Mitochondrial-DNA Phylogenetic Information and the Reconstruction of Human Population History: The South American Case

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Abstract

Mitochondrial DNA (mtDNA) sequences are becoming increasingly important in the study of human population history. Here, we explore the differences in the amount of information of different mtDNA regions and their utility for the reconstruction of South American population history. We analyzed six data sets comprising 259 mtDNA sequences from South America: Complete mtDNA, Coding, Control, hypervariable region I (HVRI), Control plus cytochrome b (cytb), and cytb plus 12S plus 16S. The amount of information in each data set was estimated employing several site-by-site and haplotype-based statistics, distances among sequences, neighbor-joining trees, distances among the estimated trees, Bayesian skyline plots, and phylogenetic informativeness profiles. The different mtDNA data sets have different amounts of information to reconstruct demographic events and phylogenetic trees with confidence. Whereas HVRI is not suitable for phylogenetic reconstruction of ancient clades, this region, as well as the Control data set, displays information for the demographic reconstruction during the Holocene period, probably because of the high rate of mutation of these regions. As expected, the Complete mtDNA and Coding data sets, displaying slower rates of mutation, present suitable information to estimate the founding subhaplogroups that populated South America and for the reconstruction of ancient demographic events. Our results point out the importance of evaluating the utility of different DNA regions to respond to different questions and problems in the human population studies, mainly considering the time scale of the phenomenon and the informativeness of the molecular region in a particular geographical area.

Molecular data have become increasingly important during the last century for the study of human population histories. In recent years these studies have been revolutionized by the analysis of genome-scale data sets (Macaulay et al. 2005; Li and Durbin 2011; Rasmussen et al. 2011; Mallick et al. 2016). In particular, the analysis of mitochondrial DNA (mtDNA) genomes has had a profound impact on studies of the evolution of human populations at global and regional scales. In this context, our understanding of the early dispersion and demographic history of the humans who peopled the Americas, mainly South America, has been...
greatly impacted by these analyses (Fagundes et al. 2008b; Perego et al. 2010; Bodner et al. 2012; de Saint Pierre et al. 2012b; Llamas et al. 2016).

Despite many studies using genomic data, migratory and demographic topics are also addressed using mtDNA control region sequences and the nonrecombining region of the Y chromosome (Vigilant et al. 1991; Rubicz et al. 2007; Bisso-Machado et al. 2012; Wallace 2015; Poznik et al. 2016). In particular, several works employ sequences from hypervariable regions I and II (HVRI and HVRII) to explore hypotheses about the origin, migration route, and demographic history of aboriginal human populations that inhabited South America (e.g., Moraga et al. 2000; O’Rourke and Raff 2010; Kemp and Schurr 2010; de Saint Pierre et al. 2012a). More recently, studies exploring human evolution in the subcontinent have analyzed ancient DNA, sequencing mainly HVRI (e.g., Carnese et al. 2010; Fehren-Schmitz et al. 2011, 2014; Mendisco et al. 2014; Postillone et al. 2017). These studies have generated a large comparative database of HVRI and HVRII, as well as a more restricted sample of complete mtDNA genomic data. Therefore, a critical issue is to understand the utility of these different DNA regions or data sets for the study of past human populations in geographical regions as South America.

This is particularly important if we consider that different mtDNA regions display differences in their substitution rate (Endicott and Ho 2008; Soares et al. 2009) and therefore differences in the amount of phylogenetic information (Townsend 2007; Dornburg et al. 2014). The relevant question here is which mtDNA region is more informative about a process or event for a given time interval in the past. Whereas in interspecies studies researchers have long discussed the utility of different mitochondrial and nuclear genomic regions for phylogenetic inference (e.g., Goldman 1998; Graybeal 1998; Yang 1998; Cotton and Wilkinson 2008; Camargo et al. 2012; Dornburg et al. 2014), this problem has been scarcely explored in depth in human population studies (see Non et al. 2007). This is striking because, for example, the different human mtDNA regions are known to differ in their substitution rate and level of saturation (Soares et al. 2009). At present, there is no robust theory predicting the power of DNA regions for a particular time in the past, although the amount of information of a molecular sequence can be empirically quantified (Townsend 2007).

Here, we empirically explored differences in the amount of information of different mtDNA regions and their utility for the reconstruction of human population history. A previous study explored the utility of different mitochondrial genomic regions for phylogenetic inference in the Old World (Non et al. 2007), suggesting that the mtDNA control region has limited utility for the study of the human population history. We statistically address this problem employing samples from South America, a model system that displays a recent human peopling (ca. 15,000–20,000 years ago; Fagundes et al. 2008b; Llamas et al. 2016). Although we explored American sequences, the objective of our work was methodological, having as its goal to understand the performance of different data sets and not the examination of the peopling of America. Specifically, in this work we addressed the impact of variability and informativeness of different mtDNA regions, some of them previously employed in South American studies, on genealogical (i.e., individual phylogeny) and demographic reconstructions. Unlike previous studies, we explicitly explored the impact of differences in the amount of information of different mtDNA regions in the population dynamics estimation at a continental scale, in a model system with a more recent human peopling than previously used (Non et al. 2007). Therefore, we expected our results to differ from previous works. We explored this problem using complete mtDNA genomes, multivariate statistics, and Bayesian methods. We also discuss the best way to employ mtDNA data sets in human population studies. We expect our study to allow for a better use of the large quantity of mtDNA data and to improve genealogical and demographic inferences.

Materials and Methods

We obtained 259 DNA sequences comprising the whole mitochondrial genome (complete mtDNA) from GenBank and previous publications (Hartmann et al. 2009; Perego et al. 2009, 2010; Bodner et al. 2012; de Saint Pierre et al. 2012b; Llamas et al. 2016). Accession numbers for the mitochondrial sequences from South America are shown in
Supplementary Table S1. These sequences come from modern and prehistoric humans of South America, particularly from south-central Andes (n = 123; Hartmann et al. 2009; Perego et al. 2009, 2010; Bodner et al. 2012; de Saint Pierre et al. 2012b; Llamas et al. 2016), northeastern South America (n = 17; Hartmann et al. 2009; Perego et al. 2009, 2010; Bodner et al. 2012; Llamas et al. 2016), and Pampa-Patagonia (n = 119; Perego et al. 2009; 2010; Bodner et al. 2012; de Saint Pierre et al. 2012b; Llamas et al. 2016).

The sequences of mitochondrial genomes were aligned in the software MAFFT (version 7.012b; Katoh and Standley 2013), using the algorithm FFT-NS-2, and manually verified with the software BioEdit (version 7.0.0; Hall 2004). After alignment, the complete mtDNA data set was divided in two new data sets, the Control and Coding region data sets (Figure 1), which previously were used in studies about South American human evolution (e.g., de Saint Pierre et al. 2012a; Fagundes et al. 2008b). The Control data set comprises 1,122 base pairs (bp), including positions 1–576 and 16024–16569; the Coding data set comprises 15,391 bp, between the positions 577 and 16023 (with two intercalated noncoding segments of 25 and 31 bp; Andrews et al. 1999). We also analyzed only the 343 bp of the HVRI (Figure 1), from position 16024 to position 16365. Finally, we generated two novel data sets. The first novel data set, Control–cyt b, includes the Control region and the 1,140 bp from the cytochrome b (cyt b) gene (Figure 1; between positions 14747 and 15887). The second novel data set, cyt b–12S–16S, includes cyt b plus the 12S rRNA (between positions 648 and 1601) and 16S rRNA (between positions 1671 and 3229; Figure 1). We chose these data sets for two reasons: (a) the control, HVRI, and coding regions are widely used in studies of human population and evolution in South America (de Saint Pierre et al. 2012a; Fagundes et al. 2008b; Perez et al. 2016) and represent regions with marked differences in substitution rate and relative number of mutations (Soares et al. 2009); (b) conversely, the cyt b, 12S rRNA, and 16S rRNA regions, as well as the control region plus cyt b gene, are widely used in phylogenetic and biogeographic studies, mainly in analyses involving closely related species and subspecies (e.g., Marín et al. 2008; Lynch Alfaro et al. 2012; Metcalf et al. 2016), and therefore could be useful for human population studies. All data sets were used in the subsequent analyses.

We first explored the differences among the molecular mtDNA data sets by estimating the similarity between distance matrices and genealogical trees obtained using each data set. We estimated the maximum composite likelihood distance and employed the neighbor-joining method, implemented in MEGA (version 7.0.20; Kumar et al. 2016), to reconstruct the phylogenies. We employed the neighbor-joining method because it is more efficient for large data sets than the alternatives (Tamura et al. 2004). We then compared the matrices of distances and the genealogical trees obtained by calculating the matrix correlation (Smouse et al. 1986; Legendre and Legendre 1998) for the former and the Robinson-Foulds (RF) distance (Robinson and Foulds 1981; Kuhner and Felsenstein 1994) for the latter. To estimate the matrix correlation, the matrices were unfolded into vectors and a simple correlation was calculated. We also estimated dispersion plots, similar to empirical saturation plots (Graybeal 1994), to graphically evaluate the utility of the mtDNA regions to reconstruct a phylogeny. The RF distance or topological congruence between trees was calculated as the number of internal branches observed in one phylogeny but not in the other. The matrix of RF distances was analyzed using two multivariate analyses: the unweighted pair group method with arithmetic mean (UPGMA) cluster and the nonmetric multidimensional scaling (nMDS). RF distances were calculated in the Tree distance 3.695 program of the PHYLIP package (Felsenstein 2005), whereas
the matrix correlations and multivariate analyzes were performed in the software PAST (version 3.0; Hammer et al. 2001).

