h (1.84%). These latter haplogroups were not considered for computational analysis. Most of these sequences belong to sub-Saharan L haplogroups. Particularly, subhaplogroups L0a, L1b/c, L2a, and L3e/h were observed, which vary in their distribution throughout Africa (Salas et al., 2002; Coelho et al., 2009). Central America mainly harbors subhaplogroups of Western and West-Central Africa origin, such as L1b/c and L3e, consistent with the major source regions for American mtDNA lineages of African ancestry (Salas et al., 2004).

Median-joining network. With the aim of investigating the genetic relationship between the A2 mtDNA lineages observed in our dataset of Nicaragua, a median-joining network was constructed. The phylogeny of A2 in Nicar-

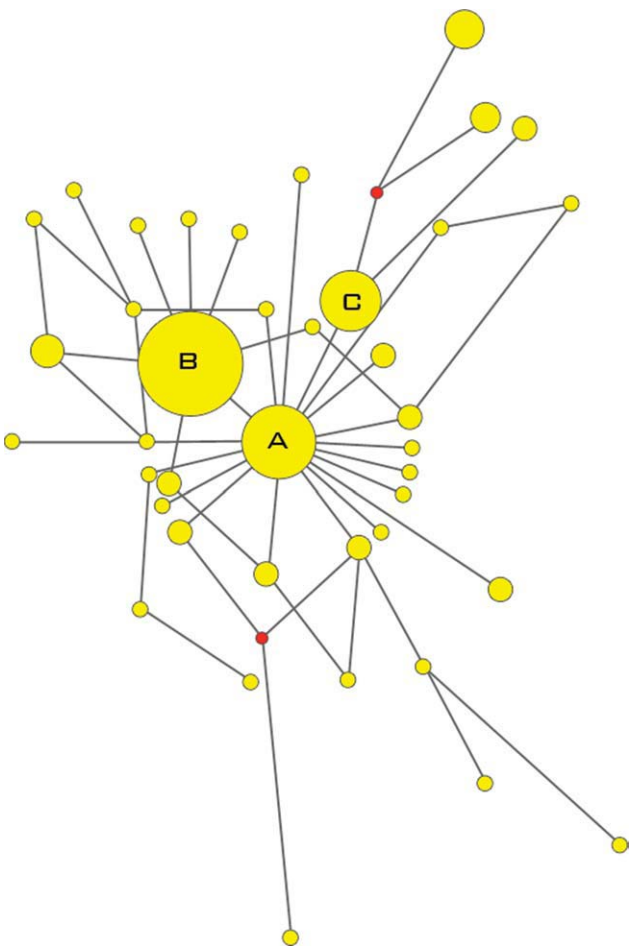

Fig. 2. Median-joining network of haplogroup A2 mtDNA sequences from Nicaragua. Only the variation contained in the HVSI region (range 16,029–16,362) was used. The areas of the circles are proportional to the number of individuals bearing the corresponding haplotype. **A**, **B,** and **C** show the haplotypes 16111T, 16223T, 16290T, 16319A, 16362C; 16111T, 16187T, 16223T, 16290T, 16319A, 16362C; and 16111T, 16223T, 16290T, 16299G, 16319A, 16362C, respectively.

agua is star-like (Fig. 2). It is rooted by the diagnostic sites C16111T, T16223C, C16290T, G16319A, and T16362C in HVSI (A in Fig. 2). Compared with the American database compiled herein, this haplotype was also observed in all American populations with the exception of Tarahumara, Necoxtla, Pima, and Wounan from Mesoamerica, and Arequipa, Araucarian, Quechua, Cayapa, Yanomami, and Waorani from South America. The haplotype referred to as B in Figure 2 of the Nicaraguan sample was also found in the Mexican populations Xochimilco and Maya, and in Ngöbé, Cuba, and El Salvador populations, all from Mesoamerica. Interestingly, haplotype referred to as C in Figure 2 bearing the transition 16299 A→G was only shared by four individuals from El Salvador. The estimated age for the haplogroup A2 based on the HVSI region was dated in 13,834 ± 7,113 Y.B.P.

AMOVA analysis. When all American populations were classified by three major geographic regions: North, Central, and South, AMOVA analysis showed that 25.22% of the variability lies among populations within groups, 72.50% within populations, and a nonsignificant 2.28% ($P > 0.05$) between groups. The results indicate genetic heterogeneity among the subcontinental regions and a lack of continental structure.

Estimation of the geographic origin. With the purpose of obtaining rough estimates for the putative geographic origin of Nicaraguan mtDNA lineages at a continental scale, the identical matching sequences in our database were searched in 29 published American populations classified by main geographical regions: North ($n = 884$), Central ($n = 287$), and South ($n = 478$). Only 18 Native American Nicaraguan lineages, representing 60.7% of the Nicaraguan individuals, were found in the American database. The distribution of the Native American Nicaraguan sequences could be described as follows: 39.5% (SD = 5.2%) to North, 55.5% (SD = 5.3%) to Central, and 4.9% (SD = 2.3%) to South. The average proportions of Nicaraguan sequences found in each geographical region can be used as an approximation to infer the relationship of this population within the continent. As expected, the Nicaraguan mtDNA pool shows a closer relationship to Central American populations. Nonetheless, it is noteworthy that a high percentage (39.5%) of Nicaraguan lineages can be ascribed to a North American origin.

