Original Research Article

The Mitochondrial DNA History of a Former Native American Village in Northern Uruguay

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Objectives: In 1828, between 8,000 and 15,000 Indians from the Jesuit Missions were brought to Uruguay. There, they were settled in a village, presently named Bella Unión, in the northwest corner of the country. According to historic sources, the Indians abandoned the settlement shortly thereafter, with the village subsequently repopulated by "criollos" and immigrants from abroad. As a first approach to reconstruct the genetic history of the population, data about the living population genetic structure will be used. Based on the analysis of the maternal lineages of the inhabitants of Bella Unión, and of those from two nearby villages, we expect to partially answer what happened with the first and subsequent inhabitants.

Methods: We analyzed the maternal lineages of the present inhabitants of Bella Unión and neighboring localities through the sequencing of the mitochondrial DNA control region.

Results: A total of 64.3%, 5.7%, and 30% of the mtDNAs were of Native, African, and West Eurasian origin, respectively. These figures are quite similar to that of the population of Tacuarembó, which is located in northeastern Uruguay. The four main Native American founding haplogroups were detected, with B2 being the most frequent, while some rare subhaplogroups (B2h, C1b2, D1f1) were also found. When compared with other Native American sequences, near- matches most consistently pointed to an Amazonian Indian origin which, when considered with historical evidence, suggested a probable Guaraní-Missionary-related origin.

Conclusions: The data support the existence of a relationship between the historic and present inhabitants of the extreme northwest Uruguay, with a strong contribution of Native Americans to the mitochondrial DNA diversity observed there. Am. J. Hum. Biol. 00:000–000, 2014.

During the 19th century, several different events changed the composition of the Uruguayan population. These included waves of immigration from Europe, the end of the slave trade from Africa, and the persecution of some indigenous (Native) populations, such as the Charrúa Indians, which occurred almost simultaneously with the entrance of Natives from the Jesuit Missions.

At the moment of the European conquest, three or four Native American ("pueblos originarios") ethnic groups were living in what today is Uruguayan territory, although only two of them—the Charrúa and the Guaraní—are mentioned in the sources dating to the 19th century. While the Charrúa Indians were subjected to genocide, basically because of economic reasons, being the Indians accused of robbery of cattle by the Spanish or "criollo" landowners (see a revision in Acosta y Lara, 1989, among others), the Guaraní population in the territory increased steadily. For a variety of reasons, the Uruguayan territory received natives from the Jesuit Missions (also known as "Guaraní reductions") during historical times in the form of single individuals escaping in search of a more independent life (González Rissotto and Rodríguez Varese, 1982). Additionally, massive movements of Indians involved in civil and military collaborations and, later, the voluntary abandonment of the initial territories after the end of the Jesuit system (1750) that culminated in the Guaraní Wars of 1756, increased the number of natives in the region (González Rissotto and Rodríguez Varese, 1982, 1989; Pi Hugarte and Vidart, 1969).

González Rissotto and Rodríguez Varese (1982) mention that, for at least two centuries, Guaraní from the Missions left the region of the Jesuit reductions and settled in the Rio de la Plata area, establishing the first migration wave into the Uruguayan territory. Three well-defined influxes of thousands of Native Americans from the former missions into Uruguayan territory occurred in 1813, 1820, and 1828, respectively. These natives were relocated on the east side of the Uruguay River in the present-day Departments (major administrative divisions) of Artigas and Salto, in northwestern Uruguay (Barreto and Curbelo, 2009; Cabrera Pérez and Curbelo, 1988; González Rissotto and Rodríguez Varese, 1990, 1991; Poenitz and

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

Contract grant sponsor: PICT (Argentina); Contract grant number: 2008 N° 715; Contract grant sponsor: PIP (Argentina); Contract grant number: 2010 N° 1 (to C.M.B.); Contract grant sponsor: US National Institute of Justice (Interagency Agreement); Contract grant number: 2005-DN-R-086.

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Received 13 May 2014; Revision received 24 October 2014; Accepted 11 November 2014

DOI: 10.1002/ajhb.22667
Published online 00 Month 2014 in Wiley Online Library (wileyonlinelibrary.com).

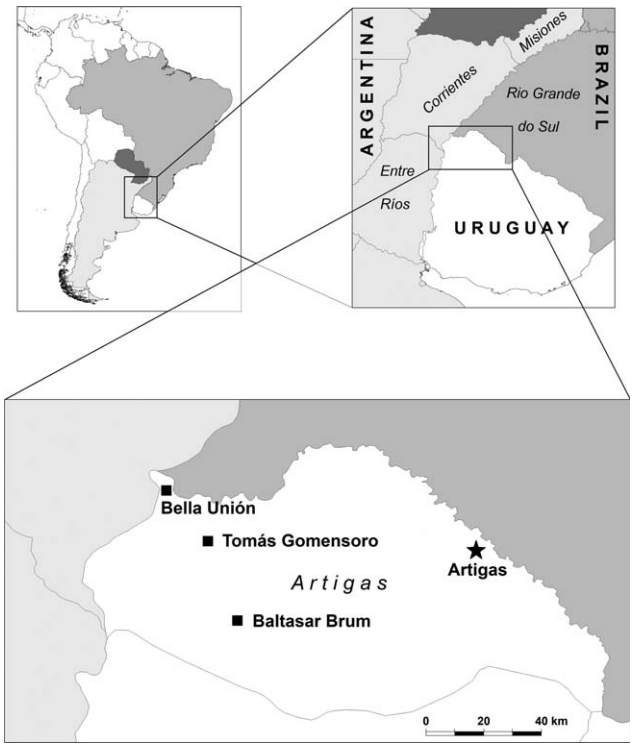

Fig. 1. Map of the region, populations analyzed and others mentioned in the text.

Poenitz, 1993). The last wave was induced by the conquest of the former "Seven Missions" by General Fructuoso Rivera in 1828, which caused approximately 8,000 to 15,000 Indians from the region to join Rivera's army and move to Uruguay. With them, Rivera founded Santa Rosa del Cuareim, at which today is named Bella Unión, but the population was poorly attended by the authorities and left to their own fate (Cabrera Pérez and Curbelo, 1988).

This study aims to analyze the history of the population of the village Santa Rosa del Cuareim, founded with some thousands of Indians brought from the Jesuit Missions. As a first approach to delineating the history of its first inhabitants and the subsequent process of re-peopling of the village, we chose to analyze the maternal lineages (mitochondrial DNA) of the inhabitants of present city of Bella Unión, originated in the oldest village. Additionally, due to the possible spread of the initial village's inhabitants, we selected two more modern cities in the area, Baltasar Brum and Tomás Gomensoro, which are close to Bella Unión and are tied to the development of the Norton Western Railway that reached the area in 1886. This is the first time that a native-descendant origin population from Uruguay has been analyzed and the results provide new information about the historic process of formation of the population.

MATERIAL AND METHODS

Geographic and historical data

The research was conducted in three localities in the northwestern corner of Uruguay: Bella Unión, Tomás Gomensoro and Baltasar Brum. These localities are geographically located in a straight line from west to east extending 62 km (Fig. 1). The largest and oldest of the three is Bella Unión, located in what is called the "triple border" between Uruguay, Argentina, and Brazil, where the Cuareim River flows into the Uruguay River. Bella Unión was founded in 1829 and re-founded in 1853. At present, the city has become an agro-industrial center, specializing in fruit, corn, and sugar cane production. It has a population of 17,377 inhabitants, including its surrounding villages, and is the second largest city of the Department of Artigas, after its capital city, Artigas (INE, 2011).

The other two populations were selected because the historic sources mentioned above have pointed out that, after lacking of government support, the town was supposedly abandoned and the Indians spread into the region. Soon afterwards, the old village was reoccupied by "criollos" (Uruguayans with European or mixed ancestry) and immigrants from abroad (Cabrera Pérez and Curbelo, 1988; González Rissotto and Rodríguez Varese, 1990, 1991; Poenitz and Poenitz, 1993). Tomás Gomensoro is the third largest city of the department, with 2,659 inhabitants (INE 2011). Founded in 1883 by immigrants from northern Italy who settled there during the construction of the railway it is located 27 km from Bella Unión. Some time later, the village received Russian and German immigrants. At present, the area is mostly dedicated to rice and cattle and sheep husbandry. Baltasar Brum is the fourth largest city in the department, with a population of 2,531 inhabitants (INE, 2011), and was also founded in response to the train expansion, which began with the installation of a rail station in 1886. It also received Italian immigrants, and has a strong agricultural focus, especially rice, having its own industry to process the crop.