We further described the differences among the molecular mtDNA data sets by calculating several site-by-site and haplotype-based statistics (Rozas 2009). For the site-by-site analyses, we calculated the number of variable positions or segregating sites \(S\) in each data set. Because this statistic is sensitive to the number of sites in the sequences \(N_s;\) Rozas 2009), we calculated the number of segregating sites on the total number of sites in the sequence \(S/N_s\). We also estimated the mean number of nucleotide differences between sequences \(k\) and the average number of nucleotide differences per site or the nucleotide diversity \(\pi\), defined as \(k/N_s\) divided by the number of sites in the sequence, excluding the sites with alignment gaps (Rozas 2009). Finally, we estimated the number of different DNA sequences or haplotypes \(h\) and the mean of the haplotype proportions or haplotype diversity \(H;\) Rozas 2009). Because the definition of haplotypes is related to the number of sites in the sequence \(N_s\), we estimated the number of haplotypes divided by the number of site in the sequences \(h/N_s\).

Second, we used the six mtDNA data set to estimate the demographic trajectories of the South American populations employing the Bayesian skyline plot (BSP) method (Drummond et al. 2005) implemented in the software BEAST (version 1.6.1; Drummond and Rambaut 2007). The BSP method uses the shape of a genealogy estimated with molecular data to reconstruct the demographic dynamics of a population in the past (Drummond et al. 2005; Ho and Shapiro 2011). The method simultaneously estimates genealogy, coalescence time, and population size through time using a Markov chain Monte Carlo sampling model. We set the tree priors as a coalescent Bayesian skyline. The number of generations was established at 50,000,000 and the sample frequency at 5,000 for the Markov chain Monte Carlo sampling. We employed two widely used substitution rates in South American studies (Fagundes et al. 2008b; de Saint Pierre et al. 2012a; Perez et al. 2016): 3.02E–7 substitutions per site per year (Endicott and Ho 2008) for the control, HVRI, and control plus cyt\(b\) regions, and 1.26E-08 substitutions per site per year (Fagundes et al. 2008b) for complete mtDNA, cyt\(b\) plus 12S plus 16S, and coding region. The use of ancient DNA can influence our demographic estimations, so we alternatively included and excluded the ancient sequences in the analyses. The BSPs were reconstructed using the estimated genealogies in the software Tracer (version 1.5; Rambaut and Drummond 2007). Tracer also was used to test for the convergence in the parameters of the Bayesian analyses.

Finally, we explicitly tested for the phylogenetic informativeness of the different data sets. We employed the online application PhyDesign (López-Giráldez and Townsend 2011), which implements the Townsend (2007) phylogenetic informativeness profile. This method provides a quantitative measurement of the utility of a molecular region or data set to reconstruct a phylogeny at different times in the past (Townsend 2007; López-Giráldez and Townsend 2011). Phylogenetic informativeness relates nucleotide saturation in a molecular region to the estimated divergence time (Townsend 2007; Dornburg et al. 2014). Specifically, the informativeness profile is estimated based on the ratio of the observed rate of substitution to the optimal rate of substitution for genealogical inference at different times in the past (Dornburg et al. 2014).

**Results**

The relationships among mtDNA lineages estimated by the neighbor-joining trees are similar for the Complete mtDNA, Control, Control–cyt\(b\), and Coding data sets (Figure 2). The complete mtDNA tree shows that haplogroup B, including the subhaplogroups B2 and B2i, is related to subhaplogroup A2. Haplogroup D displays large variability and shows four well-defined clades or subhaplogroups (note that we use the terms...
haplogroup, subhaplogroup, and haplotype only as
a convenience in this work; for a broader discussion
of the terminology, see Kemp and Schurr 2010), all
of them monophyletic (D1, D4h3, D1g, and D1j). The
tree also displays large diversity in haplogroup C,
showing the monophyly of subhaplogroups C1b,
C1c, and C1d. The trees based on the Control and
Control–cyt b data sets display slight differences
from the Complete mtDNA tree. In particular, in
these data sets some subhaplogroups, such as D1
and D4h3, are not monophyletic. Conversely, the
trees based on HVRI and cyt b–12S–16S data sets
display large differences. In particular, the HVRI
shows that almost all the subhaplogroups are not
monophyletic, displaying different relationships
among them. Finally, the tree based on the Coding
data set displays some similarities with the Com-
plete mtDNA tree, showing only subhaplogroups
D1g and B2 as polyphyletic.

The pattern of similarity between the data
sets can be better observed in the nmMDS (stress
= 0) and UPGMA results (Figure 3). These results
confirm that the Control and Control–cyt b data
sets generate trees more similar to the Complete
mtDNA tree, whereas the HVRI and cyt b–12S–16S
trees display large differences compared to each
other and to the Complete mtDNA tree. We also
explored the variation in a data set displaying
sequences from all America, including the 259
South American sequences and 174 sequences
from North America (Supplementary Table S2;
Just et al. 2008; Hartmann et al. 2009; Perego et
al. 2009, 2010; Achilli et al. 2013; Llamas et al.
2016), to compare with the South American case.
The results show a pattern of differences among
the mtDNA data sets for the American sample
(Supplementary Figure S1) that is similar to the
pattern observed for the South American sample,
suggesting that our results could be generalized to
the entire peopling of America. When we observed
the correlations between distance matrices (Table
1), the Control, Control–cyt b, and Coding data sets
show correlations between 0.90 and 0.98, whereas
the cyt b–12S–16S and HVRI data sets display corre-
lation values of 0.84 and 0.87, respectively. Despite
the global similarities, the dispersion plot showed
in Figure 4 graphically suggests that the HVRI
and Control data sets change at a much higher
rate than do the Complete mtDNA, Coding, and
cyt b–12S–16S data sets.

FIGURE 2. Phylogenetic trees estimated employing the neighbor-joining method and the maximum
composite likelihood distance for each data set.
A similar pattern of differences among the data sets arises from the analyses of site-by-site and haplotype variation (Table 2). The number of segregating sites was relatively large in the Complete mtDNA and Coding data sets, intermediate in the Control and HVRI data sets, and low in Control–cytb and cyt–12S–16S data sets. However, the Control and HVRI data sets display the largest values of proportional segregating sites ($S/N_s$). We observed the same pattern when we calculated the mean number of nucleotide differences between sequences ($k$) and the nucleotide diversity or the proportion of $k$ on $N_s$ (Table 2). When we consider the haplotype analyses, we also observed a pattern similar to the one we detected in the site-by-site analyses: whereas the Complete mtDNA and Coding data sets display the largest number of haplotypes ($h$), the Control and HVRI data sets display the largest values of proportional number of haplotypes ($h/N_s$).

In concordance with the pattern of similarities in sequence variability and rate of substitution, the BSP result displays a clear pattern of differences between data sets. In particular, the noncoding data sets (Control and HVRI) show that the female effective population size was constant from the initial peopling until ca. 7,500 years ago and increased between 7,500 and 4,000 years ago (Figure 5). The Control–cytb data set result displays a similar pattern of demographic change. Conversely, the Complete mtDNA and Coding data sets show more complex demographic dynamics, with an additional increase in the population size ca. 17,000 years BP (Figure 5). These data sets also display a later increase in population size ca. 5,500 years BP, suggesting that the largest data sets present more
information for demographic studies. The results shown that the exclusion of ancient DNA in the BSP analyses does not influence the demographic estimations (Supplementary Figure S2), although they represent more than the 30% of the samples used in the database. Perhaps this may relate to the fact that most of them correspond to the late Holocene (300–700 years BP; Llamas et al. 2016).