Pairwise Fst distances. To compare the Nicaraguan population with each of the American populations from our database, pairwise *F*st distances were estimated (Supporting Information Table S2). The lower *F*st values were for Nicaragua with Triqui, Mixtec (nonsignificant value, $P > 0.05$), Xochimilco, and Zitlala from Mexico, Ngöbé and El Salvador populations from Central America.

Y chromosome

Haplotype diversity. From a total of 165 Nicaraguan individuals, there were 158 distinct haplotypes observed (based on 17 Y chromosome STR polymorphisms). Y chromosome STR haplotypes and their corresponding haplogroups are presented in Supplementary file 1. Most common haplotypes and their frequency in the Nicaraguan sample are shown in Supporting Information Table S3. Haplotype diversity was high, estimated as 0.9993 ± 0.0008, explained by the high polymorphic value of this set of markers.

Haplogroup composition. Figure 1B shows the haplogroup frequencies in the Nicaraguan dataset. The majority of Nicaraguan lineages can be traced back to the West Eurasian gene pool. The haplogroup with the highest frequency was haplogroup R1b (43.63%), followed by other Eurasian haplogroups at lower frequencies, J2 (8.48%), J1 (4.24%), G2a (3.63%), R1a (3.03%), T (2.42%), I1 (1.21%), I2 (3.03%), L (1.21%), and H (0.6%). African (E1b1a, E1b1b) and Native American (Q) haplogroups represent the 14.54% and 13.93% of the Nicaraguan sample, respectively.

Multidimensional scaling (MDS) analysis of genetic distances based on Y-STR pairwise *F*st estimates (Fig. 3) show a close correspondence between Nicaraguan Mestizos and European populations. In terms of pairwise distance estimates, Nicaragua appears to be much closer to Spain (*F*st = 0.0144) and Italy (*F*st = 0.0140) (Supporting Information Table S4). These results are in agreement with the haplogroup frequencies found in Nicaragua. R1b, the haplogroup with the highest frequency in our sample (43.63%), is the most common in Western Europe (Rosser et al., 2000; Semino et al., 2000), and approaches 66% in Spain (Adams et al., 2008). Moroccan populations show a lower genetic distance (Supporting Information Table S4) with Nicaragua than the other African populations. This result could be explained because the Iberian Peninsula and Balearic Island populations show 10.6% North African ancestry (Adams et al., 2008), because of the Islamic occupation from the 8th to the 15th century. Thus, Spanish conquerors could have introduced genetic traces of North African origin during the colonization of Nicaragua.

Among African haplogroups found in Nicaragua, E1b1b (or E3b) is the most frequent haplogroup (9.09%). This haplogroup is the most seen in North Africa (Arredi et al., 2004), but it has also been observed in Europe (Semino et al., 2000) and Western Asia (Underhill et al., 2000). On the other hand, E1b1a is restricted to Africa, being distributed at different frequencies throughout the continent, particularly in Western Africa at higher frequencies (Coelho et al., 2009). This proportion of E1b1a lineages found in Nicaragua (5.45%) may be because of the Western African slaves introduced by the Spanish during colonial times (Rout, 1976).

Haplogroup J is also present in the Nicaraguan sample at similar frequencies to E1b1a/b haplogroups (J1: 4.24%; J2: 8.48%). J2 lineages seem to display a decreasing frequency gradient from the Near East toward Western Europe, whereas J1 shows its highest frequencies in the Middle East, North Africa, and Ethiopia, and its lowest in Europe, having been observed only in the Mediterranean area (Semino et al., 2004).

Autosomal STRs

Autosomal STR genotypes and allele frequencies are provided in Supplementary file 1. Genetic diversity was high, estimated as 0.792 ± 0.06, according to expectations for admixed populations. Admixture proportions based on 15 autosomal STRs were calculated using Spanish, African from Equatorial Guinea and Angola, and Mexican Amerinds as ancestral populations. Admixture proportions in Nicaraguan Mestizos were (0.690 ± 0.113) Spanish, (0.203 ± 0.089) African, and (0.106 ± 0.079) Amerindian. Large standard deviations are a reflection of short genetic distances among the source populations.