Samples

The scope and purpose of the project was explained to the volunteers before obtaining their signed informed consent. The Project was approved by the Ethics Committee of the Facultad de Humanidades y Ciencias de la Educación, Universidad de la República. Blood or buccal swabs were obtained from 70 individuals who were born in the locality where they were sampled, including 32 from Bella Union, 27 from Tomás Gomensoro, and 11 from Baltasar Brum. The sample size is similar to other studies in the country and the region (e.g. Alves-Silva et al., 2000; Corach et al., 2010; Bonilla et al., 2004; Sans et al., 2011). In order to avoid sampling temporary residents, participants were selected only if they reported having at least three generations in the cities (either parents and grandparents, or children and grandchildren). The samples were taken from public and private health institutions, with the number of samples from each health center taken in proportion to the population served by each center, following the National Statistics Institute of Uruguay (INE, 2011).

DNA extraction, sequencing, and analysis

Vacutainer tubes were used to collect 5 mL of whole blood from the participants living in Bella Unión and Tomás Gomensoro, while buccal swabs were used for DNA collection in Baltasar Brum. Total genomic DNA was extracted from blood samples along with previously dried

buccal swabs according to the "salting out" method of Miller et al. (1988).

The complete mitochondrial DNA (mtDNA) control region (nucleotide positions 16,024–573) as well as the adjacent 5' (15,878–16,023) and 3′segments (574–649) were amplified and sequenced at the Armed Forces DNA Identification Laboratory (AFDIL) using a high-throughput strategy suggested by Brandstätter et al. (2004) and Irwin et al. (2007). In addition to the quality control checks of the data performed at AFDIL, sequences were also checked by EMPOP (EDNAP mitochondrial DNA population database: www.empop.org; Parson and Dur, 2007). Sequences were aligned using Genedoc 2.7.0 (Nicholas et al., 1997). To analyze the quality of the sequencing data, the ratio of the number of weighty transitions (WTTI) to the number of transversions plus indels was calculated (Bandelt et al., 2002).

Primary haplogroup assignment was performed using the control region motifs listed in Bandelt et al. (2012), and afterwards refined or modified with the last updated version of Phylotree (mtDNA tree Build 15, 30 September, 2012; van Oven and Kayser, 2009) along with an in-house database of complete mtDNA sequences of Native American origin compiled for comparative studies. In this database, we basically expanded the list of 148 sequences analyzed in Achilli et al. (2008) with those published afterwards (see Supporting Information Text S.1 for references), while also including several sequences submitted to GenBank by FTDNA, a commercial genealogy-by-genetics company.

One sequence (UryAr030), presumably belonging to haplogroup D1 but lacking the diagnostic 16325C mutation, was tested for mutations 5178A (D) and 2092T (D1). The detection of the 5178A mutation was performed by screening the sample for the 5176 *Alu*I(−) restriction site polymorphism, while the presence of the 2092T was determined by amplification of the region carrying the mutation. For this purpose, primers 2069F/mism (5'-CTCTAAA TCCCCTTGTAAAGTTAA-3') and 2583R (5'- GTTAGGGT ACCGCGGCCGTTA −3'), designed by one of us (C.M.B.), were used. The first carries a mismatch at np 2087 that generates a polymorphic *Hin*cII restriction site. The double digestion of the 516 bp PCR products with *Hae*III and *Hin*cII generates two possible fragment patterns recognized through polyacrylamide gel electrophoresis. The non-D1 2092C state, with a functional *Hin*cII restriction site at np 2089, generates five bands of 248, 146, 83, 23, and 16 bp, whereas the D1 2092T transition, which produces the lack of the restriction site, results in four bands of 248, 146, 106, and 16 bp.

The confirmation of putative haplogroup H membership in individuals UryAr009, 017, 019, 020, 024, 056, 057, and 059 was determined through PCR-RFLP analysis of the ALUI 7025 site. For this purpose, primers designed by Martínez-Cruzado et al. (2005) were used according to the specifications of Sans et al. (2011). Haplogroup C1b2 was confirmed by testing samples for the absence of the RsaI 70113 restriction pattern site, using the same amplified segment as for haplogroup H.

To explore the phylogeographical affinities of the Native American sequences, we analyzed an in-house database of sequences derived from both Native and admixed populations (updated to papers published through February 2013) for the presence of matches and near-matches, i.e., haplotypes sharing the presence of specific mutations in addition to the nodal (founding) sequence for each haplogroup. Regions of known homoplasy including length variation at polyC tracts 16,184–16,193, 303–315, and 568–573, as well as hotspot mutations such as 16,519,16,182, and 16,183 and CA repeats variation at 514–523 were not considered for the analyzes. Despite those exclusions, given the high level of homoplasy expected for the mtDNA control region, these results should be cautiously interpreted when matches or near-matches are based on single SNPs and/or a combination of fast evolving sites, which is why coding region SNP typing or whole mitogenome sequencing is important. Nonetheless, the (near) matching analysis should also consider other variables such as geographical or ethnic distribution of haplotypes.

Haplogroup frequencies were compared with those of native and admixed populations living south of parallel 20°S. Given the great quantity of data available, mixed populations from Argentina were grouped (or re-grouped by us) according to geographical regions (references about populations compared are detailed in Fig. 2).

Statistical methods

All statistical parameters were calculated with Arlequin 3.5 (Excoffier and Lischer, 2010). Genetic diversity within populations was assessed by computing haplotype diversity (H) and nucleotide diversity (p) (Nei, 1987). Haplotype diversity (also known as gene diversity) represents the probability that two randomly sampled haplotypes are different, while nucleotide diversity is defined as the average number of nucleotide differences per site in pairwise comparisons between DNA sequences (Nei, 1987).

Intra- and interpopulation regional differences (southern Brazil, Argentina, and Uruguay) on the basis of geographic origin of haplogroups (European, Native American, or African), as well as the frequencies of the four Amerindian haplogroups, were evaluated with $R \times C$ contingency tables that are robusts for this purpose (Miller, 1997).

To evaluate the distances between populations based exclusively on the native-origin sequences (HVRI positions 16,051–16,380), we calculated pairwise FST estimates based on Tamura and Nei's distance model (1993) with a gamma value of 0.26. The UPGMA tree based on pairwise FST matrix values was produced using the Phylip 3.67 software (Felsenstein, 2005). Admixed populations were grouped into regions (Northeastern, Northwestern, Center, and Southern Argentina, and South and Southestern Brazil) (Alves-Silva et al., 2000; Bobillo et al., 2010; Catelli et al., 2011; Marrero et al., 2007a; Tamm et al., 2007), and two Uruguayan populations were also included (Tacuarembó: Bonilla et al., 2004; Artigas: the present study). Indigenous populations were grouped considering regions (Chaco, Southern Chile, and Patagonia) (Ginther et al., 1993; Cabana et al., 2006; de Saint Pierre et al., 2012) or included separately (Kaingang, Aymara, Guaraní) (de Saint Pierre et al., 2012; Marrero et al., 2007b).