Figure 6 displays the results of the informativeness profiles for each data set. The Complete mtDNA and Coding data sets show the largest values of net informativeness in the more distant

| Datasets          | Sample size | N     | N_s  | S     | S/N_s | k     | m     | π     | h     | h/h-max | h/N_s | H     |
|-------------------|-------------|-------|------|-------|-------|-------|-------|-------|-------|---------|--------|-------|
| Complete-mtDNA    | 259         | 16593 | 643  | 0.039 | 32.066| 0.002 | 233.000| 1.000 | 0.014 | 0.999   |
| Coding            | 259         | 15450 | 500  | 0.032 | 23.562| 0.002 | 212.000| 0.910 | 0.014 | 0.998   |
| Control           | 259         | 1144  | 143  | 0.125 | 8.504 | 0.009 | 169.000| 0.725 | 0.148 | 0.994   |
| HVRI              | 259         | 348   | 74   | 0.213 | 4.688 | 0.016 | 108.000| 0.464 | 0.310 | 0.964   |
| Control-cytb      | 259         | 2280  | 198  | 0.087 | 11.641| 0.006 | 189.000| 0.811 | 0.083 | 0.996   |
| cyt-b-12S-16S     | 259         | 3648  | 97   | 0.027 | 5.157 | 0.001 | 90.000 | 0.386 | 0.025 | 0.960   |

N: number of sequences; N_s: number of sites; S: number of segregating sites or number of polymorphic sites; S/N_s: the proportion of S on N_s; k: mean number of nucleotide differences between sequences; m: nucleotide diversity or the proportion of k on N_s; h: number of haplotypes or the different DNA sequences; h/h-max: the proportion of h on the maximum value of h; h/N_s: the proportion of h on the number of sites; H: haplotype diversity.
past, ca. 17,000 years BP, decaying quickly from that time until the present. The Control and Control–cyt b data sets, together with the Complete mtDNA, display the largest informativeness during the last 2,000 years (Figure 6). The pattern of differences among the data sets observed in the informativeness profiles look similar to that observed in BSP results and in the other analyses, suggesting the importance of considering informativeness estimations in reconstructing population dynamics at different times in the past.

Discussion

Previous work in the Old World has shown that phylogenetic trees based on the mtDNA control region are poorly resolved (Non et al. 2007). In the same way, the works performed in America have suggested that data sets such as the mtDNA control region—including the HVRI—have limited utility for studying human population history in the continent (Tamm et al. 2007; Fagundes et al. 2008b; de Saint Pierre et al. 2012b). In particular, these studies have pointed out that the lineage genealogy or phylogeny of the mtDNA variants in America could not be reconstructed using the HVRI or control region, making it difficult to define the founding clades or subhaplogroups that arrived on the continent during the early peopling (Fagundes et al. 2008a; de Saint Pierre et al. 2012b). More generally, it was suggested that the time and mode of the early American peopling cannot be reconstructed with confidence when the HVRI or control region is used because they both display limited information—mainly due to the small size of these sequences—and high frequency of recurrent mutations (Tamm et al. 2007; Non et al. 2007; Fagundes et al. 2008b). Conversely, previous studies pointed out that the complete coding region and the complete mtDNA should be more appropriate for genealogical reconstruction and the study of population history in America (Tamm et al. 2007; Fagundes et al. 2008a, 2008b; de Saint Pierre et al. 2012b). Many of these studies assume that these large sequences are most useful than the noncoding ones because they present changes that are unique and irreversible (but see Non et al. 2007). This assumption is in line with more general discussions in studies of phylogenetic inference about the utility of unique and irreversible changes versus characters that present recurrent states (Townsend 2007; Yang 1998). To the best of our knowledge, although many statistics have been generated to measure the utility of different sequence data in phylogenetic inference, there have been few systematic explorations of this problem in anthropological studies (Non et al. 2007), and there has been no systematic research at small temporal scales, such as the observed in the South American peopling.

Contrary to suggestions of previous studies, our results display a complex scenario, where different mtDNA regions have variable usefulness for the study of different problems. In particular, the results suggest that sequence length is not the relevant dimension to discuss the utility of the different data sets for the study of human population history in South America. We demonstrate that data sets with different sequence lengths display very similar distance matrices and almost identical phylogenetic trees (Table 1, Figures 2 and 3). The Control and Control–cyt b data sets generated trees more similar to the Complete mtDNA data set tree. When we compare the distance matrices, we can observe that the Coding, Control, and Control–cyt b data sets display a pattern of differences among cases similar to that of the Complete mtDNA data set. These results suggest that the control region has sufficient information to reconstruct the lineage
genealogy or phylogeny of the mtDNA variants in South America. This result contrasts with the most general expectation that the length of sequence is important in experimental design (Goldman 1998).

Conversely, the tree and distance matrix obtained with the HVRI data set display important differences with the other data sets. Therefore, we argue that this mtDNA region does not provide sufficient information to reconstruct a reliable phylogenetic tree and define the founding subhaplogroups that peopled South America, as has been previously suggested (Fagundes et al. 2008a; de Saint Pierre et al. 2012b).

However, the problem discussed here is not simple. As we point out above, previous studies also suggested that the high mutation and/or substitution rate of the noncoding HVRI and full control region could be a problem for the study of ancestral population dynamics in America, particularly because of the high frequency of recurrent mutations (Tamm et al. 2007; Fagundes et al. 2008b). Nevertheless, our results suggest that HVRI and the control region display a convenient rate of mutation for studying details of the Holocene demographic change in South America (Figures 4 and 5). In particular, when we analyze these molecular data sets, and when we explore the Control–cytb data set, the BSP analyses show a quick population increase ca. 7,000–6,000 years ago, which is similar to the results shown for South America by previous works employing molecular and archaeological data (Marquet et al. 2012; Goldberg et al. 2016; Perez et al. 2016). The results also show the impact of the European colonization during the last 500 years on the mtDNA molecular variation. Conversely, the Coding and Complete mtDNA data sets show less detail for the dynamics of population increase after ca. 7,000–6,000 years ago. The analyses of these data sets also suggest that this event was more recent, ca. 5,000 years ago, than the interpretation based on the BSP analysis of the noncoding sequences. Although this temporal difference in the time of the Holocene population increase could be related to uncertainties in the estimation of the substitution rate employed (Llamas et al. 2016), the BSP based on Coding and Complete mtDNA data sets shows another important difference: an additional population increase event ca. 19,000 years ago. This population increase has been described previously in DNA studies employing different data (Fagundes et al. 2008a, 2008b; Kitchen et al. 2008; Llamas et al. 2016; Poznik et al. 2016). However, it is important to remark that the estimated mean time of this event varies between 15,000 and 25,000 years ago, depending on the substitution rate employed by each study (Llamas et al. 2016).

All the results obtained in this work make sense if we consider the time scale of South American peopling and the values of sequence informativeness. The informativeness profiles show that only three data sets, Complete mtDNA, Control, and Control–cytb, display relatively high values of net informativeness during the last 2,000 years (Figure 6). In particular, the Control and Control–cytb data sets display relatively high values of informativeness mainly in times close to the present, whereas the Complete mtDNA data displays high values in this period and during the initial divergence time ca. 17,000 years ago. The Coding data set also displays high values of informativeness for the earliest times analyzed. Both the Coding and Complete mtDNA data sets are very useful to study relatively ancient processes and events in America, but this large quantity of information about ancient events could cause problems in the estimation of more recent events, as suggested by our BSP results (Figure 5). These results, together with our other results, do not support the opinion that the mtDNA control region presents limited information for genealogical reconstruction and for the study of population history in America (Tamm et al. 2007; Fagundes et al. 2008a, 2008b). Conversely, the HVRI and the cyt–12S–16S data sets show low values of informativeness over time, corroborating its previously hypothesized limited value for the American human population studies. Our results also suggest that mtDNA regions widely used previously to explore phylogenetic relationships among closely related species or subspecies, such as cyt, 12S, and 16S, do not display sufficient information to investigate the population processes in the time frame of South America human evolution, or in similar problems such as the peopling of the entire America, probably related to the fact that a relatively small number of mutations can be observed in this mtDNA region for our data set (Table 2, Figure 6).

In summary, we demonstrate the complex behavior of the different mtDNA data sets used in previous studies. They have different degrees
of information to reconstruct phylogenetic trees with reliability and infer without error the founding monophyletic clades or subhaplogroups of America. Our results indicate that HVRI is not suitable for phylogenetic reconstruction of ancient clades because this mtDNA region does not have sufficient information of events that occurred in the distant past, that is, during the Pleistocene. Moreover, the HVRI and Control data sets display information for the demographic reconstruction during the Holocene period, where the high rate of mutation seems to be a valuable characteristic. Conversely, the Complete mtDNA, Coding, Control, and Control–cyt b data sets display sufficient information for phylogenetic reconstruction in this geographic region during the time span of the human peopling. As expected, the complete mtDNA and coding region, displaying slower rates of mutation, present better information for the reconstruction of ancient demographic events.

**Final Remarks**

During the last decades it became obvious that inference of evolutionary patterns from molecular sequences is a statistical problem and requires the use of experimental design (Goldman 1998; Townsend 2007; Yang and Rannala 2012). Nevertheless, human population studies have paid little attention to this problem. Previous studies have followed the “empirical folklore” and a few world-scale studies (Non et al. 2007), such as occurred decades ago in the interspecies works (Goldman 1998), about the best molecular sequences to use and the sampling of individuals for population analyses. They have not systematically employed formal methods to explore the utility of different sequences or to sample individuals to test different population problems. Our results indicate the importance of evaluating the utility of different DNA regions to address different questions and problems in human population studies, mainly considering the time scale of the phenomenon and the informativeness of the molecular region in a particular geographical area.