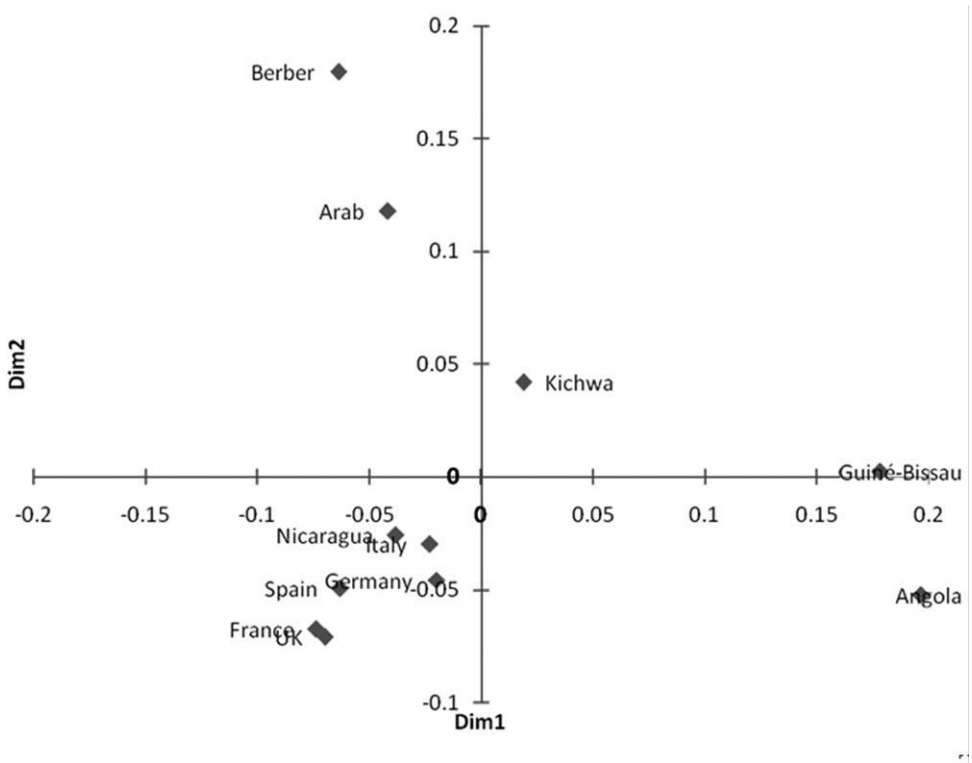

Fig. 3. Multidimensional scaling (MDS) analysis based on Y-STR pairwise Fst estimates among European (Spain, Italy, France, Germany, and United Kingdom), African (Arabic and Berber-speaking populations from Morocco, Guiné-Bissau, and Angola), and American populations (Nicaraguan Mestizos and Amerindian Kichwa). Stress value = 0.12.

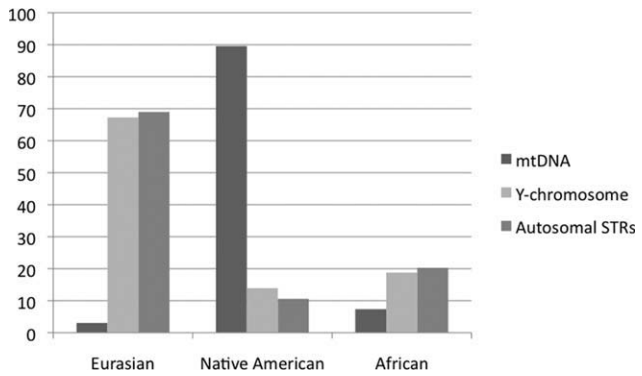

Fig. 4. Admixture estimates based on mtDNA, Y-chromosome STRs, and autosomal STRs data.

There are currently several existing panels of markers for ancestry analysis. To obtain accurate admixture estimates, the average allele frequency difference among ancestral populations and the choice of these populations are factors to take into consideration (Barnholtz-Sloan et al., 2008). Although representative populations have been used as ancestral populations in this study, the average allele frequency difference between ancestral populations is not >0.1 (data not shown). Thus, these results should be regarded as a rough ancestry approximation.

Admixture estimates

Admixture estimates of the present Nicaraguan population are shown in Figure 4. Mitochondrial DNA and Y-chromosome admixture proportions were based on haplogroup frequencies, grouping haplogroups by Native American, Eurasian, or African origin. The results indicate a vast Eurasian contribution on the basis of both autosomal STRs (69%) and Y chromosome haplogroup frequencies (67.27%), whereas the Amerindian component is mainly due to maternal contribution (89.57%). An African contribution was also detected, being higher with autosomal STRs (20.3%) and Y-chromosome (18.78%) than with mtDNA estimates (7.36%).

DISCUSSION

When Spanish conquerors arrived in Nicaragua, they found a densely populated area with several ethno-linguistic groups. Some of the major groups that inhabited Nicaragua before Columbian times may have migrated south from Mexico, being descendants of Aztec and Mayan cultures from Mesoamerica. Also, other groups belonging to the Chibcha linguistic family were generally present in the Central Mountain region and the Caribbean coast of Nicaragua. Mitochondrial DNA data obtained here supports that these groups could be the ancestors of the current Nicaraguan population, mainly composed of Mestizos. Haplotypic comparisons and pairwise Fst values show a relation of Nicaraguan lineages to Triqui, Mixtec (Oto-manguean linguistic family), Xochimilco, Zitlala (Uto-Aztecan linguistic family), Maya Mexican populations, and Ngobé (Chibcha linguistic family) from Panamá. In addition, when our Native American mtDNA sequences were analyzed within the

continental landscape, 39.5% of the lineages can be attributed to a North American origin.

Haplogroup A2 shows the highest frequency in Nicaragua (73.62%), and a significant percent of the Native American lineages (39.3%) remain unobserved in the other American populations used in this study. The high frequency of A2 haplogroup in Nicaragua is in agreement with the frequencies of this haplogroup in other Mesoamerican populations (Santos et al., 1994; Batista et al., 1995; Boles et al., 1995; Kolman et al., 1995; Kolman and Bermingham, 1997; Salas et al., 2009; Sandoval et al., 2009). It is worth mentioning that there is a substantial heterogeneity of haplogroup frequency patterns in America, even between genetically and geographically close populations (Salas et al., 2009). In the case of Mesoamerica, although haplogroup A2 shows a wide distribution, haplogroup B2, C1, and D1 seem to show discontinuities (Santos et al., 1994; Batista et al., 1995; Boles et al., 1995; Kolman et al., 1995; Kolman and Bermingham, 1997; Green et al., 2000; Salas et al., 2009; Sandoval et al., 2009). In the case of haplogroup C1, it is found at high frequencies in Mexico (Green et al., 2000; Sandoval et al., 2009) and Panamá, and at lower frequencies in Guatemala and El Salvador, whereas in Nicaragua and Costa Rica it is absent. The absence of haplogroup C1 in the dataset may reflect the Chibchan origin of some Nicaraguan lineages since other Chibchan groups lack this haplogroup as well (Santos et al., 1994; Batista et al., 1995; Kolman et al., 1995). European and African mtDNA haplogroups were also observed at low frequencies in our dataset (3.06% and 7.34%, respectively). Correspondingly, the impact of non-American populations on the mtDNA pool of Nicaragua is very low.