We also the calculated distances based on the frequency on the Native American origin haplogroups, as D_A distances (Nei, 1987). In this case, an extended majority rule consensus UPGMA tree (Gronau and Moran, 2007; Sneath and Sokal, 1973) was constructed from 1,000 bootstrap D_A distances. The distance computation, the bootstrap procedure, and the construction of the resulting

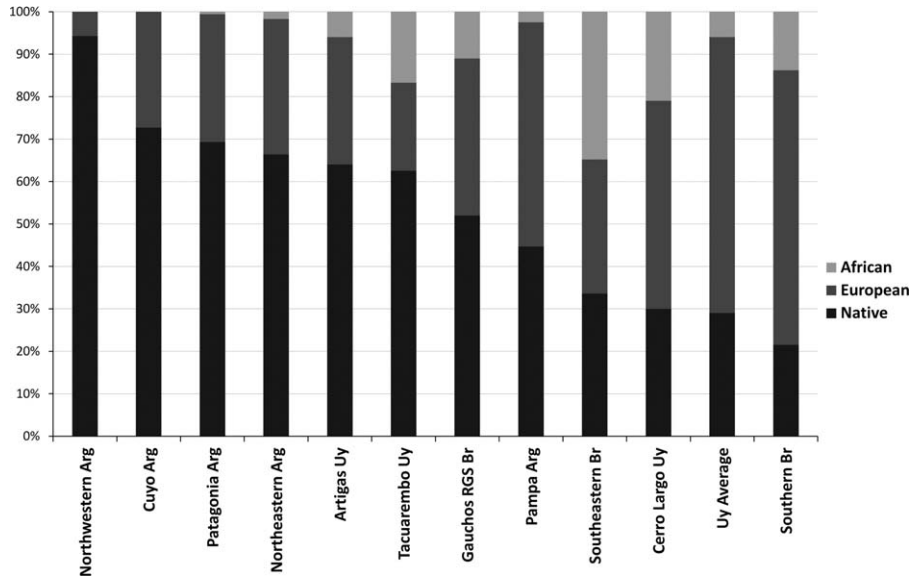

Fig. 2. African, European and Native maternal lineages contribution in South American populations. Arg: Argentine, Uy: Uruguay, Br: Brazil. References: northwestern Arg: Dipierri et al 1998, Catelli et al., 2011; Cuyo Arg: Catelli et al., 2011; Patagonia Arg: Avena et al., 2009, 2010, Bobillo et al., 2010, Catelli et al., 2011; northeastern Arg: Corach et al., 2010, Bobillo et al., 2010, Catelli et al., 2011; Artigas: present study; Tacuarembó: Bonilla et al., 2004; Gauchos RGS: Marrero et al., 2007a; Pampa Arg: Martínez-Marignac et al., 1999, Dejean et al., 2003, Avena et al., 2007, Salas et al., 2008, Bobillo et al., 2010, Catelli et al., 2011; Southeastern Br: Alves-Silva et al., 2000; Cerro Largo: Sans et al., 2006; Uy average: modified from Pagano et al., 2005; Southern Br: Alves-Silva et al., 2000.

trees were implemented using the Phangorn 1.7-1 package (Schliep, 2011) in the R 2.14.1 environment (R Development Core Team, 2011). We also performed a Nonmetric Multidimensional Scaling (NMDS) analysis of the distance matrix, using the MASS 7.3–16 package (Venables and Ripley, 2002) in the R environment, reducing the distance data to a two-dimensional configuration conveniently visualized through a scatterplot.

RESULTS

mtDNA control region sequences are provided in Supporting Information Table S.1, and will be available in EMPOP under accession number EMP00543. The indexes used to evaluate the quality of the sequences gave results as expected. The WTTI, considering only HVRI for the purpose of comparison, was 4.5, while the speedy transitions/weighty transitions ratio was 9.3.

No significant differences were found when the three sampled localities (Baltasar Brum, Bella Unión and Tomás Gomensoro) were compared based on the geographical origin of the haplogroups (Indigenous, European, African) ($P = 0.523$), the combined haplogroups ($P = 0.150$), or only Native-origin haplogroups (A,B,C,D) ($P = 0.158$). No X2a mtDNAs were found, consistent with previous studies, e.g. Dornelles et al. (2005). However, the three samples from northwestern Uruguay showed striking similarities according to the F_{ST} and pairwise population differentiation test ($F_{ST} = 0.00$). Consequently, all of the subsequent analyzes considered the three samples as belonging to a single population, which henceforth we designate as "Artigas." No significant differences were found between the Artigas (northwest) and Tacuarembó (northeast) samples in Uruguay. Nonetheless, significant F_{ST} values were observed when the "Gauchos" from Rio Grande do Sul, in the neighboring area of Brazil, were compared with both the Artigas ($F_{ST} = 0.025$; $P < 0.05$) and Tacuarembó ($F_{ST} = 0.026$; $P < 0.05$) samples.

Among the 70 Artigas mtDNA sequences, 48 different haplotypes were observed in the HVRI region and 42 different haplotypes in the HVRII region, adding up to 57 different haplotypes for the whole control region when variation at the polyC tracts and CA repeats was disregarded (Supporting Information Table S.1). We identified 80 and 48 polymorphic sites in the HVRI and HVRII, respectively. The genetic diversity was higher in the HVRI compared with the HVRII. Nucleotide diversity was relatively low in both regions, with the lowest value in HVRII (Table 1). The values obtained for both regions were similar to the ones obtained by Palencia et al (2010) in the neighboring region of Santa Catarina (southern Brazil).

Haplogroup distributions are detailed in Supporting Information Table S.1, and a summary is presented in Table 2. Forty-five (64.3%) sequences were classified as Native American origin, four (5.7%) of African origin, and the remaining 21 (30%) of West Eurasian (i.e., Europe plus Middle East and North Africa) origin. Among the native mtDNAs, the four main haplogroups (A2, B2, C1, D1) were observed, with C1 being represented by subgroups C1b, C1c, and C1d. Although no private control region mutations were available for Native American clade B2, we assigned all of our haplogroup B4b lineages—defined by the presence of the 499A mustation to B2, since available information derived from complete mtDNAs points to B2 as the only branch of B4b present in Native Americans. Asian haplogroup B4b1—the only sister clade currently known for B2—is also easily distinguishable since it carries a control region transition at np 16136 (PhyloTree build 15, van Oven and Kayser, 2009).

TABLE 1. Nucleotide and haplotype diversity in Bella Unión

	n	*k*	PS	Hd	*p*	n.d.
HVR I	70	48	80	0.972 ± 0.011	0.020 ± 0.002	6.67
HVR II	70	42	48	0.954 ± 0.018	0.0085 ± 0.0008	4.064

n: sample size, *k*: number of different haplotypes, PS: number of polymorphic sites Hd: haplotype diversity, *p*: nucleotide diversity, n.d.: average number of nucleotide differences per region.

TABLE 2. Summary of mtDNA haplogroups and subtypes

ID (UryAr)	N	ST	ID (UryAr)	N	ST
003, 015, 021, 026, 051, 076, 080	7	A2	009,017, 019, 020	4	H[a]
001, 005, 006, 012, 013, 036, 043, 045, 046, 047, 049, 055, 061, 063, 090	15	B2[b]	057	1	H1a
085	1	B2b	024	1	H1b/b1d
008, 023, 052	3	B2b	056	1	HV0
016, 065	2	B2h	059	1	HV15
029, 033, 037, 048, 053, 060	6	C1b	031	1	K1a
072	1	C1b2	022	1	K1b1a2
058	1	C1c	038, 077	2	T2b
010	1	C1d1	060	1	T2e1
030[a], 034, 066, 088, 089	5	D1	011	1	U2e1
032, 039	2	D1f1	035	1	U5a2a
039	1	D1/1a1	014, 027	2	U5b
044	1	L0a1	042	1	U6a1a1
018	1	L0d1'2	040	1	U6a1b1a
041	1	L1b1a	050	1	X2
004	1	L1c1			

ID: sample identification; ST: probable haplogroup and subtype.
[a]Confirmed with RFLPs.
[b]See Results for classification.

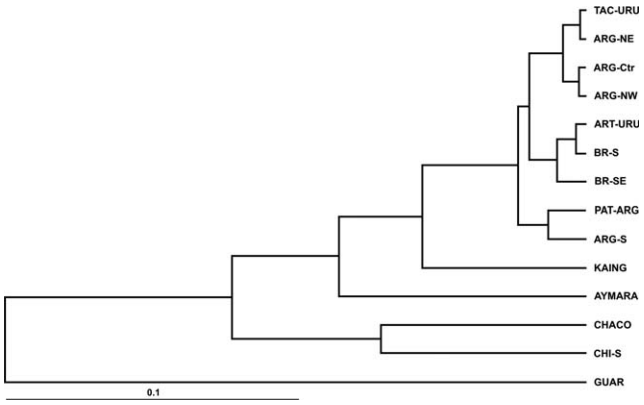

Fig. 3. UPGMA tree based on pairwise FST matrix values computed using Tamura and Nei distance model (1993). TAC-URU: Tacuarembó, Uruguay (Bonilla et al., 2004); ARG-NE, -Cr, - S: Argentinean regions northeast, center, northwest, south (Bobillo et al., 2010; Catelli et al., 2011); ARG-NW: Argentinean northwest (Catelli et al., 2011; Tamm et al., 2007); ART-URU: Artigas, Uruguay, present study; BR-S, -SE: Brazilian regions south and southeast (Alves-Silva et al., 2000); PAT-ARG: Native peoples from Patagonia, Argentina (de Saint-Pierre et al., 2012; Ginther et al., 1993); KAING: Kaingang, Brazil (Marrero et al., 2007b); AYMARA: Natives from Chile (de Saint-Pierre et al., 2012); CHACO: Native populations from Chaco, Argentina (Cabana et al., 2006); CHI-S: Native populations from southern Chile (de Saint-Pierre et al., 2012).