In this study, we explored the utility of sequences for the reconstruction of human population history. However, the discussions about experimental design in molecular studies go beyond sequence informativeness (Goldman 1998; Graybeal 1998; Geuten et al. 2007). Therefore, future studies are needed to explore the impact of the use of multiple sequences and the sampling of individuals from different times and geographical areas in the reconstruction of human population history. This is particularly relevant if we consider that the mtDNA databases for regions such as HVRI or the control region are considerably larger than the sample of complete mtDNA genomes.

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SUPPLEMENTARY FIGURE S1. Nonmetric multidimensional scaling (nmMDS) and unweighted pair group method with arithmetic mean (UPGMA) cluster analyses of topological differences among the phylogenetic trees for all American sequences. The analyses used the Robinson-Foulds (RF) distance to estimate the similarity between the data sets.

SUPPLEMENTARY FIGURE S2. Demographic changes in South America estimated based on the modern molecular sequences of the six data sets studied. The times are scaled.
Supplementary Table S1. Data Set of 259 Complete Mitochondrial DNA (mtDNA) Sequences from Modern and Ancient Humans of South America (South-Central Andes, \( n = 123 \); Northeast, \( n = 17 \); Pampa-Patagonia, \( n = 119 \)) Used in This Study

| Genebank Accession # | This Study ID | Haplogroup/Haplotype | Geographic Region       | Paper                      |
|----------------------|---------------|----------------------|-------------------------|----------------------------|
| JN253392.1           | 1.D1          | D1                   | Argentina, Río Negro    | Bodner et al. 2012         |
| JN253393.1           | 2.D1g         | D1g                  | Chile, Los Lagos        | Bodner et al. 2012         |
| JN253394.1           | 3.D1g1a       | D1g1a                | Argentina, Buenos Aires | Bodner et al. 2012         |
| JN253395.1           | 4.D1g1a       | D1g1a                | Argentina, Río Negro    | Bodner et al. 2012         |
| JN253396.1           | 5.D1g1a       | D1g1a                | Argentina, Río Negro    | Bodner et al. 2012         |
| JN253397.1           | 6.D1g1a       | D1g1a                | Argentina, Río Negro    | Bodner et al. 2012         |
| JN253398.1           | 7.D1g1b       | D1g1b                | Chile, Tarapacá         | Bodner et al. 2012         |
| JN253399.1           | 8.D1g1b       | D1g1b                | Argentina, Chubut       | Bodner et al. 2012         |
| JN253400.1           | 9.D1g1b       | D1g1b                | Argentina, Río Negro    | Bodner et al. 2012         |
| JN253401.1           | 10.D1g2       | D1g2                 | Chile, Biobio           | Bodner et al. 2012         |
| JN253402.1           | 11.D1g2       | D1g2                 | Argentina, Buenos Aires | Bodner et al. 2012         |
| JN253403.1           | 12.D1g2       | D1g2                 | Argentina, Río Negro    | Bodner et al. 2012         |
| JN253404.1           | 13.D1g2a      | D1g2a                | Argentina, Río Negro    | Bodner et al. 2012         |
| JN253405.1           | 14.D1g2a      | D1g2a                | Argentina, Río Negro    | Bodner et al. 2012         |
| JN253406.1           | 15.D1g2a      | D1g2a                | Argentina, Río Negro    | Bodner et al. 2012         |
| JN253407.1           | 16.D1g3       | D1g3                 | Chile, Maule            | Bodner et al. 2012         |
| JN253408.1           | 17.D1g3       | D1g3                 | Chile, Valparaiso       | Bodner et al. 2012         |
| JN253409.1           | 18.D1g3       | D1g3                 | Argentina, Río Negro    | Bodner et al. 2012         |
| JN253410.1           | 19.D1g4a      | D1g4a                | Chile, Valparaiso       | Bodner et al. 2012         |
| JN253411.1           | 20.D1g4a      | D1g4a                | Argentina, Río Negro    | Bodner et al. 2012         |
| JN253412.1           | 21.D1g4       | D1g4                 | Chile, Atacama          | Bodner et al. 2012         |
| JN253413.1           | 22.D1g5       | D1g5                 | Chile, Aisen            | Bodner et al. 2012         |
| JN253414.1           | 23.D1g5       | D1g5                 | Argentina, Buenos Aires | Bodner et al. 2012         |
| JN253415.1           | 24.D1g5       | D1g5                 | Argentina, Río Negro    | Bodner et al. 2012         |
| JN253416.1           | 25.D1g5       | D1g5                 | Argentina, Río Negro    | Bodner et al. 2012         |
| JN253417.1           | 26.D1g6       | D1g6                 | Chile, Araucania        | Bodner et al. 2012         |
| JN253418.1           | 27.D1g6       | D1g6                 | Argentina, Neuquén      | Bodner et al. 2012         |
| JN253419.1           | 28.D1j        | D1j                  | Chile, Biobio           | Bodner et al. 2012         |
| JN253420.1           | 29.D1j        | D1j                  | Brazil, Río Grande do Sul | Bodner et al. 2012         |
| JN253421.1           | 30.D1j1       | D1j1                 | Argentina, Buenos Aires | Bodner et al. 2012         |
| JN253422.1           | 31.D1j1       | D1j1                 | Argentina, Catamarca    | Bodner et al. 2012         |
| JN253423.1           | 32.D1j1a      | D1j1a                | Argentina, Córdoba      | Bodner et al. 2012         |
| JN253424.1           | 33.D1j1a      | D1j1a                | Chile, Maule            | Bodner et al. 2012         |
| JN253425.1           | 34.D1j1a      | D1j1a                | Chile, Valparaiso       | Bodner et al. 2012         |
| JN253426.1           | 35.D1j1a      | D1j1a                | Brazil, São Paulo       | Bodner et al. 2012         |
| JN253427.1           | 36.D1j1a      | D1j1a                | Argentina, Catamarca    | Bodner et al. 2012         |
| JN253428.1           | 37.D1j1a      | D1j1a                | Argentina, Catamarca    | Bodner et al. 2012         |
| JN253429.1           | 38.D1j1a1     | D1j1a1               | Argentina, Tucumán      | Bodner et al. 2012         |
| JN253430.1           | 39.D1j1a1     | D1j1a1               | Argentina, Buenos Aires | Bodner et al. 2012         |
| JN253431.1           | 40.D1j1a1     | D1j1a1               | Argentina, Buenos Aires | Bodner et al. 2012         |
| JN253432.1           | 41.D1j1a1     | D1j1a1               | Argentina, Buenos Aires | Bodner et al. 2012         |
| JN253433.1           | 42.D1j1a1     | D1j1a1               | Argentina, Salta        | Bodner et al. 2012         |
| JN253434.1           | 43.D1j1a2     | D1j1a2               | Argentina, Buenos Aires | Bodner et al. 2012         |
| JN253435.1           | 44.D1j1a2     | D1j1a2               | Argentina, Corrientes   | Bodner et al. 2012         |
| JN253391.1           | 45.D1        | D1                   | Argentina, Buenos Aires | Bodner et al. 2012         |
| JX413011.1           | 46.B2i2a      | B2i2a                | Argentina, Río Negro    | de Saint Pierre et al. 2012 |
| JX413012.1           | 47.B2i2a      | B2i2a                | Argentina, Neuquén      | de Saint Pierre et al. 2012 |
| JX413013.1           | 48.B2i2a      | B2i2a                | Argentina, Neuquén      | de Saint Pierre et al. 2012 |
| JX413014.1           | 49.B2i2a      | B2i2a                | Chile, Isla de Chiloé   | de Saint Pierre et al. 2012 |
| JX413015.1           | 50.B2i2a      | B2i2a                | Chile, Isla de Chiloé   | de Saint Pierre et al. 2012 |
| Genebank Accession # | This Study ID | Haplogroup/Haplotype | Geographic Region | Paper |
|----------------------|--------------|----------------------|------------------|-------|
| JX413016.1           | 51.B2i2a     | B2i2a                | Chile, Isla de Chiloé | de Saint Pierre et al. 2012 |
| JX413017.1           | 52.B2i2a     | B2i2a                | Chile, Aconcagua   | de Saint Pierre et al. 2012 |
| JX413018.1           | 53.B2i2a     | B2i2a                | Chile             | de Saint Pierre et al. 2012 |
| JX413019.1           | 54.B2i2a     | B2i2a                | Argentina, Río Negro | de Saint Pierre et al. 2012 |
| JX413020.1           | 55.B2i2a     | B2i2a                | Chile             | de Saint Pierre et al. 2012 |
| JX413021.1           | 56.B2i2a     | B2i2a                | Chile, Aconcagua   | de Saint Pierre et al. 2012 |
| JX413022.1           | 57.B2i2a     | B2i2a                | Chile             | de Saint Pierre et al. 2012 |
| JX413023.1           | 58.B2i2a     | B2i2a                | Chile, Aconcagua   | de Saint Pierre et al. 2012 |
| JX413024.1           | 59.B2i2b     | B2i2b                | Chile, Aconcagua   | de Saint Pierre et al. 2012 |
| JX413025.1           | 60.B2i2b     | B2i2b                | Chile, Aconcagua   | de Saint Pierre et al. 2012 |
| JX413026.1           | 61.B2i2b     | B2i2b                | Chile             | de Saint Pierre et al. 2012 |
| JX413027.1           | 62.B2i2b     | B2i2b                | Chile, Valparaíso  | de Saint Pierre et al. 2012 |
| JX413028.1           | 63.B2i2b     | B2i2b                | Chile             | de Saint Pierre et al. 2012 |
| JX413029.1           | 64.B2i2b     | B2i2b                | Chile, Aconcagua   | de Saint Pierre et al. 2012 |
| JX413030.1           | 65.B2i2b     | B2i2b                | Chile, Isla de Chiloé | de Saint Pierre et al. 2012 |
| JX413031.1           | 66.B2i2b     | B2i2b                | Argentina, Río Negro | de Saint Pierre et al. 2012 |
| JX413032.1           | 67.B2i2b     | B2i2b                | Chile, Aconcagua   | de Saint Pierre et al. 2012 |
| JX413033.1           | 68.B2i2b     | B2i2b                | Chile, Aconcagua   | de Saint Pierre et al. 2012 |
| JX413034.1           | 69.B2i2b     | B2i2b                | Chile, San Juan de la Costa | de Saint Pierre et al. 2012 |
| JX413035.1           | 70.B2i2b     | B2i2b                | Chile, Santiago    | de Saint Pierre et al. 2012 |