Despite the numerous Native American people inhabiting Nicaragua during the colonial period, historical records report that the arrival of the Spanish caused a drastic demographic decline, estimated at 90–95% of the indigenous population, and subsequently a new group developed, the Mestizos (Tous, 2003), currently the most abundant group in Nicaragua. This group originated primarily by admixture of Native American women and European men. The Nicaraguan mtDNA and Y chromosome lineages clearly show a strong unidirectional sex bias in European–Native American admixture. The mtDNA is mostly of Native American origin and Y chromosome lineages have a predominantly west Eurasian ancestry. Pairwise Fst distances for Y-STR markers show a close relationship between Nicaragua and European populations studied, particularly with Spain. Y chromosome haplogroup R1b, which is present in a particularly high frequency in the Spanish population (Adams et al., 2008), is the most frequent in Nicaragua. The influence of the other European populations may reflect more recent migrations to the country. Y chromosome subhaplogroups E1b1a/b and J1 account together for 18.78% of the total haplogroups. Because of their distinct distributions throughout Africa, these two subhaplogroups may have been the result of Spanish soldiers with North African ancestry or West African male slaves transported to the region, since West Africa was one of the most important sources of slaves to the Americas (Rout, 1976). Further analysis using Y chromosome SNPs need to be performed to confirm and deepen into these results.

Y chromosome STR haplotypes show a substantial interpopulation differentiation both on a worldwide and continental scale (Kayser et al., 2001; Roewer et al., 2005), and their variability is partitioned by haplogroup background (Bosch et al., 1999). Although SNPs can allow us to identify deep lineages of Y chromosomes and provide a better Y haplogroup resolution, Y-STRs can be highly informative markers for studies of recent evolutionary (or historical) events (Roewer et al., 1996; Pérez-Lezaun et al., 1997).

Admixture proportions based on autosomal STRs show a predominantly Spanish contribution (69%), followed by 20.3% African and 10.6% Amerindian to the genetic pool of Nicaraguan Mestizos. Similar proportions of Spanish ancestry have been found in Mestizo Mexicans (Cerda-Flores et al., 2002; Simms et al., 2008), and in areas with relatively low pre-Columbian Native population density (Central Valley of Costa Rica, Medellin from Colombia, and Rio Grande do Sul from Brazil) (Wang et al., 2008). However, this is not true for Nicaragua, which was highly populated when the Spanish arrived (Tous, 2003). Admixture estimates obtained for mtDNA contrast sharply with those obtained for Y chromosome and autosomal markers, which show a similar admixture pattern. Initial asymmetric mating involving mainly immigrant male and Native American women would explain the proportions seen for mtDNA and Y chromosome, but not for autosomal STRs. Thus, subsequent asymmetry in mating primarily between Mestizo women and European male, within a background of substantial decrease of Native American population, need to be referred to explain the results.

Finally, the African ancestry proportion is also evident for autosomal markers (20.3%). The highest African ancestry based on autosomal microsatellites has been determined in Mestizos from circum-Caribbean areas and in South American regions with past large African immigrations (Zúñiga et al., 2006; Mendizabal et al., 2008; Simms et al., 2008; Wang et al., 2008). Nowadays, the important proportion of African ancestry in Nicaragua is also evident within the census (VIII Censo de Población y IV de Vivienda, 2005). Afro-indigenous groups such as Miskitos, Sumus, Garifundas, and Ramas account for ~30% of the Nicaraguan population distributed throughout the country.

LITERATURE CITED

Achilli A, Perego UA, Bravi CM, Coble MD, Kong QP, Woodward SR, Salas A, Torroni A, Bandelt HJ. 2008. The phylogeny of the four pan-American MtDNA haplogroups: implications for evolutionary and disease studies. PLoS One 3:e1764.

Adams SM, Bosch E, Balaresque PL, Ballereau SJ, Lee AC, Arroyo E, López-Parra AM, Aler M, Grifo MS, Brion M, Carracedo A, Lavinha J, Martínez-Jarreta B, Quintana-Murci L, Picornell A, Ramon M, Skorecki K, Behar DM, Calafell F, Jobling MA. 2008. The genetic legacy of religious diversity and intolerance: paternal lineages of Christians, Jews, and Muslims in the Iberian Peninsula. Am J Hum Genet 83:725–736.

Alves C, Gusmao L, Lopez-Parra AM, Soledad Mesa M, Amorim A, Arroyo-Pardo E. 2005. STR allelic frequencies for an African population sample (Equatorial Guinea) using Amp FlSTR Identifiler and Powerplex 16 kits. Forensic Sci Int 148:239–242.

Arredi B, Poloni ES, Paracchini S, Zerjal T, Fathallah DM, Makrelouf M, Pascali VL, Novelletto A, Tyler-Smith C. 2004. A predominantly neolithic origin for Y-chromosomal DNA variation in North Africa. Am J Hum Genet 75:338–345.

Athey TW. 2005. Haplogroup prediction from Y-STR values using an allele frequency approach. J Genet Geneal 1:1–7.