Among Native American maternal lineages, haplogroup B2 was the most frequent (46.7%; $n = 21$), with the other three haplogroups being at approximately similar frequencies to one another: 20% for haplogroup C1 ($n = 9$); 17.8% for haplogroup D1 ($n = 8$); and 15.5% for haplogroup A2 ($n = 7$). Among the West Eurasian haplogroups, the higher frequency corresponded to haplogroup H (33.3%, $n = 6$), while the same frequency was found in haplogroup U, pertaining in all cases to a broad variety of subtypes. Among African haplogroups, only L0 and L1 were observed, each occurring at equal frequencies 50% ($n = 2$).

Admixture proportions were based on the phylogeographic origin (Native American, African, European) of each haplogroup. The population of Artigas was significantly different from all other Uruguayan populations except Tacuarembó, and similar to other three locations in the neighboring region, two in Argentina (northeastern Argentina and Patagonia), and one from Brazil ("Gauchos" from Rio Grande do Sul), with Patagonia the least similar ($P = 0.044$). When considering only African ancestry, Artigas was similar to the entire region except from Southeast Brazil. With regard to only the Amerindian contribution, it was similar to some Argentinean locations (northeastern, Patagonia, and Cuyo), and from Gauchos from Brazil (Fig. 2)

The UPGMA dendrogram of Native-American origin sequences from mixed and native populations of the southern region of South America, based on pairwise FST estimates, showed that admixed populations from the neighboring regions of Brazil and Argentina formed a big cluster. On the other hand, native populations (Kaingang, Aymara, Guaraní, Chaco, and Southern Chile), with the only exception of those from Patagonia, did not fall into that cluster. Artigas, the focus of this study, formed a small cluster within the big cluster mentioned above, together with groups from South and Southern Brazil (see Fig. 3).

When the frequency of each native haplogroup was analyzed using Nei's DA distance UPGMA, the dendrogram presented two well-differentiated clusters. The first main cluster included two sub-clusters, with one including Artigas (present study) and natives from the northern region of Argentina (including Chaco) and Chile, other Uruguayan population (Trinidad), and northwestern Argentina, while the other included most of the other populations, including those from regions neighboring with Artigas such as Gauchos, south and southeastern Brazil, and northeastern Argentina. The second main cluster also included the populations from the southern part of the continent. Surprisingly, the Guaraní and Kaingang from Brazil, and the Ache from Paraguay, were not included in the two above mentioned clusters (Supporting Information Fig. S.1).

Similarly, the NMDS analysis based on the same haplogroup frequencies showed a close relationship between Artigas and Chacoan populations (Supporting Information Fig. S.2). All samples, except those from the Ache from Paraguay and the Kaingang and Guaraní Indians from Brazil, seemed to be relatively close, with the southernmost populations grouping in a same cluster. In addition, it was possible to identify a first subgroup that included the southernmost populations, and a second one that included part of the Uruguayan populations and other geographically close populations, while some populations, including Artigas and Trinidad, appeared in an intermediate position within the cluster (Supporting Information Fig. S.2).

mtDNA sequences related to haplogroups A-D were further analyzed in depth to help shed light on the history of the"Pueblos originarios" that inhabited the region. Nine of 45 sequences did not show any variants besides the typical SNPs defining the founder haplotypes. Of the remaining 36 sequences, five did not have "near-matches" with other native-origin sequences, while nine were found only in cosmopolitan locations (one in the capital city of Uruguay, two in cities in the northeastern Argentina, four in cities from Brazil, and two abroad). Consequently, 22 sequences had near-matches with other sequences of native origin. One was found mostly in Brazilian natives but also in one Quechua; another appeared in a Kolla from Salta, Argentina; a third occurred in a Quechua from Peru; and three more originated in the Chaco region. The remaining 13 sequences were consistent with an Amazonian origin, with six being related to Tupí speakers, four to Jé speakers, and the others being shared between them and/or other ethnic groups (Supporting Information Table S.2 and Text S.2).

DISCUSSION

As expected, considering the foundation of Bella Unión with natives brought from the Jesuit Missions and the subsequent spread of these populations in the region, the northwestern part of the department of Artigas shows a high Amerindian genetic background when compared with other populations from Uruguay. However, this genetic contribution is similar to that observed in Tacuarembó, which has a different history and origin (Bonilla et al., 2004). As expected due to the geographical position of Artigas, the African contribution is intermediate between that found in southern Brazil and the populations of Argentina.

While a bias in the mtDNA data due to sampling size is possible, the characteristics of the mtDNA lineages in Artigas are concordant with those of others observed in the region, especially admixture proportions and Native American haplogroup composition. In the case of European and African origin sequences, the scarcity of non-Native American haplotypes in Artigas reflects aspects of its population history that will be discussed below.

European and African origin mtDNA haplotypes

MtDNAs of European origin exhibit a relatively low frequency of haplogroup H and high frequency of haplogroup U, both appearing at 33.3% in Artigas. Even when sampling errors cannot be excluded, some explanations for this pattern can be posed In the case of haplogroup H, its frequency is low compared with that observed in most European countries, but similar with some regions of Spain and Italy (Achilli et al., 2004). This pattern is consistent with the Spanish and Portuguese origin of the colonizing population and the immigration waves received from Spain and Italy during the second half of the 19th century and the first half of the 20th century (Vidart and Pi, 1969). This value is also in agreement with data from the department of Cerro Largo in the northeast of Uruguay and from a general sample of Uruguay, as well as from southern Brazil and central Argentina (32.4%, calculated from data by Alves Silva et al., 2000; Catelli et al., 2011; Pagano et al., 2005; Sans et al., 2006).

By contrast, the frequency of haplogroup U mtDNAs in Artigas is high (33.33%) and similar to that observed in north/northeastern Argentina (39.3%) but different from the other Argentinean, Brazilian and Uruguayan regions analyzed. In particular, this high frequency is due to the fact that subhaplogroups U5 and U6 were relatively high in our sample (see Table 2 and Supporting Information Table S.1). Haplogroup U6 has a unique distribution, being found primarily in North Africa and the Canary Islands (Pereira et al., 2010). Its presence in Uruguay, as also seen in Cerro Largo, has been discussed before and is most likely explained by the Spanish-Canary Islands migration, not with the slave trade (Sans et al., 2006). However, in this previous study the two sequences observed belonged to U6b, while in our study, they belong to U6a, a haplogroup highly dispersed throughout North Africa and related to the first African expansion from the Maghreb in Paleolithic times (Maca-Meyer et al., 2003; Pereira et al., 2010).

Haplogroup U5 has been proposed as an important lineage associated with the repopulation from the southwestern refuge in the Late Upper Paleolithic (Pereira et al., 2010). The haplogroup is relatively rare in Europe, but occurs at high frequencies in Basques from Navarra as well as in the Saami, Finns and Karelians (Cardoso et al., 2011; Pereira et al., 2010). Artigas shows a high frequency of U5b, which has been previously found in lower frequencies in different regions in Uruguay and Argentina, and is rare in Brazil. Disregarding length variation in the 303 to 309 polyC tract, the three sequences are identical and suggest that the high frequency of U5b is attributed to a founder effect.