| JX413036.1           | 71.C1b13a    | C1b13a               | Chile, San Juan de la Costa | de Saint Pierre et al. 2012 |
| JX413037.1           | 72.C1b13a    | C1b13a               | Chile, Aconcagua   | de Saint Pierre et al. 2012 |
| JX413038.1           | 73.C1b13a    | C1b13a               | Chile, Aconcagua   | de Saint Pierre et al. 2012 |
| JX413039.1           | 74.C1b13a    | C1b13a               | Chile             | de Saint Pierre et al. 2012 |
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| JX413042.1           | 77.C1b13b    | C1b13b               | Chile, Isla de Chiloé | de Saint Pierre et al. 2012 |
| JX413043.1           | 78.C1b13b    | C1b13b               | Chile, Talagante   | de Saint Pierre et al. 2012 |
| JX413044.1           | 79.C1b13c    | C1b13c               | Argentina, Neuquén | de Saint Pierre et al. 2012 |
| JX413045.1           | 80.C1b13c    | C1b13c               | Chile, Isla de Chiloé | de Saint Pierre et al. 2012 |
| JX413046.1           | 81.C1b13c    | C1b13c               | Chile             | de Saint Pierre et al. 2012 |
| JX413047.1           | 82.C1b13c    | C1b13c               | Chile             | de Saint Pierre et al. 2012 |
| JX413048.1           | 83.C1b13c    | C1b13c               | Chile, Trapa Trapa | de Saint Pierre et al. 2012 |
| JX413049.1           | 84.C1b13d    | C1b13d               | Chile, Aconcagua   | de Saint Pierre et al. 2012 |
| JX413050.1           | 85.C1b13d    | C1b13d               | Chile             | de Saint Pierre et al. 2012 |
| JX413051.1           | 86.C1b13e    | C1b13e               | Chile, Isla de Chiloé | de Saint Pierre et al. 2012 |
| JX413052.1           | 87.C1b13e    | C1b13e               | Chile             | de Saint Pierre et al. 2012 |
| JX413053.1           | 88.C1b13e    | C1b13e               | Chile, Isla de Chiloé | de Saint Pierre et al. 2012 |
| JX413054.1           | 89.C1b13e    | C1b13e               | Chile, Aconcagua   | de Saint Pierre et al. 2012 |
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| EU597534.1           | 95.B2        | B2                   | Andes              | Hartmann et al. 2009 |
| EU597546.1           | 96.D4h       | D4h                  | Brazil, Guyana, Venezuela | Hartmann et al. 2009 |
| EU597569.1           | 97.B2        | B2                   | Colombia           | Hartmann et al. 2009 |
| EU597580.1           | 98.B2        | B2                   | Colombia           | Hartmann et al. 2009 |
| FJ168713.1           | 99.D4h3a1    | D4h3a1               | Chile, Atacama     | Peregó et al. 2009 |
| FJ168714.1           | 100.D4h3a1   | D4h3a1               | Chile, Coquimbo    | Peregó et al. 2009 |
| FJ168715.1           | 101.D4h3a1   | D4h3a1               | Chile, O’Higgins   | Peregó et al. 2009 |
| FJ168716.1           | 102.D4h3a1   | D4h3a1               | Chile, Biobio      | Peregó et al. 2009 |
| FJ168717.1           | 103.D4h3a1   | D4h3a1               | Chile, Biobio      | Peregó et al. 2009 |
| FJ168718.1           | 104.D4h3a1   | D4h3a1               | Chile, Coquimbo    | Peregó et al. 2009 |
| Genebank Accession # | This Study ID | Haplogroup/Haplotype | Geographic Region     | Paper                        |
|----------------------|--------------|----------------------|-----------------------|------------------------------|
| FJ168719.1           | 105.D4h3a1   | D4h3a1               | Chile, Talcahuano     | Perego et al. 2009           |
| FJ168720.1           | 106.D4h3a1   | D4h3a1               | Chile, Santiago       | Perego et al. 2009           |
| FJ168721.1           | 107.D4h3a1   | D4h3a1               | Chile, Los Lagos      | Perego et al. 2009           |
| FJ168722.1           | 108.D4h3a1   | D4h3a1               | Chile, Biobio         | Perego et al. 2009           |
| FJ168723.1           | 109.D4h3a1   | D4h3a1               | Chile, Biobio         | Perego et al. 2009           |
| FJ168724.1           | 110.D4h3a2   | D4h3a2               | Chile, Biobio         | Perego et al. 2009           |
| FJ168725.1           | 111.D4h3a2   | D4h3a2               | Chile, Biobio         | Perego et al. 2009           |
| FJ168726.1           | 112.D4h3a2   | D4h3a2               | Chile, Coquimbo       | Perego et al. 2009           |
| FJ168727.1           | 113.D4h3a2   | D4h3a2               | Chile, Biobio         | Perego et al. 2009           |
| FJ168728.1           | 114.D4h3a3   | D4h3a3               | Chile, Tarapacá       | Perego et al. 2009           |
| FJ168735.1           | 115.D4h3a4   | D4h3a4               | Perú, Suyu            | Perego et al. 2009           |
| FJ168736.1           | 116.D4h3a4   | D4h3a4               | Perú, Arequipa        | Perego et al. 2009           |
| FJ168737.1           | 117.D4h3a4   | D4h3a4               | Perú, Arequipa        | Perego et al. 2009           |
| FJ168738.1           | 118.D4h3a5   | D4h3a5               | Perú, La Libertad     | Perego et al. 2009           |
| FJ168739.1           | 119.D4h3a5   | D4h3a5               | Chile, Maule          | Perego et al. 2009           |
| FJ168740.1           | 120.D4h3a5   | D4h3a5               | Chile, Los Lagos      | Perego et al. 2009           |
| FJ168741.1           | 121.D4h3a5   | D4h3a5               | Chile, Santiago       | Perego et al. 2009           |
| FJ168743.1           | 122.D4h3a3   | D4h3a3               | Perú, Arequipa        | Perego et al. 2009           |
| FJ168744.1           | 123.D4h3a   | D4h3a                | Perú, Arequipa        | Perego et al. 2009           |
| FJ168747.1           | 124.D4h3a   | D4h3a                | Perú, Loreto          | Perego et al. 2009           |
| FJ168748.1           | 125.D4h3a   | D4h3a                | Perú, Loreto          | Perego et al. 2009           |
| FJ168749.1           | 126.D4h3a   | D4h3a                | Perú, Piura           | Perego et al. 2009           |
| FJ168750.1           | 127.D4h3a   | D4h3a                | Bolivia, La Paz       | Perego et al. 2009           |
| FJ168751.1           | 128.D4h3a   | D4h3a                | Perú, Apurimac        | Perego et al. 2009           |
| FJ168752.1           | 129.D4h3a   | D4h3a                | Perú, Ancash          | Perego et al. 2009           |
| FJ168754.1           | 130.D4h3a   | D4h3a                | Brazil, Maranhão      | Perego et al. 2009           |
| HM107309.1           | 131.C1d     | C1d                  | Argentina, Salta      | Perego et al. 2010           |
| HM107310.1           | 132.C1d     | C1d                  | Argentina, Salta      | Perego et al. 2010           |
| HM107311.1           | 133.C1d2a   | C1d2a                | Colombia              | Perego et al. 2010           |
| HM107312.1           | 134.C1d2a   | C1d2a                | Colombia              | Perego et al. 2010           |
| HM107313.1           | 135.C1d2a   | C1d2a                | Colombia              | Perego et al. 2010           |
| HM107314.1           | 136.C1d2a   | C1d2a                | Colombia              | Perego et al. 2010           |
| HM107315.1           | 137.C1d2    | C1d2                 | Colombia              | Perego et al. 2010           |
| HM107316.1           | 138.C1d     | C1d                  | Argentina, Buenos Aires| Perego et al. 2010          |
| HM107317.1           | 139.C1d     | C1d                  | Colombia              | Perego et al. 2010           |
| HM107323.1           | 140.C1d1b   | C1d1b                | Argentina, Salta      | Perego et al. 2010           |
| HM107324.1           | 141.C1d1b   | C1d1b                | Argentina, Catamarca  | Perego et al. 2010           |
| HM107325.1           | 142.C1d1b   | C1d1b                | Argentina, Salta      | Perego et al. 2010           |
| HM107326.1           | 143.C1d1b   | C1d1b                | Argentina, Catamarca  | Perego et al. 2010           |
| HM107327.1           | 144.C1d1b   | C1d1b                | Argentina, Buenos Aires| Perego et al. 2010          |
| HM107328.1           | 145.C1d1b   | C1d1b                | Argentina, Río Negro  | Perego et al. 2010           |
| HM107329.1           | 146.C1d1b   | C1d1b                | Argentina, Buenos Aires| Perego et al. 2010          |
| HM107330.1           | 147.C1d1b   | C1d1b                | Argentina, Corrientes | Perego et al. 2010           |
| HM107331.1           | 148.C1d1b   | C1d1b                | Uruguay, Flores       | Perego et al. 2010           |
| HM107332.1           | 149.C1d1b   | C1d1b                | Argentina, Buenos Aires| Perego et al. 2010          |
| HM107333.1           | 150.C1d1b   | C1d1b                | Argentina, Salta      | Perego et al. 2010           |
| HM107338.1           | 151.C1d     | C1d                  | Brazil, Río Grande do Sul| Perego et al. 2010        |
| HM107339.1           | 152.C1d     | C1d                  | Perú, Lima            | Perego et al. 2010           |
| HM107340.1           | 153.C1d     | C1d                  | Argentina, Buenos Aires| Perego et al. 2010          |
| HM107341.1           | 154.C1d     | C1d                  | Perú, Loreto          | Perego et al. 2010           |
| HM107342.1           | 155.C1d     | C1d                  | Perú, Loreto          | Perego et al. 2010           |
| HM107343.1           | 156.C1d     | C1d                  | Ecuador, Imbabura     | Perego et al. 2010           |
| HM107344.1           | 157.C1d     | C1d                  | Colombia              | Perego et al. 2010           |
| HM107345.1           | 158.C1d     | C1d                  | Colombia              | Perego et al. 2010           |
| Genebank Accession # | This Study ID | Haplogroup/Haplotype | Geographic Region | Paper |
|----------------------|--------------|----------------------|-------------------|-------|
| HM107346.1           | 159.C1d1d    | C1d1d                | Argentina, Buenos Aires | Perego et al. 2010 |
| HM107347.1           | 160.C1d1d    | C1d1d                | Brazil, Rio Grande do Sul | Perego et al. 2010 |
| HM107348.1           | 161.C1d1d    | C1d1d                | Uruguay           | Perego et al. 2010 |