Athey TW. 2006. Haplogroup prediction from Y-STR values using a Bayesian-allele-frequency approach. J Genet Geneal 2:34–39.

Baeta M, Nuñez C, González-Andrade F, Sosa C, Casalod Y, Bolea M, Zweynert S, Vacas O, González-Solorzano J, Geppert M, Roewer L, Martínez-Jarreta B. 2009. Mitochondrial analysis revealed high homogeneity in the Waorani population—the last nomadic group of hunter-gatherers from Ecuador. Forensic Sci Int: Genet Supp Ser 2:313–314.

Ballard DJ, Phillips C, Thacker CR, Robson C, Revoir AP, Syndercombe Court D. 2005. Y chromosome STR haplotypes in three UK populations. Forensic Sci Int 152:289–305.

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

Barnholtz-Sloan JS, McEvoy B, Shriver MD, Rebbeck TR. 2008. Ancestry estimation and correction for population stratification in molecular epidemiologic association studies. Cancer Epidemiol Biomarkers Prev 17:471–477.

Barrot C, Sanchez C, Ortega M, Gonzalez-Martin A, Brand-Casadevall C, Gorostiza A, Huguet E, Corbella J, Gene M. 2005. Characterisation of three Amerindian populations from Hidalgo State (Mexico) by 15 STR-PCR polymorphisms. Int J Legal Med 119:111–115.

Batista O, Kolman CJ, Bermingham E. 1995. Mitochondrial DNA diversity in the Kuna Amerinds of Panama. Hum Mol Genet 4:921–929.

Beleza S, Alves C, Reis F, Amorim A, Carracedo A, Gusmao L. 2004. 17 STR data (AmpF/STR Identifiler and Powerplex 16 System) from Cabinda (Angola). Forensic Sci Int 141:193–196.

Bertorelle G, Excoffier L. 1998. Inferring admixture proportions from molecular data. Mol Biol Evol 15:1298–1311.

Boles TC, Snow CC, Stover E. 1995. Forensic DNA testing on skeletal remains from mass graves: a pilot project in Guatemala. J Forensic Sci 40:349–355.

Bortolini MC, Salzano FM, Thomas MG, Stuart S, Nasanen SP, Bau CH, Hutz MH, Layrisse Z, Petzl-Erler ML, Tsuneto LT, Hill K, Hurtado AM, Castro-de-Guerra D, Torres MM, Groot H, Michalski R, Nymadawa P, Bedoya G, Bradman N, Labuda D, Ruiz-Linares A. 2003. Y-chromosome evidence for differing ancient demographic histories in the Americas. Am J Hum Genet 73:524–539.

Bosch E, Calafell F, Santos FR, Perez-Lezaun A, Comas D, Benchemsi N, Tyler-Smith C, Bertranpetit J. 1999. Variation in short tandem repeats is deeply structured by genetic background on the human Y chromosome. Am J Hum Genet 65:1623–1638.

Brandstatter A, Zimmermann B, Wagner J, Gobel T, Rock AW, Salas A, Carracedo A, Parson W. 2008. Timing and deciphering mitochondrial DNA macro-haplogroup R0 variability in Central Europe and Middle East. BMC Evol Biol 8:191.

Budowle B, Allard MW, Fisher CL, Isenberg AR, Monson KL, Stewart JE, Wilson MR, Miller KW. 2002. HVI and HVII mitochondrial DNA data in Apaches and Navajos. Int J Legal Med 116:212–215.

Camacho MV, Benito C, Figueiras AM. 2007. Allelic frequencies of the 15 STR loci included in the AmpFlSTR Identifiler PCR amplification kit in an autochthonous sample from Spain. Forensic Sci Int 173:241–245.

Capelli C, Arredi B, Baldassari L, Boschi I, Brisighelli F, Caglia A, Dobosz M, Scarnicci F, Vetrugno G, Pascali VL. 2006. A 9-loci Y chromosome haplotype in three Italian populations. Forensic Sci Int 159:64–70.

Carvalho-Silva DR, Santos FR, Rocha J, Pena SD. 2001. The phylogeography of Brazilian Y-chromosome lineages. Am J Hum Genet 68:281–286.

Cerda-Flores RM, Budowle B, Jin L, Barton SA, Deka R, Chakraborty R. 2002. Maximum likelihood estimates of admixture in Northeastern Mexico using 13 short tandem repeat loci. Am J Hum Biol 14:429–439.

CIA World Factbook. 2007.https://www.cia.gov/library/publications/the-world-factbook/

Coelho M, Sequeira F, Luiselli D, Beleza S, Rocha J. 2009. On the edge of Bantu expansions: mtDNA. Y chromosome and lactase persistence genetic variation in southwestern Angola. BMC Evol Biol 9:80.

Constenla A. 2002. Acerca de la relación genealógica de las lenguas lencas y las lenguas misumalpa. First Archaeological Congress of Nicaragua. Revista de Filología y Lingüística de la Universidad de Costa Rica 28:189–205.

Dupanloup I, Bertorelle G. 2001. Inferring admixture proportions from molecular data: extension to any number of parental populations. Mol Biol Evol 18:672–675.

Easton RD, Merriwether DA, Crews DE, Ferrell RE. 1996. mtDNA variation in the Yanomami: evidence for additional New World founding lineages. Am J Hum Genet 59:213–225.

Excoffier L, Laval G, Schneider S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Fuselli S, Tarazona-Santos E, Dupanloup I, Soto A, Luiselli D, Pettener D. 2003. Mitochondrial DNA diversity in South America and the genetic history of Andean highlanders. Mol Biol Evol 20:1682–1691.