The other European haplogroups found in Artigas (HV, K1, T2) are branches of haplogroup R, as expected in European-derived populations (Torroni et al., 2006). The only exception was one individual carrying a X2 haplotype. Haplogroup X is subdivided into two major branches, X1, which is restricted to the populations of North and East Africa and the Near East, and X2, which is related to a recent population expansion in Eurasia (Reidla et al., 2003). The sequence from Artigas was classified as belonging to the X2d subgroup, one that is present mostly in the Mediterranean region, from Italy to Turkey as well as in western Asia (Reidla et al., 2003) (Supporting Information Table S.1).

The African sub-Saharan contribution was represented exclusively by two haplogroups, L0 and L1. Haplogroups L2 and L3, which are usually more frequent in the region, were not found in the present study. This includes haplogroup L3e, the most frequent subhaplogroup in Brazil (Alves-Silva et al., 2000; Bandelt et al., 2001). Due to changes in mtDNA nomenclature, haplogroup L0 (frequent in west-central and southeastern regions of Africa, Salas et al., 2004) was not previously identified in Uruguay. However, some sequences found in Tacuarembó and the southern region, as well as in Brazil, can now be classified as belonging to L0 (data from Alves Silva et al., 2000; Bonilla et al., 2004; Pagano et al., 2005).

The other two sequences belong to subhaplogroups L1b and L1c, which have been previously observed in the region, the former being more frequent in West Africa, and the latter more common in west-central Africa (Salas et al., 2004). West-central and southeast Africa is the area of dispersal of Bantu groups, and is noted by Isola (1975) as the most important focus of the Uruguayan slave trade. The author also mentions that 32% of the slaves that entered Montevideo were from the Guineo-Sudanese area, in the

western region of the African continent, in which L1b mtDNAs are commonly observed. The exclusive presence of these two African haplogroups could be probably caused by sampling error, as only four sequences from that origin were identified. However, this issue needs to be explored further in future studies.

Native American origin mtDNA haplotypes

A major focus of this paper was to analyze what happened to the Indians brought from the Jesuit Missions to help create Bella Unión as well as those populating the neighboring region (the cities of Tomás Gomensoro and Baltasar Brum). The first point to be emphasized is that all three localities show no significant genetic differences despite the fact that one of the locations is considered more "ancient" and directly related with the Missionary Indians and the other two locations are more recent in origin and are associated with the building of the railway. At present, Bella Unión attracts people from different parts of the country as an active agro-industrial center (Cardozo Soto et al., 1988; Oyhantçabal and Carámbula, 2011).

According to a report written at the beginning of the 1830 decade,"hunger and misery" were present in the village (the recently founded Bella Unión, or Santa Rosa del Cuareim) and soon after its foundation the situation devolved to the point where the population rebelled, but was quickly defeated. After this incident, the natives from the former Missions began to spread to the south through the Arapey and Cuareim River basins, populating the region and founding new villages and hamlets (J. I. Aubouin cited by González Rissotto and Rodríguez Varese, 1990). Thus, this initial population spread throughout the region and probably contributed to the population of different villages that were founded at later times, like Tomás Gomensoro and Baltasar Brum.

Moreover, the three localities showed no genetic differences with Tacuarembó in terms of mtDNA sequence origin and diversity, frequency of native haplogroups, and the extent of the Native contribution. Interestingly, Tacuarembó is 346 km southeast of Bella Unión and its population has traditionally been considered to be more related to the Charrúa Indians because of military campaigns organized by the Uruguayan government against them, with the most important conflict that occurred in 1831 at Salsipuedes (Acosta y Lara, 1979, 1985). However, some references also mention the presence of Guaraní Indians there (Acosta y Lara, 1979; Saint-Hilaire, 1961).

Another point of emphasis is the origin of Native American lineages, although the picture here is unclear. When analyzing haplogroup frequencies, the samples from the three Artigas' populations appear to be more related to native populations from the Chaco region, followed by those from Patagonia, while the Guaraní appear to be fairly related (Supporting Information Fig. S.1 and S.2). While Guaraní samples from southern Brazil and northeastern Argentina have high frequencies of A (>40%) and low B (<18%) haplogroups (data from Marrero et al., 2007b and Sala et al., 2010; Supporting Information Table S.2), haplogroup A occurs in Artigas at the lowest frequency (15.6%) and B at the highest (46.7%) among the Native haplogroups. In fact, Artigas presents the highest frequency of haplogroup B in the entire region, which is difficult to explain and can be due to microevolutionary factors as well as sampling error.

In reality, little is known about the missionary Indians that founded the present day Bella Unión. Data about the city of Salto, located not far from Bella Unión, in the decade of 1830, indicates that the population had come from different Missions, including Yapeyú, San Nicolás, San Carlos, San. Xavier, La Cruz, Mandisoví, San. Borja, Angeles, and Espíritu Santo (C. López, 1900, in González Rissotto, 1989). Moreover, according to the Archives of the Catholic Church, marriages involving missionary males and females were common (Gonzalez Rissotto and Rodríguez Varese, 1982). These endogamic patterns could explain the unusual characteristics of the mtDNA sequences from Artigas.

When analyzing pairwise FST based on HVRI sequences, the Guaraní are positioned at some distance from Artigas, and only indigenous groups from Patagonia, as well as Aymara and Kaingang, share the same cluster with admixed populations from Argentina, Brazil, and Uruguay including the populations of Artigas. It should be noted that, despite the number of individuals sampled (i.e., 200 Guaraní, 78 Kaingang), the intrapopulational variation is usually low. According to Marrero et al. (2007b), in the case of Guaraní, sampled in southern Brazil, they could possibly have lost part of their intrapopolation variation on their southern route of migration due to a bottleneck.

A more accurate strategy would be to look beyond general analyzes of HVRI mtDNA sequences or haplogroup frequencies and focus on private, or rare mutations. This type of analysis would potentially recover the most basal variation in the Native American mtDNA haplogroups with the purpose to clarify ancient migrations as suggested by Bodner et al. (2012). In this case, the situation seems to contradict what is shown through analyses of haplogroup frequencies and clarify the relations found with southern and southern Brazil based on HVRI sequences, as most of the haplotypes with rare mutations seem to have Amazonian affinities. However, this statement should be qualified for several reasons. First, despite belonging to one of the four main founder haplogroups, more than half of the sequences (26 in 45) were not included in the analysis because of lacking rare mutations or because they did not show coincidences with other sequences in the region. In addition, the quantity of data associated with mtDNA of native populations in the region from published sources is scarce, and sometimes limited to the frequency of haplogroups, or hypervariable region I (see Bisso-Machado et al., 2012 for a review). Finally, we consider that the information analyzed in this study comes from present-day populations, which have been subjected to different evolutionary and demographic processes, especially migratory movements and genetic drift, factors that may have modified its characteristics after approximately seven generations (that is, around 180 years).

Taking these ideas into consideration, we can start to draw some conclusions about our results. The first concerns the presence of particular mtDNA subtypes. One of these, D1j, was recently defined by Bodner et al. (2012), who pointed out its local high frequencies, essentially restricted to populations from the Southern Cone of South America (Chile and Argentina), with an estimated age that agrees with the earliest archaeological sites in South America. This subtype was first reported in Uruguay in a Basque descendant from a city in the country, Trinidad

(Sans et al., 2011), and was also found further north, in southern Brazil (São Paulo) and in a Quechua from Bolivia, and south in southern Chile (Concepción) and in a Mapuche individual from Argentina (Bodner et al., 2012; Ginther et al. 1993), attaining its highest frequency in central Argentina (18% in Santiago del Estero, 16% in Córdoba (García et al., 2012). Moreover, we observed another subtype, C1b2, identified by a transition in the coding region (np 7,013) and the gain of the rCRS allele at the control region np 263. Originally defined by Achilli et al. (2008) based on 12 complete mtDNAs of US Hispanic origin (Just et al., 2008) and one from the Canarian Islands (Maca-Meyer et al., 2001), its presence in the Amazonian Yanomama, Macushi and Marubo can be inferred on the grounds of high resolution RFLP analysis, i.e. the loss of *RsaI* site at position 7013 (Achilli et al., 2008; Torroni et al., 1993). Based on the diagnostic SNP at np 263 alone, its putative presence in further samples of US Hispanics (e.g. FBI database, Monson et al., 2002; Saunier et al., 2008) and Venezuelans (Castro de Guerra et al., 2012) can also be proposed, while complete mitongenome sequences of several Puerto Ricans attest to its Caribbean distribution (Zheng et al., 2012).