| HM107349.1           | 162.C1d1     | C1d1                 | Brazil, Minas Gerais | Perego et al. 2010 |
| HM107350.1           | 163.C1d1e    | C1d1e                | Chile, Biobio      | Perego et al. 2010 |
| HM107351.1           | 164.C1d1e    | C1d1e                | Argentina, Rio Negro | Perego et al. 2010 |
| HM107352.1           | 165.C1d1     | C1d1                 | Perú, Cajamarca    | Perego et al. 2010 |
| HM107353.1           | 166.C1d1     | C1d1                 | Perú, Huanucu      | Perego et al. 2010 |
| HM107354.1           | 167.C1d1     | C1d1                 | Perú, Puca Puca    | Perego et al. 2010 |
| HM107355.1           | 168.C1d1     | C1d1                 | Argentina, Buenos Aires | Perego et al. 2010 |
| HM107356.1           | 169.C1d1     | C1d1                 | Brazil, Mato grosso do Sul | Perego et al. 2010 |
| HM107357.1           | 170.C1d1     | C1d1                 | Paraguay          | Perego et al. 2010 |
| HM107358.1           | 171.C1d1     | C1d1                 | Argentina, Salta   | Perego et al. 2010 |
| HM107359.1           | 172.C1d1     | C1d1                 | Perú, Piura       | Perego et al. 2010 |
| HM107360.1           | 173.C1d1     | C1d1                 | Perú, Huancavelica | Perego et al. 2010 |
| HM107361.1           | 174.C1d1     | C1d1                 | Argentina, Corrientes | Perego et al. 2010 |
| HM107362.1           | 175.C1d1     | C1d1                 | Chile, Los Lagos  | Perego et al. 2010 |
| HM107363.1           | 176.C1d1     | C1d1                 | Chile, Los Lagos  | Perego et al. 2010 |
| EU095222.1           | 177.C1d1     | C1d1                 | Brazil           | Perego et al. 2010 |
| KU523264.1           | 178.A2       | A2                   | Perú, Wari       | Llamas et al. 2016 |
| KU523265.1           | 179.A2       | A2                   | Perú, Wari       | Llamas et al. 2016 |
| KU523266.1           | 180.A2       | A2                   | Perú, Lima       | Llamas et al. 2016 |
| KU523267.1           | 181.A2       | A2                   | Perú            | Llamas et al. 2016 |
| KU523268.1           | 182.A2       | A2                   | Perú, Tiwanaku   | Llamas et al. 2016 |
| KU523269.1           | 183.A2       | A2                   | Argentina, Arroyo Seco | Llamas et al. 2016 |
| KU523270.1           | 184.A2       | A2                   | Chile           | Llamas et al. 2016 |
| KU523271.1           | 185.A2       | A2                   | Perú            | Llamas et al. 2016 |
| KU523272.1           | 186.A2       | A2                   | Chile           | Llamas et al. 2016 |
| KU523273.1           | 187.A2       | A2                   | Chile           | Llamas et al. 2016 |
| KU523274.1           | 188.A2       | A2                   | Perú            | Llamas et al. 2016 |
| KU523275.1           | 189.B2b      | B2b                  | Perú            | Llamas et al. 2016 |
| KU523276.1           | 190.B2       | B2                   | Perú            | Llamas et al. 2016 |
| KU523277.1           | 191.B2b      | B2b                  | Perú            | Llamas et al. 2016 |
| KU523278.1           | 192.B2b      | B2b                  | Perú            | Llamas et al. 2016 |
| KU523279.1           | 193.B2       | B2                   | Perú            | Llamas et al. 2016 |
| KU523280.1           | 194.B2b      | B2b                  | Perú, Wari      | Llamas et al. 2016 |
| KU523281.1           | 195.B2b      | B2b                  | Perú, Wari      | Llamas et al. 2016 |
| KU523282.1           | 196.B2b      | B2b                  | Perú, Wari      | Llamas et al. 2016 |
| KU523283.1           | 197.B2b      | B2b                  | Perú, Lima      | Llamas et al. 2016 |
| KU523284.1           | 198.B2       | B2                   | Perú            | Llamas et al. 2016 |
| KU523285.1           | 199.B2b      | B2b                  | Perú            | Llamas et al. 2016 |
| KU523286.1           | 200.B2       | B2                   | Perú, Lima      | Llamas et al. 2016 |
| KU523287.1           | 201.B2       | B2                   | Perú, Lima      | Llamas et al. 2016 |
| KU523288.1           | 202.B2       | B2                   | Perú, Lima      | Llamas et al. 2016 |
| KU523289.1           | 203.B2       | B2                   | Chile           | Llamas et al. 2016 |
| KU523290.1           | 204.B2       | B2                   | Chile           | Llamas et al. 2016 |
| KU523291.1           | 205.B2       | B2                   | Chile           | Llamas et al. 2016 |
| KU523292.1           | 206.B2       | B2                   | Perú            | Llamas et al. 2016 |
| KU523293.1           | 207.B2       | B2                   | Perú            | Llamas et al. 2016 |
| KU523294.1           | 208.B2       | B2                   | Perú            | Llamas et al. 2016 |
| KU523295.1           | 209.B2b      | B2b                  | Perú            | Llamas et al. 2016 |
| KU523296.1           | 210.B2b      | B2b                  | Perú            | Llamas et al. 2016 |
| KU523297.1           | 211.B2       | B2                   | Perú            | Llamas et al. 2016 |
| KU523298.1           | 212.B2b      | B2b                  | Perú            | Llamas et al. 2016 |
| Genebank Accession # | This Study ID | Haplogroup/Haplotype | Geographic Region | Paper               |
|----------------------|---------------|----------------------|-------------------|---------------------|
| KU523299.1           | 213.B2        | B2                   | Bolivia           | Llamas et al. 2016  |
| KU523300.1           | 214.B2        | B2                   | Bolivia           | Llamas et al. 2016  |
| KU523301.1           | 215.B2        | B2                   | Bolivia           | Llamas et al. 2016  |
| KU523302.1           | 216.B2        | B2                   | Bolivia           | Llamas et al. 2016  |
| KU523303.1           | 217.B2        | B2                   | Bolivia           | Llamas et al. 2016  |
| KU523308.1           | 218.B2        | B2                   | Perú              | Llamas et al. 2016  |
| KU523309.1           | 219.B2        | B2                   | Perú              | Llamas et al. 2016  |
| KU523310.1           | 220.B2        | B2                   | Perú              | Llamas et al. 2016  |
| KU523311.1           | 221.B2        | B2                   | Perú              | Llamas et al. 2016  |
| KU523312.1           | 222.C1b       | C1b                  | Argentina, Llullaillaco | Llamas et al. 2016 |
| KU523313.1           | 223.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523314.1           | 224.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523315.1           | 225.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523316.1           | 226.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523317.1           | 227.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523318.1           | 228.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523319.1           | 229.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523320.1           | 230.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523321.1           | 231.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523322.1           | 232.C1c       | C1c                  | Perú              | Llamas et al. 2016  |
| KU523323.1           | 233.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523324.1           | 234.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523325.1           | 235.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523326.1           | 236.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523327.1           | 237.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523328.1           | 238.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523329.1           | 239.C1c       | C1c                  | Perú              | Llamas et al. 2016  |
| KU523330.1           | 240.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523331.1           | 241.C1c       | C1c                  | Perú              | Llamas et al. 2016  |
| KU523332.1           | 242.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523333.1           | 243.C1b       | C1b                  | Perú              | Llamas et al. 2016  |
| KU523334.1           | 244.C1c       | C1c                  | Perú              | Llamas et al. 2016  |
| KU523335.1           | 245.C1c       | C1c                  | Bolivia           | Llamas et al. 2016  |
| KU523336.1           | 246.C1       | C1                   | Bolivia           | Llamas et al. 2016  |
| KU523337.1           | 247.C1b       | C1b                  | Bolivia           | Llamas et al. 2016  |
| KU523339.1           | 248.C1d       | C1d                  | Perú              | Llamas et al. 2016  |
| KU523340.1           | 249.C1d       | C1d                  | Perú              | Llamas et al. 2016  |
| KU523341.1           | 250.D1        | D1                   | Argentina, Llullaillaco | Llamas et al. 2016 |
| KU523342.1           | 251.D1        | D1                   | Perú              | Llamas et al. 2016  |
| KU523343.1           | 252.D         | D                    | Perú              | Llamas et al. 2016  |
| KU523344.1           | 253.D1        | D1                   | Perú              | Llamas et al. 2016  |
| KU523345.1           | 254.D         | D                    | Perú              | Llamas et al. 2016  |
| KU523346.1           | 255.D1        | D1                   | Perú              | Llamas et al. 2016  |
| KU523347.1           | 256.D1        | D1                   | Perú              | Llamas et al. 2016  |
| KU523348.1           | 257.D1        | D1                   | Perú              | Llamas et al. 2016  |
| KU523349.1           | 258.D1        | D1                   | Argentina, Arroyo Seco | Llamas et al. 2016 |
| KU523350.1           | 259.D1        | D1                   | Perú              | Llamas et al. 2016  |
### Supplementary Table S2. Data Set of 174 Complete Mitochondrial DNA (mtDNA) Sequences from Modern Humans of North America Used in This Study