Gonzalez-Andrade F, Sanchez D, Gonzalez-Solorzano J, Gascon S, Martinez-Jarreta B. 2007. Sex-specific genetic admixture of Mestizos. Amerindian Kichwas, and Afro-Ecuadorans from Ecuador. Hum Biol 79:51–77.

Green LD, Derr JN, Knight A. 2000. mtDNA affinities of the peoples of North-Central Mexico. Am J Hum Genet 66:989–998.

Hammer MF, Behar DM, Karafet TM, Mendez FL, Hallmark B, Erez T, Zhivotovsky LA, Rosset S, Skorecki K. 2009. Extended Y chromosome haplotypes resolve multiple and unique lineages of the Jewish priesthood. Hum Genet 126:707–717.

Helms M. 1996. "Miskito". Encyclopedia of World Cultures. Retrieved April 06, 2010 from Encyclopedia.com,http://www.encyclopedia.com/doc/1G2–3458001376.html

Horai S, Kondo R, Nakagawa-Hattori Y, Hayashi S, Sonoda S, Tajima K. 1993. Peopling of the Americas, founded by four major lineages of mitochondrial DNA. Mol Biol Evol 10:23–47.

Jobling MA, Tyler-Smith C. 2003. The human Y chromosome: an evolutionary marker comes of age. Nat Rev Genet 4:598–612.

Karafet TM, Mendez FL, Meilerman MB, Underhill PA, Zegura SL, Hammer MF. 2008. New binary polymorphisms reshape and increase resolution of the human Y chromosomal haplogroup tree. Genome Res 18:830–838.

Kayser M, Caglià A, Corach D, Fretwell N, Gehrig C, Graziosi G, Heidorn F, Herrmann S, Herzog B, Hidding M, Honda K, Jobling M, Krawczak M, Leim K, Meuser S, Meyer E, Oesterreich W, Pandya A, Parson W, Penacino G, Perez-Lezaun A, Piccinini A, Prinz M, Schmitt C, Schneider PM, Szibor R, Teifel-Greding J, Weichhold G, de Knijff P, Roewer L. 1997. Evaluation of Y-chromosomal STRs: a multicenter study. Int J Legal Med 110:125–133,141–149.

Kayser M, Krawczak M, Excoffier L, Dieltjes P, Corach D, Pascali V, Gehrig C, Bernini LF, Jespersen J, Bakker E, Roewer L, de Knijff P. 2001. An extensive analysis of Y-chromosomal microsatellite haplotypes in globally dispersed human populations. Am J Hum Genet 68:990–1018.

Keyser-Tracqui C, Ricaut FX, Blandin P, Ludes B. 2003. French allele frequencies and haplotypes of nine Y-specific STRs. J Forensic Sci 48:242–244.

Kolman CJ, Bermingham E. 1997. Mitochondrial and nuclear DNA diversity in the Choco and Chibcha Amerinds of Panama. Genetics 147:1289–1302.

Kolman CJ, Bermingham E, Cooke R, Ward RH, Arias TD, Guionneau-Sinclair F. 1995. Reduced mtDNA diversity in the Ngobe Amerinds of Panama. Genetics 140:275–283.

Lee HY, Song I, Ha E, Cho SB, Yang WI, Shin KJ. 2008. mtDNAmanager: a Web-based tool for the management and quality analysis of mitochondrial DNA control-region sequences. BMC Bioinformatics 9:483.

Martin P, Garcia-Hirschfeld J, Garcia O, Gusmao L, Garcia P, Albarran C, Sancho M, Alonso A. 2004. A Spanish population

study of 17 Y-chromosome STR loci. Forensic Sci Int 139:231–235.

Mendizabal I, Sandoval K, Berniell-Lee G, Calafell F, Salas A, Martinez-Fuentes A, Comas D. 2008. Genetic origin, admixture, and asymmetry in maternal and paternal human lineages in Cuba. BMC Evol Biol 8:213.

Mesa NR, Mondragón MC, Soto ID, Parra MV, Duque C, Ortíz-Barrientos D, García LF, Velez ID, Bravo ML, Múnera JG, Bedoya G, Bortolini MC, Ruiz-Linares A. 2000. Autosomal, mtDNA, and Y-chromosome diversity in Amerinds: pre- and post-Columbian patterns of gene flow in South America. Am J Hum Genet 67:1277–1286.

Mulero JJ, Chang CW, Calandro LM, Green RL, Li Y, Johnson CL, Hennessy LK. 2006. Development and validation of the AmpFlSTR Yfiler PCR amplification kit: a male specific, single amplification 17 Y-STR multiplex system. J Forensic Sci 51:64–75.

Nei N. 1987. Molecular evolutionary genetics. New York: Columbia University Press.

Paul LM, editor. 2009. Ethnologue: languages of the world, 16th ed. Dallas, TX: SIL International.

Perego UA, Achilli A, Angerhofer N, Accetturo M, Pala M, Olivieri A, Kashani BH, Ritchie KH, Scozzari R, Kong QP, Myres NM, Salas A, Semino O, Bandelt HJ, Woodward SR, Torroni A. 2009. Distinctive Paleo-Indian migration routes from Beringia marked by two rare mtDNA haplogroups. Curr Biol 19:1–8.

Perez-Lezaun A, Calafell F, Seielstad M, Mateu E, Comas D, Bosch E, Bertranpetit J. 1997. Population genetics of Y-chromosome short tandem repeats in humans. J Mol Evol 45:265–270.