The extended mtDNA region analyzed in this study permits the determination of two other subtypes showing mutations in the extended 5' region. These include D1f1, which is characterized by mutations at np 16,179, 16,295, 16,142, as well as 16,497, and B2h, which is characterized by a mutation at np 16,468 (van Oven and Kayser, 2009, build 15). D1f1 was previously found in different ethnic groups from the Amazonian region, including the Mbyá Guaraní (Fagundes et al., 2008; Mazières et al., 2008; Sala et al., 2010), while B2h was found in one Ache (Paraguay) and one Guaraní Indian (Parana State, Brazil).

In light of these findings, we would like to underscore the importance of analyzing sequences beyond the hypervariable regions, stressing the importance of private mutations to analyze the origin of, and migration routes taken by, individuals and populations (Bodner et al., 2012; Garcia et al 2012; Sans et al., 2012). The mtDNAs of living individuals, together with those of prehistoric populations, will ultimately provide the clues for understanding the past and present of Native American populations.

As for the origin of the Amerindian contribution in northwestern Uruguay, two possible alternatives may be proposed. First, the present day lineages from the northwestern region of Uruguay do not reflect the historical populations of the region, especially, those with whom Bella Unión (Santa Rosa del Cuareim) was founded. Alternatively, the observed patterns of diversity are in agreement with those from the Jesuit Mission Indians, and their variability is related to the different ethnic groups that historically inhabited the Missions—depending on the historical moment and geographical location. It is interesting to note that a substantial majority of the mtDNA sequences with (near) matches are related to Amazonian ethnic groups, with at least 61.9% (if considering near-matches with Natives only), or 66.7% (when considering also cosmopolitan locations) having Amazonian affinities. When analyzing the mtDNA sequence origins, the similarities are mostly with Tupí speakers such as the Guaraní Indians.

A final issue to be mentioned concerns the Indians living in the Jesuit Missions. While some authors refer to the people as "Guaraní-Missionaries," and to the Reductions as "Guaraní Missions" (see for example González Rissotto and Rodríguez Varese, 1982, 1989), it is important to recognize that not all natives living at these Jesuit Missions were Guaraní. For example, the southern Missions of Yapeyú and San Borja were also founded with Charrúa and Guenoa Indians from the "Pampas" (Del Techo, 1897; Furlong, 1978; Torre Revello, 1932). Different ethnic groups used the Guaraní language before the Spanish conquest, thus generating confusion about their ethnicity. For this reason, Curbelo and Barreto (2010) have suggested the use of the term "Indians from the Missions" ("indígenas misioneros") instead of Guaraní from the Missions ("Guaraní misioneros") to reflect the fact that, although living in the missions they were part of different ethnic groups. It must be emphasized that these natives had a relatively homogeneous culture due both to the imposition of the Catholic religion, and to their similar political positions as their adhesion to General José G. Artigas, the Uruguayan independence hero (Meliá, 1986; Padrón, 1991, 1996; Poenitz and Poenitz, 1993). It is therefore most probable that these natives were not genetically homogeneous, due to their different origins, and part of that genetic variability that we are now detecting in their descendants reflects this fact, with a majority of Native American DNAs having an Amazonian origin but others coming from Chaco, northwestern Argentina, Patagonia and the Andean regions.

ACKNOWLEDGMENTS

The authors thank the people of Bella Unión, Tomás Gomensoro, and Baltasar Brum for their kind collaboration giving samples and data. They also thank Bernardo Bertoni (Department of Genetics, UDELAR, Uruguay) for his collaboration in DNA extraction, Jodi Irwin, Jessica Saunier, Melissa Scheible, Kimberly Sturk-Andreaggi, Rachel Rubenstein, Erin Gorden, Christopher May, and Kimberly Root lab staff (AFDIL Research Section, USA) for assistance with data analysis and review. The authors also thank the anonymous reviewers and the editors for their constructive comments. The opinions or assertions presented herein are the private views of the authors and should not be construed as official or as reflecting the views of any institution.

LITERATURE CITED

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

Achilli A, Rengo C, Magri C, Battaglia V, Olivieri A, Scozzari R, Cruciani F, Zeviani M, Briem E, Carelli V, Moral P, Dugoujon JM, Roostalu U, Loogväli EL, Kivisild T, Bandelt HJ, Richards M, Villems R, Santachiara-Benerecetti AS, Semino O, Torroni A. 2004. The molecular dissection of mtDNA haplogroup H confirms that the Franco-Cantabrian glacial refuge was a major source for the European gene pool. Am J Hum Genet 75:910–918.

Acosta y Lara E. 1979. Los Guaraníes en el antiguo territorio de la Republica Oriental del Uruguay. Ultimas campañas contra los Charrúas. Montevideo: Impr. Monteverde y Cia., p 203.

Acosta y Lara E. 1985. Salsipuedes 1831: los lugares. Rev Fac Hum Ciencias (Montevideo), serie Ciencias. Antropológicas 1:65–88.

Acosta y Lara E. 1989. La guerra de los Charrúas en la Banda Oriental. Montevideo: Impr. Linardi y Risso.

Alves-Silva J, da Silva Santos M, Guimarães PE, Ferreira AC, Bandelt HJ, Pena SD, Prado VF. 2000. The ancestry of Brazilian mtDNA lineages. Am J Hum Genet 67:444–461.

Avena SA, Goicoechea AS, Bartomioli M, Fernández V, Cabrera A, Dugoujon JM, Dejean CB, Fabrykant G, Carnese FR. 2007. Mestizaje en