| Genbank Accession # | Haplogroup/Haplotype | Geographic Region | Paper          |
|---------------------|----------------------|-------------------|----------------|
| KC710999            | A2a                  | Alaska            | Achilli et al. 2013 |
| KC711000            | A2a3                 | Greenland         | Achilli et al. 2013 |
| KC711001            | A2a4                 | New Mexico        | Achilli et al. 2013 |
| KC711002            | A2a4                 | New Mexico        | Achilli et al. 2013 |
| KC711003            | A2a4                 | Arizona           | Achilli et al. 2013 |
| KC711004            | A2a4                 | New Mexico        | Achilli et al. 2013 |
| KC711005            | A2a4                 | Chihuahua, Mexico | Achilli et al. 2013 |
| KC711006            | A2a4                 | Chihuahua, Mexico | Achilli et al. 2013 |
| KC711007            | A2a4                 | Arizona/ New Mexico | Achilli et al. 2013 |
| KC711008            | A2a                  | Northwest Canada  | Achilli et al. 2013 |
| KC711009            | A2a5                 | New Mexico        | Achilli et al. 2013 |
| KC711010            | A2a5                 | Texas             | Achilli et al. 2013 |
| KC711011            | A2a5                 | California        | Achilli et al. 2013 |
| KC711012            | A2a5                 | Arizona           | Achilli et al. 2013 |
| KC711013            | A2a5                 | Arizona           | Achilli et al. 2013 |
| KC711014            | A2a5                 | California        | Achilli et al. 2013 |
| KC711015            | A2a5                 | Arizona           | Achilli et al. 2013 |
| KC711016            | A2a5                 | New Mexico        | Achilli et al. 2013 |
| KC711017            | A2a5                 | Canada            | Achilli et al. 2013 |
| KC711018            | A2a5                 | Northwest Canada  | Achilli et al. 2013 |
| KC711019            | A2a5                 | New Mexico        | Achilli et al. 2013 |
| KC711020            | A2a5                 | New Mexico        | Achilli et al. 2013 |
| KC711021            | B2a                  | Northwest Canada  | Achilli et al. 2013 |
| KC711022            | B2a                  | Chihuahua, Mexico | Achilli et al. 2013 |
| KC711023            | B2a1a                | Mexico            | Achilli et al. 2013 |
| KC711024            | B2a1a                | Chihuahua, Mexico | Achilli et al. 2013 |
| KC711025            | B2a1b                | Chihuahua, Mexico | Achilli et al. 2013 |
| KC711026            | B2a1                 | New Mexico        | Achilli et al. 2013 |
| KC711027            | B2a1                 | Mexico            | Achilli et al. 2013 |
| KC711028            | B2a2                 | New Mexico        | Achilli et al. 2013 |
| KC711029            | B2a2                 | Colorado          | Achilli et al. 2013 |
| KC711030            | B2a2                 | Colorado          | Achilli et al. 2013 |
| KC711031            | B2a3                 | Chihuahua, Mexico | Achilli et al. 2013 |
| KC711032            | B2a3                 | Durango, Mexico   | Achilli et al. 2013 |
| KC711033            | B2a4a                | Sinaloa, Mexico   | Achilli et al. 2013 |
| KC711034            | B2a4a1               | Chihuahua, Mexico | Achilli et al. 2013 |
| KC711035            | B2a4a1               | Jalisco, Mexico   | Achilli et al. 2013 |
| KC711036            | B2a4a1               | Durango, Mexico   | Achilli et al. 2013 |
| KC711037            | B2a5                 | Arizona           | Achilli et al. 2013 |
| KC711038            | B2a5                 | Utah              | Achilli et al. 2013 |
| KC711039            | B2a5                 | Arizona           | Achilli et al. 2013 |
| EU597533            | C1c                  | Pima, Mexico      | Hartmann et al. 2008 |
| EU597545            | C1b                  | Pima, Mexico      | Hartmann et al. 2008 |
| EU597557            | C1b                  | Pima, Mexico      | Hartmann et al. 2008 |
| FJ168729            | D4h3a3               | Nuevo Leon, Mexico| Perego et al. 2009 |
| FJ168730            | D4h3a3               | California, United States | Perego et al. 2009 |
| FJ168731            | D4h3a3               | Chihuahua, Mexico | Perego et al. 2009 |
| FJ168732            | D4h3a3               | Chihuahua, Mexico | Perego et al. 2009 |
| FJ168733            | D4h3a3               | Chihuahua, Mexico | Perego et al. 2009 |
| FJ168734            | D4h3a3               | Tarahumara, Mexico| Perego et al. 2009 |
| FJ168742            | D4h3a                | Veracruz, Mexico  | Perego et al. 2009 |
| Genbank Accession # | Haplogroup/Haplotype | Geographic Region       | Paper                  |
|---------------------|-----------------------|-------------------------|------------------------|
| FJ168745            | D4h3a                 | Sonora, Mexico          | Perego et al. 2009     |
| FJ168746            | D4h3a                 | Chihuahua, Mexico       | Perego et al. 2009     |
| FJ168753            | D4h3a                 | San Luis Potosi, Mexico | Perego et al. 2009     |
| FJ168755            | D4h3a                 | California, United States| Perego et al. 2009    |
| HM107306            | C1d                   | Tamaulipas, Mexico      | Perego et al. 2010     |
| HM107307            | C1d                   | Guanajuato, Mexico      | Perego et al. 2010     |
| HM107308            | C1d                   | Chihuahua, Mexico       | Perego et al. 2010     |
| HM107318            | C1d1a1                | Oklahoma, United States | Perego et al. 2010     |
| HM107319            | C1d1a1                | Montana, United States  | Perego et al. 2010     |
| HM107320            | C1d1a1                | Quebec, Canada          | Perego et al. 2010     |
| HM107321            | C1d1a1                | Zacatecas, Mexico       | Perego et al. 2010     |
| HM107322            | C1d1a                 | Sonora, Mexico          | Perego et al. 2010     |
| HM107334            | C1d1c                 | Oaxaca, Mexico          | Perego et al. 2010     |
| HM107335            | C1d1c1                | Texas, United States    | Perego et al. 2010     |
| HM107336            | C1d1c                 | Texas, United States    | Perego et al. 2010     |
| HM107337            | C1d1c                 | Michigan, United States | Perego et al. 2010     |
| HM107344            | C1d1                  | Oklahoma, United States | Perego et al. 2010     |
| DQ282387            | A2                    | United States           | Just et al. 2008       |
| DQ282388            | A2                    | United States           | Just et al. 2008       |
| DQ282389            | A2                    | United States           | Just et al. 2008       |
| DQ282390            | A2                    | United States           | Just et al. 2008       |
| DQ282391            | A2                    | United States           | Just et al. 2008       |
| DQ282392            | A2                    | United States           | Just et al. 2008       |
| DQ282393            | A2                    | United States           | Just et al. 2008       |
| DQ282394            | A2                    | United States           | Just et al. 2008       |
| DQ282395            | A2                    | United States           | Just et al. 2008       |
| DQ282396            | A2                    | United States           | Just et al. 2008       |
| DQ282397            | A2                    | United States           | Just et al. 2008       |
| DQ282398            | A2                    | United States           | Just et al. 2008       |
| DQ282399            | A2                    | United States           | Just et al. 2008       |
| DQ282400            | A2                    | United States           | Just et al. 2008       |
| DQ282401            | A2                    | United States           | Just et al. 2008       |
| DQ282402            | A2                    | United States           | Just et al. 2008       |
| DQ282403            | A2                    | United States           | Just et al. 2008       |
| DQ282404            | A2                    | United States           | Just et al. 2008       |