Quintana-Murci L, Bigham A, Rouba H, Barakat A, McElreavey K, Hammer M. 2004. Y-chromosomal STR haplotypes in Berber and Arabic-speaking populations from Morocco. Forensic Sci Int 140:113–115.

Rickards O, Martinez-Labarga C, Lum JK, De Stefano GF, Cann RL. 1999. mtDNA history of the Cayapa Amerinds of Ecuador: detection of additional founding lineages for the Native American populations. Am J Hum Genet 65:519–530.

Rodig H, Grum M, Grimmecke HD. 2007. Population study and evaluation of 20 Y-chromosome STR loci in Germans. Int J Legal Med 121:24–27.

Rodriguez-Delfin LA, Rubin-de-Celis VE, Zago MA. 2001. Genetic diversity in an Andean population from Peru and regional migration patterns of Amerindians in South America: data from Y chromosome and mitochondrial DNA. Hum Hered 51:97–106.

Roewer L, Croucher PJ, Willuweit S, Lu TT, Kayser M, Lessig R, de Knijff P, Jobling MA, Tyler-Smith C, Krawczak M. 2005. Signature of recent historical events in the European Y-chromosomal STR haplotype distribution. Hum Genet 116:279–291.

Roewer L, Kayser M, Dieltjes P, Nagy M, Bakker E, Krawczak M, de Knijff P. 1996. Analysis of molecular variance (AMOVA) of Y-chromosome-specific microsatellites in two closely related human populations. Hum Mol Genet 5:1029–1033.

Roewer L, Krawczak M, Willuweit S, Nagy M, Alves C, Amorim A, Anslinger K, Augustin C, Betz A, Bosch E, Cagliá A, Carracedo A, Corach D, Dekairelle AF, Dobosz T, Dupuy BM, Füredi S, Gehrig C, Gusmaõ L, Henke J, Henke L, Hidding M, Hohoff C, Hoste B, Jobling MA, Kärgel HJ, de Knijff P, Lessig R, Liebeherr E, Lorente M, Martínez-Jarreta B, Nievas P, Nowak M, Parson W, Pascali VL, Penacino G, Ploski R, Rolf B, Sala A, Schmidt U, Schmitt C, Schneider PM, Szibor R, Teifel-Greding J, Kayser M. 2001. Online reference database of European Y-chromosomal short tandem repeat (STR) haplotypes. Forensic Sci Int 118:106–113.

Rosa A, Ornelas C, Brehm A, Villems R. 2006. Population data on 11 Y-chromosome STRs from Guine-Bissau. Forensic Sci Int 157:210–217.

Rosser ZH, Zerjal T, Hurles ME, Adojaan M, Alavantic D, Amorim A, Amos W, Armenteros M, Arroyo E, Barbujani G, Beckman G, Beckman L, Bertranpetit J, Bosch E, Bradley DG, Brede G, Cooper G, Côrte-Real HB, de Knijff P, Decorte R, Dubrova YE, Evgrafov O, Gilissen A, Glisic S, Gölge M, Hill EW, Jeziorowska A, Kalaydjieva L, Kayser M, Kivisild T, Kravchenko SA, Krumina A, Kucinskas V, Lavinha J, Livshits LA, Malaspina P, Maria S, McElreavey K, Meitinger TA, Mikelsaar AV, Mitchell RJ, Nafa K, Nicholson J, Nørby S, Pandya A, Parik J, Patsalis PC, Pereira L, Peterlin B, Pielberg G, Prata MJ, Previderé C, Roewer L, Rootsi S, Rubinsztein DC, Saillard J, Santos FR, Stefanescu G, Sykes BC, Tolun A, Villems R, Tyler-Smith C, Jobling MA. 2000. Y-chromosomal diversity in Europe is clinal and influenced primarily by geography, rather than by language. Am J Hum Genet 67:1526–1543.

Rout L. 1976. The African experience in Spanish America: 1502 to the present days. Cambridge, England: Cambridge University Press.

Salas A, Lovo-Gomez J, Alvarez-Iglesias V, Cerezo M, Lareu MV, Macaulay V, Richards MB, Carracedo A. 2009. Mitochondrial echoes of first settlement and genetic continuity in El Salvador. PLoS One 4:e6882.

Salas A, Richards M, De la Fe T, Lareu MV, Sobrino B, Sanchez-Diz P, Macaulay V, Carracedo A. 2002. The making of the African mtDNA landscape. Am J Hum Genet 71:1082–1111.

Salas A, Richards M, Lareu MV, Scozzari R, Coppa A, Torroni A, Macaulay V, Carracedo A. 2004. The African diaspora: mitochondrial DNA and the Atlantic slave trade. Am J Hum Genet 74:454–465.

Sampietro ML, Caramelli D, Lao O, Calafell F, Comas D, Lari M, Agusti B, Bertranpetit J, and Lalueza-Fox C. 2005. The genetics of the pre-Roman Iberian Peninsula: a mtDNA study of ancient Iberians. Ann Hum Genet 69(Pt 5):535–548.

Sánchez-Albornoz N. 1977. La población de America Latina: desde los tiempos precolombinos al año 2000, 2nd ed. Madrid: Alianza Editorial.

Sandoval K, Buentello-Malo L, Penaloza-Espinosa R, Avelino H, Salas A, Calafell F, Comas D. 2009. Linguistic and maternal genetic diversity are not correlated in Native Mexicans. Hum Genet 126:521–531.