el sur de la región pampeana (Argentina). Su estimación mediante el análisis de marcadores proteicos y moleculares uniparentales. Rev Arg Anthropol Biol 9:59–76.
Avena SA, Parolin ML, Boquet M, Dejean CB, Postillone MB, Alvarez Trentini Y, Di Fabio Rocca F, Mansilla F, Jones L, Dugoujon JM, Carnese FR. 2010. Mezcla génica y linajes uniparentales en Esquel (Pcia. de Chubut): Su comparación con otras muestras poblacionales argentinas. J Basic Appl Genet 21:1–14.
Avena SA, Parolin ML, Dejean CB, Ríos Part MC, Fabrikant G, Alicia S. Goicoechea AS, Dugoujon JM, Carnese FR. 2009. Mezcla génica y linajes uniparentales en Comodoro Rivadavia (Provincia de Chubut, Argentina). Rev Arg Antrop Biol 11:25–41.
Bandelt HJ, Alves-Silva J, Guimarães PE, Santos MS, Brehm A, Pereira L, Coppa A, Larruga JM, Rengo C, Scozzari R, Torroni A, Prata MJ, Amorim A, Prado VF, Pena SD. 2001. Phylogeography of the human mitochondrial haplogroup L3e: a snapshot of African prehistory and Atlantic slave trade. Ann Hum Genet 65:549–563.
Bandelt HJ, Quintana-Murci L, Salas A, Macaulay V. 2002. The fingerprint of phantom mutations in mitochondrial DNA data. Am J Hum Genet 71:1150–1160.
Bandelt HJ, van Oven M, Salas A. 2012. Haplogrouping mitochondrial DNA sequences in legal medicine/forensic genetics. Int J Legal Med 126: 901–916.
Barreto I, Curbelo C. 2009. Presencia indígena en el Uruguay: movilidad, estructura demográfica y conformación familiar al norte del Río Negro en el primer tercio del siglo XIX. Revista Digital de Estudios Históricos CDHRP (Rivera) 1:1–19.
Bisso-Machado R, Bortolini MC, Salzano FM. 2012. Uniparental genetic markers in South Amerindians. Genet Mol Biol 35:365–387.
Bobillo MC, Zimmermann B, Sala A, Huber G, Röck A, Bandelt HJ, Corach D, Parson W. 2010. Amerindian mitochondrial DNA haplogroups predominate in the population of Argentina: towards a first nationwide forensic mitochondrial DNA sequence database. Int J Legal Med 124: 263–268.
Bodner M, Perego UA, Huber G, Fendt L, Röck AW, Zimmermann B, Olivieri A, Gómez-Carballa A, Lancioni H, Angerhofer N, Bobillo MC, Corach D, Woodward SR, Salas A, Achilli A, Torroni A, Bandelt HJ, Parson W. 2012 Rapid coastal spread of First Americans: novel insights from South America's Southern Cone mitochondrial genomes. Genome Res 22:811–820.
Bonilla C, Bertoni B, González S, Cardoso H, Brum-Zorrilla N, Sans M. 2004. Substantial native American female contribution to the population of Tacuarembó, Uruguay, reveals past episodes of sex-biased gene flow. Am J Hum Biol 16:289–297.
Brandstätter A, Niederstätter H, Pavlic M, Grubwieser P, Parson W. 2007. Generating population data for the EMPOP database - an overview of the mtDNA sequencing and data evaluation processes considering 273 Austrian control region sequences as example. Forensic Sci Int 166:164–175.
Brandstätter A, Peterson CT, Irwin JA, Mpoke S, Koech DK, Parson W, Parsons TJ. 2004. Mitochondrial DNA control region sequences from Nairobi (Kenya): inferring phylogenetic parameters for the establishment of a forensic database. Int J Legal Med 118:294–306.
Cabana G, Merriwether AD, Hunley K, Demarchi DA. 2006. Is the genetic structure of Gran Chaco populations unique? Interregional perspectives on Native South American mitochondrial DNA variation. Am J Phys Anthropol 131:108–119.
Cabrera Pérez LL, Curbelo MC 1988. As Missoes Jesuítico-Guaranis: Cultura e Sociedade. Santa Rosa (RGS): Proceedings of the VI Simposio Nacional de Estudos Missioneiros. p 117–141.
Cardoso S, Alfonso-Sánchez MA, Valverde L, Odriozola A, Pérez-Miranda AM, Peña JA, de Pancorbo MM. 2011. The maternal legacy of Basques in northern Navarre: new insights into the mitochondrial DNA diversity of the Franco-Cantabrian area. Am J Phys Anthropol 145:480–488.
Cardozo Soto SC, Chanquet M, Fernández Ramos VM, Galeano N, Hernández Faccio JM, Wettstein G. 1988. La región de Bella Unión, cooperativismo y desarrollo agro-industrial. Montevideo: Ed. Indice. p 135.
Castro de Guerra D, Figuera Pérez C, Bravi CM, Saunier J, Scheible M, Irwin J, Coble MD, Rodríguez-Larralde A. 2012. Sequence variation of mitochondrial DNA control region in North Central Venezuela. Forensic Sci Int Genet 6:e131–133.
Catelli ML, Alvarez-Iglesias V, Gómez-Carballa A, Mosquera-Miguel A, Romanini C, Borosky A, Amigo J, Carracedo A, Vullo C, Salas A. 2011. The impact of modern migrations on present-day multi-ethnic Argentina as recorded on the mitochondrial DNA genome. BMC Genet 12:77.
Corach D, Lao O, Bobillo C, van Der Gaag K, Zuniga S, Vermeulen M, van Duijn K, Goedbloed M, Vallone PM, Parson W, de Knijff P, Kayser M. 2010. Inferring continental ancestry of argentineans from Autosomal, Y-chromosomal and mitochondrial DNA. Ann Hum Genet 74:65–76.
Curbelo C, Barreto I. 2010. Misiones jesuíticas e indígenas misioneros en Uruguay. Conocimiento aplicado para la integración al turismo cultural regional. 4to. Congreso Latinoamericano de Investigación Turística). Montevideo: Facultad de Humanidades y Ciencias de la Educación and Ministerio de Deporte y Turismo (CD ROM).
de Saint Pierre M, Bravi CM, Motti JM, Fuku N, Tanaka M, Llop E, Bonatto SL, Moraga M. 2012. An alternative model for the early peopling of southern South America revealed by analyses of three mitochondrial DNA haplogroups. PLoS One 7:e43486.
Del Techo N. 1897. Historia de la Provincia del Paraguay de la Compañía de Jesús, Vol. 3. Madrid: Editorial A. de Uribe y Cia. p 204.
Dejean CB, Goicoechea AS, Avena SA, Salaberry MT, Slepoy AS, Carnese FR. 2003. Linajes mitocondriales amerindios en una muestra poblacional de la región metropolitana de Buenos Aires. RAAB 5:65.
Dipierri JE, Alfaro E, Martínez-Marignac VL, Bailliet G, Bravi CM, Cejas S, Bianchi NO. 1998. Paternal directional mating in two Amerindian subpopulations located at different altitudes in northwestern Argentina. Hum Biol 70:1001–1010.
Dornelles CL, Bonatto SL, De Freitas LB, Salzano FM. 2005. Is haplogroup X present in extant South American Indians? Am J Phys Anthropol 127:439–448.
Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Res 10:564–567.
Fagundes NJ, Kanitz R, Bonatto SL. 2008. A reevaluation of the Native American mtDNA genome diversity and its bearing on the models of early colonization of Beringia. PLoS One 3:e3157.
Felsenstein J. 2005. PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Seattle: Department of Genome Sciences, University of Washington.
Furlong G. 1978. Misiones y sus pueblos de guaraníes. Buenos Aires: Balmes. p 788.
García A, Pauro M, Nores R, Bravi CM, Demarchi DAl 2012. Phylogeography of mitochondrial haplogroup D1: an early spread of subhaplogroup D1j from Central Argentina. Am J Phys Anthropol 149:583–590.
Ginther C, Corach D, Penacino GA, Rey JA, Carnese FR, Hutz MH, Anderson A, Just J Salzano FM, King MC. 1993. Genetic variation among the Mapuche Indians from the Patagonian region of Argentina: mitochondrial DNA sequence variation and allele frequencies of several nuclear genes. EXS 67:211–219.
González Rissotto R, Rodríguez Varese S. 1982. Contribuciones al estudio de la influencia guaraní en la formación de la sociedad uruguaya. Rev Histórica (Montevideo) 54:199–316.
González Rissotto R, Rodríguez Varese S. 1989. La importancia de las Misiones Jesuíticas en la formación de la sociedad uruguaya. Estudos Ibero-Americanos (PUCRS) 15:191–214.
González Rissotto R, Rodríguez Varese S. 1990. Los últimos pueblos de guaraníes en la Banda Oriental del Uruguay entre los años de 1820 y 1862. In: Missões: Trabalho e Evangelização. Santa Rosa, Brazil: Facultad de Filosofía, Ciências e Letras Dom Bosco. p 203–241.
González Rissotto R., Rodríguez Varese S. 1991. Guaraníes y Paisanos. Colección Nuestras Raíces, Vol. 3. Montevideo: Editorial Nuestra Tierra. p 68.
Gronau I, Moran S. 2007. Optimal implementations of UPGMA and other common clustering algorithms. Inform Process Lett 104:205–210.
INE 2011. Censo 2011, Instituto Nacional de Estadística. Montevideo: Instituto Nacional de Estadística. Available at: http://www.ine.gub.uy/socio-demograficos/pobhogyviv2008.asp - Censos_T4.xls. Accessed April 3, 2014.
Irwin JA, Saunier JL, Strouss KM, Sturk KA, Diegoli TM, Just RS, Coble MD, Parson W, Parsons TJ. 2007. Development and expansion of high-quality control region databases to improve forensic mtDNA evidence interpretation. Forensic Sci Int Genet 1:154–157.
Isola E. 1975. La Esclavitud en el Uruguay desde sus Comienzos hasta su Extinción (1943-1852). Montevideo: Talleres Gráficos Monteverde y Cia. p 334.
Just RS, Diegoli TM., Saunier JL, Irwin JA, Parsons TJ. 2008. Complete mitochondrial genome sequences for 265 African American and U.S. 'Hispanic' individuals. Forensic Sci Int Genet 2:e45–e48.
Maca-Meyer N, González AM, Larruga JM, Flores C, Cabrera VM. 2001. Major genomic mitochondrial lineages delineate early human expansions. BMC Genet 2:13.
Maca-Meyer N, González AM, Pestano J, Flores C, Larruga JM, Cabrera VM. 2003. Mitochondrial DNA transit between West Asia and North Africa inferred from U6 phylogeography. BMC Genet 4:15.
Marrero AR, Bravi C, Stuart S, Long JC, Pereira das Neves Leite F, Kommers T, Carvalho CM, Pena SD, Ruiz-Linares A, Salzano FM, Bortolini MC. 2007a. Pre- and post-Columbian gene and cultural continuity: the case of the Gaucho from southern Brazil. Hum Hered 64:160–171.
Marrero AR, Silva-Junior WA, Bravi CM, Hutz MH, Petzl-Erler ML, Ruiz-Linares A, Salzano FM, Bortolini MC. 2007b. Demographic and