| DQ282405            | A2                    | United States           | Just et al. 2008       |
| DQ282406            | A2                    | United States           | Just et al. 2008       |
| DQ282407            | A2                    | United States           | Just et al. 2008       |
| DQ282408            | A2                    | United States           | Just et al. 2008       |
| DQ282409            | A2                    | United States           | Just et al. 2008       |
| DQ282410            | A2                    | United States           | Just et al. 2008       |
| DQ282411            | A2                    | United States           | Just et al. 2008       |
| DQ282412            | A2                    | United States           | Just et al. 2008       |
| DQ282413            | A2                    | United States           | Just et al. 2008       |
| DQ282414            | A2                    | United States           | Just et al. 2008       |
| DQ282415            | A2                    | United States           | Just et al. 2008       |
| DQ282416            | A2                    | United States           | Just et al. 2008       |
| DQ282417            | A2                    | United States           | Just et al. 2008       |
| DQ282418            | A2                    | United States           | Just et al. 2008       |
| DQ282419            | A2                    | United States           | Just et al. 2008       |
| DQ282420            | A2                    | United States           | Just et al. 2008       |
| DQ282421            | A2                    | United States           | Just et al. 2008       |
| DQ282422            | A2                    | United States           | Just et al. 2008       |
| DQ282423            | A2                    | United States           | Just et al. 2008       |
| Genbank Accession # | Haplogroup/Haplotype | Geographic Region | Paper               |
|---------------------|-----------------------|-------------------|--------------------|
| DQ282424            | A2                    | United States     | Just et al. 2008   |
| DQ282425            | A2                    | United States     | Just et al. 2008   |
| DQ282426            | A2                    | United States     | Just et al. 2008   |
| DQ282427            | A2                    | United States     | Just et al. 2008   |
| DQ282428            | A2                    | United States     | Just et al. 2008   |
| DQ282429            | A2                    | United States     | Just et al. 2008   |
| DQ282430            | A2                    | United States     | Just et al. 2008   |
| DQ282431            | A2                    | United States     | Just et al. 2008   |
| DQ282432            | A2                    | United States     | Just et al. 2008   |
| DQ282433            | A2                    | United States     | Just et al. 2008   |
| DQ282434            | B2                    | United States     | Just et al. 2008   |
| DQ282435            | B2                    | United States     | Just et al. 2008   |
| DQ282436            | B2                    | United States     | Just et al. 2008   |
| DQ282437            | B2                    | United States     | Just et al. 2008   |
| DQ282438            | B2                    | United States     | Just et al. 2008   |
| DQ282439            | B2                    | United States     | Just et al. 2008   |
| DQ282440            | B2                    | United States     | Just et al. 2008   |
| DQ282441            | B2                    | United States     | Just et al. 2008   |
| DQ282442            | B2                    | United States     | Just et al. 2008   |
| DQ282443            | B2                    | United States     | Just et al. 2008   |
| DQ282444            | B2                    | United States     | Just et al. 2008   |
| DQ282445            | B2                    | United States     | Just et al. 2008   |
| DQ282446            | B2                    | United States     | Just et al. 2008   |
| DQ282447            | C1                    | United States     | Just et al. 2008   |
| DQ282448            | C1                    | United States     | Just et al. 2008   |
| DQ282449            | C1                    | United States     | Just et al. 2008   |
| DQ282450            | C1                    | United States     | Just et al. 2008   |
| DQ282451            | C1                    | United States     | Just et al. 2008   |
| DQ282452            | C1                    | United States     | Just et al. 2008   |
| DQ282453            | C1                    | United States     | Just et al. 2008   |
| DQ282454            | C1                    | United States     | Just et al. 2008   |
| DQ282455            | C1                    | United States     | Just et al. 2008   |
| DQ282456            | C1                    | United States     | Just et al. 2008   |
| DQ282457            | C1                    | United States     | Just et al. 2008   |
| DQ282458            | C1                    | United States     | Just et al. 2008   |
| DQ282459            | C1                    | United States     | Just et al. 2008   |
| DQ282460            | C1                    | United States     | Just et al. 2008   |
| DQ282461            | C1                    | United States     | Just et al. 2008   |
| DQ282462            | C1                    | United States     | Just et al. 2008   |
| DQ282463            | C1                    | United States     | Just et al. 2008   |
| DQ282464            | C1                    | United States     | Just et al. 2008   |
| DQ282465            | C1                    | United States     | Just et al. 2008   |
| DQ282466            | C1                    | United States     | Just et al. 2008   |
| DQ282467            | C1                    | United States     | Just et al. 2008   |
| DQ282468            | C1                    | United States     | Just et al. 2008   |
| DQ282469            | C1                    | United States     | Just et al. 2008   |
| DQ282470            | C1                    | United States     | Just et al. 2008   |
| DQ282471            | C1                    | United States     | Just et al. 2008   |
| DQ282472            | C1                    | United States     | Just et al. 2008   |
| DQ282473            | C1                    | United States     | Just et al. 2008   |
| DQ282474            | C1                    | United States     | Just et al. 2008   |
| DQ282475            | C1                    | United States     | Just et al. 2008   |
| DQ282476            | C1                    | United States     | Just et al. 2008   |
| DQ282477            | D1                    | United States     | Just et al. 2008   |
| Genbank Accession # | Haplogroup/Haplotype | Geographic Region | Paper       |
|--------------------|----------------------|-------------------|-------------|
| DQ282478           | D1                   | United States     | Just et al. 2008 |
| DQ282479           | D1                   | United States     | Just et al. 2008 |
| DQ282480           | D1                   | United States     | Just et al. 2008 |
| DQ282481           | D1                   | United States     | Just et al. 2008 |
| DQ282482           | D1                   | United States     | Just et al. 2008 |
| DQ282483           | D1                   | United States     | Just et al. 2008 |
| DQ282484           | D1                   | United States     | Just et al. 2008 |
| DQ282485           | D1                   | United States     | Just et al. 2008 |
| DQ282486           | D1                   | United States     | Just et al. 2008 |
| DQ282487           | D1                   | United States     | Just et al. 2008 |
| KU523304           | B2                   | United States     | Just et al. 2008 |
| KU523305           | B2                   | United States     | Just et al. 2008 |
| KU523306           | B2                   | United States     | Just et al. 2008 |
| KU523307           | B2                   | United States     | Just et al. 2008 |
| KU523338           | C1b                  | United States     | Just et al. 2008 |