Santos M, Ward RH, Barrantes R. 1994. mtDNA variation in the Chibcha Amerindian Huetar from Costa Rica. Hum Biol 66:963–977.

Séjourné L. 1972. América Latina. Antiguas culturas precolombinas , Vol.21. Madrid: Historia Universal Siglo XXI.

Semino O, Magri C, Benuzzi G, Lin AA, Al-Zahery N, Battaglia V, Maccioni L, Triantaphyllidis C, Shen P, Oefner PJ, Zhivotovsky LA, King R, Torroni A, Cavalli-Sforza LL, Underhill PA, Santachiara-Benerecetti AS. 2004. Origin, diffusion, and differentiation of Y-chromosome haplogroups E and J: inferences on the neolithization of Europe and later migratory events in the Mediterranean area. Am J Hum Genet 74:1023–1034.

Semino O, Passarino G, Oefner PJ, Lin AA, Arbuzova S, Beckman LE, De Benedictis G, Francalacci P, Kouvatsi A, Limborska S, Marcikiae M, Mika A, Mika B, Primorac D, Santachiara-Benerecetti AS, Cavalli-Sforza LL, Underhill PA. 2000. The genetic legacy of Paleolithic Homo sapiens sapiens in extant Europeans: a Y chromosome perspective. Science 290:1155–1159.

Short Tandem Repeat DNA Internet DataBase.http://www.cstl.nist.gov/strbase/

Simms TM, Garcia C, Mirabal S, McCartney Q, Herrera RJ. 2008. The genetic legacy of the Transatlantic Slave Trade in the island of New Providence. Forensic Sci Int Genet 2:310–317.

Soares P, Ermini L, Thomson N, Mormina M, Rito T, Rohl A, Salas A, Oppenheimer S, Macaulay V, Richards MB. 2009. Correcting for purifying selection: an improved human mitochondrial molecular clock. Am J Hum Genet 84:740–759.

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

Tamm E, Kivisild T, Reidla M, Metspalu M, Smith DG, Mulligan CJ, Bravi CM, Rickards O, Martinez-Labarga C, Khusnutdinova EK, Fedorova SA, Golubenko MV, Stepanov VA, Gubina MA, Zhadanov SI, Ossipova LP, Damba L, Voevoda MI, Dipierri JE, Villems R, Malhi RS. 2007. Beringian standstill and spread of Native American founders. PLoS One 2:e829.

Tous, M. 2003. De la Gran Nicoya precolombina a la Provincia de Nicaragua, siglos XV y XVI. Un estudio de la subárea arqueológico-cultural de la Gran Nicoya y de sus transformaciones derivadas de la conquista española, PhD Thesis, Universitat de Barcelona.http://www.tdx.cesca.es/TDX-0505103–110130/

Underhill PA, Shen P, Lin AA, Jin L, Passarino G, Yang WH, Kauffman E, Bonné-Tamir B, Bertranpetit J, Francalacci P, Ibrahim M, Jenkins T, Kidd JR, Mehdi SQ, Seielstad MT, Wells RS, Piazza A, Davis RW, Feldman MW, Cavalli-Sforza LL, Oefner PJ. 2000. Y chromosome sequence variation and the history of human populations. Nat Genet 26:358–361.

van Oven M, Kayser M. 2009. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat 30:E386–E394.

Vigilant L, Stoneking M, Harpending H, Hawkes K, Wilson AC. 1991. African populations and the evolution of human mitochondrial DNA. Science 253:1503–1507.

Wallace DC, Brown MD, Lott MT. 1999. Mitochondrial DNA variation in human evolution and disease. Gene 238:211–230.

Walsh PS, Metzger DA, Higuchi R. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506–513.

Wang S, Ray N, Rojas W, Parra MV, Bedoya G, Gallo C, Poletti G, Mazzotti G, Hill K, Hurtado AM, Camrena B, Nicolini H, Klitz W, Barrantes R, Molina JA, Freimer NB, Bortolini MC, Salzano FM, Petzl-Erler ML, Tsuneto LT, Dipierri JE, Alfaro EL, Bailliet G, Bianchi NO, Llop E, Rothhammer F, Excoffier L, Ruiz-Linares A. 2008. Geographic patterns of genome admixture in Latin American Mestizos. PLoS Genet 4:e1000037.

Ward RH, Frazier BL, Dew-Jager K, Paabo S. 1991. Extensive mitochondrial diversity within a single Amerindian tribe. Proc Natl Acad Sci USA 88:8720–8724.

Ward RH, Redd A, Valencia D, Frazier B, Paabo S. 1993. Genetic and linguistic differentiation in the Americas. Proc Natl Acad Sci USA 90:10663–10667.

Zegura SL, Karafet TM, Zhivotovsky LA, Hammer MF. 2004. High-resolution SNPs and microsatellite haplotypes point to a single, recent entry of Native American Y chromosomes into the Americas. Mol Biol Evol 21:164–175.

Zuniga J, Ilzarbe M, Acunha-Alonzo V, Rosetti F, Herbert Z, Romero V, Almeciga I, Clavijo O, Stern JN, Granados J, Fridkis-Hareli M, Morrison P, Azocar J, Yunis EJ. 2006. Allele frequencies for 15 autosomal STR loci and admixture estimates in Puerto Rican Americans. Forensic Sci Int 164:266–270.

VIII Censo de Población y IV de Vivienda. Cifras oficiales del censo 2005.http://www.inide.gob.ni