evolutionary trajectories of the Guarani and Kaingang natives of Brazil. Am J Phys Anthropol 132:301–310.
Martínez-Cruzado JC, Toro-Labrador G, Viera-Vera J, Rivera-Vega MY, Startek J, Latorre-Esteves M, Román-Colón A, Rivera-Torres R, Navarro-Millán IY, Gómez-Sánchez E, Caro-González HY, Valencia-Rivera P. 2005. Reconstructing the population history of Puerto Rico by means of mtDNA phylogeographic analysis. Am J Phys Anthropol 128:131–155.
Martínez-Marignac VL, Bravi CM, Lahitte HB, Bianchi NO.1999. Estudio del ADN mitocondrial de una muestra de la ciudad de la Plata. RAAB 2: 281–300.
Mazières S, Guitard E, Crubézy E, Dugoujon JM, Bortolini MC, Bonatto SL, Hutz MH, Bois E, Tiouka F, Larrouy G, Salzano FM. 2008. Uniparental (mtDNA, Y-chromosome) polymorphisms in French Guiana and two related populations–implications for the region's colonization. Ann Hum Genet 72:145–156.
Meliá, B. 1986. El guaraní conquistado y reducido. Asunción: Biblioteca Paraguaya de Antropología 5.
Miller MP. 1997. R x C: Program for the analysis of contingency tables. Flagstaff: Northern Arizona University. http://www.marksgeneticsoftware.net/rxc.htm.
Miller SA, Dykes DD, Polesky HF. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215.
Monson KL, Miller KWP, Wilson MR, DiZinno JA, Budowle B. 2002. The mtDNA Population Database: an integrated software and database resource for forensic comparison. Forensic Sci Comm 4:2. Available at: http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/april2002/miller1.htm. Accesed February 13, 2014.
Nei M. 1987. Molecular evolutionary genetics. New York: Columbia University Press.
Nicholas KB, Nicholas HB Jr, Deerfield DWII. 1997. GeneDoc: analysis and visualization of genetic variation. EMBNEW News 4:14.
Oyhantçabal G, Carámbula M. 2011. Lucha por la tierra en el norte de Uruguay. Astrolabio, Nueva Epoca (Córdoba) 7:284–312.
Padrón O. 1991. Tres documentos de los Siete Pueblos Orientales, 1828–1829. Estudos Ibero-Americanos (PUCRS) 17:17–29.
Padrón O. 1996. Ocaso de un pueblo indio. Historia del éxodo guaraní-misionero al Uruguay. Montevideo: Editorial Fin de Siglo. p 323.
Pagano S, Sans M, Pimenoff V, Cantera AM, Alvarez JC, Lorente JA, Peco JM, Mones P, Sajantila A. 2005. Assessment of HV1 and HV2 mtDNA variation for forensic purposes in an Uruguayan population sample. J Forensic Sci 50:1239–1242.
Palencia L, Valverde L, Alvarez A, Cainé LM, Cardoso S, Alfonso-Sánchez MA, Pinheiro MF, de Pancorbo MM. 2010. Mitochondrial DNA diversity in a population from Santa Catarina (Brazil): predominance of the European input. Int J Legal Med 124:331–336.
Parson W, Dür A.2007. EMPOP-a forensic mtDNA database. Forensic Sci Int Genet. 1:88–92.
Pereira L, Silva NM, Franco-Duarte R, Fernandes V, Pereira JB, Costa MD, Martins H, Soares P, Behar DM, Richards MB, Macaulay V. 2010. Population expansion in the North African late Pleistocene signaled by mitochondrial DNA haplogroup U6. BMC Evol Biol 10:390.
Pi Hugarte R, Vidart D. 1969. El legado de los inmigrantes, I. Montevideo: Ed. Nuestra Tierra, p 60.
Poenitz E, Poenitz A. 1993. Misiones Provincia Guaranítica. Defensa y disolución. Posadas: Editorial Universitaria UNM. p 282.
R Development Core Team. 2011. R: a language and environment for statistical computing. Vienna: The R Foundation for Statistical Computing. Available at: http://www.r-project.org/. Accesed June 24, 2014.
Reidla M, Kivisild T, Metspalu E, Kaldma K, Tambets K, Tolk HV, Parik J, Loogväli EL, Derenko M, Malyarchuk B, Bermisheva M, Zhadanov S, Pennarun E, Gubina M, Golubenko M, Damba L, Fedorova S, Gusar V, Grechanina E, Mikerezi I, Moisan JP, Chaventré A, Khusnutdinova E, Osipova L, Stepanov V, Voevoda M, Achilli A, Rengo C, Rickards O, De Stefano GF, Papiha S, Beckman L, Janicijevic B, Rudan P, Anagnou N, Michalodimitrakis E, Koziel S, Usanga E, Geberhiwot T, Herrnstadt C, Howell N, Torroni A, Villems R. 2003. Origin and diffusion of mtDNA haplogroup X. Am J Hum Genet 73:1178–1190.
Saint-Hilaire A de 1961. Voyage a Rio Grande do Sul (1820–1821). Anales Históricos de Montevideo 4:307–532.
Salas A, Jaime JC, Alvarez-Iglesias V, Carracedo A. 2008. Gender bias in the multiethnic genetic composition of central Argentina. J Hum Genet 53:662–674.
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.
Sans M, Merriwether DA, Hidalgo PC, Bentancor N, Weimer TA, Franco MH, Alvarez I, Kemp BM, Salzano FM. 2006. Population structure and admixture in Cerro Largo, Uruguay, based on blood markers and mitochondrial DNA polymorphisms. Am J Hum Biol 18:513–524.
Sans M, Figueiro G, Ackermann E, Barreto I, Egaña A, Bertoni B, Poittevin-Gilmet E, Maytia D, Hidalgo PC. 2011. Mitochondrial DNA in Basque descendants from the city of Trinidad, Uruguay: Uruguayan- or Basque-like population? Hum Biol 83:55–70.
Sans M, Figueiro G, Hidalgo PC. 2012. A new mitochondrial C1 lineage from the prehistory of Uruguay: population genocide, ethnocide, and continuity. Hum Biol 84:287–305.
Saunier JL, Irwin JA, Just RS, O'Callaghan J, Parsons TJ. 2008 Mitochondrial control region sequences from a U.S. "Hispanic" population sample. Forensic Sci Int Genet 2:e19–e23.
Schliep KP. 2011. Phangorn: phylogenetic analysis in R. Bioinformatics 27:592–593.
Sneath P, Sokal R. 1973. Numerical Taxonomy: the principles and practice of numerical classification. San Francisco: W H Freeman. p 373.
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.
Tamura K, Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526.
Torre Revello J. 1932. Informe sobre misiones de indios existentes en la segunda mitad del siglo XVIII, en las provincias del Paraguay (de los padres jesuitas) y de la Asunción (de los padres franciscanos). Boletín del Instituto de Investigaciones Históricas (Buenos Aires) 13:99–123.
Torroni A, Schurr TG, Margaret F. Cabell MF, Brown MD, Neel JV, Larsen M, Smith DG, Carlos M. Vullo CM, Wallace DC. 1993. Asian affinities and continental radiation of the four founding Native American mtDNAs. Am J Hum Genet 53:563–590.
Torroni A, Achilli A, Macaulay V, Richards M, Bandelt HJ. 2006. Trends Genet 22:339–345.
van Oven M, Kayser M. 2009. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat 30:e386–e394.
Venables WN, Ripley BD. 2002. Modern applied statistics with S, 4th ed. New York: Springer. p 497.
Zheng HX, Yan S, Qin ZD, Jin L. 2012. MtDNA analysis of global populations support that major population expansions began before Neolithic Time. Sci Rep 2:745.